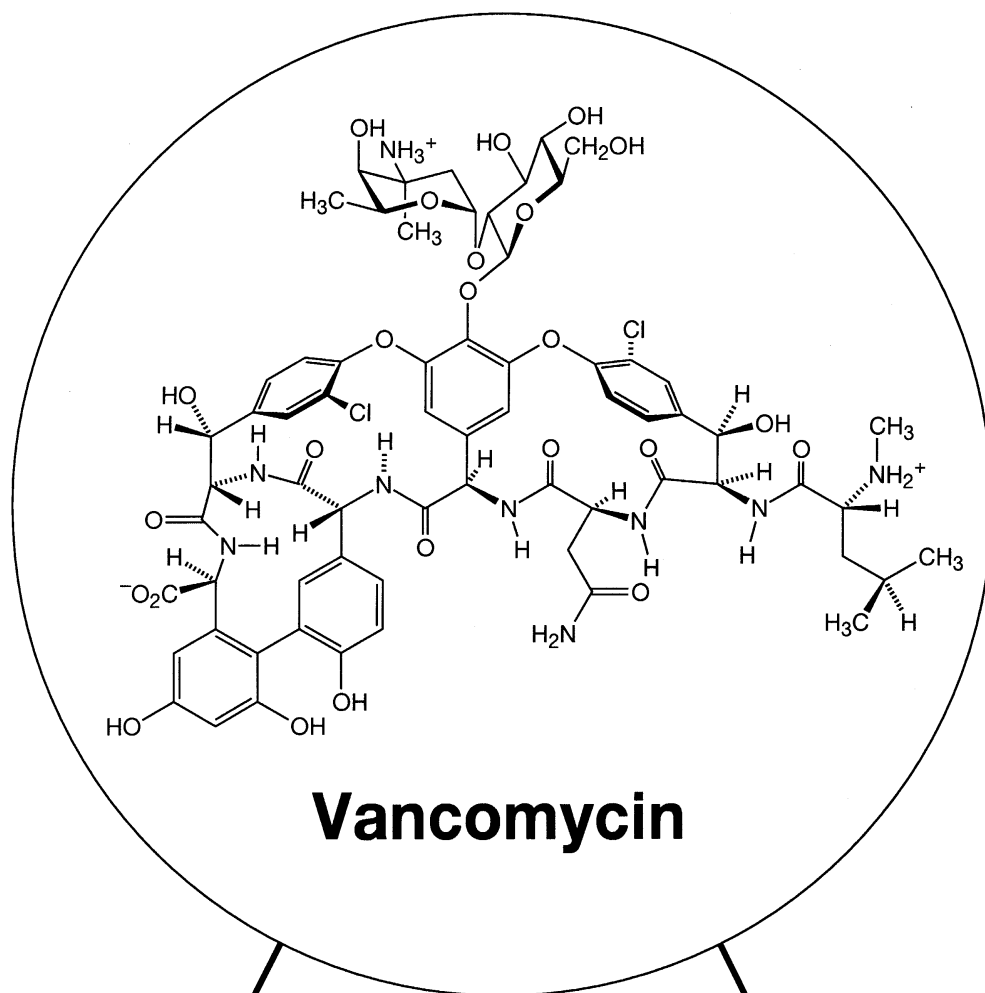
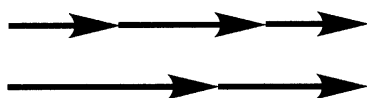


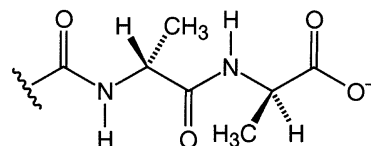


Structure and Biosynthesis



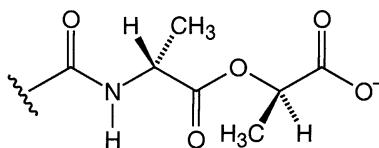
Genes encoding
Biosynthetic
Apparatus

Mode of Action



Binding to
-D-Ala-D-Ala

Bacterial Resistance



Cell Wall Precursors
Terminating in
-D-lactate

The Vancomycin Group of Antibiotics and the Fight against Resistant Bacteria

Dudley H. Williams* and Ben Bardsley

This review is an account of the discoveries in the Cambridge group of the structures and mode of action of the glycopeptide antibiotics of the vancomycin group. These antibiotics are now of enormous clinical importance, for among their members are two (vancomycin and teicoplanin) of the three antibiotics of last resort against resistant bacterial pathogens (particularly methicillin-resistant *Staphylococcus aureus* (MRSA, or “superbugs”)) in our hospitals, which would otherwise often be lethal. Their combined sales are of the order of US\$1 billion per annum. The structure determination in our laboratory started just over 25 years ago. Within ten years the first

glycopeptide structures had been determined. This was quickly followed by the determination of the molecular basis of their action, through the making of five hydrogen bonds to a cell wall peptide precursor that terminated in -Lys-D-Ala-D-Ala. In the early 1990s, we established that all the glycopeptides so far examined (other than teicoplanin) form dimers, and shortly after showed that dimerization promotes antibiotic activity. Concurrently, we were able to demonstrate that teicoplanin possesses a membrane anchor that can act to promote antibiotic activity in lieu of dimerization. The devices of dimerization and membrane anchoring, when acting concurrently,

appear to be able to account at least in part for the remarkable activity of a new semi-synthetic glycopeptide—developed at Eli Lilly—against bacteria that are resistant even to vancomycin. A full understanding of these devices is important, since occurrences of the “nightmare scenario” that clinicians have feared over recent years—that resistance to vancomycin will appear in new forms of MRSA—have recently been reported.

Keywords: antibiotics • cooperative effects • dimerizations • resistant bacteria • vancomycin

1. Introduction

One of the most important steps in fighting the microbial pathogens that pose a serious threat to human health came with the improvement of sanitation in the cities, by improving systems for drainage and removal of human waste. A second crucial step was the beginning of the antibiotic era in the 1930s, for example, the use of the sulfonamides against pneumonia, and the use of penicillin at the time of the Second World War. These antibiotics and their successors proved extremely effective in eradicating infectious diseases that had previously been potentially lethal. However, selectional processes can work rapidly in bacteria because of their short generation time and their ability to pass genetic information between species through a process called conjugation (Figure 1). These properties led to the widespread development of resistance to many of the new antibiotics, including penicillin, and by the late 1950s up to 85% of clinical isolates of

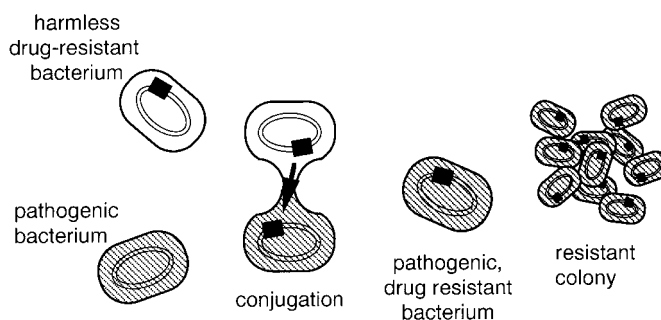


Figure 1. Drug resistance can be passed horizontally between species of bacteria. For example, a nonpathogenic, but antibiotic-resistant bacterium can physically join with a disease-causing bacterium and pass on the genetic information required for antibiotic resistance through a process called conjugation. With the advantage of drug resistance, the pathogenic bacterium can then proliferate into an untreatable, disease-causing colony.

staphylococci (disease-causing bacteria) were found to be penicillin-resistant.^[1] Since then, despite the isolation and development of new antibacterial agents (including naturally-occurring, semi-synthetic and fully synthetic compounds), bacterial strains resistant to virtually all known antibiotics have emerged. In the past decade the problem has become more acute with resistance to even the antibiotics of last resort, the vancomycin group, developing in certain species,

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and the prospect of a return to the pre-antibiotic days, with some infections being effectively untreatable, is becoming a reality.^[1–3]

It is against this background that we will describe the role that the vancomycin group of glycopeptide antibiotics have played in therapy during the last 40 years and how, in the research group in Cambridge, we elucidated the molecular basis for their mode of action. We then discuss how, in recent times, bacterial resistance to members of this group has developed and how the principles of action of a new semi-synthetic glycopeptide, which is active against these bacteria, emerged from our work. We also discuss the biosynthetic origins of these antibiotics and how, in the future, it may be possible to engineer the gene clusters encoding the enzymes responsible for this biosynthesis in order to produce novel, and possibly more efficient, antibiotics. Finally, we will discuss aspects of cooperativity in the mode of action of the glycopeptides that we have identified, and which can be used as a model for cooperative binding in more complex biological systems. The present review complements that produced by one of us (D.H.W.) in 1996.^[4]

2. The Vancomycin Group of Antibiotics

Vancomycin was first isolated from a soil sample collected by the American pharmaceutical company Eli Lilly in the mid-1950s.^[5] It is produced by the microorganism *Amycolatopsis orientalis* and was first used clinically in 1959. Its early use was somewhat limited, however, by side effects that resulted from imperfections in the purification process.^[6] For example, when vancomycin was administered intravenously phlebitis would sometimes occur near the point of injection. Its use was also not recommended in patients with any history of hearing difficulties or kidney failure.^[7] When improved purification techniques for vancomycin became available in later years, these side-effects were much reduced and it

became more widely prescribed, particularly in combating infections arising from *Staphylococcus aureus*, the majority of clinically-isolated strains of which were resistant to penicillin. The acquisition by methicillin-resistant *Staphylococcus aureus* (MRSA, a serious pathogen commonly found in hospitals and responsible for large numbers of deaths) of resistance to virtually all antibiotics in clinical use, including cephalosporins, tetracyclines, aminoglycosides, erythromycin, and the sulfonamides, has propelled the vancomycin group of glycopeptide antibiotics to the forefront of the fight against these bacteria. Two members of the group are in clinical use today, vancomycin^[8] and teicoplanin,^[9] and these glycopeptides, along with gentamycin, are currently the antibiotics of last resort in our hospitals.

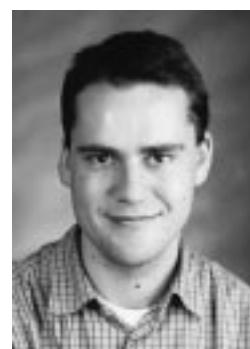
3. Glycopeptide Structure Determination

The first attempt to determine the structure of vancomycin, reported in 1965, indicated the presence of *N*-methyleucine, glucose, and chlorophenols within the molecule.^[10] Further progress was slow, however, because the solving of such a complex structure required techniques that had not been developed at this point. Our efforts to solve the structure of vancomycin began in 1970 and utilized developing techniques, such as NMR spectroscopy, to achieve the target. In particular, the use of the negative nuclear Overhauser effect (NOE) in proton NMR spectroscopy to derive structural information represented a major advance at the time.^[11] We were able to use this technique to obtain approximate interproton distances in the solution conformation of vancomycin for protons that were located close to each other in the vancomycin structure and were thus able to obtain a partial structure of vancomycin by 1977.^[12] A further advance was then provided by solution of the crystal structure of CDP-1, a degradation product of vancomycin.^[13] The resulting putative structures for vancomycin were incorrect, however, in that they mis-

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D. Williams

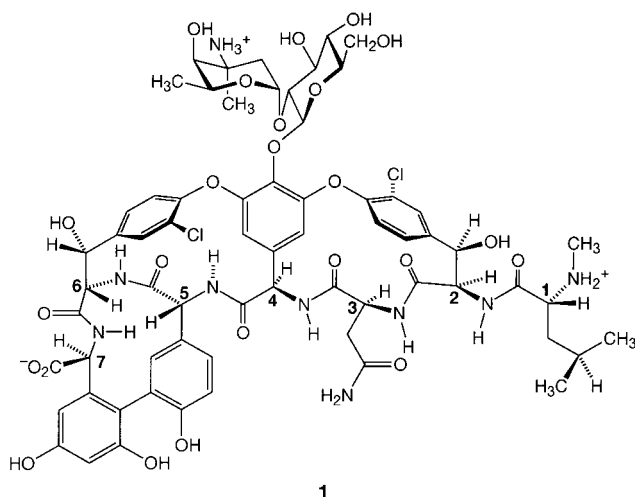


B. Bardsley

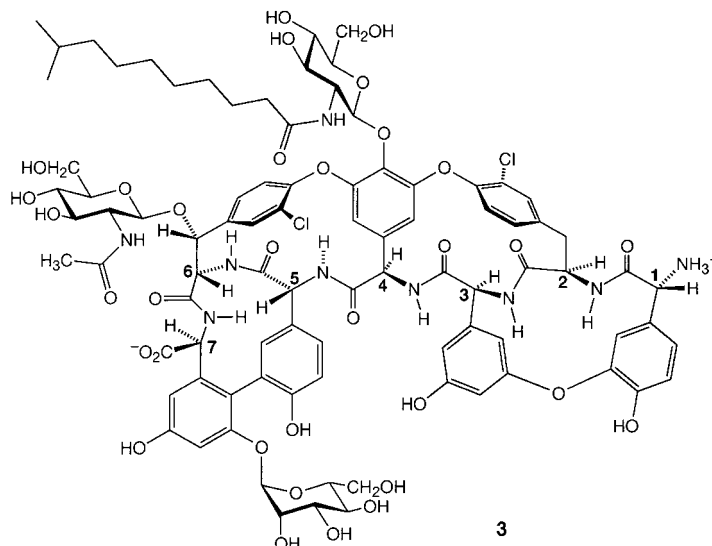
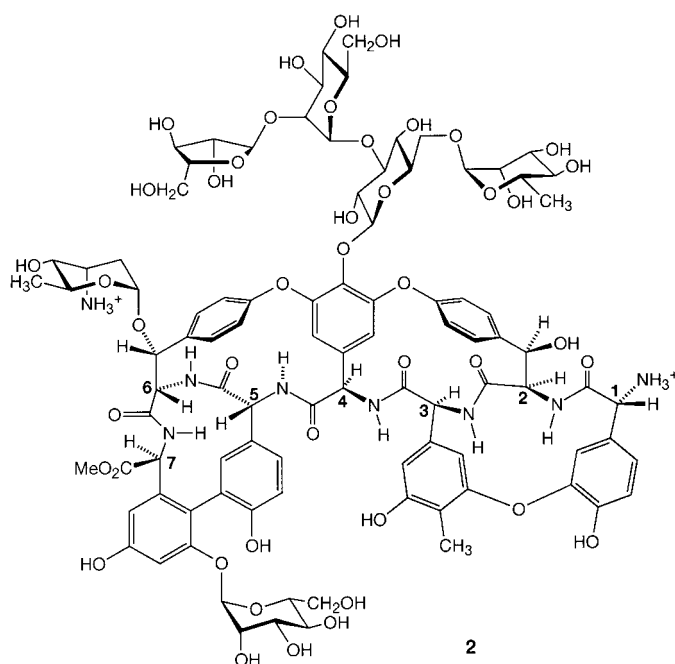
Ben Bardsley was born in Norwich in 1972 and received his bachelor degree in Natural Sciences at the University of Cambridge in 1994. He gained his Ph D. degree under the supervision of Dudley Williams at the University of Cambridge in 1998, studying aspects of cooperativity in molecular recognition and, in particular, cooperativity in the mode of action of the vancomycin group antibiotics. He is currently undertaking further research in this area with Dudley Williams.

represented the conformation of a chlorinated aromatic ring and incorporated an isoasparagine residue (rather than an asparagine residue). The former error was corrected by ourselves^[14] and the latter by Harris and Harris^[15] to give the now-accepted structure **1**.

The increasing power of proton NMR spectroscopy meant that other structural determinations of members of the vancomycin group could be carried out relatively quickly compared to the pioneering work on vancomycin. In the



structure determination of ristocetin A (**2**), important work on the sugars was carried out in Hungary,^[16] but the structure of the peptide portion was determined in our own group.^[17] This was followed by the structure determination of teicoplanin (**3**), which was carried out in our group and completed in 1984.^[18] Of these two antibiotics, teicoplanin is important as the only other member of the group apart from vancomycin in current clinical use. Ristocetin A was introduced into some hospitals in the late 1950s but was withdrawn soon afterwards



because of the deaths from side-effects of a number of patients being treated with it. These two antibiotics typify the structural variation within the group: ristocetin A is the only member of the group so far discovered to possess a tetrasaccharide (as opposed to a disaccharide or monosaccharide) attached to the sidechain of the fourth amino acid residue of the peptide backbone, whereas a C₁₁ acyl side chain attached to a glucosamine residue is incorporated at the equivalent position of teicoplanin. This last feature confers favorable pharmacological properties on teicoplanin (see later for details). At the time of writing, the structures of over 100 glycopeptide antibiotics have been established.

All the known glycopeptide structures consist of a peptide backbone constituted from seven amino acids. If these are numbered 1 to 7 from the N- to the C-terminus, then residues 4 and 5 are always constituted from *p*-hydroxyphenylglycines, and residues 2 and 6 from tyrosine, whereas residue 7 is 3,5-dihydroxyphenylglycine. In many of the antibiotics described to date, residues 1 and 3 are both aliphatic amino acids. For example, residue 1 is commonly *N*-methyleucine, and residue 3 is asparagine (for example, vancomycin (**1**)). On the other hand, both these residues may occur as aromatic amino acids, as in the case of ristocetin A (**2**) and of teicoplanin (**3**); in these cases, residue 1 is again *p*-hydroxyphenylglycine and residue 3 is 3,5-dihydroxyphenylglycine (also *p*-methylated in the case of ristocetin A). Where residues 1 and 3 are aromatic, they may, or may not, be cross-linked. In all cases where the stereochemistry has been examined, the stereochemistry at the α -C centers of the amino acids 1 to 7 are *R,R,S,R,R,S,S*. Other structural features of note, which vary within different members of the group, include chlorine atoms attached to the aromatic rings of residues 2 and/or 6 (for example, **1** and **3**), a β -hydroxy group on residues 2 and 6 (for example, **1** and **2**) and sugar residues attached to the sidechains of residue 4 (for example, **1**–**3**), residue 6 (for example, **2** and **3**), and residue 7 (for example, **2** and **3**). The combination of unusual structural features exhibited by these antibiotics means that as well as being of crucial clinical importance they provide a formidable challenge to synthetic chemists. Recently, however, total syntheses

of the aglycone of vancomycin have been completed and described by the groups of Evans^[19] and Nicolaou.^{[20][*]}

4. Observations on the Biosynthetic Origins of the Glycopeptides

An examination of the structures **1–3** allows some reasonable speculation as to their biosynthetic origins. It is a plausible expectation that in **2** and **3** the cross-linking of all the amino acid side chains (1 → 3, 2 → 4, 4 → 6, and 5 → 7) occurs by means of phenolic oxidative coupling. The first two of these hypothesized couplings are of oxygen to carbon atoms, but the 5 → 7 coupling is carbon to carbon. The biosynthetic experiments that we performed on vancomycin and ristocetin A are consistent with these proposals. To aid these studies, we first assigned the ¹³C NMR spectra of vancomycin^[21] and ristocetin A.^[22] A fermentation of the vancomycin-producing organism was then supplemented with [1,2-¹³C₂]acetate. The ¹³C spectrum of the derived product revealed enrichment with [1,2-¹³C₂]acetate at all eight carbon atoms of residue 7 in a manner that was consistent with its derivation from four units of acetate.^[23] A similar feeding experiment with the producing strain of ristocetin A revealed a similar origin of residue 7 in this antibiotic, and also that its residue 3 was derived in the same manner.^[24] The production of both these antibiotics was also investigated in media supplemented with isotopically-labeled tyrosine and phenylglycine. Analysis of the products established that:

- 1) residues 2 and 6 are, in both cases, derived from tyrosine
- 2) residues 4 and 5 are, in both cases, also derived from tyrosine (as is residue 1 of ristocetin A).

Evidently, the producing organisms can derive the *p*-hydroxyphenylglycine residues from tyrosine. The study on vancomycin also established that the benzylic hydroxy groups found in residues 2 and 6 are introduced with retention of configuration.^[23]

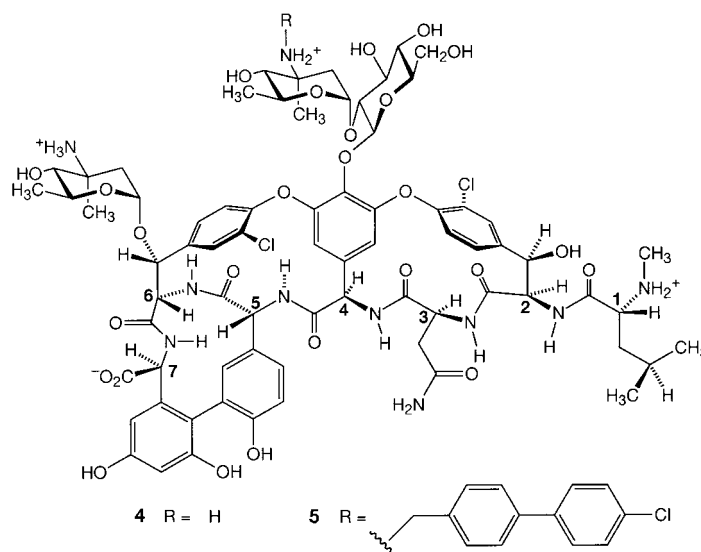
A consequence of the sequence of *R,R,S,R,R,S,S* stereochemistries at the α-C centers of residues 1 to 7 is that the peptide backbone is not of the β-sheet type often found in extended backbones of polypeptides where all residues have the *S* stereochemistry. As we will see later, natural selection has moulded this backbone into something much more subtle.

5. Sequencing and Analysis of the Genes Involved in the Biosynthesis of a Vancomycin Group Antibiotic

It is clear from the above-described experiments that the biosynthesis of vancomycin group antibiotics is complex, and involves the incorporation of units derived both from a polyketide (acetate) pathway and from tyrosine. Also, the amino sugars such as vancosamine, 4-*epi*-vancosamine and

ristosamine present on some members of the group (for example, **1** and **2**) would have to be biosynthesized by the producing organism prior to attachment to the antibiotic heptapeptide backbone. It is pertinent to enquire whether the genes that code for the biosynthesis of these amino sugars are clustered with those coding for the biosynthesis of the core heptapeptide, and to enquire as to the nature of the enzymes that catalyze the cross-linking of the side chains by means of phenol oxidative coupling and those that catalyze the regiospecific introduction of chlorine atoms. Additionally, through the cloning of selected enzymes that are involved in antibiotic biosynthesis, or direct engineering of the cluster of biosynthetic genes, it may become possible to produce novel, and possibly more efficient, antibiotics that are designed to incorporate structural features that have been shown to be beneficial in their mode of action. This method may be especially useful since selective chemical modifications of the antibiotic structures are not trivial. It was therefore desirable to sequence the DNA that codes for the biosynthesis of at least one of the glycopeptide antibiotics.

The first problem in attaining the above goal was to locate the genes that are responsible for glycopeptide biosynthesis. The key steps towards solving this problem were taken by Zmijewski and Briggs at Eli Lilly, who removed the vancosamine and glucose sugars from residue 4 of vancomycin (by a well-established acid-catalyzed cleavage), to give aglucovancomycin.^[25] A cell-free extract of enzymes from the vancomycin-producing strain was prepared and this mixture of enzymes then subjected to chromatographic fractionation. An enzyme (a glucosyl transferase) of the antibiotic biosynthetic pathway was then located by identifying the fraction that was able to catalyze the addition of glucose to residue 4 of aglucovancomycin. A limited amount of the peptide sequence of this purified enzyme was determined, and cDNA probes corresponding to this sequence were then synthesized and used to locate the biosynthetic cluster of genes for the production of vancomycin. The genes coding for the production of chloroeremomycin (**4**, also known as chloroorienticin, LY264826, and A82846B) were subsequently similarly locat-



[*] A review of the chemistry, biology, and medicinal applications of vancomycin antibiotics will appear in the near future: K. C. Nicolaou, C. N. C. Boddy, S. Bräse, N. Winssinger, *Angew. Chem.* **1991**, *111*, in press; *Angew. Chem. Int. Ed.* **1999**, *38*, in press.

ed. At Eli Lilly in Indianapolis, the genes for chloroeremomycin production were then isolated in two cosmids. Workers at Eli Lilly used one of these cosmids to sequence 5.7 kilobases of DNA that codes for the production of three enzymes.^[26] These enzymes (collectively known as glycosyl transferases) are responsible for the addition of the three sugars to the heptapeptide core of chloroeremomycin to give the fully glycosylated antibiotic. Eli Lilly then kindly made these cosmids available to our research group and, in collaboration with the Sanger Centre at Hinxton Hall, Cambridge, we have determined the sequence of 72 kilobases of DNA that codes for essentially the complete biosynthetic pathway of chloroeremomycin.^[27]

Analysis of these 72 kilobases of genomic DNA (for example, by identification of “start” and “stop” codons, and by analogy to known (homologous) protein sequences) led to the location of 39 putative genes (Figure 2). Of the 39 genes, 33 are found on the same DNA strand, whereas six of the genes (numbered as 17, 32, and 36–39 in Figure 2) are located on the opposite strand. About half of the anticipated protein products of these genes have clearly implicated roles in the biosynthesis of the antibiotic. Thus, natural selection has led to the clustering of genes that code for the production of the many enzymes in this biosynthetic pathway. These enzymes, constituted from of the order of 10^4 amino acids, catalyze the biosynthesis of molecules with molecular weights in the range of approximately only 1400–2300 Daltons, which surely indicates the importance of the biosynthetic product to the producing strain. The roles of some of these enzymes are now considered.

6. Peptide Synthetases: Synthesis of the Linear Heptapeptide Core

Conclusions concerning the biosynthetic pathway for the formation of the heptapeptide core can be deduced because the proteins that are implicated show a significant homology to known peptide synthetases. These peptide synthetases are a class of large multi-functional enzymes that catalyze the formation of small peptides (for example, of the secondary metabolites gramicidin and surfactin)^[28] by a nonribosomal route.^[29–31] In the case of chloroeremomycin biosynthesis these enzymes contain a total of approximately 9000 amino acids and they are responsible for the biosynthesis of the heptapeptide backbone of the antibiotic prior to the addition of sugars, and very probably also prior to any cross-linking.

The biosynthesis involves the condensation of seven amino acids and proceeds from the N- to the C-terminus by a process

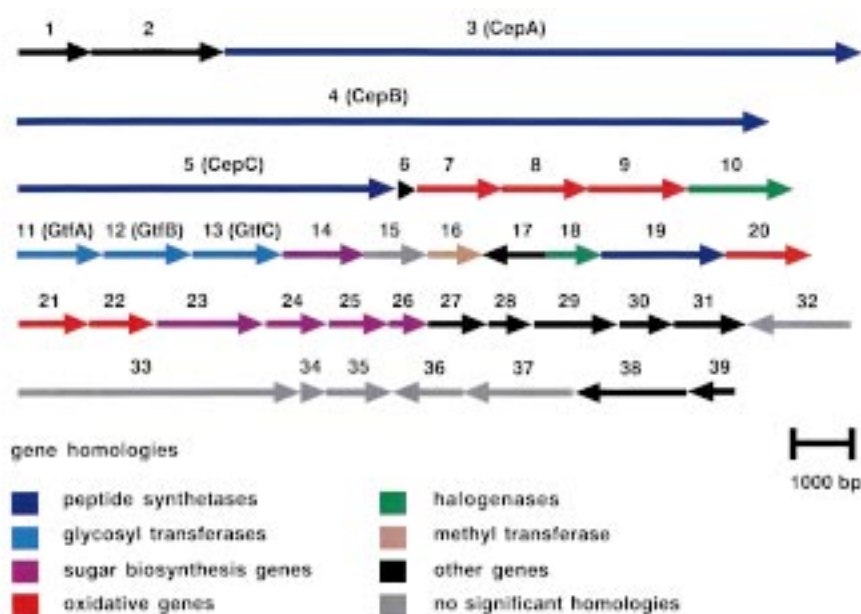


Figure 2. Pictorial representation of the genes located on cosmids pCZA363 (1–9) and pCZA361 (10–39) and putative enzymatic roles for their protein products in the biosynthesis of chloroeremomycin (4). (We thank Dr. P. F. Leadlay for pointing out that the product of gene 16 shows the characteristics expected of an *N*-methylase (putatively involved in the *N*-methylation of leucine). In the light of this observation, and sequence homology, the product of gene 14 is likely to be the C-methyltransferase responsible for C-methylation of 4-*epi*-vancosamine (see also Scheme 1), and not a hydroxylase as originally suggested by Solenberg et al.^[26])

known as the multi-enzyme thiotemplate mechanism.^[29, 31, 32] In the first step, the component amino acids are activated as aminoacyl adenylates. Each amino acid is then covalently linked to an enzyme-attached 4'-phosphopantetheine cofactor by thioesterification.^[33] Peptide synthesis then occurs by amide bond formation at the contact sites of the activating domains. The growing peptide chain is transferred from the 4'-phosphopantetheine of one module to the next, each time increasing in length by one amino acid.

Within the three multi-functional peptide synthetase enzymes, seven “modules” can be recognized (Figure 3). Each of these “modules” is responsible for the recognition of a specific amino acid, its activation (prior to attachment to another amino acid), inversion of its stereochemistry at the α -carbon atom where necessary, and its condensation (through amide bond formation) to give peptides of increasing length. The domains that recognize the amino acids show homologies to known domains in other peptide synthetases, and in some cases to each other (4, 5, and 7 to each other; 2 to 6). This evidence, taken in conjunction with the previously described feeding experiments, allows us to infer that the amino acids that are utilized for residues 2 to 7 are, respectively, (*S*)-tyrosine, (*S*)-asparagine, (*S*)-*p*-hydroxyphenylglycine, (*S*)-*p*-hydroxyphenylglycine, (*S*)-tyrosine, and (*S*)-3,5-dihydroxyphenylglycine. However, until further experiments are carried out, we cannot preclude the possibility that the (*S*)-tyrosine residues are hydroxylated and/or chlorinated prior to incorporation. The domain that recognizes the N-terminal amino acid does not show good homology to other (*S*)-leucine-recognizing domains, but does show 49% identity with the (*S*)-aminobutyric acid-recogniz-

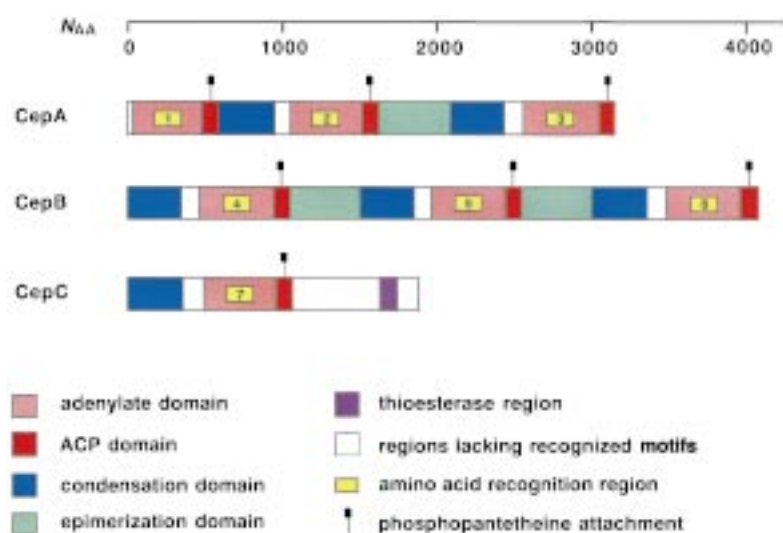


Figure 3. Schematic representation of the functional organization of the three peptide synthetases, CepA, CepB, and CepC, which are responsible for construction of the heptapeptide backbone of chloroeremomycin (**4**). The numbers 1–7 indicate sequentially the amino acid-activating domains on each peptide synthetase. The number of amino acids (N_{AA}) in each domain of the enzymes can be estimated from the scale.

ing domain (which is also associated with an epimerization domain) involved in pristinamycin biosynthesis.^[34] In conjunction with other evidence (see below), it is possible that the substrate for residue 1 is (*R*)-leucine (or its *N*-methyl analogue).

Since the stereochemistry of the backbone is *R,R,S,R,R,S,S*, it might be expected that modules 1, 2, 4, and 5 would contain an enzyme for inverting the stereochemistry from *S* to *R*. In fact, this is true for modules 2, 4, and 5, but not for module 1 (Figure 3).^[27] Two points of interest are evident from these findings. First, that although the phenylglycines at positions 4 and 5 can be biosynthesized from tyrosine, they must initially be available as *S* isomers (it has been concluded that epimerization generally occurs at the peptidyl stage for (*R*)-amino acids within the chain).^[29, 35] Second, it appears that (*R*)-leucine, or its *N*-methyl analogue, (residue 1) may be directly loaded on to the biosynthetic apparatus (see above).^[27]

A further point to note is that the multi-functional enzymes are three in number (genes 3, 4, and 5 in Figure 2, where they are also annotated as CepA, CepB, and CepC). This finding could not have been predicted, and is perhaps a quirk of the evolution of the biosynthetic apparatus. The first (CepA) is responsible for the biosynthesis of the N-terminal tripeptide. The condensation domain that catalyzes the condensation of the tripeptide to the next residue in the chain is located at the beginning of the following multi-enzyme (CepB). Overall, the second (CepB) is responsible for extending the tripeptide to a hexapeptide. The condensation domain that converts the hexapeptide to the final heptapeptide is located at the beginning of the third (CepC). CepC is also responsible for the recognition and activation of the seventh amino acid, and for the cleavage of the heptapeptide from the biosynthetic apparatus (to which it is attached as a thioester) through the action of a thioesterase.

7. Oxidative Reactions

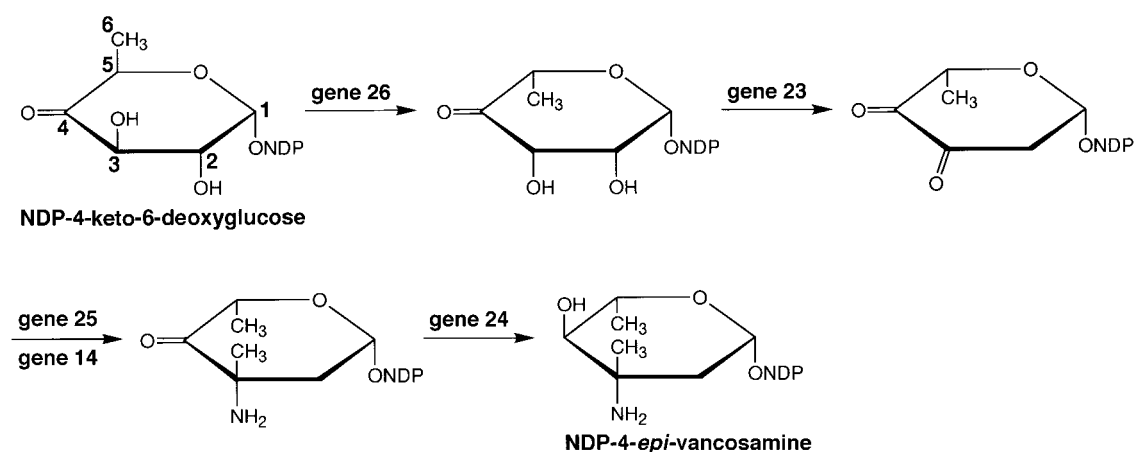
Conversion of the linear heptapeptide, derived as described above, into the chloroeremomycin nucleus which lacks sugars, requires seven oxidative processes. These are the introduction of β -hydroxy groups onto the tyrosine residues 2 and 6, coupling of rings 5 $\rightarrow$ 7, 4 $\rightarrow$ 6, and 4 $\rightarrow$ 2, and introduction of chlorine atoms on to rings 2 and 6. The DNA sequence in fact shows evidence for eight enzymes (genes 7–10, 18, and 20–22) that might be involved in oxidative processes.^[27] Of these, genes 10 and 18 show homology to non-heme haloperoxidases, and therefore appear likely to be involved in the introduction of the two chlorine atoms into the antibiotic. As indicated above, it cannot be precluded that the hydroxylation and/or chlorination may precede linear heptapeptide production.

8. Biosynthesis of 4-*epi*-Vancosamine and Attachment of Sugars to the Oxidized and Cyclized Heptapeptide Core

Once the cyclized and oxidized peptide core is available, two molecules of 4-*epi*-vancosamine and one of glucose must be added to it. The 4-*epi*-vancosamine is an L-amino-2,6-dideoxysugar. Although L-amino-2,6-dideoxysugars are relatively common in secondary metabolites, they are otherwise not widespread in nature. It was therefore likely that the genes that code for its biosynthesis would be clustered with those of the other biosynthetic genes, and this has indeed proved to be the case.^[27] Based on analogy to the biosynthesis of other 6-deoxysugars such as daunosamine and mycarose,^[36–38] the biosynthesis should proceed through an NDP-4-keto-6-deoxyglucose intermediate (NDP = nucleotide diphosphate). Enzymes for the production of this key intermediate were not identified in the cluster, but it is possible that general cellular pools of this precursor are utilized for the biosynthesis of 4-*epi*-vancosamine, an idea recently postulated by Summers et al. for the biosynthesis of the mycarose and desosamine sugars of erythromycin.^[36]

Given NDP-4-keto-6-deoxyglucose as a precursor, homologies to enzymes identified in the biosynthetic pathways for daunosamine and mycarose^[36, 38, 39] allowed a probable route to 4-*epi*-vancosamine to be deduced (Scheme 1).^[27] Five putative enzymes were identified. The protein corresponding to gene 26 was assigned as a 3,5-epimerase on the basis of its strong homology to DnmU (60% identity) of the daunosamine pathway of *Streptomyces peucetius*^[38] and EryBVII (55% identity) of the mycarose pathway of *Saccharopolyspora erythraea*.^[36]

The enzyme corresponding to gene 23 is closely related to DnmT (49% identity) from the daunosamine pathway of *S. peucetius*^[39] and EryBVI (45% identity) from the mycarose



Scheme 1. Postulated reaction scheme for the biosynthesis of NDP-4-*epi*-vancosamine starting from NDP-glucose, showing the corresponding putative enzymes involved by reference to the genes indicated in Figure 2.

pathway of *S. erythraea*, both of which have been linked with C-2 deoxygenation. In particular, EryBVI was assigned as a 2,3-dehydratase and a similar role is proposed for the product of gene 23. The product of gene 25 has a high homology (72% identity) to DnrJ, an enzyme found in daunorubicin biosynthesis and implicated in the introduction of an amine functionality,^[37, 40] probably with the aid of pyridoxamine or glutamic acid as a cofactor. The recent confirmation of EryBIII as a C-3 methyltransferase in mycarose biosynthesis^[41] suggests that the product of gene 14, which has 28% identity to this enzyme, is responsible for this step of the pathway. The proposed sugar biosynthesis is completed by C-4 ketone reduction with the enzyme corresponding to gene 24, which possesses strong homology only to EryBIV (49% identity), the 4-ketoreductase of the mycarose pathway of *S. erythraea*.^[36] Finally, the attachment of two molecules of 4-*epi*-vancosamine, and one of glucose, at three distinct sites on the antibiotic requires three glycosyl transferase enzymes, and the genes that code for these enzymes are 11–13.^[26, 27]

9. The Complex of the Glycopeptides with Bacterial Cell-Wall Precursors

An important finding, even before the structure of vancomycin was known, was that of Perkins who showed that the antibiotic binds to bacterial cell wall mucopeptide precursors that terminate in the sequence L-lysyl-D-alanyl-D-alanine.^[42] This peptide is described as a mucopeptide precursor because it is an intermediate in the process of bacterial cell-wall biosynthesis. Bacterial cell wall is built up on the outside of the cell in two main steps.^[43] First, disaccharide units with pendant peptides are exported from the cytoplasm to the outside of the cell where they are joined together by a transglycosylase enzyme (Figure 4). Second, for mechanical strength, these long polysaccharide chains are cross-linked through their peptide chains by a transpeptidase enzyme. This enzyme recognizes the sequence D-alanyl-D-alanine at the end of the peptide chain, cleaves off the terminal alanine, and joins the remainder to a peptide chain from an adjacent polysaccharide. At the time of Perkins' finding (1969), little

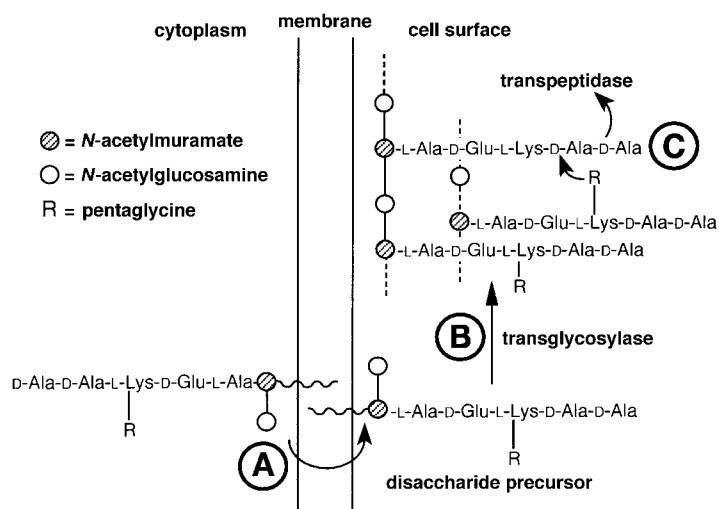
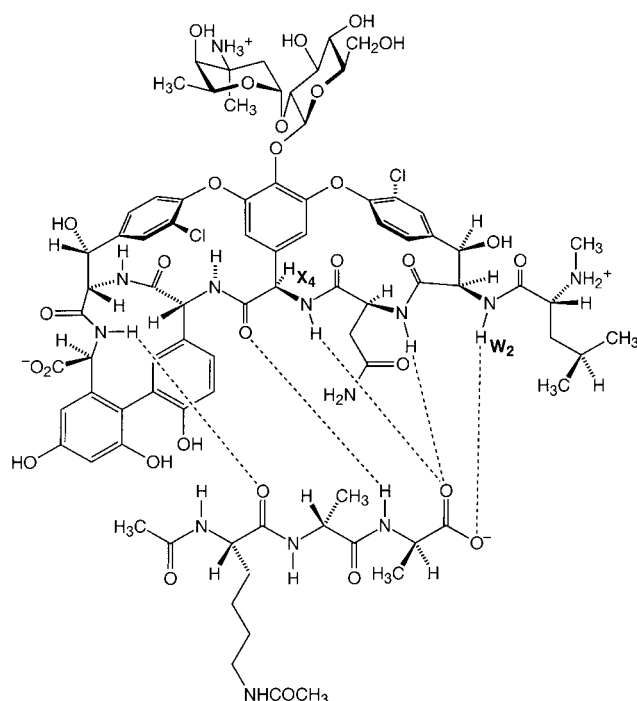


Figure 4. Schematic outline of the biosynthesis of peptidoglycan. In (A), the disaccharide precursor is transported across the membrane after which it is appended to the growing polysaccharide chain by a transglycosylase (B). Mechanical strength is conferred to the cell wall through the cross-linking of adjacent polysaccharide chains (C). The cross-linking step proceeds with a transpeptidase enzyme removing the C-terminal D-alanine residue of the pentapeptide. The carboxylate group released by this process then forms a peptide bond to the free N-terminal group of the bridging residue R. The constitution of the pendant peptide shown is that found in *Staphylococcus aureus*.

about the molecular basis of action of vancomycin could be inferred because the structure of the antibiotic was as yet unknown.

When the structure of vancomycin was finally elucidated, the molecular basis for the binding of the antibiotic to mucopeptide precursors terminating in Lys-D-Ala-D-Ala could then be determined. This was achieved using proton NMR spectroscopy of the complexes formed between the antibiotic and analogues of bacterial mucopeptide precursors (*N*-acetyl-D-alanyl-D-alanine (Ac-DADA) or *N*- α -acetyl-*N*- ϵ -acetyl-lysyl-D-alanyl-D-alanine (Ac₂-KDADA)). A number of key experiments were important in this determination. The first involved measurement of the chemical shifts of the methyl groups of Ac-DADA when free in solution and when bound to antibiotic. The upfield changes in chemical shift

observed for these resonances in the bound state indicated the methyl groups were located over aromatic rings of the antibiotics. Second, the antibiotic amide NH protons involved in hydrogen bonding to the ligand carboxylate were determined by observation of the chemical shifts in the NMR spectra, with the amide NH resonances suffering a downfield shift in the antibiotic–ligand complex characteristic of the formation of such a hydrogen bond. The other antibiotic amide NH groups involved in hydrogen bonding to ligand were determined by measurement of the solvent exposure of each of the amide NH protons. Finally, approximate inter-proton distances for antibiotic protons that lie close in space to ligand protons were determined using the NOE. This appears to have been the first use of the intermolecular NOE to determine the structure of a bimolecular complex.^[11] Such determinations were carried out for the binding of D-Ala-D-Ala-terminating peptides to ristocetin A,^[44] vancomycin,^[45] and teicoplanin^[46] in the early 1980s and led to the model of the binding interaction shown in Scheme 2. This model, with



Scheme 2. Exploded view of the binding interaction between the glycopeptide antibiotics (in this case vancomycin) and the cell-wall analogue Ac₂-KDADA. Hydrogen bonds between the two are indicated by dotted lines. The binding is also promoted by hydrophobic interactions, notably of the Ala methyl groups to the aromatic rings of the antibiotic. The amide NH proton w₂ and the α-proton x₄, both mentioned in the text, are labeled.

the carboxylate anion of the ligand binding to three adjacent antibiotic amide protons has recently been confirmed by X-ray crystallography.^[47–49]

The binding interaction of the antibiotics **1–3** to the cell wall mucopeptide precursor Ac₂-KDADA in aqueous solution at room temperature is in the region of 10⁶ M^{−1}, and to the dipeptide Ac-DADA approximately 10⁵–10⁶ M^{−1}.^[50] Clearly, the main binding affinity lies in the recognition of the N-acetylated dipeptide. In trying to understand the origins of this affinity (with reference to Scheme 2), the interactions that must be considered are:

- 1) the binding of the carboxylate anion of the C-terminal D-alanine into the pocket of the three amide NH groups of residues 2–4 of the antibiotic (the formation of this pocket is crucially dependent on the *R,R,S,R* stereochemistries of residues 1–4).^[51]
- 2) the formation of two amide–amide hydrogen bonds between the acetylated dipeptide and the antibiotic.
- 3) the hydrophobic interactions formed by the alanine methyl groups in their contacts with hydrocarbon portions of the antibiotic.

The relative importance of these contributions can only be addressed in a semi-quantitative manner because of the complex nature of cooperativity between weak interactions. However, from our studies of the problem it is clear that productive binding is crucially dependent on binding of the carboxylate (factor 1 above). It is this interaction that provides the main source of exothermicity (20–30 kJ mol^{−1}) in the association.^[52] The stronger binding of the carboxylate group by the pocket of the three antibiotic NH groups than by water seems likely to find its origin in the fact that the three repulsive NH ends of the associated CONH dipoles can be pushed together by the expenditure of energy during the process of antibiotic biosynthesis. Together, they are better able to solvate the negative charge of the carboxylate ion than water molecules (which cannot have their oxygen atoms held closely together (in the absence of a template to hold them in such a position) without paying a cost in enthalpy). Given the binding of the carboxylate, the two amide–amide hydrogen bonds do little to directly promote further binding affinity, that is, their intrinsic binding affinities are probably close to zero. This is evidenced, for example, by the fact that the binding of *N*-acetyl-glycine is greater than that of acetate by a factor of only approximately three.^[53] Indirectly, however, they do play important roles. First, in orienting the acetylated dipeptide so that the hydrophobic interactions of the two Ala methyl groups can promote binding by a further factor of about 10³. Second, by virtue of the energy barriers that exist towards breaking these two amide–amide hydrogen bonds, the motion of the peptide ligand in its antibiotic binding site is reduced and the binding of the carboxylate anion into its binding site is thereby increased.

Evidence for the last effect mentioned above is found from ¹H NMR spectroscopic studies. The chemical shift of the amide NH (w₂, Scheme 2) of residue 2 of the antibiotics is moved markedly downfield upon binding the carboxylate oxygen atom of di- and tripeptide cell wall precursor analogues. The assumption is made that the extent of this downfield shift (measured for the completely bound state of an antibiotic to a given cell-wall analogue) increases with increased bonding of the carboxylate group to w₂. Experimentally, we find that the limiting downfield shift of w₂ increases as shown in Figure 5 for a variety of ligands (chosen as a series of increasing affinity for the antibiotic, which is represented by the vertical axis and which records the Gibbs free energies of binding for the ligands). It is clear from these data that as the ligands are changed in the series acetate → *N*-acetyl-D-alanine (Ac-DA) → Ac-DADA → Ac₂-KDADA, the chemical shift of w₂ in the fully bound state increases dramatically. This is because the making of the adjacent

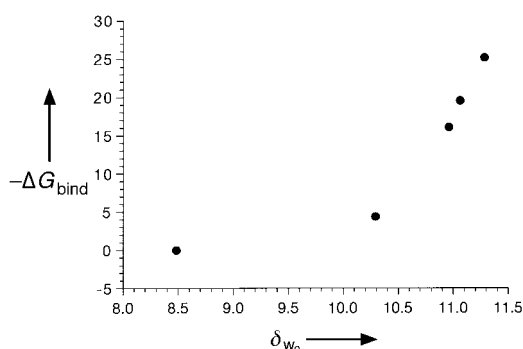
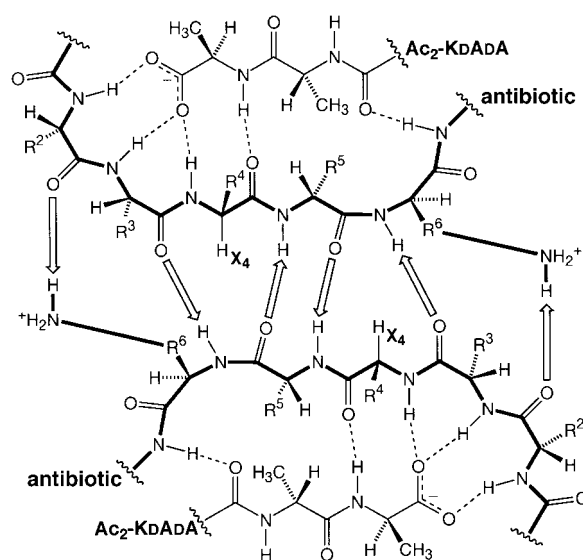


Figure 5. Plot of the Gibbs free energy of binding of peptide ligands ($-\Delta G_{\text{bind}}$ [kJ mol⁻¹]) to a vancomycin group antibiotic versus the chemical shift of the NH proton w_2 . As the ligand becomes progressively longer the Gibbs free energy of binding increases and the chemical shift of w_2 appears further downfield: an indication that the hydrogen bond between w_2 and the carboxylate group is becoming stronger. In order of increasing magnitude of ΔG_{bind} , the points represent binding of the following ligands: none, acetate, Ac-DADA, Ac₂-KADA, Ac₂-KDADA.

hydrophobic interactions,^[54] and of the adjacent amide–amide hydrogen bonds,^[55] increases the binding affinity of the carboxylate anion into its antibiotic receptor pocket. This phenomenon is probably one of general relevance to weak interactions—the addition of neighboring interactions can strengthen an existing interaction by limiting the dynamic motion of a ligand at its receptor site. Thus, cooperativity derives not only from the entropic advantage of putting several interactions on the same template (the chelate effect), but also the bonding (enthalpic) advantage of doing this in a strain-free system. Not only can an enthalpic barrier to breaking a bond restrict the motion at an adjacent site, but by so doing, it can improve the bonding at that adjacent site.

10. Dimerization of the Glycopeptides

In 1989 we discovered that the antibiotic ristocetin A forms a dimer in aqueous solution.^[56] We were also able to elucidate the relative orientations of the two monomer units of this dimer and the hydrogen bonding network formed between them. In the dimer structure of chloroeremomycin (**4**) bound to Ac₂-KDADA, (Scheme 3), the peptide backbones of two antibiotic molecules are bound together by four amide–amide hydrogen bonds, and the ammonium ion of the amino sugar (4-*epi*-vancosamine), which is attached to residue 6 of the antibiotic, forms a hydrogen bond to an amide carbonyl group in the other half of the dimer to give a total of six hydrogen bonds at the dimer interface.^[56] In vancomycin, where this amino sugar is not present, there are only four intermolecular hydrogen bonds at the dimer interface. The overall structure of the dimer that we determined by proton NMR spectroscopy has now been independently confirmed by X-ray crystallography.^[47, 48, 57, 58] Of all the glycopeptides so far examined, only teicoplanin and some of its derivatives have failed to show any evidence of dimerization in aqueous solution.^[59]



Scheme 3. Backbone structure of the chloroeremomycin dimer bound to the cell wall precursor analogue Ac₂-KDADA. The peptide backbone is shown in bold and hydrogen bonds at the dimer interface are indicated by open arrows. Hydrogen bonds at the antibiotic–ligand interface are indicated by dashed lines. The α -proton x_4 , mentioned in the text, is labeled. The central four hydrogen bonds at the dimer interface are common to all glycopeptide dimers, whereas the two outer hydrogen bonds can only be made by antibiotics that possess an amino sugar attached to residue 6.

11. Cooperativity Between Dimerization and the Binding of Cell Wall Precursor Analogues

An interesting feature of antibiotic dimerization is that for all the glycopeptides so far examined, except for ristocetin A, dimerization constants are greater when bacterial cell wall precursor analogue is bound to the antibiotic than when it is not. That is, dimerization is cooperative with ligand binding.^[60] Similarly, antibiotic dimers bind ligands more strongly than antibiotic monomers. This can be seen from a thermodynamic cycle showing the formation of a ligand-bound dimer from its constituent parts, that is, two antibiotic monomers and two bacterial cell wall precursor analogue molecules.^[60] An example is the dimerization of chloroeremomycin (**4**). The dimerization constant in the presence of the cell wall precursor analogue Ac₂-KDADA is a factor of about 100 greater than the value in the absence of ligand.^[60] Such a value implies that two cell wall precursor analogues binding with equal affinity to an antibiotic dimer should each bind to a dimer approximately ten times more strongly than to a monomer.

A number of interactions appear to be important in the expression of cooperativity between ligand binding and dimerization. First, the binding of ligand to antibiotic monomer (or dimerization of free antibiotic) should result in increased polarization of the antibiotic amide groups involved in hydrogen bonding. Since the same amide (CONH) groups are involved in hydrogen bonding at the dimer interface (or ligand binding interface), they should form stronger hydrogen bonds as a consequence of their increased polarization and resulting stronger dipoles. Second, the presence of an initial

set of hydrogen bonds with the antibiotic backbone (for example, from ligand binding) should have the effect of restricting the rocking motions of the peptide dipoles. This motional restriction should lead to stronger bonding at the second interface. Third, it can be seen from Scheme 3 that the process of dimerization brings the ammonium ion of the 4-*epi*-vancosamine residue into the proximity of the carboxylate anion of a bacterial cell-wall precursor bound to the other half of the dimer. The electrostatic attraction between these groups, which is mediated by a peptide bond, can be thought of as a pseudo-salt bridge. This last interaction can only occur in antibiotics that possess an amino sugar attached to residue 6 (for example, chloroeremomycin (**4**) and ristocetin A (**2**), but not vancomycin (**1**)).

Our work on quantitation of antibiotic dimerization^[61] has established that dimerization is promoted both by the saccharides that are frequently attached to residue 4 of the antibiotics, and by the amino sugars that are often attached to the benzylic hydroxy group of residue 6. If the view is taken that the antimicrobial action of the glycopeptides under discussion represents the evolved function of these substances, then it seems reasonable to view the sugars as adornments added to the peptide backbones to improve this function. Given this outlook, the observation that the sugars are of such structures and so positioned as to promote dimerization leads to the proposal that dimerization may well be important in antibacterial action. This view is supported by the fact that some glycopeptides possess a chlorine atom attached to residue 2 and that these antibiotics can dimerize more strongly than the equivalent antibiotics that do not have this feature. These observations are consistent with the idea of a selectional pressure to produce antibiotics that dimerize, a selectional pressure that would only operate if dimerization had a functional role in aiding the survival of the producing strain.

12. Asymmetry of the Glycopeptide Dimers

A remarkable feature of all the vancomycin group glycopeptide dimer structures so far examined (by both proton NMR spectroscopy and X-ray crystallography) is that although the peptide backbones of the two constituent antibiotic molecules are head-to-tail relative to each other, the sugars attached to residue 4 are arranged in a head-to-head manner.^[47, 48, 57, 62–64] This feature accounts for the fact that the dimers are asymmetric. For example, in the ristocetin A dimer (Figure 6), the 6-methyl resonance of the rhamnose, which is part of the tetrasaccharide attached to residue 4, occurs with two distinct chemical shifts ($\delta = 0.86$ and 1.27).^[62] This is because the rhamnose sugar of one half of the dimer is in a different environment to the rhamnose in the other half of the dimer; one rhamnose is remote from the N-terminus of the antibiotic of which it is part, whereas the other is much closer to the N-terminus of the antibiotic of which it is part (Figure 6).^[62] This result implies that the dimers have two distinct binding sites for bacterial cell wall mucopeptide precursors (Figure 6). However, for binding of free cell wall peptide analogues in aqueous solution, it is only in the case of

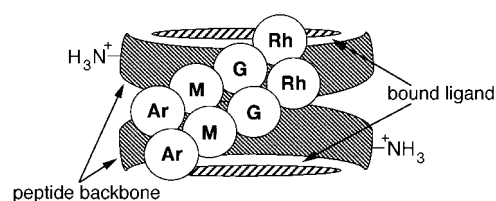


Figure 6. Schematic illustration of the ristocetin A dimer, with a molecule of a peptide cell-wall analogue in each of its two binding sites. Each of the two sets of four colinear circles represents a tetrasaccharide (Ar = arabinose, M = mannose, G = glucose, Rh = rhamnose) attached to amino acid number 4 of the peptide backbone (see **2**). Note that the sugars are arranged head-to-head, in contrast to the head-to-tail arrangement of the peptide backbone. This property, found for all ring 4-glycosylated antibiotics so far examined, means that the dimers are asymmetric.

ristocetin A that we have so far been able to demonstrate that these two sites have significantly different affinities.^[65, 66] In the cases of the other antibiotics that we have examined, any differences in binding affinities of these two sites in aqueous solution are very small.^[67]

It is interesting to speculate as to the evolutionary basis for the asymmetry of the dimer structures and whether this is related to the structure and orientation of strands of immature peptidoglycan cell wall. Schäfer et al. have postulated previously^[47] that the head-to-tail nature of the peptide backbones in an antibiotic dimer and the consequent binding of two cell-wall precursors in a head-to-tail manner relative to each other suggest that vancomycin is inserted between different immature peptidoglycan strands. By implication, the adjacent strands of peptidoglycan bound by the two halves of the dimer must also be oriented head-to-tail (antiparallel) relative to each other. We would like, however, to put forward an alternative hypothesis. Previous evidence regarding the binding of glycopeptides to cell wall precursor analogues indicates that the N-terminal portion, -L-alanyl-D- γ -glutamyl-, of the pentapeptide projects towards the sugars attached to residue 4 of the antibiotic.^[64] This would imply that the antibiotic binds with these sugar residues oriented towards the bacterial surface relative to the peptide portion of the molecule. Herein may lie the origin of the dimer asymmetry. Since the strands of peptidoglycan are constituted of polysaccharide chains there is likely to be some favorable sugar-sugar interaction between these strands and the antibiotic residue 4 saccharides. The parallel orientation of interacting saccharides on the antibiotic dimer would thus require a similar parallel orientation of the polysaccharide strands for a repeated interaction between the two saccharides of an antibiotic dimer and two polysaccharide strands. Such interactions between the polysaccharide of immature peptidoglycan and the antibiotic saccharides have not been investigated but they may be an influential factor in either location of the antibiotic at its site of action or in promoting the binding of the antibiotic to its target peptide. Thus, although no direct evidence for the parallel or antiparallel nature of adjacent immature strands of cell wall has been produced, sugar-sugar interactions between antibiotic dimers and the cell-wall polysaccharides and the parallel nature of the sugars in the dimers suggests that the strands of cell wall may also be biosynthesized with a parallel orientation relative to each other.

13. Roles for Dimerization and Membrane Anchoring in the Action of Glycopeptides

When the glycopeptides were first shown to dimerize, we postulated that dimerization might provide an enhancement in activity through the simultaneous delivery of two antibiotic molecules to the site of action.^[56] Additionally, in 1990, Good et al. showed that the antibiotic eremomycin, which binds $\text{Ac}_2\text{-KDADA}$ more weakly than vancomycin, is actually more active than vancomycin.^[68] This finding establishes that there is more to antibiotic activity than the affinities for free cell-wall analogues suggest (even allowing for cooperative enhancements from dimerization). It struck us that eremomycin dimerizes much more strongly than vancomycin, and that its greater activity might therefore be associated with its larger dimerization constant. We therefore proposed that dimerization could benefit antibacterial action by facilitating effectively “intramolecular” binding at the surface of bacteria. This is a consequence of the fact that the cell-wall precursors are tethered to the bacterial surface by C_{55} lipid chains that are inserted into the bacterial membrane. The binding of a dimer to two such anchored precursors results in the formation of an “intramolecular” complex (Figure 7B, to be contrasted with

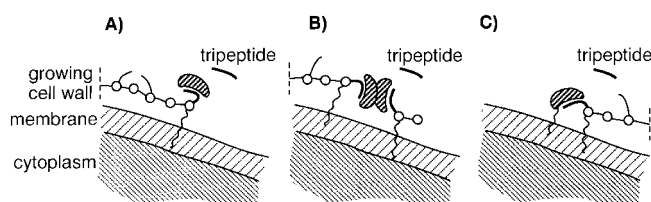


Figure 7. Antibiotics binding as A) monomer, B) dimer, and C) with a membrane anchor. Open circles represent saccharide units, and the end portion of the attached bold line represents the sequence -Lys-D-Ala-D-Ala. The bold line not attached to the saccharide represents $\text{Ac}_2\text{-KDADA}$ in solution. A) The binding of a single antibiotic molecule to the growing cell wall is a simple bimolecular association (such that externally-added ligand can replace the cell-wall peptide). B) The antibiotic dimer can benefit from an essentially intramolecular association at the surface of a bacterium (and it is more difficult to disrupt by the externally-added ligand). C) The benefit from intramolecularity can also be exploited if the antibiotic has a membrane anchor (again, the complex is more difficult to antagonize).

Figure 7A).^[60, 69] This hypothesis was extended to consider the activity of teicoplanin (**3**), which does not dimerize measurably. Instead, teicoplanin possesses a C_{11} acyl chain, which may be able to insert into the bacterial membrane itself, both locating this antibiotic at its site of action and facilitating the formation of an “intramolecular” complex at the cell surface (Figure 7C).^[60, 69] Hence, the vancomycin group of antibiotics can utilize either dimerization (Figure 7B) or membrane anchoring (Figure 7C) to enhance their antibacterial activity.

We first began to investigate whether any binding enhancement from dimerization or membrane anchoring could be observed in the mid-1990s.^[69] This involved attempting to antagonize antibacterial action against bacteria (*Bacillus subtilis*) on agar plates by the addition of external $\text{Ac}_2\text{-KDADA}$. The basis for these experiments is shown in Figure 7. If the binding in an “intramolecular” complex (as in B and C) is stronger than for an intermolecular complex (A), it should

be harder to antagonize such “intramolecular” binding with externally-added $\text{Ac}_2\text{-KDADA}$. This was indeed the case, as approximately five times more external $\text{Ac}_2\text{-KDADA}$ was required to antagonize the action of teicoplanin relative to the antibiotic $\text{TA}_3\text{-1}$ (teicoplanin lacking the C_{11} acyl chain) and, more dramatically, approximately one thousand times more external $\text{Ac}_2\text{-KDADA}$ was required to antagonize the activity of eremomycin compared to $\text{TA}_3\text{-1}$ (Figure 8).^[69] Further

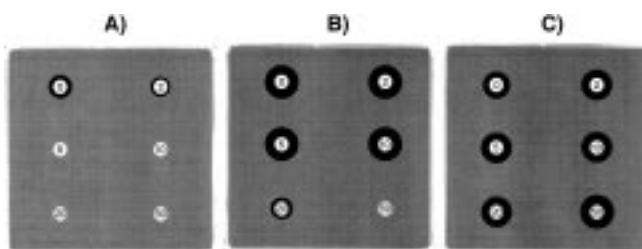


Figure 8. Three agar diffusion plates illustrating the effect of externally-added $\text{Ac}_2\text{-KDADA}$ on the potencies of three representative antibiotics in inhibiting the growth of *Bacillus subtilis*. Each dark circle represents an area where the bacteria are killed by the antibiotic placed on the paper disk (white circles). The size of the dark circle is a measure of the potency of the antibiotic. Each paper disk contained 1 mg of antibiotic. A) $\text{TA}_3\text{-1}$ (does not dimerize or possess a membrane anchor), B) vancomycin (dimerizes weakly), and C) eremomycin (dimerizes strongly). The number superimposed on each disk is the amount of $\text{Ac}_2\text{-KDADA}$ (mg) added to that disk. Incidentally, the small increase in the radius of the dark circles in C), as a function of added $\text{Ac}_2\text{-KDADA}$, shows the remarkable potentiation effect reported in references [68,69].

experiments have shown that there is in fact a striking correlation between the amount of external $\text{Ac}_2\text{-KDADA}$ required to antagonize antibiotic action and the antibiotic dimerization constant.^[69] The increases in the former quantity, for more strongly dimerizing antibiotics (Figure 9), measure

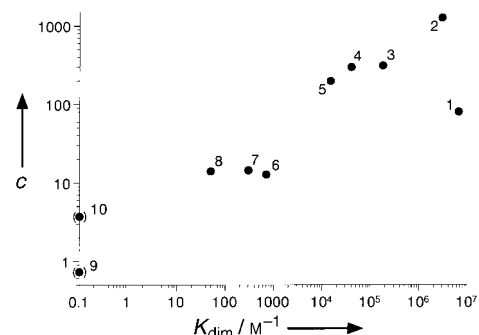


Figure 9. Plot of the amount of $\text{Ac}_2\text{-KDADA}$ required to give a 50% reduction in the potency of glycopeptide antibiotic against *Bacillus subtilis* [$\mu\text{g per disk}$] versus dimerization constant K_{dim} of the antibiotic. Teicoplanin (10), which possesses a membrane anchor, and $\text{TA}_3\text{-1}$ (9), without a membrane anchor, show no evidence for dimerization (that is $K_{\text{dim}} < 1 \text{ M}^{-1}$). The strongly dimerizing antibiotics (1–5) include eremomycin (numbered 2 in this figure) and those of moderate dimerizing tendency (6–8) include vancomycin and ristocetin A. In detail, the antibiotics corresponding to the numerical code are: 1: decaplanin (also known as MM47761), 2: eremomycin, 3: chloroeremomycin, 4: dechloroeremomycin, 5: eremomycin- ψ , 6: vancomycin, 7: ristocetin A, 8: ristocetin- ψ , 9: $\text{TA}_3\text{-1}$, and 10: teicoplanin.

the cooperative binding enhancement achieved from the binding of the dimer at the bacterial surface relative to the binding of a monomer.^[70]

14. Cooperativity in Antibiotic Binding to Micelle-Associated Peptides

Once we had shown that an advantage was gained in antibacterial activity for antibiotics that were able to dimerize or anchor to membranes, we attempted to determine the extent to which binding was enhanced when it occurred “intramolecularly” at a membrane surface (templated binding) relative to the equivalent binding event occurring intermolecularly. Our first experiments to measure this enhancement employed a crude model system for the bacterial surface that consisted of sodium dodecyl sulfate micelles as an analogue of the bacterial membrane (Figure 10A) and *N*-decanoylated cell wall precursor analogues.

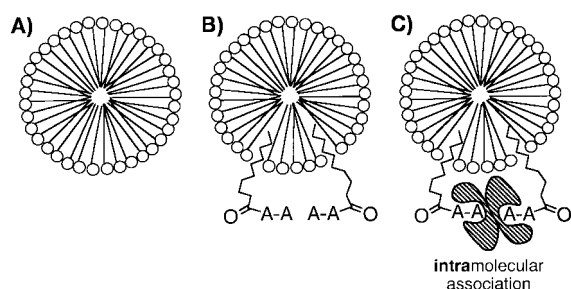


Figure 10. Schematic representation of A) a micelle, B) Dec-DADA using its membrane anchor, and C) an antibiotic dimer bound to Dec-DADA at the surface of a micelle.

The decanoyl chain of these precursor analogues was designed to be able to insert into the hydrophobic interior of the micelles with the polar C-terminus of the peptide projecting into solution (Figure 10B). Note that since the hydrocarbon side chains that form the interior of the micelle are able to pass each other (that is they can behave in the manner of a liquid crystal), *N*-decanoylated peptides that are inserted into the micelle are able to change their relative positions on the micelle. Therefore, if an antibiotic dimer now binds a micelle-bound peptide at one of its binding sites, then a second micelle-bound peptide has the possibility to adjust its position so that in principle it can bind “intramolecularly” into the second binding site of the dimer (Figure 10C). The binding affinity of the antibiotic to the peptide in such a templated assembly should be greater than that for the corresponding association in the absence of the micelle, providing this assembly can be formed in a relatively strain-free manner.

The above conjectures have been confirmed experimentally. The binding constant of free Ac-DADA to ristocetin A in solution is $4.1 \times 10^4 \text{ M}^{-1}$ (in the presence of SDS micelles, which are passive in this case, but present to give the necessary control experiment), whereas the binding constant for membrane-templated *N*-decanoyl-D-alanyl-D-alanine (Dec-DADA, as in Figure 10C) is $1.6 \times 10^7 \text{ M}^{-1}$, an increase in the binding constant of approximately 400.^[71] Moreover, the chemical shift of w_2 (which was shown earlier to occur at lower field the more strongly the ligand was bound to it) in the latter assembly occurs further downfield ($\delta = 11.65$) than for the former association ($\delta = 11.43$).^[71] Similar results were achieved for teicoplanin binding to the peptides Ac₂-KDADA

and *N*- α -decanoyl-*N*- ϵ -acetyl-lysyl-D-alanyl-D-alanine (Dec-KDADA) in the presence of micelles. As for ristocetin A binding to Dec-DADA and Ac-DADA, the binding constant of teicoplanin to Dec-KDADA was greater than that to Ac₂-KDADA and w_2 was shifted further downfield for the binding of Dec-KDADA than for the binding of Ac₂-KDADA.^[71] Thus, in the formation of templated assemblies, not only is there the advantage of a relatively small adverse entropy (the binding being effectively intramolecular), but the motional restriction associated with binding at a template (the micelle surface) is also associated with stronger bonding of the peptide carboxylate into its receptor pocket.

We have thus been able to demonstrate the operation of two distinct forms of cooperativity in the binding of bacterial cell wall precursor analogues to antibiotic dimers. First, an intrinsic form of cooperativity that arises from the influence of the two binding events (ligand binding and dimerization) on each other. This cooperativity occurs when all the components are free in solution (as opposed to being membrane-bound). Second, a form of cooperativity that arises from the restriction of motion of the component parts of binding as a consequence of their simultaneous location at the bacterial membrane.

15. Resistance to Vancomycin: A New Species of “Superbug”

Since its introduction into clinical use in the 1950s, vancomycin was notable among antibacterial agents in use because of the lack of resistance developed against it by bacteria. This lack of resistance helped it reach the forefront of the fight against infections due to MRSA, an increasingly prevalent pathogen. In 1988, however, the first report of vancomycin-resistant enterococci (VRE) appeared.^[72] Although enterococci are not normally pathogenic, such infections can be lethal if they are contracted by immunodeficient patients. Causes of immuno-deficiency include chemotherapy, AIDS, or simply weakness caused by old age, severe illness, or a recent operation. Such infections are becoming increasingly common and there is a possibility that the genes that code for resistance could be transferred to more deadly bacterial species such as MRSA (Figure 1).

The genetics and enzymology of vancomycin resistance have been elucidated in respective major contributions from the groups of Courvalin in Paris and Walsh in Cambridge, Massachusetts.^[73, 74] It appears that resistant enterococci have been able to obtain genes from other bacteria such that the precursors from which their cell wall is built no longer terminate in -D-alanyl-D-alanine, but rather terminate in -D-alanyl-D-lactate (-D-Ala-D-Lac).^[73, 74] Resistance is associated with the expression of five genes.^[74] In the absence of vancomycin, cell wall can be built in the normal way, using precursors terminating in -D-Ala-D-Ala. In the presence of vancomycin, the five genes for resistance (named *vanS*, *vanR*, *vanH*, *vanA*, and *vanX*) are switched on.^[74] The product (VanS) of the gene *vanS* becomes phosphorylated on a histidine residue. This phosphorylated protein then interacts with a protein (VanR) which is the product of the second gene, *vanR*, and this protein in turn becomes phosphorylated.

Phosphorylated VanR causes transcriptional activation of the other three genes (*vanH*, *vanA*, and *vanX*). The product of *vanH* is an α -keto reductase, which can reduce pyruvate to D-lactate. This D-lactate is then used as a substrate (along with D-alanine and ATP as co-factor) by the enzyme VanA (the product of expression of the gene *vanA*). The enzymatic activity of VanA produces D-Ala-D-Lac, which is then used in the bacterial cell-wall precursors (instead of D-Ala-D-Ala). The fifth gene (*vanX*) codes for an enzyme (VanX), which acts as a D-Ala-D-Ala peptidase, so that the D-Ala-D-Ala that is produced to provide a constituent of normal cell wall biosynthesis can now be broken down to provide D-Ala as a precursor for D-Ala-D-Lac. The arrangement of these genes, and the functions of their products, are indicated in Figures 11 and 12, respectively.

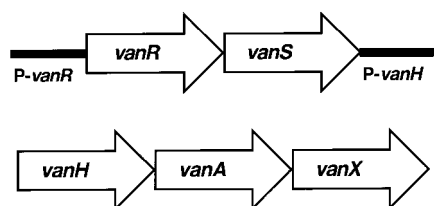


Figure 11. Arrangement of the gene cluster coding for resistance to vancomycin. The thick solid lines indicate the promoter regions for the *vanR*, *vanS* and *vanH*, *vanA*, *vanX* regions. The wide, open arrows indicate the open reading frames. These five genes are necessary for high level antibiotic resistance, as shown by Arthur and colleagues.^[73] Diagram adapted from reference [74b].

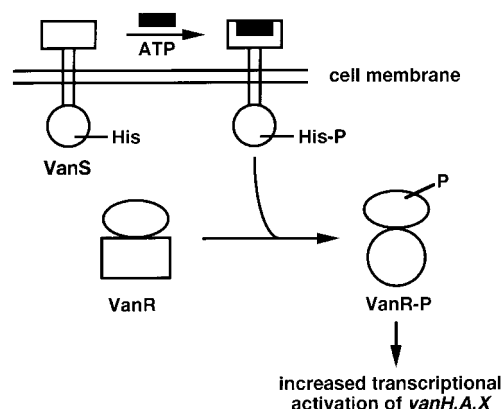
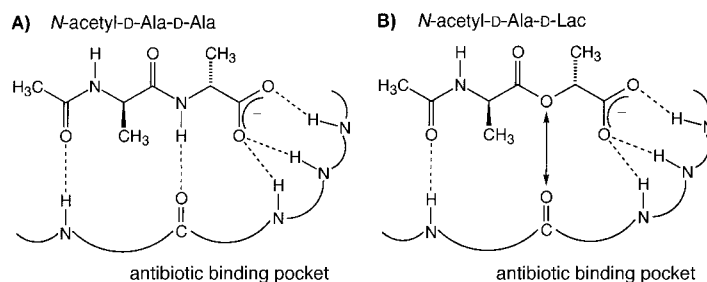


Figure 12. Functions of five gene products that code for resistance to vancomycin. VanS is a trans-membrane sensor kinase, which can bind a sensory ligand (represented by a solid block, and available when the bacterium is exposed to vancomycin). When activated by binding to such a ligand, VanS undergoes phosphorylation at a His residue. This product in turn causes phosphorylation of VanR, which promotes its dimerization and binding to DNA. Binding of phosphorylated VanR (VanR-P) to the *vanH*, *vanA*, *vanX* promoter (*P-vanH*, Figure 11), results in transcriptional activation and expression of the genes *vanH*, *vanA*, *vanX*, which code for enzymes to produce D-lactate from pyruvate, to ligate this D-lactate to D-alanine to give D-Ala-D-Lac, and to hydrolyse D-Ala-D-Ala, respectively. Diagram adapted from reference [74b].

As a consequence of the expression of the five genes associated with resistance, and the biosynthesis of cell-wall precursors that terminate in D-Ala-D-Lac, the hydrogen bond which is normally made between the NH of the terminal D-alanine group of the precursors and a carbonyl group of the antibiotic is replaced by a repulsive interaction between the

oxygen of the C-terminal D-lactate group and the carbonyl group of the antibiotic (Scheme 4). The affinity of glycopeptide antibiotics for precursor analogues terminating in D-Ala-D-Lac is thus decreased by a factor of the order of 1000



Scheme 4. In vancomycin resistant enterococci the terminal D-alanine of the immature cell wall has been replaced in part, or essentially completely, by D-lactate. This change drastically reduces the binding constant to vancomycin, as an NH that can form a hydrogen bond is replaced by an oxygen atom that cannot.

relative to the binding to precursors terminating in D-Ala-D-Ala.^[75] As a consequence of this much lower binding constant, vancomycin shows little activity against bacteria that biosynthesize such precursors.^[76] Inhibition of VRE requires 100–1000 times as much vancomycin as is required to inhibit the growth of sensitive organisms. Vancomycin is not, therefore, a viable antibiotic in these cases.

16. Proposed Operation of Dimerization and Membrane Anchoring in the Action of a New Antibiotic Active Against VRE

The nature of the difference between resistant and sensitive bacteria, and the reduction in affinity of vancomycin for precursors from resistant bacteria that results, suggests that the glycopeptide antibiotics may not be able to exhibit useful activity against such bacteria. However, attempts have been made at Eli Lilly in Indianapolis and Gruppo Lepetit-SpA in Gerenzano, Italy, to modify naturally-occurring glycopeptides to produce new antibiotics with increased activity against vancomycin-resistant bacteria.^[77] At Eli Lilly, scientists have recently taken the antibiotic chloroeremomycin (**4**) and added to this a hydrophobic *p*-chloro-*p*-phenylbenzyl group to give the resulting structure **5**, which is known as LY333328.^[78] This new molecule shows remarkable activity against VRE, being typically 50 times more active than vancomycin.^[79] It is also more active, by a factor of 4–8 in vitro, than vancomycin against MRSA and its in vivo potency is comparable with its in vitro potency.^[79] The similar program at Gruppo Lepetit-SpA has also yielded a glycopeptide that shows promising activity, both in vitro and in vivo, against vancomycin-resistant enterococci.^[80]

An answer to the question of how the modified antibiotic LY333328 is able to kill resistant bacteria was not immediately obvious. This was especially so, given that LY333328 (**5**) and vancomycin both have low affinity (binding constants in the range 300–500 M⁻¹) for the precursor analogue of resistant bacteria *N*- α -acetyl-*N*- ϵ -acetyl-lysyl-D-alanyl-D-lactate (Ac₂-

KDAdLac) in aqueous solution.^[75] These values are in contrast to the binding constant of $1.5 \times 10^6 \text{ M}^{-1}$ for vancomycin binding to Ac₂-KDADA.^[50] This similarity in binding affinity of Ac₂-KDAdLac for vancomycin and for LY333328 was not entirely surprising, however, given that the semi-synthetic modification to LY333328 was made at a position remote from the ligand binding pocket of the antibiotic. We therefore hypothesized that LY333328 was gaining extra binding affinity for -D-lactate-terminating ligands, relative to vancomycin, through the operation of the cooperative phenomena outlined earlier in this article. It was felt that the weak binding interaction between LY333328 and Ac₂-KDAdLac might be enhanced at the surface of a bacterium through the simultaneous operation of *both* dimerization (LY333328 is derived from the antibiotic chloroeremomycin, which dimerizes strongly) and membrane anchoring (by the hydrophobic *p*-chloro-*p*-phenylbenzyl chain inserting into the bacterial membrane).

17. ¹H and ¹⁹F NMR in Measurements of Binding Enhancements at Membrane Surfaces

We recently attempted to accumulate evidence in support of the above hypothesis through ¹H and ¹⁹F NMR spectroscopic studies of membrane-anchored peptides terminating in -D-lactate. In these experiments we have used phosphatidylcholine vesicles to mimic the bacterial membrane rather than SDS micelles. Vesicles are much larger lipid assemblies than are micelles, and consist of lipid bilayers in which the polar head groups exist both in the inside and the outside of the spherical structure. Cell wall precursor peptides possessing *N*-docosanoyl (a linear C₂₂ chain) lipid chains were then synthesized such that the docosanoyl chains could insert into the vesicles, as with the decanoylated peptides used earlier, in order to mimic the surface of the bacterium. Such vesicle assemblies have the advantage of being closer in size to bacteria than micelles, and the possibility of greater cooperativity being expressed therefore exists. Yet this advantage is bought at a price—the vesicles are so large that if an antibiotic becomes bound to these bacterial mimics, its proton NMR signals are no longer observable. This is because the bound antibiotic takes on the slow tumbling time that is characteristic of a large molecular assembly, and its protons then undergo fast nuclear relaxation, with a consequent severe broadening of the lines, so much so that they effectively disappear. Therefore, the w₂ resonance, whose chemical shift can be used as a semi-quantitative measure of binding affinity (Figure 5), cannot be observed in the bound state.

However, this line-broadening effect can be put to advantage in competition experiments. Suppose a -D-Lac-terminating peptide, possessing a docosanoyl group at its N-terminus, is anchored into the vesicle. It may therefore form the vesicle-templated assembly shown in Figure 13A. According to our hypotheses the antibiotic/peptide complex should be stronger in this assembly than it would be if both components were free in solution (as exemplified by the association between free chloroeremomycin and Ac₂-KDAdLac in solution, where the binding constant is approximately 240 M^{-1}).^[81] If there is

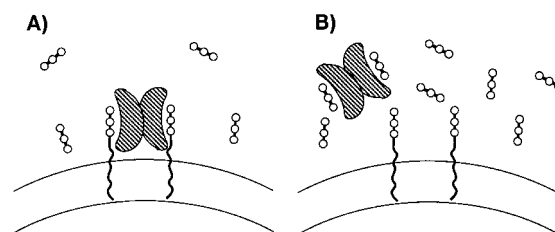


Figure 13. Schematic illustration of the competition experiment used in determining surface binding affinities of membrane-anchored cell wall precursor analogues. A) Antibiotic dimer bound to a vesicle-anchored -D-Lac-terminating peptide, with a nonanchored -D-Ala-terminating peptide present free in solution at a concentration at which it is unable to antagonize the templated binding. B) The same experiment at an increased concentration of the free -D-Ala-terminating peptide, such that the antibiotic dimer is now bound to this peptide in solution.

indeed extra binding affinity in the vesicle-attached assembly, then it should be possible to compete with such binding by the addition of a -D-Ala-terminating peptide that is only able to bind in solution, for example, Ac₂-KDADA which lacks a membrane anchor. In such a competition experiment it is possible to adjust the concentration of added Ac₂-KDADA until it is sufficiently high to “steal” the antibiotic (Figure 13B) from its vesicle-attached state (Figure 13A) where it is bound to *N*-docosanoyl-glycyl-L-alanyl-D-γ-glutamyl-L-lysyl-D-alanyl-D-lactate (Docos-GADEKDAdLac; an extended cell wall precursor analogue, where the glycine residue is used as a spacer unit in lieu of the sugar unit that is present in the natural cell-wall precursors). Removal of the antibiotic from its vesicle-attached state means that its ¹H NMR signals are no longer severely line-broadened and resonances arising from the antibiotic/Ac₂-KDADA complex can be observed in 1D ¹H NMR spectra. Integration of the resultant complex signals allows quantitation of the binding constant for the antibiotic binding to the vesicle-anchored ligand in a templated complex. We have carried out such a competition experiment using the strongly dimerizing antibiotic chloroeremomycin and the cell wall analogue components mentioned above; details of this experiment are given in Figure 14. It is found that a 5.7-fold excess of Ac₂-KDADA over vesicle-attached Docos-GADEKDAdLac is required to approximately equi-partition the binding of chloroeremomycin between these two peptides. Qualitatively, it can therefore be seen that the templated -D-Lac-terminating peptide binds the antibiotic approximately six times more strongly than does Ac₂-KDADA. Since Ac₂-KDADA has a binding constant of approximately 10^6 M^{-1} to free chloroeremomycin in solution, this corresponds to a binding constant of approximately $6 \times 10^6 \text{ M}^{-1}$ for this strongly dimerizing antibiotic to the model of a vancomycin-resistant bacterium (a more quantitative treatment of the data gives a binding constant of $7 \times 10^6 \text{ M}^{-1}$).^[82] The binding constant of the equivalent non-docosanoylated ligand, *N*-acetyl-glycyl-L-alanyl-D-γ-glutamyl-L-lysyl-D-alanyl-D-lactate (Ac-GADEKDAdLac), was measured by a different NMR method to be 2000 M^{-1} .^[83] The binding constant to Docos-GADEKDAdLac thus corresponds to a remarkable cooperative enhancement of the binding of the antibiotic to the membrane-anchored precursor analogue of VRE of approximately 3500 over the binding observed in the absence of the docosanoyl membrane anchor.

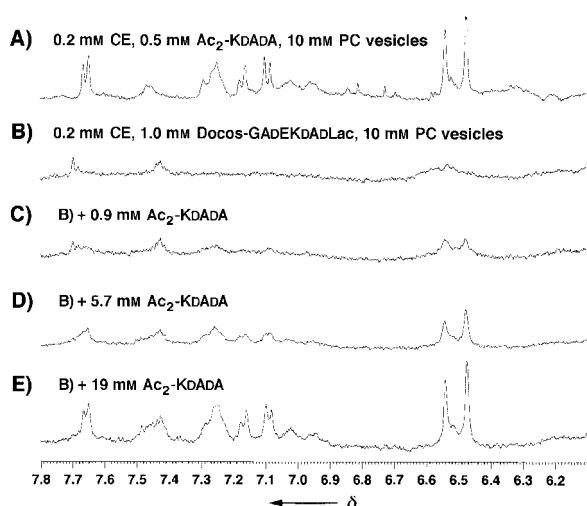


Figure 14. Competition experiment (see Figure 13) at pH 7 to determine the binding constant of chloroeremomycin (**4**) to a model of the surface of VRE. A) Proton resonances of chloroeremomycin when bound to the nonmembrane-bound cell wall precursor analogue $\text{Ac}_2\text{-KDADA}$ in the presence of phosphatidylcholine vesicles. B) The severe broadening of the same resonances when the antibiotic is bound to the -D-Lac -terminating-hexapeptide cell wall precursor analogue Docos-GADEKDADLac that is anchored into phosphatidylcholine vesicles. C) The solution from (B) to which $\text{Ac}_2\text{-KDADA}$ has been added to a concentration roughly equal to that of the membrane-bound Docos-GADEKDADLac. Note that most of the antibiotic is still bound to the membrane-bound -D-Lac -terminating peptide (from the contrasting signal intensities in (A) and (C)). D) The solution from (C), but to which more $\text{Ac}_2\text{-KDADA}$ has been added. In (D), comparable amounts of antibiotic are bound to the free -D-Ala terminating peptide in solution and to the membrane-bound -D-Lac terminating peptide. E) A large excess of $\text{Ac}_2\text{-KDADA}$ over the membrane-bound Docos-GADEKDADLac is required for most of the antibiotic to bind to the former.

We have employed a second technique that utilizes competition experiments to provide a further measurement of the binding enhancement that can be achieved through templated binding at a vesicle surface. This involved competing the antibiotic molecules from their vesicle-templated complexes with Docos-GADEKDADLac using the ligand $N\text{-}\alpha\text{-acetyl-}N\text{-}\epsilon\text{-trifluoroacetyl-lysyl-D-alanyl-D-alanine}$ (TFac-KDADA).^[84] This ligand is labeled with a [^{19}F]trifluoroacetyl group at a position not expected to interfere significantly with the binding of the ligand to the antibiotic. ^{19}F NMR spectroscopy can therefore be used to examine and quantify the binding to antibiotics. This method has the advantage over ^1H NMR spectroscopy that only two signals are seen in the ^{19}F NMR spectra during the competition experiments; one signal from free (unbound) TFac-KDADA and one from TFac-KDADA complexed with antibiotic not on the vesicle surface (Figure 15). Integration of the free and bound peaks, and comparison to the integral of an external reference peak allows quantitation of the apparent binding constant of chloroeremomycin bound to Docos-GADEKDADLac in a templated complex on the vesicle surface. The value thus obtained using this method, for the apparent binding constant of chloroeremomycin to vesicle-bound Docos-GADEKDADLac was $2.5 \times 10^5 \text{ M}^{-1}$, which is, as with the ^1H NMR-derived value, much greater than the solution binding constant of chloroeremomycin to free Ac-GADEKDADLac (2000 M^{-1}),

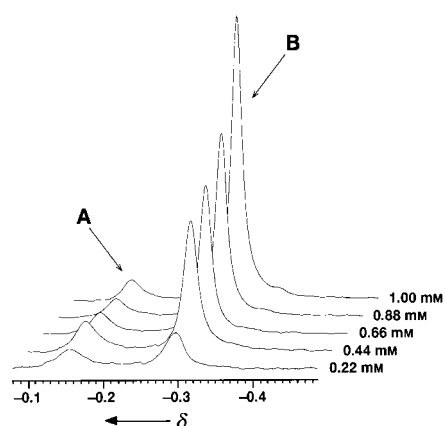


Figure 15. ^{19}F NMR spectra from a titration of a solution of TFac-KDADA into a solution containing 0.2 mM chloroeremomycin (**4**), 1.0 mM Docos-GADEKDADLac, and 10 mM phosphatidylcholine at 298 K. The concentrations of added TFac-KDADA are shown at the right-hand side of each of the corresponding 1D spectra. The peaks labeled (A) are those of unbound TFac-KDADA, while the peaks labeled (B) are those of TFac-KDADA complexed with chloroeremomycin not attached to the surface of a vesicle. The TFac-KDADA (binding constant to chloroeremomycin of $7.7 \times 10^5 \text{ M}^{-1}$) competes effectively with Docos-GADEKDADLac for the binding sites of chloroeremomycin. The binding constant of Docos-GADEKDADLac to chloroeremomycin calculated from analysis of this data is $2.5 \times 10^5 \text{ M}^{-1}$. Chemical shift values are relative to an external reference of trifluoromethanesulfonic acid ($\delta = -3.60$).

which confirms that a large enhancement to the binding of -D-Lac -terminating ligands can be achieved at a surface as a result of antibiotic dimerization.^[84]

Recall that it is proposed that the cooperativity which can be gained by simultaneous strong dimerization and membrane anchoring of the antibiotic may provide the basis for the remarkable activity of LY333328 (**5**) against bacteria utilizing -D-Lac -terminating peptides in their cell-wall biosynthesis. The experiments described thus far, although demonstrating the benefit of binding at a surface provided by dimerization, fail to quantify the cooperativity gained by simultaneous operation of these two effects. Two problems have been encountered in attempts to obtain this number. First, when micelle-associated peptides are used to bind **5**, the solution containing both these components becomes cloudy, presumably because of aggregation, and a binding constant cannot be obtained by UV difference spectroscopy. Second, the competition experiments, described above for chloroeremomycin with vesicles, cannot be simply used with **5**, since the $p\text{-chloro-}p\text{-phenylbenzyl}$ group will influence the antibiotic towards always remaining vesicle-bound and thus direct NMR measurements of the populations of bound species are not possible. Nevertheless, the templated-enhancement to binding of a factor of the order of 10^4 , cited above, from dimerization alone, is sufficiently dramatic to suggest that the remarkable activity of **5** against VRE can be accounted for, not by a different binding mode of **5** compared to vancomycin, but because of the simultaneous exercise of the cooperative benefits of dimerization and membrane-anchoring. This value of 10^4 is likely to represent a lower limit for the enhancement that will be experienced by **5**, which should also be able to achieve a benefit as a result of membrane anchoring on top of the benefit from dimerization. The operation of the two

effects together may also result in a further cooperative enhancement from the mutual motional restriction caused by the two membrane-locating devices.

A note of caution must be expressed here, however. Although the model membrane systems that we have used have unambiguously allowed the expression of enhanced binding affinities in these systems, and the dimerization propensity of the antibiotics has been correlated with the difficulty of antagonizing antibiotic action against *B. subtilis*, we cannot preclude the additional operation of as yet undiscovered factors in the remarkable activity of **5**. For example, the possibility that antibiotic dimerization can affect antibacterial activity through the cross-linking of adjacent cells may merit further investigation. Additionally, it must be borne in mind that where the antibiotics incorporate hydrocarbon groups that enhance binding at model membranes containing mucopeptide precursors, we can so far only surmise that identical effects operate at the surfaces of bacteria. For example, we cannot yet preclude that the hydrocarbon groups of the antibiotics are alternatively associating with hydrophobic clusters in membrane-bound proteins, and thereby deriving cooperative binding energy from such interactions. Nor can we yet understand the often quite marked differences in activities of the various glycopeptide antibiotics against the wide diversity of Gram-positive pathogens that are found in the clinic. The crucial message for drug design, however, is that if binding efficacy is lost as a result of a mutation in a binding site, binding of similar efficiency can be restored in principle without making any modifications directly in the binding site. A direct corollary of this conclusion is of course that the prediction of binding constants in complex networks of interactions is at best a formidably difficult task! In the present case important antibacterial activity has been recouped from a situation that may initially have appeared hopeless.

18. Implications for the Functions and Evolution of Secondary Metabolite Structures

The occurrence of two complex binding sites within the glycopeptide antibiotics (one for binding cell wall peptide precursors and the other for dimerization) is remarkable. It is a feature that strongly suggests that the evolutionary origin of the glycopeptide structures (at least in the origins of their most recently selected molecular features) lies in the antimicrobial action described. In several articles,^[85, 86] we have argued that secondary metabolites (which are defined as having no known role in the internal economy of the producer, and are typically excreted from the producer) have evolved to increase the survival fitness of the producer. This increase in survival fitness can be expressed in principle by either symbiotic or antagonistic interactions with other organisms. In either case, a receptor site at another organism is necessary. This very frequently involves a specific molecular interaction and, where the details of such interactions have been elucidated (as in the case presently described), they rival enzyme/substrate interactions in their sophistication.^[85]

Against the above view, it is sometimes argued that remarkably complementary interactions of secondary metabolites to other molecules in a second organism may be found, but yet it might be difficult initially to accept that these interactions are the ones that have evolved through natural selection. A case in point is found in the molecular basis of action of the immunosuppressive compounds cyclosporin A, FK506, and rapamycin.^[87] It certainly seems impossible to justify that these three secondary metabolites, the first produced by a fungus and the last two by *Streptomyces* bacteria, have evolved to inhibit the activation or proliferation of human T cells! Yet,

- 1) this inhibition occurs through the binding of the secondary metabolites to intracellular receptor proteins,^[87] and in the cases of cyclosporin A and FK506, the resulting complexes then interact with calcineurin.^[88]
- 2) exactly the same sequence of events occurs in yeast cells, through which the secondary metabolites exercise antifungal properties.^[89]

Thus, we see in the second point a possible selectional force for the production of the secondary metabolites by the producing strains, because these producers have a weapon to combat other microorganisms with which at some stage they may have competed. The same pathways for activity against T cells may exist because of the common origins of T cells and yeast cells, with the consequence that their primitive signalling pathways may be the same.

One of the most cogent points in favor of the key importance of secondary metabolites to the survival of their producers lies in the impressively large amount of DNA that is frequently necessary to code for their production. The pioneering work of Hopwood^[90] in investigating this DNA, and the details of its functions, is now being extended by others in many parts of the world. The use of several tens of enzymes in the production of an antibiotic is not uncommon,^[91] and thus their production is coded within many tens of kilobases of DNA. The vancomycin group of glycopeptides are no exception in this remarkable feat of evolution.^[27, 92]

19. Comments on Cooperativity: the Enthalpic Chelate Effect

During the course of our work on the mode of action of the glycopeptide antibiotics, we have been able to identify and comment upon a number of features regarding cooperativity in molecular recognition that have a general relevance to studies of binding processes in nature. In this respect, the glycopeptides have proved to be a simple and useful model for understanding aspects of binding that may be more difficult to study in more complex systems.

We have recently commented upon the fact that the decreased motion of a ligand in a binding site works to improve the electrostatic interactions that are formed in that binding site.^[55, 93] In a reciprocal manner, features that improve the electrostatics of binding in a ligand/receptor interaction likewise clearly reduce the degree of motion in the binding site. This is a demonstration of the property of enthalpy–entropy compensation;^[94] the two effects can be

regarded as working iteratively on each other. For example, if **A** binds in isolation to part of a binding site with an exothermicity X , and **B** binds in isolation to another part of the binding site with an exothermicity Y , then attachment of **A** to **B** so that they can simultaneously bind into the binding site in a strain-free manner (that is as the entity **A–B**) is not only advantageous through the operation of the classical chelate effect (an entropic benefit), but leads to an exothermicity of binding greater than $X + Y$. This effect we call the enthalpic chelate effect.^[55] An example of the enthalpic chelate effect was shown earlier with the increasing strength of the hydrogen bond between the carboxylate anion of a bacterial cell wall precursor analogue and the amide NH proton w_2 of a glycopeptide antibiotic as extra functional groups were added to the ligand (Figure 5). The extra functional groups and corresponding extra hydrogen bonds between ligand and antibiotic, relative to the binding of, say, acetate, result in an increased downfield chemical shift of w_2 upon ligand binding. Although the extra functional groups are remote from w_2 and therefore do not exert a direct influence on its chemical shift, they serve to anchor the ligand into its binding site and thus reduce the motion of the ligand carboxylate relative to the antibiotic, leading to a stronger (more exothermic) hydrogen bond to w_2 and a more downfield resonance.^[55]

20. Equilibrium Constants and the Parameters Describing Bound States

The experiments described above for measuring the chemical shift of w_2 illustrate a principle that we considered might be both general and important in terms of its implications for studies of other molecular recognition events. This is that a parameter representing the bound state of a system (for example, the limiting chemical shift of w_2) may vary according to the equilibrium constant for the association (Figure 5). Further, if we could examine the interface for a specified association, a relatively high value for the equilibrium constant would result in relatively strong bonds at the interface with correspondingly short bond lengths; the interface could be described as “tight”. Conversely, a weak association would be characterized by long interfacial bond lengths; the interface could be described as “loose”. Accordingly, we attempted to measure the “tightness” of the dimer interfaces of the glycopeptide antibiotics with respect to their dimerization constants. All glycopeptides, despite exhibiting a wide range of dimerization constants, share a common arrangement of at least four hydrogen bonds at the dimer interface (Scheme 2). A proton, x_4 (Figures 5 and 7), at the dimer interface undergoes a relatively large downfield shift upon dimerization and the extent of this *limiting* downfield shift upon dimerization ($\Delta\delta_{x_4}$) was used to probe the tightness of the dimer interface as a function of the dimerization constant. The change in chemical shift of x_4 on dimerization ($\Delta\delta_{x_4}$) is much larger for strongly dimerizing antibiotics than for weakly dimerizing antibiotics (Figure 16).^[95]

In summary, the data relating the chemical shifts of w_2 and x_4 to ligand binding and dimerization constants, respectively, indicate that as the equilibrium constants for the respective

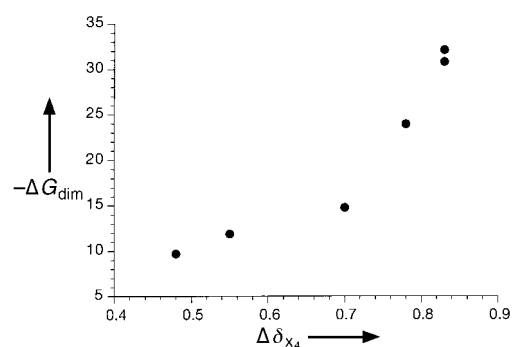


Figure 16. Plot of the Gibbs free energy of dimerization ($-\Delta G_{\text{dim}}$ [kJ mol^{-1}]) versus change in chemical shift of the proton x_4 ($\Delta\delta_{x_4}$) upon dimerization for some glycopeptide antibiotics. In order of increasing magnitude of ΔG_{dim} , the points represent the dimerization of the following antibiotics: ristocetin- ψ , monodechlorovancomycin, vancomycin, chloroeremomycin, eremomycin, and *p*-phenylbenzylchloroeremomycin.

associations increase, the associating entities come into more intimate contact. A general conclusion to be derived from this data is given in Figure 17. This finding has implications for the study of transitions between bound (or folded) and free (or unfolded) states that are considered to occur by means of a two-state process. Such studies typically involve measurement of a parameter and comparison of this to values expected for

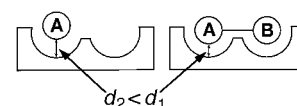


Figure 17. Schematic representation of the association of an entity **A** with its receptor (left) and the association of the same entity **A** when attached through a strain-free linker to a second entity **B** that binds to a separate site on the same receptor (right). In the latter case the association constant of the ligand with the receptor is high as a consequence of the cooperative enhancement to the binding of **A** provided by **B**. The complex formed is relatively tight and relatively few bound states are accessible to the ligand. As a result, the distance d_2 is typically less than the distance d_1 .

the bound (or folded) and free (or unfolded) states. The measured value then allows calculation of the relative proportions of the states. Typically, the parameter values representing free (or unfolded) states may be relatively simply defined. For example, in considering ligands that bind to a glycopeptide antibiotic, the free state is that where no ligand is bound (free antibiotic) and a parameter (w_2) representing this state can be easily measured. Our work shows, however, that there is no single parameter value that represents the bound (or folded) state.^[96] As the equilibrium constant for the association increases so does the parameter representing the bound (or folded) state, for example, the limiting chemical shift of w_2 .

Systematic errors can arise from the above complication. An example is the calculation of the fractional population of a short peptide (known to form an α -helix when part of a full-length protein) occupying an α -helical conformation in aqueous solution. The equation used to calculate this fraction x is generally of the form (1), where p_{unf} is a parameter

$$x = \frac{p_{\text{obs}} - p_{\text{unf}}}{p_{\text{fol}} - p_{\text{unf}}} \quad (1)$$

characteristic of the fully unfolded (“random coil”) state of the peptide, p_{fol} is the parameter characteristic of the fully folded (helical) state of the peptide, and p_{obs} is the measured value of the parameter under the conditions of interest (for example, aqueous solution).

In order to obtain an estimate for p_{fol} , it is common to introduce trifluoroethanol (TFE) as a solvent, which encourages the formation of α -helices. However, the very act of diluting the TFE solution with water would be expected, by analogy to our work on glycopeptides, to lead not only to a gradually increasing population of the unfolded state, but simultaneously to a folded state which becomes increasingly loose relative to that existing prior to dilution. Thus, the parameter p_{fol} will be reduced in magnitude as the dilution proceeds. The error inherent in such analyses therefore generally results in an underestimate of the population of the (increasingly dynamic) folded state.

21. Analysis of the Origins of Cooperative Binding Energy

Returning to the experiments described above which indicate the variation of the tightness of the dimer interface (identified by the magnitude of $\Delta\delta_{x_4}$) with the variation of the dimerization constant, we have extended these studies further to probe the interfacial origins of cooperative binding energy within systems of ligand-bound glycopeptide dimers.

Our hypothesis was that if one of the interfaces (dimer interface or antibiotic–ligand interface) was initially loose (small association constant), then formation of a cooperative tetrameric assembly (two ligands bound to an antibiotic dimer) would potentially result in a significant tightening of that interface. Conversely, if one of the interfaces was initially tight (large association constant), then formation of a tetrameric assembly would allow little scope for further tightening through cooperative interactions. If this hypothesis is true, then if the dimer interface, for example, is loose in the absence of ligand (small dimerization constant K_{dim}), an important contribution to the increase in K_{dim} in the presence of ligand will come from changes associated with the tightening of the dimer interface (which can be observed by an increase in limiting chemical shift of x_4). Alternatively, if the dimer interface is tight even in the absence of ligand (large value of K_{dim}), then the major portion of the favorable Gibbs free energy change that causes an increase in K_{dim} in the presence of ligand should actually come from changes associated with the tightening of the ligand–antibiotic interface, and there should be little accompanying change in the limiting chemical shift of x_4 . The way in which the cooperative Gibbs free energy of dimerization can be partitioned into changes associated with the dimer interface or with the ligand antibiotic interface is indicated by sets of hypothetical points in Figure 18.^[95] The expectation is that a weakly dimerizing compound will largely exercise cooperativity by tightening the dimer interface (arrows joining open and filled circles for the same antibiotic at a shallow angle to the horizontal, for example, W in Figure 18A). In contrast, a strongly dimerizing compound will largely exercise cooperativity by tightening the

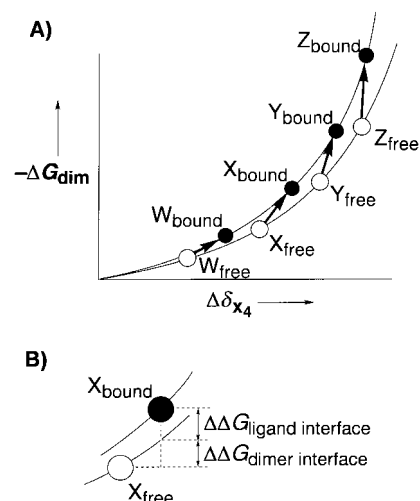


Figure 18. A) Hypothetical plots of $-\Delta G_{\text{dim}}$ versus $\Delta\delta_{x_4}$ for the dimerization of four antibiotics W, X, Y, and Z when free ($\circ$) and as antibiotic–ligand complexes ($\bullet$). The arrows connect the hypothetical points for a given antibiotic, and the series of antibiotics $W \rightarrow X \rightarrow Y \rightarrow Z$ have increasing dimerization constants. B) Since the Gibbs free energy of dimerization associated with changes at the dimer interface is defined by the curve connecting the points ($\circ$) for the dimerization of antibiotic alone, the extent to which $\bullet$ lies vertically above this curve gives the Gibbs free energy of dimerization associated with changes in the ligand–antibiotic interfaces.

interface with the ligand (arrows joining open and closed circles for the same antibiotic at a very steep angle to the horizontal, for example, Z in Figure 18A).

The experimental data (Figure 19) follow the postulated expectation from Figure 18 remarkably closely.^[95] The weakly dimerizing antibiotic ristocetin- ψ dimerizes more strongly in

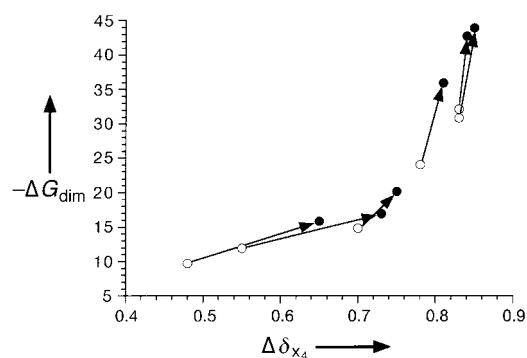


Figure 19. Combined plot of the Gibbs free energy of dimerization ($-\Delta G_{\text{dim}}$ [kJ mol⁻¹]) versus $\Delta\delta_{x_4}$ for free ($\circ$) and ligand-bound ($\bullet$) glycopeptide antibiotics. For any one antibiotic the arrows represent the changes in $-\Delta G_{\text{dim}}$ and $\Delta\delta_{x_4}$ that occur upon ligand binding. In order of increasing magnitude of ΔG_{dim} (for free antibiotics), the points represent the dimerization of the following antibiotics: ristocetin- ψ , monodechlorovancomycin, vancomycin, chloroeremomycin, eremomycin, and *p*-phenylbenzylchloroeremomycin.

the presence of $\text{Ac}_2\text{-KbADA}$ than in its absence essentially because the Gibbs free energy of binding associated with changes at the dimerization interface is more favorable. The more strongly dimerizing antibiotics chloroeremomycin, *p*-phenylbenzylchloroeremomycin (LY307599), and eremomy-

cin dimerize more strongly in the presence of Ac₂-KDA than in its absence largely because the Gibbs free energy of binding associated with changes at the antibiotic–ligand interface is more favorable in the ligand-bound dimer than in the ligand-bound monomer. In this case, there is little change in the Gibbs free energy of binding associated with changes at the dimer interface. The behavior of vancomycin is between these two extremes with the cooperative Gibbs free energy expressed partially at each of the ligand binding and dimerization interfaces.

These findings have implications for the study of protein–protein interactions and for drug design. In both areas it is common practice (though not of course uniquely the practice) to seek the origins of binding affinity at the interface formed between the associating entities.^[97, 98] Our data (Figure 19) show that the increase in the equilibrium constant for dimerization of weakly-dimerizing antibiotics in the presence of ligand (relative to when the ligand is absent) arises largely from changes associated with the tightening of the interaction between ligand and antibiotic. By analogy, where proteins (or, more specifically, receptors) have loose structures prior to binding another protein (or in the specific case of a receptor, its natural ligand or a drug), then a portion of the binding affinity can be derived by the tightening of the internal structures of the proteins in the resulting bound state. Based on this reasoning the thermodynamic parameters for protein–protein associations, which are perplexing when analyzed in terms of interfacial interactions,^[98] are realized to have much more complex origins.

22. Summary and Outlook

Despite the advances detailed in this article regarding our increased knowledge of glycopeptide activity, there is no room for complacency as evidenced by recent reports^[3, 99, 100] of the appearances in hospitals of *S. aureus* infections that are resistant to vancomycin. Although the resistance in MRSA so far seen is at a low level (and appears to involve an augmented rate of cell-wall synthesis, rather than the synthesis of a modified -D-Ala-D-Lac precursor^[99]), there is still the possibility that more high-level resistance to vancomycin will develop in *S. aureus*. It is clear that any approach that opens new possibilities for increasing the activity of glycopeptides is of the utmost importance.^[101]

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on the structural work of the Cambridge group, it is of course entirely a result of the work of scientists at Eli Lilly and Lepetit that vancomycin and teicoplanin, respectively, have made their clinical impacts.

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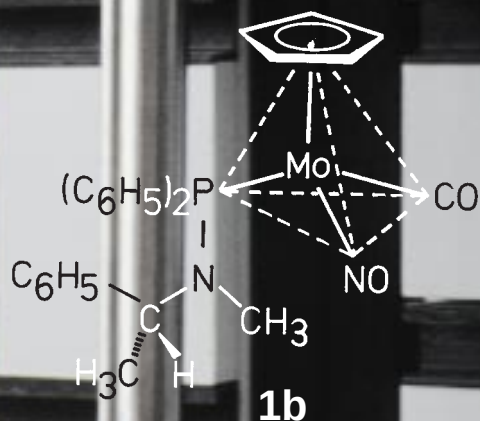
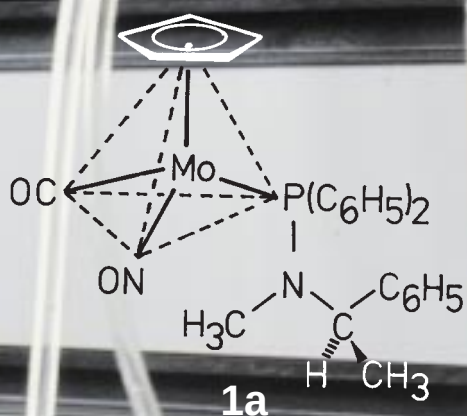
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Deposition of Data from X-Ray Structure Analyses

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Chromatographic separation of diastereomers on a Merck–Lobar column of type B, exemplified with the diastereomers of $[(\eta^5\text{-C}_5\text{H}_5)\text{-Mo(CO)(NO)\{PPh}_2\text{NMe(CHMePh)\}}]$ (**1a** and **1b**), which differ in the configuration at the Mo atom and contain the same *S*-configured amino-phosphane ligand.

Optically Active Organometallic Compounds of Transition Elements with Chiral Metal Atoms

Henri Brunner*

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

Chemistry is stereochemistry. In transition metal chemistry the optically active tris-chelating complexes, which have been known since the beginning of this century, were supplemented over the last thirty years by organometallic half-sandwich complexes of three- and four-legged piano-stool structure whose metal atom is stereogenic ("chiral transition metal atoms"). A few of the new optically active compounds proved to be configurationally stable at the metal atom even at higher temperatures. These complexes have been tools in the elucidation of the spatial course of follow-up reactions, as shown in the 1930s by Hughes and Ingold for nucleophilic

substitution at a carbon atom. There is still much to do in this area. Some of the other new optically active compounds are configurationally labile at the metal and racemize or epimerize in solution. In these cases the mechanism of the change of configuration at the metal atom and the resulting intermediates can be investigated. Complexes that are configurationally stable at the metal atom could be used as optically active auxiliaries in organic synthesis. An example is the enolate chemistry of the commercially available compounds (+)- and (-)- $[(\eta^5\text{-C}_5\text{H}_5)\text{Fe}(\text{CO})(\text{PPh}_3)(\text{COMe})]$. Chiral metal atoms also play a role in enantioselective catalysis. The metal centers

often become stereogenic in the catalytic cycle of metal-catalyzed reactions. Their chirality usually disappears upon product cleavage in the final reductive elimination of the cycle. However, it is regained in the next catalytic cycle. Control of the metal chirality during the catalysis has not yet been possible, although the use of half-sandwich complexes with chiral centers in enantioselective catalysis is beginning to show up.

Keywords: asymmetric catalysis • chirality • coordination chemistry • optical activity • reaction mechanisms

1. Introduction

Organometallic chemistry of the transition metals is intermediate between inorganic and organic chemistry, and is characterized by a manifold of structures and a multitude of reaction possibilities. Typical of a field matured by intense research for decades, transition metal chemistry today deals with subtle problems and effects, in particular with respect to the synthesis of organic compounds and catalysis of organic reactions. While the chemistry of metal carbonyl complexes developed mainly during the first half of our century, initiated with the discovery of tetracarbonylnickel by Ludwig Mond more than 100 years ago, the starting signal for modern transition metal chemistry was the discovery and structural elucidation of ferrocene in the early 1950s. It was followed by the golden period of preparative transition metal chemistry, in

which many of the basic compound types were found which still today are used as starting materials. This was the situation of transition metal chemistry in the 1960s, when I chose as the topic for my Habilitation the preparation of optically active transition metal compounds in which a tetrahedrally coordinated transition metal M is surrounded by four different ligands L^1 , L^2 , L^3 , and L^4 (Figure 1).^[1]

A simple chiral anion which derives from tetrahedral $[\text{NiCl}_4]^{2-}$ would be $[\text{NiFCIBr}]^{2-}$. Nobody would try to resolve a racemate of this anion, because rapid ligand exchange

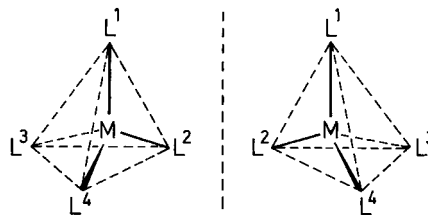


Figure 1. Image and mirror image of a transition metal complex with four different ligands.

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reactions would not only quickly interconvert image and mirror image, but symmetrization reactions would form all possible mixed species including the pure tetrahalo complexes from $[\text{NiF}_4]^{2-}$ to $[\text{NiI}_4]^{2-}$. In contrast, analogous organometallic compounds are kinetically inert, because the ligands are bound strongly to the metal atoms by the combination of σ -donor and π -acceptor bonds. Synthesis and ligand exchange reactions generally are slow, they can be controlled and planned, and there is an arsenal of methods in synthetic organometallic chemistry that is comparable to that in organic chemistry and allows specific transformations. Transition metal compounds are mainly low-spin complexes in which the metal atoms adopt a noble gas configuration. They are diamagnetic, and therefore, besides X-ray structure analysis, NMR spectroscopy is available as an efficient method of investigation.

2. Image–Mirror Image Isomerism

Image–mirror image isomerism of the asymmetric carbon atom has dominated stereochemistry since its discovery by van't Hoff and LeBel in 1874.^[2, 3] Also tetrahedral compounds of other main group elements, for example silicon, with four different substituents have been obtained in optically active form. The first reports on the resolution of racemates of silicon compounds appeared already at the beginning of this century.^[4] However, the field was investigated systematically only in the 1950s by L. H. Sommer and then R. J. P. Corriu et al.^[5–7] Resolution of tetrahedral compounds with four different substituents was extended to the higher elements of the carbon group (Group 14) and to the elements of the nitrogen group (Group 15). Today even optically active compounds with bismuth carrying four different substituents are known.^[8, 9]

Especially important are chiral compounds of the main group elements in which one of the “substituents” is a free electron pair. In the ammonia derivatives $\text{NR}^1\text{R}^2\text{R}^3$ these pseudotetrahedral compounds are notoriously configurationally labile because of inversion of the nitrogen atom through the plane of the substituents.^[10] In the corresponding phosphanes $\text{PR}^1\text{R}^2\text{R}^3$, however, the process of pyramidal inversion is so slow, that they can be prepared in optically active form (Horner phosphanes).^[11] Also other classes of pseudotetrahedral compounds such as sulfoxides $\text{S}(\text{O})\text{R}^1\text{R}^2$ show high configurational stability.

Phosphanes are variable ligands in organometallic complexes, for example in hydrogenation reactions with rhodium catalysts of the Wilkinson type. Optically active phosphanes with a chiral phosphorus atom already played a role in the beginnings of enantioselective hydrogenation in 1968.^[12, 13] The chelating ligand 1,2-ethanediylbis[(2-methoxyphenyl)-phenylphosphane] (dipamp), which contains two chiral phosphorus atoms, was introduced in the 1970s into the industrial production of L-dopa, a drug used against Parkinson's disease (Monsanto amino acid process).^[14]

Image–mirror image isomerism in the series of transition metals is dominated by the octahedral tris-chelating type, which after the asymmetric carbon atom is the next important element of chirality.^[15, 16] Metal tris-chelates with three identical symmetric ligands, for example $[\text{Co}(\text{en})_3]\text{X}_3$, are not asymmetric. They belong to the point group D_3 , and contain a C_3 and three C_2 axes, but no symmetry plane, no inversion center, and no higher improper axis, which would remove chirality. $[\text{Co}(\text{en})_2(\text{NH}_3)\text{Cl}]\text{Cl}_2$ was the first octahedral complex to be prepared in optically active form by A. Werner in 1911.^[17] Until the end of the 1960s, however, no optically active compounds existed in which a transition metal M was surrounded by four different ligands L^1 , L^2 , L^3 , and L^4 .

In this review, the term “optically active” is used to distinguish between dextro- and levorotatory isomers. “Stereoogenic” is used when the number of isomers and isomeric possibilities are discussed, whereas “asymmetric” is used when symmetry characteristics are important. The term “chiral”, whose use in the literature is not uniform, incorporates both optically active and stereoogenic, since formulations such as “chiral metal atom”, “chiral-at-metal”, and “chiral-at-iron”, etc. have meanwhile become accepted terminology.

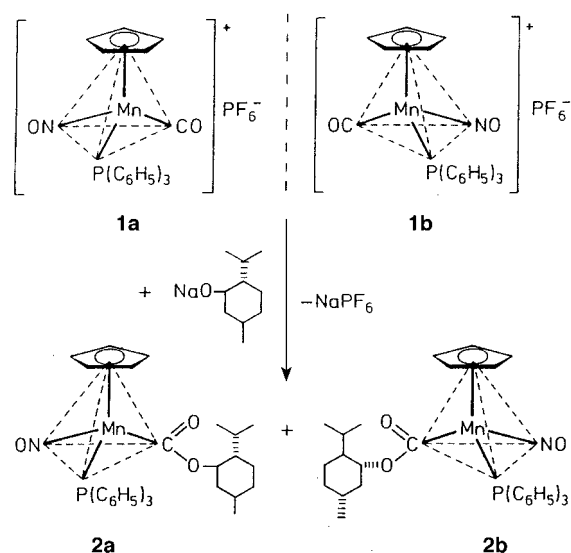
3. The First Optically Active Transition Metal Compounds with Four Different Substituents

Thirty years ago, the first resolution of a transition metal complex with four different substituents was achieved with the manganese complex **1** (Scheme 1).^[18, 19] In the cation of this complex the manganese atom is surrounded by a cyclopentadienyl, a carbonyl, a nitrosyl, and a triphenylphosphane ligand. It can be obtained in two steps from the commercially available compound $[(\eta^5\text{-C}_5\text{H}_5)\text{Mn}(\text{CO})_3]$, and is formed as



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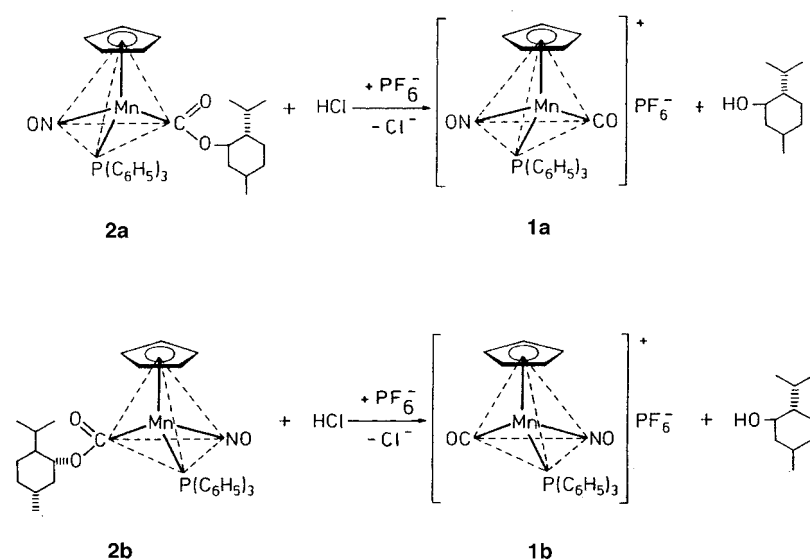
Henri Brunner, born in 1935, studied chemistry at the Universität München, Germany, where he received his Diplom in 1960. He completed his dissertation in the research group of Professor E. O. Fischer in 1963. After a one-year postdoc at the University of California, Los Angeles, with Professor H. D. Kaesz, he finished his Habilitation at the Technische Universität München. In 1971 he accepted a position at the Institut für Anorganische Chemie at the Universität Regensburg, where he is still working. His areas of research are the stereochemistry of organometallic complexes and reactions, enantioselective catalysis, and the investigation of platinum complexes in cancer chemotherapy. He has received many awards and fellowships, including the German–French Alexander von Humboldt Award in 1993, the Max Planck Research Award for International Cooperation in 1997, and the Horst Pracejus Award of the German Chemical Society in 1999.



Scheme 1. Complexes **2a** and **2b**, the first optically active transition metal complexes with four different ligands.

the racemate **1a/1b**.^[20–23] To convert the pair of enantiomers **1a/1b** into a pair of diastereomers, it is allowed to react with the sodium derivative of the optically active alcohol (1*R*,3*R*,4*S*)-menthol. The mentholate ion adds to the carbon atom of the carbonyl ligand in the cationic starting material. The neutral “esters” **2a/2b** are formed, which differ only in the configuration of the manganese atom. The two diastereomers can be separated on the basis of their solubility differences. The (+)₅₇₉ diastereomer dissolves in petroleum ether, whereas the (–)₅₇₉ diastereomer is only sparingly soluble in this solvent.^[18]

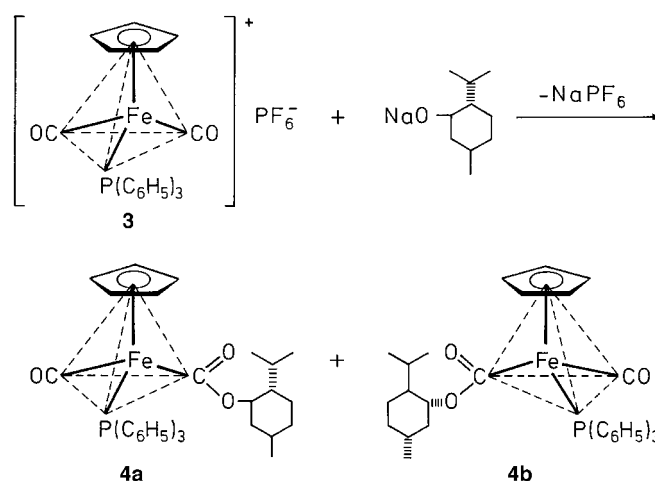
To complete the resolution the optically active auxiliary has to be removed from the separated diastereomers. This occurs upon bubbling of HCl through solutions of **2a** and **2b** in benzene. In this reaction the C–O_{menthyl} bond is cleaved. Menthol is formed, and the ester group is retransformed into the carbonyl group of the starting cation. The counterion is first Cl[–]; it is then replaced by PF₆[–] (Scheme 2).^[19] Starting



Scheme 2. Formation of the enantiomers **1a** and **1b** from the diastereomers **2a** and **2b**.

from the (+)₅₇₉ diastereomer **2a** the (+)₅₇₉ PF₆[–] salt **1a** is formed, while the (–)₅₇₉ diastereomer **2b** is transformed into the (–)₅₇₉ PF₆[–] salt **1b**.^[19] The diastereomers **2a** and **2b** contain four asymmetric centers each, the asymmetric manganese atom and three asymmetric carbon atoms in the menthyl part of the molecule. After removal of menthol the stereogenic manganese atom is the only source of chirality in complexes **1a** and **1b**. The optical rotations of the two enantiomers have opposite signs and, within the limits of error, the same magnitude.^[18, 19, 24]

The addition of mentholate ions to form diastereomers which only differ in the metal configuration is confined to cationic carbonyl complexes. Whereas the cationic manganese complexes in Scheme 1 are enantiomers, the cation in the complex $[(\eta^5\text{-C}_5\text{H}_5)\text{Fe}(\text{CO})_2\text{PPh}_3]\text{PF}_6$ (**3**)^[25] is prochiral. It contains a symmetry plane which passes through the cyclopentadienyl ligand, the triphenylphosphine ligand, and the iron atom and bisects the angle between the two carbonyl groups. It is on addition of the mentholate ion that the iron atom becomes an asymmetric center (Scheme 3). The attack

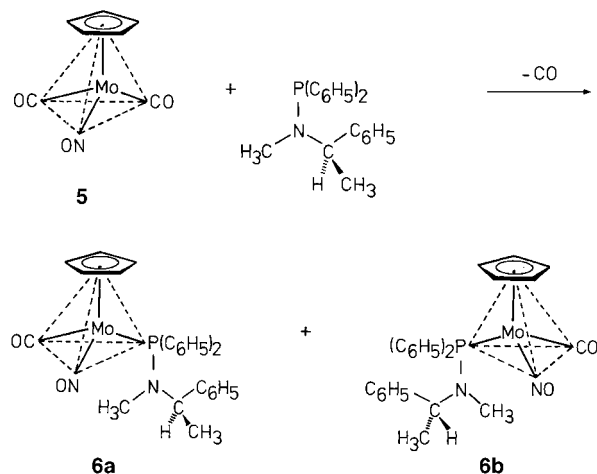


Scheme 3. Transformation of the prochiral complex **3** into the two diastereomers **4a** and **4b** with the help of menthol.

on the two enantiotopic carbonyl groups results in the two diastereomers **4a** and **4b** with opposite configuration at the iron atom. As with **2a** and **2b**, **4a** and **4b** can be separated on the basis of solubility differences.^[26, 27] However, an acid cleavage of the ester groups as in the case of **2** would lead to a loss of chirality and stereogenicity at the iron atom.^[27]

Phosphanes are excellent ligands for organometallic compounds. Therefore, optically active phosphanes should be universally suitable for the production of diastereomeric complexes which only differ in the metal configuration. Aminophosphanes of the type (*R*)-Ph₂P–NH(CHMePh) and (*S*)-Ph₂P–NMe(CHMePh) can easily be prepared from Ph₂PCl and the corresponding optically active amines.^[28, 29] In terms of size and

electronic effect, they resemble the standard ligand triphenylphosphane.^[30] The anisotropy beams which are connected with their phenyl groups usually cause the diastereomeric metal complexes to differ in their ^1H NMR spectra. In these cases the diastereomer ratio can be determined by integration of suitable signals, for example in the molybdenum complexes of Scheme 4. If in complex **5** one of the two enantiotopic CO



Scheme 4. Transformation of the prochiral complex **5** into the two diastereomers **6a** and **6b** with the help of a chiral phosphane ligand.

groups is replaced, the diastereomeric complexes **6a** and **6b** are formed with the same *R* configuration at the asymmetric carbon atom of the ligand and with mirror-image configurations at molybdenum.^[29, 31] The two diastereomers **6a** and **6b**, which can be separated by chromatography as well as by fractional crystallization, show distinctly different ^1H NMR spectra.

All the complexes in Schemes 1–4 are half-sandwich complexes with the structure of a three-legged piano stool. Indeed, they contain four different ligands. Therefore, they are pseudotetrahedral, an aspect which is emphasized by the dotted frame in the formulas. However, the $\eta^5\text{-C}_5\text{H}_5$ ligand occupies three facial coordination sites, which means that the geometry of these complexes is in fact octahedral. This is also clear from the angles between the monodentate ligands. These angles are frequently close to the octahedral angle of 90° .^[32]

4. Square-Pyramidal, Octahedral, and Tetrahedral Complexes with an Asymmetric Metal Atom

Half-sandwich complexes with the structure of a four-legged piano stool can contain up to five different ligands. While permutation of the four ligands $L^1\text{--}L^4$ at the corners of a tetrahedron results in only two isomers (image and mirror image), permutation of five ligands at the corners of a square pyramid yields 30 isomers, which subdivide into 15 pairs of enantiomers.^[33] The isomer situation of a square pyramid with five different ligands is identical to that of an octahedron with six different ligands, because the free coordination site (a phantom ligand) in the square pyramid is equivalent to the sixth ligand in the octahedron. The total of 30 isomers which is possible for the most general case of a square pyramid with

five different ligands can be reduced to only two, image and mirror image, with three constraints which can easily be realized experimentally.^[33]

The first constraint is to always leave ligand L^1 at the top of the pyramid; the second constraint is to make two ligands on the base identical ($L^4 = L^5$). These two constraints reduce the number of 30 isomers to only three, which are shown at the top of Figure 2: one pair of enantiomers with the ligands L^2

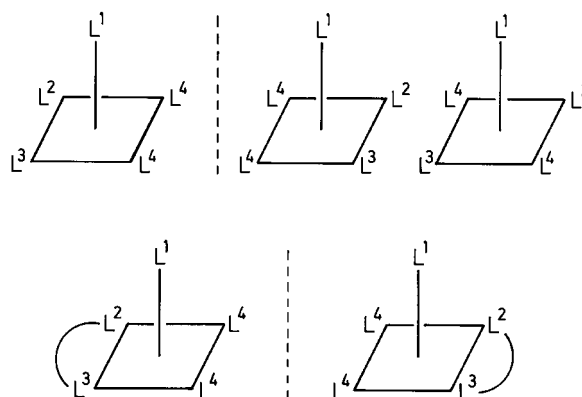
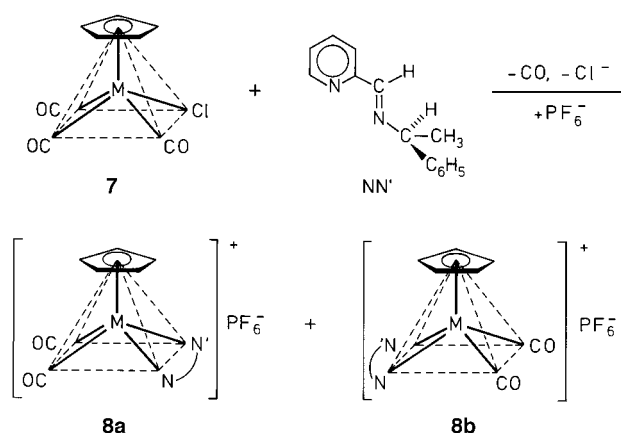


Figure 2. Reduction of the number of isomers for complexes with a square-pyramidal structure by restriction on the ligands used. See text for details.

and L^3 *cis* to each other and a third isomer with the ligands L^2 and L^3 *trans* to each other. The *trans* isomer contains a symmetry plane which passes through L^1 , L^2 , and L^3 . To exclude this achiral *trans* isomer, a third constraint is introduced. Instead of two different monodentate ligands L^2 and L^3 an unsymmetrical bidentate ligand $L^2\text{--}L^3$ is used. Such a chelating ligand can only occupy *cis* positions at a square. Therefore, the *trans* isomer is excluded, and the two *cis* isomers at the bottom of Figure 2 are the only ones left.^[33]

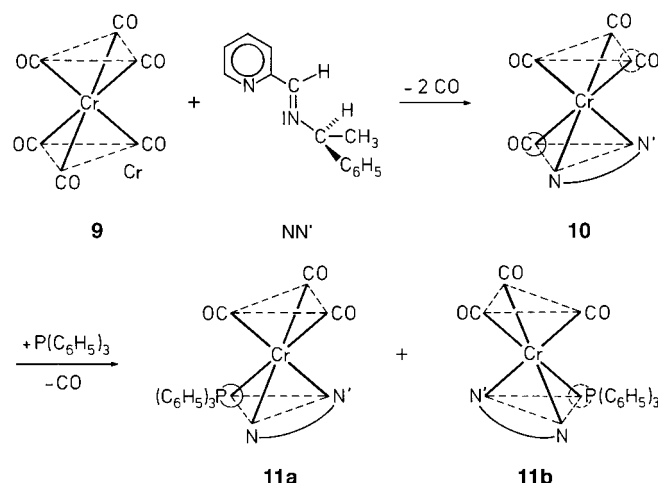
These three constraints can be realized in one synthetic step starting from the commercially available complexes $[(\eta^5\text{-C}_5\text{H}_5)\text{M}(\text{CO})_3\text{Cl}]$ ($\text{M} = \text{Mo}, \text{W}$; **7**), which have a square-pyramidal structure, that is, the four-legged piano-stool geometry (Scheme 5). In these complexes the cyclopentadienyl ring always occupies the top of the pyramid. As an unsymmetrical chelating ligand the pyridine-imine ligand NN'



Scheme 5. Synthesis of the diastereomeric complexes **8a** and **8b** with square-pyramidal structure. NN' = unsymmetric chelating ligand, $\text{M} = \text{Mo}, \text{W}$.

is used, which can be synthesized by a Schiff base condensation of 2-pyridinaldehyde and the optically active primary amine (*S*)-1-phenylethylamine.^[33, 34] In the reaction of the chelating ligand NN' with **7**, a carbonyl ligand and the covalently bound chloro substituent are displaced from the complex; the latter becomes the counterion Cl[−], which is replaced by PF₆[−] in a metathesis reaction. The two empty coordination sites on the base of the square pyramid are occupied by the chelating ligand NN'. There are only two possibilities for this. The diastereomers **8a** and **8b** are obtained, which differ only in the configuration at the metal atom.^[33–35] They could be separated on the basis of solubility differences for M = Mo and W.^[33, 34]

With the help of the unsymmetrical, optically active chelating ligand NN' and use of the *trans* effect of the carbonyl ligands in the introduction of triphenylphosphane, it is possible to convert the highly symmetric hexacarbonylchromium (**9**) into the *cis*-trisubstituted diastereomers **11a** and **11b** in two steps (Scheme 6).^[36] First, two *cis* carbonyl



Scheme 6. Conversion of hexacarbonylchromium (**9**) into the diastereomeric complexes **11a** and **11b**. NN' = unsymmetric chelating ligand.

ligands are replaced by the NN' ligand. In the reaction with the complex [Cr(CO)₄(NN')] (**10**) thus formed, the ligand triphenylphosphane does not enter *trans* to N or N', where the carbonyl ligands are bound tightly, but in the two positions marked in Scheme 6, where the two carbonyl groups are mutually labilized by their strong *trans* effect. Substitution in these two positions leads to the diastereomers **11a** and **11b**, which differ only in the configuration at the metal atom. The diastereomers could be separated by fractional crystallization, and analogous reactions were carried out for [Mo(CO)₆] and [W(CO)₆].^[36] As the three remaining carbonyl groups occupy facial coordination positions, the octahedral complexes **11** correspond to the half-sandwich complexes in Schemes 1–4, in which a cyclopentadienyl ligand binds to three *cis* coordination sites.

Complexes with four different ligands of tetrahedral, and not only pseudotetrahedral, structure were also resolved with respect to the metal configuration. Examples are the iron complexes **12**^[37] and the carbonyl(nitrosyl)cobalt complexes

with unsymmetrical chelating ligands, for example 1-methyl-1,2-ethanediylbis(diphenylphosphane) (prophos; **13a**^[38]), or two different ligands, for example triphenylphosphane and (*S*)-1-phenylethylisocyanide (**14**^[39]); Figure 3 shows only one diastereomer of each.

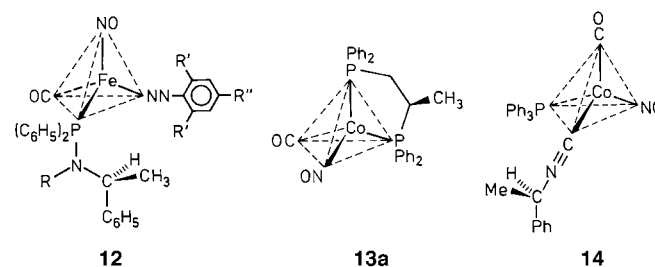


Figure 3. Examples of tetrahedral complexes with four different ligands whose isomers can be separated with respect to the metal configuration. **12**: R = benzyl, R' = R'' = methyl.

5. Absolute Configuration and Chiroptical Properties

Organometallic compounds of the transition metals crystallize excellently. Therefore, the absolute configurations at the metal atom in optically active complexes can be determined by X-ray crystallography either by means of anomalous X-ray scattering or by internal comparison with a known absolute configuration in the ligand. For assignment of the configuration symbols *R/S* the Cahn–Ingold–Prelog system^[40–42] is extended to π -bonded ligands.^[43, 44] Polyhapto-bound ligands are taken as pseudoatoms with an atomic number equivalent to the sum of the atomic numbers of the atoms bound to the metal atom. According to this definition η^6 -C₆H₆ and η^5 -C₅H₅ are pseudoatoms with atomic numbers 36 and 30.

For the (−)₅₇₉ diastereomer **4a** (see Scheme 3) the absolute configuration at the iron atom has been determined to be S_{Fe}, the priority of the ligands being η^5 -C₅H₅ > PPh₃ > COO-menthyl > CO.^[45, 46] According to Ruch^[47] tetrahedral complexes and half-sandwich complexes of the three-legged piano-stool type belong to chirality class *a*, for which an achiral border between *R* and *S* exists. In contrast, complexes with square-pyramidal and octahedral skeletons belong to chirality class *b*, for which there is no such achiral border.

The areneruthenium complexes **15a** and **15b** in Figure 4 contain as an unsymmetrical ON chelating ligand the anion of the salicylaldimine derived from (*S*)-1-phenylethylamine. Thus, in the ligand an *S*-configured carbon atom is fixed, whereas *R* and *S* configuration can result at the metal atom. As explained later on, the diastereomer equilibria of these complexes are shifted strongly to the side of the *R*_{Ru}, *S*_C diastereomers. For **15** the equilibrium ratio *R*_{Ru}, *S*_C:*S*_{Ru}, *S*_C in CDCl₃ at room temperature is 95:5.^[48, 49] Depending on the crystallization conditions the *R*_{Ru}, *S*_C isomer **15a** is obtained in two modifications. Crystallization in chloroform at 5 °C preferentially gives tetrahedron-like crystals; crystallization in chloroform at room temperature yields small plates. X-ray structure analyses confirm the *R*_{Ru}, *S*_C configuration in both cases, the priority of the ligands being η^6 -C₆H₆ > PPh₃ > O >

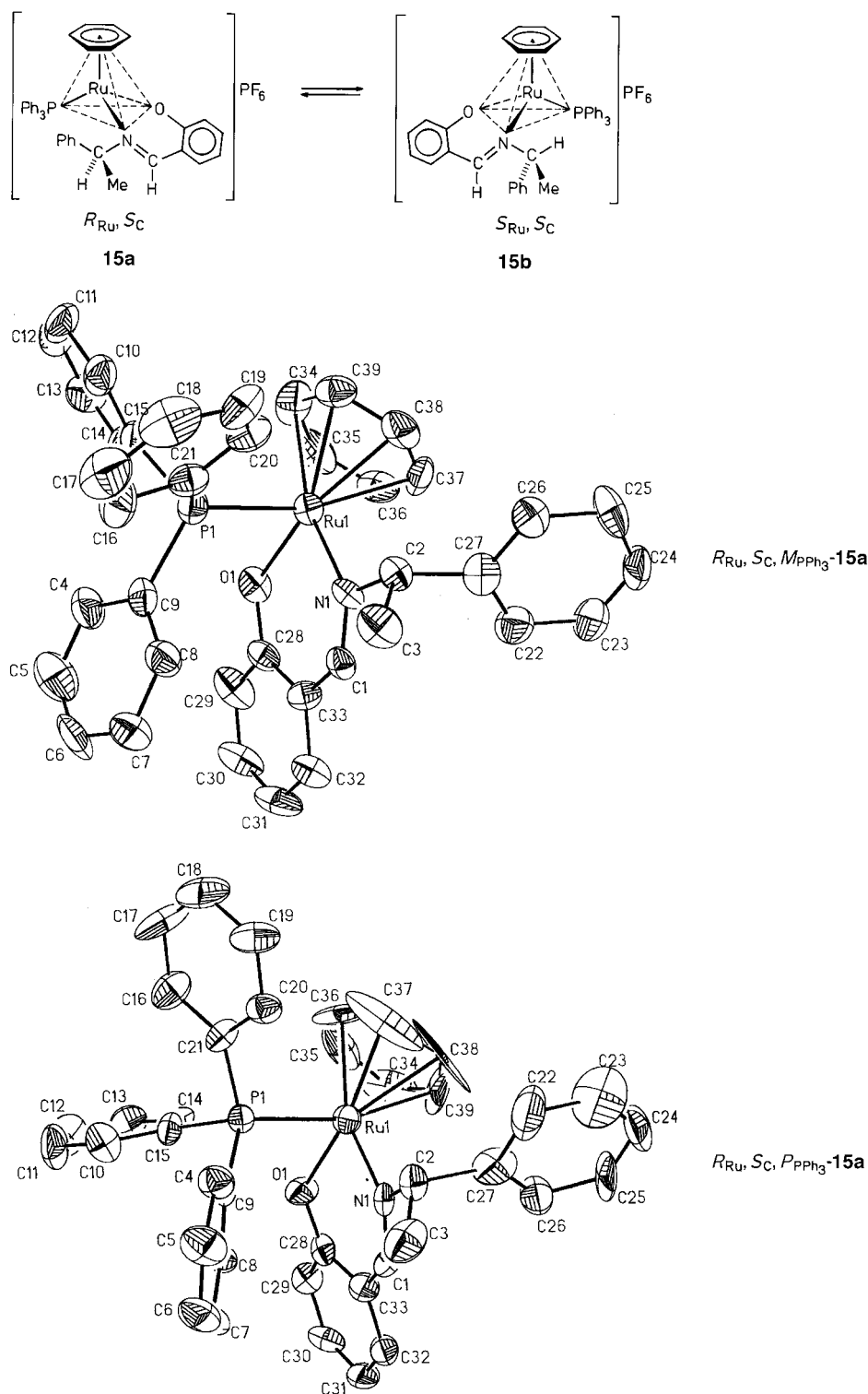


Figure 4. Top: Complexes **15a** and **15b** are in equilibrium with each other (**15a:15b** = 95:5 in CDCl_3 at room temperature). Bottom: ORTEP plots of the isomers of the cations of **15a**, which differ only in the propeller sense of the triphenylphosphane ligand.

N. They demonstrate that both isomers differ only in the propeller sense of the triphenylphosphane ligand.^[48, 49] In the tetrahedron-like crystals the triphenylphosphane ligand has an *M* conformation (*M* = minus),^[41] whereas in the small plates it has a *P* conformation (*P* = plus)^[41] (Figure 4, bottom). Up to now, the ruthenium complexes (*R_{Ru}*, *S_C*, *M_{PPh₃}*)-**15a** and (*R_{Ru}*, *S_C*, *P_{PPh₃}*)-**15a** are the only exam-

ple of a pair of diastereomers whose components have opposite chirality of the triphenylphosphane propeller.

In general, transition metal compounds are strongly colored. This leads to Cotton effects in the visible part of the spectrum. Therefore, the optical rotatory dispersion (ORD) and circular dichroism (CD) spectra are characterized by many passages through zero. Owing to the change in sign of

the bands the ORD and CD spectra contain more information than the UV/Vis spectra. However, their potential for assignments has not been used up to now. The ORD and CD spectra are dominated by the metal chromophores. Therefore, diastereomers which contain asymmetric carbon atoms of the same configuration in the ligand but differ in the configuration of the metal often have almost mirror-image chiroptical properties. An example are the CD spectra of the diastereomeric manganese complexes **2a** and **2b** (Figure 5).

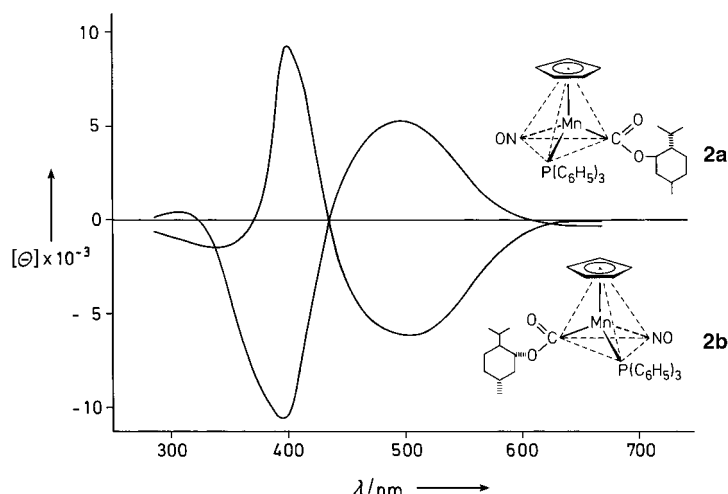


Figure 5. CD spectra of complexes **2a** and **2b** (concentrations about 10^{-3} mol L $^{-1}$ in toluene).

As a rule, the rotational values of optically active organometallic compounds at the Na_D line and the mercury lines in the visible part of the spectrum are large.

6. Elucidation of Reaction Mechanisms

Schemes 1–6 have demonstrated that transition metal complexes with chiral metal atoms can be prepared with many different ligand combinations and geometries, and resolved with respect to the metal configuration.^[24, 50–52] An attractive application of these complexes, which are stereochemically labeled at the metal atom, is the elucidation of the spatial course of reactions.^[35, 51–53]

Stereochemical investigations have played and continue to play a prominent role in the establishment of reaction mechanisms in organic chemistry. In their classic studies in the 1930s Ingold and Hughes demonstrated that either racemization or inversion takes place in nucleophilic substitution reactions at the tetrahedral carbon atom, depending on whether the reactions proceed according to an S_N1 mechanism via a planar carbenium ion or according to an S_N2 mechanism by a Walden inversion.

In the nucleophilic substitution at the asymmetric silicon atom the S_N1 mechanism can be excluded. These reactions proceed either by an S_N2–Si inversion mechanism, which is comparable to the organic S_N2 reaction, or by an S_Ni–Si retention mechanism, whose analogue in organic chemistry is rare.^[2] Thus, the homologues carbon and silicon behave in a distinctly different way. With the help of the new, optically

active transition metal complexes the stereochemical course of organometallic reactions can be studied. The crucial point is the configurational stability at the metal atom. Some of the optically active complexes are configurationally stable; their metal configuration does not change even at higher temperatures. Other complexes are labile with respect to the metal configuration; the separated diastereomers interconvert more or less rapidly. In both cases different mechanistic studies are possible.

7. Complexes That Are Configurationally Stable at the Metal Atom: Mechanisms

The reaction of the menthyl ester **4a** (see Scheme 3) with methyllithium provides an example of an unusual inversion mechanism. It leads to the elimination of lithium mentholate, and at the iron atom an acetyl substituent is formed. Starting from the (+)₅₄₆ menthyl ester **4a** the acyl complex **16b** is obtained with (–)₅₄₆ rotation (Figure 6); the (–)₅₄₆ menthyl ester **4b** yields the (+)₅₄₆ acyl complex **16a** (not shown).^[54] The optical rotations of starting material and product have

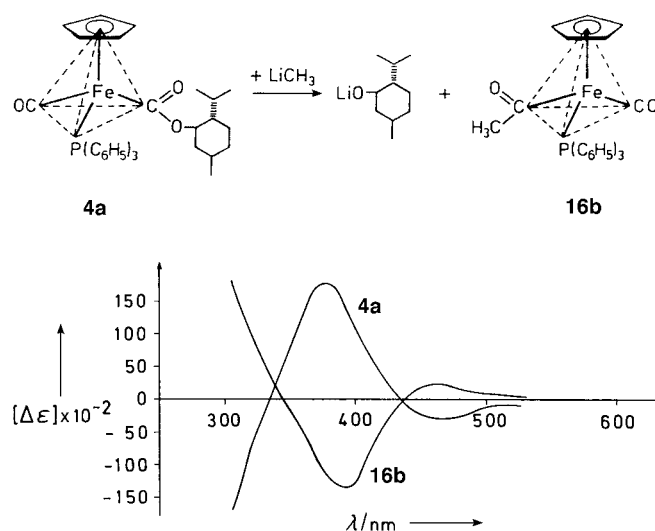


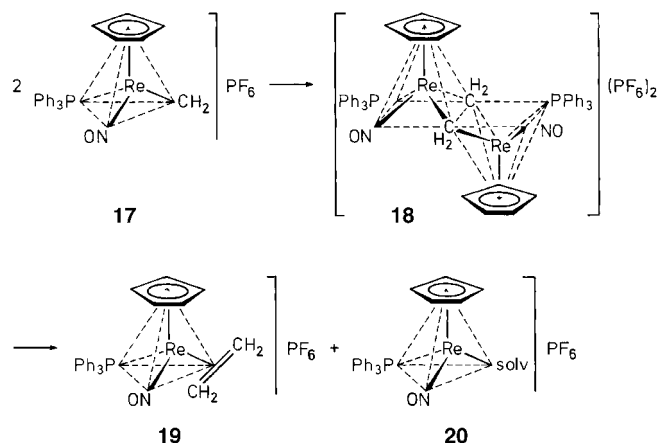
Figure 6. Top: Inversion of the configuration in the reaction of **4a** with LiCH₃ to give **16b**. Bottom: CD spectra of **4a** and **16b** (concentrations about 10^{-3} mol L $^{-1}$ in benzene).

opposite signs, and the CD spectra of **4a** and **16b** are nearly mirror images (Figure 6, bottom).^[54] These chiroptical data suggest an image–mirror image relationship between starting material and product and therefore an inversion mechanism. This has been confirmed by the X-ray determination of the absolute configuration at the iron atom in starting material and reaction product.^[45, 46, 55]

The reaction mechanism can be explained as follows. The attack of methyllithium does not occur as expected at the ester group of the starting material, but at the carbonyl group, which is transformed into the new functional group, the acetyl substituent. The mentholate ion dissociates from the ester group, the former functional group, transforming it into the new carbonyl group of the product. Thus, in the reaction of Figure 6 two substituents of the iron complex **4a**, the carbonyl

group and the functional group, change their roles. This is equivalent to an inversion of the metal configuration upon going from **4** to **16**, although none of the bonds to the iron atom is broken. Only attack of methyllithium at the carbonyl group can explain the inversion at the iron atom, as attack at the ester group would lead to retention of configuration.^[55]

$[(\eta^5\text{-C}_5\text{H}_5)\text{Re}(\text{NO})(\text{PPh}_3)]$ proved to be a versatile chiral fragment; J. A. Gladysz et al. used it for a “stereochemical deep-hole drilling”.^[56–58] A special role was played by chiral Lewis acids, conformational analyses, and molecular recognition; an example of this is the coupling of two methylene ligands to an ethene ligand shown in Scheme 7. The methyldiene complex **17**^[60] couples to the ethene complex **19** at room



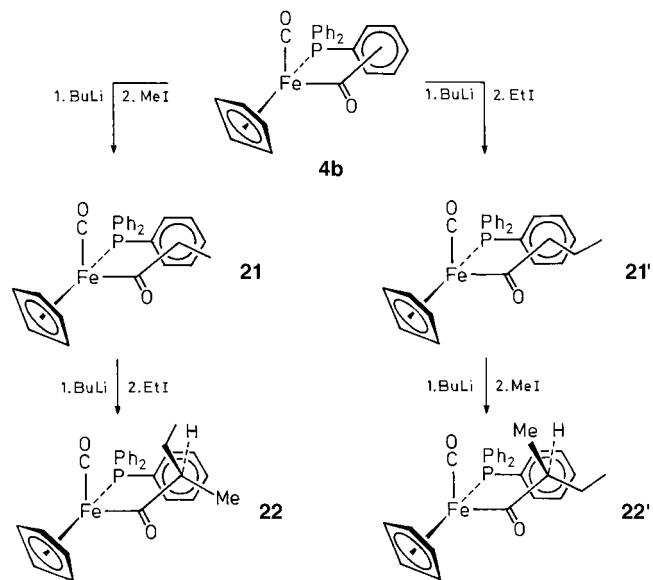
Scheme 7. Molecular recognition in the coupling of two methyldiene ligands in **17** to an ethene ligand in **19**.

temperature in dichloromethane in about 50% yield, that is, quantitatively. The reaction is second-order. Surprisingly, the enantiomerically pure complexes react 2.3 times faster than their racemic mixture. Cross-coupling experiments confirm that in the coupling the homochiral *R,R* and *S,S* transition states, for example **18**, are preferred over the heterochiral *R,S* transition state (self-recognition of enantiomers).^[59] This is surprising because nonlinearity and chiral amplification in enantioselective catalysis are based on the formation of binuclear complexes, for which *R,S* dimers are more stable than *R,R* and *S,S* dimers.^[61–63] The *meso* dimers contain an inversion center. In these heterochiral dimers the substituents of the two halves of the molecule can best avoid steric interactions; in homochiral dimers such as **18** this is not possible. The coupling product **19** forms with greater than 98% retention at the rhenium atom, and the solvated intermediate **20a** can be trapped in the presence of nitriles with retention of configuration at the rhenium atom.^[59]

8. Complexes That Are Configurationally Stable at the Metal Atom: Organic Synthesis

Optically active transition metal complexes can also be used in reactions which do not occur at the chiral metal atom but at the ligands, and thus find applications in enantioselective organic synthesis. The complexes (+)- and (–)- $[(\eta^5\text{-C}_5\text{H}_5)\text{Fe}(\text{CO})(\text{PPh}_3)(\text{COMe})]$

were developed as optically active auxiliaries; they are highly selective stoichiometric reagents that are commercially available and allow the synthesis of a variety of interesting compounds by the enolate chemistry of the acyl substituent.^[64–68] This is demonstrated in the double alkylation sequence of Scheme 8.



Scheme 8. $[(\eta^5\text{-C}_5\text{H}_5)\text{Fe}(\text{CO})(\text{PPh}_3)(\text{COMe})]$ as a chiral auxiliary: synthesis of both configurational series of carboxylic acids that are chiral at the α -C atom.

Complex **4b** can be deprotonated with butyllithium at the methyl group of the acetyl ligand to provide the enolate.^[69] The reaction with methyl iodide to give **21** is almost quantitative. If **21** is subjected to another cycle of metalation and alkylation, a new chiral center is formed in the acyl substituent, provided an alkylation reagent is used which has a different alkyl group than the first alkyl halide. The use of ethyl iodide in the second alkylation gives the 2-methylbutyryl group (**22**). If this double alkylation is carried out with **4b**, which is *R*-configured at the iron atom, **22** is formed with *R* configuration in the acyl fragment with an optical purity of greater than 200:1.^[70–72] If the sequence of alkylation is inverted, the complex **22'**, which is *S*-configured in the acyl substituent, is also formed with an optical purity of greater than 200:1.^[70–72] With $\text{Br}_2/\text{H}_2\text{O}$ the iron–acyl bond is cleaved, and the corresponding carboxylic acids can be isolated in almost optically pure form. Thus, double metalation/alkylation yields carboxylic acids, which are chiral at the α -C atom, of both configurational series.

The X-ray structure analysis of **4b** showed that one of the phenyl rings of the triphenylphosphane ligand completely shields one side of the acetyl group. Thus, attack is only possible from the other side (Scheme 8). This shielding is one of the prerequisites for the observed unusually high stereoselectivities. Furthermore, the alkyl group of the acyl fragment in the complex prefers a uniform conformation. The acetyl oxygen atom and carbonyl ligand occupy *anti* positions, and the β -C atom of the alkyl chain is located *syn* with respect to the small acyl oxygen atom (and *anti* with respect to the

large $[\text{CpFe}(\text{CO})(\text{PPh}_3)]$ fragment), as shown in Scheme 8 both for the *n*-propionyl intermediate **21** and for the *n*-butyryl intermediate **21'**. Similarly, the chirality at the Fe atom controls the formation of new asymmetric carbon atoms in the α -, β -, and γ -position of the acyl fragment in aldol condensations and Michael reactions with high enantioselectivity.^[68–72]

9. Complexes That Are Configurationally Labile at the Metal Atom: Mechanisms of the Change of the Metal Configuration

Optically active organometallic compounds with chiral transition metal atoms are configurationally stable in the solid state. In many cases, however, a change of the metal configuration takes place in solution by different mechanisms.

The configurational changes at the metal atom can be monitored by polarimetric measurements. In these reactions enantiomeric complexes which contain only chiral metal atoms approach the 1:1 racemization equilibrium, and optical activity is lost. If in these studies diastereomers are used which contain configurationally stable asymmetric carbon atoms in addition to the configurationally labile metal atom, the epimerization at the metal atom results in diastereomer equilibria with optical rotations other than zero and equilibrium ratios differing from 1:1. Figure 7 shows an example for

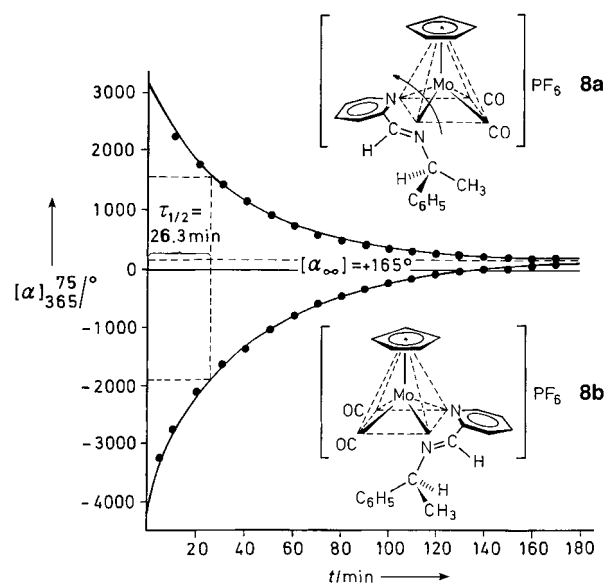


Figure 7. Decrease of the optical rotation in the epimerization of complexes **8a** and **8b** in dimethylformamide at 75 °C.

the drop of optical activity^[73] and the approach to epimerization equilibrium for the square-pyramidal complexes **8a** and **8b** ($M = \text{Mo}$), which differ in the metal configuration (see Scheme 5 for their synthesis^[33, 34]) and whose absolute configurations are known.^[74, 75]

Because optically active organometallic complexes are mostly colored and absorb strongly in the visible section, the transparency in the polarimetric measurements is limited. Therefore, only dilute solutions can be studied, and their air sensitivity represents a possible source of error. As mentioned

before, diastereomers frequently differ in their ^1H NMR spectra. Therefore, the kinetics of the configurational change at the metal atom can be followed by integration of suitable signals as a function of time. In this technique larger quantities can be used, the possibilities for exclusion of air are better, and therefore the results are more reliable than those obtained from polarimetry. Figure 8 shows the change in the

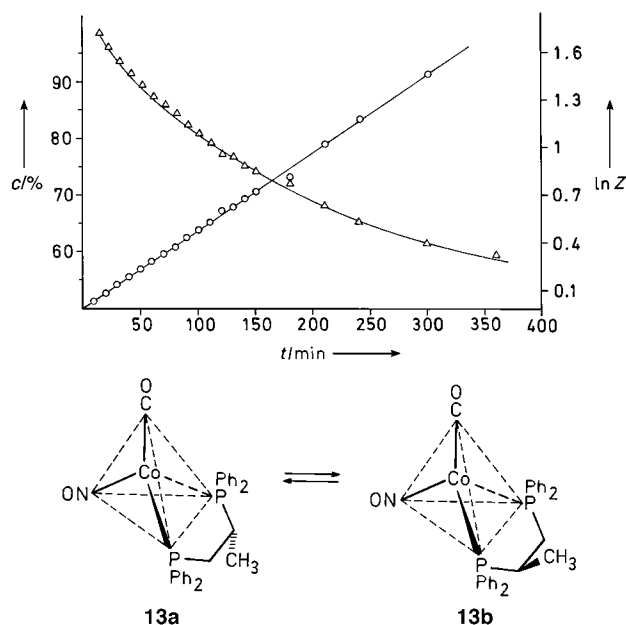


Figure 8. Epimerization of complex **13a** in $[\text{D}_6]$ benzene at 35 °C (equilibrium ratio **13a**:**13b** = 50:50) and interpretation according to first order. The change in the concentration c of **13a** was measured by integration of the methyl signal in the ^1H NMR spectrum. $Z = ([\text{13a}]_0 - [\text{13a}]_\infty) / ([\text{13a}] - [\text{13a}]_\infty)$.

concentration of the ($S_{\text{Co}}, R_{\text{C}}$)-cobalt–prophos complex **13a** as a function of time measured by ^1H NMR spectroscopy during approach of the epimerization equilibrium and its interpretation according to first order.^[38]

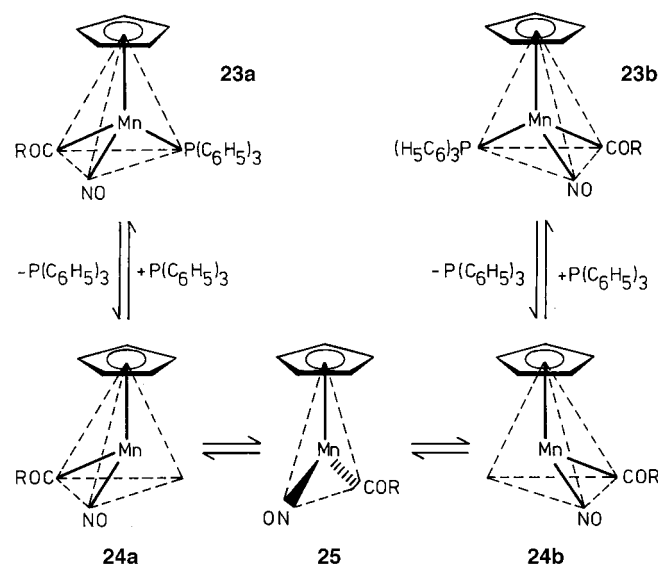
In most cases the configurational change at the metal atom is a first-order reaction. Therefore, the half-lives are a gauge for the configurational stability. The square-pyramidal complexes **8** (Figure 7) have a half-life of 26.3 min in DMF at 75 °C.^[33] Thus, solutions of these complexes can be handled at room temperature for a short time without a change of configuration at the metal atom. The half-life for the approach to the epimerization equilibrium for the cobalt–prophos complexes **13a** and **13b** (Figure 8) is 141 min in $[\text{D}_6]$ benzene at 30 °C. In this case a slow change of configuration at the cobalt atom has to be expected at room temperature in solution.^[38]

All the previous results indicate that the change of configuration of the square-pyramidal complexes takes place intramolecularly by a pseudorotation mechanism, which ultimately leads to rotation of the unsymmetrical chelating ligand by 180°, as shown by the arrow in the upper formula of Figure 7.^[35, 50, 73, 76, 77] Such intramolecular isomerizations at the metal atom can also be investigated by ^1H NMR spectroscopy with the help of diastereotopic probes, a resolution with respect to the metal atom being unnecessary. These coales-

cence measurements, however, cover relatively rapid reactions and thus a rate range completely different from that of the kinetic data accessible by integration of suitable diastereomer signals as a function of time.

10. Complexes That Are Configurationally Labile at the Metal Atom: Stereochemistry and Mechanisms

If intermediates are formed upon the change of the configuration at the metal atom, more information can be gained from racemization and epimerization experiments with the help of stereochemical labeling by optical activity than from dynamic NMR spectroscopy. Examples are the acylmanganese complexes **23** (Scheme 9), the racemization of



Scheme 9. Change in the configuration of complexes **23a** and **23b** via the chiral intermediates **24a** and **24b** and the achiral transition state **25**. R = methyl, phenyl, *para*-substituted benzene ring.

which involves the cleavage of the manganese–triphenylphosphane bond. The half-life of the benzoyl compound **23a** (R = Ph) is 49.4 min at 20 °C in toluene.^[78]

Kinetic and stereochemical arguments indicate that in the dissociation of the triphenylphosphane ligand from **23a** a pyramidal intermediate **24a** is first formed^[78–84] which still has the chirality of the starting material. This pyramidal intermediate can invert via the planar transition state **25** (as with an ammonia derivative). Addition of triphenylphosphane to the inverted intermediate **24b** leads to the opposite configuration at the metal atom in **23b** and subsequently to racemization. Stereochemical proof for the formation of chiral intermediates in the racemization of the manganese complexes **23a** and **23b** is provided by the formation of optically active substitution products when the intermediates are trapped with suitable phosphane ligands.^[79, 80] The products have the same relative configuration as the starting material. The formation of pyramidal intermediates of the type **24** was supported by calculations,^[85] which have been confirmed recently.^[86]

Instead of the unsaturated intermediates **24a** and **24b**, which have a certain pyramidal stability, saturated intermediates of the type **26**, which contain η^2 -bound acyl substituents, can be formulated to explain the retention upon phosphane substitution in the manganese complexes **23a** and **23b** (Figure 9). In **26** the manganese atom would remain chiral.

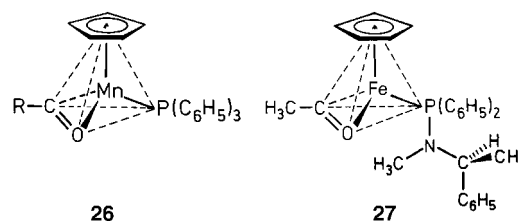
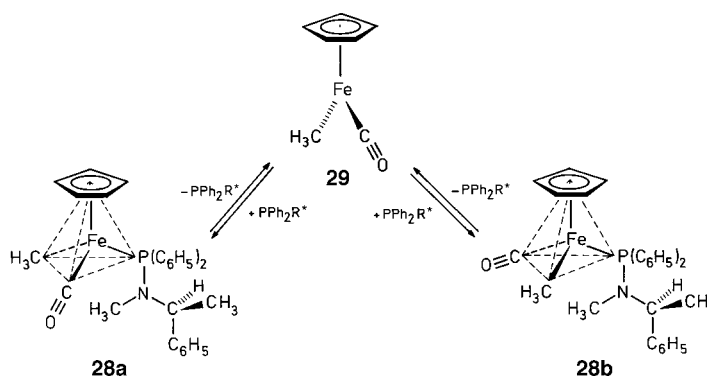


Figure 9. Possible η^2 -acyl intermediates in the change of the configuration at the metal atom. R = methyl, phenyl, *para*-substituted benzene ring.

Backside attack of the acyl oxygen atom would lead to inversion, while frontside attack would give retention. In the reaction of the η^2 -acyl intermediate with a phosphane ligand the processes would occur in the opposite direction (microscopic reversibility). Originally, this explanation had not been taken into consideration; the first stable η^2 -acyl complex was published only after the described experiments had been carried out.^[87] That this kind of stabilization cannot be neglected is obvious from the fact that retention in substitution reactions up to now could only be observed in complexes with acyl and ester ligands, but not in complexes whose substituents lack the ability to enter into η^2 bonding. Thus, the acyl(aminophosphane)iron complexes $[(\eta^5\text{-C}_5\text{H}_5)\text{Fe}(\text{CO})\text{-(COR)}(\text{PPh}_2\text{NR}'\text{R}^*)]$,^[88–90] which correspond to the manganese complexes **23**, also react in the phosphane substitution with partial retention of configuration; η^2 -acyl intermediates of type **27** (Figure 9) have been discussed.^[91] However, there has been no evidence for the formation of chiral intermediates in the phosphane substitution of the corresponding alkyl(aminophosphane)iron complexes $[(\eta^5\text{-C}_5\text{H}_5)\text{Fe}(\text{CO})(\text{Me})\text{-(PPh}_2\text{NR}'\text{R}^*)]$ (**28a**^[92, 93] and **28b**,^[94] Scheme 10). Phosphane



Scheme 10. Change in the configuration of complexes **28a** and **28b** via the planar intermediate **29** with loss of the stereochemical information at the metal atom.

substitution in **28a** and **28b** occurs with loss of the stereochemical information at the iron atom. It proceeds via the planar intermediate **29**, which can be attacked by the phosphane from the frontside or from the backside, leading

to the two diastereomers with opposite iron configuration in the diastereomer equilibrium.^[94]

The epimerization of the alkyliron complexes **28a** and **28b** in $[D_6]$ benzene at 70 °C takes place with a half-life of 70 min. However, electron transfer catalysis^[95] in the case of an oxidative start with $[Cp_2Fe]PF_6$ at 20 °C leads to complete epimerization within 5 min. This means that by the catalytic formation of a 17-electron species the epimerization at the Fe atom can be drastically accelerated compared to the thermal reaction of the 18-electron species.^[94]

11. Diastereomer Equilibria

If the synthesis temperatures of organometallic complexes are distinctly below the temperatures at which epimerization at the metal atom takes place, the optically active metal complexes are formed under kinetic control. Then, the diastereomer ratio is a measure for the optical induction of the stable chiral centers within the ligands for the formation of the metal configuration during the reaction. Various successive or decomposition reactions of the frequently air-sensitive diastereomers may modify the isomer ratios as well as fractionations which take place during workup of the reaction mixtures.^[96]

It is much better to investigate diastereomer equilibria which result in the change of the configuration at the metal atom. If specific intramolecular interactions are lacking, the

diastereomer equilibria are frequently about 50:50.^[96] With the use of special effects, for example C–H $\cdots$ π interactions^[97] such as the β -phenyl effect (see below), extreme diastereomer ratios can be reached.^[77] The shift of the diastereomer equilibria of thioamidato and amidinato complexes of four-legged piano-stool structure from 50:50 to 99:1 by simple substituent variation has already been reported.^[77, 96] Also the areneruthenium complexes,^[48, 49, 98–107] which represent catalyst precursors^[108–114] and have been intensely studied recently, show extreme diastereomer ratios. The equilibrium ratio of the pair of complexes **15a/15b** (Figure 4) in chloroform at room temperature is $R_{Ru}, S_C : S_{Ru}, S_C = 95:5$. In these complexes the β -phenyl effect^[77, 115] is the reason for the stabilization of the R_{Ru}, S_C diastereomer **15a** relative to the S_{Ru}, S_C diastereomer **15b**. In **15a** the phenyl ring of the 1-phenylethyl substituent is oriented towards the π -bound benzene ligand to give a distorted T-shaped structure.^[116] This geometry can be seen in the two ORTEP plots in Figure 4.^[48, 49] The ^1H NMR spectra demonstrate that this structural element is maintained as the preferred conformation in solution. There is a high-field shift of the signal of the π -bound arene lying within the inner anisotropy cone of the phenyl ring of the 1-phenylethyl substituent. This additional stabilization through the C–H $\cdots$ π interaction^[97] results in the observed preference of the R_{Ru}, S_C isomer **15a** in the diastereomer equilibrium.

A recent example for this C–H $\cdots$ π interaction and its consequences is the complex pair **30a/30b** (Figure 10), the components of which only differ in the ruthenium config-

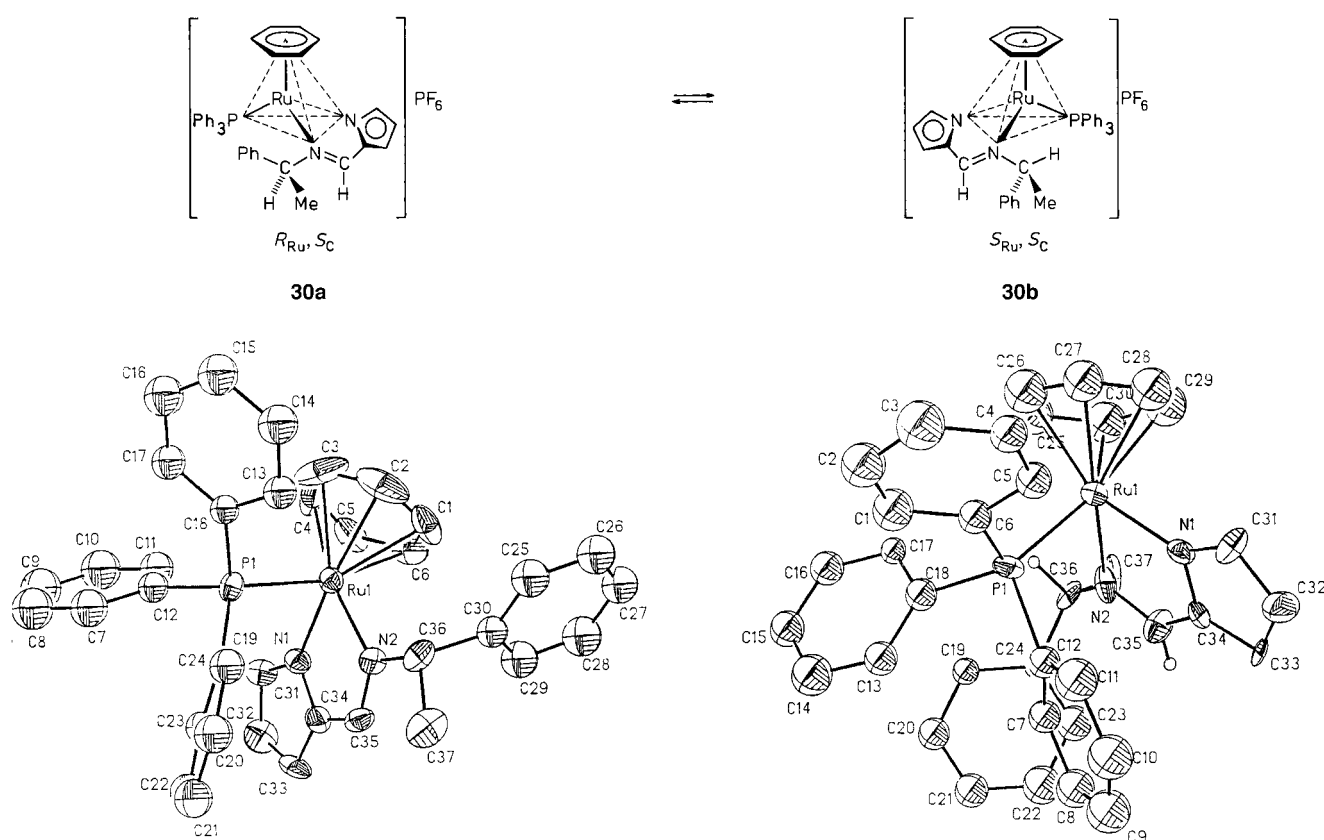


Figure 10. Complexes **30a** and **30b** (the equilibrium ratio in $[D_3]$ nitromethane at 85 °C is 93.5:6.5) and ORTEP plots of the cations of **30a** and **30b**, which differ only in the configuration at the ruthenium atom.

uration.^[101] The R_{Ru},S_C isomer **30a** dominates in the diastereomer equilibrium (93.5:6.5). The C–H $\cdots\pi$ interaction of the π -bound benzene ligand with the phenyl ring C25–C30 of the 1-phenylethyl substituent is clear from the ORTEP plot (Figure 10, bottom left). In the S_{Ru},S_C isomer **30b** this stabilizing interaction is lacking; the phenyl ring C19–C24 of the 1-phenylethyl substituent in the ORTEP plot “hangs downwards” and is far away from the π -bound benzene ring (Figure 10, bottom right). In solution, the C–H $\cdots\pi$ interaction shows itself in the signal intensities as well as the chemical shifts: Whereas the “normal” position for the signal of the π -bound arene in **30b** is at $\delta = 6.37$, the β -phenyl effect in the R_{Ru},S_C diastereomer **30a** leads to a high-field shift to $\delta = 5.68$. Compounds **30a** and **30b** are areneruthenium complexes with a relatively high configurational stability.^[48, 49, 101, 104] The half-life for the epimerization in nitromethane at 85 °C is 58 min.

12. Complexes with Chiral Metal Atoms in Enantioselective Catalysis

In the formation of chiral products from prochiral precursors the distance between inductor and substrate plays an important role. The smaller this distance is, the better the

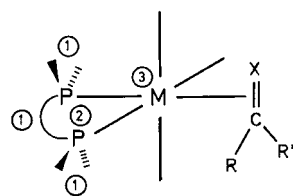


Figure 11. An octahedral phosphanemetal complex catalyst, in which a prochiral substrate $RR'C=X$ is transformed into a chiral product. The inducing chirality can be located in the chelating ring or in the phosphorus substituents (①), at the coordinating phosphorus atoms (②), or at the metal atom (③).

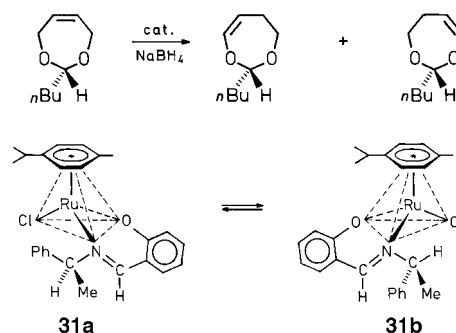
chirality transfer and thus the optical induction should be. In general, for enantioselective catalysis with transition metal compounds optically active ligands are used whose chirality is mostly in the substituents or in the chelating skeleton (① in Figure 11). Therefore, the inducing chirality is rather far away from the metal atom at which catalysis takes place. Nevertheless, special mechanisms of chirality transfer ensure that many of these reactions proceed with extraordinarily high enantioselectivity.

In the hydrogenation of dehydroamino acids a specific chiral conformation in the chelating skeleton of the ligand and, as a consequence, the arrangement of the phenyl substituents at the phosphorus atoms in plane/edge orientation are responsible.^[117–119]

In the Horner phosphanes^[120] and in ligands such as dipamp^[14] the inducing chirality is at the phosphorus atoms (② in Figure 11) and thus closer to the metal atom. The synthesis of compounds that are chiral at phosphorus is tedious, however, and unfavorable for a broad use. If the metal atom (③ in Figure 11) of the catalyst would be the inducing chirality, this would be the shortest possible distance. Strong effects on the transformation of prochiral substrates into chiral products within the coordination sphere of the metal atom would be the consequence. As organometallic complexes of the kind presented here are similar to the common enantioselective catalysts, there were repeated attempts to use transition metal complexes with chiral metal

atoms as catalysts in enantioselective reactions. However, there is still no solution to this problem.^[121] The complexes were either too stable or unsuitable as catalysts for the reactions planned.

At present, the efforts to utilize optically active complexes with chiral metal atoms for enantioselective catalytic transformations are concentrated on areneruthenium complexes.^[108–114] Whereas in Noyori's catalyst precursor $[(\eta^6\text{-arene})Ru(\text{binap})X]^+$ (binap = 1,1'-binaphthalene-2,2'-diylbis(diphenylphosphane), $X = \text{halogen}$) the arene is lost during hydrogenation,^[122] in the olefin isomerization shown in Scheme 11 it remains in the complex.^[114] This olefin isomerization starts from 2-*n*-butyl-4,7-dihydro-1,3-dioxepin, which



Scheme 11. Desymmetrization of the prochiral 2-*n*-butyl-4,7-dihydro-1,3-dioxepin. The reaction is catalyzed by complexes **31a** and **31b**.

can easily be obtained from *cis*-1,4-butanediol and valeraldehyde.^[123] It contains a symmetry plane which disappears in the catalytic isomerization. In this desymmetrization the olefinic double bond migrates from the allyl to the vinyl position. The *n*-butyl-substituted carbon atom becomes a stereogenic center. In this reaction enantiomeric excesses of 12–13 % were obtained^[123] with rhodium and ruthenium catalysts of the ligand 4,5-bis(diphenylphosphanylmethyl)-2,2-dimethyl-1,3-dioxolane (diop),^[124] which is derived from tartaric acid. Recently, the enantioselectivity (for the *tert*-butyldihydro-dioxepin) with nickel–chiraphos catalysts^[125] (chiraphos = 1,2-dimethyl-1,2-ethanediylbis(diphenylphosphane)) could be increased to 67 %.^[126]

Areneruthenium complexes **31a** and **31b**^[48, 49] proved to be catalytically active in the olefin isomerization shown in Scheme 11. With this catalyst optical inductions of up to 61 % *ee* were obtained.^[114] Areneruthenium complexes with chiral metal atoms and optically active chelating ligands were also used successfully as enantioselective catalysts in Diels–Alder reactions.^[112, 113, 127] Although the diastereomer equilibrium **31a** $\rightleftharpoons$ **31b** is established for complex **31** under the conditions of the olefin isomerization (20 °C, 24 h), such equilibria are, as mentioned, strongly shifted to one side. How the configurationally favored and disfavored diastereomers contribute to the enantioselectivity of the catalytic olefin isomerization and how the two diastereomers differ in their reactivities are yet to be explained. In the hydrogenation of dehydroamino acids with rhodium catalysts according to Halpern and Brown, the diastereomer which is disfavored in the equilibrium determines the product configuration.^[128]

13. Future Prospects

In the hydrogenation of dehydroamino acids with complexes similar in type to Wilkinson's catalyst, the rate-determining step is the oxidative addition of hydrogen to the square-planar complexes **32a** and **32b** (Figure 12). These

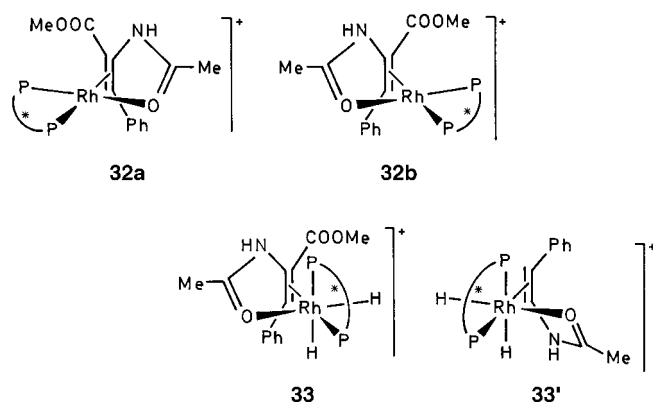


Figure 12. Intermediates in the enantioselective hydrogenation of methyl α -N-acetylaminocinnamate. Top: the diastereomers **32a** and **32b** with different coordination of the olefinic double bond. Bottom: the intermediates **33** and **33'** (formed in the oxidative addition of hydrogen to **32b**), which differ in the metal configuration. In **33'** the COOMe group has been omitted for the sake of clarity.

complexes are in equilibrium with each other and are diastereomeric to each other (same optically active chelating phosphane P*P and frontside or backside coordination of the prochiral olefin). In the oxidative addition of H₂ to **32a** and **32b** octahedral complexes are formed, to which structures such as **33** are ascribed.^[128] In these complexes the metal atom is stereogenic. For **33** a diastereomer with mirror-image metal configuration (**33'**) is possible without a change in the *cis/trans* arrangement of the ligand skeleton.^[77, 129–131] The two diastereomers **33** and **33'** belong to the product-determining branch, which starts from the disfavored but very reactive square-planar complex **32b**. The chiral metal complexes **33** arise anew in each catalytic cycle. However, the products are formed by reductive elimination, leading back to the square-planar rhodium complexes **32**, in which the rhodium atom is no longer stereogenic. Thus, in each catalytic cycle controlled by the P*P ligand chiral metal complexes are built up and disappear, albeit in a passive manner that cannot be influenced with respect to the metal configuration. Therefore, active use of chirality at the metal atom in enantioselective catalysts remains a challenge for the future.

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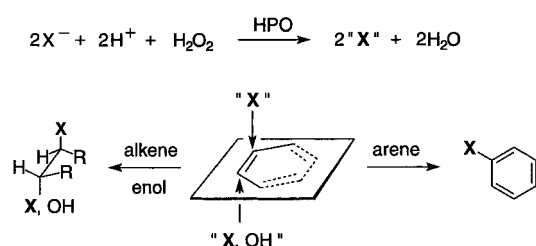
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The Biosynthesis of Barbamide—A Radical Pathway for “Biohalogenation”?

Jens Hartung*

In natural product research naturally occurring organohalogens have evolved within the last few years from exotics to firmly established compounds—and there are good reasons for this! Halogenated organic compounds occur widely in the biosphere, they are structurally diverse, and some of these compounds are formed in significant quantities. Fluorine, chlorine, bromine, and iodine, the four most important halogens, exist in natural products which act as numerous important physiological agents, for instance as thyroid hormones, as repellents, and as toxins.^[1] Halogens are deposited in nature as minerals or are dissolved as negatively charged ions in oceans. They can be activated from these sources by organisms for biosynthetic purposes. A multitude of so called “biohalogenations” is achieved by haloperoxidases (classification number for haloperoxidases: EC1.11.1.X, halide: hydrogen peroxide oxidoreductases; for example, chloroperoxidase: EC1.11.1.10). Haloperoxidases are enzymes that utilize hydrogen peroxide as an electron acceptor. According to the maximally oxidizable halide they are subdivided into chloroperoxidases, bromoperoxidases, and iodoperoxidases. Some haloperoxidases require iron porphyrin complexes or peptide-bound vanadium(v) as cofactors, while others function without transition metal catalysts.^[2] In general, haloperoxidases allow the activation of halides by hydrogen peroxide and their preferential incorporation into substrates with electron rich C–C double bonds (Scheme 1).



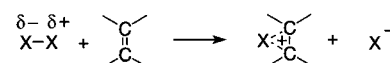
Scheme 1. Haloperoxidase reaction: enzymatic oxidation of halides by hydrogen peroxide and the incorporation of the halogen in a substrate with electron-rich C–C double bonds. X = Cl, Br, I. HPO = haloperoxidase. “X•” = $\frac{1}{2}X_2$ or X^+ in HOX.

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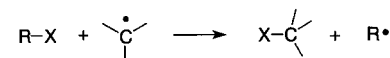
The oxidational power of haloperoxidases is solely dependent on the nature of the active centre. Chloroperoxidases of all three types (iron-porphyrin-containing, vanadium-dependent, and transition metal-free) are known.^[1, 2] In spite of intensive research, the nature of the halogenating species in haloperoxidase reactions is not fully established. Experimental results point to elemental halogens or hypohalite (for example, hypobromous or hypochlorous acid) in a free or coordinatively bound form as the halogen source in “biohalogenations”.^[1–3] However, the formation of monohalogenomethanes from *S*-adenosyl methionine,^[4] as well as reactions of NADH-dependent halogenases,^[5] seem to be easier to rationalize because such processes lead to the incorporation of halides into organic residues through nucleophilic substitution. It is likely that the last mentioned methods also account for the synthesis of fluorometabolites in vivo, because elemental fluorine or reagents that contain positively polarized fluorine cannot be generated under physiological conditions.^[6]

Laboratory synthesis takes profit from three mechanisms for halogenation: electrophilic, radical, or nucleophilic processes (Scheme 2). In terms of mechanistic considerations,

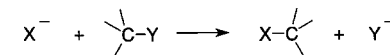
• electrophilic halogenation



• radical halogenation



• nucleophilic halogenation



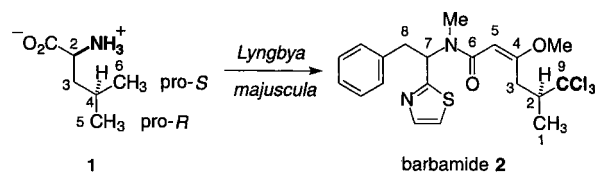
Scheme 2. Mechanisms for the synthesis of organohalogen compounds.

electrophilic halogenations correspond to haloperoxidase reactions. Alternatively, well elaborated free radical halogenation methods became available in the last few years and can be efficiently applied in synthesis.^[7] Nucleophilic incorporation of halogens into target compounds can be achieved by substitution reactions that utilize halides as nucleophiles.

New results obtained by Gerwick and co-workers indicate, however, that the picture that has been drawn about “biohalogenation” may not yet be complete.^[8] The authors

studied constituents of a species of cyanobacteria. These organisms are photosynthetically active procaryotic blue green algae that lack cellular organelles. Before their studies, cyanobacterial metabolites attracted attention as a result of the tricyclic guanidines, cyclic peptides, and a large number of structurally diverse isonitriles that were isolated from these organisms.^[9] Gerwick and co-workers, however, purified a trichloromethyl substituted metabolite barbamide (**2**) from a lipophilic fraction of extracts of cyanobacterium *Lyngbya majuscula*. This result afforded valuable information about the symbiosis between sponges and cyanobacteria.

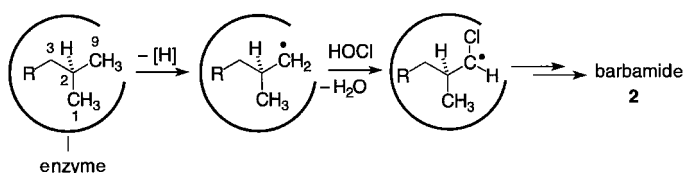
Further, by using isotopically labelled precursors the authors were able to show that carbon atoms 1–4 and 9 of the lipophilic moiety of **2** originate from the amino acid L-leucine (**1**) and correspond to atoms 2–6 (Scheme 3). The



Scheme 3. Biosynthesis of barbamide (**2**) from L-leucine (**1**).

carbon atom of the carboxylic acid group of **1** is not incorporated into **2**. The C5 and C6 atoms in **2** originate from an acetate molecule.^[10] While determination of the absolute geometry at C7 is still under investigation, the 2-*S*-configuration at C2 was unambiguously determined. Gerwick and co-workers prepared two stereoselectively labelled amino acids **1** in order to determine which methyl group from **1** is transformed in the course of the biosynthesis of **2** into a trichloromethyl group. The first sample contained a ¹³C label at the pro-*R*-methyl group, the second one at the pro-*S*-methyl substituent. These precursors were fed in two parallel sets of experiments over a certain period of time to culture media of *L. majuscula*. After ten days the organisms were harvested and metabolite **2** was isolated from both batches. Results from ¹³C NMR spectroscopy surprisingly indicated that only the pro-*S*-methyl group of **1** is chlorinated in the course of the barbamide synthesis! By what mechanism could such a chlorination reaction take place? The feeding of perdeuterated **1** afforded a new labelled product **2**. ²H NMR spectroscopy indicated that the ratio of deuterium in position 3 and 2 taken together with respect to the non-chlorinated perdeuteromethyl group C1 was 2.77:3.00. These experiments seem to rule out any oxidation reactions of **1** or derivatives thereof in the course of catabolism of **1**. In other words: no temporary C=C double bond should be formed between C2 and C3 in the course of the barbamide synthesis. These findings however led to new questions: Which enzyme in *L. majuscula* is able to stereoselectively chlorinate a nonactivated methyl group? Does this generally rule out a common electrophilic chlorination reaction using a chloroperoxidase? Gerwick and co-workers proposed a free-radical chlorination for this step of the biosynthesis without going further into details of this reaction. At a closer look it becomes clear that such a radical chlorination must be

exceptional in two ways. The required stereoselectivity of hydrogen abstraction from **1** or a derivative thereof is the first issue and the energetics of this process the second. If free-radical chlorination of 2-methylbutane were taken as an example for the chlorination that leads to barbamide (**2**) it is clear from bond dissociation energies^[6] that hydrogen abstraction from tertiary hydrogen bonds ($\text{BDE}_{\text{C-H}_{\text{tert}}} = 404 \pm 2 \text{ kJ mol}^{-1}$) is favored over homolytic cleavage of primary C-H bonds ($\text{BDE}_{\text{C-H}_{\text{prim}}} = 423 \pm 2 \text{ kJ mol}^{-1}$). A simple example illustrates the problem. In the gas-phase chlorination of 2-methylbutane by elemental chlorine at $T = 300^\circ\text{C}$, the chlorine atoms attack the tertiary C-H bond of the substrate 4.4 times faster than the C-H bonds from methyl groups (namely 5-H or 6-H in **1**). A hypothetical decrease of the reaction temperature from $T = 300^\circ\text{C}$ to physiological conditions will even further increase the preference for abstraction of tertiary hydrogen in 2-methylbutane that corresponds to 4-H in **1**. [1,2] Migrations of atoms via radical intermediates are forbidden reactions because they are 3-center-3-electron processes. Thus, the pro-*S*-methyl group should also be the location of the initial hydrogen abstraction. The answer to the question of the chlorination mechanism that is part of the biosynthesis of **2** remains to be uncovered by new decisive experiments. This topic however is reminiscent of the principle of negative catalysis by enzymes.^[11] This principle states that a pathway of a reaction between an organic compound and a reactive intermediate in solution can be completely altered by the binding of the substrate to an enzyme. If applied to the biosynthesis of **2** the first step of the chlorination reaction should be the binding of a biosynthetic precursor of **2** to an enzyme in such a way that the pro-*R*-methyl group and the tertiary hydrogen are sterically shielded. Only the pro-*S*-methyl group would then be exposed to attacking reagents (Scheme 4). If hypochlorous acid (chloroperoxidase?) is generated in the vicinity of the coordinated



Scheme 4. Proposed pathway for the stereoselective chlorination of a barbamide-precursor using free radical chemistry.

substrate, spontaneous or induced homolysis of the weak O-Cl bond in HOCl ($\text{BDE}_{\text{HO-Cl}} = 251 \pm 13 \text{ kJ mol}^{-1}$)^[6] would afford chlorine atoms and hydroxyl radicals. Further, only the hydrogen atoms of the nonshielded methyl group would be available for hydrogen abstraction. The newly formed alkyl radical would be trapped by additional HOCl to afford a carbon-chlorine bond and a hydroxyl radical. The latter would have to be detoxified or could be used for further hydrogen abstraction from the CH₂Cl group.

The challenge for further studies in this field of research will be to verify the radical nature of intermediates in the chlorination reaction in the biosynthesis of barbamide (**2**) by using mechanistic probes and to answer the following

questions: Which chlorinating enzyme is available in *L. majuscula* and which cofactor is required? Will other amino acids except **1** be suitable substrates for a stereoselective free-radical "biochlorination"? If these assumptions were confirmed, a second enzyme-induced radical process besides coenzyme B₁₂ mediated reactions would have been uncovered. The new halogenation reaction could have a significant influence on modern radical chemistry comparable to the role of the alkyl cobalamines,^[11] and should stimulate important new research in the field of stereo- and regioselective free radical chemistry.

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Keywords: biosynthesis • chlorine • chloroperoxidase • cyanobacteria • radicals

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Monovalent Group 13 Organometallic Compounds: Weak Association to Monomeric, Versatile Two-Electron Donors**

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Dedicated to Professor S. S. Krishnamurthy on the occasion of his 60th birthday

There is currently an immense enthusiasm among chemists to stabilize low-valent, multiply bonded compounds containing low-coordinate elements from Groups 13, 14, and 15. Especially the last few years have been an exciting period in which reports concerning the synthesis of several unusual compounds have appeared in the literature. To name a few, a) a tetrasil-1,3-butadiene,^[1a] b) the first silaarene,^[1b] c) compounds with unsupported Bi=Bi^[2a] and Sb=Sb double bonds,^[2b] d) silylium cations,^[3a] e) a cyclotrigermanium cation with a 2 π -electron system,^[3b] f) an Al₇₇ cluster ion with concentric spheres of Al atoms,^[4] g) compounds containing a discrete metal–chalcogen double bond such as Sn=Se,^[5] and h) multiply bonded species involving gallium^[6] have been discovered.

During this period another exciting facet of main group chemistry has been unraveled, and there have been important developments in the stabilization of monovalent Group 13 organometallic compounds. These efforts have resulted in the isolation of compounds of the type M^IR (M = Group 13 metal), which, while being monomeric in the gas phase or in solution, show interesting aggregation properties in the solid state. Recently in an important breakthrough Schnöckel and co-workers have shown that although [GaCp*] is hexameric in the solid state, the Ga...Ga distances are very long and only weakly influence the aggregation.^[7] Instead, these clusters are believed to be formed as a result of van der Waals interactions of the organic envelope around the metal. In another development, Power, Niemeyer, and Haubrich have succeeded in preparing examples of unassociated Group 13 mono-alkyl compounds that exist as monomers in the solid state.^[8] Continuing their studies on the use of In^I compounds as ligands, Uhl et al. have reported an unusual Ni⁰ complex bearing only monomeric indium moieties as ligands.^[9] Also Jutzi and co-workers have prepared a variety of metal carbonyl clusters containing monomeric [GaCp*] groups as terminal as well as bridging ligands.^[10] This article is aimed to highlight these new results and to provide a brief summary of other important results in Group 13 M^I–organometallic chemistry obtained recently.

While subvalency is common among the inorganic and organometallic compounds of heavier Group 13 elements

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such as In and Tl,^[11] low-valent compounds of the lighter elements B, Al, and Ga have been synthesized only recently. For example, the common oxidation state for thallium centers in inorganic compounds of thallium is +1, and several species of the type $[\text{RTl}]_n$ are very stable and are often synthesized in aqueous medium. There have also been reports on the stabilization of Tl^{I} compounds in other situations. Thus the interesting Tl^{I} amide $[(2,6\text{-}i\text{Pr}_2\text{C}_6\text{H}_3)(\text{SiMe}_3)\text{NTl}]_4$ was synthesized by Roesky and co-workers in 1994.^[12] This compound is monomeric in the gas phase. In the solid state, a weak interaction of Tl with the aryl group of a neighboring molecule is seen along with weak $\text{Tl}\cdots\text{Tl}$ interactions (406.0 pm), leading to a loosely associated tetramer. The $(2,6\text{-}i\text{Pr}_2\text{C}_6\text{H}_3)\text{N}(\text{SiMe}_3)$ group used to stabilize this molecule was also useful later in the development of a new class of kinetically stabilized silanetriols.^[13] Even though it has long been believed that +3 is the most stable oxidation state for B, Al, and Ga, a number of divalent compounds of these elements have been reported; this topic has already been comprehensively reviewed by Uhl.^[14] However, the developments in this area have been rather slow, and it was only in the beginning of the 1990s that reports concerning the isolation and unambiguous structural characterization of stable and discrete monovalent M^{I} organometallic compounds of the lighter Group 13 elements started to appear in the literature.

One of the key requirements for the stabilization of monovalent Group 13 compounds is a suitable R group on the fragment $\text{M}^{\text{I}}\text{R}$. The substituent has to provide steric bulk and also be electronically stabilizing. The types of R groups that have been found to be suitable and have been used so far are a) cyclopentadienyl (Cp) groups (including C_5H_5 (Cp), C_5Me_5 (Cp^*), and $\text{C}_5(\text{CH}_2\text{Ph})_5$ (Cp^{B})), b) bulky alkyl or persilylated alkyl groups (including $\text{C}(\text{SiMe}_3)_3$, $t\text{Bu}$, CH_2CMe_3 , and $\text{Si}(t\text{Bu})_3$), and c) 2,6-disubstituted aryl groups. The first notable breakthrough in this area was achieved by two different synthetic strategies. While Paetzold and co-workers assembled the organo-B^I compound $[\{\text{BrBu}\}_4]$ by the reductive dehalogenation of $t\text{BuBF}_2$ with Na/K alloy,^[15] Schnöckel et al. reported the synthesis of the first organo-Al^I compound $[\{\text{AlCp}^*\}_4]$ by a metathetical exchange of halide in a reaction involving the metastable $[\text{AlCl}]$ and $[\text{MgCp}_2^*]$.^[16] Quite recently Linti and co-workers have shown that the reaction of metastable $[\text{Ga}]$ with $\text{LiSi}(\text{SiMe}_3)_3$ affords the tetragallane $[\text{R}_4\text{Ga}_4\text{I}_3]^-$ and the polyhedral nonagallane $[\text{R}_6\text{Ga}_9]^-$ ($\text{R} = \text{Si}(\text{SiMe}_3)_3$).^[17] Roesky and co-workers successfully employed the reductive dehalogenation method to prepare synthetically useful quantities of $[\{\text{AlCp}^*\}_4]$ in the reaction of $[\text{Cp}^*\text{AlCl}_2]$ with potassium in toluene.^[18a] These authors have quite recently adopted this synthetic strategy for the reduction of the aluminum diiodide $[(\text{Me}_3\text{Si})_3\text{CAI}_2 \cdot \text{THF}]$ with Na/K alloy to afford $[\{\text{AlC}(\text{SiMe}_3)_3\}_4]$.^[18b] Jutzi and co-workers have also shown that reductive dehalogenation of $[\text{Cp}^*\text{GaI}_2]$ with potassium metal and the use of ultrasonic metal activation affords $[\{\text{GaCp}^*\}_6]$.^[10] Through the use of these two synthetic strategies, many monovalent compounds containing Al, Ga, In, and Tl have been assembled.

The X-ray crystal structures of these monovalent compounds show interesting aggregates in the solid state. $[\{\text{AlCp}^*\}_4]$, $[\{\text{Al}(\text{Si}t\text{Bu}_3)_4\}]$,^[19] and $[\{\text{MC}(\text{SiMe}_3)_3\}_4]$ ($\text{M} =$

Al ,^[18b] Ga ,^[20] In ,^[21] Tl ^{[22])} are tetrameric, while $[\text{MCp}^*]$ ($\text{M} = \text{Ga}$ ^[7] and In ^{[23])} are hexameric. In contrast, $[\text{InCp}]$, $[\text{TlCp}]$, and $[\text{InCp}^*]$ are polymeric.^[24] $[\text{InCp}^{\text{B}}]$ and $[\text{TlCp}^{\text{B}}]$, obtained with bulky cyclopentadienyl substituents, are dimeric.^[25] Accumulated experimental evidence suggests that these compounds are monomeric in solution or in the gas phase.

What holds the clusters together in the solid state? The $\text{M}\cdots\text{M}$ bond distances observed for the tetrameric derivatives $[\{\text{AlCp}^*\}_4]$, $[\{\text{Al}(\text{Si}t\text{Bu}_3)_4\}]$, and $[\{\text{MC}(\text{SiMe}_3)_3\}_4]$ ($\text{M} = \text{Al}$, Ga , In , Tl) are comparable to that in the native metals, suggesting that this fairly strong interaction is responsible for the formation of the cluster. However, the situation is different for compounds of monovalent Ga, In, or Tl containing Cp-type ligands. The recent X-ray structural investigation of $[\text{GaCp}^*]$ has thrown new light on the nature of association of these compounds in the solid state.^[7] Although $[\text{GaCp}^*]$ was prepared in 1993, single crystals of this compound were only recently obtained by Schnöckel and co-workers by cooling of a molten solution at $+4^\circ\text{C}$. It is found that $[\text{GaCp}^*]$ is hexameric and made up of two distinct Ga_3 units (Figure 1).

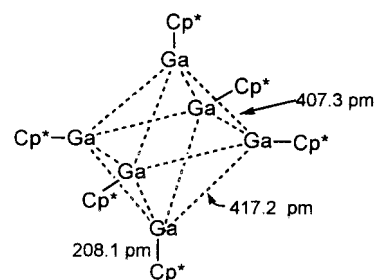


Figure 1. Schematic representation of the weakly associated structure of a $[\text{GaCp}^*]$ hexamer in the solid state.

Interestingly, the $\text{Ga}\cdots\text{Ga}$ bond distances in these subunits (417.2 and 407.3 pm) are much longer than in Ga metal (245–307 pm). A second important finding is that the dimensions of the $[\text{GaCp}^*]$ units are similar in the solid state and in the gas phase. Thus the distance between Ga and the centroid of the Cp^* ligand is 208.1 pm in both the gas phase and the solid state. Indeed, such weak $\text{M}\cdots\text{M}$ interactions are also found for hexameric $[\text{InCp}^*]$,^[23] dimeric $[\text{MCp}^{\text{B}}]$ ($\text{M} = \text{In}$, Tl),^[25] and polymeric $[\text{InCp}]$,^[24] $[\text{TlCp}]$,^[24] $[\text{TlCp}^*]$,^[24] and $[\text{TlCp}^{\text{B}}]$.^[25] Schnöckel et al. suggest that in these compounds the various monomeric subunits are held together primarily as a result of the van der Waals interactions of the outer organic envelope rather than $\text{M}^{\text{I}}\cdots\text{M}^{\text{I}}$ interactions.^[7] Further evidence for this argument stems from the fact that the unit cell volumes of $[\text{InCp}^*]_6$ and $[\text{GaCp}^*]_6$ are very similar, although in the former the metal–ring distance is about 11 % longer than in the latter. It is possible that different packing requirements of the ligands enforce the type and structure of the aggregate formed.^[7] There is already theoretical evidence for the instability of the aggregates in comparison with the monomers. Theoretical calculations on $[\{\text{AlCp}\}_4]$ and $[\{\text{AlCp}^*\}_4]$, suggests that the tetramers are unstable with respect to the monomers. This has been primarily attributed to the $\text{Cp} \rightarrow \text{Al} \pi$ backbonding.^[26]

Indirect support for the suggestion by Schnöckel et al. comes from recent studies of Power, Niemeyer, and Hau-brich.^[8] Using entirely different and sterically encumbered Trip-type ligands (Trip = 2,4,6-*i*Pr₃C₆H₂), these researchers have isolated monocoordinated, monovalent In^I and Tl^I compounds. The packing requirements of these bulky ligands, being different from those of the other Cp-type ligands studied so far, perhaps force monocoordination. For some time, Power et al. and others^[6] have been employing several sterically overcrowded Trip ligands to stabilize unusual Group 13 organometallic compounds (Figure 2). This substituent was also used recently by Robinson et al. for the

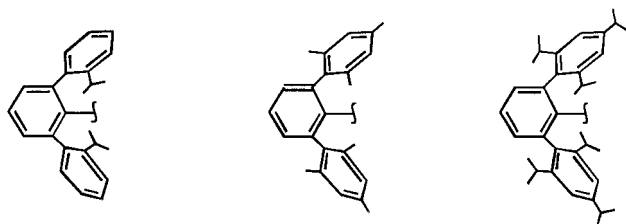
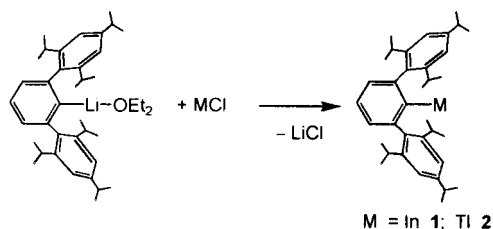


Figure 2. Bulky 2,6-disubstituted aryl ligands.

synthesis of multiply bonded gallium compounds,^[6] a discovery over which there has been strong disagreement among main group and structural chemists in recent times.^[27]

The bright orange compounds **1** and **2** were obtained in moderate to good yields by a fairly straightforward synthetic route starting from the aryllithium salt and the subvalent metal chlorides [InCl] and [TlCl], respectively (Scheme 1).^[8]



Scheme 1. Synthesis of **1** and **2**.

While the In compound **1** is thermally stable and decomposes only above 215 °C, the light-sensitive Tl^I derivative **2** is only stable for extended periods of time in solution below –40 °C. The isolated solid of **2** slowly decomposed even at low temperatures with deposition of thallium metal. Compounds **1** and **2** show characteristic absorptions at 440 and 487 nm, respectively. The single-crystal X-ray structure analyses reveal that **1** and **2** exist as monomers [RM] with monocoordinated metal centers in the solid state. While the shortest intermolecular In...In contact in **1** is 6.89 Å, the corresponding Tl...Tl distance for **2** is 6.81 Å. It is interesting to compare these values with those of “polymeric” [TlCp] (6.38 Å), [TlCp*] (5.41 Å), and [TlCp^B] (7.37 Å). Thus, the degree of association of the monomeric units in monovalent Group 13 compounds can be adjusted by the ligand used. Clearly, more data in terms of number and variety of structures as well as detailed

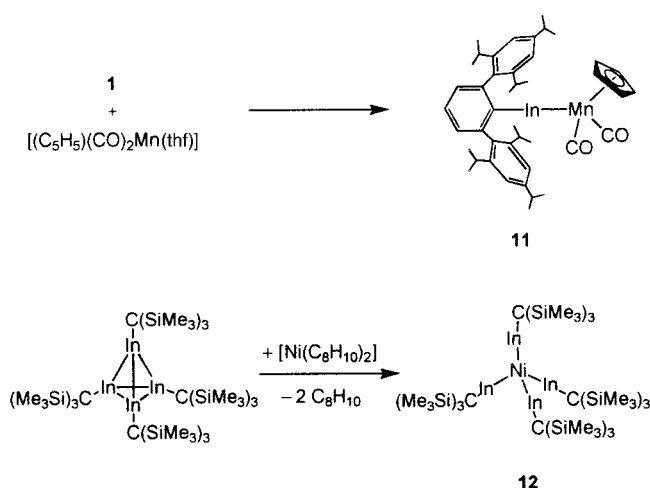
theoretical calculations are required to obtain a quantitative understanding on this ligand-driven nature of association.

Are these monovalent (associated and monomeric) Group 13 compounds useful to synthetic organometallic chemists in any way? Based on the reactivity studies of [In{C(SiMe₃)₃}]₄ with various transition metal centers reported in a series of recent papers by Uhl et al.^[9, 28] and the studies of [GaCp*]₆ with a variety of first-row transition metal carbonyl complexes reported by Jutzi and co-workers,^[10] the answer to this question seems to be “yes”. The ability of monovalent Group 13 compounds to function as Lewis bases^[29] makes them useful two-electron donor ligands in transition metal organometallic chemistry.

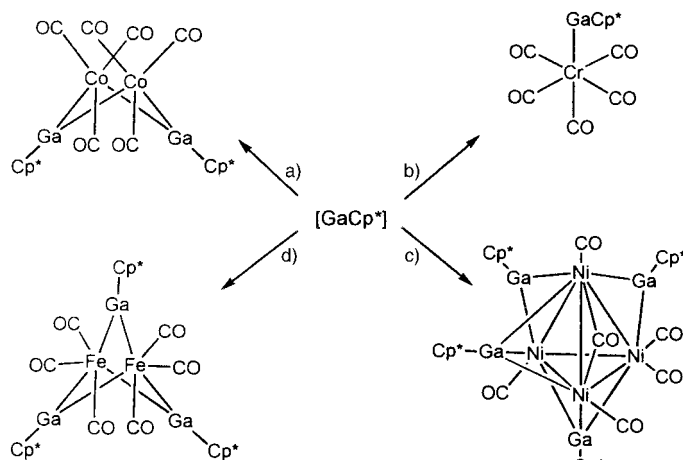
Studies on the reactivity of [RM] fragments toward soft metal centers are not new. As early as 1994 Schnöckel et al. reported on [(CpNi)₂(μ₂-AlCp*)₂] (**6**), the first transition metal complex bearing a M^IR fragment as a bridging two-electron donor in a μ₂ coordination mode.^[16b] The ability of [AlCp*] to act as a two-electron ligand was further substantiated by the synthesis of [(μ₂-AlCp*)₂Co₂(CO)₆] (**7**) through a facile reaction between [AlCp*] and [Co₂(CO)₈] in toluene at 80 °C.^[30] Similarly, the In^I compound [In{C(SiMe₃)₃}]₄ acts as a bridging ligand in [Mn₂(CO)₈(μ₂-InC(SiMe₃)₃)] (**8**), [Co₂(CO)₇(μ₂-InC(SiMe₃)₃)] (**9**), and [Mn₂(CO)₆(μ₂-InC(SiMe₃)₃)] (**10**).^[28] Recently Linti and co-workers have carried out reactions of [[Ga(Cl)[Si(SiMe₃)₃]₄] with iron carbonylates Na₂[Fe(CO)₄], Na₂[Fe₂(CO)₈], and Na₂[Fe₃(CO)₁₁] to afford derivatives of [Fe₂(CO)₉] in which some or all of the bridging carbonyls have been replaced with monomeric Ga[Si(SiMe₃)₃] fragments.^[31]

The monomeric M^IR compounds formally have two empty p orbitals perpendicular to the M–C bond (or the centroid of the C₅ ring in the case of Cp ligands) apart from a lone pair of electrons at the metal. Hence the frontier orbitals of M^IR are similar to those of the CO ligand. In fact, the recent studies clearly lend evidence to the ease of substitution of CO groups by M^IR groups in compounds **6**–**10**. However, it is interesting to note that until recently there have been no reports on the synthesis of complexes where [M^IR] compounds coordinate to the metal in a terminal coordination mode, which is the most commonly observed mode for the CO ligand. Preparation of terminally coordinated M^IR ligands would, in fact, allow us to draw an isolobal relationship between CO and MR ligands.

The In^I compound **1** and the tetrameric derivative [In{C(SiMe₃)₃}]₄ have been used to synthesize low-valent transition metal complexes with terminal MR ligands. Compound **1** readily combines with [Cp(CO)₂Mn(thf)] to afford [Cp(CO)₂Mn(InR)] (**11**) in 46% yield (Scheme 2).^[8a] Similarly, the reaction between [Ni(cod)₂] (cod = cyclooctadiene) and [In{C(SiMe₃)₃}]₄ results in a facile substitution of both the alkene ligands by four [In{C(SiMe₃)₃}] units to form [Ni{In[CSiMe₃]₃}]₄ (**12**) in 76% yield.^[9] In particular, the synthesis of **12** is very interesting since this compound represents the first transition metal complex in which the metal is coordinated to only M^IR groups and no other type of ligand, as in the case of [Ni(CO)₄], thereby clearly drawing an isolobal analogy between CO and M^IR ligands. The M–M'–C bonds are linear in this molecule; the Ni–In–C bond angle in **12** is 180.0° and the Mn–In–C angle in **11** is 175.4°. Although the short Ni–In bonds

Scheme 2. Synthesis of **11** and **12**.

(2.310 Å) are indicative of π backbonding, the extent of the same is uncertain in the absence of any MO calculations.^[9, 32] On the other hand, the observed CO stretching frequencies for **11** lend evidence for a description of the the M¹R ligands as σ donors with weak π -acceptor properties.^[8a] Similar to the In^I derivative, $[\text{GaCp}^*]_6$ also interacts with transition metal carbonyl complexes to afford compounds in which the monomeric GaCp* functions as a terminal and a bridging ligand (Scheme 3).^[10] The cone angle of this ligand has been

Scheme 3. Reactions of $[\text{GaCp}^*]$: a) $[\text{Co}_2(\text{CO})_8]$; b) $[\text{Cr}(\text{CO})_5(\text{C}_8\text{H}_{14})]$; c) $[\text{Ni}(\text{CO})_4]$; d) $[\text{Fe}(\text{CO})_3(\text{cht})]$ (cht = cycloheptatriene).

estimated to be 112° , and this ligand has been shown to be predominantly an electron donor with weak π -accepting properties. This observation parallels those for the ferrogallylene complex $[(\text{CO})_4\text{Fe}[\text{Ga}(2,4,6\text{-Trip}_3\text{C}_6\text{H}_2)]]$ from Robinson et al., where the monomeric GaR unit is also believed to function with almost no π -acceptor properties.^[32]

In conclusion, the recent syntheses and structure determinations of monovalent Group 13 compounds have taken this subject one step ahead of its previous limits. The problem of understanding the ligand-mediated weak association^[7] is likely to capture the attention of theoretical and experimental

chemists further. Likewise, the demonstration of the use of these compounds as both terminal and bridging ligands in transition metal chemistry is likely to have a huge impact on the way new metal–metal bonds are made in the future. If these results are any indication and if many other cluster chemists are to adopt these results in their research, there is no doubt that we will be witnessing the synthesis of a whole range of new cluster types in the future.

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Keywords: cluster compounds • main group elements • metal–metal interactions • organometallic compounds • subvalent compounds

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Combinatorial Chemistry—Challenge and Chance for the Development of New Catalysts and Materials

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The development of new materials—that is, solids with a defined function, such as catalysts, superconductors, liquid crystals, and new polymers—together with the development of new pharmaceuticals probably represent the most important tasks for chemistry in the near future. The need for new materials and catalysts could never be satisfied with conventional methods. Large research efforts are devoted worldwide to the development of more selective new catalysts for a variety of processes (e.g. direct oxidation of benzene to phenol and of propene to propylene oxide, dehydrogenation of the C_4 fraction to isobutene and its oxidation to methacrolein or methacrylic acid) by conventional methods. The need for better superconductors, luminescent materials, photo- or thermochromic materials, new semiconductors, transparent conductors, and many other materials increases continuously. Most of the new materials have been discovered either by systematic screening or by serendipity. With respect to the discovery of new lead structures, conventional research has to be blamed for restricting itself largely to known fields and structures. Dominant here are the modification, derivatization, and preparation of analogues of well-known materials as well as the equipment-intensive and expensive detailed characterization of chemical processes on known materials and catalysts with near-atomic resolution. The strengths of conventional chemistry lie in the optimization, systematic modification, and improvement of new lead structures, not in their discovery. Discovery is the potential strength of combinatorial chemistry.

The origin of combinatorial chemistry is found in nature, where the vast diversity of life has developed through the combination of only a few building blocks, such as amino acids and nucleic acids. The immune system starts its defence against unknown organisms with the “combinatorial” production of millions of different antibodies, and then switches to exclusive production of the appropriate antibodies once they are recognized by rather complex mechanisms. Combinatorial chemistry, which started with the production of peptide and oligonucleotide libraries about 15 years ago,^[1] has meanwhile established itself in the pharmaceutical industry as

an important and rapidly growing technique for the discovery of new lead structures in drug development. This aspect of combinatorial chemistry utilizes the highly advanced concepts of organic synthesis for the elegant and efficient preparation of compound libraries. The parallelism involved as the concept to increase efficiency of the experimental work in the area of drug development may well be a major motivation for the strong engagement of the pharmaceutical industry as well as for the reservation of academic researchers in the area of combinatorial chemistry.

In my opinion an important argument for a more intensive engagement of academic research in this area is the huge potential of combinatorial chemistry for new discoveries and new lead structures. This can be elucidated with a few numbers. Bohacek et al. have recently attempted to estimate, based on structural considerations, how many stable organic structures are possible from the elements C, O, N, S (and H).^[2] They came to the astronomical number of 10^{63} stable structures for molecules with up to 30 of the above elements. Under the assumption that this number is correct, a total mass of 10^{60} g would be obtained when only 1 mg of each compound would become available. The total mass of the earth is about 5×10^{27} g, and that of our sun is about 2×10^{33} g. This means that the mass of this organic library would be equivalent to the mass of 10^{27} suns! The total mass of the known universe is estimated to be about 10^{63} g. There are neither so many suns in the known universe, nor that much carbon! When we generously assume a total of 20 million known organic compounds, with 1 mg of each a total mass of just 20 kg represents our present “chemical universe”, which is small compared with the mass of 10^{27} suns. It can be concluded that although mankind has opened a nice field of organic chemistry, it is negligibly small compared to the organic diversity that is theoretically possible. Even with combinatorial methods this diversity can never be explored comprehensively; these methods can only help to find new lead structure more often than in the past.

In the area of drug development systematic synthesis concepts of organic chemistry could be ideally combined with combinatorial principles. Through the use of well-defined synthetic building blocks and synthetic strategies libraries of almost any class of compounds have been or can be prepared. However, the major disadvantage is that only known structural elements can be combined. Combinatorial chemistry in

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the area of drug development only allows systematic access to structural diversity within the limits of our known chemistry. Approaches to access new areas and structures, which according to the above numbers should exist in vast numbers, are not yet known (for example systematic permutation of the elements O, C, N, S, and/or P or alteration of rings, ring sizes, and/or connectivities in any moderately complex organic structure immediately leads to plentiful new structures, most of which are not accessible by "linear" combinatorial chemistry).

The situation becomes even more problematic when we move from organic diversity to the diversity of the periodic table. Under the assumption that there are 75 useful and stable elements, about 5600 binary, 4×10^5 ternary, 3×10^7 quaternary, and over 10^{18} decanary compositions are possible, and this without even considering stoichiometric and structural diversity. Here it should also be considered that the composition is only one aspect of the vast chemical diversity (CH = one binary component = all hydrocarbons, CHO = all molecules containing just C, H, and O, independent of structure and stoichiometry; analogous for CHN, CHNO, CHS, CHNOS, etc.). The structural and stoichiometric diversity that is potentially contained in every individual combination of elements brings a second astronomical dimension to this simple-minded view of chemical diversity. Since the total number of known inorganic structures is much smaller than for the organic counterparts, it can be concluded that the little that is known in inorganic chemistry becomes negligible when compared with the possibilities contained in the periodic system. Combinatorial chemistry is presently the only rational concept offering a more effective access to this diversity, whereby it seems obvious that a systematic and comprehensive access to chemical diversity will be impossible. Any combinatorial synthesis of new materials should readily lead to new materials within a relatively short time. For the sceptic I may point out the superconductors, which were detected by conventional methods but whose existence had not been expected by traditional chemistry and physics. It can be expected that through the combination of elements of the periodic table any number of new materials with completely new, or at least extremely improved, properties can be obtained.

Combinatorial solid-state chemistry and materials synthesis differ significantly from organic chemistry. Here no advanced synthesis concepts exist, and the preparation of a materials library will almost automatically generate new solids. The following tasks of such a combinatorial solid-state chemistry are therefore of equal importance:

1. development of synthesis methods for the preparation of libraries of solid-state components
2. development of new and effective methods for the detailed characterization of library components
3. development of procedures for the simultaneous detection of desired properties of library components

There are only a few generalizable synthetic strategies in inorganic solid-state chemistry. In contrast to the organic synthesis of defined molecules, the preparation of homogeneous inorganic solids (defined phases) is dominated by the "primitive" heating of defined mixtures (mixing, milling, and

heating or decomposition of fine powders). Such reactions are based upon two basic steps, the solid-state diffusion of the reactants and the nucleation of crystalline intermediates or products. The rate-determining step of such a reaction is the diffusion, owing to the high activation barrier and the large distance of diffusion as a result of the macroscopic size of common powder particles. An elegant and rarely used synthesis technique is based on the sequential deposition of thin films (thickness in the range of submonolayer to a few atomic layers) by controlled evaporation. Thus, any stoichiometry can be produced, and diffusion resistance and diffusion distances are extremely reduced. As a result new kinetically stable phases that were not yet accessible by conventional techniques have been prepared. Johnson has advanced this technique,^[3] and with it many new solid phases have been identified already.

Combination of thin-film deposition with state-of-the-art masking readily provides access to materials libraries with spatial resolution. The first such library, prepared by Schultz et al. in 1995, was an array of 128 components prepared by masking and radio frequency (RF) sputtering, and led to the discovery of new superconducting materials.^[4] Just two years later, searching for new luminescent materials, Weinberg and co-workers from Symyx technologies reported on investigations on 25000-component libraries prepared by the combination of masking with physical vapor deposition (PVD).^[5] The advantage of such thin-film techniques is controlled access to nearly all element combinations of the periodic system. Of disadvantage is, however, that the laboratory or bulk synthesis of newly discovered compounds has to be developed subsequently.

The methods of liquid-phase synthesis—such as sol–gel and hydrothermal synthesis, polymerization, and precipitation reactions—are free of such upgrading problems and also suitable for library syntheses. Conventional synthesis robots can be used for library preparation. The use of ink-jet technology, as applied with high precision in standard color printers, has been appealing. A library of new luminescent materials has been prepared from aqueous solutions of La, Eu, Gd, and Al nitrates.^[6] Here volumes as small as 0.5 nL can be dosed with high precision and spatial resolution. Such an ink-jet technique was also applied in the first combinatorial discovery of a new heterogeneous electrocatalyst for applications in the methanol fuel cell. Mallouk et al. projected three-dimensional phase diagrams of Pt, Os, Rh, Pd, and Ir onto planar libraries and investigated the activity of the components for methanol decomposition by the use of a H^+ -sensitive fluorescence indicator. The best phases discovered ($Pt_{44}Ru_{41}Os_{10}Ir_5$ and $Pt_{62}Rh_{25}Os_{13}$) showed a 40% higher current yield compared to the industry standard $Pt_{50}Ru_{50}$.^[7] Even hydrothermal syntheses can be carried out combinatorially for the direct preparation of materials libraries.^[8]

As far as the recognition of materials properties and catalytic activities and the characterization of materials are concerned, there are no limits yet for creativity. Video cameras are useful for the spatially resolved detection of optical properties. IR cameras record with high temperature resolution spatially resolved heats of reactions and are useful

as indicators for catalyst activities.^[9] Even X-ray powder patterns with high spatial resolution from component areas larger than 50 μm ^[8] or X-ray fluorescence spectra from component areas larger than 100 μm can be obtained in an automated fashion with commercially available systems. Furthermore, spatially resolved mass spectra for product analysis on catalyst libraries have been reported.^[10]

The strength of conventional research relative to combinatorial research has been commented on in detail by R. Schlögl.^[11] However, the combinatorial approach should be discussed as complementary and not as competitive to conventional catalysis research. The combinatorial approach does not require a sound hypothesis describing the structure–activity relationship. The huge successes of the combinatorial chemistry approach in various areas of molecular chemistry should lead to similar expectations for applications in heterogeneous catalysis. That there is “no novel scientific knowledge produced [by combinatorial methods] in comparison to a typical empirical design method” has already been contradicted by the discovery of new materials.^[4–7, 12] Schlögl is right when he emphasizes that there are no well-defined synthetic building blocks or synthetic strategies for the controlled synthesis of solids or catalysts and that the intuition of the chemist is still required. The design of useful libraries will be the key to success, since the above-outlined chemical diversity is much too large to be accessed comprehensively or systematically by sheer library size. Conventional catalysis and materials research remain the decisive instruments in development and evaluation, since any newly identified material or catalyst has to be synthesized on the laboratory scale and its special activity or property has to be confirmed and characterized in more detail under conventional conditions.

In summary it can be stated that there are already several effective methods for the preparation and characterization of significantly different materials libraries, and these have been highlighted just recently by Bein^[12] and by Liu and Schulz.^[13] In the near future a rapid development of new preparation and characterization techniques can be expected. Combina-

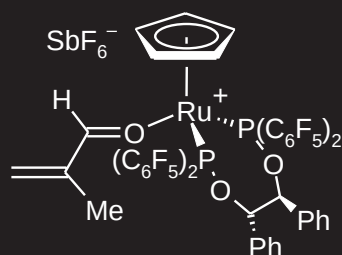
torial chemistry presents a unique chance for the development of new materials and catalysts, and its major task, at least in the academic environment, should be the discovery of new materials and not the fine-tuning and optimization of known materials.

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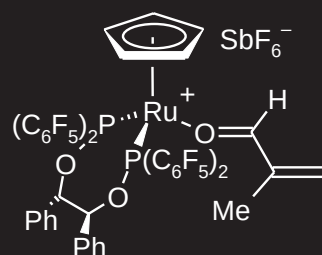
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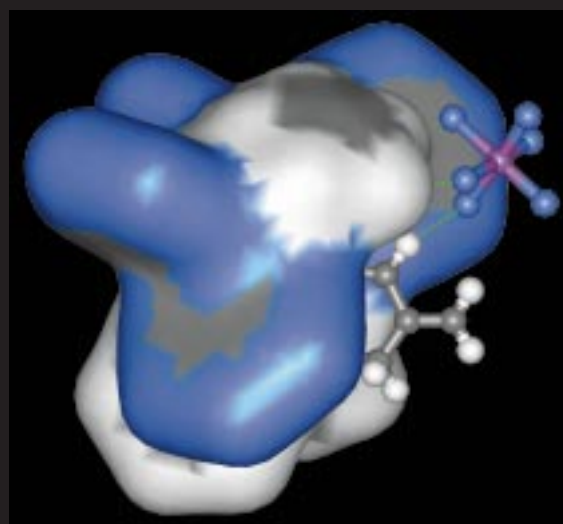
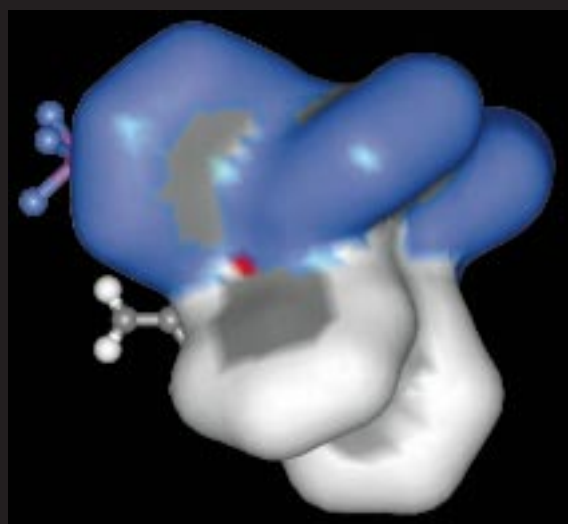
Many chiral Lewis acids catalyze asymmetric Diels–Alder reactions between enals and dienes, but a new readily accessible and thermally stable Ru Lewis acid also provides valuable structural information about catalyst–substrate interactions. Of particular interest is an X-ray structure that shows cooperative binding of the dienophile by the catalyst and the anion.



C_{α} -*si*-face view



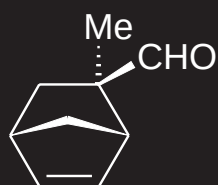
C_{α} -*re*-face view



C_{α} -*si* face
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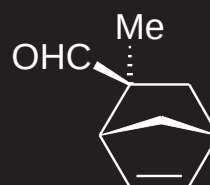


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A Stable and Recoverable Chiral Ru Lewis Acid: Synthesis, Asymmetric Diels–Alder Catalysis and Structure of the Lewis Acid Methacrolein Complex**

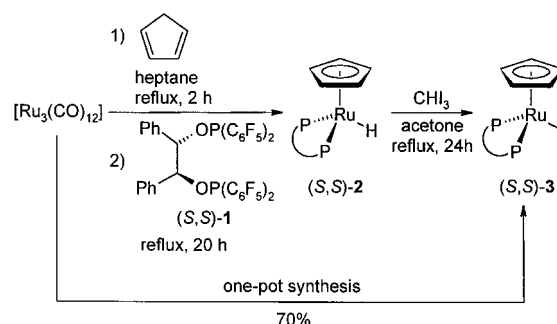
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Gérald Bernardinelli

The asymmetric Diels–Alder reaction between enals and dienes has become the test reaction for chiral Lewis acids with a single coordination site. In these reactions chiral B, Cu, and Ti compounds are often the catalysts of choice.^[1] We have recently found that the iron Lewis acid catalysts [CpFe(cyclop-F)][BF₄] and [CpFe(biphop-F)][BF₄] (cyclop-F = 1,2-bis[bis(pentafluorophenyl)phosphanyloxy]cyclopentane; biphop-F = 1,2-bis[bis(pentafluorophenyl)phosphanyloxy]-1,2-diphenylethane) can give competitive enantioselectivities.^[2] Herein we focus on Ru catalysts for this reaction, present an efficient one-pot synthesis of the catalyst precursor, and report details of reactivity and structural data of the catalyst–methacrolein complex.

The perfluoroaryldiphosphinite ligands cyclop-F^[3] and biphop-F^[2b] (**1**) used for the chiral Fe and Ru Lewis acids create the chiral environment around the coordination site of the enal. Since they are electron-poor, they offset the donor properties of the Cp ligand and thus contribute to the Lewis acidity of the metal center. An attractive feature of this family of Lewis acids is that the immediate catalyst precursors are amenable to structural characterization.^[2, 4] Interpretation of observed enantioselectivities is thereby placed on firmer ground than those advanced for in situ prepared catalysts.

Lewis acids based on the fragment CpRu⁺ are known^[5] but they have not previously found application in Diels–Alder catalysis of enals—presumably because they tend to coordinate the alkene rather than the carbonyl function.^[6] We note, however, recent reports on Diels–Alder catalysis with structurally closely related complexes containing the isoelectronic (η^6 -arene)Ru²⁺ fragment.^[4b,c] The more highly charged metal center thus prefers to bind to the enal oxygen atom to form a M–O σ bond.

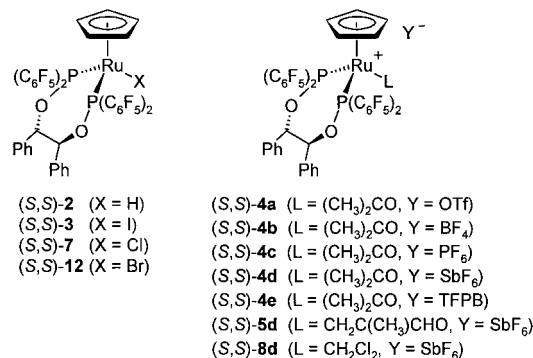
The iodoruthenium complex (*S,S*)-**3** was prepared in a one-pot procedure from [Ru₃(CO)₁₂].^[7] (Scheme 1; details in Experimental Section). Crucial to the success was the hydride-labilizing effect^[8] that enabled CO substitution in the in situ formed [CpRu(CO)₂H].^[9] The intermediate hydrido complex (*S,S*)-**2** was initially isolated. Its ¹H NMR



Scheme 1. One-pot synthesis of (*S,S*)-**3**.

spectrum (in C₆D₆) showed a triplet (*J* = 33.5 Hz) at δ = –11.08 for the Ru–H proton. Reflux in acetone in the presence of iodoform^[10] afforded the air-stable iodo complex (*S,S*)-**3**. In subsequent preparations, complex (*S,S*)-**2** was not isolated.

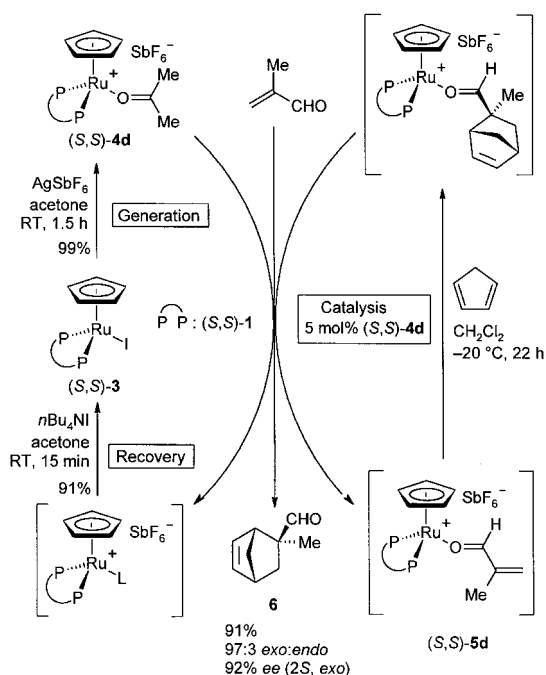
Reaction of a solution of (*S,S*)-**3** in acetone with the appropriate silver salt generated the cationic complexes (*S,S*)-**4a–d**.^[11] The cationic complex (*S,S*)-**4e** was obtained from (*S,S*)-**4d** by metathesis^[12] with sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB).^[13] In CH₂Cl₂, methacrolein readily displaced the coordinated acetone in (*S,S*)-**4d** to give complex (*S,S*)-**5d** (Scheme 2). In contrast to the corresponding Fe Lewis acid aldehyde complexes, which slowly



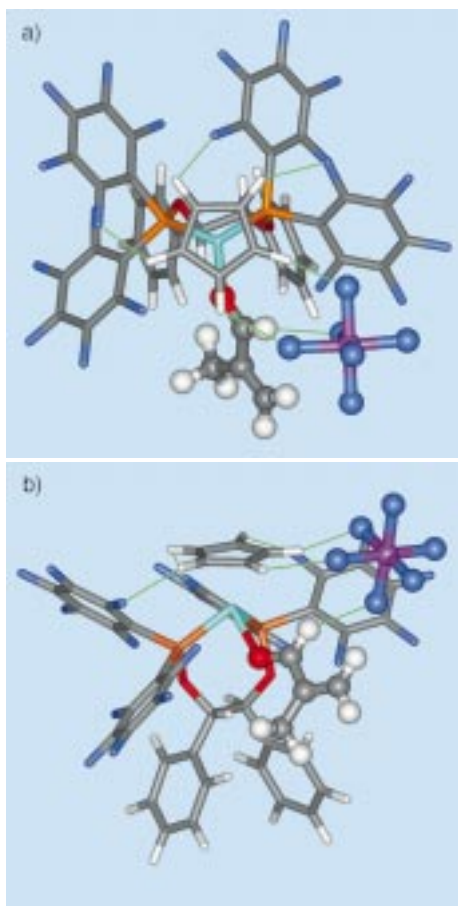
decompose in CH₂Cl₂ above –20 °C,^[2] complexes (*S,S*)-**4d** and (*S,S*)-**5d** are stable in solution at room temperature. The ¹H NMR and IR spectra (CD₂Cl₂) of (*S,S*)-**5d** showed coordinated (δ = 9.76, $\tilde{\nu}$ = 1606 cm^{–1}) and free (δ = 9.53, $\tilde{\nu}$ = 1695 cm^{–1}) methacrolein and its NOESY spectrum at –20 °C indicated an *s-trans* coordinated methacrolein with the formyl proton pointing towards the cyclopentadienyl ring.^[14] This preferred structure in solution coincides with that found in the solid state by X-ray diffraction analysis (Figure 1).^[15,16] The asymmetric unit contains two molecules of the same absolute configuration (*S,S*) and which adopt almost identical conformations. Both cationic complexes are associated in pairs with SbF₆[–] ions; the Ru–Sb distances are 5.785(1) and 5.798(1) Å, respectively. In each ion pair, three fluorine atoms of the anion are involved in hydrogen bond interactions (H–F distances less than 2.6 Å^[17]): two with the cyclopentadienyl ring and one with the formyl hydrogen atom^[18] of the methacrolein moiety (Figure 1). These hydrogen bonds fix the substrate in the chiral pocket and impede rotation around the Ru–O bond. Other hydrogen atoms of the Cp ligand are involved in intramolecular hydrogen bond interactions with

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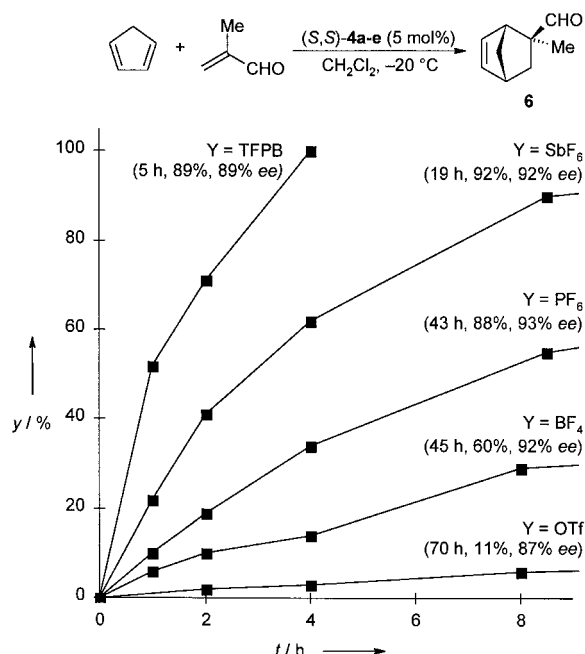
Scheme 2. Catalyst generation and recovery.


 Figure 1. a) Top view and b) front view of one of the ion pairs present in the asymmetric unit of (S,S)-5d. The C–H...F hydrogen bonds are represented by green lines. (WebLab ViewerPro^[28])

fluorine atoms of the pentafluorophenyl substituents.^[19] The *s-trans* conformation of the methacrolein was determined without ambiguity.

The Diels–Alder reaction of methacrolein with cyclopentadiene catalyzed by (S,S)-4d (5 mol %, CH₂Cl₂, –20 °C, 22 h) gave the cycloadduct 6 in 91 % yield with a diastereomeric ratio of *exo:endo* of 97:3 and an enantioselectivity of 92 % *ee* (*exo*), and 2*S* absolute configuration (Scheme 2). We infer that product 6 was formed through a diene approach from the less hindered top side (cyclopentadienyl side) and addition to the hindered *C_α-re* face of the *s-trans* conformer of the dienophile.^[20] The methacrolein conformation found in both the solid state and in solution is close to that which we presume to lead to the transition state of the cycloaddition.

The nature of the counterion had a large effect on the rate (TfO[–] < BF₄[–] < PF₆[–] < SbF₆[–] < TFPB[–]; TfO[–] = trifluoromethanesulfonate) but not on the enantioselectivity of the reaction (Figure 2).^[4a, 21] The previously advanced interpretation for a similar phenomenon in dicationic Cu catalysts

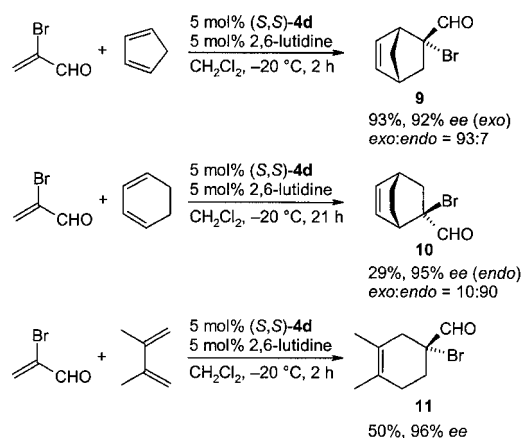

 Figure 2. Plot of GC yield (*y*) as a function of reaction time (*t*) for the catalyzed reaction of methacrolein with cyclopentadiene in presence of (S,S)-4a–e. In parentheses: total reaction time, yield of isolated product and *ee* value of *exo*-cycloadduct 6.

invoked competition of the anion and the substrate for the Lewis acid site.^[21] Figure 1 prompts another explanation. By interacting simultaneously with catalyst and coordinated aldehyde, the anion slows down turnover frequency. Though weak, these hydrogen bonding interactions (green in Figure 1) must be highly sensitive to the nature of the anion.^[22] With catalyst (S,S)-4d, a fivefold increase in diene had little effect on the rate, indicating that the rate-limiting step is not the cycloaddition reaction but may be ligand exchange.

Diels–Alder reactions with the Ru complex (S,S)-4b are slower than those with the analogous Fe complex and gives product 6 with a lower enantioselectivity (Fe: 97 % *ee*, Ru: 92 % *ee*). The difference in product *ee* can be attributed to the increased size of the chiral pocket on going from the first row to the second row transition metal.

Chiral Lewis acid catalysts are generally lost during workup and at best the chiral ligand is recovered. The stability of our catalyst makes recovery straightforward. Addition of *n*Bu₄NI to the reaction mixture generated the catalyst precursor complex (*S,S*)-**3** but separation from **6** was difficult. This reaction also afforded the chloro complex (*S,S*)-**7** as minor product, presumably the result of iodide attack on coordinated CH₂Cl₂ in (*S,S*)-**8d**.^[23, 24] A more general procedure for catalyst recovery involved addition of hexane to the reaction mixture and filtration over celite. Catalyst extraction from celite with acetone followed by addition of *n*Bu₄NI yielded (*S,S*)-**3** (91 %).

A limitation of the use of our Ru Lewis acid became apparent in reactions with α -bromoacrolein. Reactions with this dienophile had to be carried out in the presence of a base (2,6-lutidine). Without it, the catalyst decomposed and a racemic cycloadduct was obtained. More importantly, while the reaction with cyclopentadiene afforded cycloadduct **9** in good yield and enantioselectivity (Scheme 3), with the less



Scheme 3. Diels–Alder reactions of α -bromoacrolein with dienes catalyzed by (*S,S*)-**4d**: catalyst deactivation by the product (see text).

reactive cyclohexadiene and dimethylbutadiene, yields of **10** and **11** dropped to 29 % and 50 %, respectively, although *ee* values remained high. The isolation of the bromo complex (*S,S*)-**12** as sole Ru product recovered (in 83 % and 91 %, respectively) from these reactions points to catalyst deactivation by the cycloaddition products **10** and **11**.^[25] The highly acidic organic cation formed by bromide abstraction from **10** and **11** may explain the formation of racemic Diels–Alder product in the absence of the amine.

To summarize, the key features of the new chiral Ru Lewis acid described here are its straightforward synthesis, a well defined structure, and high stability which makes easy recycling possible. First results in its use as Diels–Alder catalyst are promising and cycloadduct enantioselectivities are high although limitations exist with brominated cycloadducts.

Experimental Section

(*S,S*)-**3**: Cyclopentadiene (11 mL, 134 mmol) was added to [Ru₃(CO)₁₂] (1.065 g, 1.67 mmol) in refluxing heptane (300 mL). After 2 h, (*S,S*)-**1** (5.187 g, 5.50 mmol) was added and the reaction mixture was refluxed for

20 h. The solution was concentrated and maintained at 0 °C overnight. After decantation and drying of the pale yellow solid under vacuum, iodoform (2.166 g, 5.50 mmol) and acetone (50 mL) were added and the mixture was refluxed for 24 h. Volatiles were removed under vacuum and the residue was washed in air with MeOH. Recrystallization from CH₂Cl₂/MeOH afforded (*S,S*)-**3** as red-orange crystals (4.389 g, 70 %). M.p. > 250 °C; [α]_D²⁰ = −126.3 (*c* = 1.0 in CH₂Cl₂); ¹H NMR (400 MHz, C₆D₆, 20 °C, TMS): δ = 7.49 (d, *J* = 7.9 Hz, 2H; H_{ar}), 6.60–7.00 (m, 9H; H_{ar}, CH), 5.22 (t, *J* = 7.4 Hz, 1H; CH), 4.61 (s, 5H; C₅H₅); ³¹P NMR (162 MHz, C₆D₆, 20 °C, H₃PO₄): δ = 123.9 (br. d_{AB}, ²*J*(P,P) = 72 Hz, 1P), 116.4 (br. d_{AB}, ²*J*(P,P) = 71 Hz, 1P); elemental analysis calcd for C₄₃H₁₇F₂₀IO₂P₂Ru (%): C 41.80, H 1.39; found: C 41.79, H 1.60.

(*S,S*)-**4d**: 11.8 mL (1.17 mmol) of AgSbF₆ (695 mg, 1.98 mmol) in CH₂Cl₂ (20 mL) were added to (*S,S*)-**3** (1.235 g, 1.00 mmol) in acetone (15 mL). The mixture was stirred for 90 min. After filtration through celite and evaporation of the solvent under vacuum the residue was twice purified by dissolution in acetone, precipitation by addition of Et₂O, decantation, dissolution in acetone, and filtration through celite. (*S,S*)-**4d** was obtained as a yellow solid (1.392 g, 99 %). M.p. > 170 °C (decomp); [α]_D²⁰ = −81.5 (*c* = 0.60 in CH₂Cl₂); ¹H NMR (400 MHz, CD₂Cl₂, 20 °C, TMS): δ = 7.23–6.94 (m, 6H; H_{ar}), 6.85–6.45 (m, 2H; H_{ar}), 6.77 (d, *J* = 7.1 Hz, 2H; H_{ar}), 5.27 (dd, *J* = 7.9, 14.3 Hz, 1H; CH), 5.01 (t, *J* = 8.1 Hz, 1H; CH), 4.92 (s, 5H; C₅H₅), 2.56 (s, 6H; CH₃); ³¹P NMR (162 MHz, CD₂Cl₂, 20 °C, H₃PO₄): δ = 130.1 (br. d_{AB}, ²*J*(P,P) = 71 Hz, 1P), 124.9 (br. d_{AB}, ²*J*(P,P) = 66 Hz, 1P); IR (CH₂Cl₂): $\tilde{\nu}$ = 1657 cm^{−1}.

(*S,S*)-**5d**: (*S,S*)-**4d** (141 mg, 0.10 mmol) was dissolved in CH₂Cl₂ (2 mL), and methacrolein (160 μ L, 1.95 mmol) was added. After stirring the mixture for 5 min, volatiles were removed under vacuum. This treatment was repeated twice to give (*S,S*)-**5d** as a yellow solid (142 mg, 99 %). M.p. > 150 °C (decomp); [α]_D²⁰ = −17.7 (*c* = 0.60 in CH₂Cl₂); ¹H NMR (400 MHz, CD₂Cl₂, 20 °C, TMS): δ = 9.76 (s, 1H; CHO), 6.95–6.25 (m, 8H; H_{ar}), 6.89–6.83 (m, 2H; H_{ar}, CH₂), 6.76–6.70 (m, 2H; H_{ar}, CH₂), 5.20 (dd, *J* = 7.9, 14.8 Hz, 1H; CH), 5.03 (t, *J* = 7.9 Hz, 1H; CH), 4.96 (s, 5H; C₅H₅), 1.90 (s, 3H; CH₃); ³¹P NMR (162 MHz, CD₂Cl₂, 20 °C, H₃PO₄): δ = 127.4 (br. d_{AB}, ²*J*(P,P) = 71 Hz, 1P), 125.4 (br. d_{AB}, ²*J*(P,P) = 70 Hz, 1P); IR (CH₂Cl₂): $\tilde{\nu}$ = 1606 cm^{−1}.

6: Methacrolein (82 μ L, 1.00 mmol) and cyclopentadiene (100 μ L, 1.22 mmol) were successively added to (*S,S*)-**4d** (69.9 mg, 0.05 mmol) in CH₂Cl₂ (1 mL) at −20 °C. After stirring the mixture at −20 °C for 22 h, hexane (10 mL) was added and the mixture filtered through celite. Chromatography of the filtrate on silica gel (pentane:CH₂Cl₂ = 8:1 to 2:1) gave cycloadduct **6** as a deliquescent white solid (127 mg, 91 %) with 97:3 *exo:endo* ratio (determined before chromatography) and 92 % *ee* (*exo*). Catalyst recovery: celite was eluted with acetone and *n*Bu₄NI (32 mg, 0.09 mmol) was added. After the mixture had been stirred for 15 min, evaporation of the solvent and chromatography on silica gel (hexane:CH₂Cl₂ = 4:1 to 2:1) gave (*S,S*)-**3** (56 mg, 91 %).

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- [15] Crystal structure determination of (S,S)-**5d**: [$\text{C}_{47}\text{H}_{23}\text{F}_{20}\text{O}_3\text{-P}_2\text{Ru}$][SbF_6]; $M_r = 1414.4$; $\mu = 1.018\text{ mm}^{-1}$, $F(000) = 1376$, $\rho_{\text{calc}} = 1.844\text{ g cm}^{-3}$, triclinic, $P1$, $Z = 2$, $a = 13.021(1)$, $b = 13.189(1)$, $c = 15.167(2)\text{ Å}$, $\alpha = 89.68(1)$, $\beta = 89.22(1)$, $\gamma = 78.05(1)^\circ$, $V = 2548.0(4)\text{ Å}^3$, yellow plate $0.18 \times 0.38 \times 0.64\text{ mm}$ mounted on a quartz fiber with RS3000 perfluoropolyether oil. Intensities were measured at 150 K on a STOE IPDS diffractometer with graphite-monochromated MoK_α radiation ($\lambda = 0.71069\text{ Å}$). $2\theta_{\text{max}} = 56^\circ$. A total of 43036 measured reflections, 21885 unique reflections ($R(\text{int.}) = 0.033$) of which 21123 were observable ($|F_o| > 4\sigma(F_o)$). Data were corrected for Lorentz and polarization effects and for absorption ($T_{\text{min}} = 0.6212$, 0.8437). The structure was solved by direct methods using MULTAN 87,^[26] all other calculations used XTAL^[27] system and WebLab ViewerPro^[28] program. The absolute configuration was determined and the Flack parameter^[29] converged to $x = -0.01(2)$. Full-matrix least-squares refinement based on F using weight of $1/[\sigma^2(F_o) + 0.0003(F_o^2)]$ gave final values $R = 0.045$, $\omega R = 0.055$, and $S = 2.61(2)$ for 1436 variables and 21123 contributing reflections. Hydrogen atoms were placed in calculated positions and other non-disordered atoms were refined with anisotropic displacement parameters. The final difference electron density map showed a maximum of $+1.60$ and a minimum of -1.47 e Å^{-3} . One of the two molecules shows an *in-plane* disorder of a phenyl group (angle between the two phenyl mean planes $= 3.0(6)^\circ$). This disorder was resolved by refinement of two tilted positions (tilt angle of about 12°) with population parameters of 0.60 and 0.40, respectively, and restraints on bond lengths, bond angles, and torsional angles (40 restraints). 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-112276. Copies of the data can be obtained free of charge on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
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Synthesis and Biological Evaluation of a Cyclo- β -tetrapeptide as a Somatostatin Analogue

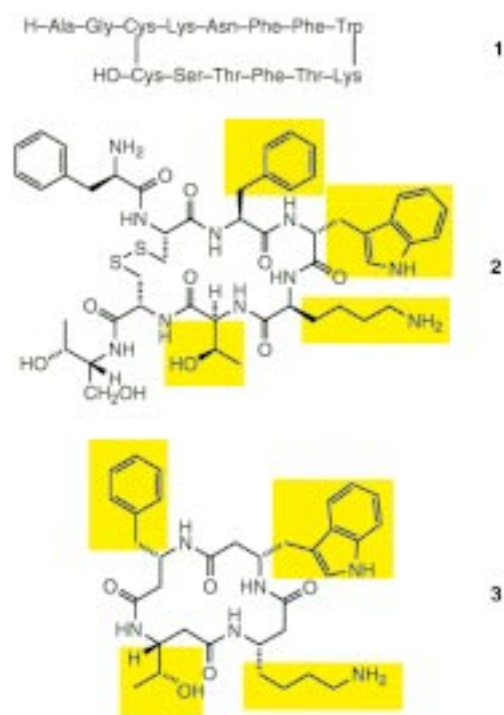
Karl Gademann, Martin Ernst, Daniel Hoyer, and Dieter Seebach*

In contrast to small α -peptides, short-chain β -peptides (oligomers of β -amino acids) show a remarkable ability to fold into well defined secondary structures in solution as well as in the solid state.^[1] The main three structural elements of proteins (helices, pleated sheets, and turns) have been identified in β -hexapeptides in solution.^[2] Cyclic β -peptides arrange in the solid state as tubular structures with a tight net of pleated-sheet-type hydrogen bonds (“nanotubes”).^[3] The second fundamental difference between natural peptides and β -peptides is the latter’s excellent stability against degradation by proteases and peptidases, including the most aggressive ones, such as pronase and proteinase K.^[4]

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The enzymatic stability and the variety of secondary structures discovered push the door wide open for the application of β -peptides as peptidomimetics in medicinal chemistry. β -Amino acids (in two synthetic steps from the corresponding α -amino acids),^[5] as well as β -peptides are readily prepared (by solution or solid-phase methods),^[6] allowing their synthesis by combinatorial methods. β -Amino acids have been introduced in pharmacologically active compounds for a long time,^[7] and recently the synthesis of conjugates of α - and β -peptides as MHC-class-I protein-binding peptides was reported.^[8] However, to the best of our knowledge, there is no report in the literature about the pharmacologic potential of β -peptides as biologically active compounds. Thus, the question whether β -peptides can mimic natural α -peptides in their recognition by (human) receptors is hitherto unanswered.

We chose the natural α -peptide hormone somatostatin (SRIF₁₄, **1**), isolated in 1973 by Guillemin et al. from 500 000 hypothalami. This peptide has various important biological functions, for example the regulation of the release of growth hormone and insulin.^[9] Octreotide (SANDOSTATIN, **2**), a cyclic α -octapeptide derived from the tetradecapeptide somatostatin, is clinically in use to treat acromegaly and certain



intestine cancers.^[10] However, the elimination half-life of **2** (90 min) is still rather short; it is therefore of great importance to find nonpeptidic derivatives with higher bioavailability. The biologically active conformation of octreotide, a β -turn, was revealed by structure–activity relationships; the amino acids in the turn (Phe-Trp-Lys-Thr) are required for activity.^[10] For the design of a β -peptidic analogue by molecular modeling, we substituted the side chains in the powder X-ray structure of a cyclic all-(*S*)- β -tetrapeptide^[3a] with those of Phe, Trp, Lys, and Thr. Superposition of the resulting model (Figure 1,

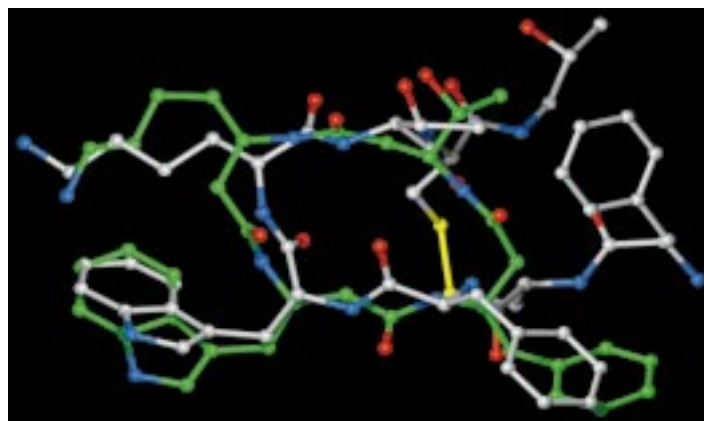
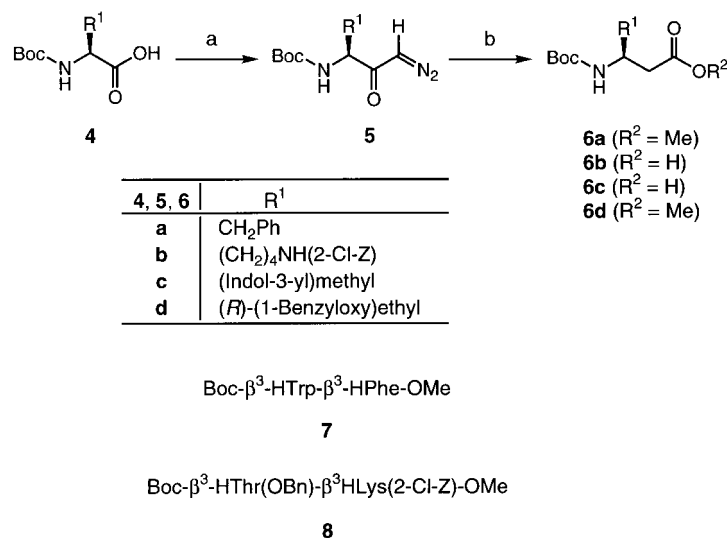


Figure 1. Top view of the superposition of the solution structure of octreotide (**2**) (C atoms in gray)^[13] and a model of the somatostatin-analogue **3** derived from the powder X-ray structure of cyclo-(*S*)-(β -HAla)₄^[3a] (C atoms in green). The side chains of **2** and **3** overlap well, a requirement for high affinity.

green) with the solution structure of octreotide^[11] (Figure 1, gray) gave a good spatial overlap of the side chains, a requirement for biological activity. Therefore, the cyclic β -tetrapeptide **3** was chosen as target for our synthesis.

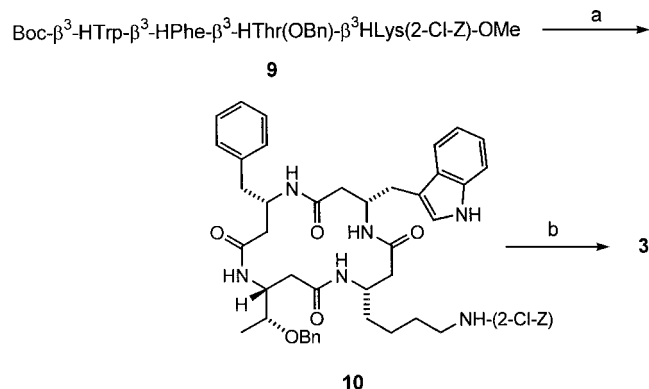
The β -amino acid building blocks **6a–d** were prepared from the corresponding *N*-Boc- α -amino acids **4a–d** using the Arndt–Eistert reaction via the diazoketones **5a–d** and Wolff rearrangement (Scheme 1). We chose the standard Boc/



Scheme 1. Synthesis of β^3 -HPhe, β^3 -HLys, β^3 -HTrp, and β^3 -HThr derivatives **6a–d** and of dipeptides **7** and **8**. a) ClCO₂Et, Et₃N, THF, then CH₂N₂, Et₂O; **5a**: 53 %, **5b**: 50 %, **5c**: 59 %, **5d**: 69 %; b) CF₃CO₂Ag (cat.), Et₃N, THF, R²OH; **6a**: 94 %, **6b**: 89 %, **6c**: 89 %, **6d**: 88 %. Bn = benzyl; Boc = *tert*-butoxycarbonyl; Z = benzyloxycarbonyl.

benzyl-protecting group strategy for peptide synthesis in solution. Boc-deprotection of β -amino ester **6a** and coupling with **6c** gave dipeptide **7**, esterification and Boc-deprotection of **6b**, and coupling with **6d** gave dipeptide **8**; **7** was C- (NaOH solution) and **8** was N-terminally deprotected (CF₃CO₂H), and the resulting fragments were coupled to give the β -tetrapeptide **9** in 93 % yield. Compound **9** was deprotected at

the C-terminus and allowed to react with pentafluorophenol to give the active ester, which, after Boc-deprotection, was cyclized (Hünig base, CH₃CN) to give the cyclo- β -tetrapeptide **10** in good yield (Scheme 2). This peptide is essentially



Scheme 2. The β -tetrapeptide precursor **9** and its cyclization to give **10** and subsequent deprotection to give **3**. a) 1) 50 equiv NaOH, H₂O, DMF, 45 °C, 92 %; 2) C₆F₅OH, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, DMF/CH₂Cl₂, quant; 3) CF₃CO₂H, CH₂Cl₂, (CH₂SH)₂, quant; 4) *i*PrNEt₂, CH₃CN, 70 °C, syringe pump, 48 %; b) Pd/C, H₂, LiCl, THF, MeOH, 48 h, 9 %.^[14]

insoluble in all standard solvents used in peptide chemistry.^[12] Therefore, we were forced to apply a new method for the hydrogenolysis of the benzyl- and 2-Cl-Z-protecting groups: Peptides can be solubilized in organic solvents such as THF, by the addition of LiCl,^[13] but, to the best of our knowledge, hydrogenolysis has never been carried out under these conditions; the cyclo- β -tetrapeptide **10** was solubilized in THF by the addition of six equivalents of LiCl, and could, indeed, be deprotected albeit in poor yields.^[14] Purification by reversed-phase HPL chromatography produced **3**.

The affinity of the cyclo- β -tetrapeptide **3** for five different human somatostatin receptors (hsst 1–5, expressed in CHO-39 or CCL-39 cell lines) was measured by using radioligand-binding assays (Table 1). Displacement experiments for the specific binding of [¹²⁵I]LTT-SRIF₂₈ to these receptors were carried out as described previously,^[15] and the affinities are reported as $pK_d \pm \text{SEM}$ values. The data in Table 1 clearly show that **3** has affinity, although the concentrations are in the micromolar and not in the nanomolar range as for **1** and **2** (the affinity is reduced by a factor of 10 (hsst 4) to 10⁵ (hsst 2)). Using a glucose-derived somatostatin mimic, a comparable affinity in the micromolar range had been observed.^[16]

We have demonstrated for the first time, that a small β -peptide (consisting of only four β -amino acids) can mimic a natural peptide hormone and display biological activity and micromolar affinity for human receptors. Thus, β -peptides can, in principle, be considered as peptidomimetics, also due to their excellent stability against peptidases that might allow for oral bioavailability (corresponding experiments with **3** are underway). It appears feasible to use short-chain β -peptides with their compact and well-defined secondary structures as metabolically stable vaccines that could be recognized by human receptors and the immune system.

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Table 1. Comparison of the affinities of cyclo- β -tetrapeptide **3** and octreotide **2** by radioligand-binding assays (displacement experiments of the specific binding of [¹²⁵I]LTT-SRIF₂₈) with the five different human somatostatin receptors (hsst 1–5, expressed in two different cell lines). The values are reported as $pK_d \pm \text{SEM}$ (SEM = standard error). The affinities of **3** are in the micromolar range, at least one order of magnitude lower than those of octreotide **2**.^[a]

Receptor	β -Peptide 3	Octreotide (2)
hsst-1 CCL39	4.85 $\pm$ 0.04	6.65
hsst-2 CCL39	4.44 $\pm$ 0.09	9.19
hsst-3 CHO	5.48 $\pm$ 0.01	7.88
hsst-4 CCL39	5.00 $\pm$ 0.05	6.40
hsst-5 CCL39	3.73 $\pm$ 0.15	7.17

[a] CCL-39 = Chinese hamster lung fibroblasts; CHO = Chinese hamster ovaries; hsst = human somatostatin receptor subtype; SRIF = somatotropin release inhibiting factor.

Keywords: hormones • peptides • peptidomimetics • somatostatin

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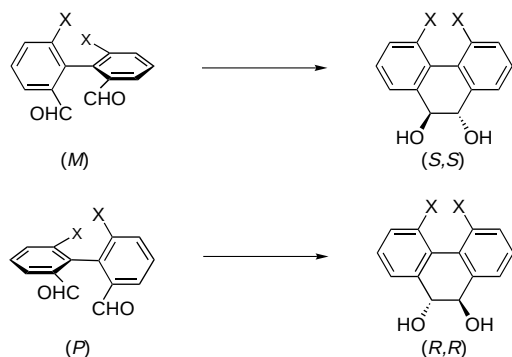
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From Axial Chirality to Central Chiralities: Pinacol Cyclization of 2,2'-Biaryldicarbaldehyde to *trans*-9,10-Dihydrophenanthrene-9,10-diol**

Ken Ohmori, Mitsuru Kitamura, and Keisuke Suzuki*

In memory of Vladimir Prelog

We describe herein two salient features of the pinacol cyclization of 2,2'-biaryldicarbaldehydes (Scheme 1): 1) the stereoselectivity to give only the *trans*-diol, and 2) the stereospecificity to transmit the axial chirality (in case the starting



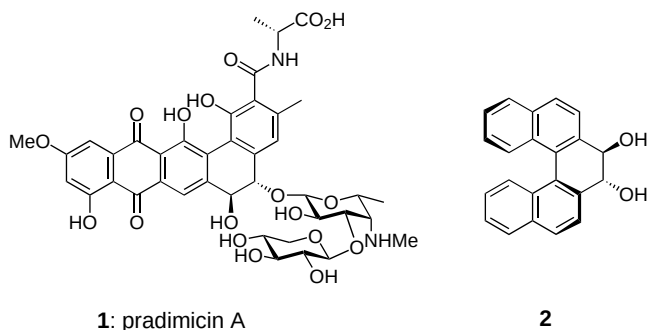
Scheme 1. Chiral transmission.

biphenyl is configurationally stable) onto two stereogenic centers of the product. The accessibility of the *trans*-9,10-dihydrophenanthrene-9,10-diol structure has consequences

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not only to the synthesis of natural products (e.g. **1**),^[1] but also to the creation of a new compound such as **2** with potential utility in asymmetric synthesis.^[2]



Our initial attempt was centered on the reaction of 2,2'-biphenyldicarbaldehyde (**3**),^[3] which upon treatment with SmI₂^[4] (2 equiv, THF, 0 °C, 5 min) was cleanly converted into the *trans*-diol **4**^[5] as the sole product in 99% yield (Table 1, run 1). To our knowledge, the *intramolecular* pinacol coupling of two aromatic aldehydes is unprecedented,^[6] as opposed to many *intermolecular* examples.^[7] The observed *trans* selectivity stands in contrast to the *cis* selectivity generally seen for aliphatic 1,6-dicarbonyl compounds.^[7, 8]

Table 1. Pinacol cyclization of **3** with various reductants.

Run	Reductant	Yield [%]	<i>trans/cis</i> of 4
1	SmI ₂	99	> 99/1
2	SmI ₂ , HMPA	93	> 99/1
3	[VCl ₃ (THF) ₃], Zn ^[a]	93	> 99/1
4	TiCl ₄ , <i>n</i> BuLi ^[b]	80	> 99/1
5	TiCl ₄ , Zn ^[c]	96	20/1
6	TiCl ₄ , Mg(Hg) ^[c]	94	16/1
7	Ce, I ₂	81	16/1

[a] In CH₂Cl₂. [b] In Et₂O. [c] At –10 °C.

It turned out that consistently high selectivities and yields were attained with various reducing agents (Table 1). Especially, SmI₂ or [VCl₃(THF)₃]/Zn^[9a] led to perfect *trans* selectivity (runs 1 and 3). Use of a dipolar aprotic cosolvent (hexamethyl phosphoramide, HMPA) did not affect the reaction course (run 2). Use of low-valent titanium or cerium^[9b–c] gave a slight decrease in either the selectivity or the yield (runs 4–7).

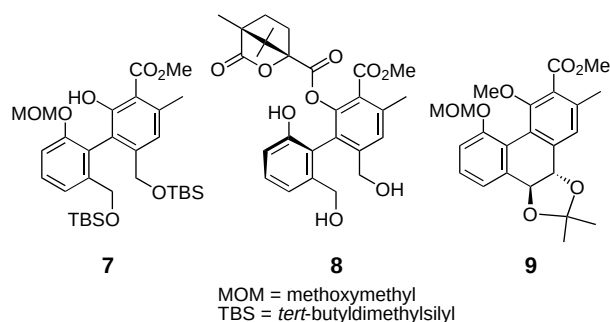
At this stage, we made an analysis on the origin of the selectivity. Taking the *M* isomer as an example (Scheme 2), emergence of the *trans* selectivity could be traced back to two possible modes of reaction with respect to the mutual relation of two aldehyde faces, both with a *like* topicity, *Re,Re* and/or *Si,Si*. Given that the reaction proceeded uniquely by one of



Scheme 2. Two possible modes of the pinacol cyclization.

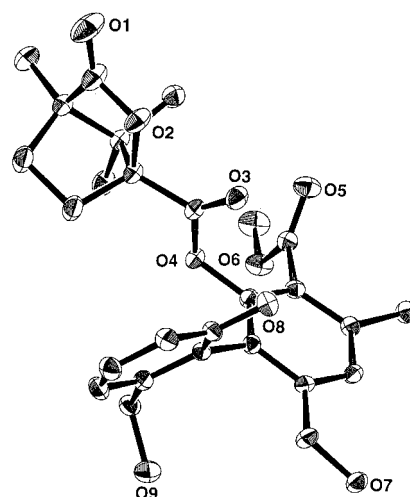
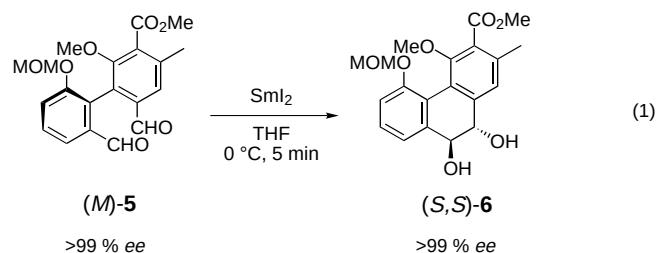
these modes, the chiral information due to the atropisomerism can be transmitted to the diol stereogenicities of the product. Of course, prerequisite for such a scenario is the configurational stability of the starting biphenyl (cf. **3**) before participation in the pinacol cyclization.^[10]

As a model compound to check out this possibility, chiral dialdehyde **5** [see Eq. (1)] was chosen, considering the relevance to the projected natural product synthesis.^[1] Optical resolution was carried out for phenol **7** by esterification ((-)-(1*S*,4*R*)-camphanoyl chloride) and separation of the resulting



diastereomers by column chromatography (SiO₂, hexane/EtOAc 4/1). Subsequent four steps (1. K₂CO₃/MeOH, 2. MeI, K₂CO₃, 3. *n*Bu₄NF, 4. MnO₂) gave the enantiomeric dialdehydes (*P*)-**5** and (*M*)-**5**, which proved to be configurationally stable at room temperature, in line with the general behavior for 2,2',6,6'-tetrasubstituted biphenyls.^[10] The more polar isomer of **7** was converted into the crystalline derivative **8**, which was suitable for an X-ray analysis (Figure 1).^[11]

Upon reaction of (*M*)-**5** with SmI₂ [Eq. (1)], we observed quantitative formation of the *trans*-diol (*S,S*)-**6**, which proved to be diastereo- and enantiomerically pure.^[12] The absolute configuration was determined by the CD spectrum of the


 Figure 1. X-ray crystal structure of **8**.

derived acetone **9**.^[13, 14] Thus, among two possible modes of chiral transmission, mode A [(*M*) → (*S,S*)] proved to be uniquely operative (Scheme 2). The same situation was, of course, true for the antipodal series [(*P*) → (*R,R*)].

This outcome implies two points: 1) The reaction proceeds by the *synclinal* mode,^[15] and 2) the initial conformation of the cyclized product is diequatorial.^[15] However, the latter information is generally lost at the stage of cyclized products, as they undergo a rapid conformational change (diequatorial ⇌ diaxial) due to the pronounced lowering of the rotational barrier of the “2,2'-tethered biphenyl” with a two-atom bridge.^[10, 16] Indeed, the diacetate **10** derived from **6** has a low barrier for this interconversion (ca. 15 kcal mol⁻¹), as estimated by variable-temperature NMR spectroscopy (Figure 2).

However, our attempt to snapshot the “kinetic conformation” of the product was nicely achieved by using a conformationally restricted substrate **11** [racemate; Eq. (2)].^[17] Treatment of **11** with SmI₂ gave the diequatorial isomer **12a** as the only *trans*-diol, consistent with the above mentioned data.^[18]

The synthetic scope of the present process is not restricted to natural product synthesis,^[1] but has relevance to asymmetric synthesis: Application of the reaction to the enantiopure dialdehyde **14**, readily obtained from the 2,2'-(*S*)-(-)-binaphthol-derived diester **13**^[19] in two steps (1. LiAlH₄, 93%,

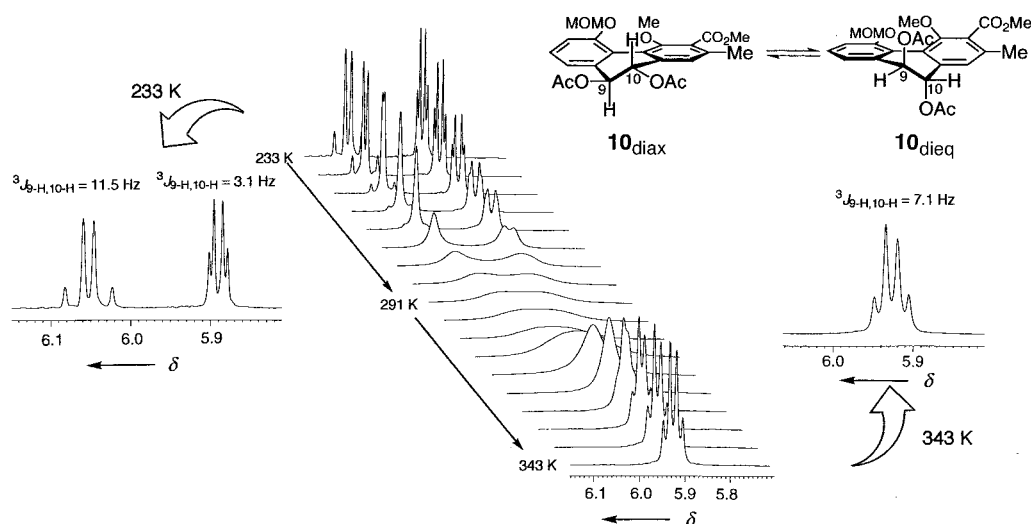
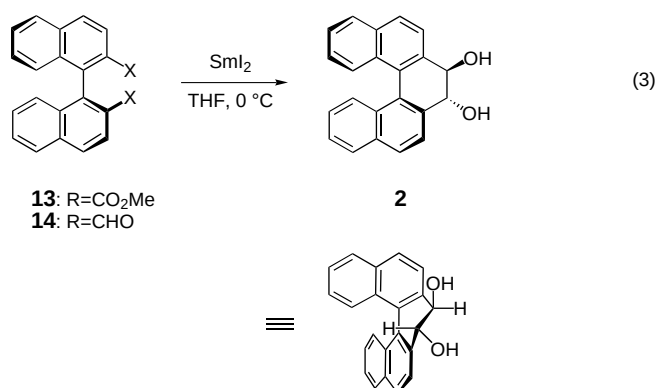
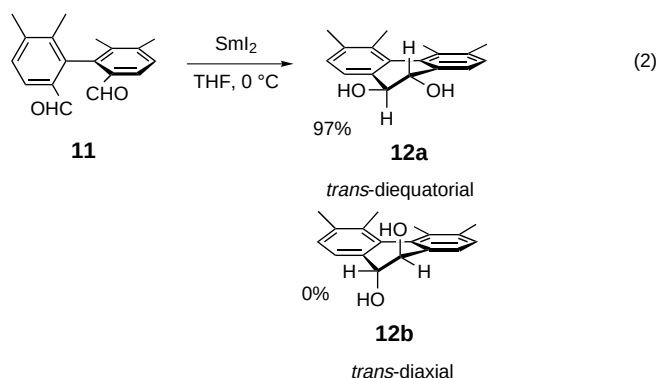


Figure 2. Variable-temperature NMR spectrum of **10** (500 MHz, CDCl₃, sealed tube). At low temperature (233 K), two sets of doublets were observed, assignable to **10**_{dieq} (³J_{9-H,10-H} = 11.5 Hz) and **10**_{diaz} (³J_{9-H,10-H} = 3.1 Hz), suggesting their slow interconversion. The ratio of **10**_{dieq} to **10**_{diaz} was about 1:1 (233 K). Upon warming, these peaks became closer with broadening and finally coalesced (*T*_c = 291 K), and further warming (343 K) led to a set of sharp doublets (³J_{9-H,10-H} = 7.1 Hz).

2. MnO₂, 96%) gave a novel C₂-symmetric diol **2** in 94% yield with full stereochemical integrity [*trans*, diequatorial, >99% *ee*; Eq. (3)].^[20] The utility of **2** and its derivatives in asymmetric reactions is the subject of our current investigation.



Experimental Section

In a typical experiment for pinacol cyclization, SmI₂ (0.1 M in THF, 5.0 mL, 0.50 mmol) was added to a solution of **3** (50.0 mg, 0.238 mmol) in THF (2 mL) at 0 °C. After 5 min of stirring at 0 °C, the reaction was quenched by addition of 1 M HCl, and the mixture was extracted with EtOAc (3 ×). The

combined organic extracts were washed with water and brine, dried (Na₂SO₄), and concentrated in vacuo. The residue was purified by preparative thin-layer chromatography (TLC; hexane/EtOAc 1/1) to afford **4** (49.9 mg, 99%) as white solid.

2: Colorless needles (EtOAc); *R*_f = 0.28 (silica gel, EtOAc/hexane 1/1); m.p. 277–278 °C (decomp.); [*α*]_D²⁵ = +689 (*c* = 1.00, MeOH); IR (KBr): *ν* = 3356 (br), 3055, 1593, 1507, 1359, 1339, 1113, 820, 761, 745 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ = 3.29 (brs, 2H; OH), 4.73 (brs, 2H; CHOH), 7.25 (ddd, 2H, *J* = 8.5, 7.0, 1.5 Hz; ArH), 7.45 (ddd, 2H, *J* = 8.0, 7.0, 1.0 Hz; ArH), 7.54 (brd, 2H, *J* = 8.5 Hz; ArH), 7.89 (brd, 2H, *J* = 8.0 Hz; ArH), 7.90 (d, 2H, *J* = 8.5 Hz; ArH), 7.94 (d, 2H, *J* = 8.5 Hz; ArH); ¹³C NMR (125 MHz, CDCl₃): δ = 136.0, 133.6, 130.0, 128.9, 128.33, 128.29, 127.4, 125.4, 125.2, 121.3, 74.8; HR-MS (EI): *m/z*: 312.1155 [*M*⁺] (calcd for C₂₂H₁₆O₂: 312.1150); elemental analysis calcd for C₂₂H₁₆O₂ · 0.5 CH₃CO₂C₂H₅: C 80.87, H 6.06; found: C 80.88, H 5.66.

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Keywords: chirality • cyclizations • NMR spectroscopy • pinacol coupling • samarium

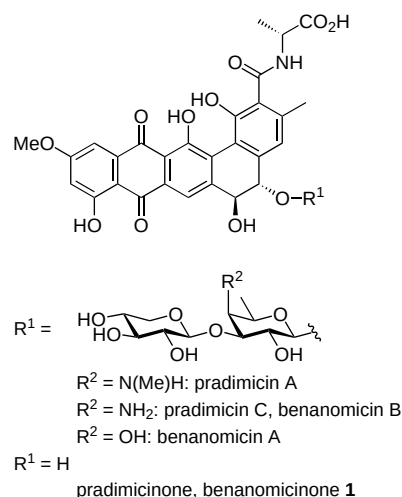
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Total Synthesis of Pradimicinone, the Common Aglycon of the Pradimicin–Benanomycin Antibiotics**

Mitsuru Kitamura, Ken Ohmori, Toshihisa Kawase, and Keisuke Suzuki*

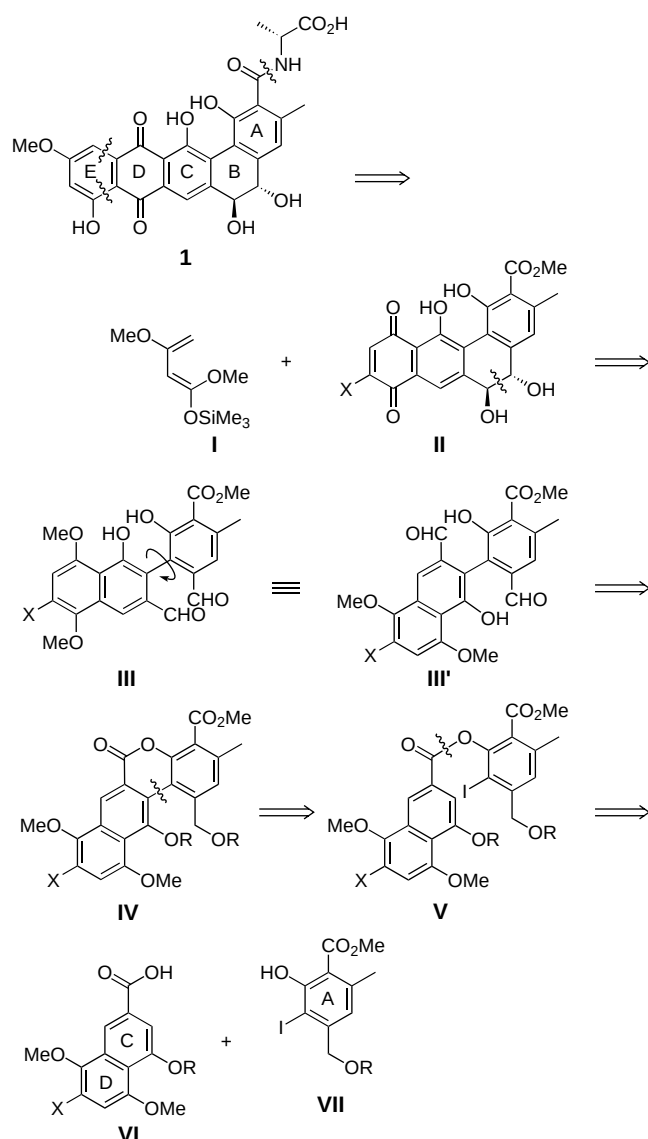
The pradimicin–benanomycin antibiotics^[1] constitute an emerging class of natural products with a unique structure consisting of a benzo[*a*]naphthacenequinone core, an amino acid, and a disaccharide. The important biological activities shown by these compounds, antifungal and anti-HIV, are attributed to the potentially specific binding to oligosaccharides of fungi or viral surfaces.^[1, 2] Stimulated by the unique structure and the significant bioactivities, we initiated a synthetic study of these compounds.^[3] Herein, we report the first total synthesis of pradimicinone (benanomycinone, **1**), the common aglycon of these antibiotics, based on the chiral transmission approach.^[4]



Scheme 1 outlines the synthesis plan. Disconnection of the D-alanine moiety from **1** leads to an intact pentacycle, which can presumably be obtained from the simpler tetracyclic haloquinone **II** (X = halogen) by Diels–Alder reaction with siloxydiene **I**.^[5] Given that the key pinacol-forming reaction^[4] worked well, the diol could be derived from dialdehyde **III**. Formal rotation of the molecule around the biphenyl axis as in **III'** suggests tetracyclic lactone **IV** as a precursor. As another key step, the sterically encumbered biaryl bond could hopefully be formed by the Pd-catalyzed internal C–C bond formation^[6] of ester **V**, which in turn could

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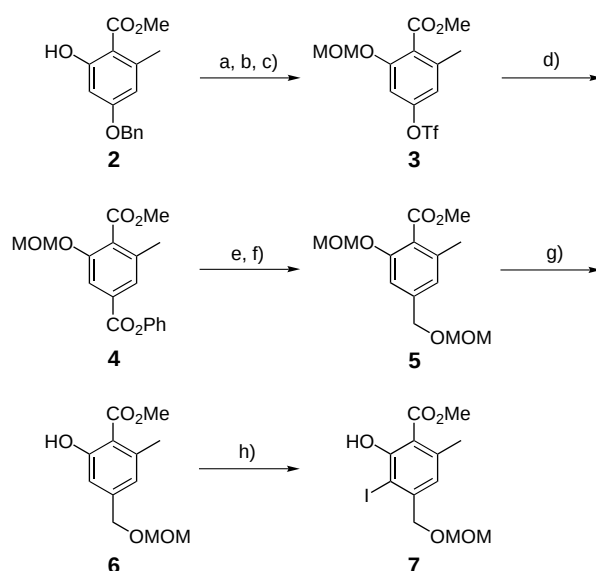


Scheme 1. Retrosynthesis of pradimicinone (benanomycinone, **1**).

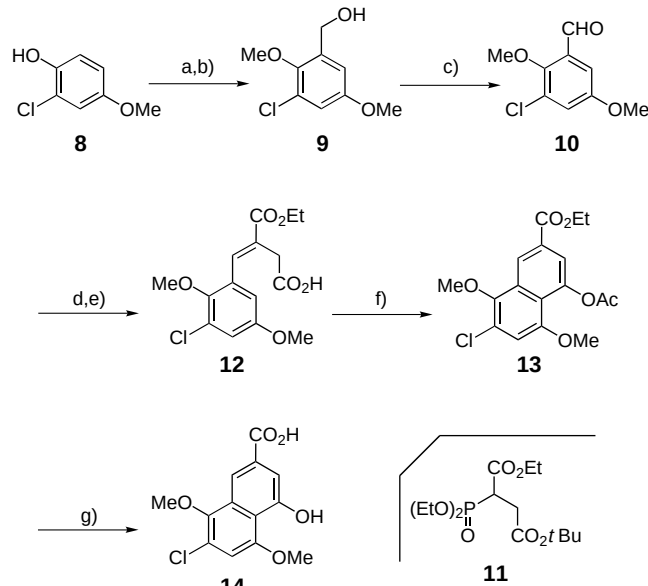
be derived from naphthalene carboxylic acid **VI** and phenol **VII**.

Synthesis of the A ring fragment **7** (Scheme 2) began with the orsellinic acid derivative **2**,^[7] which, after conversion into triflate **3** in three steps, was carbonylated in the presence of phenol to afford phenyl ester **4**.^[8] Selective reduction of the phenyl ester moiety in **4** was nicely achieved with NaBH₄ without touching the methyl ester moiety to give the alcohol, which was protected to give the bis-MOM ether **5**. Acid treatment allowed selective removal of the MOM protection of the phenol to give **6**, and its iodination gave iodophenol **7**.^[7]

Synthesis of the CD ring fragment started with the known compound **8** (Scheme 3).^[9] Regioselective hydroxymethylation^[10] of **8** and methylation of the phenol gave benzyl alcohol **9**, which was oxidized to aldehyde **10**. Wittig–Horner reaction with use of phosphonate **11**^[11] gave the corresponding unsaturated ester, whose *tert*-butyl ester was hydrolyzed with acid to give unsaturated acid **12**. Treatment of **12** with acetic anhydride/sodium acetate gave naphthyl acetate **13**,^[12] which was hydrolyzed to naphthol **14**.

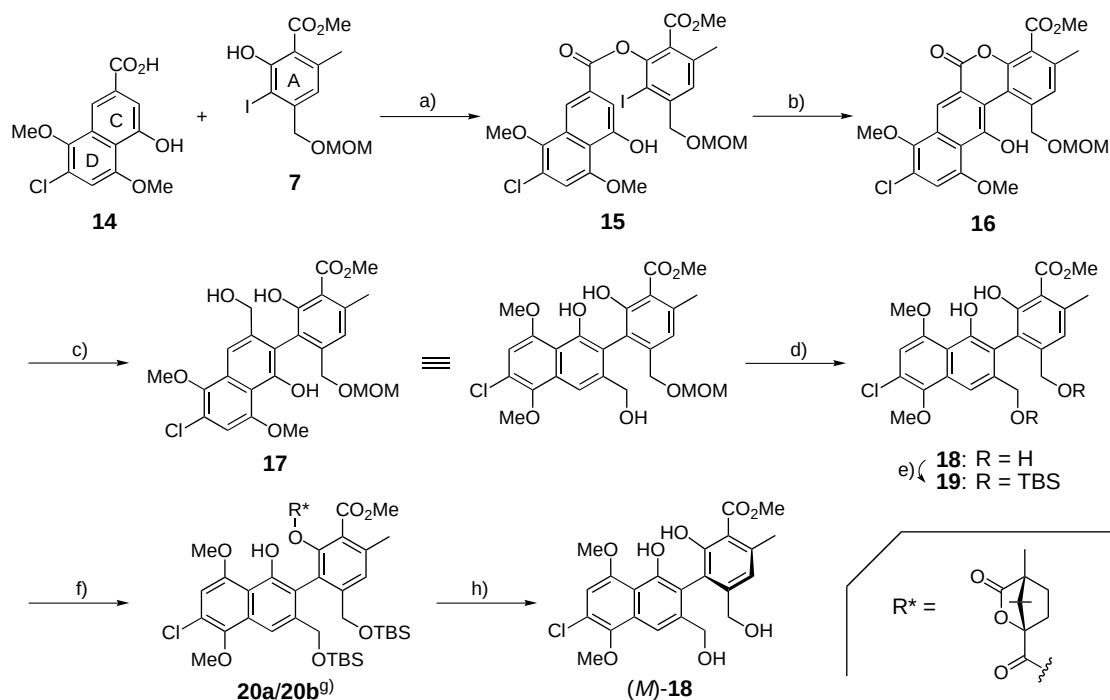


Scheme 2. Synthesis of the A ring fragment **7**. a) MOMCl, *i*Pr₂NEt/CH₂Cl₂, 40 °C, 4 h; b) H₂, Pd(OH)₂/EtOAc, 24 h; c) Tf₂O, *i*Pr₂NEt/CH₂Cl₂, –78 °C, 30 min (3 steps, 96%); d) CO, PhOH, Et₃N, Pd(OAc)₂, DPPF/DMF, 60–80 °C, 3 h (quant.); e) NaBH₄/1,4-dioxane, MeOH, 0 °C → room temperature, 2.5 h (85%); f) MOMCl, *i*Pr₂NEt/CH₂Cl₂, 3 h (88%); g) CF₃CO₂H/CH₂Cl₂, 0 °C, 3 h (99%); h) I₂, Hg(OAc)₂/CH₂Cl₂, 0 °C, 1 h (99%). Bn = benzyl; MOM = methoxymethyl; DPPF = 1,1'-bis(diphenylphosphanyl)ferrocene; Tf = trifluoromethanesulfonyl.



Scheme 3. Synthesis of the CD ring fragment **14**. a) (HCHO)_m, Me₂AlCl/CH₂Cl₂, 0 °C, 4.5 h; b) MeI, K₂CO₃/acetone, reflux, 13 h (91%); c) MnO₂/CH₂Cl₂, 40 °C, 17 h (90%); d) **11**, NaH/THF; e) CF₃CO₂H, H₂O, 1 h; f) Ac₂O, NaOAc, reflux, 1 h (3 steps, 76%); g) NaOH (aq)/THF, EtOH, 70 °C, 1 h; H₃O⁺ (87%).

Union of the A and CD ring fragments, **7** and **14**, was effected by the ester formation with a water-soluble carbo-diimide (Scheme 4). After considerable efforts to optimize the reaction conditions, we were delighted to observe that the Pd-catalyzed internal cyclization of ester **15** proceeded smoothly and regioselectively in the presence of sodium pivalate^[6b] to give tetracycle **16**. Because the product was prone to



Scheme 4. Synthesis of optically active tetraol (*M*)-**18**. a) EDCl, DMAP/CH₂Cl₂, 3 h (78 %); b) Pd(OAc)₂ (30 mol %), PPh₃ (60 mol %), *t*BuCO₂Na (3 equiv)/*N,N*-dimethylacetamide, 110 °C, 1.5 h; c) NaBH₄, THF, MeOH, –40 °C, 3 h (2 steps, 86 %); d) 6 M HCl/DME, 50 °C, 3 h (93 %); e) TBSCl, imidazole/DMF, 0.5 h (84 %); f) (–)-(1*S*,4*R*)-camphanoyl chloride, DMAP, pyridine, 20 h; g) silica gel for separation (**20a** (more polar): 38 %, **20b** (less polar): 40 %); h) HF (aq)/CH₃CN (aq), 45 min; K₂CO₃/MeOH, 19 h (97 % from **20a**). EDCl = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; DMAP = 4-dimethylaminopyridine; DME = 1,2-dimethoxyethane, TBS = *tert*-butyldimethylsilyl.

hydrolysis during purification on silica gel, we opted to reduce the crude material with NaBH₄ to obtain alcohol **17**.^[13]

After detachment of the MOM protection, tetraol **18** was resolved in the following manner: The primary hydroxyl groups were protected as TBS ethers to give **19**, which was treated with (–)-(1*S*,4*R*)-camphanoyl chloride to give diastereomeric mono-esters **20**. Separation of the diastereomers by chromatography (SiO₂, hexane/EtOAc 7/3) gave more polar **20a** (*R*_f = 0.34; 38 %) and less polar **20b** (*R*_f = 0.42; 40 %). The former diastereomer was converted in two steps into enantiopure tetraol (*M*)-**18**, which was employed for the total synthesis.^[14]

Scheme 5 shows the key cyclization of enantiopure dialdehyde (*M*)-**21**, derived from tetraol (*M*)-**18**. We were delighted to observe, upon treatment of dialdehyde (*M*)-**21** with SmI₂ (0 °C, THF), the quantitative formation of the *trans*-diol **22** as the sole product,^[4] which was proven to be enantiomerically pure.^[15] For elaborating the tetracycle to the pentacyclic full carbon skeleton, the diols were protected as diacetate, and oxidation with Ce(NH₄)₂(NO₃)₆ (CAN) gave chloroquinone **23** quantitatively. Diels–Alder reaction of **23** with siloxydiene **24**^[5a] proceeded in a fully regiocontrolled manner to give the pentacycle, where the chloro group was important in order for the regioselective cycloaddition and the smooth aromatization to occur. Selective hydrolysis of the silyl acetal with acidic SiO₂ followed by elimination of HCl with K₂CO₃ gave naphthacenequinone **25** in 90 % yield. Selective removal of the methyl ether groups proximal to the carbonyl groups was nicely achieved by treatment with BCl₃, and saponification gave the fully functionalized aromatic **27**. Condensation of

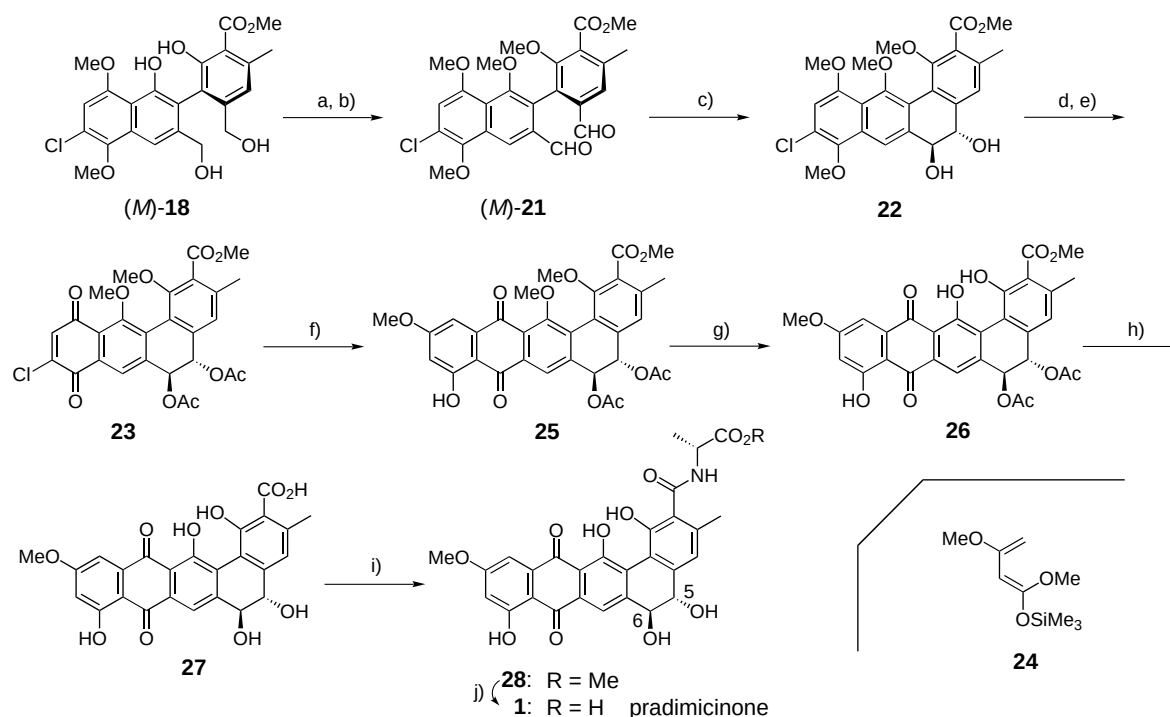
acid **27** with D-alanine methyl ester by using a benzotriazole derivative (BOP) gave pradimicinone methyl ester (**28**) in 80 % yield (from **26**); the sample was fully indistinguishable from an authentic specimen.^[16, 17] Final saponification gave pradimicinone (**1**), which was again consistent with an authentic specimen.^[16, 17]

Currently we are studying the glycosylation of **28**, aiming at the total synthesis. Also under study is the refinement of the synthetic scheme, particularly the asymmetric synthesis without resort to resolution.^[13]

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Scheme 5. Total synthesis of pradimicinone (**1**). a) MeI, K₂CO₃/acetone, 40 °C, 11 h (81 %); b) MnO₂/CH₂Cl₂, 24 h (79 %); c) SmI₂/THF, 0 °C, 5 min (quant.); d) Ac₂O, DMAP/pyridine, 0.5 h (quant.); e) Ce(NH₄)₂(NO₃)₆/CH₃CN, H₂O, 0 °C, 5 min (quant.); f) **24**/THF, 0 °C → room temperature, 2 h; SiO₂, 12 h, then K₂CO₃/CH₂Cl₂, THF, 4 h (90 %); g) BCl₃/CH₂Cl₂, –10 °C, 30 min (99 %); h) 2 M NaOH (aq), 70 °C, 2 h; H₃O⁺; i) D-Ala-OMe · HCl, BOP, Et₃N/DMF, 1.5 h (2 steps, 80 %); j) 0.1 M NaOH, 15 min; H₃O⁺ (quant.). BOP = benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate.

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- [14] That the diastereomer **20a** has the requisite chirality (*M*) was judged by the CD spectra of the two enantiomers of **18**, derived from **20a** and **20b**, respectively.
- [15] Determined by HPLC analysis (DAICEL CHIRALCEL OD-H (25 cm, 0.46 cm diameter), hexane/iPrOH 9/1).
- [16] Prepared by the degradation of benanomycin A,^[1] kindly provided by Meiji Seika, Ltd. The ¹H NMR spectra of **28** (and **1**) are highly dependent on concentration, pH, temperature, and other factors, which makes their identification difficult. However, ¹H NMR measurement on a mixed sample of synthetic and authentic materials fully coincided.
- [17] The same sequence of conversions was also applied to racemic **18**. In the samples of **28** (and **1**) thus obtained, additional peaks in the ¹H NMR spectra were observed arising from the 5,6-bis-epimer (relative to the D-alanine moiety).

Enantiomerically Pure Cyclic *trans*-1,2-Diols, Diamines, and Amino Alcohols by Intramolecular Pinacol Coupling of Planar Chiral Mono-Cr(CO)₃ Complexes of Biaryls**

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Enantiomerically pure 1,2-diols, diamines, and amino alcohols have found widespread use as chiral ligands in asymmetric reactions.^[1] Although a reductive coupling of carbonyl or imine compounds, pinacol coupling, is the most direct way to synthesize 1,2-diols or diamines, highly stereoselective formation of these compounds is problematic.^[2] We

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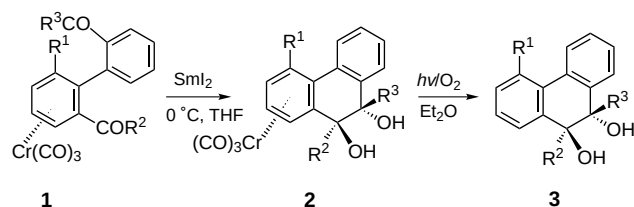
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report the stereoselective synthesis of cyclic *trans*-1,2-diols, diamines, and amino alcohols in enantiomerically pure form by an intramolecular pinacol coupling of the planar chiral mono-Cr(CO)₃ complexes of biphenyls with dicarbonyl, diimino, or iminocarbonyl groups.

Optically pure mono-Cr(CO)₃-complexed biphenyls as starting materials were prepared by palladium-catalyzed cross-coupling of the planar chiral tricarbonyl(bromobenzene)chromium complexes with phenylboronic acids according to a known procedure.^[3] This cross-coupling reaction gave the Cr(CO)₃-complexed biaryls in high selectivity depending on the *ortho* substituent of arylboronic acids. Thus, *o*-formylphenylboronic acid was coupled with the planar chiral tricarbonyl(2,6-disubstituted 1-bromobenzene)chromium complexes^[4] to afford the thermodynamically more stable products, in which the formyl group of the chromium-uncomplexed phenyl ring is oriented *anti* to the Cr(CO)₃ moiety.

An intramolecular pinacol coupling^[5] of enantiomerically pure tricarbonyl(2,2'-diformyl-1,1'-biphenyl)chromium (**1**, R¹ = R² = R³ = H) with samarium diiodide in THF at 0 °C afforded the chromium-complexed 9,10-*trans*-dihydroxy-9,10-dihydrophenanthrene (**2**, R¹ = R² = R³ = H) in 81 % yield without any formation of stereoisomers (Scheme 1; Table 1,



Scheme 1. Synthesis of enantiomerically pure *trans*-1,2-diols **3**.

entry 1). The stereochemistry of the pinacol cyclization product **2** was determined by X-ray crystallography^[6] of the corresponding racemic diacetate chromium complex. The proximal benzylic acetoxyl group to the chromium-complexed phenyl ring was found to be *anti* to the Cr(CO)₃ moiety, and the distal benzylic acetoxyl group was oriented *syn* (Figure 1). Photo-oxidative demetalation of **2** in diethyl ether gave (–)-(*S,S*)-9,10-dihydroxy-9,10-dihydrophenanthrene (**3**,^[7] R¹ = R² = R³ = H) in a quantitative yield.

Similarly, the mono-Cr(CO)₃-complexed biphenyls with keto-aldehyde functionality at the 2- and 2'-positions produced stereoselectively the corresponding cyclic *trans*-diols under the same reaction conditions (Table 1, entries 3–5). This cyclic *trans*-1,2-diol formation is in sharp contrast to the intramolecular reductive cyclization of 1,5- or 1,6-dicarbonyl

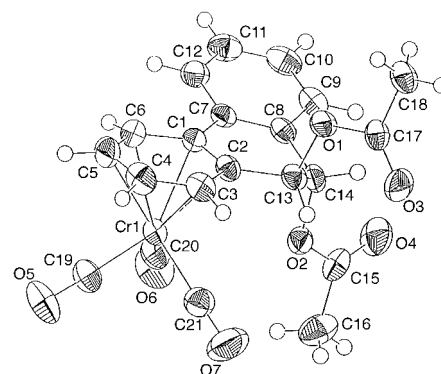
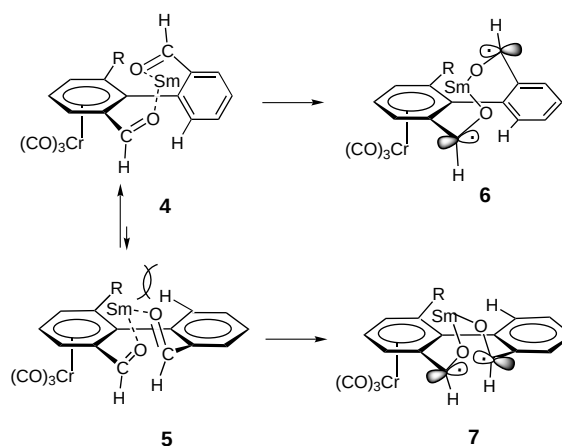


Figure 1. X-ray structure of the racemic diacetate of **2** (R¹ = R² = R³ = H).

compounds giving the corresponding five- or six-membered *cis*-diols.^[8] Suzuki et al. have found independently that axially chiral biaryldicarbonyls without the Cr(CO)₃ moiety also produced *trans*-1,2-diols by the intramolecular pinacol cyclization.^[9] Although either chromium-complexed or chromium-free biphenyls having the carbonyl group at the 2- and 2'-positions produced the stereochemically identical cyclic *trans*-1,2-diols by the intramolecular pinacol cyclization, the reductive coupling using the planar chiral Cr(CO)₃-complexed biaryls is a significant procedure for the preparation of enantiomerically pure cyclic *trans*-1,2-diols if the biaryl substrates such as 2,2'-diformyl-1,1'-biphenyl have no axial chirality or undergo easily axial isomerization.

A plausible reaction mechanism of the pinacol coupling would be as follows (Scheme 2). Samarium diiodide approaches from the *exo* side of the Cr(CO)₃ moiety, generating a bis-ketyl radical intermediate. Among two possible inter-



Scheme 2. Plausible reaction mechanism.

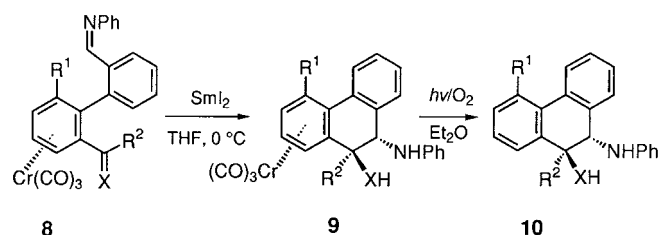
Table 1. Intramolecular pinacol coupling of mono-Cr(CO)₃ complexes of 2,2'-dicarbonyl-1,1'-biphenyls (**1**, see Scheme 1).

Entry	R ¹	R ²	R ³	2	3		
				yield [%]	yield [%]		
				[α] _D (c) ^[a]	[α] _D (c) ^[b]		
1	H	H	H	81	+ 95.4 (0.44)	98	− 81.9 (0.20)
2	OMe	H	H	85	+ 66.8 (0.38)	96	− 80.0 (0.26)
3	H	Me	H	82	+ 200.9 (0.21)	97	− 168.7 (0.20)
4	H	H	Me	82	+ 62.8 (0.24)	97	− 167.9 (0.21)
5	OMe	Me	H	79	+ 235.3 (1.08)	97	− 229.0 (0.42)

[a] In CHCl₃. [b] In EtOH.

mediates with samarium metal chelating both carbonyl groups, the transition state **4** would be more favorable because the two arene rings are required to be nearly coplanar for formation of the alternative transition state **5**, in which severe steric hindrance between the substituents at the 6- and 6'-positions is present. Therefore, the *trans*-1,2-diol chromium complexes **2** would be formed by the coupling of biradical intermediate **6** generated by the attack of the samarium to both *re* faces of aldehyde carbonyl groups.

This intramolecular pinacol coupling can be further applied for the preparation of optically pure six-membered *trans*-1,2-diamines and amino alcohols (Scheme 3, Table 2). Thus, the



Scheme 3. Synthesis of enantiomerically pure *trans*-1,2-diamines and amino alcohols **10** (X = NPh, O).

mono- $\text{Cr}(\text{CO})_3$ complex of diiminobiphenyl **8** ($\text{R}^1 = \text{R}^2 = \text{H}$, X = NPh)^[10] produced a single *trans*-1,2-diamine derivative **9** under the same reaction conditions (entry 1). The relative stereochemistry of the diaminochromium complex **9** ($\text{R}^1 = \text{OMe}$, $\text{R}^2 = \text{H}$, X = NPh) is shown in Figure 2. The benzylic

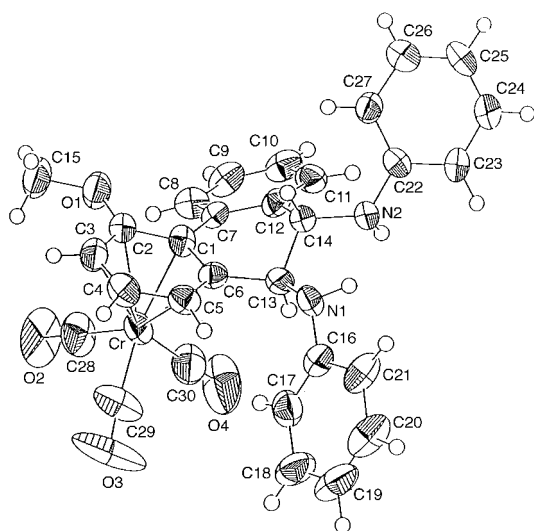


Figure 2. X-ray structure of racemic **9** ($\text{R}^1 = \text{OMe}$, $\text{R}^2 = \text{H}$, X = NPh).

nitrogen atom adjacent to the chromium-complexed arene is also *anti* to the $\text{Cr}(\text{CO})_3$ moiety.^[6] The diamino complex **9** was exposed to sunlight to give the corresponding chromium-free *trans*-1,2-diamine **10** ($\text{R}^1 = \text{R}^2 = \text{H}$, X = NPh). Similarly, the tricarbonylchromium complexes of 2-acyl-2'-phenylimino-1,1'-biphenyl produced stereoselectively 9-*exo*-hydroxy-10-*endo*-anilino chromium complexes **10** under the same reaction conditions (entries 3 and 4).

In conclusion, we have demonstrated that planar chiral mono- $\text{Cr}(\text{CO})_3$ -complexed biaryls with carbonyl or imino groups at the 2- and 2'-positions produced stereoselectively cyclic *trans*-1,2-diols, amino alcohols, or diamines in optically pure form by the samarium diiodide mediated intramolecular pinacol coupling. These enantiomerically pure 1,2-diols, diamines, and amino alcohols would be expected to be significant compounds for asymmetric reactions.

Experimental Section

Typical procedure for pinacol coupling: A solution of SmI_2 (0.1M, 5.7 mL, 0.57 mmol) in THF was added to a solution of **1** ($\text{R}^1 = \text{R}^2 = \text{R}^3 = \text{H}$; 80 mg, 0.23 mmol) in THF (0.5 mL) at 0 °C by syringe under argon. The reaction mixture was stirred for 30 min at 0 °C, and quenched with saturated aqueous NH_4Cl . The precipitate was filtrated, and the filtrate was extracted with Et_2O . The extract was washed with brine, dried over MgSO_4 , and evaporated under reduced pressure. The crude product was purified by chromatography on SiO_2 to afford 65 mg (81 %) of the $\text{Cr}(\text{CO})_3$ -complexed *trans*-1,2-diol **2** ($\text{R}^1 = \text{R}^2 = \text{R}^3 = \text{H}$). M.p. 102 °C; $[\alpha]_D^{25} = +95.4$ ($c = 0.44$ in CHCl_3); ^1H NMR (270 MHz, CDCl_3): $\delta = 2.16$ (br, 2H, OH), 4.74 (br, 2H, ArCH), 5.43 (t, 1H, $J = 6.0$ Hz, Ar(Cr)H), 5.47 (d, 1H, $J = 6.0$ Hz, Ar(Cr)H), 5.89 (d, 1H, $J = 6.0$ Hz, Ar(Cr)H), 6.02 (t, 1H, $J = 6.0$ Hz, Ar(Cr)H), 7.34–7.60 (m, 4H, ArH); IR (CHCl_3): $\tilde{\nu} = 3200, 1940, 1860 \text{ cm}^{-1}$; elemental analysis calcd for $\text{C}_{17}\text{H}_{12}\text{O}_5\text{Cr}$: C 58.63, H 3.47; found: C 58.90, H 3.76.

9 ($\text{R}^1 = \text{R}^2 = \text{H}$, X = NPh): yellow liquid; $[\alpha]_D^{25} = +45.3$ ($c = 0.76$ in CHCl_3); ^1H NMR (270 MHz, CDCl_3): $\delta = 3.87$ (d, 1H, $J = 8.1$ Hz, NH), 4.20 (d, 1H, $J = 8.1$ Hz, NH), 4.64 (t, 1H, $J = 8.0$ Hz, Ar(Cr)CH), 4.80 (t, 1H, $J = 8.0$ Hz, ArCH), 5.31 (t, 1H, $J = 6.0$ Hz, Ar(Cr)H), 5.58 (t, 1H, $J = 6.0$ Hz, Ar(Cr)H), 5.80 (d, 1H, $J = 6.0$ Hz, Ar(Cr)H), 5.89 (d, 1H, $J = 6.0$ Hz, Ar(Cr)H), 7.11–7.65 (m, 14H, ArH); IR (CHCl_3): $\tilde{\nu} = 3200, 1960, 1870 \text{ cm}^{-1}$; elemental analysis calcd for $\text{C}_{29}\text{H}_{22}\text{O}_3\text{N}_2\text{Cr}$: C 69.87, H 4.45, N 5.61; found: C 69.70, H 4.16, N 5.52.

Photooxidative demetalation: A solution of **2** ($\text{R}^1 = \text{R}^2 = \text{R}^3 = \text{H}$; 100 mg, 0.29 mol) in Et_2O (5 mL) was exposed to sunlight for 1 h. The precipitate was filtrated, and the filtrate was reduced in vacuo. The residue was purified by column chromatography on SiO_2 (Et_2O /hexane 1/1) to give **3** ($\text{R}^1 = \text{R}^2 = \text{R}^3 = \text{H}$; 60 mg, 98 %). M.p. 155 °C; $[\alpha]_D^{25} = -81.9$ ($c = 0.20$ in EtOH); ^1H NMR (270 MHz, CDCl_3): $\delta = 2.58$ (br, 2H, OH), 4.75 (s, 2H, ArCH), 7.25–7.44 (m, 4H, ArH), 7.67–7.77 (m, 4H, ArH); IR (CHCl_3): $\tilde{\nu} = 3300 \text{ cm}^{-1}$; elemental analysis calcd for $\text{C}_{14}\text{H}_{12}\text{O}_2$: C 79.22, H 5.70; found: C 79.35, H 5.49.

10 ($\text{R}^1 = \text{R}^2 = \text{H}$, X = NPh): colorless liquid; $[\alpha]_D^{25} = +163.2$ ($c = 0.30$ in CHCl_3); ^1H NMR (270 MHz, CDCl_3): $\delta = 3.88$ (br, 2H, NH), 4.78 (s, 2H, ArCH), 6.67 (d, 4H, $J = 8.2$ Hz, ArH), 6.75 (t, 2H, $J = 8.2$ Hz, ArH),

Table 2. Pinacol coupling of mono- $\text{Cr}(\text{CO})_3$ complexes of diiminobiphenyl and iminocarbonylbiphenyl (**8**, see Scheme 3).

Entry	R^1	R^2	X	9		10	
				yield [%]	$[\alpha]_D^{25} (c)^{[a]}$	yield [%]	$[\alpha]_D^{25} (c)^{[a]}$
1	H	H	NPh	86	+45.3 (0.44)	97	+163.2 (0.20)
2	OMe	H	NPh	75	+58.1 (0.38)	97	+141.2 (0.26)
3	H	Me	O	77	+96.8 (0.21)	98	–70.0 (0.20)
4	OMe	Me	O	78	+169.1 (1.08)	98	–41.4 (0.42)

[a] In CHCl_3 .

7.16–7.45 (m, 10H, ArH), 7.84 (d, 2H, $J = 8.2$ Hz, ArH); IR (CHCl₃): $\tilde{\nu} = 3200, 1360$ cm⁻¹; HR-MS (C₂₆H₂₂N₂): calcd: 362.1770; found: 362.1766.

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Imprint Coating: A Novel Synthesis of Selective Functionalized Ordered Mesoporous Sorbents**

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Dedicated to Professor T. F. Williams

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Polymerization of metal alkoxides in the presence of molecular assemblies of surfactants or related substances as structure directors has resulted in several novel classes of mesoporous and macroporous inorganic materials with extremely high surface areas and ordered mesostructure.^[1–4] These materials have now found extensive applications as catalyst supports and chromatographic resins.^[4] Recently, Feng et al.^[5] and Mercier and Pinnavaia^[6] have developed new, effective mesoporous sorbents based on mesoporous materials as supports for the removal of toxic metal ions. The essence of their methodology is to coat surfaces of hexagonally packed mesoporous silica with organic functional groups to enhance their affinities for the targeted metal ions. High capacities and fast kinetics have been observed for these new sorbents.^[5–7] The selectivity of these materials relies solely on the affinity of the surface-coated functional ligand for a specific metal ion, with no consideration of the stereochemical interactions between the ligand and metal ion. However, the stereochemical arrangement of the ligand with respect to the targeted metal ion plays a key role in molecular recognition^[8] and dative bond formation between the toxic metal ion and coordinating ligands.^[5]

We have been interested in the development of sol–gel sorbent materials based upon the potential superior performance offered by molecular imprinting.^[9] Herein, we describe a design strategy for imprint-coated, functionalized ordered mesoporous sorbents through surface molecular imprinting. This coating methodology allows precise control of the stereochemical arrangement of ligands on the surfaces of mesopores, which in turn optimizes the binding of a targeted metal ion.

Imprinting methods based on the template approach have been used in cross-linked polymers, as well as in silica gels, to prepare polymeric supports that possess organized solid-state

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structures.^[10–12] Imprinting processes generally involve three steps:

- 1) selection of a target molecule as a template,
- 2) incorporation of the template into rigid solid networks through in situ copolymerization, and
- 3) removal of the template, to leave cavities with a predetermined number and arrangement of ligands that later “recognize” or selectively rebinding the template or target molecule.

Imprinted materials thus prepared have been shown to combine the binding ability of specifically chosen functional groups or ligands for target molecules with the shape and size characteristics of cavities imprinted into a rigid polymer matrix by a template. Such imprinted organic polymers have been used to resolve racemates^[10] and separate mixtures of metal cations.^[10–12] One major drawback associated with this bulk molecular imprinting technique is that the kinetics of the sorption/desorption process are unfavorable, as the template and ligand are totally embedded in the bulk polymer matrices and the mass transfer must take place through nonpolar, microporous channels.^[10] Furthermore, molecular imprinting studies have thus far all been conducted in disordered polymers or amorphous sol–gel matrices^[10] where the inhomogeneity of the cavities produced by the molecular imprinting reduces the selectivity of the final imprinted materials.

In the present report these issues in molecular imprinting are addressed by development of surface imprinting^[12] in ordered mesoporous materials. The functional groups are introduced onto the pore surface of mesoporous silica through imprint coating. The key to the current design is to coat the mesopore surface with complexes of the ligands and target metal ions rather than just the free ligands. After removal of the metal ions, the ligand imprints of the template metal ions are created on the mesopore surfaces. This organization reflects both the size and stereochemical signature of the template ion and ultimately should lead to future ion recognition and selective rebinding of the target ion from ion mixtures. We have found that such functionalized, ordered mesoporous materials exhibit significantly greater binding selectivities of the target ion than sorbents prepared by conventional coating methods. The success of our approach is built upon the unique environments of ordered, hexagonally packed mesopore surfaces for conducting surface imprinting. These beneficial characteristics include:

- 1) circularly curved, extremely rigid pore surfaces with optimum pore diameters (20–100 Å) that match the stereochemical requirements for surface imprinting of four or six coordinate metal ions, and
- 2) a very uniform distribution of pore size, which allows the generation of the uniform imprints and limits the possible choices of coordination environments or the number of other possible complexes.^[10f]

The mesoporous ordered silica hosts used in this study were prepared by a surfactant-assembly pathway, which involves matching the charge between the surfactant and inorganic silica precursors.^[1–3] Calcined, ordered mesoporous silica samples prepared by this procedure have surface areas of over 1000 m²g^{−1}, mesopore volumes of 0.98 cm³g^{−1}, and

average pore sizes of ≈25 Å. Both powder X-ray diffraction and small-angle X-ray scattering^[13] show a peak at $2\theta \approx 2^\circ$, which agrees with the pore size determined by the method of Barrett, Joyner, and Halenda.^[14]

To illustrate the potential of this imprinting methodology Cu²⁺-selective, ordered mesoporous sorbents were chosen to demonstrate both the basic principles of the concept and the ease with which imprinted sorbents may be prepared. Furthermore, extensive literature reviews of imprinted polymers for the separation of copper already exists.^[11, 15] A critical comparison of the binding properties of our sorbents with imprinted organic polymeric matrices shows the advantages of our imprint-coated mesoporous sorbents (see below). To test the selectivity of our new sorbents we conducted competitive ion-binding experiments on aqueous Cu²⁺/Zn²⁺ mixtures. This system constitutes one of the most stringent tests for ion-binding selectivity because both ions have identical charge, similar sizes, and exhibit high affinities for amine ligands.

The two imprinting complex precursors used in this study are [Cu(apts)_x·S_{6–x}]²⁺ and [Cu(aapts)₂S₂]²⁺ ($x = 3–5$; S = H₂O or methanol; apts = H₂NCH₂CH₂CH₂Si(OMe)₃; aapts = H₂NCH₂CH₂NHCH₂CH₂CH₂Si(OMe)₃), which were synthesized by standard literature procedures.^[16] Molecular mechanics modeling studies^[17] have shown the sizes of the most probable conformations for these two complexes to be in the range of 16–25 Å, which are ideal for fitting into mesoporous substrates with pore diameters of 25 Å. Surface imprinting of the support was conducted by mixing stoichiometric amounts of an imprinting precursor with the mesoporous silica in methanol or aqueous solution.^[18] Figure 1 shows the UV/Vis spectra of the two imprinting precursors in methanol and

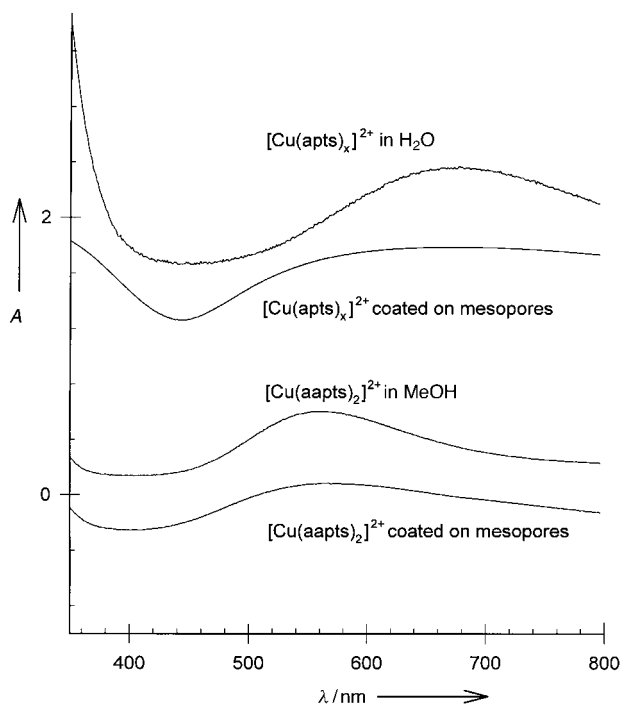


Figure 1. UV/Vis spectra of [Cu(apts)_x]²⁺ and [Cu(aapts)₂]²⁺ in methanol and aqueous solutions, respectively, and of both complexes coated on mesopore surfaces.

aqueous solutions and the corresponding diffuse reflectance spectra of washed and dried imprint-coated mesoporous silica. The close match between the spectra of $[\text{Cu}(\text{apts})_2\text{S}_2]^{2+}$ and $[\text{Cu}(\text{s-apts})_2\text{S}_2]^{2+}$ (s-apts = surface-coated apts ligand) indicates that the stereochemical environments of the copper ions in the two systems are similar and that the complex is coated on the surfaces of the mesopores.

The absorption band in the UV/Vis spectrum of $[\text{Cu}(\text{s-apts})_x\text{S}_{6-x}]^{2+}$ (s-apts = surface-coated apts ligand) is broader than that of the corresponding precursor in solution. On the basis of the average environment rule^[19] this indicates that the distribution of distinct copper complexes in the solid phase is broader than that in the solution phase. The locations of the electronic transition bands for all the complexes agree well with those of the model complexes $[\text{Cu}(\text{en})_2\text{S}_2]^{2+}$ and $[\text{Cu}(\text{H}_2\text{NR})_x\text{S}_{6-x}]^{2+}$ (en = ethylenediamine).^[20] Control samples were prepared under identical conditions except that no templating ion (Cu^{2+}) was added to the coating solutions. Amines are protonated below pH = 3 and lose their ability to complex metal cations. This characteristic provides a simple and efficient way to strip the Cu^{2+} template from the imprinted mesoporous sorbents. Thus, template-free imprinted sorbents were easily obtained by soaking the Cu^{2+} -imprinted coatings on mesoporous silica with 1M nitric acid for 20 min. Control mesoporous sorbents were also treated identically to ensure that both types of sorbents had identical treatments of their surfaces. Both imprint-coated and conventionally coated sorbents were then neutralized to pH = 7 and dried in a vacuum oven at 50 °C for 6 h before adsorption tests were conducted. A schematic illustration of our imprint-coating process is shown in Figure 2A, while the difference in the final cavity structure between our imprint-coated and conventionally coated mesoporous sorbents is shown in Figure 2B.

The ^{29}Si MAS solid-state NMR spectra of the sorbents produced by imprint coating and that of the corresponding control blank showed two groups of peaks. The peaks around $\delta = -65$ are attributed to silicon atoms tethered to amino groups on surfaces, whereas the peaks around $\delta = -110$ arise from the silicon atoms derived from the original support.^[9, 10, 21] The loadings of apts and aapts are estimated from the relative areas of the envelopes for these two groups of peaks to be 0.92 mmol g^{-1} and 0.40 mmol g^{-1} , respectively. This estimation is consistent with the elemental ICP-AE analysis of the solutions after HF digestion of the coated sorbents (ICP = inductively coupled plasma, AE = atomic emission). Both imprint-coated and blank-coated mesoporous sorbents exhibit similar ligand loadings.

Table 1 summarizes the measured adsorption capacities of imprint-coated and conventionally coated (control) mesoporous sorbents for Cu^{2+} . Both imprinted sorbents exhibit distinctly higher effective capacities for Cu^{2+} than their respective control blanks. Furthermore, their capacity does not change after repeated cycles of loading and elution. These

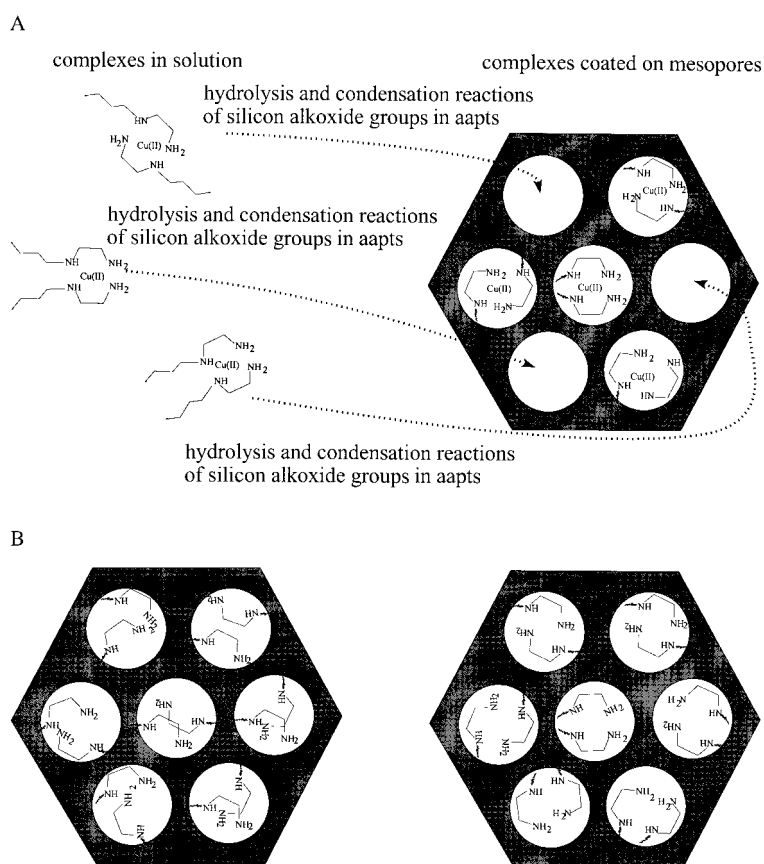


Figure 2. A) Schematic diagram of the imprint-coating process: first the complexes are introduced between target metal ions and bifunctional ligands, then the siloxane groups in the bifunctional ligands are hydrolyzed, and finally covalently coated on the mesopore surfaces. B) Schematic representation of the difference between the cavities generated by conventional coating (left) and imprint coating (right).

Table 1. Capacity of imprint-coated and conventionally coated mesoporous sorbents with respect to Cu^{2+} uptakes at specific Cu^{2+} concentrations and pH 5.0 (acetic acid/acetate buffer).^[a]

Sorbent	Solution	% Cu abs	K_d of Cu^{2+} [c]	Cu capacity [mmol g ⁻¹]
nonimprinted apts ^[d]	0.001M Cu	98.2	5300	0.098
imprinted apts ^[e]	0.001M Cu	99.6	26000	0.1
nonimprinted aapts	0.001M Cu	98.5	6800	0.099
imprinted aapts	0.001M Cu	99.7	39000	0.1
nonimprinted apts	0.0001M Cu	99.2	14000	0.0099
imprinted apts	0.0001M Cu	100 ^[b]	-	-

[a] In all experiments 0.1 g of each sample was equilibrated with 10 mL of the buffer solution in stoppered plastic vials and these mixtures were stirred for 1 h at room temperature. The uptake of Cu^{2+} by sorbents was measured with a Perkin-Elmer Plasma 400 ICP/AE spectrophotometer. [b] No detectable copper ions were found in the solution after treatment. [c] $K_d = \{(C_i - C_f)/C_f\} \times \{\text{volume of solution [mL]}/\{\text{mass of gel [g]}\}\}$; where C_i and C_f represent the initial and final solution concentrations, respectively. [d] Nonimprinted apts = sorbent coated by the conventional method. [e] Imprinted apts = sorbent coated by the surface molecular imprinting.

properties make them ideal for many separation applications that require recyclable solid phases.

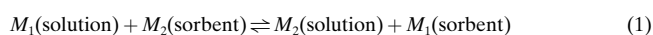
The selectivities of these sorbents were investigated using aqueous solutions of zinc and copper. If a sorbent is simultaneously exposed to mixtures of different metal ions, those ions with the highest binding affinity will be preferential

Table 2. Competitive loading of M_1 (Cu^{2+}) and M_2 (Zn^{2+}) by copper-imprinted and control blank mesoporous sorbents at pH 5.0 (acetic acid/acetate buffer).

Type	Solution concentrations ^[a]		% Cu abs	% Zn abs	$K_d(\text{Cu})$	$K_d(\text{Zn})$	k	k'
	Cu [mol L^{-1}]	Zn [mol L^{-1}]						
nonimprinted apts	0.001	0.01	35.4	63.3	55	170	0.3	–
imprinted apts	0.001	0.01	53.2	68	110	210	0.51	1.7
nonimprinted apts	0.001	0.001	98.5	96.6	6500	2900	2.3	–
imprinted apts	0.001	0.001	99.8	82.9	44000	480	91	40
nonimprinted apts	0.001	0.005	98.5	70	6500	230	28	–
imprinted apts	0.001	0.005	99.6	52.6	24000	110	220	7.7

[a] Initial concentrations of the solutions.

bound. The selectivity coefficient k for the binding of a specific metal ion in the presence of competitor species [Eq. (1)] can be obtained from equilibrium binding data^[11, 22] according to Equation (2), where K_d is the distribution



$$k = \frac{[M_2]_{\text{solution}} [M_1]_{\text{sorbent}}}{[M_1]_{\text{solution}} [M_2]_{\text{sorbent}}} = K_d(\text{Cu})/K_d(\text{Zn}) \quad (2)$$

coefficient. A comparison of the k values of the imprint-coated sorbents with those for blank control samples allows an estimation of the effect of imprinting on selectivity. Following the work by Kuchen and Schram,^[11] a relative selectivity coefficient k' [Eq. (3)] can be defined.

$$k' = k_{\text{imprinting-coated}}/k_{\text{control}} \quad (3)$$

Table 2 summarizes K_d and k' values obtained in these selectivity tests for zinc and copper. A comparison of the K_d values for the Cu^{2+} -imprinted samples with the control samples for the apts-coated sorbent system shows a sixfold increase in K_d for Cu^{2+} , while K_d for Zn^{2+} decreases by the same amount. The value of k' is greater than 1 for all imprint-coated sorbents. A k' value of 40 for the apts-imprint-coated sorbent is, to the best of our knowledge, the highest value currently reported for the molecular imprinting of metal ions in similar conditions.^[11, 15] The value of k' (1.71) for the apts-imprinted sorbent is smaller than that for the apts ligand. This observation may be attributed to the flexibility of the propyl chain that links the monodentate amine to the support, which allows for an easy redirection of the ligand and a larger range of coordination geometries for these ligands. Accordingly, coordination to a metal ion is less specific and therefore less selective for the target ion. In fact, this also agrees with the UV/Vis spectra (Figure 1) that indicate a broad distribution of the ligand imprints in the apts-imprint-coated sorbent.

In contrast to the results described above, imprint coating on the surfaces of commercial, amorphous silica gel (Aldrich, $\bar{d} = 60 \text{ \AA}$; surface area = $600 \text{ m}^2 \text{ g}^{-1}$) showed very little evidence of imprinting effects in selectivity experiments.^[23] This is consistent with the idea that the shape and curvature of the pore surfaces play pivotal roles in the success of the surface imprinting of inorganic coordination complexes.

In conclusion, surface imprinting has, for the first time, been conducted on ordered, cylindrical surfaces of mesopores, thereby organizing ligand functional groups successfully according to the sizes and stereochemical signatures of template ions. We view these sorbents as solid-state analogues

of crown ether-type ligands, which can be tailored for a specific target ion. The simplicity of this technique should lead to a wide variety of new, highly selective sorbents, the properties of which can be optimized for many metal ions with the proviso that they form stable coordination complexes with a suitable bifunctional ligand containing a silane group.^[24] Furthermore, this surface imprinting methodology should not be limited to the binding of metal ions. If complexes or molecules can be formed between targeted organic molecules and functional groups that contain a silane group then application of the above methodology should lead to the synthesis of sorbents that exhibit molecular recognition of organic molecules.

Experimental Section

Preparation of ordered mesoporous silica support:^[1–3] The typical procedure involves mixing cetyltrimethylammonium bromide (CTAB), water, and base (NaOH). The molar ratio of CTAB: H_2O :NaOH is 0.12:130:0.7. Tetraethylorthosilicate (TEOS) was added to this solution at room temperature and the mixture was then heated at 100°C for 24 h. The solid product was recovered by filtration.

Preparation of imprint-coated ordered mesoporous sorbent:^[18] In a typical preparation $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ (0.567 g) is added under stirring to MeOH (50 mL). After the $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ has completely dissolved, apts is added (1.02 mL). After stirring the mixture for 1 h, mesoporous silica (0.25 g) is added. The solution is stirred and evaporated to dryness. The residue is washed with copious amounts of 1M HNO_3 to remove all the copper and any excess ligand. The resulting gel is placed in deionized water and titrated with 2M NaOH to pH 7.5, filtered, washed with deionized water, and placed under vacuum at 80°C for 4 h. The control blank samples were prepared using an identical procedure but without the addition of $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$. The procedure for coating using water as a solvent is the same as that reported in the literature.^[18]

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- [23] The highest enhancement of K_d for Cu^{2+} through imprint coating on commercial, amorphous silica is 1.54, which is much less than the best value obtained on the ordered mesopore surfaces.
- [24] We have also developed sorbents coated with the imprints of $[\text{Zn}(\text{aapts})_2]^{2+}$ and $[\text{Hg}(\text{aapts})_2]^{2+}$ for the selective adsorption of Zn^{2+} and Hg^{2+} . The adsorption capacities of both sorbents were increased by surface imprinting. In the case of the Zn^{2+} -imprinted sorbent a 100% adsorption of Zn^{2+} in 10 mL of a 10^{-3}M solution by 0.1 g of the sorbent was observed. The selectivity of the sorbent imprinted with $[\text{Zn}(\text{aapts})_2]^{2+}$ was tested using the $\text{Zn}^{2+}/\text{Cu}^{2+}$ system. The value of k' is 3.3.

Strong N–H...O Hydrogen Bonding in a Model Compound of the Catalytic Triad in Serine Proteases**

Jacob Overgaard, Birgit Schiøtt, Finn K. Larsen, Arthur J. Schultz, John C. MacDonald, Bo B. Iversen*

The cleavage of peptide bonds is an important reaction in nature that involves the catalytic triad of residues (Ser-His-Asp) in the active site of the serine proteases class of enzymes (Figure 1).^[1] A number of studies have suggested that a low-barrier hydrogen bond (LBHB) is involved in the reaction

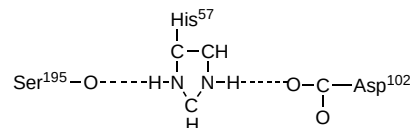


Figure 1. Schematic drawing of the catalytic triad in serine proteases. No formal charges or bond orders have been assumed in the sketch.

mechanism as a partial proton transfer between His⁵⁷ and Asp¹⁰².^[2] Formation of a LBHB can account for many of the special physicochemical observations found in some enzymes, but it is still a matter of controversy whether a LBHB contributes significantly toward the catalytic activity of the triad.^[3] Much research has been carried out to examine the nature of short, strong hydrogen bonds, and the principal tools of analysis have been ab initio computations on small model systems,^[4] crystal structure correlations,^[5] and spectroscopic investigations.^[6] It is increasingly evident that a number of factors besides the distance between heteroatoms influences the formation of a LBHB. Factors such as matching of pK_a values, involvement in other hydrogen bonds, and steric strain are also of importance. Analysis of charge densities (CDs) in model systems provides an alternative method for studying hydrogen bonds. Charge densities in hydrogen bond systems can be determined experimentally from combined analysis of low-temperature X-ray and neutron diffraction data.^[7] Contrary to ab initio theory, experimental CD studies have far fewer restrictions on the size of the model system that can be studied, and the results inherently reflect all interactions in the crystal. Detailed quantitative information about the

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bonding can be obtained by Bader topological analysis of the CD.^[8]

In order to investigate the electronic structure of short N–H···O bonds, we have carried out combined low-temperature (28(1) K) X-ray and neutron diffraction studies of the cocrystal between betaine, imidazole, and picric acid (Figure 2). The complex serves as a model for the catalytic triad,

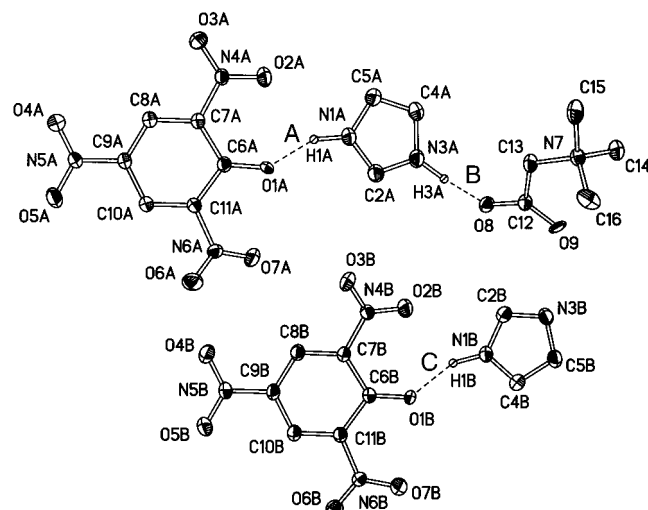


Figure 2. Cocrystal of betaine, imidazole, and picric acid (ORTEP drawing at 95% probability level based on neutron diffraction data at 28 K). Hydrogen atoms have been omitted for clarity. A short C–H···O interaction exists between C8B–H8B···O7A (3.070(3) Å), which is important for the crystal packing.

and the system is attractive a priori since it contains three different short N–H···O contacts (<2.7 Å) in the same crystal. This enables us to investigate factors such as matching of pK_a values and three center hydrogen bonding without the effects of differences in systematic errors between different experiments normally encountered in crystal structure correlations.

To achieve high resolution within strict neutron beam time limitations we employed the white beam time-of-flight neutron diffraction technique at the SCD station at the Intense Pulsed Neutron Source.^[9] The structure contains 75 unique atoms in the asymmetric unit making it one of the

largest structures investigated to high resolution ($d_{\min} > 0.38$ Å) by neutron diffraction. High resolution is critical to define the thermal motion of the atoms properly. Refinement of anharmonic thermal parameters provides an experimental probe for the shape of the potential energy surface for proton transfer.^[7] X-ray data at matching temperatures were collected on two different crystals using both synchrotron radiation and conventional sealed tube radiation. The synchrotron data were collected to high resolution at beam line X3A1 at the National Synchrotron Light Source using image plate detectors, but for technical reasons the data was not complete in the low-order region. Therefore, complete low-order data were collected on a four-circle sealed tube diffractometer. Since extinction in both crystals was negligible, the two data sets were refined simultaneously in the CD modeling.

None of the atoms showed significant anharmonic thermal parameters in the refinement against the neutron data. Furthermore the atomic mean square displacements are not conspicuous in size. This result indicates that the potential energy surfaces of all the hydrogen bonds in the complex consist of two separate harmonic potential wells. The structural parameters for the 25 hydrogen atoms derived from the neutron data were used at fixed values in the refinement against the X-ray data. The charge density was modeled with the Hansen–Coppens multipole model,^[10] which accurately describes aspherical density distributions for compounds containing second row elements.

Bond lengths and topological properties for the three short hydrogen bonds are listed in Table 1. The neutron data reveal a significant lengthening of the N–H distances relative to weakly bonded systems (1.0 Å), and the lengthening fits well with the N–H versus H···O distance correlations established by Steiner.^[5b] The data show that all the hydrogen atoms are localized at the nitrogen atoms, and that none are involved in LBHBs. The absence of LBHBs is supported by the topological analysis, which shows the O···H interactions to be predominantly electrostatic interactions ($\nabla^2\rho > 0$) between closed-shell atoms (Figure 3). It is, however, clear that especially bond B has a considerable polarization of the Laplacian (Laplacian operator of the electron density function) towards the acceptor atom. Madsen et al. have shown that in LBHBs the fundamental nature of the interaction is

Table 1. Topological properties of the short, strong hydrogen bonds. ρ is the electron density and $\nabla^2\rho$ the Laplacian at the bond critical points. q_1 and q_2 are atomic charges derived from the charge density. V and T are the potential energy density and the kinetic energy density, respectively. E_{HB} is the hydrogen bond energy.

Bond		D_{neutron} [Å]	$\angle \text{N–H} \cdots \text{O}$ [°]	ρ [e Å ⁻³]	$\nabla^2\rho$ [e Å ⁻⁵]	q_1 ^[a]	q_2 ^[a]	V ^[b] [kcal mol ⁻¹]	T ^[b] [kcal mol ⁻¹]	$E_{\text{HB}} = -\frac{1}{2}V$ ^[c] [kcal mol ⁻¹]
A	N1A–O1A	2.613(2)	154.5(5)							
	N1A–H1A	1.046(4)		2.00	–30.7	–0.04	0.23			
	H1A–O1A	1.631(4)		0.37	3.2	0.23	–0.20	–34.9 (–33.8) ^[d]	28.0 (33.5)	17.4 (16.9)
B	N3A–O8	2.685(2)	171.2(4)							
	N3A–H3A	1.056(4)		1.87	–29.9	–0.08	0.28			
	H3A–O8	1.637(4)		0.41	1.8	0.28	–0.38	–37.8 (–33.0)	24.8 (32.9)	18.9 (16.5)
C	N1B–O1B	2.676(2)	157.2(4)							
	N1B–H1B	1.049(4)		2.00	–34.6	–0.05	0.28			
	H1B–O1B	1.678(4)		0.30	3.0	0.28	–0.20	–26.2 (–28.4)	23.0 (29.4)	13.1 (14.2)

[a] Based on spherical kappa refinement. [b] Y. A. Abramov, *Acta Crystallogr. Sect. A* **1997**, 53, 264–272. [c] E. Espinosa, E. Molins, C. Lecomte, *Chem. Phys. Lett.* **1998**, 285, 170–173. [d] Values in parentheses are calculated based on correlations established in [c] for normal hydrogen bonds.

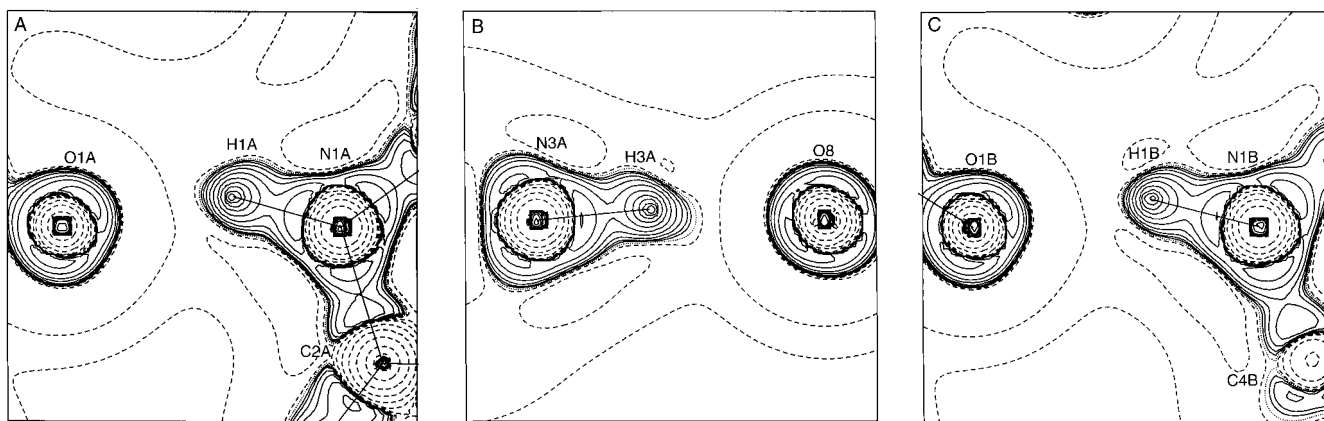


Figure 3. Contour plot of the negative of the Laplacian of the electron density in the planes of the three strong N–H...O hydrogen bonds. A) N1A–H1A...O1A; B) N3A–H3A...O8; C) N1B–H1B...O1B. The contours are drawn at logarithmic intervals of $1.0 \times 2^N \text{ e } \text{\AA}^{-5}$. The dotted line is the zero contour. Solid lines are positive contours, and broken lines are negative contours.

different than in longer hydrogen bonds, and the hydrogen atom becomes partly covalently bonded to both heteroatoms.^[7]

If the heteroatom distance uniquely defines the nature of the hydrogen bonds, then the present study could be used to establish an upper limit for the distance of N–H...O LBHBs in enzymes (2.613(2) Å). There are, however, subtleties in the N–H...O bond properties that indicate other factors are important. For example, the N–H distance is marginally longer in bond B than in bond A, even though the N...O and O...H distances are longer in bond B (Figure 1, Table 1). Furthermore bond B is a two-center bond, whereas bonds A and C are three-center bonds with longer secondary hydrogen bond interactions (O2A...H1A 2.312(5) Å, O2B...H1B 2.242(5) Å). The secondary interactions will further lengthen the N–H distances in bonds A and C. The longer N–H distance in bond B may be due to a better pK_a match between betaine (1.8) and the imidazolium ion (7.0) than between the imidazolium ion and picric acid (0.4) ($pK_a(\text{imidazole}) = 10.5$). The topological analysis reveals that the strongest hydrogen bond is formed in bond B, although all three hydrogen bonds are strong with bonding energies exceeding those of normal hydrogen bonds (2–10 kcal mol^{−1}). In fact the bond energies (E_{HB}) are very similar to the LBHB of benzoylacetone (16 kcal mol^{−1}).^[4c] This study demonstrates that short, strong hydrogen bonds do not have to be low-barrier. The estimate of the bond energy E_{HB} is, however, only strictly valid for electrostatic interactions; the present hydrogen bonds deviate from the correlations established previously for a large number of normal hydrogen bonds.^[11] Our data therefore indicates that the strong hydrogen bonds in the present complex are not entirely electrostatic in character. The smaller positive value of $\nabla^2\rho$ in bond B indicates that bond B has most covalency and thus is closest to being a LBHB system. Furthermore the atomic charges of bond B are larger than in the two other hydrogen bonds (Table 1), although still smaller than in the LBHB of benzoylacetone (O(−0.51(5)), H(+0.40(3)), O(−0.45(4))).^[7] The transition to covalency appears to occur without abrupt changes in the character of the hydrogen bond interaction. It is notable that the bond that best mimics the His⁵⁷–Asp¹⁰² structure (bond B), also is closest to being a LBHB. It seems reasonable that to attain a

LBHB in bond B more charge needs to be added to N3A, and to a lesser extent removed from H3A. If low-barrier hydrogen bonds in fact are present in enzymes, then other factors not active in the present model complex must account for this charge migration. A possible explanation could be the electron-donating CH₂ groups present in histidine that are absent in imidazole. We are currently performing high-level ab initio calculations on our model system to gain further insights about the triad and to provide a detailed comparison with the experimental results.

Experimental Section

The cocrystal of C₅N₂O₂H₁₁, C₃N₂H₅, and C₆N₃O₇H₂ (C₂₃N₁₁O₁₆H₂₅ in the asymmetric unit, FW = 711.51 g mol^{−1}) belongs to the monoclinic space group *C2/c*. 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-112286. 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).

Neutron diffraction: $T = 28(1) \text{ K}$, $a = 33.536(5) \text{ \AA}$, $b = 7.636(1) \text{ \AA}$, $c = 25.066(4) \text{ \AA}$, $\beta = 114.90(1)^\circ$, $V = 5822(2) \text{ \AA}^3$, $V_{\text{crystal}} = 13.2 \text{ mm}^3$, $0.7 < \lambda < 4.2 \text{ \AA}$, complete data out to $(\sin\theta/\lambda)_{\text{max}} = 0.81 \text{ \AA}^{-1}$, partial data out to $(\sin\theta/\lambda)_{\text{max}} = 1.3 \text{ \AA}^{-1}$, μ_a (true absorption at $\lambda = 1.8 \text{ \AA}$) = 1.22 cm^{−1}, μ_s (total scattering) = 1.15 cm^{−1} (absorption correction performed), No. of measured reflections = 29014.

Synchrotron X-ray diffraction: $T = 28(1) \text{ K}$, $a = 33.54(2) \text{ \AA}$, $b = 7.64(2) \text{ \AA}$, $c = 24.98(4) \text{ \AA}$, $\beta = 114.76(5)^\circ$, $V = 5813(30) \text{ \AA}^3$, $V_{\text{crystal}} = 0.001 \text{ mm}^3$, $\lambda = 0.643(1) \text{ \AA}$, $(\sin\theta/\lambda)_{\text{max}} = 1.08 \text{ \AA}^{-1}$, $\mu_1 = 0.08 \text{ cm}^{-1}$ (no absorption correction), No. of measured reflections = 98 132, No. of unique = 15 657, $R_{\text{int}} = 0.030$.

Sealed tube X-ray diffraction: $T = 10(1) \text{ K}$, $a = 33.57(1) \text{ \AA}$, $b = 7.640(2) \text{ \AA}$, $c = 25.031(8) \text{ \AA}$, $\beta = 114.84(2)^\circ$, $V = 5826(6) \text{ \AA}^3$, $V_{\text{crystal}} = 0.058 \text{ mm}^3$, $\lambda = 0.5616 \text{ \AA}$, $(\sin\theta/\lambda)_{\text{max}} = 0.51 \text{ \AA}^{-1}$, $\mu_1 = 0.06 \text{ cm}^{-1}$ (no absorption correction), No. of measured reflections = 16 382, No. of unique = 3187, $R_{\text{int}} = 0.016$.

Neutron refinement: $N_{\text{obs}} = 8867$ ($I > 3\sigma(I)$), $N_{\text{par}} = 708$, GOF = 1.79, $R_F = 0.082$, $R_{\text{wF}} = 0.063$.

Multipole refinement: The O, N, and C atoms were modeled with multipole expansions up to octupole level with two radial kappa parameters for each chemically unique type of atom (12 different kappa sets). The hydrogen atoms had multipole expansions to quadrupole level with positions and anisotropic thermal parameters fixed at values from the neutron refinement. The accuracy of the model can be tested by comparing thermal parameters for the heavy atoms derived separately from the X-ray and neutron data.^[12] In the present case we obtain $\langle \Delta U_{\text{X-N}} \rangle = 0.0009 \text{ \AA}^2$ and $\langle U_{\text{X}}/U_{\text{N}} \rangle = 1.05$. This demonstrates that the systematic errors are minimal,

and thus we can have confidence in the static model charge densities. $N_{\text{obs}} = 11182$ (only multiple measured reflections), $N_{\text{par}} = 1128$, $\text{GOF} = 1.13$, $R_{\text{F2}} = 0.035$, $R_{\text{wF2}} = 0.046$.

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Pentamethylcyclopentadienylrhodium(III) and -iridium(III) Complexes Showing P,O Coordination: Unprecedented Insertion of tcne and tcnq into a C–H Bond**

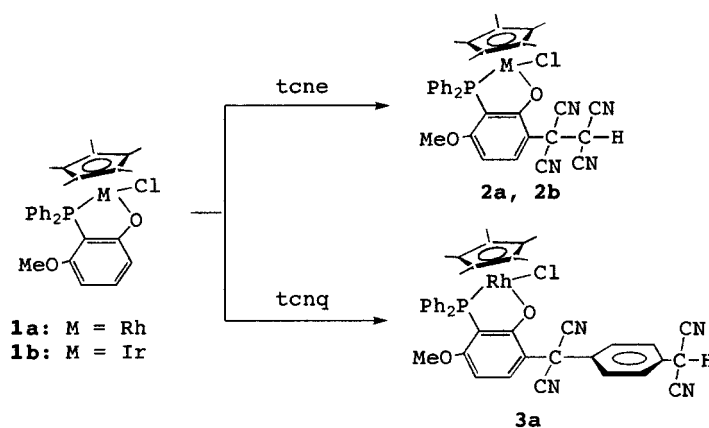
Yasuhiro Yamamoto,* Xiao-Hong Han, Ken-ichiro Sugawara, and Saho Nishimura

Tetracyanoethylene (tcne) forms a variety of charge transfer and organometallic complexes as a result of its strong electrophilicity.^[1] It is widely used as a precursor of organic

magnets, the tetracyanoethylene-based organic magnets.^[2] Reactions of tcne with metal–acetylide, metal–hydride, or metal– η^1 -dienyl complexes exhibit characteristic features: 1) insertion into M–C bonds^[3a, b] and into M–H bonds to afford cyano(dicyanomethyl)keteniminato complexes;^[3c–e] 2) [2+2] addition to acetylide groups to give cyclobutene–metal complexes;^[4] 3) [4+2] or [3+2] addition to diene moieties to produce cyclohexene– or cyclopentane–metal complexes;^[5] 4) α,β addition of dicyanomethylene fragments, derived from cleavage of the double bond of tcne, to an acetylide group.^[6] In all cases except for (1), tcne is reactive to strongly activated unsaturated groups on the ligands.

Recently we reported that one of the *ortho*-methoxy groups in (2,6-dimethoxyphenyl)diphenylphosphane (MDMPP) is demethylated upon reaction with bis[dichloro(η^6 -arene)rhodium(III)] or bis[dichloro(η^5 -pentamethylcyclopentadienyl)rhodium(III)] to give $[(\eta^6\text{-arene})\text{RuCl}(\text{MDMPP-}P,O)]$ ^[7a, b] and $[(\eta^5\text{-C}_5\text{Me}_5)\text{RhCl}(\text{MDMPP-}P,O)]$,^[8] respectively (MDMPP- P,O = PPh₂(2-O-6-MeO-C₆H₃), a P,O chelating phosphane). These complexes react with Lewis bases (L) in the presence of PF₆[−] to give the corresponding cationic complexes $[(\eta^6\text{-arene})\text{Ru}(\text{MDMPP-}P,O)(L)][\text{PF}_6]$ ^[7c] and $[(\eta^5\text{-C}_5\text{Me}_5)\text{Rh}(\text{MDMPP-}P,O)(L)][\text{PF}_6]$.^[8] While investigating the interactions of these complexes with small molecules such as olefins and alkynes, we found that tcne inserted into a weakly activated C–H bond on the substituted phenyl ring of the phosphane ligand upon reaction with the rhodium(III) or iridium(III) complexes $[(\eta^5\text{-C}_5\text{Me}_5)\text{MCl}(\text{MDMPP-}P,O)]$ (**1a**: M = Rh,^[8] **1b**: M = Ir^[9]). This represents novel reactivity for tcne.

When tcne was added to **1a** or **1b** in CH₂Cl₂ at room temperature (Scheme 1), the solution became brown or yellow. In each case a 1:1 adduct was isolated, which on the



Scheme 1. Reactions of complexes **1** with tcne or tcnq.

basis of fast atom bombardment (FAB) mass spectrometry was assigned as **2a** (m/z 709 [M^+], orange-brown) or **2b** (m/z 798 [M^+], yellow; see the Experimental Section). In the IR spectrum of **2a** a very weak C≡N stretching frequency appeared at 2250 cm^{−1}, which is at higher energy than that of free tcne (2207 cm^{−1}) and π -complexed tcne (2170–2235 cm^{−1}).^[1] However, no IR signal was observed for **2b** as bands in the ν_{CN} region are extremely weak. In the ¹H NMR spectra of **2** three characteristic resonances around $\delta = 1.50$,

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3.50, and 6.40 were assigned to the $\eta^5\text{-C}_5\text{Me}_5$ (Cp^*), methoxy, and $\text{HC}(\text{CN})_2$ protons, respectively.

The structure of **2b** was determined by X-ray crystal structure analysis (Figure 1). The tcne molecule has been inserted into the C–H bond adjacent to the Ir–O σ bond. The

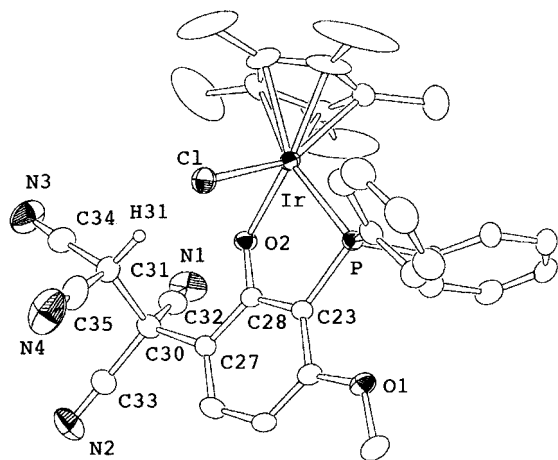


Figure 1. ORTEP diagram of the structure of **2b** with thermal ellipsoids drawn at the 30% probability level (see Table 1).

C30–C31 bond length of 1.592(7) Å is in the range of a usual C–C single bond (Table 1). The $\text{CH}(\text{CN})_2$ and $\text{C}(\text{CN})_2\text{R}$ groups adopt a staggered conformation (torsion angles C27–C30–C31–C34 168.8(5)°, C27–C30–C31–C35 –67.1(6)°). The $\text{Cl}\cdots\text{H31}$ and $\text{Cl}\cdots\text{C31}$ interatomic contacts are 2.78 and 3.63 Å, respectively, suggesting the presence of a very weak interaction.

Table 1. Selected bond lengths [Å] and angles [°] for **2b**.

Ir–Cl	2.411(2)	Ir–P	2.311(1)
Ir–O2	2.096(3)	O1–C24	1.352(6)
O2–C28	1.311(6)	C27–C30	1.517(7)
C30–C31	1.592(7)	C30–C32	1.494(8)
C32–N1	1.116(7)	C30–C33	1.489(8)
C33–N2	1.127(7)	C31–C34	1.452(8)
C34–N3	1.134(8)	C31–C35	1.450(9)
C35–N4	1.125(9)		
Cl–Ir–P	89.99(5)	Cl–Ir–O2	80.88(10)
P–Ir–O2	81.56(9)	Ir–P–C23	99.7(2)
Ir–O2–C28	117.9(3)	O2–C28–C23	122.3(4)
P–C23–C28	113.4(3)	C27–C30–C31	110.1(4)
C27–C30–C32	112.0(5)	C31–C30–C33	108.7(5)
C32–C30–C33	107.0(5)	C30–C31–C34	112.9(5)
C30–C31–C35	110.1(5)	C34–C31–C35	110.6(5)

These results prompted us to investigate the reaction with 7,7,8,8-tetracyano-*p*-quinodimethane (tcnq). Complex **1a** readily reacts with tcnq to form orange crystals formulated as the 1:1 adduct **3a** (Scheme 1), based on elemental analysis and FAB mass spectrometry (m/z 785 [M^+]; see the Experimental Section). The IR spectrum of **3a** showed a $\text{C}\equiv\text{N}$ stretching frequency at 2247 cm^{-1} , which is about 20 cm^{-1} higher in energy than that for free tcnq. The ^1H NMR spectrum showed three characteristic resonances at δ = 1.35 (d), 3.44 (s), and 5.08 (s), assignable to the Cp^* , methoxy, and

$\text{HC}(\text{CN})_2$ protons, respectively. These chemical shifts appeared upfield in comparison with those for **2**; this is a result of the strong electron withdrawing ability of the $\text{C}(\text{CN})_2\text{–C}(\text{CN})_2$ moiety. The signal for the *para*-proton is an AB-type quartet, suggesting aromatization of the quinone group.

The crystal structure analysis of **3a** verified the conversion of the quinone ring into an aromatic ring upon insertion of tcnq into the C–H bond (Figure 2). The bulky $\text{C}_6\text{H}_4\text{C}(\text{CN})_2\text{H}$

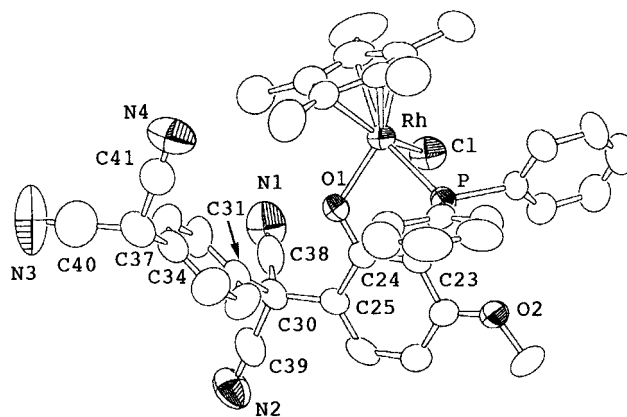


Figure 2. ORTEP diagram of the structure of **3a** with thermal ellipsoids drawn at the 50% probability level (see Table 2).

moiety is pointing in the opposite direction as the Cl ligand, relieving steric repulsion between the two groups. The C30–C31 and C34–C37 bond lengths are 1.54(1) and 1.56(1) Å, respectively, in agreement with a C–C single bond (Table 2). The $\text{C30}(\text{CN})_2\text{R}$ and $\text{C37}(\text{CN})_2\text{H}$ groups adopt a staggered conformation, similar to the case in **2a**.

Table 2. Selected bond lengths [Å] and angles [°] for **3a**.

Rh–Cl	2.393(2)	Rh–P	2.306(3)
Rh–O1	2.085(7)	C25–C30	1.54(1)
C30–C31	1.54(1)	C34–C37	1.56(2)
C30–C38	1.49(2)	C38–N1	1.18(2)
C30–C39	1.48(2)	C39–N2	1.14(1)
C37–C40	1.53(2)	C40–N3	1.12(2)
C7–C41	1.47(2)	C41–N4	1.13(2)
Cl–Rh–P	85.0(1)	Cl–Rh–O1	86.3(2)
P–Rh–O1	82.1(2)	Rh–P–C23	99.3(3)
Rh–O1–C24	118.5(6)	O1–C24–C23	122.3(9)
P–C23–C24	113.3(7)	C25–C30–C31	112.1(8)
C25–C30–C38	108.6(8)	C25–C30–C39	110.2(9)
C31–C30–C38	112.4(9)	C31–C30–C39	107.1(8)
C38–C30–C39	106.3(9)	C34–C37–C40	111(1)
C40–C37–C41	109(1)		

Complexes **1a** and **1b** were treated with moderately electron withdrawing olefins such as fumaronitrile and dimethyl fumarate in methanol at room temperature or at reflux. The starting materials were recovered quantitatively.

To our knowledge these results represent the first examples of insertion reactions of tcne and tcnq into a C–H bond on an aromatic ring. These reactions demonstrate the novel C–H bond activation by organometallic complexes in connection with insertion of strongly electron withdrawing cyanoolefins.

Experimental Section

2a: To a solution of **1a** (53.2 mg, 0.092 mmol) in CH₂Cl₂ (10 mL) was added tene (13 mg, 0.10 mmol) at room temperature. After 4 h the solvent was removed, and the solids were washed with diethyl ether. The residue was crystallized from CH₂Cl₂ and diethyl ether to give orange-brown crystals of **2a** (43.2 mg, 66.3 %). IR (nujol): $\tilde{\nu}$ = 2250 cm⁻¹ (C≡N); UV/Vis (CH₂Cl₂): λ_{max} = 392, 329 nm; ¹H NMR (250 MHz, CDCl₃): δ = 1.50 (d, J(P,H) = 5.0 Hz, Cp*), 3.48 (s, OMe), 5.27 (s, CH₂Cl₂), 6.36 (s, CH), ca. 6.06 and 7.3–8.0 (m, ArH); ³¹P NMR (100 MHz, CDCl₃): δ = 45.2 (d, J(Rh,P) = 142.7 Hz); FAB-MS: *m/z*: 709 [M⁺], 673 [M⁺ – Cl]; elemental analysis calcd for C₃₅H₃₁N₄O₂PClIr·CH₂Cl₂: C 54.46, H 4.19, N 7.06; found: C 54.87, H 4.08, N 6.84.

2b: Yellow crystals of **2b** (41.4 mg, 42.9 %) were obtained from **1b** (80 mg, 0.121 mmol) and tene (21.8 mg, 0.170 mmol) by a procedure similar to that for **2a**. UV/Vis (CH₂Cl₂): λ_{max} = 324 nm; ¹H NMR (250 MHz, CDCl₃): δ = 1.53 (s, Cp*), 3.50 (s, OMe), 5.24 (s, CH₂Cl₂), 6.31 (s, HC(CN)₂), ca. 6.13 and 7.2–7.9 (m, ArH); ³¹P NMR (100 MHz, CDCl₃): δ = 26.2 (s); FAB-MS: *m/z*: 798 [M⁺]; elemental analysis calcd for C₃₅H₃₁N₄O₂PClIr: C 52.66, H 3.91, N 7.02; found: C 52.98, H 3.84, N 7.00. Crystal data: C₃₅H₃₁N₄O₂PClIr, monoclinic, space group *P*₂₁/*n* (no. 14), *a* = 12.123(5), *b* = 14.314(7), *c* = 20.407(3) Å, β = 95.34(2)°, *V* = 3525(1) Å³, *Z* = 4, ρ_{calcd} = 1.504 g cm⁻³, *R* = 0.028 and *R*_w = 0.037 [*w* = 1/ σ^2 (*F*_o)] for 4487 reflections [*I* > 3.0σ(*I*)] with 397 variables. The structure was solved by Patterson methods (DIRDIF92) and refined by full-matrix least-squares techniques using the teXsan program package.^[10]

3a: Orange crystals of **3a** (28 mg, 32 %) were obtained from **1a** (60 mg, 0.103 mmol) and tcnq (25 mg, 0.13 mol) by a procedure similar to that for **2a**. IR (nujol): $\tilde{\nu}$ = 2247 cm⁻¹ (C≡N); UV/Vis (CH₂Cl₂): λ_{max} = 398, 330 nm; ¹H NMR (250 MHz, CDCl₃): δ = 1.35 (d, J(P,H) = 3.0 Hz, Cp*), 3.44 (s, OMe), 5.08 (s, CH), 7.51 (ABq, *J* = 10.0 Hz), ca. 6.00 and 7.3–8.0 (m, ArH); ³¹P NMR (100 MHz, CDCl₃): δ = 8.4 (d, J(Rh,P) = 150.0 Hz); FAB-MS: *m/z*: 785 [M⁺], 750 [M⁺ – Cl]; elemental analysis calcd for C₄₁H₃₅N₄O₂PClIr: C 62.73, H 4.49, N 7.14; found: C 62.55, H 4.55, N 7.29. Crystal data: C₄₁H₃₅N₄O₂PClIr, monoclinic, space group *P*₂₁/*n* (no. 14), *a* = 13.70(1), *b* = 19.076(7), *c* = 15.965(10) Å, β = 101.31(6)°, *V* = 4092(4) Å³, *Z* = 4, ρ_{calcd} = 1.274 g cm⁻³, *R* = 0.055 and *R*_w = 0.080 for 2961 reflections [*I* > 4.0σ(*I*)] with 451 variables. The structure was solved by Patterson methods (DIRDIF92) and refined by full-matrix least-squares techniques using the teXsan program package.^[10]

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New Tripodal, “Supercharged” Analogues of Adenosine Nucleotides: Inhibitors for the Fhit Ap₃A Hydrolase**

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Diadenosine polyphosphates, discovered over 30 years ago,^[1] are ubiquitous components of all cells. Recently, diadenosine triphosphate and tetraphosphate, Ap₃A and Ap₄A, have assumed vital significance as ligands for the tumor suppressor protein, Fhit,^[2a] which is an Ap₃A hydrolase^[2b] whose signalling appears to depend on Ap_nA binding.^[3] We are interested in the specific enzymes of dinucleoside polyphosphate catabolism,^[2a, 4, 5] in the chemistry^[6] of those compounds, and also in bisubstrate analogues for phosphoglycerate^[7] and other kinases, and present here the synthesis

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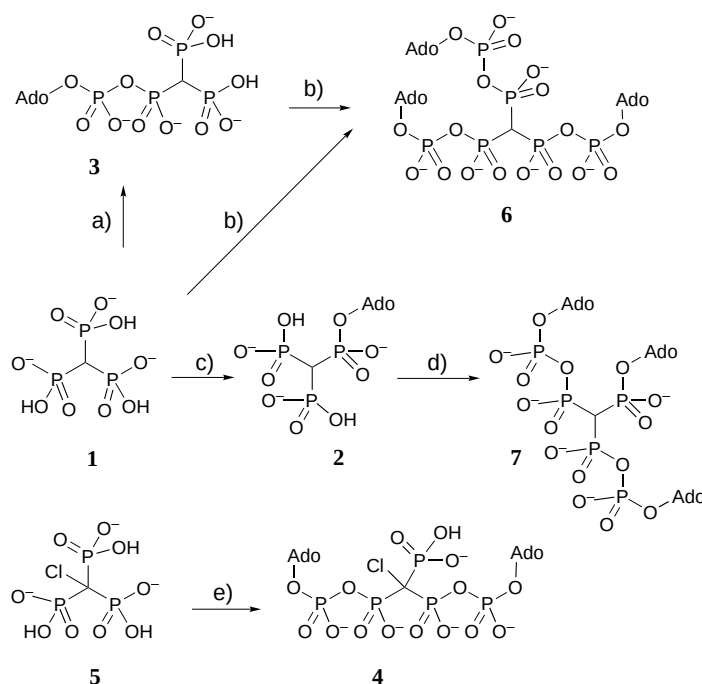
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of novel bisubstrate analogues that are isopolar^[8] to the transition state for Ap₄A hydrolysis.

A key problem in these endeavors is the unavoidable diminution in anionic charge of a linear bisubstrate nucleotide analogue relative to that in the transition state for the corresponding phosphoryl transfer reaction. For example, adenylate kinase (AK) interconverts two ADP molecules (net substrate charge -6) with AMP plus ATP via a trigonal-bipyramid transition state of charge -6 . The bisubstrate analogue for this reaction, Ap₄A, has a charge of only -4 . Not surprisingly, Ap₅A ($K_i = 30 \text{ nM}$) is a superior inhibitor of AK compared to Ap₄A ($K_i = 10 \text{ }\mu\text{M}$),^[9] though we note that phosphate chain conformational factors may also contribute to this difference. Likewise, the lupine Ap₄A hydrolase cleaves Ap₄A to give AMP plus ATP via an in-line trigonal-bipyramid transition state^[10] of charge -5 . There is thus a good opportunity to generate improved bisubstrate analogue inhibitors for many phosphoryl transfer enzymes through the use of pyrophosphate mimics that incorporate additional negative charge by branching rather than by phosph(on)ate chain elongation. Previously we have described the synthesis of some such "supercharged" analogues of pyrophosphoric acid (**1**, **5**) and their incorporation into analogues of ATP.^[11] We now report their incorporation into analogues of ADP and of diadenosine polyphosphates and especially the novel use of the C₃ symmetry branch point of methanetrissphosphonic acids to create new tripodal adenosine nucleotide analogues.

These products show differential competitive inhibition between two diadenosine triphosphate hydrolases. Ap_nA hydrolases appear to comprise at least two evolutionary groups. The human *fhit* gene product is a member of the histidine triad superfamily^[12a] of nucleotide binding proteins which has homologues in animals and fungi. Other Ap_nA hydrolases are related to mutT^[12b] and have been found in all forms of life. Because the Fhit protein is a tumor suppressor protein that is inactivated early in the development of epithelial cancers^[12c] but is not the only enzyme that can cleave Ap₃A, new chemical entities that distinguish between Fhit and other Ap_nA hydrolases have great potential in cancer diagnostics.

Methanetrissphosphonic acid (**1**) was described by Gross et al. in 1993.^[13] Key improvements in that synthesis have enabled us now to prepare chloro- and fluoromethanetrissphosphonic acids for the first time and in usable quantity.^[14] Compound **1** was readily transformed into an ADP analogue (**2**) by the method of Poulter et al.^[15] and into an analogue of ATP (**3**) by the phosphoromorpholidate procedure of Khorana and Moffatt^[16] (Scheme 1). For ATP analogue synthesis^[11] AMP morpholidate was used as the limiting reagent, while in the case of diadenosine tetraphosphate analogue **4**, prepared from chloromethanetrissphosphonate (**5**), AMP morpholidate was used in excess. The two primary phosphonic acid functionalities in the ADP analogue **2** and the ATP analogue **3** were sites for double adenylation by a modification to the procedure of Khorana and Moffatt in the synthesis of two novel tripodal nucleotide analogues (Scheme 1). The reaction of the tris-tri-*n*-butylammonium salt of **1** with AMP morpholidate was extremely sluggish, and only a small amount of the desired trisadenylylated product **6** was obtained after seven



Scheme 1. Synthesis of the nucleotide analogues **2**–**7**. a) AMP-morpholidate (0.8 equiv), tetrazole, pyridine; b) AMP-morpholidate (5 equiv), tetrazole, pyridine; c) 5'-O-tosyl adenosine, MeCN; d) excess AMP-morpholidate, pyridine; e) AMP-morpholidate (2.2 equiv), tetrazole, pyridine. All counterions are tri-*n*-butylammonium. Ado = 5'-adenosyl.

days. Evidently the incorporation of the third adenylate moiety was extremely slow since both the intermediate ATP analogue **3** and the Ap₄A analogue **4** (with H instead of Cl) were isolated as major products. Tetrazole proved to be an excellent catalyst,^[17] and reaction was complete in two days to give P¹,P²,P³-tris(5'-adenylyl) methanetrissphosphonate (**6**) in 59% yield. Similarly, 5'-adenosyl methanetrissphosphonate (**2**) was treated with an excess of AMP morpholidate with tetrazole catalysis to give the tripodal nucleotide analogue P¹-5'-adenosyl P²,P³-bis(5'-adenylyl) methanetrissphosphonate (**7**) in 58% yield. These provide the first two examples of three adenylate moieties linked together by a methanetrissphosphonate core.^[18] The pK_a values for these species were determined and clearly show the benefit of α -halogenation for increasing the acidity of the deprotonated phosphonic acids (Table 1).

We envisaged that these novel nucleotide analogues might be substrates for Ap_nA hydrolases, which cleave the P¹,P²-pyrophosphate linkage in Ap_nAs.^[5, 19] Assays with two *asymmetrical* Ap₄A hydrolases (EC 3.6.1.17), one from lupine^[4] and a recombinant human Ap₄A hydrolase,^[20] showed that none of the nucleotide analogues behaved as substrates while they are all strong, nondiscriminatory inhibitors of these Ap₄A hydrolases (data not presented). However, these analogues did not inhibit the activity of a lupine adenosine 5'-tetraphosphate hydrolase.^[21] We then examined members of two different classes of Ap₃A hydrolase (EC 3.6.1.29), Fhit protein^[2b] and an Ap₃A hydrolase from yellow lupine seeds.^[22] The test nucleotides were not hydrolyzed, and we now found that they inhibited these two enzymes in very contrasting ways (Figure 1).

Table 1. The pK_a values, inhibition constants, and net charges calculated for inhibition of human Fhit and yellow lupine Ap_3A hydrolases by a range of methanetrissphosphonates and their adenosine nucleotide derivatives.^[a]

Inhibitor	pK_a	Human Fhit Ap_3A hydrolase K_i [μM]	net charge pH 6.9	Lupine Ap_3A hydrolase K_i [μM]	net charge pH 8.2	δ_p D ₂ O, pD 7
PCF(P)P (5, F instead of Cl)	$pK_{a's}$ 5.77, 8.86	n.d.	3.93	1.0	4.18	8.89; s
PCCl(P)P (5)	$pK_{a's}$ 5.92, 9.08	n.d.	3.91	1.0	4.12	11.42; s
PCH(P)P (1)	$pK_{a's}$ 6.46, 9.90	n.d.	3.74	1.3	3.98	13.17; s
AdoPCH(P)P (2)	$pK_{a's}$ 7.48	18.3	3.21	6.1	3.84	15.15, 12.94; ABB'
AdoPPCH(P)P (3)	$pK_{a's}$ 7.50	4.0	4.20	2.8	4.83	13.23, 7.85, -9.41; AA'MX ^[11]
AdoPPCCl(P)PPAdo (4)	$pK_{a's}$ 8.86	1.9	5.01	16.2	5.18	8.18, 3.75, 1.36, -9.33; AA'MM'X
AdoPCH(PPAdo) ₂ (7)	n.a.	1.2	5.00	3.0	5.00	13.48, 5.08, -9.78; AA'MM'X
(AdoPP) ₃ CH (6)	n.a.	0.4	6.00	5.4	6.00	5.22, -9.46; AA'A'XXX"

[a] The pK_a values were measured in the range $3.5 < pH < 10.5$. n.a.: no ionization change in this pH range. n.d.: no inhibition detectable at an inhibitor concentration of 200 μM or less. Each enzyme was assayed at the pH at which it shows maximum activity.

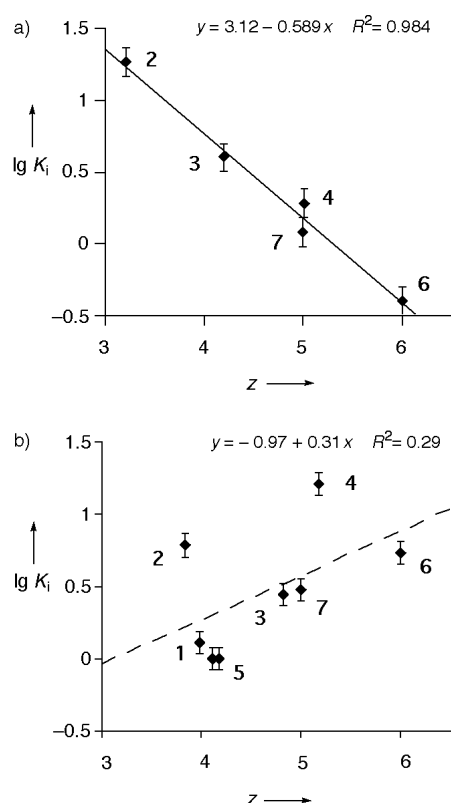


Figure 1. Linear free energy plots of $\lg K_i$ versus inhibitor charge z for the binding of a range of trisphosphonates and their nucleotide analogues to Ap_3A hydrolases from human Fhit (a) and yellow lupine sources (b). The K_i values are in μM . Charge is calculated from pK_a values (Table 1) respective to the pH optimum for enzyme activity: a) Fhit, pH 6.9; b) lupine Ap_3A hydrolase, pH 8.2.

Methanetrissphosphonate (**1**) and its chloro (**5**) and fluoro derivatives (**5**, F instead of Cl) do not detectably inhibit human Fhit, but are strong inhibitors of the lupine enzyme with K_i values similar to the K_m value for Ap_3A (1.2 μM , Table 1).^[23] By contrast, most of the adenylated polyphosphonates strongly and competitively inhibit Fhit with K_i values similar^[3b] to the K_m value for Ap_3A (1.9 μM) while they are less effective as inhibitors of the lupine enzyme (Table 1). Most of the difference in inhibition constants for these two hydrolases evidently stems from contrasting charge dependencies of ligand binding. The competitive inhibition of Fhit shows a

rather good linear free energy relationship to the anionic charge for five adenosine phosphonate analogues (calculated at the pH optimum of the enzyme assay), providing that at least one adenosine moiety is available for binding (Figure 1a). That requirement accords with results from X-ray crystal structures of two diastereoisomers of 5',5''-diadenosyl P^1 -thio- P^2 , P^3 -methylenetrissphosphate complexed to a wild-type and to an active-site mutant of Fhit. These both show^[3b] one adenosine in a deep cleft while the second is in surface contact with bulk solvent. By contrast, the strongest inhibitors of the lupine enzyme are the three methanetrissphosphonic acids lacking any adenosine moiety. Moreover, increasing the charge on the inhibitors broadly tends to a decrease in affinity for the lupine enzyme, though the correlation is weak (Figure 1b).

These novel tripodal adenine nucleotides have provided a first example of differential inhibition of human Fhit from an alternative Ap_3A hydrolase, with a better than tenfold selectivity ratio (Table 1). Detection of levels of Fhit protein is an important cancer problem that currently is complicated by the activity of unrelated enzymes that cleave Ap_3A . For this reason, Fhit-selective inhibitors such as **4** and **6** will be valuable as Fhit diagnostics, while the greater than 1000-fold selectivity for competitive inhibition of the lupine enzyme by the non-nucleotide species **1** and **5** is expected to fulfill a complementary role in inhibiting lupine-related Ap_3A hydrolytic activities in human cells. This differential selectivity could provide a valuable tool for further study of Ap_3A hydrolases and their relationship to tumor suppression.

Experimental Section

Enzyme assays: Inhibitory properties of the "supercharged" phosphonates were determined in an incubation mixture (final volume 0.050 mL) containing either 50 mM Mes/KOH, pH 6.9 (for the human Fhit protein, its pH optimum) or 50 mM Hepes/KOH, pH 8.2 (for the lupine Ap_3A hydrolase, its pH optimum), 0.1 mM dithiothreitol, 0.1 mM [^3H]- Ap_3A (about 400 000 cpm), 1 mM $MgCl_2$, 1 % of glycerol, 0.1 mg of bovine serum albumin per mL, various concentrations of the inhibitor phosphonate, and a rate-limiting amount of the enzyme. The incubation was carried out at 30 °C. Rates were determined by taking 0.005-mL aliquots at set times (5, 10, 15, and 20 min) and spotting them onto thin layer aluminium plates precoated with silica gel containing fluorescent indicator (Merck). An ADP standard was added, and the chromatogram developed in dioxane/ammonia/water (6/1/4). ADP spots were cut out and the radioactivity counted. The K_i values were calculated from Dixon plots.

Enzyme preparation: Human Fhit protein was overexpressed in *Escherichia coli* as described.^[3a] Purification from the crude bacterial extract comprised ammonium sulfate fractionation, ion-exchange chromatography on DEAE-Sephacel, gel filtration on Sephadex G-100, and immobilized-metal-affinity chromatography on TALON (Clontech) resin from which adsorbed protein was eluted with imidazole (50 mM). Homogeneous dinucleoside triphosphate hydrolase from yellow lupine seeds was obtained as described previously.^[22]

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Highly Regio- and Stereoselective Synthesis of Mannose-Containing Oligosaccharides with Acetobromo Sugars as the Donors and Partially Protected Mannose Derivatives as the Acceptors via Sugar Orthoester Intermediates**

Wei Wang and Fanzuo Kong*

Many biologically important natural products such as glycoproteins,^[1] the ubiquitous components of the cell membrane, contain an oligomannopyranose core, while the cell wall of yeast contains branched mannans.^[2] Mannose-containing oligosaccharides have been synthesized by well-established methods^[3] that involve multistep selective protection and deprotection procedures. The use of unprotected or partially protected mannose and acetobromo sugars as raw materials in glycosylations is very attractive for organic chemists because the synthetic routes can be substantially simplified. In previous work^[4a] we described a new method for regio- and stereoselective synthesis of oligosaccharides by an orthoester^[4d-m] formation/rearrangement procedure with unprotected glucopyranosides as the glycosyl acceptors and acetobromo sugars as the donors which gave 1→6-linked oligosaccharides in satisfactory yields. In addition, 3-selective glycosylation was achieved with partially protected glucose acceptors containing unprotected 2,3- or 3,4-hydroxy groups. It was found, however, that the glycosylation with unprotected glycosides as the acceptors was rather slow and difficult to monitor owing to the poor solubility of the acceptors in the reaction media. We now report a new strategy for highly regio- and stereoselective synthesis of mannose-containing di- and oligosaccharides via orthoester intermediates by coupling acetobromo sugars with partially protected mannose derivatives as the acceptors, in particular naked mannose 1,2-*O*-ethylidenate.

It is well known that 3,6-branched mannotrisaccharide Manpa1→6(Manpa1→3)Man is present in all asparagine-

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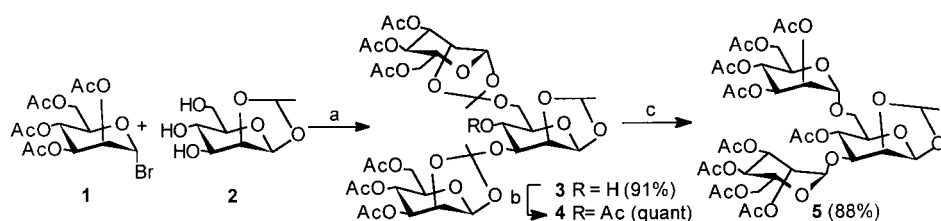
 Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.

linked oligosaccharides, and it is the major Con A binding epitope on oligomannose-type carbohydrates.^[5] The syntheses of the trisaccharide involve the use of different protective groups and lengthy reaction routes leading to the product in low yields.^[6] With the newly developed orthoester formation/rearrangement strategy the trisaccharide was readily synthesized^[7] by coupling 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl bromide (**1**, 2.4 equiv) with 1,2-*O*-ethylidene- β -D-mannopyranose (**2**, 1 equiv) in the presence of silver trifluoromethanesulfonate (AgOTf, 2.2 equiv) and 2,4-lutidine (2.4 equiv) followed by rearrangement (Scheme 1).

The formation of trisaccharide diorthoester **3** is highly regioselective; no 4-substitution was found, and **3** and **4** were obtained as white crystals. The structure of **3** was unambiguously verified by its ¹H NMR spectrum,^[8] which showed two characteristic signals at $\delta = 5.54$ and 5.50 for H1' and H1'' respectively, and by its acetylation product **4**, which exhibited a new triplet at $\delta = 5.12$, a clear indication^[8] of 3,6-branched glycosylation. Rearrangement^[4a-c, 9] of **4** in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) gave the 3,6-branched mannotrisaccharide **5** in high yield. Its 2D ¹H NMR spectrum also gave a clear indication^[8] of 3,6-branched glycosylation.

The orthoester **3** is a very important intermediate. It can be modified at the reducing ends to couple sugar donors at the 2'- and 2''-positions, and its transformation to **5** followed by removal of the 1,2-ethylidene group can lead to suitable glycosyl donors such as the Schmidt reagent and phenyl thioglycoside. Subsequent coupling with sugar acceptors affords α -^[3c] and β -linked^[10] oligosaccharides respectively.

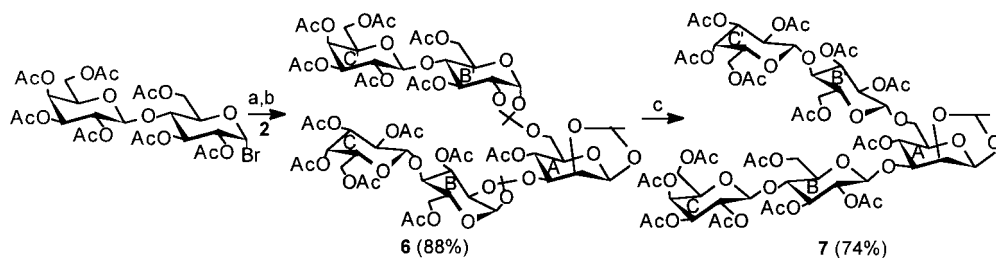
Encouraged by the success in coupling **2** with **1**, we tried the glycosylation of **2** with disaccharide as the glycosyl donor. Thus, acetobromolactose (2.2 equiv) was coupled with **2** (1 equiv) under the same conditions as described for the prepara-



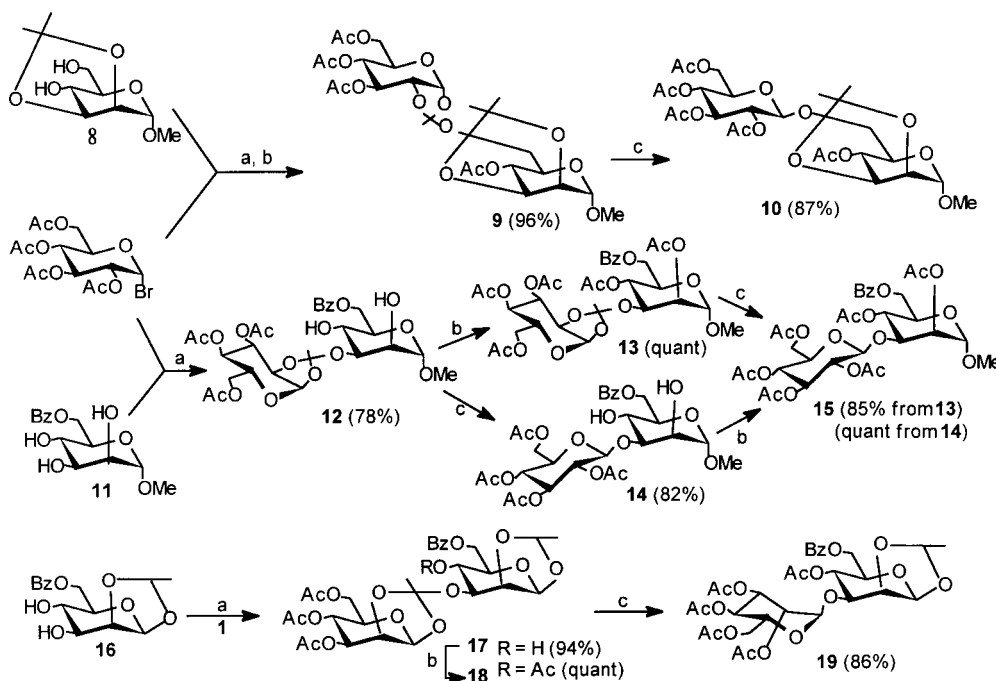
Scheme 1. Conditions and reagents: a) AgOTf/2,4-lutidine, CH₂Cl₂, molecular sieves (4 Å), 3 h; b) Ac₂O/anhydrous pyridine; c) TMSOTf/CH₂Cl₂, molecular sieves (4 Å), -30°C, 30 min.

tion of **3** to afford the 3,6-branched pentosaccharide diorthoester **6** in 88% yield, the rearrangement of which gave the 3,6-branched pentosaccharide **7** in 74% yield (Scheme 2). These transformations revealed that the regio- (3,6) and stereoselectivity (1,2-*trans*) was dependent on the acceptor rather than the donor structure. The structure of **6** was verified by its 2D ¹H NMR spectrum^[8] which showed H4 as a low-field triplet.

To investigate the application of the new strategy to more general mannose acceptors, a number of partially protected mannose derivatives was examined (Scheme 3). Thus, with methyl 2,3-*O*-isopropylidene- α -D-mannopyranoside (**8**), a



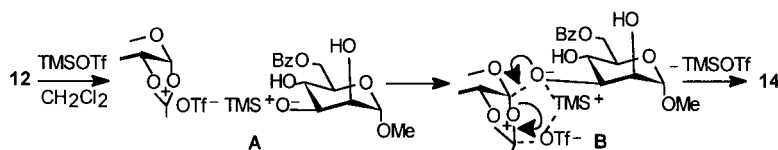
Scheme 2. Conditions and reagents: a) AgOTf/2,4-lutidine, CH₂Cl₂, molecular sieves (4 Å), 4 h; b) Ac₂O/anhydrous pyridine; c) TMSOTf/CH₂Cl₂, molecular sieves (4 Å), N₂, 30 min.



Scheme 3. Conditions and reagents: a) AgOTf/2,4-lutidine, CH₂Cl₂, molecular sieves (4 Å), 2–3 h; b) Ac₂O/anhydrous pyridine; c) TMSOTf/CH₂Cl₂, molecular sieves (4 Å), N₂, 30–40 min.

6-linked orthoester was formed as the sole product in almost quantitative yield. Its acetylation to **9** followed by rearrangement readily furnished **10**. With methyl 6-*O*-benzoyl- α -D-mannopyranoside^[11] (**11**) as the acceptor, the 3-linked orthoester **12** was obtained in a high yield. Its acetylation to **13** followed by rearrangement, or its rearrangement to **14** followed by acetylation produced the same compound **15**, indicating that both orthoester formation and rearrangement were regio- and stereoselective. The same 3-selectivity was obtained with 6-*O*-benzoyl-1,2-*O*-ethylidene- β -D-mannopyranose (**16**) as the acceptor, when the 1,3-linked disaccharide **19** was formed in a satisfactory yield.

Since the rearrangement of **12** to **14** (82%) and the rearrangement of **13** to **15** (85%) afforded the respective disaccharides in similar yields, and the rearrangement of **4** afforded the trisaccharide **5**, also in a high yield, we rationalized the mechanism of the rearrangement as shown in Scheme 4. When TMSOTf was added to the solution of **12** in dichloromethane, selective bond cleavage of the orthoester linkage rather than 2- or 4-OH bond cleavage occurred to give a closely associated ion pair (form **A**). When this ion pair reaches an appropriate six-membered ring geometry (form **B**) rearrangement occurs to afford the required disaccharide **14**. We still consider that steric factors are mainly responsible for the high regioselectivity in orthoester formation.^[4]



Scheme 4.

In summary, here we present a very effective regio- and stereoselective glycosylation method with 1,2-*O*-ethylidenated mannose or partially protected mannosides as glycosyl acceptors and simple acetobromo sugars as the glycosyl donors by an orthoester formation/rearrangement procedure. A number of 1 $\rightarrow$ 6-, 1 $\rightarrow$ 3-, and 3,6-branched oligosaccharides with exclusive 1,2-*trans* linkage were readily synthesized by the new strategy. This approach, in terms of yields, simplicity, and efficiency, will be a general one for the synthesis of mannose-containing oligosaccharides.

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[7] Typical conditions for orthoester preparation: To a stirred solution of 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl bromide (**1**, 1.02 g, 2.5 mmol), 2,4-lutidine (276 μ L, 2.4 mmol), and 1,2-*O*-ethylidene-*(R,S)*- β -D-mannopyranose (**2**, 206 mg, 1 mmol) in dichloromethane (20 mL) under nitrogen atmosphere was added AgOTf (565 mg, 2.2 mmol) in a dark room. The reaction was carried out at room temperature and monitored by TLC (petroleum ether/ethyl acetate, 1/1.5). After completion of the reaction the mixture was partitioned between dichloromethane and water, the organic phase was concentrated, dried, and subjected to column chromatography on silica gel with petroleum ether/ethyl acetate (1/1) as the eluent to give the trisaccharide diorthoester **3** (91% yield based on acceptor **2**) as the sole product. The procedure for the preparation of the disaccharide orthoesters is the same as described above except for the amounts of the acetoglycobromide donors ($\approx$ 1.2 equiv) and the promoters ($\approx$ 1.2 equiv). Typical rearrangement conditions: To a stirred solution of sugar-sugar orthoester **4** (580 mg, 0.64 mmol) in dichloromethane (10 mL) was added TMSOTf (12 μ L, 0.1 equiv) under nitrogen atmosphere and the reaction was monitored by TLC (petroleum ether/ethyl acetate, 1/1). After completion of the reaction the mixture was treated with triethylamine (20 μ L), filtered, and the filtrate was washed with CH_2Cl_2 . The combined solution was washed with 1M HCl (10 mL), saturated aqueous NaHCO_3 (10 mL), and aqueous NaCl (2×10 mL), dried over anhydrous Na_2SO_4 , and concentrated. The residue was subjected to column chromatography on silica gel with petroleum ether/ethyl acetate (1/1) as the eluent to give **5** (510 mg, 88%).

- [8] Physical data and ^1H NMR (400 MHz, CDCl_3 , 25°C, TMS) data for **3–6**. **3**: m.p. 110–112°C; $[\alpha]_D = +5.2$ ($c = 1.5$ trichloromethane); ^1H NMR: $\delta = 5.54, 5.50$ (2d, ^3J ($\text{H}_{1(1'')}, \text{H}_{2(2'')}) = 2.7$ Hz, 2H; $\text{H}-1'$, 1''), 5.30–5.15 (m, $\text{H}-1$, 3', 3'', 4', 4'', 6H; CH_3CH), 4.75, 4.63 (2dd, ^3J (H_2, H_3) = 3.8 Hz, ^3J ($\text{H}_{2(2'')}, \text{H}_{3(3'')}) = 3.9$ Hz, 2H; $\text{H}-2'$, 2''), 4.23–4.13 (m, 4H; $\text{H}-6'$, 6''), 4.06 (dd, ^3J (H_1, H_2) = 2.4 Hz, ^3J (H_2, H_3) = 3.6 Hz, 1H; $\text{H}-2$), 3.84–3.68 (m, 6H; $\text{H}-3$, 4, 5', 5'', 6), 3.40–3.35 (m, 1H; $\text{H}-5$), 2.11, 2.10, 2.07, 2.07, 2.06, 2.05 (6s, 18H; 6 CH_3CO), 1.85, 1.74 (2s, 6H; 2 CH_3CO), 1.47 (d, ^3J (H,H) = 5.0 Hz, 3H; CH_3CH). **4**: m.p. 135–138°C; $[\alpha]_D = +0.4$ ($c = 0.1$, trichloromethane); ^1H NMR: $\delta = 5.49, 5.43$ (2d, ^3J (H_1, H_2), ^3J ($\text{H}_{1(1'')}, \text{H}_{2(2'')}) = 2.8$ Hz, 2H; $\text{H}-1'$, 1''), 5.30–5.16 (m, 6H; $\text{H}-1$, 3', 3'', 4', 4'', CH_3CH), 5.12 (t, ^3J (H,H) =

- 9.6 Hz, 1 H; H-4), 4.66 (dd, ^3J ($H_{1'}$, $H_{2'}$) = 2.9 Hz, ^3J ($H_{2'}$, $H_{3'}$) = 3.9 Hz, 1 H; H-2''), 4.58 (dd, ^3J (H_1 , H_2) = 2.8 Hz, ^3J (H_2 , H_3) = 4.0 Hz, 1 H; H-2'), 4.24–4.08 (m, 5 H; H-2, 6', 6''), 4.01 (dd, ^3J (H_2 , H_3) = 4.6 Hz, ^3J (H_3 , H_4) = 9.6 Hz, 1 H; H-3), 3.75–3.65 (m, 2 H; H-5', 5''), 3.60 (d, ^2J (H_{6a} , H_{6b}) = 4.5 Hz, 2 H; H-6), 3.55–3.50 (m, 1 H, H-5), 2.12, 2.10, 2.07, 2.06, 2.06, 2.05, 2.04 (7 s, 21 H; 7 CH_3CO), 1.77, 1.71 (2 s, 6 H; 2 CH_3CO_2), 1.49 (d, ^3J (H,H) = 4.9 Hz, 3 H; CH_3CH). **5**: m.p. 177–178 °C; $[\alpha]_D^{25} = +16.9$ ($c = 0.15$, trichloromethane); ^1H NMR: $\delta = 5.36$ –5.21 (m, 8 H; H-1, 2', 3', 3'', 4, 4', 4'', CH_3CH), 5.14 (dd, ^3J ($H_{1'}$, $H_{2'}$) = 1.9 Hz, ^3J ($H_{2'}$, $H_{3'}$) = 3.1 Hz, 1 H; H-2''), 4.98 (d, ^3J ($H_{1'}$, $H_{2'}$) = 1.9 Hz, 1 H; H-1''), 4.78 (d, ^3J (H_1 , H_2) = 1.7 Hz, 1 H; H-1'), 4.34–4.27 (m, 4 H; H-2, 5', 6''), 4.10–4.01 (m, 3 H; H-5'', 6'), 3.91 (dd, ^3J (H_2 , H_3) = 3.8 Hz, ^3J (H_3 , H_4) = 9.7 Hz, 1 H; H-3), 3.80 (dd, ^3J (H_5 , H_{6a}) = 6.3 Hz, ^3J (H_{6a} , H_{6b}) = 10.4 Hz, 1 H; H-6a), 3.62–3.56 (m, 2 H; H-5, 6b), 2.16, 2.15, 2.15, 2.11, 2.09, 2.06, 2.06, 2.00, 1.98 (9 s, 27 H; 9 CH_3CO), 1.53 (d, ^3J (H,H) = 5.0 Hz, 3 H; CH_3CH). **6**: $[\alpha]_D^{25} = +2.4$ ($c = 0.15$, trichloromethane); ^1H NMR: $\delta = 5.69$ (d, ^3J (H_{1B} , H_{2B}) = 5.1 Hz, 1 H; H-1B), 5.65 (d, ^3J (H_{1B} , H_{2B}) = 5.2 Hz, 1 H; H-1B'), 5.50 (dd, ^3J (H_{2B} , H_{3B}) = 2.4 Hz, ^3J (H_{3B} , H_{4B}) = 1.2 Hz, 1 H; H-3B), 5.42 (t, ^3J (H,H) = 1.9 Hz, 1 H; H-3B'), 5.38 (2d, ^3J (H,H) = 3.3 Hz, 2 H; H-4C, 4C'), 5.26 (d, ^3J (H_{1A} , H_{2A}) = 1.6 Hz, 1 H; H-1A), 5.26 (q, ^3J (H,H) = 5.0 Hz, 1 H; CH_3CH), 5.16 (2 dd, ^3J ($H_{1C(C)}$, $H_{2C(C)}$) = 8.0 Hz, ^3J ($H_{2C(C)}$, $H_{3C(C)}$) = 9.6 Hz, 2 H; H-2C, 2C'), 5.10 (t, ^3J (H,H) = 9.5 Hz, 1 H; H-4A), 5.00 (2dd, 2 H; H-3C, 3C'), 4.60, 4.55 (2d, ^3J ($H_{1C(C)}$, $H_{2C(C)}$) = 8.0 Hz, 2 H; H-1C, 1C'), 4.41 (dd, 1 H; H-2B), 4.35 (dd, 1 H; H-2B'), 4.30–4.20 (m, 3 H; H-2A, 6B_a, 6B'_a), 4.13–4.05 (m, 6 H; H-6B_b, 6B'_b, 6C, 6C'), 3.98–3.88 (m, 3 H; H-3A, 5C, 5C'), 3.85–3.78 (m, 2 H; H-5B, 5B'), 3.64 (2t, ^3J (H,H) = 9.5 Hz, 2 H; H-4B, 4B'), 3.60–3.58 (m, 3 H; H-5A, 6A), 2.17–1.98 (13 s, 39 H; 13 CH_3CO), 1.74, 1.68 (2 s, 6 H; 2 CH_3CO_2), 1.47 (d, ^3J (H,H) = 5.0 Hz, 3 H; CH_3CH).
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Chemoenzymatic Synthesis of a Characteristic Glycophosphopeptide from the Transactivation Domain of the Serum Response Factor**

Jörg Sander and Herbert Waldmann*

Phosphorylation^[1] and O-glycosidic attachment of *N*-acetylglucosamine (GlcNAc)^[2] to serine and threonine residues are key events in the cellular transduction of mitogenic signals. For instance, the serum response factor (SRF), a widely found transcription factor, is phosphorylated at various serine and threonine residues in response to extracellular stimuli. It then translocates to the nucleus, binds to the serum response element (SRE, which occurs in the promoters of numerous genes), and induces gene transcription.^[3] In addition, SRF is glycosylated at four different sites,^[4] where the major glycosylation site—most probably serine 383 in the

transactivation domain—is flanked by several phosphorylation sites (Figure 1). These findings suggest that the correct orchestration of SRF phosphorylation and glycosylation as well as the controlled removal of these covalent protein modifications appear to be paramount to the regulation of gene transcription under the control of SRF.

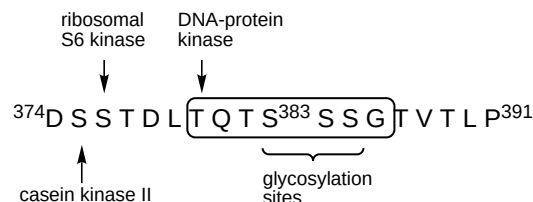


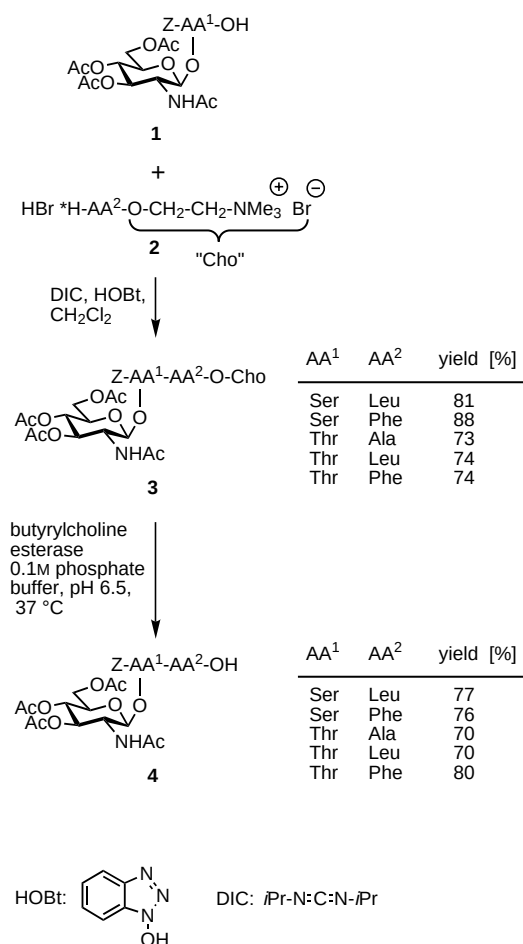
Figure 1. Phosphorylation and glycosylation sites in the transactivation domain of the serum response factor (underlined amino acid symbols). A kinase responsible for the phosphorylation of a certain amino acid is shown. The phosphorylated and glycosylated heptapeptide **17** synthesized in the context of this work is highlighted in the box.

Peptide conjugates embodying the characteristic structural elements of the naturally occurring protein conjugates (namely, phosphates and O-glycosides) may be efficient tools for the study of such biological processes in molecular detail.^[5, 6] However, glycopeptides are very sensitive to acid and base, and phosphopeptides are even more base labile, with carbohydrate and phosphotriester groups being lost in a β -elimination reaction at pH values greater than 8–9.^[5] In the synthesis of glycophosphopeptides these problems potentiate each other and consequently protecting groups have to be used that can be removed with complete selectivity under the mildest conditions, preferably at pH 6–8 and room temperature.^[5] We now report that such labile peptide conjugates can successfully be built up by employing the enzymatic removal of the choline ester and the *p*-phenylacetoxycarbonyloxycarbonyl (PhacOZ) group as the key steps.

The choline ester was previously investigated as a possible blocking group for glycopeptide synthesis, but under the conditions required for its cleavage (pH 10–11) the sensitive O-glycosylated peptides were destroyed.^[7] This problem can, however, be solved efficiently by employing the enzyme butyrylcholine esterase from horse serum for the removal of the protecting group. To investigate the suitability of the enzymatic choline ester cleavage for glycopeptide synthesis O-GlcNAc-modified serine/threonine building blocks **1**^[8] were coupled with amino acid choline esters **2**^[7] to give glycopeptides **3** in high yield. Glycodiester esters **3** were subsequently treated with butyrylcholine esterase in phosphate buffer at pH 6.5 (Scheme 1). In the ensuing enzymatic reaction the C-terminal choline esters were saponified exclusively. An attack on the acetate or the N-terminal urethane groups and an anomerization or β -elimination of the carbohydrate did not occur. The enzyme accepts different amino acid combinations and also tolerates sterically demanding residues such as phenylalanine at the C-terminus. The desired, selectively unmasked, glycodiester esters **4** were isolated in yields of 70–80%. These results clearly indicate that the enzymatic removal of choline esters can be applied advantageously to glycopeptide chemistry. Particularly re-

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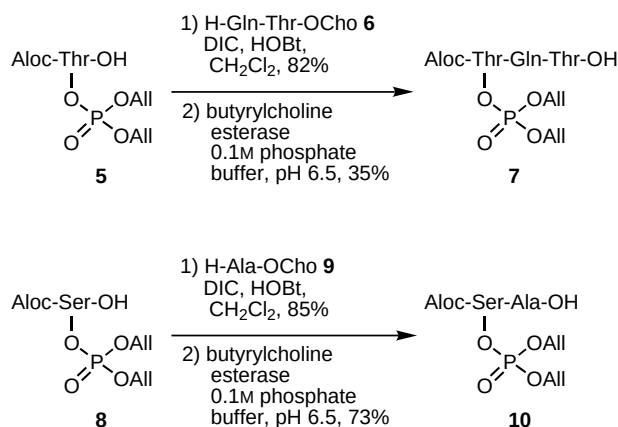


Scheme 1. Synthesis and selective enzymatic deprotection of glycopeptide choline esters by butyrylcholine esterase from horse serum. AA = amino acid, DIC = diisopropylcarbodiimide, HOBT = 1-hydroxy-1H-benzotriazole, HOCho = choline, Z = benzyloxycarbonyl.

warding is the high solubility of the charged substrates in aqueous buffer; a solubility enhancing cosolvent that might denature the enzyme is not required.

The suitability of the choline ester for phosphopeptide synthesis was investigated after coupling the O-phosphorylated threonine **5**^[9] and the serine derivative **8** with the peptide choline ester **6** and the alanine choline ester **9**,^[10] respectively (Scheme 2). The subsequent deprotections under the conditions described above proceeded once more without any undesired attack on the side-chain functionality and a β -elimination of the phosphate group could also not be observed.^[11] Thus, the choline ester group can also be applied advantageously to the synthesis of very base-sensitive phosphopeptides.

To demonstrate the full capacity of the enzymatic protecting group technique a phosphorylated and glycosylated heptapeptide was built up. This peptide (see Figure 1) represents the central part of the multiphosphorylated and glycosylated substructure of the transactivation domain of the serum response factor. In planning the synthesis we intended to combine the enzyme-labile choline ester as a C-terminal blocking function with the enzymatically removable *p*-phenylacetoxycarbonyl urethane group. This urethane

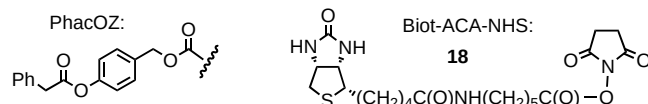


Scheme 2. Synthesis and selective enzymatic deprotection of phosphopeptide choline esters by butyrylcholine esterase from horse serum. All = allyl, Aloc = allyloxycarbonyl.

group can be cleaved under very mild conditions by a saponification of the built-in phenylacetate unit mediated by penicillin G acylase and subsequent spontaneous fragmentation of the phenolate liberated thereby.^[12]

To this end, O-glycosylated PhacOZ-serine **11**^[12] was condensed with serine choline ester **12** and the choline group was removed from the resulting PhacOZ-masked glycopeptide ester in 88 % yield (Scheme 3). Again, in this process no undesired side reaction was observed. The substrate specificity of the choline esterase and the mild reaction conditions guarantee that the glycoside and the phenol ester of phenylacetic acid incorporated into the N-terminal protecting group are not attacked. After elongation of the peptide chain with dipeptide **14** the PhacOZ urethane was selectively cleaved by fragmentation of the urethane blocking group by means of a reaction initiated by penicillin G acylase to yield selectively unmasked glycotetrapeptide **15** in high yield. The peptide chain was further elongated by coupling with phosphopeptide **7**, which had been synthesized by means of selective C-terminal removal of the choline ester protecting group (see Scheme 2). Glycophosphopeptide **16** thereby obtained in high yield was then completely deprotected. To this end, all allylic protecting groups were removed simultaneously by treatment with formic acid/*n*-butylamine in the presence of a Pd⁰ catalyst at room temperature. Subsequently, the *tert*-butyl blocking functions were cleaved with trifluoroacetic acid, and the unmasking of the hydroxyl groups of the carbohydrate was achieved with hydrazine hydrate in methanol.

Finally, the glycophosphopeptide **17** was equipped with a biotin label by selective N-acylation with biotinylaminocaproic acid-*N*-hydroxysuccinimide (**18**; Biot-ACA-NHS; Scheme 3). The biotinylated peptide conjugate **19** thus formed may serve as an efficient molecular probe. The biotin label can be traced by means of complex formation with the protein streptavidin, which is available in fluorescently labeled form or modified with colloidal gold and thus allows the study of a glycosylated and phosphorylated model protein in eukaryotic cells. For instance, such protein conjugates can be detected after microinjection by fluorescence microscopy and electron microscopy.^[13]



In conclusion we have devised a new and efficient strategy for the synthesis of glycosylated and phosphorylated peptides based on the combination of suitable enzyme-labile protecting groups. By means of this methodology acid- and base-sensitive biologically relevant labeled peptide conjugates can be built up, which may open up new avenues of research in biology and bioorganic chemistry. In particular, they should serve to unravel the chemical biology of the serum response factor and the importance of its posttranslational modification in molecular detail.

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**[(TeMe₂)Mn(CO)₄(μ₅-Te)(μ₄-Te)Mn₄(CO)₁₂]⁻:
A Pentacoordinate Bridging Tellurido Ligand
in a Square-Pyramidal Geometry****

Minghuey Shieh,* Horng-Sun Chen, Huey-Yea Yang,
and Chuen-Her Ueng

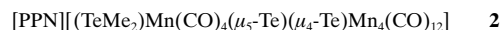
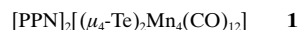
Chalcogen-containing transition metal carbonyl complexes have attracted much attention recently due to their versatile bonding modes and reactivity.^[1] Nevertheless, Mn–Te–CO clusters have remained unknown despite considerable advances in the corresponding chemistry of Fe–Te–CO clusters.^[2, 3] We have discovered a simple and efficient route to this new family of clusters that involves the direct thermal reaction of the common reagents [Mn₂(CO)₁₀] and K₂TeO₃. In contrast to the well-studied Fe system, the new Mn–Te clusters are based on octahedra with μ_4 -Te centers.

The parent cluster **1** (PPN=[P(C₆H₅)₃]₂N⁺) was synthesized by the reaction of K₂TeO₃ with [Mn₇(CO)₁₀] in methanol

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followed by precipitation with [PPN]Cl. The Te-metalated complex **2** was obtained from **1** and MeSO₃CF₃ in CH₂Cl₂. The cluster anions of **1** and **2** represent the first examples of tellurido manganese carbonyl clusters; each contains four manganese–manganese bonds.



A variety of coordination modes are observed for tellurido ligands in transition metal complexes.^[4, 5] Doubly and triply bridging tellurido ligands are common, but quadruply bridging tellurido ligands are much rarer^[1, 2, 4, 5] due to the decreased basicity of the tellurium atom compared to its congeners. The only two previous examples of complexes with the bridging $\mu_5\text{-Te}$ ligands in a pyramidal mode are [Ni₉Te₆(PEt₃)₈] and [Ni₂₀Te₁₈(PEt₃)₁₂].^[6] Here we describe for the first time the ability of the Te²⁻ ligand in the pyramidal TeMn₄ unit to bind another metal center in an axial fashion.

X-ray structure analysis shows that the anion of **1** has an octahedral geometry with two $\mu_4\text{-Te}$ ligands and four seven-coordinate manganese atoms (Figure 1).^[7] Structurally char-

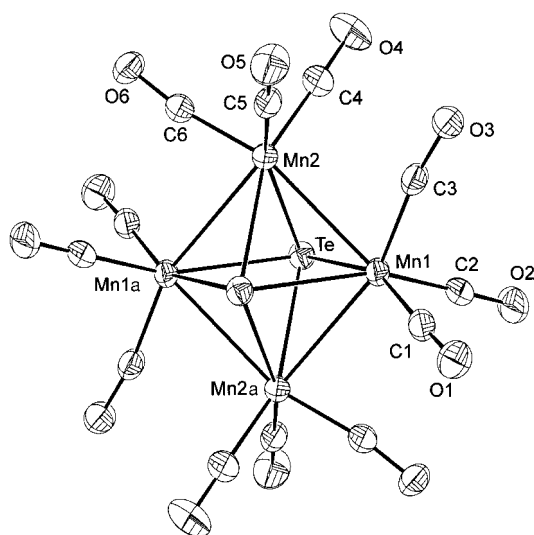


Figure 1. Crystal structure of the dianion of **1** (30% thermal ellipsoids). Selected bond lengths [Å] and angles [°]: Te–Mn1 2.6363(8), Te–Mn1a 2.6355(6), Te–Mn2 2.6482(8), Te–Mn2a 2.6444(7), Mn1–Mn2 2.7976(8), Mn1–Mn2a 2.8237(9); Mn1–Te–Mn1a 97.42(2), Mn2–Mn1–Mn2a 90.40(2), Mn1–Mn2–Mn1a 89.60(2).

acterized complexes containing $\mu_4\text{-Te}$ ligands include [(\mu₄-Te)₂Ru₄(CO)₁₁],^[8] [(\mu₄-Te)₂Ru₂Fe₂(CO)₁₁],^[9] [(\mu₄-Te)(\mu₃-Te)-Fe₂Os₃(CO)₁₇],^[10] [[Fe₂(CO)₆](\mu₄-Te)(\mu₃-Te){Ru₃(CO)₁₁}],^[11] [(\mu₄-Te)₂Co₄(CO)₆(Et₃P)₄],^[12] [Co₁₁Te₇(CO)₁₀]²⁻,^[13] [Ru₆(Te₂)₇(CO)₁₂]²⁻,^[14] and [(\mu₄-Te)₂Fe₄(CO)₁₁].^[15] However, the lone pair of the $\mu_4\text{-Te}$ atom was not shown to be chemically active, and its basicity has not been demonstrated in transition metal complexes.

The question arises whether the basicity of Te is affected by the transition metal centers. Increased basicity of Te might be expected owing to the more electropositive character of Mn. We therefore attempted the alkylation of **1**. Careful methyl-

ation of **1** with MeSO₃CF₃ led to the isolation of the unexpected Te-metalated complex **2**, in which a quadruply bridging tellurido ligand is externally bonded to a Mn(CO)₄-TeMe₂ fragment, presumably by formation of a Te→Mn donor–acceptor bond. The structure of **2** was determined by single-crystal X-ray analysis (Figure 2).^[7] The ¹H NMR spec-

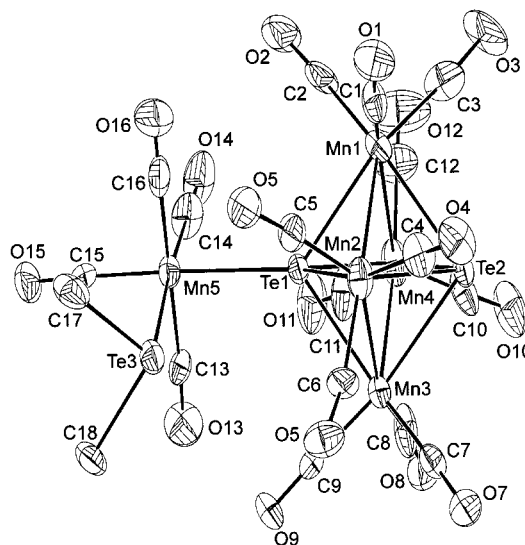


Figure 2. Crystal structure of the anion of **2** (30% thermal ellipsoids). Selected bond lengths [Å] and angles [°]: Te1–Mn1 2.601(3), Te1–Mn2 2.577(3), Te1–Mn3 2.602(3), Te1–Mn4 2.577(3), Te1–Mn5 2.643(3), Te2–Mn1 2.632(3), Te2–Mn2 2.647(3), Te2–Mn3 2.638(3), Te2–Mn4 2.642(4), Te3–Mn5 2.614 (3), Te3–C av 2.15(2), Mn1–Mn2 2.858(4), Mn1–Mn4 2.837(5), Mn2–Mn3 2.810(5), Mn3–Mn4 2.854(5); Mn2–Mn1–Mn4 89.4(1), Mn1–Mn2–Mn3 90.5(1), Mn2–Mn3–Mn4 90.0(1), Mn1–Mn4–Mn3 90.1(1).

trum of the anion of **2** showed signals at δ = 2.18 and 2.14, similar to that of the methyltellurido ligands in [Fe₄(Te₂)₂(Te)₂(TeMe₂)₂(CO)₈]²⁻ (δ = 2.2).^[16]

A detailed study showed that the mononuclear cationic species [Mn(CO)₄(TeMe₂)]⁺ was produced in the course of this reaction, which was monitored by IR spectroscopy.^[17] Hence, it is reasonable to assume that the anion of **2** results from the interaction of the dianion of **1** through the $\mu_4\text{-Te}$ atom with the moiety [(TeMe₂)Mn(CO)₄]⁺, which could be derived from the fragmentation of cluster **1** upon methylation. However, the role of MeSO₃CF₃ is crucial because the attempted reaction of **1** with [Mn(CO)₅]Br failed to give the expected metalated product.

There are several interesting features in **2**. First, the geometry of the $\mu_5\text{-Te}$ ligand is close to square pyramidal, but slight differences in the Te1–Mn distances and the Mn5–Te1–Mn angles cause a deviation from perfect C_{4v} symmetry. The crystal packing of **2** shows no significant interaction between the cation and the anion or between the anions. The bulky Te₂Mn₄(CO)₁₂ group and the TeMe₂ moiety are *cis* rather than *trans* to each other. This is explained in terms of the *trans* influence of the Te₂Mn₄(CO)₁₂ moiety. Since Te₂Mn₄(CO)₁₂ is a not a strong σ -bonding ligand, a strongly σ bonding CO ligand is preferred to a TeMe₂ group in the position *trans* to the Te₂Mn₄(CO)₁₂ ligand. The ($\mu_4\text{-Te}$)–Mn

bond lengths of **2** and **1** are almost equal (2.632–2.648 Å). The (μ_5 -Te)–Mn bonds of **2** were expected to be significantly longer, but the opposite effect is observed: these bonds are unusually short (2.577–2.602 Å).

We have discovered a new family of Te–Mn–CO clusters and a novel μ_5 -Te^{2–} bonding mode. Our results strongly suggest the existence of μ_5 -Te^{2–} ligands in other early transition metal clusters, and the investigation of this possibility is currently underway.

Experimental Section

All experiments were performed under a nitrogen atmosphere.

1: To a mixture of [Mn₂(CO)₁₀] (0.725 g, 1.86 mmol) and K₂TeO₃·H₂O (0.236 g, 0.930 mmol) was added MeOH (40 mL). The solution was heated to reflux (110 °C) for 98 h to give a red-brown solution, which was filtered and concentrated. Dropwise addition of a solution of [PPN]Cl (0.705 g, 1.23 mmol) in MeOH precipitated a solid, which was washed several times with deionized water. The residue was recrystallized from CH₂Cl₂/MeOH several times to give 0.44 g (0.233 mmol) of **1** (50% yield based on Te). IR (CH₂Cl₂): $\tilde{\nu}_{\text{CO}}$ = 1941 (s), 1884 cm^{–1} (m); elemental analysis calcd for C₈₄H₆₀Mn₄N₂O₁₂P₄Te₂: C 53.43, H 3.20, N 1.48; found: C 53.44, H 3.15, N 1.47. Compound **1** is soluble in CH₂Cl₂, THF, and MeCN, but insoluble in hexanes, Et₂O, and MeOH. Crystals suitable for X-ray analysis were grown from a solution in MeCN.

2: MeSO₃CF₃ (0.283 mL, 2.20 mmol) was added to **1** (0.520 g, 0.275 mmol) in CH₂Cl₂ (30 mL) at 0 °C. The solution was allowed to warm to room temperature and stirred for 26 h. The resulting solution was filtered, the solvent was removed under vacuum, and the residue was extracted with CH₂Cl₂ and then recrystallized from CH₂Cl₂/hexane several times to give 0.210 g (0.130 mmol) of **2** (78% yield based on Te). IR (THF): $\tilde{\nu}_{\text{CO}}$ = 2078 (w), 2008 (m), 1960 (s), 1905 cm^{–1} (m); elemental analysis calcd for C₃₄H₃₆Mn₅NO₁₆P₂Te₃: C 38.74, H 2.17, N 0.84; found: C 38.72, H 2.09, N 0.94; ¹H NMR (400 MHz, CDCl₃, 295 K): δ = 2.18, 2.14. Compound **2** is soluble in Et₂O, CH₂Cl₂, THF, MeCN, and MeOH, but insoluble in hexane. Crystals suitable for X-ray analysis were grown from a solution in Et₂O/CH₂Cl₂.

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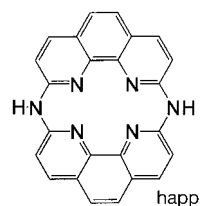
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- [7] a) Crystal data for **1**: C₈₄H₆₀Mn₄N₂O₁₂P₄Te₂, M_r = 1888.24, triclinic, space group $P\bar{1}$, Z = 1, a = 12.200(2), b = 13.158(3), c = 13.791(2) Å, α = 66.96(2), β = 89.63(2), γ = 77.46(2)°, V = 1981.0(6) Å³, ρ_{calcd} = 1.583 g cm^{–3}, MoK α radiation, λ = 0.70930 Å, μ = 1.46 mm^{–1}. A total of 6973 unique reflections were collected on a Nonius CAD-4 diffractometer at 298 K in the range $2.0 < 2\theta < 50^\circ$ with $\theta/2\theta$ scans, and an absorption correction by azimuthal ψ scans was applied. The structure was solved by direct methods and refined (NRCC-SDP-VAX packages) by full-matrix least-squares methods to R = 0.025 and R_w = 0.022 for 5885 observed reflections ($I > 2.5\sigma(I)$) and GOF = 2.27. b) Crystal data for **2**: C₃₄H₃₆Mn₅NO₁₆P₂Te₃, M_r = 1674.30, triclinic, space group $P\bar{1}$, Z = 2, a = 11.491(4), b = 16.581(5), c = 18.032(4), α = 112.20(3), β = 105.19(3), γ = 92.86(3)°, V = 3027.0(2) Å³, ρ_{calcd} = 1.837 g cm^{–3}, MoK α radiation, λ = 0.70930 Å, μ = 2.51 mm^{–1}. A total of 10625 unique reflections were collected on a Nonius CAD-4 diffractometer at 298 K in the range $2.0 < 2\theta < 50^\circ$ by using $\theta/2\theta$ scans, and an absorption correction by azimuthal ψ scans was applied. The structure was solved by direct methods and refined (NRCC-SDP-VAX packages) by full-matrix least-squares to R = 0.053 and R_w = 0.046 for 4109 observed reflections ($I > 6\sigma(I)$) and GOF = 2.84. c) Further details of the crystal structure investigations may be obtained from the Fachinformationszentrum Karlsruhe, D-76344 Eggenstein-Leopoldshafen, Germany (fax: (+49) 7247-808-666; e-mail: crysdata@fiz-karlsruhe.de) on quoting the depository numbers CSD-408889 (**1**) and -408890 (**2**).
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A New Co^{II} Complex as a Bulge-Specific Probe for DNA **

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Kuo-Shen Chuang, Chi-Fong Luo, and Wen Jwu Wang*

Bulge structures in nucleic acids are crucial motifs in the recognition of DNA by proteins that bind nucleic acids in biological systems.^[1] Despite their importance, the structural details of only a few DNA bulges have been reported.^[2] These structures are expected to be less stable. The melting temperature of DNA containing a single-base DNA bulge is destabilized by 2.0–3.6 °C relative to that of the corresponding totally Watson–Crick base-paired structure.^[3]

Although many synthetic chemical nucleases have been developed to explore a variety of DNA/RNA structures,^[4] probes specific for the DNA bulge site are still rare. Recently, two types of DNA-targeting antitumor drugs, neocarzinostatin chromophore (NCS-C) and bleomycin (BLM), were shown to have a preference for a DNA nick site near the bulge.^[5] Likewise, DNA intercalators, such as ethidium bromide^[6] and [Pt(terpy)(het)]⁺ (terpy = 2,2':6',2''-terpyridine, het = "2-hydroxyethylenethiol" = 2-sulfanylethanol),^[7] are also known to target specifically to the bulge site of nucleic acids.^[6] However, these molecules bind to the double-stranded DNA domains so strongly that it is inevitable that they induce an alteration in the DNA conformation in the locale where they bind. Here, we report the novel octahedral Co^{II} complex [Co^{II}(tfa)₂(happ)] (tfa = trifluoroacetate), which can serve as a specific probe for DNA bulges through its ability toward specific oxidative cleavage of these structures. This complex also has the advantage that it exhibits only a low affinity towards double-stranded DNA. Moreover, it does not show the cleavage reactivity toward single-stranded DNA.



the happ ligand was prepared by a modification of the procedure^[8] for the condensation of two molecules of 2,9-dichloro-1,10-phenanthroline at 200 °C under a flowing ammonia gas.^[9] Its Co^{II} complex was prepared by treating the happ ligand with cobalt(II) acetate in TFA/MeOH. The X-ray crystal structure of the [Co^{II}(tfa)₂(happ)] complex (Figure 1)

reveals that the complex contains two fused 1,10-phenanthroline moieties, with all four pyridinium nitrogen atoms sitting on the same coordination plane, and two labile axial tfa ligands.^[10] The average Co–N bond length is approximately 1.86 Å. The EPR spectrum of the Co^{II} complex gave a *g*_{av} value of 2.005–2.331 in methanol, which is indicative of an octahedral Co^{II} complex. The addition of one equivalent of pyridine rapidly displaces one of the axial tfa ligands under ambient conditions, as monitored by EPR spectroscopy, which suggests that the tfa ligands are labile enough to be substituted by nucleophiles.

Since the 1,10-phenanthroline ligand in the tetrahedral [Cu^I(phen)₂] complex is known to intercalate into DNA, it might be expected that [Co^{II}(tfa)₂(happ)], which also contains this flat macrocyclic ligand, might also act as a DNA intercalator. However, in the absence of H₂O₂ and under noncleavage conditions (see below), neither the topoisomerase I assay^[11] showed any sign of DNA unwinding associated with DNA intercalation, nor did the native gel mobility shift

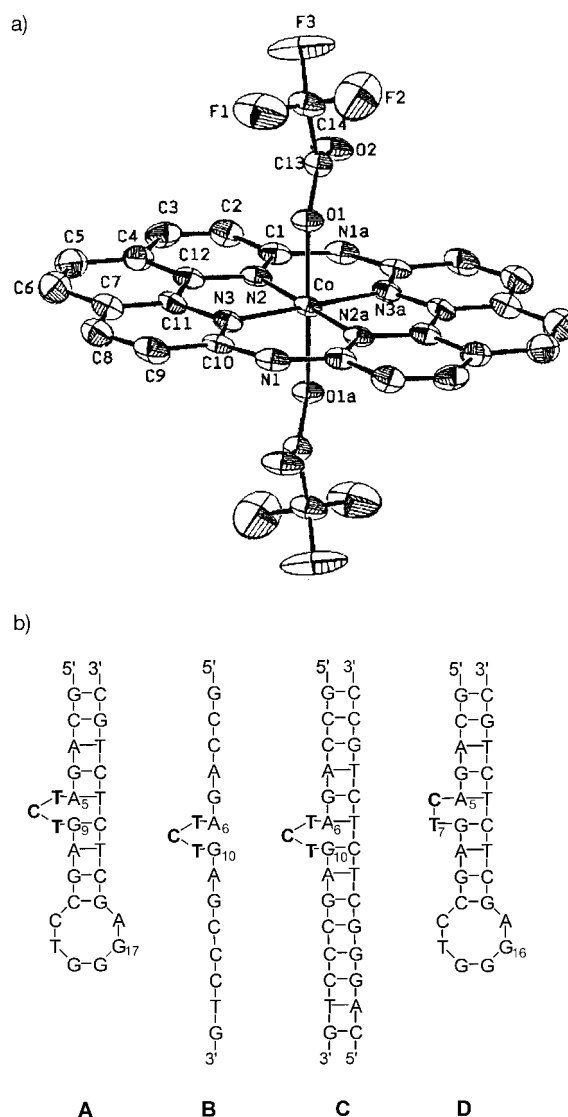


Figure 1. a) ORTEP representation of [Co^{II}(tfa)₂(happ)] in the crystal showing the numbering of the atoms. b) The various DNA substrates used in this study.

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assay reveal the presence of high-molecular-weight bands attributable to the $[\text{Co}^{\text{II}}(\text{happ})]^{2+}$ -DNA adducts in polyacrylamide gel electrophoresis (PAGE). In addition, the melting temperature of calf thymus DNA (60 μM per nucleotide) incubated with $[\text{Co}^{\text{II}}(\text{happ})]^{2+}$ (8 μM) displayed only a modest change of 0.5–1.0 $^{\circ}\text{C}$. The incubation of DNA with ethidium bromide under similar conditions resulted in a DNA adduct with a melting temperature T_m that differed by 12–13 $^{\circ}\text{C}$ from the control. The DNA-binding constant of $[\text{Co}^{\text{II}}(\text{happ})]^{2+}$ with calf thymus DNA was determined by spectral titration at 399 nm as approximately $1 \times 10^4 \text{ M}^{-1}$ in 10 mM phosphate buffer at pH 7. In contrast, the corresponding four-coordinated copper complex, $[\text{Cu}^{\text{II}}(\text{happ})]^{2+}$, is a stronger intercalator in DNA with a binding constant of 10^5 M^{-1} , as determined by the ethidium displacement assay.^[9] It is evident that the axial ligands of the octahedral $[\text{Co}^{\text{II}}(\text{happ})(\text{X})_2]$ ($\text{X} = \text{tfa}$ or H_2O) complex provides a significant steric hindrance to limit the insertion of the Co^{II} complex between the adjacent bases of the double-stranded DNA.

The $[\text{Co}^{\text{II}}(\text{tfa})_2(\text{happ})]$ complex cleaves DNA catalytically in the presence of H_2O_2 . Reactions of $[\text{Co}^{\text{II}}(\text{happ})]^{2+}$ (0.6 μM) with various 5'-end ^{32}P -labeled oligonucleotides^[12] (4–5 μM) were performed in the presence of H_2O_2 (0.005–0.05 %) for 5 min at ambient temperature. The DNA fragments produced were analyzed by high resolution PAGE. These experiments were carried out on the 27-mer DNA substrate 5'-GCA-GATCTGA-GCCTGGGAGC-TCTCTGC-3', whose secondary structure is comprised of a three-base bulge, a six-base single-stranded loop, as well as double-stranded domains (**A** in Figure 1). This DNA sequence was based on the RNA hairpin from the *trans*-activation response element (TAR-RNA).^[5, 13–14] After piperidine treatment the strand scission was found to occur predominantly at the DNA-bulge site (T6, C7, and T8) and only very weakly at the DNA hairpin loop (C13–A18), despite the fact that both sites contain the same DNA 5'-CTG-3' sequence, as shown in Figure 2.

Minor cleavage was also found at the sites near the flanking junctions of these nucleobases. Interestingly, no significant oxidative cleavage was observed at the 5'-GGG-3' region in the DNA hairpin loop. The guanine residues in this DNA loop have been reported to be the sites most susceptible to oxidative cleavage as a result of its accessibility^[13] and low reduction potential.^[15] The equivalent DNA cleavage in the absence of H_2O_2 required a higher Co^{II} complex concentration (>50 μM) as well as a longer reaction time (>40 min). Moreover, competitive inhibition by the DNA intercalator $[\text{Pt}(\text{terpy})(\text{het})]^+$, which targets the DNA bulge site, was found to reduce the extent of cleavage at the bulge site remarkably. These observations support the bulge-specific DNA cleavage by the $[\text{Co}^{\text{II}}(\text{happ})]^{2+}$ complex in the presence of H_2O_2 .

The complex $[\text{Co}^{\text{II}}(\text{tfa})_2(\text{happ})]$ shows a low reactivity toward single-stranded DNA. When $[\text{Co}^{\text{II}}(\text{happ})]^{2+}$ was allowed to react with a single-stranded 16-mer DNA containing the sequence 5'-GCCAGATCTG-AGCCTG-3' (**B** in Figure 1) in the presence of H_2O_2 under identical conditions, no specific cleavage was observed at the 5'-TCT-3' site, even when a 20-fold excess of the Co^{II} complex was used. Upon annealing this single-stranded DNA with its complementary

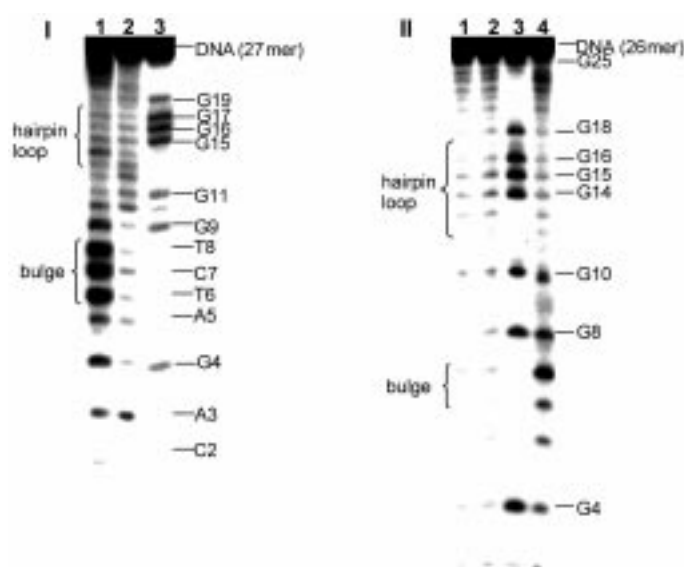


Figure 2. The autoradiogram of a denaturing polyacrylamide gel (20 %; 7 M urea) showing the results of oxidation of a 5'-end-labeled DNA (4 μM) with $[\text{Co}^{\text{II}}(\text{tfa})_2(\text{happ})]$ activated by H_2O_2 (0.005 %) in 10 mM sodium phosphate buffer (pH 6.9) at 25 $^{\circ}\text{C}$ for 5 min. All reactions were heated with 0.7 M piperidine at 90 $^{\circ}\text{C}$ for 30 min. I) DNA substrate **A** (see Figure 1). Lane 1: DNA with Co^{II} (0.6 μM) and H_2O_2 ; lane 2: DNA with H_2O_2 ; lane 3: G-reaction of the Maxam–Gilbert sequence. II) DNA substrate **D** (see Figure 1). Lane 1: DNA only; lane 2: DNA with H_2O_2 ; lane 3: G-reaction of the Maxam–Gilbert sequence; lane 4: DNA with Co^{II} (0.6 μM) and H_2O_2 .

DNA strand, a three-base bulge DNA duplex (**C** in Figure 1) was formed, as monitored by native PAGE. An enhanced DNA cleavage was observed at the 5'-TCT-3' site in the DNA bulge region upon subjecting this duplex to the same DNA cleavage reaction with the $[\text{Co}^{\text{II}}(\text{happ})]^{2+}$ complex. These results further support the conclusion that $[\text{Co}^{\text{II}}(\text{happ})]^{2+}$ serves as a DNA bulge-specific cleavage reagent without the reactivity toward the corresponding domain in the single-stranded DNA.

When magnesium monoperoxyphthalic acid (MMPP) or oxone (KHSO_5) were used instead of H_2O_2 , no significant DNA cleavage was observed with supercoiled plasmid DNA. Since the addition of superoxide dismutase and D_2O into the reaction medium did not reduce the concentration of circular DNA (Type II) formed in the DNA cleavage products mediated by this Co^{II} complex, it is unlikely that superoxide and singlet oxygen species are involved in this process. However, when mannitol, a hydroxyl radical scavenger, was added into the DNA-cleavage assay medium, the amount of circular DNA (type II) was found to be reduced by half. These results suggest that the hydroxyl radical, induced by the reaction of $[\text{Co}^{\text{II}}(\text{happ})]^{2+}$ with H_2O_2 , is likely to be one of the reactive species responsible for the DNA cleavage. In general, the hydroxyl radical is a diffusible species and lacks specificity in the oxidative DNA cleavage. Nevertheless, the DNA-strand scission induced by $[\text{Co}^{\text{II}}(\text{happ})]^{2+}$ was found to be directed specifically at the bulge site. Consequently, this specific DNA cleavage must be derived from a specific interaction between the Co^{II} complex, most probably through their ligands, and the bulge region in the DNA. Recently, the solution structure of a two-base DNA bulge has been

determined by NMR spectroscopy. This structure has been reported to produce a triangular prism pocket to bind with the enediyne analogues.^[16] Indeed, when the two-base bulge of a 26-mer DNA containing one base (T6) less than the three-base bulge of a 27-mer DNA (**D** in Figure 1) was used as the DNA substrate, enhanced cleavage activity and specificity was observed towards the T7 residue of the 26-mer DNA bulge shown in Figure 2. Thus, the shape and size of the binding pocket of the DNA bulge are probably important controlling factors in the specific recognition by the $[\text{Co}^{\text{II}}(\text{tfa})_2(\text{happ})]$ complex. The addition of the intercalator $[\text{Pt}(\text{terpy})(\text{het})]^+$ to DNA substrate **D**^[12] was, as for DNA substrate **A**, found to significantly inhibit the cleavage at the bulge site induced by $[\text{Co}^{\text{II}}(\text{happ})]^{2+}$ in the presence of H_2O_2 .

This study provides the first attempt in utilizing a novel Co^{II} complex to show specific targeting and cleavage of a DNA bulge site. No significant reactivity was observed toward the corresponding sequence in a single-stranded DNA region. We assume that the intercalation of the 1,10-phenanthroline ligand of $[\text{Co}^{\text{II}}(\text{tfa})_2(\text{happ})]$ toward double-stranded DNA is probably inhibited by the two axial ligands in this octahedral complex. On the other hand, it is possible that $[\text{Co}^{\text{II}}(\text{tfa})_2(\text{happ})]$ recognizes a binding pocket in the DNA bulge of a specific shape and size, and this recognition may be responsible for the observed bulge-specific DNA cleavage by the diffusible hydroxyl radical produced from the reaction between the cobalt(II) complex and H_2O_2 .

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- [10] Crystal data for $[\text{Co}^{\text{II}}(\text{tfa})_2(\text{happ})] \cdot 4\text{tfa}$: $\text{C}_{36}\text{H}_{14}\text{N}_6\text{O}_{12}\text{F}_{18}\text{Co}$, crystal dimensions $0.05 \times 0.25 \times 0.25 \text{ mm}^3$, triclinic, space group $P\bar{1}$, $a = 8.5242(2)$, $b = 8.850(3)$, $c = 14.293(5) \text{ \AA}$, $\alpha = 79.57(3)^\circ$, $\beta = 79.00(3)^\circ$, $\gamma = 88.81(3)^\circ$, $V = 1040.9(6) \text{ \AA}^3$, $Z = 1$, $F(000) = 558$, $\rho_{\text{calc}} = 1.792 \text{ g cm}^{-3}$, $\mu = 8.432 \text{ cm}^{-1}$, $R_{\text{f}} = 0.072$, $R_{\text{w}} = 0.072$, $\text{GOF} = 1.21$, $2\theta_{\text{max}} = 45^\circ$, $T = 295 \text{ K}$, $\text{MoK}\alpha$ ($\lambda = 0.71073 \text{ \AA}$), 2944 measured reflections, of which 1654 had $I_0 > 2\sigma(I_0)$, Nonius diffractometer, NRCVAX refinement program (E. J. Gabe, Y. Le Page, F. L. Lee in *Crystallographic Computing 3: Data Collection, Structure Determination, Proteins and Database* (Eds.: G. M. Sheldrick, C. Kreuger, R. Goddard), Clarendon, Oxford, **1985**, pp. 167–175). 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-108608. 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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Functional Monolayers with Coordinatively Embedded Metalloporphyrins**

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In memory of Jacqueline Libman

The expression of porphyrin functions in supramolecular systems depends on their immediate environment and mutual orientation.^[1] Incorporation of porphyrins into artificial bilayers^[2] or their assembly onto solid supports^[3] neither resulted in uniform orientation of the chromophores, nor

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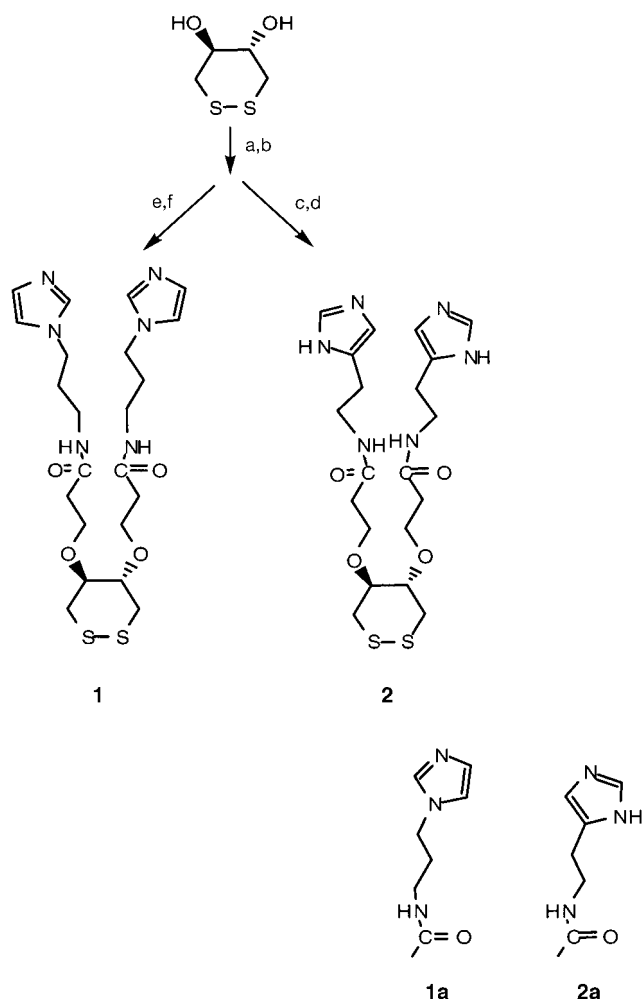
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prevented stacking interactions and chromophore aggregation. In nature ligands tend to coordinate axially to metalloporphyrins. It may be that by mimicking these biological trends one could locate the porphyrin in a well-defined environment while avoiding stacking and aggregation. If such an assembly would also retain the capability to transfer electrons to a conducting surface it could be used in future optoelectronic and light-harvesting applications.

Herein we present a novel arrangement of iron porphyrins on gold surfaces. The monolayer prepared differs from other systems in that the metalloporphyrin is not in direct contact with the surface, but rather is held in a perpendicular orientation through an interconnected ligand that also protects its open faces, thus eliminating π stacking to other porphyrin moieties. The bifunctional ligands **1** and **2** (Scheme 1) were



Scheme 1. Schematic representation of the bifunctional ligands **1** and **2** and their respective model compounds **1a** and **2a**. Synthesis: a) acrylonitrile/ OH^- ; b) HCl ; c) pentachlorophenol; d) histamine; e) oxalyl chloride; f) 1-(3-aminopropyl)imidazole.

designed to integrate two essential components: A cyclic disulfide for surface attachment,^[4] and two extended imidazolyl residues capable of binding metalloporphyrins in an axial coordination mode.

The cyclic disulfide offers several advantages as an anchor: It prohibits chain segregation even upon possible S–S bond

cleavage during self assembly, and reduces the degrees of freedom for tilting of surface-attached molecules towards the S–S axis.^[4a] The two imidazole rings provide the “hinges” that hold the porphyrin moiety in a defined orientation, while its axial coordination to the central Fe^{3+} ion masks the porphyrin from neighboring molecules. This molecular arrangement dictates a perpendicular orientation of the metalloporphyrins with respect to the S–S bond as well as to the surface (see Figure 1, bottom). Molecules **1** and **2** differ in the imidazolyl

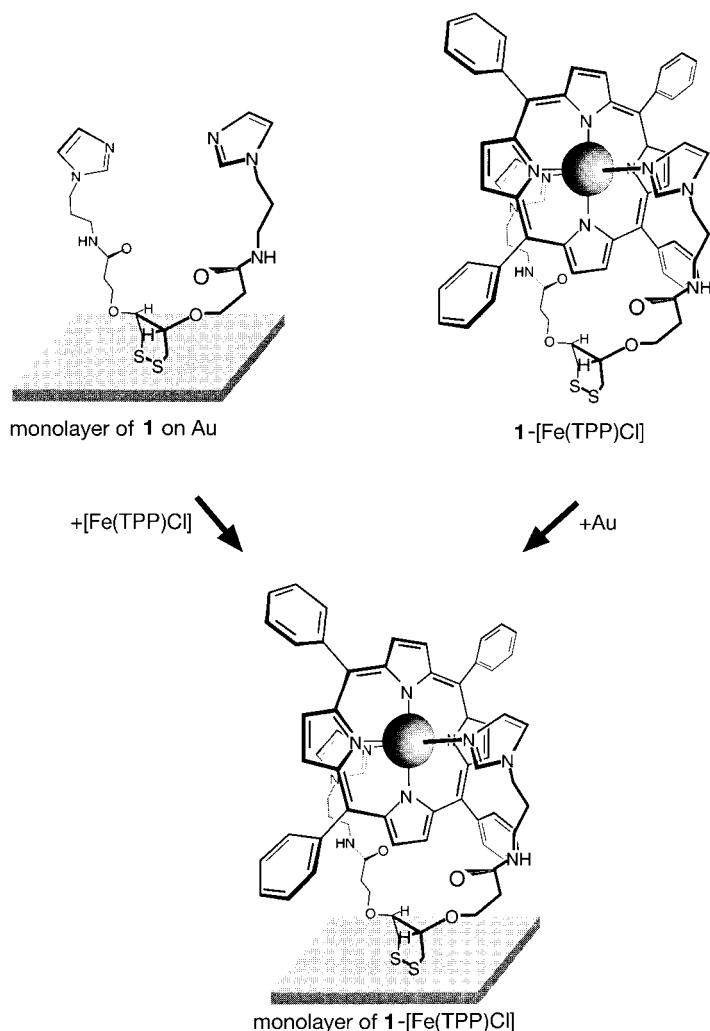


Figure 1. Schematic illustration of the stepwise (left) and preformed (right) methods for the adsorption of the complex **1**-[Fe^{III}(TPP)Cl] onto a gold surface.

residues, possessing either *N*-alkyl (**1**) or free NH groups (**2**), and the length of the chains connecting the cyclic disulfide anchor and the imidazolyl groups.

The formation of metalloporphyrin complexes of **1** and **2** and of their single-chain model compounds **1a** and **2a** was investigated in solution. The UV titration of iron porphyrins with ligands **1**, **1a**, **2**, and **2a** provided the formation constants K_1 and β_2 (Table 1).^[5] The β_2 values of the dipodal ligands are higher than those for the model compounds. Titration of iron tetraphenylporphyrin chloride [Fe^{III}(TPP)Cl] with **1** and **2** showed no formation of the monoimidazolyl complex, although such intermediates were observed with the single-stranded *N*-alkylimidazole models **1a** and with *N*-methyl-

Table 1. Binding constants for the axial coordination of the iron porphyrin [Fe^{III}(TPP)Cl] by imidazole ligands **1**, **1a**, **2**, and **2a** in DMF.

Ligand	K_1 [M ⁻¹]	β_2 [M ⁻²]
1a	ca. 50	4.02×10^3
2a	–	1.32×10^4
1	–	1.09×10^4
2	–	2.19×10^4

[a] The binding constants were calculated with respect to the number of imidazolyl residues present in the system.

imidazole.^[5a] These results demonstrate not only the well-known diimidazolyl complex formation with [Fe^{III}(TPP)Cl], but also the strong tendency of the dipodal ligands **1** and **2** to undergo intramolecular reactions, to form complexes of 1:1 stoichiometry (chelation effect).^[5c]

The ¹H NMR spectra of the low-spin [Fe^{III}(TPP)Cl] complexes of ligands **1** and **2** showed a single set of signals, which indicated the formation of single complex species. Complete assignment of the proton signals was accomplished by comparison with literature values^[6] and from 2D COSY NMR spectra recorded at –30 °C. The chemical shifts suggest an axial coordination of each imidazolyl residue to the iron porphyrin and a perpendicular orientation relative to each other.^[7] Measurements between 295 K and 193 K revealed separate signals for the diastereotopic CH₂ protons in the chain of **1**–[Fe^{III}(TPP)Cl] and **2**–[Fe^{III}(TPP)Cl],^[8] which is attributed to a slowing down of the ligand exchange process.^[9] Nonequivalent pyrrolic proton signals,^[10] which arise from an anisotropy of the ligand-bound porphyrin ring, strongly suggests there is restricted rotation of the metalloporphyrin around the Fe–N_{im} bonds (the “hinges”) in the shorter **2**–[Fe^{III}(TPP)Cl], but not in the longer **1**–[Fe^{III}(TPP)Cl] complex. A representation of the **1**–[Fe^{III}(TPP)Cl] complex in solution, consistent with the experimental data and molecular modeling studies,^[11] is shown in Figure 1 (top right).

Two methods were used to prepare metalloporphyrin monolayers of **1**–[Fe^{III}(TPP)Cl] and **2**–[Fe^{III}(TPP)Cl] on Au (1000 Å, evaporated on (111)Si,^[12]): 1) One-step formation (Figure 1, right), where the preformed, characterized (see above) ligand–metalloporphyrin complex is assembled directly onto the gold surface; 2) stepwise formation (Figure 1, left), which is achieved by preparing the ligand monolayer followed by introduction of a metalloporphyrin capable of axial ligation to the imidazolyl groups. The possibility of inter-ligand complexation in the stepwise method cannot be ruled out;^[13] characterization of the complex in solution (as described above) strongly suggests that this configuration is unfavorable when a preformed complex is used.

The thickness of the monolayers were measured ellipsometrically^[4a] (Table 2), and also calculated from a theoretical model.^[14, 15] Good agreement between the measured and the theoretical monolayer thickness is obtained for **1**–[Fe^{III}(TPP)Cl]. The ellipsometry of the adsorbed ligand **2** suggests formation of a bilayer, which is attributed to ligand–ligand hydrogen bonding.^[16] Successive treatment with an EtOH/H₂O solution (1/1; 25 min) resulted in the removal of the second layer (breaking of the hydrogen bonds), as evident from a change in the thickness of the monolayer to $9.4 \pm$

Table 2. Theoretical and experimental thickness d of monolayers of the bifunctional ligands and their iron porphyrin complexes.

Compound	Adsorption procedure	d [Å]	
		calcd	found
1	–	13–14	12 ± 2
1 –[Fe ^{III} (TPP)Cl]	preformed	17–18	17 ± 2
1 –[Fe ^{III} (TPP)Cl]	stepwise	17–18	17 ± 3
2	–	11–12	16 ± 1
2 –[Fe ^{III} (TPP)Cl]	preformed	16–17	15 ± 1
2 –[Fe ^{III} (TPP)Cl]	stepwise	16–17	14 ± 1

1 Å.^[17] The bilayer appears to break upon introduction of [Fe^{III}(TPP)Cl] and form the complex monolayer and free excess ligand. In both cases (namely with both methods) and using the two ligands the binding of the porphyrin adds approximately 4–5 Å to the monolayer thickness, which is approximately equal to half the diameter of a porphyrin ring and consistent with a perpendicular orientation of the porphyrin.

Analysis of the systems by X-ray photoelectric spectroscopy (XPS) showed the expected elements on the surface (Table 2). Both complex monolayers (but not the ligand monolayers) showed the iron peak at 711 eV. Transmission spectroscopy in the visible region of preformed and stepwise-formed monolayers of **1**–[Fe^{III}(TPP)Cl] on thin gold films (approximately 25 Å on mica)^[18] showed peaks at 424 and 554 nm, which is similar to those measured for the same complex in solution (our results) and for other [Fe^{III}(TPP)Im₂] complexes.^[5a] The absorption peaks of the stepwise-formed **1**–[Fe^{III}(TPP)Cl] monolayer are broader and smaller than those of the preformed monolayer, which is in agreement with the voltammetric results (see below).

It is evident from steric considerations (Figure 1) that the binding of the disulfide group to the gold surface forces the porphyrin rings into a perpendicular orientation (hence isolation), even if a certain tilt exists. This conclusion is supported by 1) the 1:1 ligand:iron porphyrin stoichiometry and the axial coordination of the imidazole to the porphyrin found in the complex in solution, 2) the thickness of the complex monolayer relative to that of the ligand monolayer, and 3) the existence of only bis-ligated species on the surface.

Cyclic voltammetry (CV) of **1**–[Fe^{III}(TPP)Cl] monolayers on gold (Figure 2) shows peaks that generally agree with previous measurements of porphyrinic systems in solution,^[3c, 19] while CV of a monolayer of **1** shows no identifiable peaks in the measured range.^[20] The peak–peak separation for the preformed complex monolayer is approximately 200 mV smaller than that of the monolayer formed stepwise, which indicates a more facile electron transfer for the former. The difference in peak height between the reduction and oxidation waves does not reflect chemical irreversibility, but rather a smearing of the oxidation wave, as evident from repetitive cycles (not shown). This is likely to indicate a certain distribution of structures in the reduced state.

Integration of the CV reduction peaks (Figure 2), as well as the absorbance intensity in the UV/Vis spectra (see above), indicate a considerably smaller amount of bound iron porphyrin in the monolayers formed stepwise, and shows

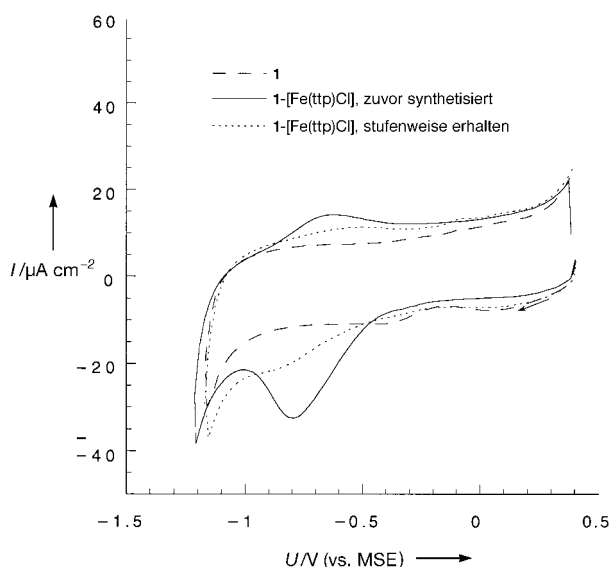


Figure 2. Cyclic voltammetry (first scan) of **1** and **1**-[Fe^{III}(TPP)Cl] monolayers on gold in aqueous 0.1M Na₂SO₄ at 200 mV s⁻¹ (using a Solartron 1286 potentiostat, reference electrode: Mercurous sulfate electrode (MSE); 0.400 V versus the standard calomel electrode)).

the advantage of the one-step procedure in obtaining stoichiometric amounts of bound iron porphyrin. This advantage reflects the different nature of the two self-assembled monolayers. The stepwise method produces monolayers that satisfy the lower energy arrangement of the ligand molecules, and are only partially useful for iron porphyrin incorporation. Self-assembly of the preformed complex, on the other hand, allows the massive complex molecules to arrange optimally in the monolayer, by taking into account the necessity for intramolecular interactions. This also implies that the density of ligand molecules is higher in the monolayer formed stepwise, which accounts for the similar ellipsometric thickness measured for the two (Table 2) despite the different number of bound porphyrins. This difference in packing arrangements would also account for the difference, noted above, in voltammetric peak–peak separation between the preformed and stepwise-formed monolayers.

The systems presented here allow the anisotropic assembly of metalloporphyrins perpendicular to conducting surfaces while avoiding stacking of the porphyrin rings. These unique features may be of substantial importance, for example, in solar energy conversion systems where they may help to reduce lateral energy dissipation while retaining the possibility of electron transfer between the surface and the chromophore (as evident from the electrochemical results). Modifications of the present methodology, already in progress, will provide monolayers containing diamagnetic metalloporphyrins (for example, Mg²⁺ and Zn²⁺) as well as multilayers containing metalloporphyrins in controllable relative orientations.

Experimental Section

Synthesis of **1** and **2**: *trans*-1,2-dithiane-4,5-diol (racemic mixture) was treated with acrylonitrile (2.2 equiv) and NaOH, and stirred overnight at room temperature. The mixture was then hydrolyzed for 3 h by refluxing with HCl to give the *trans*-1,2-dithiane dicarboxylic acid derivative (yield:

90 %). The extended diacid was coupled at room temperature, through the acyl chloride, to 1-(3-aminopropyl)imidazole to give **1** (yield: 60 %), or through the pentachlorophenolate to histamine, to give **2** (yield: ca. 30 %). **1**: ¹H NMR (CD₃OD): δ = 7.72 (s, 1 H), 7.12 (s, 1 H), 6.98 (s, 1 H), 4.07 (t, 2 H, *J* = 6.2 Hz), 3.90 (m, 2 H), 3.40 (br, 1 H), 3.21 (br, 3 H), 2.85 (m, 1 H), 2.48 (t, *J* = 5.7 Hz, 2 H), 1.99 (quint., *J* = 6.2 Hz, 2 H); IR (CHCl₃): ν̄ = 1667 cm⁻¹, 1108 cm⁻¹; MS (FAB): *m/z* = 509 [MH⁺]. **2**: ¹H NMR (CD₃OD): δ = 7.58 (s, 1 H), 6.85 (s, 1 H), 3.78 (m, 2 H), 3.42 (t, *J* = 7.2 Hz, 2 H), 3.24 (br, 1 H), 3.14 (ABq, ³*J* = 3.1 Hz, Δ(AB) = 13 Hz, 1 H), 2.75 (t, *J* = 7.2 Hz, 2 H), 2.69 (m, 1 H), 2.39 (t, *J* = 6.2 Hz, 2 H); MS (FAB): *m/z* = 482 [M⁺].

NMR spectra of the low-spin complexes: 20–30 mM; 1:1 mixtures of [Fe^{III}(TPP)Cl] with **1** or **2** in CDCl₃ or CD₂Cl₂/CD₃OD, respectively.

Monolayer assembly: a) Stepwise method: **1** or **2** were adsorbed onto evaporated gold (1–10 mm in CHCl₃ or DMF, overnight). The monolayers were then immersed in [Fe^{III}(TPP)Cl] solution (8–15 mm in CHCl₃, 1–3 min). b) Preformed method: **1**-[Fe^{III}(TPP)Cl] and **2**-[Fe^{III}(TPP)Cl] were adsorbed from 15–18 mm solution in CHCl₃ or DMF for 6 h. All monolayers were rinsed successively for 10–15 min in the adsorption solvents and in EtOH, and dried under a stream of purified nitrogen.

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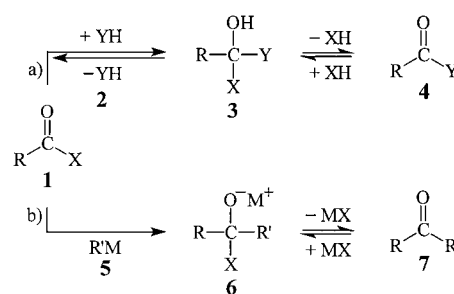
[(Ph)₂(NMe₂)C(OLi)·THF]₂: Crystal Structure of the Tetrahedral Intermediate Formed in the Reaction of *N,N*-Dimethylbenzamide and Phenyllithium**

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Norma Sbarbati Nudelman, and Gernot Boche*

*Dedicated to Professor Reiner Sustmann
on the occasion of his 60th birthday*

The tetrahedral intermediate RC(OH)XY (**3**) formed in reactions of carboxylic acid derivatives RC(O)X (**1**) with nucleophiles YH (**2**) to give RC(O)Y (**4**; Reaction a in Scheme 1) is of continued interest, for example, because of its significance for in vivo acylation reactions.^[1] Only recently, the solid-state structure of the (protonated) intramolecular amine adduct of a carboxylic acid was determined in a rather special case.^[2]

Similarly, reactions of **1** with organometallic compounds R'M (**5**) proceed via a tetrahedral intermediate **6** (Reaction b in Scheme 1). In the case of X = Hal, SR, and OR, **6**



Scheme 1. Reaction of carboxylic acid derivatives **1** with nucleophiles YH (**2**; a) and R'M (**5**; b). R = H, alkyl, aryl; X = Hal, SR, OR, NR₂; YH = R'SH, R'OH, R'NH₂; R' = H, alkyl, aryl; R' = alkyl, aryl; M = Li, MgX, etc.

undergoes a very fast 1,2-elimination of MX to give aldehyde or ketone **7**, which may react further with **5**. In the case of X = NR₂, **6** is more stable, a fact that has been utilized for the preparation of ketones **7** (**1** + **5** → **6**; **6** + H₃O⁺ → **7**)^[3] as well as for the protection of aldehydes and ketones (**7** + MNR₂ → **6**).^[4] What is the structure of a compound of type **6**, and in particular what is the environment at the new tetrahedral carbon atom? The X-ray crystal structure of [(Ph)₂(NMe₂)C(OLi)·THF]₂ (**10**),^[5] which is prepared from *N,N*-dimethylbenzamide (**8**) and phenyllithium (**9**) and crystallized from tetrahydrofuran/diethyl ether [Eq. (1)], gives an answer to these questions (Figure 1).

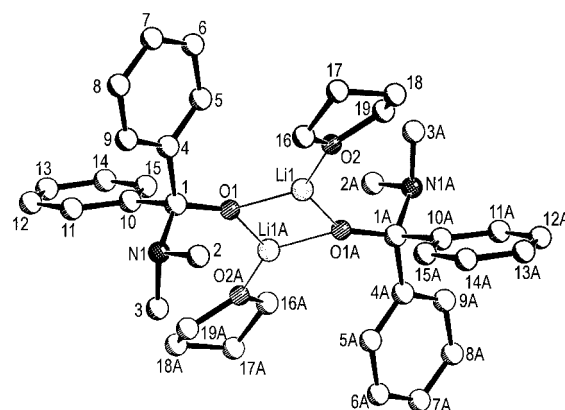
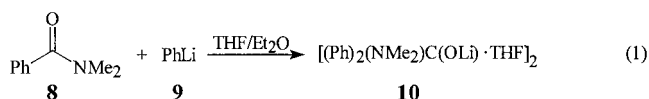


Figure 1. Crystal structure of **10**. Important bond lengths [pm] and bond angles [°]: C1–O1 137.1(2), C1–N1 150.0(3), C1–C4 154.8(3), C1–C10 154.9(3), Li1–N1 375.1(4), Li1–N1A 373.7(4); O1–C1–N1–C2 –58.4(2), O1–C1–N1–C3 63.8(2), C4–C1–N1–C2 65.3(2), C10–C1–N1–C3 –60.8(2).

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In the dimeric structure the Li atoms Li1 and Li1A are bound to the anionic O atoms O1 and O1A and to the O atom of a THF molecule (O2 and O2A, respectively), leading to three-coordinate Li cations, which is rarely observed. The C1–O1 bond is 137.1(2) pm long, which corresponds to the C–O bond length in an α -alkoxyamine (137 pm).^[6] In (CH₃)₃C–OLi a length of 139.2 pm has been determined for

the C–O bond.^[7] In amides such as **8** the C=O bond measures 123.1 pm,^[8] and in aromatic ketones 123.0 pm.^[8] The C1–N1 bond is 150.0(3) pm long, which corresponds to the value of 149 pm determined for the above-mentioned α -alkoxyamine.^[6] The mean value for C_{sp}–N bonds is 146.9 pm.^[8] In carboxamides the C–N bond is even shorter (134.6 pm).^[8] The bonds from C1 to C4 and C10 of the phenyl rings are 154.8(3) and 154.9(3) pm long. The mean value for C_{sp}–C_{aryl} bonds amounts to 151.3 pm.^[8] Thus, the bonds C1–C4, C1–C10, and C1–N1 are slightly elongated, whereas the C1–O1 bond is somewhat shortened.

Dunitz, Bürgi, and Shefter investigated the geometrical reaction coordinates of the intramolecular addition of amine and alcohol nucleophiles to carbonyl groups finally leading to the tetrahedral intermediate (e.g. **1** + **2** $\rightleftharpoons$ **3**).^[9] In agreement with their investigations, the structure of **10** indicates the beginning of the decomposition of an analogous tetrahedral intermediate, namely, the 1,2-elimination of LiNMe₂ (the best leaving group in **10**) to give benzophenone. This is shown by the bond lengths C1–N1 and C1–O1 discussed above. It is also in agreement with the angles between the C1–C4–C10 plane and the vectors C1–N1 (60.3°) and C1–O1 (52.8°): O1 is on the way towards the C1–C4–C10 plane, whereas N1 is removed from **10** along the trajectory.

A further point of interest is the conformation of the dimethylamino groups; their N atoms are not coordinated to the Li cations, which is normally very favorable.^[10] In **10** the distances Li1...N1 and Li1...N1A are 375.1(4) and 373.7(4) pm, whereas normal Li–N bonds are approximately 200 pm long.^[11] Bonding of N1 and N1A to Li1 and Li1A would require a rotation of the amino groups in **10** by about 180°, as shown by the torsional angles O1–C1–N1–C2 –58.4(2)°, O1–C1–N1–C3 63.8(2)°, C4–C1–N1–C2 65.3(2)°, and C10–C1–N1–C3 –60.8(2)°. This, however, would lead to unfavorable eclipsed conformations of the methyl groups at N1 and N1A with the phenyl groups at C1 and C1A. Furthermore, the electron lone pairs at the N atoms in **10** are perfectly placed for an interaction with the σ^* orbitals of the C–O bonds (anomeric effect^[12]). The balance between N...Li complex-

ation, conformational strain, and anomeric effect is shown by the data in Table 1, which summarizes the calculated bond lengths, bond angles, and relative energies of the compounds **11**–**15** in the conformations **A** and **B**.

Thus, **11B** is 8.0 kcal mol^{–1} higher in energy than **11A**, whose conformation at the C–N bond corresponds to that in **10**. The anomeric effect in **11A** is nicely shown by the bond lengths: The C–O bond is longer (137.0 pm) and the C–N bond is shorter (153.2 pm) than in **11B** (135.9 and 159.0 pm, respectively). A similar effect is observed in compounds **12**–**15**.^[13] Interestingly the torsional angles O–C–N–R³ (–158.0°) and O–C–N–R⁴ (73.3°) in **11B** are almost equal to the corresponding angles in a recently investigated α -amino-lithium alkoxide (158.9/–71.7° and 154.9/–73.9°, respectively).^[14] Apparently, to avoid eclipsed interactions, rotation about the C–N bond takes place if a Li–O–C–N four-membered ring is formed. A decrease in conformational strain (**11** $\rightarrow$ **12** $\rightarrow$ **13** $\rightarrow$ **14**) provides a reduction in the destabilization of conformers **B** from 4.3 kcal mol^{–1} (**12B**) to 1.5 kcal mol^{–1} (**13B**). In the case of **14** the **B** isomer is –2.7 kcal mol^{–1} more stable than **14A**, and the torsional angles O–C–N–R³ and O–C–N–R⁴ ideally amount to –120.1° and 121.1°. Isomer **15B** is even more stable than **15A** (–4.5 kcal mol^{–1}). In the case of **15** the energies and geometries of the conformers **A** and **B** were also determined by the MP2 method, and the results confirm the tendency shown in Table 1.

In conclusion, the bond lengths and bond angles at the tetrahedral C1(C1A) atom in **10** indicate the tendency for 1,2-elimination of LiNMe₂ to give benzophenone, even though the anomeric effect stabilizes **10**.

Experimental Section

10: A solution of *N,N*-dimethylbenzamide (500 mg, 3.35 mmol) in diethyl ether (60 mL) was allowed to react with 1.8 *N* phenyllithium in cyclohexane/diethyl ether (1.86 mL, 3.35 mmol) at room temperature under an argon atmosphere. The white precipitate was washed with diethyl ether (2 $\times$ 10 mL) under argon and dissolved in THF (10 mL). A 4-mL aliquot of this solution was treated with diethyl ether (2 mL) and kept at –16°C for 8 h. Colorless rhombic crystals were obtained (121 mg, 44% yield).

Table 1. Calculated bond lengths [pm], torsion angles [°], and relative energies [kcal mol^{–1}] of the conformers **A** and **B** of compounds **11**–**15** (RHF/3–21G//PM3). The MP2/6-31+G**//MP2/6-31+G* values of **15** are given in parentheses.

Compd	R ¹	R ²	R ³	R ⁴			O–C–N–R ³	O–C–N–R ⁴	<i>E</i> _{rel}
					C–O	C–N			
11A	Ph	Ph	Me	Me	137.0	153.2	58.4	–70.9	0.0
11B	Ph	Ph	Me	Me	135.9	159.0	–158.0	73.3	8.0
12A	Ph	H	Me	Me	136.7	152.0	59.2	–71.1	0.0
12B	Ph	H	Me	Me	135.4	157.2	–146.4	86.0	4.3
13A	H	H	Me	Me	136.5	150.4	64.9	–65.8	0.0
13B	H	H	Me	Me	134.9	156.7	–117.3	116.6	1.5
14A	H	H	H	H	136.9	148.4	58.7	–61.3	0.0
14B	H	H	H	H	135.2	154.5	–120.1	121.1	–2.7
15A	Me	Me	H	H	138.3	150.4	59.9	–60.2	0.0
					(139.3)	(147.1)	(56.7)	(–56.7)	(0.0)
15B	Me	Me	H	H	136.2	155.9	–126.0	115.2	–4.5
					(137.3)	(154.8)	(–129.7)	(112.5)	(–6.0)

¹H NMR (200 MHz, [D₈]THF, 298 K): δ = 7.78 (4H), 7.12 (4H), 6.93 (2H), 2.20 (6H); ¹³C NMR (50 MHz, [D₈]THF, 298 K): δ = 154.6, 128.0, 127.6, 125.2, 94.3,^[16] 39.7.

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Total Synthesis of (–)-Mucocin**

Stefan Bäurle, Sabine Hoppen, and Ulrich Koert*

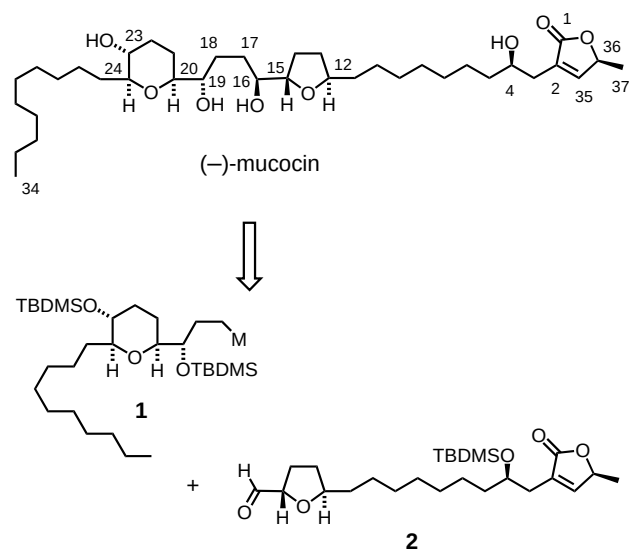
The acetogenins of *Annonaceae* are characterized by their variety in structure and their potent biological features, for example, as antitumor agents or immunosuppressants.^[1] Therefore, this class of compounds is subject to intensive synthetic efforts.^[2] Within the acetogenins a group of compounds exists that also bear a tetrahydropyran (THP) ring additional to the usual tetrahydrofuran rings in the molecular scaffold. A representative member of that group is (–)-mucocin, which was isolated from *Rollinia Mucosa*.^[3] The molecule shows a highly selective inhibitory effect against A-549 (lung cancer) and PACA-2 (pancreatic cancer) cell lines with a selectivity up to 10000 times that of the known antitumor agent adriamycin. A blockage of the mitochondrial complex I is discussed as the mode of action.^[1]

Here we report on a total synthesis of (–)-mucocin that follows a highly convergent synthetic strategy.^[4] According to our retrosynthetic analysis (disconnection at C16–C17) mucocin could be constructed by the addition of a THP organometallic compound **1** to a THF aldehyde **2** (Scheme 1).

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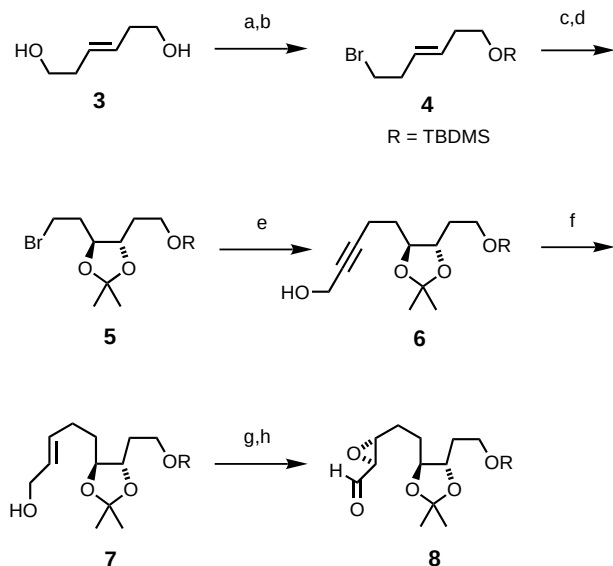
Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.



Scheme 1. Retrosynthetic analysis of (-)-mucocin. TBDS = *tert*-butyl-dimethylsilyl.

The compatibility of the butenolide with the reaction conditions in regard to a possible epimerization as well as the stereocontrol of the coupling reaction were considered as critical to the strategy.

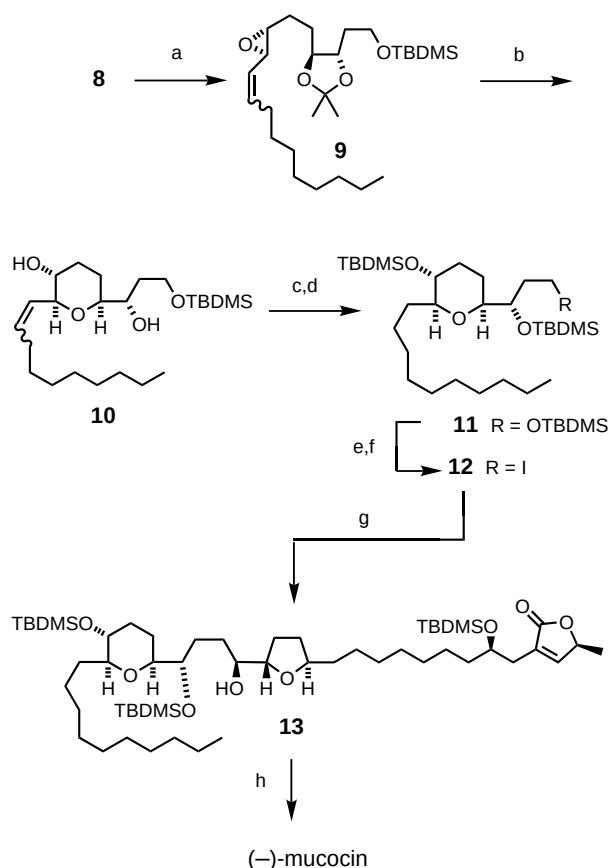
The synthesis of the left part of the molecule (Scheme 2) started with the diol **3**. After monosilylation^[5] and subsequent bromination to **4**, the sequence of dihydroxylation/acetonide^[6] protection gave the bromide **5**. The alkylation to **6**, followed by an *E*-selective reduction^[7] of the resulting alkyne



Scheme 2. a) NaH (1.0 equiv), TBDMSCl (1.0 equiv), THF, 0°C, 1 h, 54%; b) 1. *p*-TsCl (2.0 equiv), Py (10 equiv), CH₂Cl₂, 0°C, 12 h; 2. LiBr (4.0 equiv), acetone, 12 h, 85%; c) AD-mix *a*, MeSO₂NH₂, H₂O/*t*BuOH (1/1), 0°C → RT, 24 h; d) *p*-TsOH (5 mol %), 3,3-dimethoxypropane (4.0 equiv), CH₂Cl₂, 1 h, 92% over two steps; e) propargylic alcohol (3.0 equiv), *n*BuLi (6.0 equiv), NH₃/THF/DMPU (5/5/2), -40°C, 6 h, 91%; f) Red-Al (2.0 equiv), THF, 0°C, 4 h, 95%; g) TBHP (2.0 equiv), (-)-DIPT (12 mol %), Ti(O*i*Pr)₄ (10 mol %), CH₂Cl₂, -20°C, 3.5 h, 85%; h) Dess–Martin periodinane (2.0 equiv), Py (10 equiv), CH₂Cl₂, 0°C, 3 h, 89%. *p*-Ts = *p*-toluenesulfonyl, Py = pyridine, DMPU = *N,N'*-dimethylpropylene urea, TBHP = *tert*-butylhydroperoxide, DIPT = diisopropyl tartrate.

to the alkene **7** opened the way for the elaboration of the THP ring. A 6-*endo* attack of the C20 oxygen atom on a C23–C24 epoxide by an activation of the C24 position by a C25–C26 double bond was envisaged for the construction of the THP ring.^[8] Sharpless epoxidation^[9] of the allylic alcohol **7** afforded an epoxy alcohol that was oxidized to the aldehyde **8** by using the Dess–Martin protocol.^[10]

The precursor for the cyclization to the THP ring was obtained after Wittig reaction of **8** with the C25–C34 phosphonium ylide (Scheme 3). This reaction (**9** → **10**) deserves further comments: an acid-catalyzed intramolecular



Scheme 3. a) H₁₉C₉PPh₃Br (1.4 equiv), NaHMDS (1.2 equiv), THF, -78°C, 5 min, 85%, *E*:*Z* = 1:1; b) CSA (8 mol %), CH₂Cl₂/*i*PrOH (30/1), -40 → 0°C, 3.5 h, 89%; c) 5% Pt/C, H₂ (1 bar), EtOAc, 0°C, 5 h, 95%; d) TBDMSCl (3.0 equiv), 2,6-lutidine (5.0 equiv), CH₂Cl₂, 0°C, 1 h, 92%; e) CSA (0.25 equiv), CH₂Cl₂/MeOH (2/1), 0°C, 30 min, 73%; f) I₂ (1.2 equiv), PPh₃ (1.1 equiv), imidazole (3.0 equiv), CH₂Cl₂, 0°C, 3 h, 80%; g) **12** (1.2 equiv), *t*BuLi (2.2 equiv), Et₂O, -105°C, 5 min; then MgBr₂·OEt₂ (2.4 equiv), -100 → -35°C, 1.5 h; → -78°C, **2** (1.0 equiv), → -15°C, 1.5 h, 56%, 34% of **2** was also recovered; h) HF (5 equiv), CH₃CN/CH₂Cl₂, 20°C, 1 h, 91%. NaHMDS = sodium hexamethyldisilazide, CSA = camphersulfonic acid.

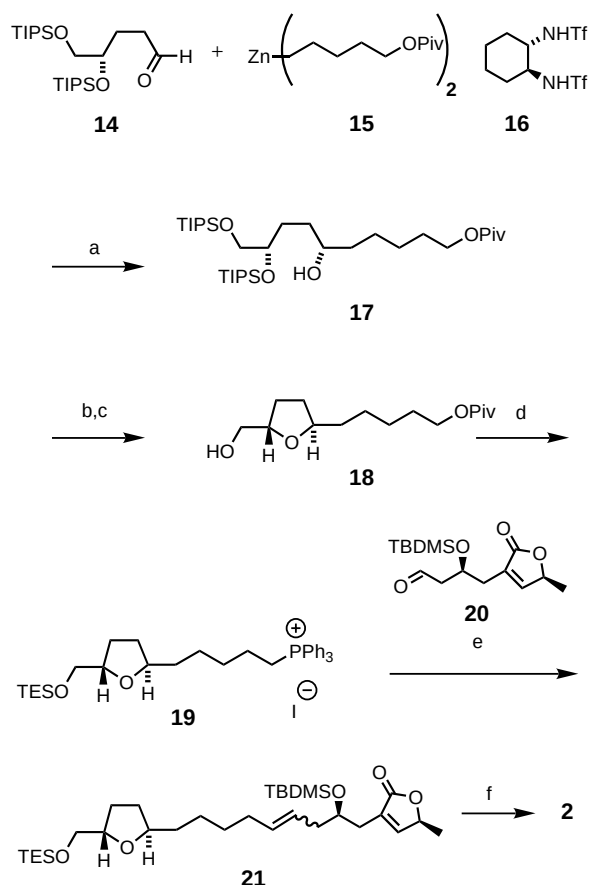
6-*endo* attack on the alkenyl epoxide of the acetonide had to occur, while possible intermolecular side reactions at the epoxide^[11] and cleavage of the silyl group had to be suppressed. Only when isopropyl alcohol was used as a weak nucleophilic protic cosolvent was a clean conversion accomplished.^[12] The cleavage of the acetonide and the opening of the epoxide could possibly happen in a concerted manner without the free diol as an intermediate. After the selective

opening of the epoxide the double bond at C25–C26 was removed. A standard sequence (**10**→**11**→**12**) led to the iodide **12**, the precursor to the convergent final step of the synthesis. An iodine–lithium exchange in **12** was realized at -105°C ^[13] followed by a transmetalation to magnesium. The precise choice of temperature and time was crucial for the success of the reaction. If the transmetalation did not take place within five minutes after the halogen–metal exchange and if the temperature rose above -100°C a retro-Brook migration^[14] from the silyl group at O–C19 to C17 was observed as a side reaction. Treatment of the aldehyde **2** with the organomagnesium compound and subsequent warming to -15°C gave the desired product **13**. Noteworthy, only 1.2 equivalents of iodide **12** to 1 equivalent of aldehyde **2** were necessary. The stereoselectivity of the reaction was 4:1 in favor of the desired epimer **13**. The synthetically undesired, but pharmacologically interesting, C16 minor epimer could be separated by chromatography. Cleavage of the silyl protecting groups provided (–)-mucocin ($[\alpha]_{\text{D}}^{25} = -12.7$, $c = 0.27$ in CH_2Cl_2), which was found to be identical to the naturally occurring product in respect to the spectroscopical data (Table 1).

Table 1. Selected analytical and spectroscopic data of **13** and (–)-mucocin.

13: HPLC: $R_t = 10.3$ min (Rainin Si 60, *i*PrOH/*n*-hexane (1/99), 1.0 mL min^{–1}); $[\alpha]_{\text{D}}^{25} = -19.2^{\circ}$ ($c = 0.60$, CHCl_3); ^1H NMR (300 MHz, CDCl_3): $\delta = 7.09$ (d, $J = 1.1$ Hz, 1H), 4.98 (dq, $J = 6.8$, 1.1 Hz, 1H), 3.94 (m, 1H), 3.88–3.79 (m, 1H), 3.75 (dt, $J = 14.2$, 6.7 Hz, 1H), 3.62 (dt, $J = 10.5$, 5.3 Hz, 1H), 3.38–3.27 (m, 1H), 3.27–3.14 (m, 2H), 2.98 (m, 1H), 2.43 (d, $J = 2.7$ Hz, 1H), 2.40 (d, $J = 5.3$ Hz, 2H), 2.04–1.88 (m, 3H), 1.76–1.15 (m, 47H), 1.39 (d, $J = 6.8$ Hz, 3H), 0.85 (brs, 30H), 0.02 (brs, 18H); ^{13}C NMR (75 MHz, CDCl_3): $\delta = 174.0$ (C1), 151.6 (C35), 130.8 (C2), 82.4 (C24), 82.0 (C15), 79.9 (C20), 79.2 (C12), 77.4 (C36), 74.6 (C16), 74.1 (C19), 71.0 (C23), 70.1 (C4), 36.9, 35.7, 33.5, 32.7, 32.4, 31.9, 29.8, 29.7, 29.6, 29.5, 29.3, 28.8, 28.7, 28.4, 26.2, 25.5, 25.1, 22.7 (C3, C5–C11, C13, C14, C17, C18, C21, C22, C25–C33), 25.9, 25.9, 25.8, (3SiC(CH₃)₃), 19.0 (C37), 18.2, 18.0, 18.0 (3SiC(CH₃)₃), 14.1 (C34), –4.0, –4.4, –4.5, –4.6, –4.8, (6SiCH₃); HR-MS (EI): calcd (found) for $\text{C}_{55}\text{H}_{109}\text{O}_8\text{Si}_3$ [$M^+ + \text{H}$]: 981.7430 (981.7441). (–)-Mucocin: HPLC: $R_t = 8.9$ min (Rainin Si 60, *i*PrOH/*n*-hexane (30/70), 1.5 mL min^{–1}); $[\alpha]_{\text{D}}^{25} = -12.7^{\circ}$ ($c = 0.27$, CH_2Cl_2); ^1H NMR (300 MHz, CDCl_3): $\delta = 7.16$ (d, $J = 1.5$ Hz, 1H), 5.04 (dq, $J = 6.8$, 1.5 Hz, 1H), 3.95–3.67 (m, 3H), 3.52–3.34 (m, 2H), 3.32–3.18 (m, 1H), 3.18–3.08 (m, 1H), 3.02 (dt, $J = 8.8$, 2.2 Hz, 1H), 2.84 (brs, 1H), 2.71 (brs, 1H), 2.50 (d, $J = 15.1$ Hz, 1H), 2.37 (dd, $J = 15.1$, 8.3 Hz, 1H), 2.30 (brs, 1H), 2.14–2.05 (m, 1H), 2.05–1.89 (m, 2H), 1.88–1.76 (m, 1H), 1.75–1.13 (m, 41H), 1.41 (d, $J = 6.8$ Hz, 3H), 0.85 (t, $J = 6.8$ Hz, 3H); ^{13}C NMR (75 MHz, CDCl_3): $\delta = 174.6$ (C1), 151.8 (C35), 131.2 (C2), 82.0 (C24), 81.9 (C15), 80.1 (C20), 79.3 (C12), 78.0 (C36), 73.8 (C16), 73.5 (C19), 70.5 (C23), 70.0 (C4), 37.4, 35.6, 33.3, 32.6, 32.4, 32.0, 31.9, 29.7, 29.6, 29.5, 29.4, 29.4, 29.3, 28.8, 28.7, 28.3, 26.9, 26.2, 25.5, 25.5, 22.7 (C3, C5–C11, C13, C14, C17, C18, C21, C22, C25–C33), 19.1 (C37), 14.1 (C34); HR-MS (EI): calcd (found) for $\text{C}_{57}\text{H}_{67}\text{O}_8$ [$M^+ + \text{H}$]: 639.4836 (639.4838).

The synthesis of aldehyde **2** began with a stereocontrolled addition of the functionalized diorganozinc compound **15** to the aldehyde **14** by using the chiral ligand **16** (Scheme 4) to give the alcohol **17** (diastereoselectivity 95:5).^[15] A subsequent TBAF-induced intramolecular Williamson reaction afforded the 2,5-disubstituted THF-derivative **18**, which could be transformed into the phosphonium salt **19**. A Wittig reaction of **19** with the aldehyde **20** gave olefin **21**, and completed the construction of the right half of the molecule.

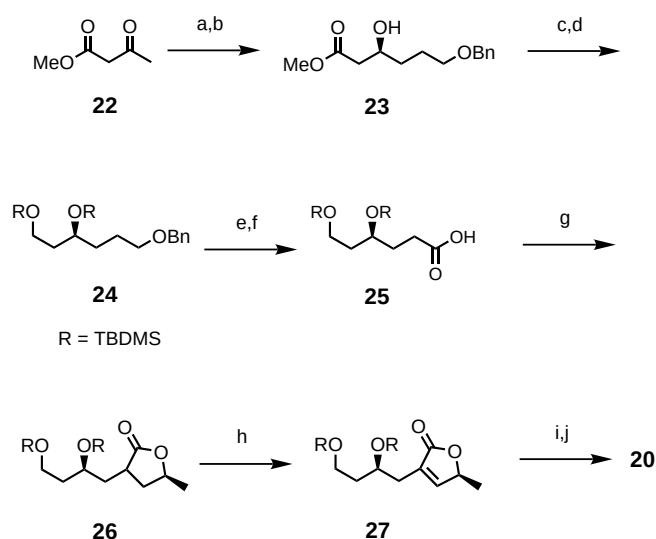


Scheme 4. a) **15** (1.8 equiv), **16** (0.1 equiv), $\text{Ti}(\text{O}i\text{Pr})_4$ (2.0 equiv), xylene, -25°C , 16 h, 65%; b) *p*-TsCl (4.0 equiv), $\text{Py}/\text{CH}_2\text{Cl}_2$ (1/1), RT, 12 h, 93%; c) TBAF (3.0 equiv), THF, RT, 45 min, 95%, *trans*:*cis* = 95:5 (HPLC); d) 1. TESCl (1.2 equiv), imidazole (2.0 equiv), CH_2Cl_2 , RT, 2 h, 86%; 2. DIBALH (2.5 equiv), THF, -20°C , 1 h, 93%; 3. I_2 (1.2 equiv), PPh_3 (1.1 equiv), imidazole (3.0 equiv), CH_2Cl_2 , $0^{\circ}\text{C} \rightarrow \text{RT}$, 1.5 h, 84%; 4. PPh_3 (5.0 equiv), $\text{CH}_3\text{CN}/\text{toluene}$ (1/1), 70°C , 20 h; e) NaHMDS (1.0 equiv), THF, 0°C , 30 min, then **20**, $-70 \rightarrow 0^{\circ}\text{C}$, 20 min, 60%; f) 1. $[(\text{PPh}_3)_3\text{RhCl}]$ (0.15 equiv), H_2 (1 atm), benzene, RT, 3 h, 95%; 2. CSA (0.08 equiv), $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (5/1), -20°C , 10 min, 76%; 3. Dess–Martin periodinane (2.0 equiv), Py (10 equiv), CH_2Cl_2 , $0^{\circ}\text{C} \rightarrow \text{RT}$, 4.5 h, 91%. TBAF = tetrabutylammonium fluoride, TES = triethylsilyl, DIBALH = diisobutylaluminum hydride.

Aldehyde **2** was obtained by hydrogenation of the isolated double bond of compound **21**,^[16] selective cleavage of the primary silylether, and subsequent Dess–Martin oxidation.

The synthesis of the butenolide aldehyde **20**—which should be interesting for other acetogenin syntheses as well—is summarized in Scheme 5. The hydroxyl function was prepared from the β -ketoester **22** by dianion alkylation and subsequent Noyori reduction with BINAP^[17] (**22**→**23**, *ee* = 97%). The β -hydroxyester **23** was transformed via the benzylether **24** to the carboxylic acid **25**. Treatment of **25** with (*S*)-propene oxide afforded the butyrolactone **26**. After introduction of the C2–C35 double bond (**26**→**27**) the desired butenolide aldehyde **20** was obtained by selective deprotection of the primary siloxy function and Dess–Martin oxidation.

The distinctive features of this synthesis of mucocin are the high convergence and the modular way of construction. New variations of mucocin with pharmacological importance



Scheme 5. a) NaH (1.5 equiv), *n*BuLi (1.5 equiv), THF, $-30 \rightarrow -15^\circ\text{C}$, 15 min, then Br(CH₂)₂OBn (0.85 equiv), -10°C , 2.5 h, 75 %; b) H₂ (5 bar), Ru^{II}-(S)-(-)-BINAP (0.6 mol %), 95°C , 18 h, 90 %, *ee* = 97 % (HPLC: Chiralcel OD-H, 10 % *i*PrOH/hexane (1/9)); c) BH₃·Me₂S (2.2 equiv), THF, 60°C , 30 min, 83 %; d) TBDMSCl (2.4 equiv), imidazole (3.0 equiv), DMAP (0.1 equiv), CH₂Cl₂, RT, 16 h, 88 %; e) H₂ (1 atm), 10 % Pd/C (5 mass %), EtOAc, 98 %; f) 1. (COCl)₂ (2.0 equiv), DMSO (4.0 equiv), NEt₃ (5.0 equiv), CH₂Cl₂, $-78 \rightarrow -40^\circ\text{C}$, 1.5 h, 2. NaClO₂ (3.0 equiv), NaH₂PO₄·2H₂O (4.0 equiv), methyl-2-butene (20 equiv), *t*BuOH/H₂O (1/1), 3 h, 95 %; g) 1. LDA (2.5 equiv), THF, 0°C , 45 min, then (S)-(-)-propene oxide (3.0 equiv), RT, 3 h, 2. PivCl (1.1 equiv), NEt₃ (2.0 equiv), CH₂Cl₂, RT, 10 min, 73 %; h) 1. KHMDS (3.0 equiv), THF, 0°C , 30 min, then PhSeCl (3.0 equiv), 1 h, 2. MMPP (4.0 equiv), THF/MeOH (1/1), RT, 30 min, 88 %; i) CSA (0.25 equiv), CH₂Cl₂/MeOH (1/1), 0°C , 30 min, 79 %; j) Dess–Martin periodinane (1.5 equiv), Py (10 equiv), CH₂Cl₂, $0^\circ\text{C} \rightarrow \text{RT}$, 4.5 h, 90 %. BINAP = 2,2'-bis(diphenylphosphanyl)-1,1'-binaphthyl, DMAP = 4-dimethylaminopyridine, DMSO = dimethylsulfoxide, LDA = lithium diisopropylamide, Piv = pivaloyl ((H₃C)₃CCO), KHMDS = potassium hexamethyldisilazide, MMPP = magnesium monoperoxophthalate.

should be easily available by a combination of different modules. The butenolide fragment proved to be compatible with the metalloorganic coupling reaction. This strategy should be applicable to the synthesis of other annonaceous acetogenins and presents a new, efficient, and flexible approach to this biologically important class of compounds.

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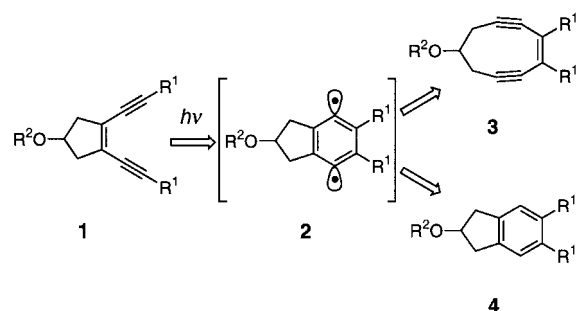
Photochemical Cycloaromatization of Non-Benzenoid Enediynes**

Toshio Kaneko, Miki Takahashi, and Masahiro Hirama*

Dedicated to Professor Satoru Masamune on the occasion of his 70th birthday

The Bergman reaction, the thermal cycloaromatization of hexa-1,5-diyne-3-ene to afford benzenoid compounds, is well-known.^[1, 2] Some photochemical examples have been reported; however, these are restricted to arene-fused enediynes.^[3–5] A photo-Bergman reaction of aliphatic enediynes could play an important role in 1,4-didehydrobenzene (*p*-benzyne) chemistry because it could be carried out at low temperature and thus allow characterization of reactive intermediates.^[6] We describe herein the successful photo-cycloaromatization of several aliphatic enediynes.

Based on our previous studies on the synthesis of nine-membered cyclic enediynes,^[7] we first examined the photo-reaction of 1,2-diethynylcyclopentene derivatives **1**. The photochemical formation of **3** was expected through the 1,4-didehydrobenzene intermediate **2** (Scheme 1). A degassed



Scheme 1. Plausible course of the photo-Bergman reaction. R² = TBS = *tert*-butyldimethylsilyl.

solution of **1a** (R¹ = H)^[8] in hexane was irradiated in a quartz flask at room temperature with a low-pressure mercury lamp (4 × 20 W) for 6 h. The enediyne **1a** disappeared completely, but the aromatic product **4a** was obtained only in very low yield (3 %) and no nine-membered enediyne **3** (R¹ = H) was detected (Table 1). Neither **1b**^[8] nor **1c**^[8] possessing the bulky substituents trimethylsilyl (TMS) and phenyl, respectively, at the alkyne termini yielded any indane derivatives under the same reaction conditions; the reactants were recovered almost quantitatively. However, photolysis of the dipropynyl

Table 1. Photoreaction of 1,2-diethynylcyclopentene derivatives **1a–1d** in hexane.

$$\text{R}^2\text{O}-\text{Cyclopentene}(\text{C}\equiv\text{C}-\text{R}^1)_2 \xrightarrow[\text{RT}]{\substack{h\nu \\ (254 \text{ nm}) \\ n\text{-C}_6\text{H}_{14} \\ (3.6 \text{ mM})}} \text{R}^2\text{O}-\text{Benzene}(\text{X})(\text{R})(\text{Y})$$

1a–d

$\text{R}^2 = \text{TBS}$

4a–d ($\text{X} = \text{Y} = \text{H}$)

5 ($\text{R} = \text{Me}$, $\text{X} = \text{H}$, $\text{Y} = \text{CH}_2\text{CN}$)

6 ($\text{R} = \text{Me}$, $\text{X} = \text{H}$, $\text{Y} = \text{CHCl}_2$)

Substrate	Reaction time [h]	Yield [%]
1a : $\text{R}^1 = \text{H}$	6	4a : 3
1b : $\text{R}^1 = \text{TMS}$	18	4b : 0
1c : $\text{R}^1 = \text{Ph}$	18	4c : 0
1d : $\text{R}^1 = \text{Me}$	18	4d : 71

derivative **1d** (R¹ = Me)^[8] gave **4d** in high yield (71 %; 80 % yield by GC analysis).^[9] Photoreduction products such as dienyne, which are formed in the case of benzenoid enediynes,^[4b] were not detected. The photo-cycloaromatization took place in a variety of solvents (but not in MeOH): *n*-hexane/1,4-cyclohexadiene (20/1; **4d**: 41 %), cyclohexane (**4d**: 52 %), THF (**4d**: 35 %), CH₃CN (**4d**: 21 %, **5**: 8 %), *i*PrOH (**4d**: 20 %), CH₂Cl₂ (**4d**: trace, **6**: 20 %).

Photolysis of **1d** was then carried out in [D₈]THF (99.5 atom % D). The deuterated product [D₂]**4d** (X = Y = D) ([D₂]: 88 %, [D₁]: 12 %, [D₀]: 0 %) was obtained in 14 % yield. These results strongly suggest that the photocyclization of **1d** proceeds through the 4,7-didehydroindane diradical intermediate **2** (R¹ = Me). Irradiation of **1d** under a high-pressure mercury lamp (400 W, Riko UVL-400HA, pyrex) in hexane did not cause any reaction, not even in the presence of triplet photosensitizers (toluene, acetophenone, benzophenone, anthracene, or naphthalene). Thus, neither pyrex-filtered light nor the triplet energy of these sensitizers is effective for the photo-cycloaromatization of **1d**.

We then irradiated the strained, ten-membered cyclic enediyne **7**^[8, 10] with a low-pressure mercury lamp (4 × 20 W) at room temperature for 3 h. Tetrahydronaphthalene (**8**) was obtained, and an appreciable amount of 1,2-diethynylcyclohexene (**9**)^[11] was formed in all solvents except *i*PrOH (Table 2). Compound **9** underwent photolysis under the same reaction conditions, and it was consumed completely upon photolysis in *i*PrOH for 3 h to give **8** in 3 % yield. Photolysis of **7** in [D₈]*i*PrOH was stopped after 30 % conversion (5 min), and the products were analyzed by 500-MHz ¹H NMR

Table 2. Photolysis of cyclodeca-1,5-diyne-3-ene (**7**).

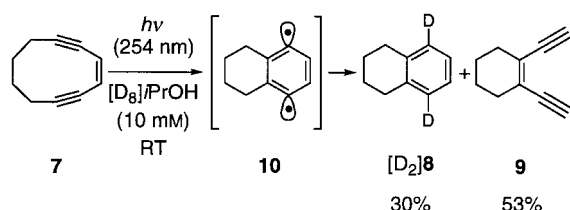
7	8	9
$h\nu$ (254 nm) $\xrightarrow{\text{solvent, 3 h}}$ (2.3 mM) RT		
Solvent	Yield of 8 [%] ^[a]	Yield of 9 [%] ^[a]
hexane ^[b]	29	6
cyclohexane	26	20
CH ₃ CN	12	24
<i>i</i> PrOH	3	— ^[c]

[a] By GC analysis. [b] Irradiated for 5 h. [c] Not detected.

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spectroscopy (Scheme 2). The yields of **[D₂]****8** and **9** were 30 and 53 %, respectively, based on recovered **7**. The formation of **9** in over 50 % yield is highly remarkable because enediyne **9** should arise from the retro-Bergman reaction of the hypothetical diradical intermediate **10**, and because **9** has never been isolated in the thermal reaction of **7**.^[10, 12]



Scheme 2. Photolysis of **7** in **[D₈]***i*PrOH for 5 min (30 % conversion).

We have established a highly effective photo-Bergman reaction of the aliphatic, ten-membered cyclic enediyne **7**. The photoreaction of **7** also showed remarkable formation of **9**, which should be a retro-Bergman product from **10**. Thus, **7** appears to be ideal for flash photolysis and for the characterization of the 1,4-didehydrobenzene diradical intermediate **10**. A mechanistic study along this line will be reported in due course.

Experimental Section

Photolysis of 1d: A solution of **1d** (50.0 mg, 0.182 mmol) in hexane (50 mL) was irradiated in a quartz flask (30 mm (inner diameter) × 230 mm) with four GL-20 low-pressure mercury lamps (National Electric Co. Ltd.) at room temperature under an argon atmosphere for 18 h. The solution was concentrated and purified by flash column chromatography on silica gel (eluent: hexane containing 0–1 % of ethyl acetate). The solid residue **4d** was recrystallized from hexane; yield: 35.8 mg (0.129 mmol, 71 %).

GC analysis of the photoreaction products of **7** was carried out with 1,3,5-trimethylbenzene as an internal standard.

¹H NMR analysis of the photoreaction of **7**: A 5.0 mm solution of **7** in **[D₈]***i*PrOH (99 + atom % D) in a quartz NMR tube (5 mm inner diameter) was placed in a quartz jacket filled with methanol and irradiated for 5 min with four GL-20 low-pressure mercury lamps (National Electric Co. Ltd.) at 25 °C under an argon atmosphere. The ¹H NMR spectra were taken directly for the reaction mixture on a Varian INOVA-500 instrument. The yields of **8** and **9** and the amount of recovered **7** were determined using the residual protons of **[D₈]***i*PrOH as an internal standard.

1d: Pale yellow crystals; m.p. 39.5–40 °C (*n*-hexane); ¹H NMR (200 MHz, CDCl₃): δ = 0.04 (6H, s), 0.87 (9H, s), 2.05 (6H, s), 2.38–2.45 (2H, m), 2.67–2.79 (2H, m), 4.37–4.51 (1H, m); ¹³C NMR (125 MHz, CDCl₃): δ = –4.82 (CH₃), 4.84 (CH₃), 18.08 (C), 25.81 (CH₃), 46.58 (CH₂), 71.05 (CH), 76.28 (C), 91.98 (C), 126.05 (C); IR (neat): ν̄ = 2958, 2932, 2860, 2224, 1473, 1437, 1363, 1257, 1216, 1195, 1104, 1069, 1007 cm^{–1}; MS (EI, 70 eV): *m/z* (%): 276 (1.1) [*M*⁺+2H], 275 (3.9) [*M*⁺+H], 274 (16) [*M*⁺], 217 (100), 143 (8.9); elemental analysis calcd for C₁₇H₂₆OSi: C 74.39, H 9.55; found: C 74.60, H 9.67; HR-MS (EI, 70 eV) calcd for C₁₇H₂₆OSi [*M*⁺]: 274.1753, found 274.1752.

4d: Pale yellow amorphous solid; ¹H NMR (500 MHz, CDCl₃): δ = 0.09 (6H, s), 0.90 (9H, s), 2.22 (6H, s), 2.81 (2H, dd, ³*J*(H,H) = 15.5, 6.0 Hz), 3.06 (2H, dd, ³*J*(H,H) = 15.5, 6.5 Hz), 4.63 (1H, quint, ³*J*(H,H) = 6.2 Hz), 6.96 (2H, s); ¹³C NMR (125 MHz, CDCl₃): δ = –4.73 (CH₃), 18.25 (C), 19.73 (CH₃), 25.93 (CH₃), 42.21 (CH₂), 74.17 (CH), 125.70 (CH), 134.49 (C), 138.55 (C); IR (neat): ν̄ = 3010, 2960, 2860, 1497, 1464, 1379, 1255, 1199, 1104, 1069 cm^{–1}; MS (EI, 70 eV): *m/z* (%): 276 (2.0) [*M*⁺], 261 [*M*⁺–Me] (3.5), 219 (100) [*M*⁺–*t*Bu], 145 (14); HR-MS (EI, 70 eV) calcd for C₁₇H₂₈OSi [*M*⁺]: 276.1909, found: 276.1901; calcd for C₁₇H₂₈OSi [*M*⁺–Me]:

261.1675, found: 261.1678; calcd for C₁₇H₂₈OSi [*M*⁺–*t*Bu]: 219.1205, found: 219.1238.

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- UV spectra of enediynes. **1a** (*n*-hexane): λ_{max} (lg ε) = 270 (4.05), 259 (4.16), 250 nm (sh, 4.05); **1b** (*n*-hexane): λ_{max} (lg ε) = 294 (4.23), 280 nm (4.15); **1c** (*n*-hexane): λ_{max} (lg ε) = 350 (sh, 4.11), 328 (4.32), 260 (4.40), 225 nm (4.31); **1d** (*n*-hexane): λ_{max} (lg ε) = 275 (4.15), 263 nm (4.22); **7** (CH₃CN): λ_{max} (lg ε) = 281 (3.76), 270 (sh, 3.83), 267 (3.84), 258 nm (sh, 3.75).
- Turro and co-worker independently found that the photolysis of 1,2-bis(1-pentynyl)cyclopentene as well as of 1,2-bis(1-phenylacetylenyl)cyclopentene yielded the indane derivatives; see citation [39] in reference [4b].
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- We confirmed that the thermal reaction of **7** proceeded smoothly at 57 °C and did not give even trace amounts of **9**. The enediyne **9** did not undergo cycloaromatization at the same temperature; this reaction did not occur at an appreciable rate until at least 100 °C.

SSZ-35 and SSZ-44: Two Related Zeolites Containing Pores Circumscribed by Ten- and Eighteen-Membered Rings**

Paul Wagner,* Stacey I. Zones, Mark E. Davis, and Ronald C. Medrud

Large and extra-large pore high-silica microporous materials are in demand as catalysts in the petrochemical and pharmaceutical industries because of their combined properties of shape selectivity, acid strength, and high thermal stability.^[1–3] Important insights into the catalytic properties of microporous materials are gained through a knowledge of their crystal structures, and these insights can, in turn, lead to the design of new materials with desirable properties.

Here we present the structure solution of two related high-silica molecular sieves, SSZ-44^[4,5] and SSZ-35,^[4,6] with unusual one-dimensional straight channels which contain pore openings that alternate between rings of ten and eighteen tetrahedral atoms (ten- and eighteen-membered rings, 10MR and 18MR). SSZ-35 and SSZ-44 are the first high-silica zeolites that possess pores with rings larger than 14MR (during the review of this paper the structure of a clathrasil, that is a nonporous silicate, was reported^[7] that is composed of cages that also contain 18MR). The experimental micropore volumes obtained for SSZ-44 (0.185 cm³ g^{−1})^[5] and SSZ-35 (0.197 cm³ g^{−1})^[6] are significantly larger than any other known one-dimensional high-silica zeolite including the 14MR zeolites UTD-1^[2] and CIT-5^[3] that both have micropore volumes less than 0.14 cm³ g^{−1}. SSZ-35 and SSZ-44 demonstrate the potential for a dramatic increase in the pore size of thermally stable high-silica zeolites beyond the currently available 14MR materials. These extra-large pore zeolites are highly desired because they permit catalysis and adsorption to be carried out on large molecules (greater than 8 Å) that are of interest to the pharmaceutical and fine chemicals industries, as well as the petrochemical industry. In addition to possessing unique, large pores, SSZ-35 and SSZ-44 are also the pure end-member polymorphs of a new fault series and allow the complex relationship between the organic structure-directing agent (SDA) and the stacking-fault probability of the zeolite framework to be investigated.

The structure of SSZ-44 (Figure 1a) was solved by the application of the FOCUS^[8] Fourier recycling algorithm to the synchrotron powder X-ray data (SPXRD) and confirmed from the Rietveld refinement^[9] of the SPXRD (Figure 2). The SSZ-44 structure is seen to possess mirror plane symmetries parallel to the pore direction [001] that separate two layerlike

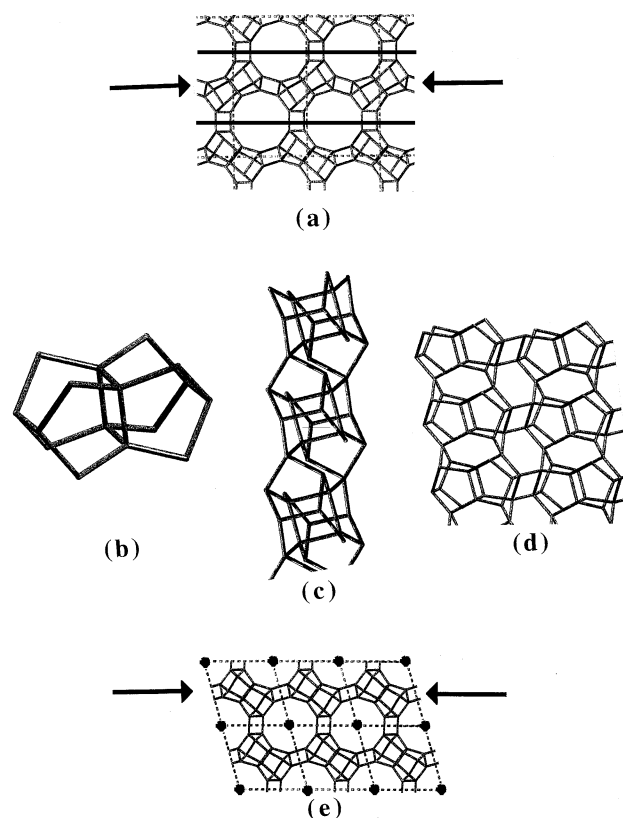


Figure 1. a) Framework topology of SSZ-44 viewed down the pores ([001] direction). The oxygen atoms have been omitted for clarity. Mirror planes are represented by solid lines separating layerlike building units. Arrows indicate the layer-like unit common to both SSZ-44 and SSZ-35. b) Basic building unit (BBU) of SSZ-35 and SSZ-44. c) Interconnection of BBUs along pore direction ([001] direction) to form stacked columns. d) Interconnection of stacked columns to form layer-like unit common to both SSZ-35 and SSZ-44. e) Framework topology of SSZ-35 viewed down the pores ([001] direction). Inversion centers are represented by black dots separating layerlike building units.

units related by AB stacking. These layer units are composed of basic building units (BBU) that contain five-membered rings (Figure 1b). The BBUs are stacked along the [001] pore direction and offset to form staircase-like columns (Figure 1c). These columns are then interconnected to create the layerlike unit (Figure 1d).

Replacement of the mirror plane symmetries possessed by the SSZ-44 structure with inversion centers generates a new topology composed of the same layerlike units found in SSZ-44. This new topology involves ABC stacking of the layers but still contains a one-dimensional pore system similar to that found in SSZ-44. The distance-least-squares (DLS^[10]) refinement indicated reasonable structural parameters for this ABC stacking topology.

Similarities between the catalytic activity of SSZ-35 and SSZ-44 led to the identification of SSZ-35 as a potential candidate for the ABC stacking polymorph of SSZ-44. Comparison of the simulated powder X-ray data^[11] for the ABC stacking polymorph to the experimental SPXRD for SSZ-35 and the subsequent Rietveld refinement (Figure 3) confirmed that SSZ-35 (Figure 1e) is the ABC stacking polymorph of SSZ-44.^[12]

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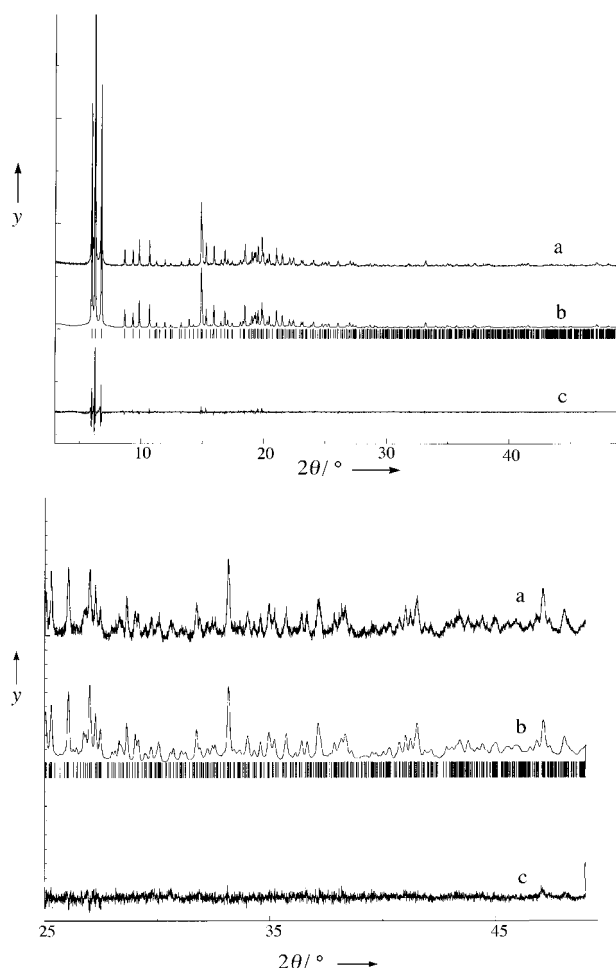


Figure 2. Synchrotron powder X-ray Rietveld refinement of SSZ-44: a) experimental, b) simulated, c) difference plots (plotted vs. relative intensity y). The lower data set shows an expansion of the 2θ range between 25 and 50° (multiplication factor $\times 10$ relative to upper data set).

SSZ-44 and SSZ-35 both possess an unusual one-dimensional straight channel system with a pore diameter that alternates between a 10MR and a contorted 18MR (Figure 4). This alternating pore diameter of the straight channels can also be viewed as a consequence of the stacking of cages with 10 MR portals at the top and bottom. The cage structures of the two related materials SSZ-44 and SSZ-35 differ from each other in their unique arrangements of the four-, five-, and six-membered rings that circumscribe the internal walls of the cage (Figure 4). The 10MR of the SSZ-35 material has a free diameter of 5.5×6.1 Å, while the 18MR has a free diameter of 12.5×9 Å. The 10MRs in the SSZ-44 material are nearly circular (free diameter of 5.8 Å), while the 18MRs possess a slightly larger free diameter (12.9×9 Å) than those in the SSZ-35 material. For comparison, the only two known extra-large pore 14MR zeolites, UTD-1 and CIT-5, contain pores with free diameters of 10×7.5 Å and 7.4 Å, respectively.

As previously observed, SSZ-35 and SSZ-44 are related to each other through the orientation of the successively stacked layerlike unit that is contained in both structures (Figure 1 d). The SSZ-44 topology results if the layers are related through mirror planes and the SSZ-35 topology results if these layers

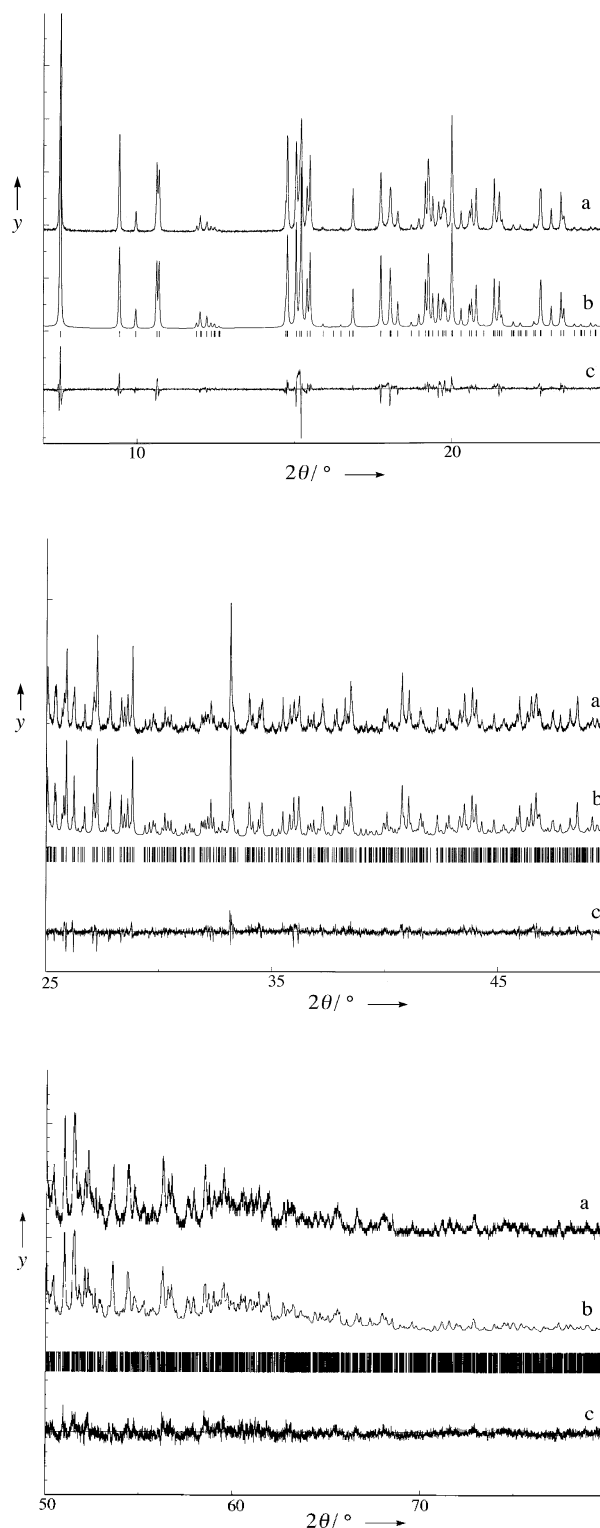


Figure 3. Synchrotron powder X-ray Rietveld refinement of SSZ-35: a) experimental, b) simulated, c) difference plots (plotted vs. relative intensity y). The middle data set shows an expansion of the 2θ range between 25–50° (multiplication factor $\times 8$ relative to upper data set), the lower data set shows an expansion of the 2θ range between 50–80° (multiplication factor $\times 32$ relative to upper data set).

are related through inversion centers (Figures 1 a and e). However, Rietveld refinement indicates that these materials are the pure end-member polymorphs which means they are neither faulted or intergrown. This is surprising since there

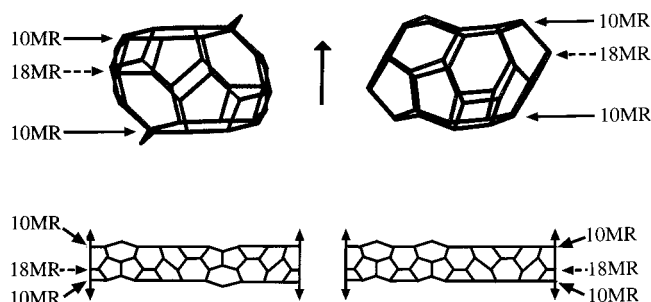


Figure 4. Illustration of the alternating 10 MR – 18 MR pore diameter for the SSZ-35 (left) and SSZ-44 (right) structures. Pore direction ([001] direction) is parallel to central vertical arrow (top half of the Figure). Arrangement of the ring system which circumscribes the internal pore surface is depicted in the bottom half of the Figure.

exist numerous examples of faulted or intergrown zeolites that are also composed of similarly related layers with varying stacking probabilities (e.g. FAU/EMT,^[14] CON/SSZ-33/SSZ-26,^[15] and zeolite beta^[16]). These faulted intermediates are encountered frequently as a result of the poor specificity of the structure-directing agent for a particular end-member polymorph.

Often the pure end-member polymorphs are highly desired for properties that are unique from the faulted intermediates. One notable example is that of the chiral end-member of the zeolite beta fault series that could have tremendous utility in the catalysis and separation of chiral molecules. However, the designed synthesis of pure end-member zeolite polymorphs from the observed faulted intermediates has met with only limited success due to the poor understanding between the properties of the organic structure-directing agent or template and the stacking probability of the resulting zeolite framework. Although studies of the EMT/FAU fault series with crown ether SDAs have provided some insight into the effects of the SDA on the FAU/EMT fault probability,^[17, 18] a greater understanding of the factors that influence zeolite faulting is needed.

The SSZ-35 and SSZ-44 materials allow the complex relationship between the SDA and resulting inorganic framework to be investigated by probing the synthetic and structural parameters that determine the stacking probabilities between these end-members.^[19] Determination of the pertinent factors that govern zeolite faulting will open the possibility of eventually designing fault- and intergrowth-free microporous materials that are of great industrial and academic interest such as chiral zeolite beta.

In addition to being the pure end-member polymorphs of a new fault series, these materials are also the first high-silica zeolites found to possess 18MR-containing pores and illustrate the potential for the synthesis of thermally stable zeolites with pores larger than 14MR. Such extra-large pore zeolites are in great demand industrially for catalytic applications on large molecules.

Experimental Section

Synthesis of the organic structure-directing agent (SDA): The quaternary ammonium compound for use in the SSZ-44 reaction, *N,N*-diethyl 2,6-*cis*-dimethylpiperidinium (SDA I), is prepared by exhaustive ethylation of 2,6-

cis-dimethylpiperidine by ethyl iodide in the presence of potassium bicarbonate in methanol. A more detailed procedure can be found in the analogous description for the 3,5-dimethylpiperidine derivative published by Nakagawa.^[20]

N-ethyl, *N*-methyl 2,6-*cis*-dimethylpiperidinium (SDA II), used to synthesize SSZ-35, is prepared by a sequential alkylation of the 2,6-*cis*-dimethylpiperidine with ethyl iodide in methanol, removal of HI with potassium bicarbonate, and subsequent reaction of the tertiary base product with methyl iodide, again with methanol as solvent. Note that a large number of other organic SDAs can also be used to crystallize SSZ-35.^[21]

Both SDAs are converted to their OH[−] forms from the iodide salts by treatment with AG1-X8 hydroxide ion-exchange resin from BioRad. The exchange can be carried out by running the compound through the resin in a column, or by simply stirring the components overnight. The molarity of the SDA solution is determined by titration.

Zeolite synthesis: Either zeolite can be synthesized from the appropriate SDA by use of the following reaction quantities. SDA (5.1 g of a 0.58 M solution) (SDA I is employed to crystallize SSZ-44 or SDA II for SSZ-35) was measured into the Teflon cup of a Parr 23 mL reactor bomb. KOH (1.5 g of a 1.0 N solution) and water (4.4 g) were also added. Reheis F-2000 (hydrated alumina, ~53 wt % Al₂O₃) (0.04 g) was dissolved in the solution. Finally Cabosil-M5 (97 % fumed SiO₂) (0.90 g) was added to the synthesis mixture and the mixture was stirred. The Teflon cup with the synthesis mixture was closed and sealed in a Parr autoclave. The autoclave was rotated at 43 rpm, while heated to 160 °C. The crystalline zeolite product was obtained after the mixture had been heated for 6–11 days. The solids were filtered, washed with approximately 100 times the water volume of the reaction, and air-dried.

The high-silica materials were prepared for synchrotron powder X-ray data (SPXRD) collection by calcination at 550 °C, then the sample was heated under vacuum in a capillary tube to 350 °C and the capillary tube was sealed under vacuum. The data were collected at the X7A beamline at the National Synchrotron Light Source at Brookhaven Laboratory (Upton, NY).

For the Rietveld refinements of SSZ-35 and SSZ-44 from the SPXRD, the background parameters (shifted Chebyshev function), the scale factor, and the zero shift were first refined, followed by the refinement of the lattice parameters and the profile parameters (Simpson's rule integration of the pseudovoigt function), and finally the atomic positions and the isotropic temperature factors were refined.

Structure and refinement details for SSZ-44: The initial unit cell parameters for the calcined SSZ-44 material were obtained from the indexation of the SPXRD and refined to $a = 11.48534(26)$, $b = 21.94579(49)$, $c = 7.38811(14)$ Å and $\beta = 94.7016(3)^\circ$ ($V = 1855.9$ Å³). Analysis of the systematic absences indicated symmetry consistent with space group $P2_1/m$ (no. 11). The observed structure factors were extracted by the LeBail extraction technique^[22] and normalized by use of the scale factor obtained from the Wilson plot of the data. Reflections with d spacings larger than 1.3 Å were used as a pseudo single-crystal data set for input into the Fourier recycling algorithm, FOCUS,^[8] for structure solution. At the 1.3 Å resolution limit employed, the reflection overlap (defined here as $\theta_1 - \theta_2 < [\text{FWHM}_1 + \text{FWHM}_2]/2$) (FWHM = full width at half maximum) is approximately 80 %.

For the Rietveld refinement of SSZ-44, 136 variables were refined over a profile range of $3.0 - 50^\circ 2\theta$ ($\lambda = 1.20106$ Å, step size = $0.005^\circ 2\theta$ which resulted in 9400 observables) and contained 731 reflections. A total of 82 soft constraints were employed (32: $d(\text{Si}-\text{O}) = 1.61(01)$ Å, 50: $d(\text{O}-\text{O}) = 2.61(01)$ Å) which resulted in $d(\text{Si}-\text{O})$ of 1.597(15) Å (max 1.627, min 1.568 Å). The average O-Si-O angle was 109.47° (max 112.4°, min 106.2°), and the average Si-O-Si angle was 151.4° (max 165.5°, min 133.0°). The weighting for the soft constraints was reduced but elimination of the constraints caused slight bond angle and distance distortions. The final residual values were $wR_p = 0.1058$ and an $R_p = 0.0892$. The Fourier difference map showed maximum peaks heights all less than $0.5 \text{ e}^- \text{ Å}^{-3}$. The atomic positions together with the isotropic temperature factors are presented in Table 1.

Structure and refinement details for SSZ-35: The highest maximal topology of the pure-silica SSZ-35 framework and the symmetry of the as-made

Table 1. Fractional atomic coordinates for SSZ-44, esds in parentheses, (monoclinic, $P2_1/m$, $a = 11.48534(26)$, $b = 21.94579(49)$, $c = 7.38811(14)$ Å, $\beta = 94.7016(3)^\circ$).^[a]

Atom	x	y	z	U(iso)
Si	0.27227(57)	0.97791(29)	0.93601(92)	– 0.0044
Si	0.13548(63)	0.62930(29)	0.10193(97)	– 0.0023
Si	0.23886(57)	0.67904(28)	0.47825(90)	0.0142
Si	0.39323(60)	0.56890(30)	0.59794(84)	0.0019
Si	0.03652(58)	0.68184(27)	0.74254(92)	0.0161
Si	0.05149(61)	0.04087(32)	0.76211(88)	0.0115
Si	0.11053(58)	0.06798(30)	0.36508(91)	– 0.0059
Si	0.35948(58)	0.02838(31)	0.29204(89)	0.0039
O	0.22578(82)	0.91806(33)	0.02742(13)	0.0110
O	0.32885(84)	0.96241(46)	0.75474(103)	0.0330
O	0.36382(71)	0.01059(45)	0.08089(86)	0.0365
O	0.16115(66)	0.02349(41)	0.89856(12)	0.0162
O	0.02146(73)	0.59436(39)	0.15514(13)	– 0.0042
O	0.19913(84)	0.66360(45)	0.27348(97)	0.0097
O	0.09682(88)	0.67737(39)	0.94529(100)	0.0453
O	0.27877(107)	0.7500	0.49498(185)	0.0128
O	0.34544(77)	0.63729(30)	0.55819(136)	0.0259
O	0.13244(80)	0.66902(52)	0.60239(139)	0.0218
O	0.36967(107)	0.53179(37)	0.41218(103)	0.0088
O	0.53084(53)	0.57271(41)	0.65389(140)	0.0194
O	0.93509(70)	0.63319(30)	0.70903(128)	0.0258
O	0.98407(103)	0.7500	0.71105(177)	0.0303
O	0.96479(79)	0.98471(36)	0.73380(146)	0.0147
O	0.09791(92)	0.06329(49)	0.57827(89)	0.0087
O	0.24371(53)	0.06267(46)	0.32726(144)	0.0365

[a] This structure solution has been accepted by the International Zeolite Association Structure Committee and the code SFF has been assigned for SSZ-44.

SSZ-35 material is monoclinic ($C2/m$) with unit cell parameters of $a = 13.9$, $b = 18.2$, $c = 7.4$ Å and $\beta = 99.4^\circ$. However, upon calculation the interaxial angles of the monoclinic cell relax to $\alpha = 90.924^\circ$, $\beta = 98.920^\circ$, and $\gamma = 89.437^\circ$ which lowers the symmetry to triclinic (space group symmetry to $P\bar{1}$). The refined unit cell parameters for the SSZ-35 reduced cell are $a = 11.411404(137)$, $b = 11.526825(136)$, $c = 7.376957(74)$ Å, $\alpha = 94.6607(7)^\circ$, $\beta = 96.2055(8)^\circ$, and $\gamma = 104.8919(6)^\circ$ ($V = 926.244$ Å³).

For the Rietveld refinement of SSZ-35, 123 parameters were refined over the profile range $7-80^\circ 2\theta$ ($\lambda = 1.20106$ Å, step size = $0.005^\circ 2\theta$ which resulted in 14600 observables). 80 soft constraints were employed (32: $d(\text{Si}-\text{O}) = 1.61(01)$ Å, 48: $d(\text{O}-\text{O}) = 2.61(01)$ Å) which resulted in $d(\text{Si}-\text{O}) = 1.599(11)$ Å (max 1.6165, min 1.5765 Å), an average O-Si-O angle of 109.466° (max 111.77° , min 107.52°), and an average Si-O-Si bond angle of 152.33° (max 162.62° , min 136.618°). The weighting for the soft constraints was reduced but elimination of the constraints caused slight bond angle and distance distortions. The final refinement resulted in $wR_p = 0.1188$ and $R_p = 0.0995$. The Fourier difference map showed maximum peaks heights less than $0.6 \text{ e}^- \text{ Å}^{-1}$. The atomic positions together with the isotropic temperature factors are presented in Table 2.

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-410595 (SSZ-35) and CSD-410596 (SSZ-44).

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Table 2. Fractional atomic coordinates for SSZ-35, esds in parentheses, (triclinic, $P\bar{1}$, $a = 11.411404(137)$ Å, $b = 11.526825(136)$ Å, $c = 7.376957(74)$ Å, $\alpha = 94.6607(7)^\circ$, $\beta = 96.2055(8)^\circ$, $\gamma = 104.8919(6)^\circ$).^[a]

Atom	x	y	z	U(iso)
Si	0.36019(35)	0.35630(33)	0.61106(50)	0.01319
Si	0.41221(34)	0.42852(33)	0.21918(49)	0.01888
Si	0.14209(37)	0.64205(36)	0.40966(53)	0.02179
Si	0.36181(37)	0.85678(37)	0.57148(54)	0.02607
Si	0.13396(37)	0.44151(35)	0.67151(54)	0.01930
Si	0.23859(38)	0.56731(36)	0.04636(53)	0.02242
Si	0.46001(38)	0.75796(38)	0.92351(53)	0.02925
Si	0.55968(37)	0.87468(35)	0.30376(52)	0.02226
O	0.36502(60)	0.37709(37)	0.40067(83)	0.02369
O	0.23168(48)	0.36461(47)	0.67114(77)	0.01125
O	0.47018(43)	0.45770(45)	0.73017(88)	0.03990
O	0.37472(50)	0.22510(37)	0.64375(83)	0.02625
O	0.30673(48)	0.47043(48)	0.10734(76)	0.03288
O	0.44890(49)	0.32523(47)	0.09908(72)	0.03153
O	0.22725(38)	0.77005(38)	0.50790(72)	0.02335
O	0.16588(60)	0.54074(44)	0.53230(76)	0.02545
O	0.17639(55)	0.61558(53)	0.20899(78)	0.03111
O	0.00178(28)	0.64477(50)	0.39598(79)	0.01427
O	0.43707(44)	0.85788(66)	0.39827(68)	0.03808
O	0.35217(52)	0.99191(30)	0.62479(81)	0.02041
O	0.43055(53)	0.81119(54)	0.73707(58)	0.02927
O	0.13629(45)	0.50548(50)	0.87315(54)	0.01517
O	0.33382(44)	0.67945(46)	0.98040(85)	0.03128
O	0.52218(51)	0.86584(43)	0.08546(49)	0.02315

[a] This structure solution has been accepted by the International Zeolite Association Structure Committee and the code STF has been assigned for SSZ-35.

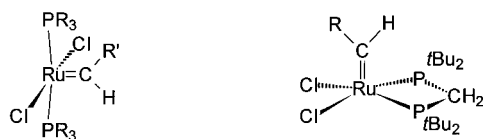
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A New Class of Ruthenium Carbene Complexes: Synthesis and Structures of Highly Efficient Catalysts for Olefin Metathesis**

S. Michael Hansen, Martin A. O. Volland, Frank Rominger, Frank Eisenträger, and Peter Hofmann*

Dedicated to Professor Hans Hofmann on the occasion of his 70th birthday

Efficient, well-defined, single-component, single-site homogeneous catalysts for olefin metathesis have recently provided new, elegant tools for C–C bond formation in organic synthesis and polymer chemistry.^[1a] Due to their stability, Grubbs-type square-pyramidal ruthenium(II) carbene complexes with *trans*-phosphane ligands (**1a–c**) have found wide application in ring opening metathesis polymerization (ROMP), in ring-closing metathesis (RCM), in acyclic diene metathesis (ADMET), and in acyclic olefin metathesis reactions.^[1–3] Attempts to improve their catalytic performance have focussed upon varying the carbene fragment,^[3,4] the phosphane^[3] or anionic ligand,^[5] replacement of the phosphane and chloro ligand by chelating Schiff-bases,^[6] on exchanging the two *trans*-phosphanes by Wanzlick–Arduengo-type carbenes,^[7] and upon Lewis acid addition.^[8a,b] Quite recently Grubbs et al. prepared neutral and cationic Ru^{II}(Tp) carbenes (Tp = tris(pyrazolyl)borate), which seem to be inactive.^[9] Based on previous experience^[10] with bis(di-*tert*-butylphosphanyl)methane (dtbpm) *t*Bu₂PCH₂P*t*Bu₂^[11] as a ligand, we recently prepared the first Grubbs-type catalyst with a rigid *cis*-stereochemistry of phosphane ligands **2a**.^[12]

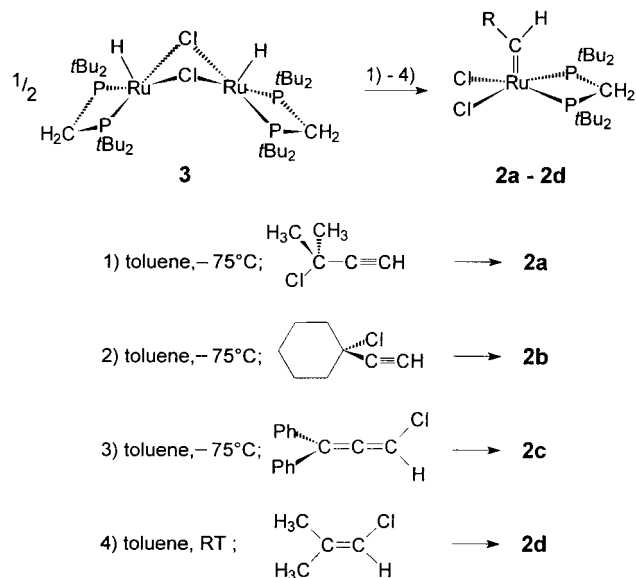


1a: R = Cy, R' = Ph
1b: R = Cy, R' = CH=CPh₂
1c: R = Ph, R' = CH=CPh₂

2a: R = CH=CMe₂
2b: R = CH=C(Ph)₂
2c: R = CH=C(Ph)₂
2d: R = CHMe₂

Here we report the synthesis, structures, and properties of a novel class of cationic Ru^{II} carbene complexes^[13] with high catalytic activity in ROMP reactions. They are easily obtained from **2a** and from its congeners **2b–d** by chloride abstraction with trimethylsilyl triflate.

Reactions of ruthenium hydrides with propargyl or vinyl chlorides yielding Grubbs-type carbene complexes are well established.^[14] The dinuclear dihydride **3**, accessible in a one-pot reaction from [(cod)RuCl₂]_x (cod = 1,5-cyclooctadiene), has been shown to be an excellent precursor for **2a** (Scheme 1),^[12] and the cyclohexylidene derivative **2b** can be



Scheme 1. Synthesis of complexes **2a–d** from **3** and propargyl, allenyl, and vinyl chlorides.

prepared from **3** and 1-chloro-1-ethynylcyclohexane^[15] in an analogous way. In the synthesis of the diphenyl derivative **2c**, we discovered that 1-chloro-3,3-diphenyl-1,2-propadiene is a more suitable substrate than the corresponding isomeric propargyl compound 3-chloro-3,3-diphenyl-1-propyne.^[16] Compound **2c** was obtained from **3** and the chloroallene in 74% yield. Chloroallenes thus are established as another convenient class of organic precursors for the synthesis of carbene complexes [(κ²-dtbpm)Cl₂Ru=CH–CH=CR'R²] from **3**. When the vinyl chloride 1-chloro-2-methyl-1-propene was employed as a substrate, the saturated carbene complex **2d** could be isolated in 41% yield. The structures of all new carbene complexes **2b–d** have been confirmed by single-crystal X-ray diffraction.^[17a] Their molecular geometries, represented in Figure 1 for **2d** as a typical case, display interesting subtle differences, which have been discussed on the basis of EH and DFT calculations elsewhere.^[12] Similar to **2a**, complexes **2b–d** are active catalysts for ROMP reactions of norbornene.

The catalytic ROMP activity of the neutral complexes, both with unsaturated and saturated carbene ligands, can be dramatically enhanced, however, by treating them with trimethylsilyl triflate in CH₂Cl₂ at ambient temperature. The irreversible formation of Me₃SiCl by chloride abstraction from **2a** and **2d** leads to air-stable, dinuclear dications **4a** and **4d** as triflates, which can be isolated in 89–94% yield (Scheme 2). The cations **4b** and **4c** were synthesized analogously from **2b** and **2c**. The molecular structure of **4a**, which is very similar to that of **4d**, is displayed in Figure 2.^[17b]

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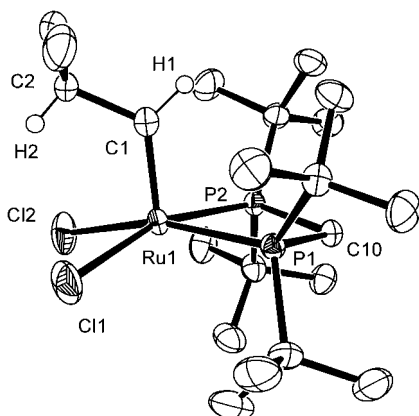


Figure 1. Molecular structure of the saturated carbene complex **2d** in the crystal (ORTEP, 50 % probability, CH hydrogen atoms except H1 and H2 of the carbene moiety omitted for clarity). Selected bond lengths [Å] and angles [°]: Ru1–P1 2.3328(5), Ru1–P2 2.3435(5), Ru1–Cl1 2.4099(6), Ru1–Cl2 2.4047(6), Ru1–C1 1.826(2), C1–C2 1.513(3); P1–Ru1–P2 73.485(17), Cl1–Ru1–Cl2 87.76(2), P1–Ru1–Cl1 95.82(2), P2–Ru1–Cl1 162.08(2), P1–Ru1–Cl2 160.46(3), P2–Ru1–Cl2 97.77(2), C1–Ru1–P1 97.62(7), C1–Ru1–P2 96.17(7), C1–Ru1–Cl1 99.53(8), C1–Ru1–Cl2 100.73(7).

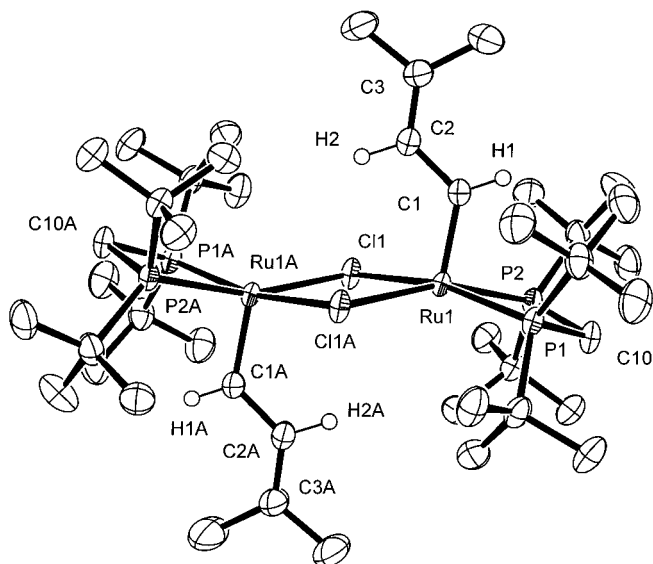
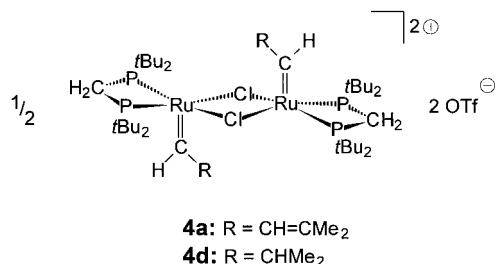
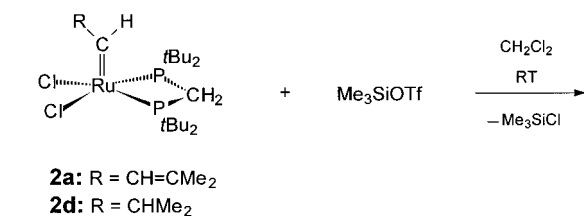


Figure 2. Molecular structure of dication **4a** in the crystal (ORTEP, 50 % probability, CH hydrogen atoms except H1, H1A, H2, and H2A of the carbene moieties omitted for clarity). Selected bond lengths [Å] and angles [°]: Ru1–P1 2.3253(9), Ru1–P2 2.336(1), Ru1–Cl1 2.4659(9), Ru1–Cl1A 2.5040(9), Ru1–C1 1.857(4), C1–C2 1.417(6), C2–C3 1.345(6); P1–Ru1–P2 73.63(3), Cl1–Ru1–Cl1A 80.95(3), P1–Ru1–Cl1 161.38(4), P2–Ru1–Cl1 100.01(3), P1–Ru1–Cl1A 101.23(3), P2–Ru1–Cl1A 166.63(4), C1–Ru1–P1 93.85(13), C1–Ru1–P2 95.08(13), C1–Ru1–Cl1 104.25(13), C1–Ru1–Cl1A 97.61(13), Ru1–Cl1–Ru1A 99.05(3).



Scheme 2. Preparation of **4a** and **4d** from **2a** and **2d** using Me₃SiOTf. OTf = OSO₂CF₃.

Each ruthenium center of **4a** and **4d** is in a square-pyramidal ligand environment with P atoms of κ^2 -dtbpm and the μ -chloro ligands in the basal plane and the carbene moiety in an apical position, nearly bisecting the P–Ru–P angle. In the solid state, the two carbene fragments are *trans* to each other. The two triflate ions are not coordinated to the ruthenium centers. ¹³C and ³¹P NMR spectra of **4a** in solution show a complete double set of signals, indicating the presence of two stereoisomers. Variable-temperature NMR spectra in CD₂Cl₂ reveal a dynamic equilibrium between two dimeric species with *cis* and *trans* arrangement of the carbene moieties. At –40 °C the ¹H NMR signals of the olefinic β protons of the two isomers are well resolved (δ = 9.18, 9.29; ³J(H _{β} , H _{α}) = 11.1 and 11.3 Hz, respectively, Figure 3).^[18]

Equilibration between both structures occurs through mononuclear, solvent-stabilized, five-coordinate cationic carbene complexes [(κ^2 -dtbpm)ClRu=CH–CH=CMe₂(Solv)]⁺.

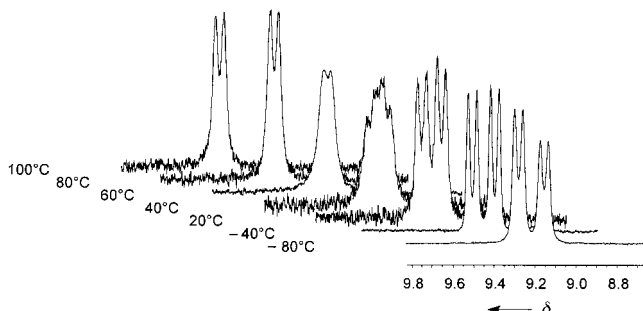
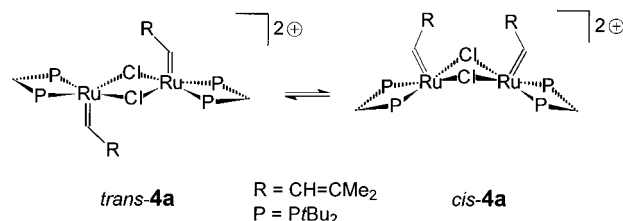
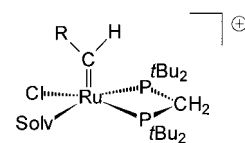


Figure 3. Variable-temperature ¹H NMR spectra of **4a** in CD₂Cl₂ (vinyl proton resonances), indicating *cis*–*trans* interconversion between the dinuclear carbene species *cis*-**4a** and *trans*-**4a**.^[18]

We are able to observe such a monomeric species **6d** in ¹H and ³¹P NMR spectra of **4d** in CD₂Cl₂. In a 1:1 mixture of **4a** and **4d** in the same solvent the signals of all dinuclear dicationic cross products (*cis* and *trans* isomers) can be detected and identified. The new cationic carbene complexes are very efficient catalysts for ROMP reactions. In con-



6a: R = CH=CMe₂, Solv = CD₂Cl₂
6d: R = CHMe₂, Solv = CD₂Cl₂

trast to the cationic carbyne systems described by Werner et al.,^[13b] they are thermally stable in solution (CD_2Cl_2), without signs of decomposition (NMR spectroscopy) for three days at ambient temperature under an argon atmosphere. The turnover frequency (TOF) for the polymerization of norbornene in CH_2Cl_2 rises from 60 h^{-1} for **2a** (71 % *trans*) to 8400 h^{-1} for **4a** (80 % *trans*); cyclopentene is also efficiently polymerized. To get a precise comparison with Grubbs' catalyst (**1a**) and Herrmann's *trans*- $[\text{L}_2\text{Cl}_2\text{Ru}=\text{CHPh}]$ (**5**, $\text{L} = N,N'$ -diisopropylimidazolin-2-ylidene),^[7] which currently are the most active Ru^{II} carbene complexes, we have studied the polymerization of cyclooctene with **4a** as catalyst by ^1H NMR spectroscopy. The relative activity of Grubbs' catalyst **1a**, which is more active than **5**,^[7] could be reproduced by our measurements conducted under identical conditions. The much higher reactivity of **4a** required an increase of the [cyclooctene]:[Ru] ratio by a factor of 50. The rates of formation of cyclooctenamer with **1** and **4a** under these conditions is compared in Figure 4.^[19] To the best of our knowledge, **4a** and its derivatives represent the most active homogeneous Ru^{II} ROMP catalysts described so far.

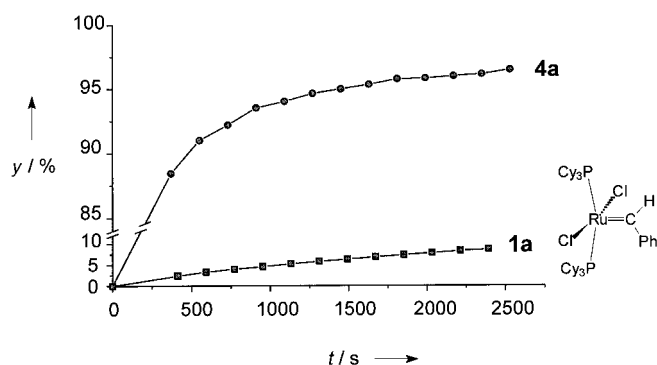


Figure 4. Kinetics of the ROMP reaction of cyclooctene. Comparison of polyoctenamer production using **4a** versus carbene complex **1a** as monitored by ^1H NMR spectroscopy. $T = 23^\circ\text{C}$; $0.5 \text{ mL } \text{CD}_2\text{Cl}_2$; [cyclooctene]/[Ru] = 12500:1.^[19] y = yield.

Unlike the neutral compounds **2a–d**, dication **4a** also catalyzes the RCM of 1,7-octadiene to cyclohexene. In ^1H NMR experiments at temperatures between -80 to 0°C (CD_2Cl_2 , [diene]:[Ru] = 24:1), the reaction already starts at -40°C , yielding cyclohexene (24 %) along with octadiene isomers (26 %).^[20] While **4a** slowly disappears, a second, transient carbene species can be identified by a carbene hydrogen quartet at $\delta = 16.97$.

Although detailed investigations will be necessary to establish the role of dinuclear $[(\kappa^2\text{-dtbpm})(\mu\text{-Cl})\text{Ru}=\text{CHR}]_2^{2+}$ species **4a–d**, we believe the observed mononuclear cations $[(\kappa^2\text{-dtbpm})\text{ClRu}=\text{CHR}(\text{Solv})]^+$ (**6**) ($\text{Solv} = \text{CD}_2\text{Cl}_2$) function as the active catalysts in ROMP and RCM reactions of **4a** and its congeners.

In summary, a facile, general two-step synthesis of neutral Ru^{II} carbenes $[(\kappa^2\text{-dtbpm})\text{Cl}_2\text{Ru}=\text{CHR}]$ from **3** and propargyl, vinyl, or allenyl chlorides, followed by chloride abstraction with trimethylsilyl triflate as a convenient and efficient reagent, has made accessible a new class of highly active, cationic Ru^{II} metathesis catalysts. Halide abstraction is

facilitated by the chelating phosphane ligand dtbpm, which forces the P-Ru-Cl *trans* arrangement. It should be noted that in these cases, halide as opposed to phosphane dissociation, claimed to be the initiating step in metathesis reactions of Grubbs-type *trans* complexes,^[5b,21] leads to remarkably increased catalytic activity. Similar effects of halide abstraction have been observed by Osborn et al. for tungsten carbenes.^[22] Efforts to tune the catalytic properties of systems **4** by modifying the ligand set, counterion, and carbene structure, along with polymer characterization, mechanistic, and computational studies are under way in order to establish the potential of these novel catalysts.

Experimental Section

All reactions were carried out under an atmosphere of dry argon with standard Schlenk tube techniques. Solvents were dried according to standard procedures and saturated with argon prior to use. Abbreviations used for NMR data are: s = singlet, d = doublet, t = triplet, "d" = pseudo-doublet, "t" = pseudotriplet, "q" = pseudoquartet, "quint." = pseudoquintet, "sept." = pseudoseptet, m = multiplet, ΣH = sum of integrals in ^1H NMR spectra for specified resonances.

2c: A solution of 1-chloro-3,3-diphenyl-1,2-propadiene (85 mg, 0.376 mmol) in toluene (6 mL) was added under stirring to a solution of complex **3** (158 mg, 0.179 mmol) in toluene (6 mL) at -75°C . The reaction mixture was allowed to warm to ambient temperature within 1 h, and then stirred for an additional 2 h at this temperature. The resulting microcrystalline green precipitate, which was separated from the brown supernatant solution by cannula filtration, was washed with toluene ($2 \times 1 \text{ mL}$) and hexane ($3 \times 2 \text{ mL}$). The remaining yellow-green solid was dried in vacuo (10^{-5} bar). Yield: 177 mg (74 %). Elemental analysis (%): calcd: C 57.48, H 7.54, P 9.26; found: C 57.37, H 7.52, P 9.31. The molecular structure was confirmed by single-crystal X-ray diffraction. ^1H NMR (CD_2Cl_2 , 300 MHz): $\delta = 1.05$ ("d", 18H, *t*Bu-H), 1.54 ("d", 18H, *t*Bu-H), 3.90 ("quint.", ABX₂ system, $^2J(\text{H,H}) = 16.7 \text{ Hz}$, $^2J(\text{H,P}) = 9.1 \text{ Hz}$, 1H, PCHHP), 4.04 ("quint.", ABX₂ system, $^2J(\text{H,H}) = 16.5 \text{ Hz}$, $^2J(\text{H,P}) = 8.8 \text{ Hz}$, 1H, PCHHP), 7.16–7.69 (m, 10H, Ar-H), 9.44 (d, $^3J(\text{H,H}) = 10.4 \text{ Hz}$, 1H, CHCH=CPh₂), 15.74 ("q", $^3J(\text{H,H}) = 10.8 \text{ Hz}$, $^3J(\text{P,H}) = 11.0 \text{ Hz}$, 1H, Ru=CH); $^{31}\text{P}\{^1\text{H}\}$ NMR (CD_2Cl_2 , 121 MHz): $\delta = 25.2$ (s, $\text{P-CH}_2\text{-P}$); IR (KBr): $\tilde{\nu} = 1526 \text{ cm}^{-1}$ (C=C).

2d: 1-Chloro-2-methyl-1-propene (338 mg, 3.73 mmol) was added to a solution of **3** (200 mg, 0.226 mmol) in toluene (20 mL) at ambient temperature. After the mixture was left to stand for 72 h a red crystalline precipitate had formed. The solid was isolated from the supernatant deep red solution by cannula filtration and washed with toluene ($5 \times 2 \text{ mL}$) and hexane ($3 \times 4 \text{ mL}$). The remaining red solid was dried in vacuo (10^{-5} bar). Yield: 98 mg (41 %). Elemental analysis (%): calcd: C 47.37, H 8.71, P 11.63, Cl 13.32; found: C 47.44, H 8.68, P 11.73, Cl 12.93; ^1H NMR (CD_2Cl_2 , 200 MHz): $\delta = 1.19$ (d, 6H, $^3J(\text{H,H}) = 6.9 \text{ Hz}$, CHCH(CH₃)₂), 1.30 ("d", 18H, *t*Bu-H), 1.56 ("d", 18H, *t*Bu-H), 4.01 ("sept.", $N = 61.0 \text{ Hz}$, 2H, PCH₂P), 5.50 (m, $N = 40.8 \text{ Hz}$, 1H, CHCH(CH₃)₂), 16.22 (dt, $^1J(\text{H,H}) = 7.9 \text{ Hz}$, $^3J(\text{H,P}) = 11.8 \text{ Hz}$, 1H, Ru=CH); $^{31}\text{P}\{^1\text{H}\}$ NMR (CD_2Cl_2 , 121 MHz): $\delta = 22.7$ (s, PCH₂P).

4a: $\text{Me}_3\text{SiOSO}_2\text{CF}_3$ (63 mg, 0.283 mmol) was added to a solution of **2a** (50 mg, 0.092 mmol) in CH_2Cl_2 (5 mL) at ambient temperature. The red-brown reaction solution was stirred for 1 h and then concentrated to 1 mL in vacuo. Upon addition of hexane (5 mL), a green solid precipitated that was separated from the mother liquid by cannula filtration. The solid was dissolved in CH_2Cl_2 (1 mL) and reprecipitated with hexane (3 mL). After this procedure had been repeated three times, the remaining green microcrystalline green solid was washed with hexane ($3 \times 3 \text{ mL}$) and dried in vacuo (10^{-5} bar). Yield: 57 mg (94 %). Elemental analysis (%): calcd: C 41.97, H 7.04; found: C 41.46, H 7.02; ^1H NMR (CD_2Cl_2 , 300 MHz, -40°C): $\delta = 1.21$ (m, 72H, $N = 20.5 \text{ Hz}$, *t*Bu-H), 1.56 ("d", 36H, *t*Bu-H), 1.64 ("d", 36H, *t*Bu-H), 1.70, 1.73, 1.80, 1.83 (each s, each 6H, CHCH=C(CH₃)₂), 4.27 ("t", $N = 18.2 \text{ Hz}$, 8H, PCH₂P), 9.18 (d, $^3J(\text{H,H}) = 11.1 \text{ Hz}$, 2H, CHCH=C(CH₃)₂), 9.29 (d, $^3J(\text{H,H}) = 11.3 \text{ Hz}$, 2H, CHCH=C(CH₃)₂), 17.32 (m, $N = 43.0 \text{ Hz}$, 4H, Ru=CH); $^{31}\text{P}\{^1\text{H}\}$ NMR (CD_2Cl_2 , 81 MHz):

$\delta = 26.7$ (s, PCH_2P), 27.4 (s, PCH_2P); $^{13}\text{C}\{^1\text{H}\}$ NMR (CD_2Cl_2 , 75 MHz): $\delta = 22.0$, 22.2, 28.8, 29.1 (each s, $\text{CH}=\text{C}(\text{CH}_3)_2$), 30.3, 30.4, 31.2, 31.3 (each s, $\text{C}(\text{CH}_3)_3$), 37.8 (m, PCH_2P), 38.6 (m, $N = 21.1$ Hz, $\text{C}(\text{CH}_3)_3$), 39.4 (m, $N = 16.5$ Hz, $\text{C}(\text{CH}_3)_3$), 148.6, 148.8 (each s, $\text{CH}=\text{C}(\text{CH}_3)_2$), 149.8, 150.2 (each s, $\text{CH}=\text{C}(\text{CH}_3)_2$), 301.5 (not well resolved, $\text{Ru}=\text{CH}$); IR (KBr): $\tilde{\nu} = 1567\text{ cm}^{-1}$ ($\text{C}=\text{C}$).

Cationic complexes **4b–d** can be prepared in an analogous manner. The structures of **4b** and **4d** were confirmed by single-crystal X-ray diffraction.

4d: Elemental analysis (%): calcd: C 40.90, H 7.18; found: C 40.69, H 7.25; ^1H NMR (CD_2Cl_2 , 500 MHz, -20°C): $\delta = 1.19$ – 1.32 (m, $t\text{Bu}-\text{H}$, $\text{CH}(\text{CH}_3)\text{CH}_3$), 1.43 (d, $^3J(\text{H,H}) = 7$ Hz, $\text{CH}(\text{CH}_3)\text{CH}_3$), 1.51– 1.56 (m, $t\text{Bu}-\text{H}$), 1.68 (“d”, $t\text{Bu}-\text{H}$) Σ_{H} (1.19–1.68) = 42 H, 4.00 (“quint.”, $^3J(\text{H,H}) = 16.9$ Hz, $^2J(\text{H,P}) = 9.4$ Hz, $[(t\text{Bu}_2\text{PCHHP}t\text{Bu}_2)\text{CIRu}=\text{CHCH}(\text{CH}_3)_2(\text{CD}_2\text{Cl}_2)]^+$), 4.19 (“quint.”, $^2J(\text{H,H}) = 16.9$ Hz, $^2J(\text{H,P}) = 9.5$ Hz, $[(t\text{Bu}_2\text{PCHHP}t\text{Bu}_2)\text{CIRu}=\text{CHCH}(\text{CH}_3)_2(\text{CD}_2\text{Cl}_2)]^+$), 4.49 (m, $N = 64.6$ Hz, *cis*- and *trans*- $[(t\text{Bu}_2\text{PCH}_2\text{PtBu}_2)\text{CIRu}=\text{CHCH}(\text{CH}_3)_2]^{2+}$) Σ_{H} (4.00, 4.19, 4.49) = 2 H, 5.28 (broad s, $[(\kappa^2\text{-dtbpm})\text{CIRu}=\text{CHCH}(\text{CH}_3)_2(\text{CD}_2\text{Cl}_2)]^+$), 6.40 (m, $N = 34.5$ Hz, *cis*- and *trans*- $[(\kappa^2\text{-dtbpm})\text{CIRu}=\text{CHCH}(\text{CH}_3)_2]^{2+}$) Σ_{H} (5.28, 6.40) = 1 H, 16.61 (“q”, $^3J(\text{H,H}) = 9.9$ Hz, $^3J(\text{H,P}) = 9.9$ Hz, “0.41 H”, $[(\kappa^2\text{-dtbpm})\text{CIRu}=\text{CHCH}(\text{CH}_3)_2(\text{CD}_2\text{Cl}_2)]^+$), 17.49 (“d”, not well resolved, $^3J(\text{H,P}) = 10.6$ Hz, “0.05 H”, *cis*- $[(\kappa^2\text{-dtbpm})\text{CIRu}=\text{CHCH}(\text{CH}_3)_2]^{2+}$), 17.55 (“q”, $^3J(\text{H,H}) = 10.6$ Hz, $^3J(\text{H,P}) = 10.6$ Hz, “0.54 H”, *trans*- $[(\kappa^2\text{-dtbpm})\text{CIRu}=\text{CHCH}(\text{CH}_3)_2]^{2+}$) Σ_{H} (16.61, 17.49, 17.55) = 1 H; $^{31}\text{P}\{^1\text{H}\}$ NMR (CD_2Cl_2 , 202 MHz, -60°C): $\delta = 22.4$ (s, *trans*-**4d**), 25.2 (s, *cis*-**4d**), 26.1 (d, $^2J(\text{P,P}) = 40.5$ Hz, $[(t\text{Bu}_2\text{PCH}_2\text{PtBu}_2)\text{CIRu}=\text{CHCH}(\text{CH}_3)_2(\text{CD}_2\text{Cl}_2)]^+$), 27.9 (d, $^2J(\text{P,P}) = 40.5$ Hz, $[(t\text{Bu}_2\text{PCH}_2\text{PtBu}_2)\text{CIRu}=\text{CHCH}(\text{CH}_3)_2(\text{CD}_2\text{Cl}_2)]^+$).

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- [17] a) Crystals suitable for X-ray diffraction were obtained from slow evaporation of a saturated solution of **2d** in CH_2Cl_2 . Crystal dimensions $0.28 \times 0.24 \times 0.23\text{ mm}^3$, crystal system orthorhombic, space group $Pca2_1$, $Z = 4$, $a = 21.5872(2)$, $b = 8.2840(1)$, $c = 14.4771(2)\text{ Å}$, $V = 2588.92(5)\text{ Å}^3$, $\rho_{\text{calcd}} = 1.366\text{ g cm}^{-3}$, $2\theta_{\text{max}} = 51.2^\circ$, radiation $\text{MoK}\alpha$, $\lambda = 0.71073\text{ Å}$, $0.3^\circ\omega$ scans with CCD area detector, $T = 200\text{ K}$, 18290 reflections measured, 4498 unique, 4328 observed ($I > 2\sigma(I)$), intensities were corrected for Lorentz and polarization effects, an empirical absorption correction was applied using the SADABS program based on the Laue symmetry of the reciprocal space, $\mu = 0.94\text{ mm}^{-1}$, $T_{\text{min}} = 0.78$, $T_{\text{max}} = 0.84$, structure solved by direct methods and refined against F^2 with a full-matrix least-squares algorithm using the SHELXTL-PLUS (5.10) software package, 257 parameters refined, hydrogen atoms were treated by using the appropriate riding models except of H1 and H2, which were refined isotropically, final residual values $R(F) = 0.017$, $wR(F^2) = 0.042$ for observed reflections, residual electron density -0.32 to 0.29 e Å^{-3} .^[17c] b) Crystals suitable for X-ray diffraction were obtained from slow diffusion of pentane into a saturated solution of **4a** in CH_2Cl_2 . Crystal dimensions $0.36 \times 0.28 \times 0.17\text{ mm}^3$, crystal system monoclinic, space group $P2_1/n$, $Z = 2$, $a = 15.2553(1)$, $b = 15.2411(2)$, $c = 17.2867(1)\text{ Å}$, $V = 3672.97(5)\text{ Å}^3$, $\rho_{\text{calcd}} = 1.497\text{ g cm}^{-3}$, $2\theta_{\text{max}} = 51.1^\circ$, radiation $\text{MoK}\alpha$, $\lambda = 0.71073\text{ Å}$, $0.3^\circ\omega$ scans with CCD area detector, $T = 200\text{ K}$, 26585 reflections measured, 6343 unique, 5444 observed ($I > 2\sigma(I)$), intensities were corrected for Lorentz and polarization effects, an empirical absorption correction was applied by using the SADABS program based on the Laue symmetry of the reciprocal space, $\mu = 0.98\text{ mm}^{-1}$, $T_{\text{min}} = 0.77$, $T_{\text{max}} = 0.91$, structure solved by direct methods and refined against F^2 with a full-matrix least-squares algorithm using the SHELXTL-PLUS (5.10) software package, 396 parameters refined, 24 geometrical restraints (disordered solvent), hydrogen atoms were treated by using appropriate riding models except of H1 and H2, which were refined isotropically, final residual values $R(F) = 0.041$, $wR(F^2) = 0.110$ for observed reflections, residual electron density -0.82 to 1.34 e Å^{-3} . c) Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-112064 (**2d**) and CCDC-112065 (**4a**). 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).
- [18] The high-temperature ^1H NMR spectra were measured in a sealed high-pressure NMR tube under a pressure of 10 bar Ar.
- [19] a) In a glovebox **4a** (1.6 mg, 1.25 μmol) was dissolved in CD_2Cl_2 (2.5 mL). From this solution 50 μL (0.025 μmol **4a**) were immediately transferred into an NMR tube and diluted with CD_2Cl_2 (0.45 mL). After addition of degassed cyclooctene (81.4 μL , 625 μmol ; >99.5% (GC); Fluka) the NMR tube was sealed and shaken. The NMR spectra were recorded at 23°C on a Bruker DRX 500 spectrometer at 500 MHz. b) Polymerization of cyclooctene with **1a** (0.05 μmol ; Strem Chemicals Inc.) as catalyst was carried out under identical reaction conditions.
- [20] The product distribution was analyzed by GC-MS.
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Spacer Control of Directionality in Supramolecular Helicates Using an Inexpensive Approach**

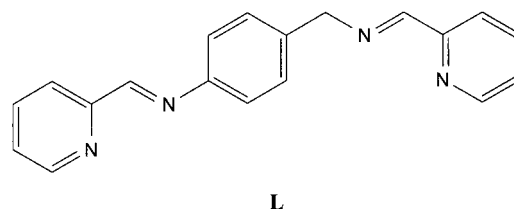
Michael J. Hannon,* Siona Bunce, Adam J. Clarke, and Nathaniel W. Alcock

The double-helical structure has provided an important forum for the evolution of metallo-supramolecular construction principles.^[1] Their application in other systems has led to a plethora of supramolecular architectures such as boxes,^[1–3] grids,^[4] and cylinders.^[5] While the basic features of the design necessary to assemble a double helix are now fairly well established,^[1] challenges in defining the precise topology or conformation of the helical superstructure remain. If a nonsymmetrical ligand is used for helicate formation, the issue of controlling the relative orientations (or directions) of the ligands within the helical superstructure arises. This issue is important because selectively orienting substituents on the outside of a helix is desirable in order to control the assembly of helicates into larger supramolecular arrays (we have previously used this principle to control the aggregation of supramolecular boxes^[2]) or to relay the chiral information inherent in the helix to an extra-helical site.

Constable et al. have shown that by careful positioning of bulky substituents on the back of a ligand, double helices of an exclusive head-to-head or head-to-tail ligand orientation can be assembled.^[6] However, such interactions seem to represent a relatively small energy term and may be relieved by a flattening of the helical pitch resulting in a mix of head-to-head and head-to-tail helices in solution, for all but the most bulky substituents.^[6] An alternative approach is to use different donor sets to control the assembly: Piguet et al.^[7] and Albrecht and Fröhlich^[8] have used ligands containing two different binding units to form directional triple helices.

We reasoned that an alternative approach might be to introduce asymmetry into a spacer placed between the metal-binding domains. Since this introduces the asymmetry directly within the helical array rather than at the periphery, it seems likely to provide a greater energetic stabilization for one orientation over the other. An additional advantage is that the extra-helical sites are left vacant for subsequent incorporation of functional substituents.

We have recently described an inexpensive and simple imine-based approach to supramolecular architecture which has the advantage that it allows us to generate ligands which can act as supramolecular building blocks rapidly in high yields from commercial materials.^[9] Applying this imine approach, we prepared the ligand **L** to investigate the effect



of incorporating an asymmetric spacer. The ligand contains two binding domains separated by a methylenephénylene spacer. The two binding domains are sterically prevented from binding to the same metal center and so the ligand is constrained to act as a dinucleating ligand. As illustrated in Figure 1, a head-to-tail configuration is anticipated when **L**

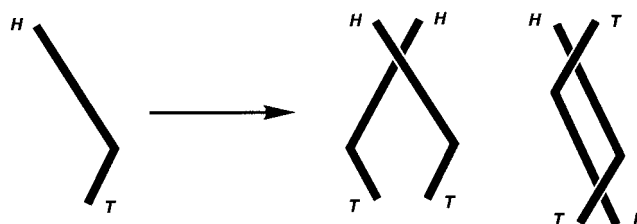


Figure 1. Schematic illustration of the formation of head-to-head and head-to-tail structures.

reacts with tetrahedral metal ions; a head-to-head configuration would result in a very small dihedral angle between the two 'head' binding units (almost square planar rather than tetrahedral) and the two phenyl rings would also be placed unacceptably close. Ligand **L** is prepared in 77% yield by mixing ethanolic solutions containing two equivalents of 2-pyridinecarbaldehyde and one equivalent of 4-aminobenzylamine. Reaction of **L** with silver(I) acetate in methanol yields a colorless solution from which a white solid is obtained on treatment with $[\text{NH}_4][\text{PF}_6]$. Mass spectrometry (FAB) shows peaks corresponding to $\{\text{Ag}_2\text{L}_2(\text{PF}_6)\}^+$ and $\{\text{Ag}_2\text{L}_2\}^+$ consistent with formation of a dimeric species of formula $[\text{Ag}_2\text{L}_2][\text{PF}_6]_2$. Such a formulation is consistent with either a helix or the alternative box structure (Figure 2) and also with

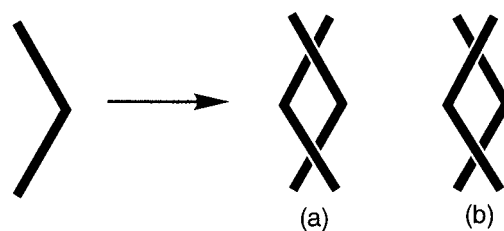


Figure 2. Schematic illustration of the formation of (a) helical or (b) bridging nonhelical (box) structures.

either a head-to-tail (HT) or head-to-head (HH) configuration of the ligands (Figure 1). The ^1H NMR spectra of the compound in CD_2Cl_2 recorded at both room temperature and low temperature (193 K) confirm the presence of a unique molecular species, which from consideration of the ligand geometry we anticipated to contain a helical arrangement.^[10]

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The imine proton resonances show coupling to Ag at both 298 K and 193 K confirming the coordination of the silver ion. The magnitude of the Ag–imine coupling is unaffected by temperature. The ^{109}Ag NMR spectrum reveals the presence of a single silver resonance at $\delta = 533$. This observation indicates that the solution species is indeed the head-to-tail isomer (the head-to-head isomer should contain two non-equivalent silver centers; it is possible that the peak might represent two coincident signals, though unlikely given the different nature of the HH and TT environments).

Recrystallization of the compound from acetonitrile by diffusion of diethyl ether afforded X-ray quality crystals and we have determined the crystal structure to confirm the molecular structure.^[11] The structure of the cation (Figure 3) is

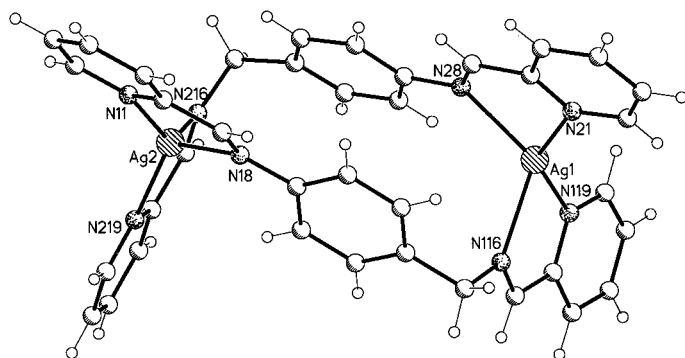


Figure 3. View of the cation $[\text{Ag}_2\text{L}_2]^{2+}$.

indeed a head-to-tail helix, containing each silver(I) ion in a pseudo-tetrahedral coordination geometry bound to the pyridyl–phenylimine donor unit of one ligand and the pyridyl–methylimine unit of the other. The two ligands wrap around the metal–metal axis giving rise to the helical structure, both enantiomers of which are present. The phenyl ring is approximately coplanar with the adjacent pyridylimine unit as expected for maximum conjugation; the helical twisting of the ligand is restricted by the design to the methylene group.

We have shown that by careful selection of spacer unit between ligand binding sites the directionality of a helicate may be controlled. We are currently extending our studies to apply this principle within more sophisticated supramolecular arrays and are also investigating incorporation of directional helicates within larger supramolecular arrays.

Experimental Section

L: 2-Pyridinecarbaldehyde (1.6 mL, 16.8 mmol) and 4-aminobenzylamine (0.9 mL, 8.0 mmol) were stirred in ethanol (25 mL) for 12 h. The orange solution was concentrated under reduced pressure and cooled in ice. An off-white solid precipitated (1.85 g, 77%) and was collected by filtration and dried over P_2O_5 . ^1H NMR (CDCl_3): $\delta = 8.68$ (1H, d, $J = 5.6$ Hz; H_6), 8.64 (1H, d, $J = 5.6$ Hz; H_6), 8.60 (1H, s; H_{im}), 8.50 (1H, s; H_{im}), 8.18 (1H, d, $J = 7.6$ Hz; H_3), 8.07 (1H, d, $J = 7.6$ Hz; H_3), 7.80 (1H, t, $J = 7.6$ Hz; H_4), 7.76 (1H, t, $J = 7.6$ Hz; H_4), 7.40–7.20 (6H, m; H_{ph} H_5 H_5), 4.76 (2H, s; CH_2); MS (EI): m/z : 300 $[M^+]$.

$[\text{Ag}_2\text{L}_2][\text{PF}_6]_2$: Ligand **L** (0.045 g, 0.15 mmol) and $\text{Ag}(\text{OAc})$ (0.025 g, 0.15 mmol) were stirred in methanol (25 mL) for 2 h. The solution was treated with methanolic $[\text{NH}_4][\text{PF}_6]$ (excess) to yield a pale yellow product (0.056 g, 68%) which was isolated by filtration. ^1H NMR^[10] (CD_2Cl_2): $\delta =$

8.79 (1H, d, $J = 6.4$ Hz; H_{im}), 8.66 (1H, d, $J = 7.4$ Hz; H_{im}), 8.62 (1H, d, $J = 4.9$ Hz; H_6), 8.53 (1H, d, $J = 4.9$ Hz; H_6), 8.08 (1H, t, $J = 7.6$ Hz; H_4), 8.02 (1H, t, $J = 7.6$ Hz; H_4), 7.96 (1H, d, $J = 7.8$ Hz; H_3), 7.78 (1H, d, $J = 7.8$ Hz; H_3), 7.64–7.54 (2H, m; H_5 H_5), 7.07 (2H, d, $J = 7.8$ Hz; H_{ph}), 6.92 (2H, d, $J = 7.8$ Hz; H_{ph}), 4.77 (2H, s; CH_2); MS (FAB): m/z : 961 $[\text{Ag}_2\text{L}_2(\text{PF}_6)]$ and 816 $[\text{Ag}_2\text{L}_2]$.

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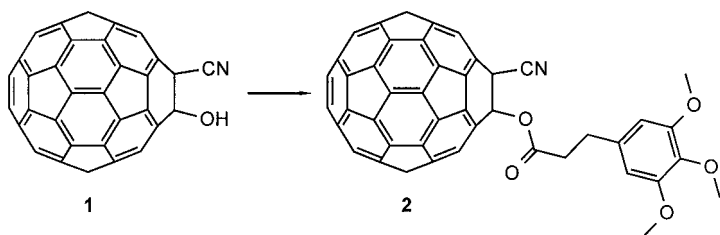
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- [10] Although the (diastereotopic) CH_2 resonance broadens at lower temperature we see no significant broadening of any other resonances at low temperature and no new peaks appear in the spectrum. This is consistent with the presence of only one solution species (albeit in two exchanging enantiomeric forms).
- [11] a) Crystal structure data for $\text{C}_{19}\text{H}_{16}\text{N}_4\text{Ag}_2\text{PF}_6 \cdot 1.5\text{C}_2\text{H}_5\text{N}$, $M_r = 614.78$, triclinic, space group $P\bar{1}$, $a = 13.1320(10)$, $b = 15.1807(5)$, $c = 15.4680(10)$ Å, $\alpha = 105.268(5)^\circ$, $\beta = 105.745(5)^\circ$, $\gamma = 113.796(5)^\circ$, $V = 2460.2(3)$ Å³, $T = 180(2)$ K, $\lambda = 0.71073$ Å, $Z = 4$, $\rho_{\text{calcd}} = 1.660$ Mg m⁻³, $F(000) = 1228$, $\mu(\text{MoK}\alpha) = 0.952$ mm⁻¹. Crystal character: golden-yellow blocks. Crystal dimensions $0.3 \times 0.3 \times 0.3$ mm, data collected with a Siemens SMART three-circle system with CCD area detector. The crystal was held at 180(2) K with an Oxford Cryosystem Cryostream Cooler; $\theta_{\text{max}} = 28.44^\circ$. A total of 14820 reflections were measured, 10802 unique [$R_{\text{int}} = 0.0175$]. Absorption correction by Psi-scan. Weighting scheme $w = 1/[\sigma^2(F_o^2) + (0.0383 P)^2]$, where $P = (F_o^2 + 2F_c^2)/3$. Goodness-of-fit on F^2 was 0.936, $R1$ [for 7511 reflections with $I > 2\sigma(I)$] = 0.0340, $wR2 = 0.0833$. Data/restraints/parameters 10802/0/643. Largest difference Fourier peak and hole 0.841 and -0.482 e Å⁻³. Refinement used SHELXL 96 (G. M. Sheldrick, **1996**). 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-102733. 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).

Determination of the Electron Density Distribution in the Bonds of a Fullerene Derivative by High-Resolution X-Ray Structure Analysis**

Hermann Irngartinger,* Anton Weber, and Thomas Oeser

Since the discovery of the synthesis of fullerenes on a macroscopic scale in 1990,^[1] their derivatization, especially that of C₆₀, has led to a multitude of compounds with interesting properties. Theoretical considerations of the bonding showed^[2] that of the 12500 possible resonance structures of C₆₀ the lowest energy is found in the one in which all double bonds are located at the contact of two six-membered rings ([6,6] bonds). Therefore no double bonds occur in five-membered rings (as [5,6] bonds) which would contribute to an increase in strain. The question of the degree of electron delocalization and therefore the degree of aromaticity of C₆₀ has been discussed controversially.^[3–6] The more than 160 X-ray structure investigations of fullerenes^[7] and their derivatives that have been published so far gave experimental insights into the nature of the bonds. These data confirm the results of spectroscopic studies and of calculations, according to which the [6,6] bonds are significantly shorter than the [5,6] bonds in [60]fullerenes.^[8] Thus we were able to solve the structure of isoxazolo[60]fullerenes, which were synthesized by the addition of nitrile oxides to C₆₀.^[9,10] In the anthryl derivative^[9] the average^[11] bond lengths of the [6,6] and [5,6] bonds are 1.391(9) and 1.452(9) Å, respectively. As C₆₀ is a highly strained, polycyclic ring system,^[3] bent bonds are to be expected. We have now determined the distribution of electron density in the bonds of a fullerene derivative for the first time by high-resolution X-ray structure determination of the difference density.

The compound we have examined is a product of an isoxazolo[60]fullerene whose dihydroisoxazole ring was opened, as we reported,^[12] which led to the fullerenol **1**. Its reaction with 3-(3,4,5-trimethoxyphenyl)propionic acid gave the novel 1,2-dihydro[60]fullerene derivative **2** (Scheme 1),



Scheme 1. Esterification of 3-(3,4,5-trimethoxyphenyl)propionic acid with fullerenol **1** (see Experimental Section).

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which crystallizes from toluene as a 1:1 clathrate in the space group $P\bar{1}$ (Figure 1). The average lengths of the [6,6] and [5,6] bonds (excluding the bonds to the site of addition) are 1.395(8) and 1.452(8) Å, respectively, and the mean esd value (σ) for the 90 bonds of the fullerene is 0.002 Å. In order to

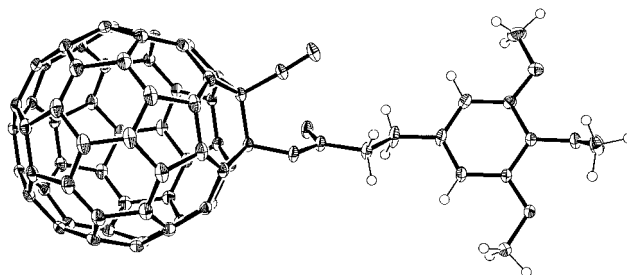


Figure 1. Molecular structure of **2** in the crystal.

make it easier to follow the discussion of the results, the numbering of the atoms mentioned is given in Figure 2. The distance between C1–C2 at the addition site (1.611(2) Å) is significantly larger than in the isoxazolo[60]fullerenes [1.584(5) Å,^[9] 1.576(3) Å,^[10] 1.564(4) Å,^[10] 1.580(4) Å^[13]]. Evidently, the short repulsive 1,4 contact C61...O1 [2.609(3) Å] of the eclipsed substituents is responsible for the bond lengthening. In the 1-fluorenyl-1,2-dihydro[60]fullerene^[14] the C1–C2 bond is much shorter (1.570(6) Å) since the 2-position is unsubstituted.

A possible attractive interaction between nucleophilic O1 and electrophilic C61, on the other hand, seems to play a minor role because owing to the torsion angle C2–C1–O1–C62 of $-72.1(2)^\circ$ the lone electron pairs at O1 do not show a favorable orientation towards the C61 of the cyanide group. Furthermore, the nucleophilicity is smaller than that of an ether oxygen atom which, for example, in 8-methoxy-1-naphthonitrile causes a deviation from the linear arrangement of the cyanide group of 6.2° for an O...C distance of 2.594(4) Å.^[15] In our case, however, an attractive interaction results with the carbonyl oxygen atom O2, to which the distance is slightly larger [2.919(3) Å], but still is significantly smaller than the van der Waals distance. The carbonyl group is twisted towards the cyanide group [C2–C1–O1–C62 $-72.1(2)^\circ$, C1–O1–C62–O2 $-2.6(3)^\circ$]. As a consequence the latter deviates from linearity by 3.7° [C2–C61–N1 $176.3(2)^\circ$], and N1 bends away from O2 [C1–C2–C61–N1 $-155(3)^\circ$]. A similar arrangement was found in 8-nitro-1-naphthonitrile.^[15]

The determination of difference density resulted in significant differences in the distribution of electron density for the [6,6] and [5,6] bonds. Below the planes of the six-membered rings (0.4 Å towards the center of the fullerene skeleton) the

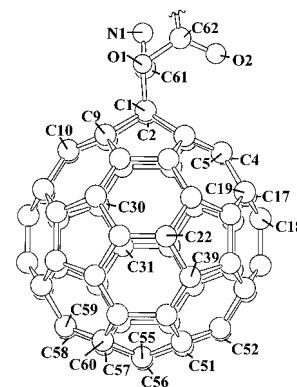


Figure 2. Numbering of the atoms in the fullerene skeleton relevant to the discussion.

mean maximum electron density in the [6,6] bonds is significantly higher ($0.19(4) \text{ e } \text{\AA}^{-3}$) than in the [5,6] bonds ($0.10(5) \text{ e } \text{\AA}^{-3}$), in the planes of the six-membered rings the differences are at the limits of significance ($0.45(7)$ versus $0.37(7) \text{ e } \text{\AA}^{-3}$), and above the planes of the six-membered rings ($0.4 \text{ \AA}$) the bonding types do not differ [$6,6/5,6: 0.22(4)/0.22(5) \text{ e } \text{\AA}^{-3}$]. The average values were determined by means of cross sections through the planes of all six-membered rings with the exception of the two rings at the addition site. In Figures 3a and 3b this is illustrated for one six-membered ring. Overall, the maxima in the [6,6] bonds are higher than those in the [5,6] bonds in agreement with the bond character. From the above-mentioned values it also follows that in the

[5,6] bonds the mean maximum electron densities are greater outside the fullerene skeleton than within, but not in the [6,6] bonds. The differences can evidently be ascribed to bent bonds. In the planes of the five-membered rings the bonds show a tendency to bend away from the five-membered ring; however, the mean distance of the maxima of electron density from the bond axes are at the limits of significance at $0.05(5) \text{ \AA}$. Figure 3c shows this for one five-membered ring. No bends are observable in the [6,6] bonds (Figure 3a).

Figure 3d shows a section through two parallel [6,6] bonds (C9–C10, C51–C52) which divides the fullerene skeleton into two halves (mirror symmetric with respect to the skeleton). The atoms C7, C20, C37, C57, C46, and C27 lie above the projection plane, and [6,6] bonds (C20–C19 and C46–C47) and [5,6] bonds (C7–C6, C36–C37, C49–C57, and C27–C28) lead vertically downward from these atoms. The figure shows that the maximum electron densities in the [6,6] bonds lie on the interatomic axes. This is also confirmed by the average value for all [6,6] bonds. Corresponding sections are also possible for parallel [5,6] bonds; these, however, do not divide the fullerene skeleton with mirror symmetry. Figure 3e shows such a section through the bonds C30–C31 and C22–C39. In this case the maximum electron densities lie significantly outside the fullerene framework in both bonds. In the [5,6] bonds (except the four leading away from C1 and C2), the shift in the maxima of the electron densities from the bond axes outwards is on average $0.07(5) \text{ \AA}$.

The outward bending of the [5,6] bonds also accounts for the fact that the electron density for this bond type is about twice as high $0.4 \text{ \AA}$ above the planes of the six-membered rings as it is $0.4 \text{ \AA}$ below them. Figure 3f shows another section through two [5,6] bonds. However, these are not parallel since the C1–C9 bond originates from the addition site. In contrast to the $\text{C}(\text{sp}^2)\text{--}\text{C}(\text{sp}^2)$ bond C51–C56, this $\text{C}(\text{sp}^3)\text{--}\text{C}(\text{sp}^2)$ bond is not bent as the ring strain is locally decreased at the addition site.

Experimental Section

2-Cyano-1,2-dihydro[60]fullerenyl 3-(3,4,5-trimethoxyphenyl)propionate **2**: 3-(3,4,5-Trimethoxyphenyl)propionic acid (6.2 mg, $2.6 \times 10^{-5} \text{ mol}$), dicyclohexylcarbodiimide (5.4 mg, $2.6 \times 10^{-5} \text{ mol}$) and 4-(dimethylamino)pyridine (0.3 mg, $2.6 \times 10^{-6} \text{ mol}$) were added to a solution of the alcohol **1**^[12] (10 mg, $1.3 \times 10^{-5} \text{ mol}$) in toluene/acetonitrile (9/1; 50 mL) under nitrogen. The mixture was stirred at ambient temperature for 72 h and subsequently concentrated in vacuo. The product was isolated by column chromatography on silica gel with toluene/acetonitrile (9/1) ($R_f = 0.5$). Compound **2** (11.8 mg, 92 %) was obtained as a dark brown solid. Single crystals suitable for X-ray structure analysis were obtained by crystallization from toluene.

^1H NMR (300 MHz, $\text{CDCl}_3/\text{CS}_2$ 1/8): $\delta = 6.51$ (s, 2H, arom.), 3.81 (s, 6H, CH_3), 3.70 (s, 3H, CH_3), 3.26 (s, 4H, CH_2); ^{13}C NMR (75 MHz, $\text{CDCl}_3/\text{CS}_2$ 1/8): $\delta = 172.49$ (CO), 153.09 (arom.), 148.13 (1C), 147.61 (1C), 146.41 (2C), 146.37 (4C), 146.04 (4C), 145.98 (2C), 145.78 (2C), 145.44 (2C), 145.24 (2C), 145.12 (2C), 145.08 (2C), 144.34 (2C), 144.29 (2C), 143.88 (2C), 143.71 (2C), 142.76 (2C), 142.54 (2C), 142.51 (2C), 142.07 (4C), 141.62 (2C), 141.27 (2C), 141.24 (2C), 140.72 (2C), 139.85 (2C), 139.46 (2C), 137.48 (2C), 136.26 (2C + arom.), 135.07 (arom.), 115.54 (CN), 105.58 (arom.), 59.92 (CH_3), 55.55 (CH_3), 36.61 (CH_2), 31.16 (CH_2) (because of the long relaxation time the signals for C1 and C2 could not be detected); FT-IR (KBr): $\tilde{\nu} = 2930$ (m, CH), 2331 (w), 2241 (w, CN), 1756 (m, CO), 1127 (s), 527 cm^{-1} (m, fullerene); UV/Vis (CHCl_3): λ_{max} (lg ϵ) = 232 nm (5.10), 256 (5.22), 318 (4.79), 580 (3.09), 680 (2.60); MALDI-TOF-MS: m/z (%):

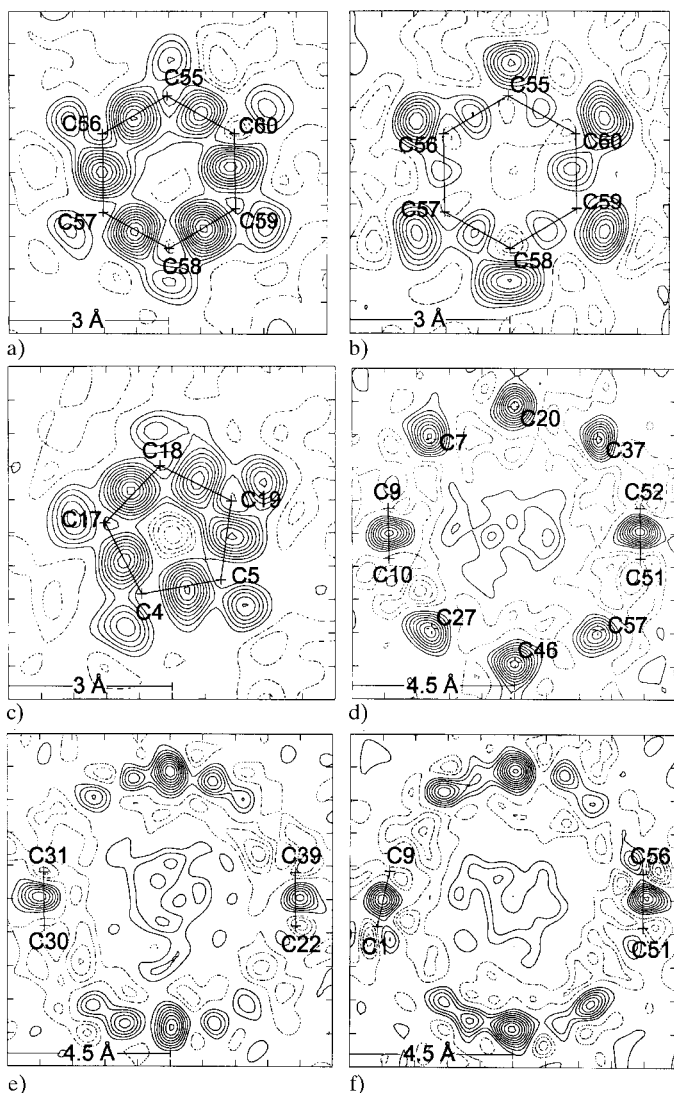


Figure 3. Difference density maps of **2**. The distance between contours is $0.05 \text{ e } \text{\AA}^{-3}$, and solid lines correspond to positive and broken lines to negative electron densities. a) Cross section through the plane of a six-membered ring. [5,6] bonds: C55–C60, C56–C57, C58–C59; [6,6] bonds: C55–C56, C57–C58, C59–C60. b) Cross section through the same six-membered ring, $0.4 \text{ \AA}$ below the plane. c) Cross section through the plane of a five-membered ring. d) Cross section through the plane of two parallel [6,6] bonds. e) Cross section through the plane of two parallel [5,6] bonds. f) Cross section through the plane of two opposite [5,6] bonds, one of which is the $\text{C}(\text{sp}^3)\text{--}\text{C}(\text{sp}^2)$ bond C1–C9 which originates at the addition site.

985.4 (18) [M^- , $C_{73}H_{15}NO_5$], 746.1 (100) [$C_{61}N^-$], 720.0 (7) [C_{60}]; HR-FAB-MS: m/z : 985.0950 [$C_{73}H_{15}NO_5^+$] calcd; 985.0942 found.

High-resolution X-ray structure analysis with low-temperature data:^[16] $C_{73}H_{15}NO_5 \cdot C_7H_8$, $M_r = 985.86$, dark brown crystal $0.44 \times 0.22 \times 0.12$ mm, triclinic, space group $P\bar{1}$, $a = 10.0417(2)$, $b = 14.5831(4)$, $c = 16.8772(4)$ Å, $\alpha = 68.277(1)^\circ$, $\beta = 75.985(1)^\circ$, $\gamma = 84.583(1)^\circ$, $V = 2227.59(9)$ Å³, $Z = 2$, $T = 123(2)$ K, $Mo_{K\alpha}$ radiation, $\lambda = 0.71073$ Å, $\rho_{\text{calcd}} = 1.607$ kg dm⁻³, $F(000) = 1100$, $\mu = 0.1$ mm⁻¹, an empirical absorption correction was carried out, Bruker SMART diffractometer, ω and φ scan, $\theta_{\text{max}} = 50.6^\circ$, $-21 \leq h \leq 20$, $-30 \leq k \leq 30$, $-32 \leq l \leq 36$, 43 s measuring time per frame; of 119 878 measured reflections, 44 299 were independent and 22 618 were observed. The structure was solved by direct methods (SHELXS-97).^[17] Refinement (F^2 after the LSQ method with SHELXL-97^[18]) and calculation of the difference density in the following order: a) With the reflections from $\theta = 5-28^\circ$, 10 592 independent, 8791 observed [$I > 2\sigma(I)$], 867 variables. Isotropic refinement of the hydrogen atoms and anisotropic refinement of the heavy atoms, $R = 0.046$, $wR = 0.11$, $R_{\text{int}} = 0.03$, $GOF = 1.07$. b) High-angle refinement with the reflections from $\theta = 28-50.6^\circ$, 33 719 independent, 13 839 observed [$I > 2\sigma(I)$], 775 variables. Anisotropic refinement of the heavy atoms, parameters of the hydrogen atoms were adopted from (a), $R = 0.105$, $wR = 0.154$. The Hirshfeld rigid bond test^[19] resulted in a mean deviation of 6.3×10^{-4} Å² for the 90 bonds of the fullerene skeleton. An R value of 0.028 was obtained for the fullerene skeleton by applying a T,L,S analysis according to Schomaker and Trueblood.^[20] c) With the reflections from $\theta = 5-50.6^\circ$, 44 299 independent, 22 618 observed [$I > 2\sigma(I)$]. Refinement of the scale factor, $R = 0.081$, $wR = 0.175$. d) Calculation of the structural factors (F_{calc}) for $\theta = 5-28^\circ$ [$I > 5\sigma(I)$] with the structure model from (b) and the scale factor from (c).

Figure 1 was generated with the program ORTEP3.^[21] The difference density sections in Figure 3 were calculated with the program WinGX-98.^[22] The rigid bond test and the T,L,S analysis were carried out with the program PLATON.^[23]

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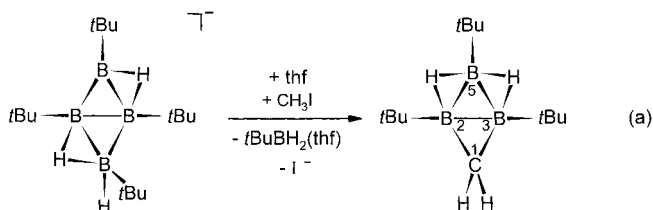
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2,3,5-Tri-*tert*-butyl-1-carba-*nido*-tetraborane**

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*Dedicated to Professor Bernt Krebs
on the occasion of his 60th birthday*

In the series of isoelectronic *nido* clusters B_4H_8 , CB_3H_7 , and NB_3H_6 , the *tert*-butyl derivatives $B_4H_4tBu_4$ ^[1] and $NB_3H_2tBu_4$ ^[2] are well known. We report here on a derivative, $CB_3H_4tBu_3$, of the missing link. It is formed by the reaction of iodomethane and the *nido*-tetraborane $[B_4H_3tBu_4]^-$ ^[1] in thf in 90% yield [Eq. (a)]; the numbering of the skeletal atoms follows the



IUPAC *nido* cluster rules that attribute the number 4 to the missing atom of the corresponding trigonal-bipyramidal *closo* cluster]. Traces of $B_4H_2tBu_4$ ^[3] and $B_4H_4tBu_4$ ^[1] cannot be removed from the product.

The structure of the product follows from the NMR spectra. C_s symmetry is concluded from the equivalence of the $tBu(2)$ and $tBu(3)$ groups and of the bridging protons. The coupling between the methylene protons in *exo/endo* position ($^2J = 13$ Hz) and between these and the bridging protons ($^3J(\mu/endo) = ^3J(\mu/exo) = 6$ Hz) are found at the expected values. Homonuclear 2D couplings are observed between B(2)/B(3) and B(5) and between the methylene protons. All of the five ^1H NMR chemical shifts give heteronuclear 2D cross peaks: the μ -H atoms are coupled to both kinds of B atoms, the methylene-H atoms are related to the methylene-C atom, and the methyl-H atoms display 2D hetcor coupling not only with each neighboring C atom, but also with the more remote C

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atoms of *t*Bu. No dynamic processes can be observed in the NMR spectra in a temperature range from -50 to $+90$ °C. Replacing the solvent thf with $[D_8]$ toluene does not significantly alter the NMR spectra.

Ab initio calculations (B3LYP/6-31++G(d,p))^[4] on the parent molecule CB_3H_7 and on its methyl derivative $CB_3H_4Me_3$ lead to minima on the potential energy hypersurfaces that represent bicyclobutane-type *nido* clusters with a CH_2 group in position 1 and hydrogen atoms that bridge B(2)/B(3) and B(5). The computed bond lengths and the angle between the planes through B(2)-B(3)-B(5) and B(2)-B(3)-C(1) for the molecules CB_3H_7 , $CB_3H_4Me_3$, and $CB_3H_4tBu_3$ are summarized in Figure 1. The geometric data for the title compound are not significantly changed by going from the 6-31G(d) to the more sophisticated 6-31++G(d,p) level. The optimized geometric parameters were used as a basis for a calculation of the NMR signals using the GIAO method.^[5] The computed chemical shifts of the skeletal atoms and the

alkyl groups than methyl. This is shown in Equation (b) for the case of bromoethane.



The isoelectronic four-vertex *nido* clusters $B_4H_4tBu_4$,^[1] $CB_3H_4tBu_3$, and $NB_3H_2tBu_4$ ^[2] can be represented by structural formulae that make use of localized two-center, two-electron (2c2e) and three-center, two-electron (3c2e) bonds and obey the octet rule (Figure 2). An equilibrium (c) between two tautomeric species has been observed in the case of $NB_3H_2tBu_4$.^[2]

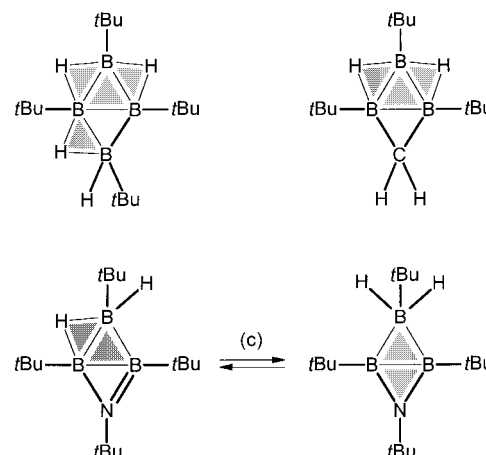


Figure 2. Localized bonds in the isoelectronic molecules $B_4H_4tBu_4$, $CB_3H_4tBu_3$, and $NB_3H_2tBu_4$ (equilibrium of two isomers) (bold lines: 2c2e bonds; gray triangles: 3c2e bonds).

The title compound can be derived from the three-vertex *nido* cluster $B_3H_4(tBu)_3$ by replacing two terminal H atoms by a bridging CH_2 group. It can be considered, therefore, to be a member of the family of *nido*-triboranes with B_3H_7 as the parent molecule. Although B_3H_7 itself has not yet been isolated, its *arachno* adducts with Lewis bases L of the type B_3H_7L have been known for many years.^[6]

Experimental Section

2,3,5-Tri-*tert*-butyl-1-carba-*nido*-tetraborane: Iodomethane (78 mg, 0.55 mmol) was added to a solution of $[K(thp)_6][B_4H_3tBu_4]$ (400 mg, 0.48 mmol; thp = tetrahydropyran)^[1] in thf (2 mL) at -78 °C. After 1 h stirring at ambient temperature, volatiles were removed in vacuo (5 mbar), and the residue was extracted with hexane (5 mL). The product was distilled into a cooled receiver (25 °C/0.001 mbar; 101 mg). According to NMR spectra, it contained equal amounts of $B_4H_2tBu_4$ and $B_4H_4tBu_4$ (ca. 5% in total; yield of main product ca. 95 mg, i.e. ca. 90%). The nonvolatile material (30 mg) that remained from the distillation gave H_2 on acidification. Apparently products of the decomposition of $(tBuBH_2)_2$ were present; this borane underwent an equilibrium with $tBuBH_2(thf)$ in thf and decomposed into nonvolatile products of unknown structure after a while.^[7] ^1H (^{11}B) NMR (500 MHz, $[D_8]$ toluene): δ = 0.307, 1.362 (2d, t, ^2J = 13 Hz, ^3J = 6 Hz; *exo*-H and *endo*-H of CH_2 ; assignment according to Table 1), 1.077, 1.093 (2s, 18H, 9H; *t*Bu), 0.781 (d, d, $^3J_1 = ^3J_2 = 6$ Hz; 2 μ -H); ^{11}B NMR (160.4 MHz, $[D_8]$ toluene): δ = 22.4, 34.5 (2:1); ^{13}C NMR (125.7 MHz, $[D_8]$ toluene): δ = 13.2 (broad, CH_2), 19.7 (C(1) of 3 *t*Bu), 31.85, 32.71 (Me of *t*Bu(2,3) and *t*Bu(5); assignment according to ^{13}C / ^1H cross peaks).

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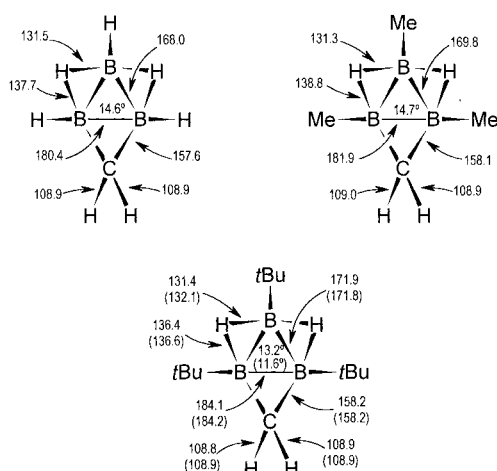


Figure 1. Calculated structure of the molecules CB_3H_7 , $CB_3H_4Me_3$, and $CB_3H_4tBu_3$ with distances [pm] and angles [°] between the triangles BBB and BBC (6-31++G(d,p) level; numbers in parentheses: 6-31G(d) level).

skeleton-bound H atoms of the molecules CB_3H_7 , $CB_3H_4Me_3$, and $CB_3H_4tBu_3$, along with the corresponding experimental values, are summarized in Table 1. The good agreement of the calculated and observed values gives clear support for the adopted structure of the title compound.

Table 1. Calculated^[a] and observed chemical shifts δ of $CB_3H_4R_3$ with R = H, Me, *t*Bu.

	CB_3H_7 calcd	$CB_3H_4Me_3$ calcd	$CB_3H_4tBu_3$ calcd	$CB_3H_4tBu_3$ exp.
^1H NMR: H_{exo}	0.721	0.143	0.226	0.307
^1H NMR: H_{endo}	2.105	1.789	1.363	1.362
^1H NMR: μ -H	-0.820	0.404	0.597	0.781
^{11}B NMR: B(2)	10.9	20.0	21.4	22.4
^{11}B NMR: B(5)	23.4	27.4	30.7	34.5
^{13}C NMR: C(1)	14.6	14.7	11.6	13.2

[a] B3LYP/GIAO at the 6-31++G(d,p) level.

The *closo*-tetraborane $B_4H_2tBu_4$ ^[3] is obtained when the *nido* anion $[B_4H_3tBu_4]^-$ is oxidized by haloalkanes with bigger

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A New In Vitro Model of Lignin Biosynthesis

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There are still many open questions concerning the structure as well as the peculiar biosynthesis of lignin.^[1–3] The most accredited theories go back to the early 1960s, when Freudenberg and co-workers proposed dehydrogenation reactions as the dominating framework of a polymerization process which involves *p*-hydroxycinnamyl alcohols as the primary precursors and building blocks of all lignins.^[4, 5] Numerous structural investigations of isolated and synthetic lignins and of model compounds then outlined a composite picture of a very complex, three-dimensional polymeric structure devoid of ordered repeating units, such as in the case of other biological macromolecules.^[1, 5–11] According to these studies lignin production is triggered by the enzymatic formation of resonance-stabilized phenoxy radicals, while the polymerization is generally known to run as a non-enzymatic process which advances in a random fashion through radical and ionic steps. We now report that under in vitro homogeneous conditions—which, in principle, could be related to the natural microenvironment^[12, 13]—a synthetic lignin is produced. The beginning of the polymerization process appears to be an alternating sequence of radical and ionic steps which generate ordered structures of oligolignols.

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The polymerization of the monomeric precursor by random coupling reactions cannot be studied in vivo; many theories on lignin structure and biosynthesis rely upon in vitro experiments on the polymerization of coniferyl alcohol (**1**, Figure 1),^[5, 7, 14] which is the most common natural lignin

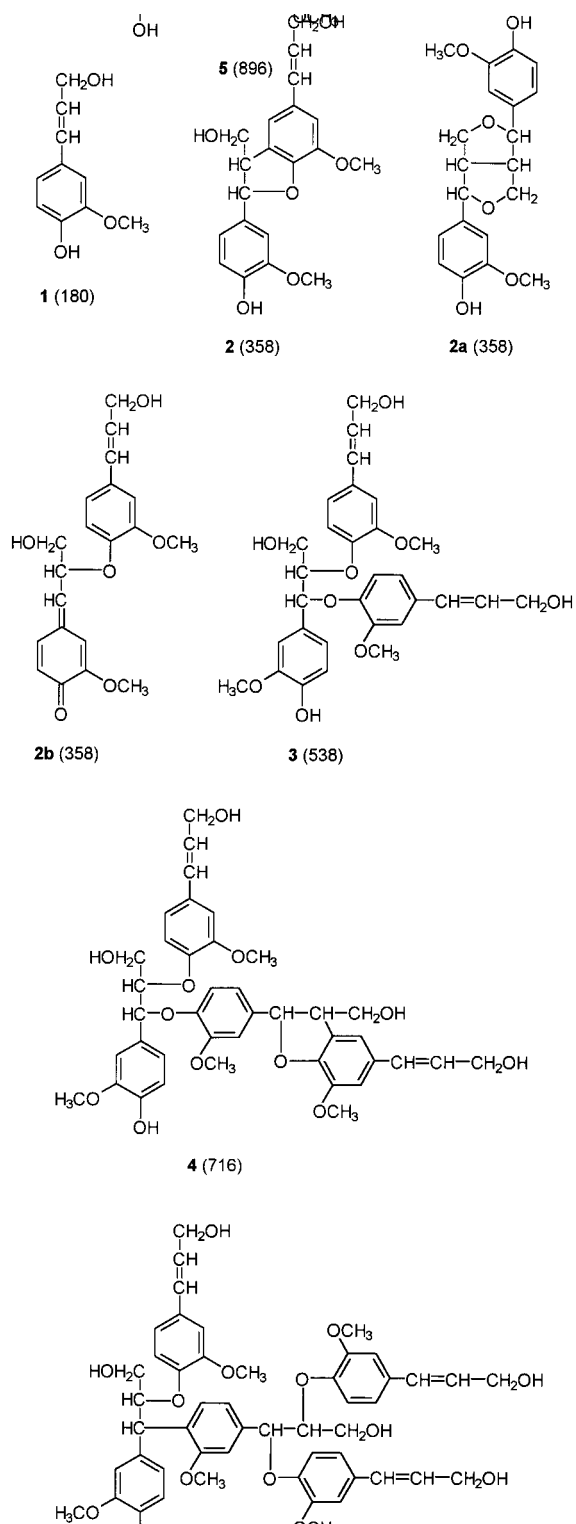


Figure 1. Schematic representation of oligolignols (molecular weights are given in parentheses).

precursor, followed by isolation and structural determination of the produced polymer. The reaction is in general conveniently accomplished^[15] by slow mixing, over a period of several hours, of a highly diluted solution of the precursor^[16] with a buffered solution of H₂O₂ in the presence of horseradish peroxidase,^[17] which serves to initiate the polymerization process. The presence of a small quantity of a polar, water-soluble material like guaiacylglycerol, which is able to copolymerize with coniferyl alcohol, is also recommended in order to keep the growing polymer in solution as long as possible. This synthetic lignin, commonly referred to as dehydrogenative polymerizate (DHP),^[5] generally very rapidly separates from the reaction medium, thus subtracting itself from a possible higher degree of polymerization. We have analyzed this material^[18] by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS)^[19] and deduced that the radical mechanism is preferred over the competing ionic mechanism for the polymerization process. Following our continuing interest in lignin biosynthesis, we have found that horseradish peroxidase is still highly active in a phosphate buffer solution of cetyltrimethylammonium sulfate ((CTA)₂SO₄) at a concentration higher than the critical micelle concentration (c.m.c. = 2.7×10^{-4} M). When tested at a surfactant concentration of 2.7×10^{-2} M with *o*-phenylenediamine as the model substrate in a buffered solution of H₂O₂, a decrease in activity of only twofold with respect to the experiment performed in the absence of surfactant is observed. We also checked that, under these experimental conditions, micelles actually formed. Thus, we were able to polymerize coniferyl alcohol, keeping the final polymer in solution for an indefinite period.

Fundamental results regarding the structures of the oligolignols formed in our experiments and the polymerization mechanism involved came from electrospray ionization mass spectrometry (ESI-MS)^[20] combined with HPLC; the reaction mixture was analyzed when the polymerization process was interrupted at the beginning, that is, after five minutes. We used a mass spectrometer with an ESI interface as a detector after HPLC separation of the mixture (Figure 2), employing a postcolumn doping of the eluate with ammonium acetate. The instrument^[21] gave the masses of the $[M+NH_4]^+$ ionic species of each oligomer (**1–6** (see Figure 1 for the structures of **1–5**)).^[22] By direct infusion of the whole mixture into the mass spectrometer ion source, even the heptamer (**7**) and the octamer (**8**) are revealed, as it is apparent from the mass spectrum (Figure 3). Also it is clearly evident that all the oligomers have definite masses, since other products with masses close to the indicated ones are practically absent (the satellite peaks at higher masses are due to the sodiated and potassiated oligomers).

What is new and totally unexpected from our experiment is the high regularity of the oligomeric units, which differ from one other by mass units of, alternately, about 178.1 and 180.1. As the molecular weight of coniferyl alcohol is

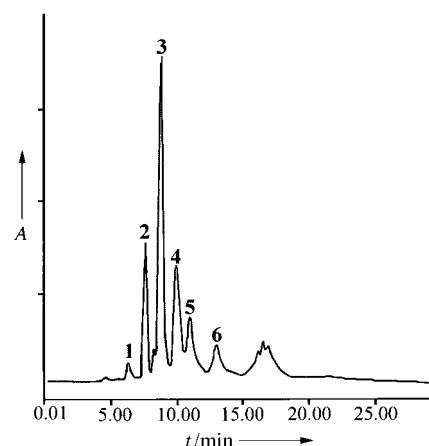


Figure 2. HPLC profile of the oligolignol mixture. Column: RP-18 (12.5 cm, 4.6 mm inner diameter); eluent A: H₂O; eluent B: CH₃CN; gradient B: 0 → 40% in 15 min; flow rate: 0.8 mL min⁻¹; UV detection at 268 nm. Compound **2a** coelutes with **2**.

180.1, these mass increments imply that the two reaction mechanisms, the radical and the ionic ones, are not operating randomly, but in parallel in a coordinate process. Coupling of two radical moieties would give a mass increment of 178.1; conversely, the ionic mechanism, formally consisting of the addition of one monomer unit to a quinone methide intermediate, leads to an increment of 180.1.

On the grounds of the ESI-MS data it is possible to propose the biosynthetic pathway which is reasonably followed to produce the oligolignols we have detected under our biomimetic conditions. Both the dimers **2** and **2a** ($M_r = 358$ each) are formed by one radical step (formally, the monomer (with 180) plus 178). In all the other cases, the key step is the production of the dimeric quinone methide intermediate (**2b**, also formed by one radical step). This compound is then able to add coniferyl alcohol (**1**) to form the trimer **3** (i.e., guaiacylglycerol- β,γ -bis-coniferyl ether, $M_r = 538$); alternatively, it can add dehydroconiferyl alcohol (**2**), or pinoresinol (**2a**), to form the tetramer **4** (i.e., guaiacylglycerol- β -coniferyl- γ -dehydro-diconiferyl ether, $M_r = 716$). In the former case, one radical step and a ionic step are involved (formally the dimer (with 358) plus 180); in the latter case, two radical steps (to form the intermediate dimers **2b** and **2**) and an ionic step are necessary (formally the trimer (with 538) plus 178). The pentamer **5** ($M_r = 896$) is formed through two ionic and two radical steps, by the addition of the trimer **3** to the quinone methide **2b** (formally the tetramer (with 716) plus 180).

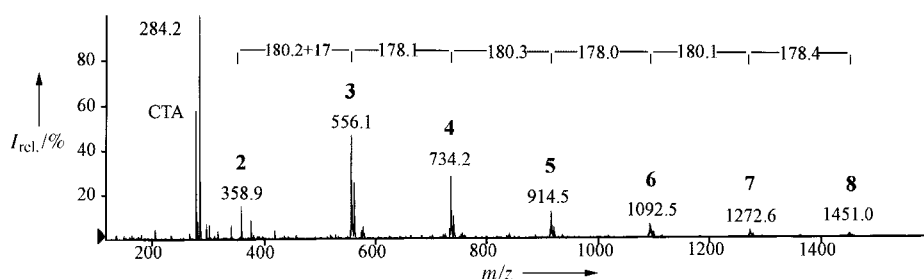


Figure 3. ESI mass spectrum of the oligolignol mixture (direct infusion); NH_4^+ adducts (H^+ adduct for **2**); CTA = cetyltrimethylammonium ion. The mass differences between the peaks are reported.

Our study is the first reported demonstration that model lignins can be efficiently produced in vitro, in an aqueous environment, under homogeneous conditions; the presence of the micelles is able to keep in solution all the lipophilic reagents and reaction products, with practically no detriment to the catalytic efficacy of the enzyme. Moreover, since the activity of an enzyme under micelle conditions could be considered more similar to the cell system than in buffer alone,^[12, 13] we propose that in our case the polymerization mechanism which drives oligolignol formation is closer to the natural process than are the other, well-established in vitro biosynthetic experiments. In conclusion, our results show that in lignin biosynthesis the ionic and the radical mechanisms operate effectively in parallel to the same extent, and they formally appear as strictly alternate steps. These results are also in favor of a more recent concept of a structural scheme for natural lignins which assumes an ordered structure with repeating units.^[23, 24]

Experimental Section

In a typical experiment 20 mg of coniferyl alcohol are dissolved in 3.6 mL of sodium phosphate buffer (0.01 M; pH 6.5) containing (CTA)₂SO₄ at a concentration of 2.7×10^{-2} M; to this solution are added sequentially 130 μ L of 3% H₂O₂ and 20 μ L of buffer containing seven purpurogallin units of horseradish peroxidase (type II, 200 U mg⁻¹, Sigma). The clear solution, where the substrate is at a concentration of 5.3 g L⁻¹, is stirred over a period of 5 min. After this time, which is sufficient for the substrate to be consumed (HPLC analysis), the reaction is stopped by the addition of a few drops of 5% Na₂S₂O₅, and the mixture thoroughly extracted with ethyl acetate. The organic layer is then washed with brine and dried, and the solvent evaporated under vacuo to give approximately 23 mg of product.

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Highly Substituted Spiro[4.4]nonatrienes from a β -Amino-Substituted α,β -Unsaturated Fischer Carbene Complex and Three Molecules of an Arylalkyne**

Heiko Schirmer, Michael Duetsch, Frank Stein, Thomas Labahn, Burkhard Knieriem, and Armin de Meijere*

Dedicated to Professor Klaus Kühlein on the occasion of his 60th birthday

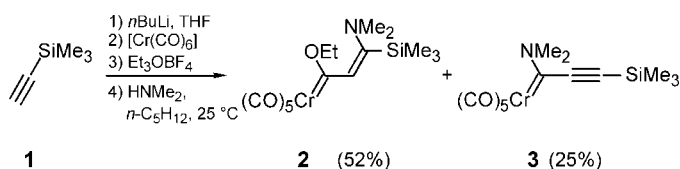
In recent years α,β -unsaturated Fischer carbene complexes have established themselves as valuable functional building blocks in organic chemistry.^[1] In particular, β -amino-substituted alkenylcarbene complexes exhibit a highly diverse reactivity depending upon the nature of the substituents and reaction conditions.^[2] These complexes are readily available from alkynylcarbene complexes by 1,4-addition of amines to 1-metalla-1-en-3-ynes^[3] and undergo inter alia formal [3+2],^[4] [2+2+1],^[5] and [4+2] cycloadditions^[4c, 6] with alkynes. In contrast, (3-dimethylaminoalkenylidene)chromium complexes with sterically demanding substituents on the alkenyl terminus, and therefore normally with a *Z* configuration, react preferentially by sequential insertion of two alkyne units and carbon monoxide and only then undergo cyclization, that is, they give cyclopenta[b]pyrans by a formal [3+4+1] cycloaddition, frequently in high yields.^[7] Although it also has a sterically demanding substituent and a *Z* configuration, the

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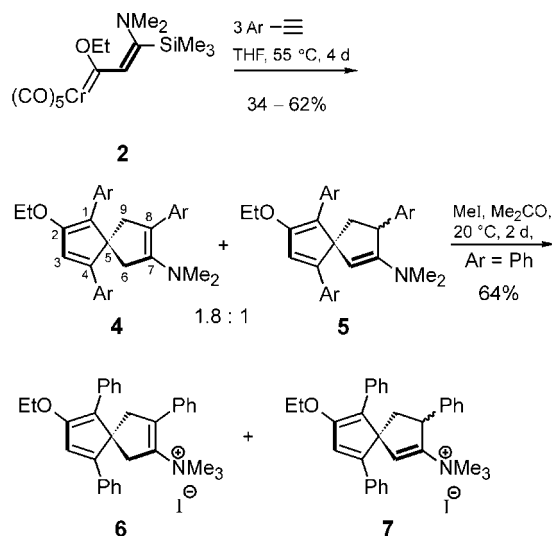
trimethylsilyl-substituted complex **2** reacts with formal insertion of three alkyne units in a manner not previously observed.^[8a]

(3-Dimethylamino-1-ethoxy-3-trimethylsilylallylidene)-pentacarbonylchromium (**2**) may be prepared by the established procedure, but in diethyl ether a 0.9:1 mixture of **2** and **3** is formed in only modest yield (27 + 30 %).^[3b, 8, 9] The best yield of **2** (52 % against 25 % **3**) is obtained when the addition of dimethylamine to the alkynyl complex (prepared from trimethylsilylethyne (**1**) by an improved method^[4c, d]) is carried out in a one-pot reaction in pentane^[9] at room temperature (Scheme 1).



Scheme 1. Synthesis of **2** from the trimethylsilylethyne **1** by the one-pot procedure.

Upon heating complex **2** with six equivalents (optimized, twofold excess) of phenylethyne in THF at 55 °C (complete conversion of **2** after four days according to thin-layer chromatography) a yellow solid which fluoresces strongly in solution is obtained after purification by column chromatography (Scheme 2). ¹H and ¹³C NMR spectra indicated that it



Scheme 2. Synthesis of **4** and **5** and their conversion (Ar = Ph) into the quaternary ammonium salts **6** and **7**.

was a mixture of three isomers (ratio 3.6:1:1), none of which contained a trimethylsilyl group. Assuming that the highest peak in the mass spectrum of the mixture at *m/z* 433 corresponded to the molecular ion peak, a compound had been formed in this reaction in 62 % yield from the protidesilylated carbene ligand and three molecules of phenylethyne without carbonyl insertion.

However, it was not possible to separate the three isomers by repeated column chromatography, nor was it possible to assign the correct structures from the NMR spectra of the mixture. All attempts at crystallizing the major isomer failed

since the compound mixture dissolved in only a small volume of pentane and decomposed rapidly during slow evaporation. To increase the polarity of all isomers at their dimethylamino groups the mixture was treated with iodomethane in acetone at room temperature. The mixture of the three isomeric quaternary ammonium iodides obtained in 64 % yield after two days was considerably more stable in solution (Scheme 2). It was poorly soluble in ethanol, but readily soluble in acetone, and slow evaporation of a solution of the mixture in ethanol/acetone allowed the isolation of single crystals. According to X-ray structural analysis these consisted of the quaternary ammonium iodide **6** of 7-dimethylamino-2-ethoxy-1,4,8-triphenylspiro[4.4]nona-1,3,7-triene (**4a**).^[10] An NMR spectrum of the single crystals showed that the product was the main isomer of the mixture (Figure 1).

A conclusive assignment of the other isomers in the mixture of products from **2** and phenylethyne was obtained by high-resolution 2D NMR correlation experiments,^[11] according to which these two minor isomers consisted of a 1:1 diastereomeric mixture of the spiro[4.4]nona-1,3,6-triene **5a**. The

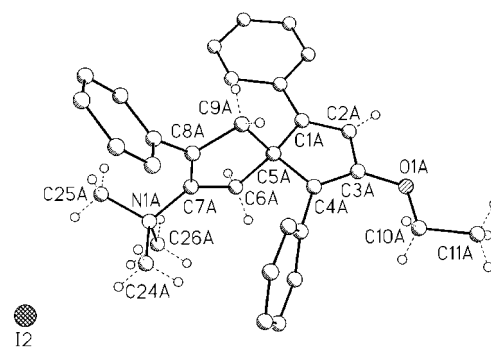


Figure 1. Crystal structure of the quaternary ammonium iodide **6**.^[10] The hydrogen atoms and numbering of the phenyl rings have been omitted for clarity.

assumption that the isomers **4** and **5** existed in a dynamic equilibrium was not confirmed. ¹H NMR spectra measured at different temperatures showed that there were no changes in the characteristic signal intensities.

To elucidate the scope of this new reaction, **2** was allowed to react with a series of alkynes. This showed that only arylethyne yielded the spiro[4.4]nonatrienes **4** and **5**. The isomeric ratio of the products thus obtained varied according to the substitution pattern of the arylethyne employed (Table 1). Terminal alkynes such as 1-pentyne and internal alkynes such as 2-butyne reacted in the known manner^[2, 4] to give the respective substituted cyclopentadienes.^[12]

The conspicuously intense fluorescence of these spiro[4.4]-nonatrienes must be attributed to the conjugation of the diarylcyclopentadiene moiety in **4** and **5**. The mixture of the

Table 1. Formation of spiro[4.4]nonatrienes **4** and **5** from **2** and three equivalents of arylethyne.

	Ar	Yield 4 + 5 [%]	4 : 5
a	C ₆ H ₅	62	1.8:1
b	C ₆ H ₄ -4-C ₆ H ₅	34	4.5:1
c	C ₆ H ₄ -4-OMe	37	2.5:1
d	C ₆ H ₃ -3,5-(Me) ₂	48	1.6:1

(3,5-dimethylphenyl)-substituted isomers **4d/5d** (1.6:1) showed an absorption maximum in the UV spectrum at $\lambda_{\text{max}} = 237$ nm (isooctane) with $\epsilon_{\text{max}} = 29500$. The absorption maximum in the fluorescence spectrum lay at $\lambda_{\text{max}} = 480$ nm (isooctane), and the relative fluorescence quantum yield was calculated to be 46 %.

All previous speculations on the mode of formation of these new cocyclization products of aryethynes with the allylidene chromium complex **2** are poorly illuminating. It is particularly unusual that the original connectivity of the carbene ligand in **2** is lost in the products **4** and **5**. (Scheme 2). In contrast, the original connectivity is maintained in all previously known products from β -dialkylamino-substituted α,β -unsaturated carbene chromium complexes.^[2] Attempts to shed light on the mechanism by introduction of terminally or internally ^{13}C -labeled phenylethyne are in progress. The possible triple insertion^[13] of an alkyne with an alkenylcarbene chromium complex described herein for the first time once more extends the broad range of cycloadducts which can be selectively obtained from α,β -unsaturated Fischer carbene complexes.

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- [10] Crystal structure analysis of **6** ($\text{C}_{32}\text{H}_{34}\text{INO} + \text{C}_2\text{H}_6\text{O}$): $Z = 2$, $M_r = 621.57$, crystal dimensions: $0.6 \times 0.6 \times 0.5$ mm, triclinic, space group $P\bar{1}$, $a = 988.95(14)$, $b = 1589.04(10)$, $c = 1033.17(18)$ pm, $\alpha = 103.842(5)^\circ$, $\beta = 96.126(10)^\circ$, $\gamma = 97.561(10)^\circ$, $V = 3.0435(5)$ nm³,

$\rho_{\text{calc}} = 1.357$ Mg m⁻³, $F(000) = 1280$, $\lambda = 71.073$ pm, $T = 133(2)$ K, $\mu(\text{MoK}\alpha) = 1.082$ mm⁻¹, $2.10 \leq 2\theta \leq 22.50^\circ$; of 30634 reflections collected, 7953 are independent and were used for structure refinement of 729 parameters with the help of 73 restraints. The R values are $R1 = 0.0445$ ($I > 2\sigma(I)$) and $wR2 = 0.1099$ (all data); min./max. residual electron density $-0.25/0.23$ e nm⁻³. The data were collected with a Stoe–Siemens–Huber four-circle diffractometer with a Siemens CCD area detector. The intensities were measured with ϕ and ω scans. Data integration was carried out with the SAINT program. The structure was solved by direct methods (SHELXS-90/97) and refined by the least-squares procedure against F^2 . All non-hydrogen atoms were anisotropically refined.^[14] All hydrogen atoms were positioned with ideal geometries and included in the refinement. All disorders were resolved with distance and ADP restraints and anisotropically refined. 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-410149. Copies of the data can be obtained free of charge by application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (Fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

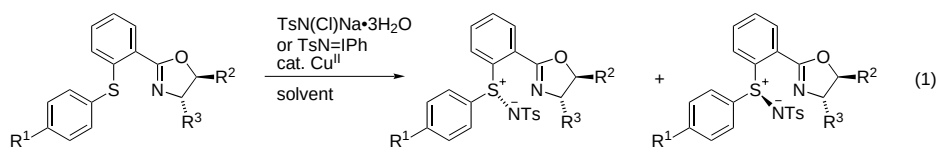
- [11] Spectroscopic data for compounds **4** and **5**: IR (KBr): $\tilde{\nu} = 3053$ cm⁻¹ (C–H), 2976 (C–H), 2850 (C–H), 2790, 1609 (C=C), 1595 (C=C), 1494, 1442, 1375, 1191, 1142, 1047, 999, 910, 846, 771, 697. **4**: ^1H NMR (500 MHz, CDCl_3): $\delta = 1.47$ (t, $^3J = 6.9$ Hz, 3H; OCH_2CH_3), 2.65 (s, 6H; $\text{N}(\text{CH}_3)_2$), 2.96 (AB, dd, $^2J = 17.1$, $^4J = 2.4$ Hz, 1H; 9-H), 3.03 (AB, dd, $^2J = 17.1$, $^4J = 2.4$ Hz, 1H; 9-H), 3.09 (AB, dd, $^2J = 17.1$, $^4J = 2.4$ Hz, 1H; 6-H), 3.18 (AB, dd, $^2J = 17.1$, $^4J = 2.4$ Hz, 1H; 6-H), 4.24 (q, $^3J = 6.9$ Hz, 1H; OCH_2CH_3), 4.25 (q, $^3J = 6.9$ Hz, 1H; OCH_2CH_3), 6.95 (s, 1H; 3-H), 7.28–7.34 (m, 9H; Ph), 7.42 (dd, $^3J = 7.6$, $^4J = 0.7$ Hz, 2H; Ph), 7.66 (dd, $^3J = 7.6$, $^4J = 0.7$ Hz, 2H; Ph), 7.90 (dd, $^3J = 7.6$, $^4J = 0.7$ Hz, 2H; Ph); ^{13}C NMR (125.7 MHz, CDCl_3 , plus DEPT): $\delta = 15.59$ (+, OCH_2CH_3), 42.30 (–, C-9), 42.57 (+, $\text{N}(\text{CH}_3)_2$), 44.55 (–, C-6), 53.11 (C_{quat} , C-5), 65.97 (–, OCH_2CH_3), 112.59 (C_{quat} , C-4), 119.46 (+, C-3), 124.70, 125.20, 125.59, 125.97, 127.20, 127.74, 127.98, 128.32, 128.69 (+, Ph), 134.01, 134.83, 138.97 (C_{quat} , Ph), 144.89 (C_{quat} , C-8), 147.04 (C_{quat} , C-1), 154.48 (C_{quat} , C-7), 156.37 (C_{quat} , C-2). **5** (1:1-diastereomeric mixture): ^1H NMR (500 MHz, CDCl_3): $\delta = 1.25$ and 1.23 (t, $^3J = 6.9$ Hz, 3H; OCH_2CH_3), 2.09 and 2.12 (AB, dd, $^2J = 13.5$, $^3J = 2.4$ Hz, 1H; 9-H), 2.37 and 2.38 (AB, dd, $^2J = 13.5$, $^3J = 8.5$ Hz, 1H; 9-H), 2.48 and 2.52 (s, 6H; $\text{N}(\text{CH}_3)_2$), 3.40 and 3.61 (dt, $^3J = 8.5$, $^4J = 1.4$ Hz, 1H; 8-H) 3.92 and 4.04 (q, $^3J = 6.9$ Hz, 1H; OCH_2CH_3), 3.93 and 4.05 (q, $^3J = 6.9$ Hz, 1H; OCH_2CH_3), 4.48 and 4.49 (d, $^4J = 1.4$ Hz, 1H; 6-H), 6.43 and 6.59 (s, 1H; 3-H), 6.92 and 7.02 (d, $^3J = 7.6$ Hz, 2H; Ph), 7.14–7.26 (m, 9H; Ph), 7.56 and 7.58 (d, $^3J = 7.6$ Hz, 2H; Ph), 7.74 and 7.79 (d, $^3J = 7.6$, 2H; Ph); ^{13}C NMR (125.7 MHz, CDCl_3 , plus DEPT): $\delta = 15.47$ and 15.52 (+, OCH_2CH_3), 40.87 and 40.88 (+, $\text{N}(\text{CH}_3)_2$), 41.58 and 42.81 (–, C-9), 50.96 (+, C-8), 65.78 and 65.91 (–, OCH_2CH_3), 66.23 (C_{quat} , C-5), 102.62 and 102.86 (+, C-6), 121.75 and 122.97 (+, C-3), 123.72 (C_{quat} , C-4), 125.38, 125.89, 127.03, 127.16, 127.34, 127.40, 127.42, 127.59, 127.62, 127.77, 127.80, 128.02, 128.09, 128.29, 128.30, 128.89, 129.55 (+, Ph), 135.73, 136.16, 136.17, 136.41, 136.48 (C_{quat} , Ph), 152.25 and 153.03 (C_{quat} , C-1), 153.79 and 155.09 (C_{quat} , C-7), 155.34 (C_{quat} , C-2). The assignments were verified with 2D correlation experiments. MS (70 eV): m/z (%): 433 (81) [M^+], 404 (100) [$M^+ - \text{C}_2\text{H}_5$], 398 (17) [$M^+ - \text{N}(\text{CH}_3)_2$], 356 (13), 202 (6), 103 (5). – $\text{C}_{31}\text{H}_{31}\text{NO}$ (433.6).
- [12] Compound **2** yields 5-dimethylamino-3-ethoxy-1-propyl-5-trimethylsilyl-1,3-cyclopentadiene (25 %) with 1-pentyne in THF after four days at 55 °C, and the analogous 1,2-dimethylcyclopentadiene derivative (44 %) with 2-butyne in pyridine at 80 °C.
- [13] The possible triple insertion can be considered as the start of an alkyne polymerization catalyzed by carbene complexes which takes place by multiple insertion into the metal–carbene carbon bond. See also: T. J. Katz, S. J. Lee, *J. Am. Chem. Soc.* **1980**, *102*, 422–424. However, as one referee has commented, it has not been confirmed whether or not the products **4** and **5** are formed in a sequence of double insertion of the aryethyne, [2+1] cycloaddition of the carbene to an aryethyne, and a subsequent transformation of the thus formed hexatrienylcyclopropene. However, an appropriate mechanism is not obvious.
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Catalytic Diastereoselective Imidation of Diaryl Sulfides Bearing a Chiral Oxazolinyl Moiety with Chloramine T Trihydrate**

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Currently, we are interested in developing a catalytic method for the enantioselective imidation of prochiral sulfides and selenides to chiral sulfimides^[1, 2] and selenimides^[3] using *[N-(p-toluenesulfonyl)imino]phenyliodinane* (TsN=IPh)^[4] in the presence of a copper salt and a chiral ligand, because these optically active products might be useful for some asymmetric reactions. Williams et al. have recently reported a highly diastereoselective oxidation of diaryl sulfides bearing a chiral oxazolinyl moiety^[5] and demonstrated that the produced optically active sulfoxides are effective ligands for asymmetric palladium-catalyzed allylic substitution.^[6] These interesting results prompted us to investigate the diastereoselective imidation of similar sulfides to prepare novel optically active sulfimides which might be suitable as chiral ligands and reagents.

First, imidation of diaryl sulfides **1** and **3**, bearing an enantiomerically pure 4-isoproploxazolinyl group derived from valinol at the *ortho* position, was carried out by the procedure developed by us [Eq. (1)];^[1] namely, **1** and **3** were treated with chloramine T trihydrate (*N*-chloro-*p*-toluenesul-



- 1** R¹ = R² = H, R³ = *i*Pr
3 R¹ = Me, R² = H, R³ = *i*Pr
5 R¹ = H, R² = Ph, R³ = CH₂OSiMe₂tBu
7 R¹ = H, R² = Ph, R³ = CH₂OH
9 R¹ = H, R² = Ph, R³ = CH₂OMe
11 R¹ = Me, R² = Ph, R³ = CH₂OSiMe₂tBu
13 R¹ = Me, R² = Ph, R³ = CH₂OMe

- 2a**
4a
6a
8a
10a
12a
14a

- 2b**
4b
6b
8b
10b
12b
14b

fonamide, sodium salt; TsN(Cl)Na·3H₂O)^[7] or TsN=IPh using a copper(II) salt as catalyst in a suitable solvent. 1,6-Asymmetric induction occurred to give the corresponding optically active *N*-tosylsulfimides **2** and **4** in about 60 % yield; however, the diastereoselectivity was moderate in all cases (40–48 % *de*, Table 1). The use of TsN(Cl)Na·3H₂O in

Table 1. Diastereoselective imidation of diaryl sulfides **1**, **3**, **5**, **7**, **9**, **11**, and **13**.^[a]

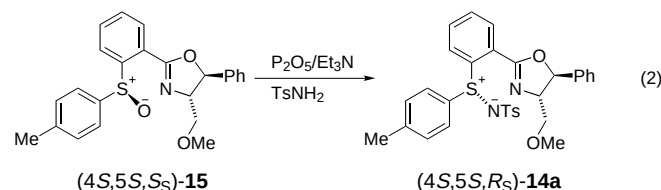
Sulfide	NTs source	Catalyst	Solvent	Yield [%]	<i>de</i> [%] ^[b]
1	TsN(Cl)Na	none	EtOH	29	34
1	TsN(Cl)Na·3H ₂ O	CuCl ₂	MeCN	56	45
1	TsN=IPh	Cu(OTf) ₂	toluene	57 ^[c]	40
3	TsN(Cl)Na·3H ₂ O	CuCl ₂	MeCN	63	48
5	TsN(Cl)Na·3H ₂ O	CuCl ₂	MeCN	76	87
5	TsN(Cl)Na·3H ₂ O	CuCl ₂	toluene	36 ^[d]	< 10
5	TsN(Cl)Na·3H ₂ O	Cu(OTf) ₂	MeCN	63	91
7	TsN(Cl)Na·3H ₂ O	Cu(OTf) ₂	MeCN	67	51
9	TsN(Cl)Na·3H ₂ O	Cu(OTf) ₂	MeCN	52	99
11	TsN(Cl)Na·3H ₂ O	Cu(OTf) ₂	MeCN	58	74
13	TsN(Cl)Na·3H ₂ O	Cu(OTf) ₂	MeCN	61	99

[a] Reactions were performed at 25 °C for 24 h with sulfide (0.2 mmol), NTs source (0.2 mmol), catalyst (10 mol %), and solvent (1 mL). Unless otherwise noted, the formation of a slight amount (<5 %) of the corresponding sulfoxide was observed. [b] Determined by ¹H NMR spectroscopy. [c] The formation of the corresponding sulfoxide was not observed at all. [d] The corresponding sulfoxide (12 %) was also isolated.

acetonitrile gave a slightly better result than the use of TsN=IPh in toluene.^[8]

In contrast, when diaryl sulfides **5**, **7**, **9**, **11**, and **13**, bearing an enantiomerically pure oxazolinyl group derived from (1*S*,2*S*)-(+)-2-amino-1-phenyl-1,3-propanediol, were employed as substrates, a much higher diastereoselectivity (up to 99 % *de*) was observed on treatment with TsN(Cl)Na·3H₂O in the presence of copper(II) salt in a suitable solvent. Here, the combination of Cu(OTf)₂ (OTf = trifluoromethanesulfonate) and acetonitrile was the best (Table 1). The use of TsN=IPh resulted in poor diastereoselectivity. In the present imidation, a higher stereoselectivity was observed for sulfides bearing a methoxymethyl or silyloxymethyl moiety at the 4-position of the oxazoline ring than for **1**, where an isopropyl moiety is present at the same position.

To determine the absolute configuration of the produced *N*-*p*-tosylsulfimides, the sulfoxide (4*S*,5*S*,*S*_S)-**15** ([α]_D²⁵ = −255.2, *c* = 1.5 in acetone), prepared by methylation of the hydroxyl group of a known compound,^[5] was treated with *p*-toluenesulfonamide [Eq. (2)] since the

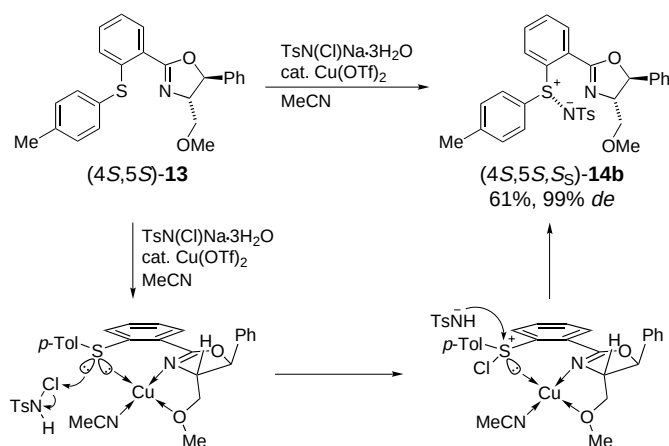


substitution of oxygen on sulfur by the NTs moiety is known to proceed with inversion of configuration.^[9] The produced *N*-tosylsulfimide **14a** ([α]_D²⁵ = −136.0, *c* = 1.5 in acetone), which is supposed to have an absolute configuration of 4*S*,5*S*,*R*_S, corresponded to the minor product of the present imidation.

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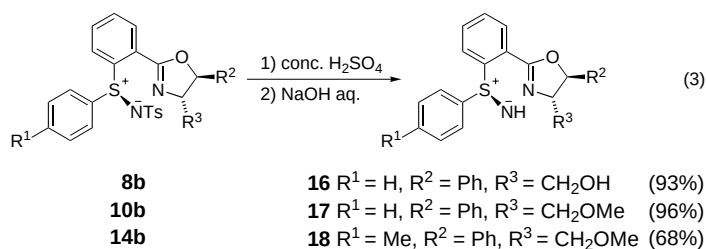
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The major product **14b** ($[\alpha]_D^{25} = +53.5$, $c = 1.5$ in acetone) should have the $4S,5S,S_S$ configuration. This evidence and the quite high diastereoselectivity observed might be explained by assuming that the reaction proceeds ionically^[10] through back-side attack of the anion of *N-p*-tosylamine on sulfur of the chlorosulfonium ion intermediate,^[11] whose lone pair interacts with Cu, which is coordinated by N, O, and a solvent molecule (Scheme 1). However, the possibility of some intervention of the nitrene transfer mechanism^[12] may not be excluded.



Scheme 1. One of the possible reaction pathways for the copper-catalyzed imidation of sulfide **13**.

Additionally, hydrolysis^[13] allowed *N-p*-tosylsulfimides **8b**, **10b**, and **14b** to be converted into the corresponding optically pure sulfimides **16**, **17**, and **18**, respectively, in high yields [Eq. (3)]. We assume that the hydrolysis proceeds without any



change in configuration, as in the case of the hydrolysis of *N*-tosylsulfoximide.^[9b]

Preliminary results of the use of these novel, optically active sulfimides and *N-p*-tosylsulfimides as chiral ligands in the palladium(II)-catalyzed allylic alkylation of 1,3-diphenyl-3-acetoxy-1-propene with dimethyl malonate show that the product is obtained quantitatively. Furthermore, sulfimide **17** works more efficiently than *N-p*-tosylsulfimide **10** in terms of stereoselectivity (90 and 46% *ee*, respectively).^[14] Further studies on the clarification of the reaction scheme as well as asymmetric reactions using these novel optically active sulfimides as chiral ligands or reagents are currently in progress.

Experimental Section

A typical experimental procedure is as follows: Acetonitrile (5.0 mL), **9** (75.5 mg, 0.20 mmol), and Cu(OTf)_2 (7.2 mg, 0.020 mmol) were placed in 10-mL flask. After 15 min chloramine T trihydrate (56.3 mg, 0.20 mmol) was added to this solution, and the mixture was stirred at 25 °C for 24 h. The mixture was then poured into water and extracted with CH_2Cl_2 . The organic layer was dried over MgSO_4 and concentrated. The residue was purified by column chromatography on silica gel, and the major fraction afforded 56.6 mg (52% yield) of **10b** with a diastereoselectivity of greater than 99%. A slight amount (<3%) of the corresponding sulfoxide was also isolated. **10b**: White solid; m.p. 62–63 °C; ^1H NMR (270 MHz, CDCl_3): $\delta = 2.34$ (s, 3H; Me), 3.45 (s, 3H; OMe), 3.55 (dd, $J = 9.8, 6.4$ Hz, 1H; CHHOMe), 3.69 (dd, $J = 9.8, 6.4$ Hz, 1H, CHHOMe), 4.12 (m, 1H; CHN), 5.38 (d, $J = 6.4$ Hz, 1H; CHO), 6.96–8.64 (m, 18H; Ar); ^{13}C NMR (67.8 MHz, CDCl_3): $\delta = 21.3, 59.4$ (CH_2O), 73.8 (OMe), 75.0 (CHN) 83.7 (CHO), 125.3, 126.2, 127.8, 128.2, 128.3, 128.7, 128.9, 129.2, 130.2, 131.2, 131.5, 132.4, 139.3, 139.8, 141.3, 141.7, 160.4 (C=N).

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Large Circular Dichroism Ellipticities for N-Templated Helical Polypeptides Are Inconsistent with Currently Accepted Helicity Algorithms**

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Among polypeptide conformations, only helices permit calculation of conformational stability from a readily measurable physical parameter. If a polypeptide exhibits a helical circular dichroism (CD) spectrum (intense positive maximum near 190 nm, negative minima at 208 and 222 nm), Equation (1) is generally used to relate the average fractional helicity^[1] (FH) to the experimental mean residue molar ellipticity^[2a,c] ($[\theta]_{222}$). The limiting ellipticity $[\theta_{H\infty}]_{222}$ of Equation (1) corresponds to a completely helical peptide of infinite length, and the term $(1 - x/N)$; where N = peptide length; $2.4 < x < 4.5$) provides a finite length correction.^[3] From studies of N-templated peptides containing more than twelve amino acid residues, we now demonstrate that literature values underestimate both $[\theta_{H\infty}]_{222}$ and its length dependence.

$$FH = \frac{[\theta]_{222}}{[\theta_{H\infty}]_{222} \left(1 - \frac{x}{N}\right)} \quad (1)$$

$$[\theta_H]_{222} = (-44000 + 250T) \left(1 - \frac{2.5}{N}\right) = 100\% \text{ helicity for length } N \quad (2)$$

Certain alanine-rich peptides containing lysine or arginine are reported to approach FH values of 0.7 to 0.8 in water at low temperature,^[2b] but no peptide has been unequivocally proven to be 100% helical. Cited $-\theta_{H\infty}]_{222}$ values of 37 000 to 44 000 deg cm² dmol⁻¹ are usually extrapolated as limits of a series of temperature- or additive-dependent CD spectra.^[2c, 4]

Ellipticities of peptides that are amide-linked to an efficient helix initiator such as the N-terminal cap AcHel^[5] should give a better approximation to $[\theta_{H\infty}]_{222}$. Figure 1 shows helical CD spectra at 5 °C intervals for the N-templated 22-residue peptide **1**, AcHel-(Ala₄Lys)₄Ala₂-NH₂, in water containing 16 mol % of ethylene glycol, which permits CD measurements at sub-zero temperatures. The value of $-\theta_{222}$ increases dramatically as the temperature is lowered, but does not converge to a limit. At the frequently studied temperature of 2 °C, $-\theta_{222}$ for **1** is 50 900 deg cm² dmol⁻¹, corresponding to 1.32 times the largest reported temperature- and length-corrected value of $[\theta_H]_{222}$ [Eq. (2)].^[4b,c] Similar values and temperature dependencies for $-\theta_{222}$ of **1** were observed at

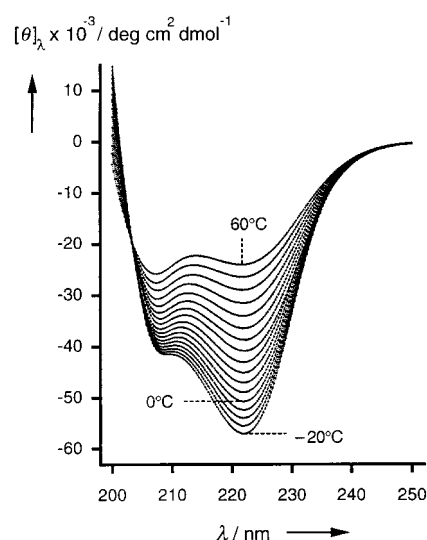


Figure 1. Overlay of 17 CD spectra of AcHel-(Ala₄Lys)₄Ala₂-NH₂ measured in 5 °C intervals between -20 and 60 °C in 16 mol % ethylene glycol in water (pH 1, 0.2 mol L⁻¹ NaClO₄). Peptide concentration: 45 μmol L⁻¹.

pH 1 in aqueous NaCl (5 mol L⁻¹) and in aqueous 2,2,2-trifluoroethanol at pH < 2.

Are the low temperature CD properties of **1** unique? Figure 2 depicts values of $-\theta_{222}$ measured in water at 2 °C for N-templated peptides AcHel-(Ala₄Lys)_nAla₂-NH₂, $n = 1$

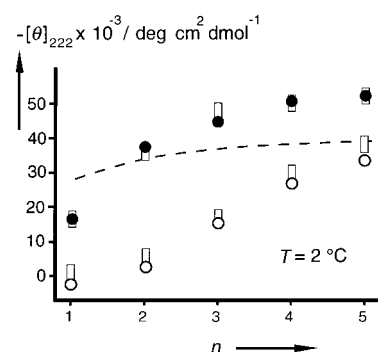


Figure 2. Effect of oligomer length n on the $-\theta_{222}$ value for N-templated AcHel-(Ala₄Lys)_nAla₂-NH₂ (filled circles) and N-protonated H₂⁺(Ala₄Lys)_nAla₂-NH₂ (open circles) (2 °C, pH 1, 0.2 mol L⁻¹ NaClO₄). The dashed line shows the calculated length dependence for $[\theta_H]_{222}$ from Equation (2). For the templated series, $n > 1$, the experimental values exceed this predicted limit. The open rectangles give $[\theta]_{222}$ values calculated from Equation (3), as described in the text and supporting information. Experimental $[\theta]_{222}$ values are corrected for the contribution of AcHel (less than 1% for $n > 2$).

to 5, and for the analogous N-protonated peptides H₂⁺(Ala₄Lys)_nAla₂-NH₂. Data are also available for the series AcHel-(Ala₄Lys)₃Ala_n-NH₂, $n = 0$ to 6, and for the conjugates AcHel-Ala₄Glu-(Ala₄Lys)₂Ala₂-NH₂ and AcHel-(Ala₄Lys)₂-Ala₇-X-NH₂ (X = Lys or Arg). For these ten partially helical AcHel peptides with $16 < N < 22$, the $-\theta_{222}$ values in water at 2 °C pH 1 exceed $-\theta_{H\infty}]_{222}$ values for 100% helical peptides predicted from Equation (2), with a mean ratio, $[\theta]_{222}/[\theta_H]_{222} = 1.14 (\pm 0.04)$.

Could the presence of the template significantly bias the CD spectra of the conjugates? The contribution of the

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template CD chromophore to $-\theta_{222}$ of highly helical peptides is small.^[5b] More significantly, low temperature CD spectra of nontemplated peptides can be superimposed on corresponding spectra of their AcHel conjugates measured at higher temperatures (Figure 3). Thus the template merely

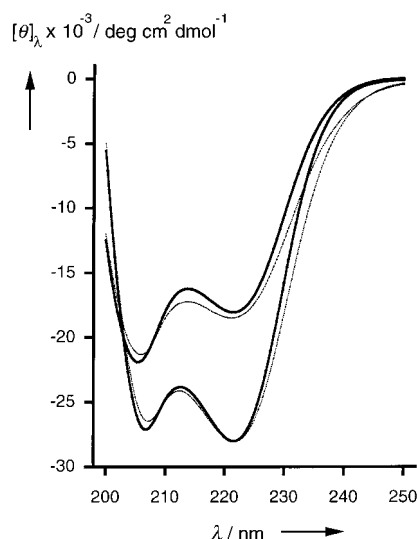


Figure 3. CD curve shape comparisons (pH 1, 0.2 molL⁻¹ NaClO₄). Light curves: AcHel-(Ala₄Lys)₄Ala₂-NH₂ at 40°C (lower spectrum) and 55°C (upper spectrum). Bold curves: H₂⁺(Ala₄Lys)₄Ala₂-NH₂ at 0°C (lower spectrum) and 20°C (upper spectrum).

stabilizes the peptide helix, while preserving all details of its CD spectrum.

Values of $[\theta]_{222}$ for templated and nontemplated peptides of Figure 2 show trends that are consistent with FH values calculated from the Lifson–Roig equation (L–R), which uses the peptide length N , an initiation constant v , and amino acid helical propensities w as variables.^[6] A L–R calculation treats a helical peptide as a manifold of nonhelical, completely helical, and partially helical conformations and assigns to each helical conformation a relative abundance, expressed as a mole fraction χ_H . The fractional helicity FH of a single helical conformation is k/N , where k is the length of its helical region, and each conformation thus contributes $\chi_H(k/N)$ to the overall FH. For the short ($n = 1$ or 2) nontemplated peptides studied under the conditions of Figure 2, the most abundant conformations are nonhelical, but for $n = 3, 4$, and 5 , the helical conformations dominate. This difference results in a dramatic increase of FH with length, as shown by the sigmoid shape of the $-\theta_{222}$ plot. The very different monotonic shape of the plot for the templated peptide series reflects the role of the template as an efficient helix initiator. For all N-templated peptides the helical conformations dominate.

The template also selectively increases the χ_H of conformations with long helical regions. This effect explains a second feature of the data in Figure 2. For $n = 4$ or 5 , the templated peptides exhibit much larger $-\theta_{222}$ values. Since k/N approaches 1 for these conformations, they contribute maximally to FH and to $-\theta_{222}$. By contrast, the helical manifolds for nontemplated peptides are dominated by many

conformations containing short helical regions. These have smaller k/N values and contribute inefficiently to FH.

The L–R equation thus models the data of Figure 2 qualitatively. For quantitative modeling, a revised version of Equation (2) is needed. The dotted line of Figure 2 gives values of $-\theta_{222}$ calculated from Equation (2) that predict the experimental $-\theta_{222}$ value expected for a hypothetical peptide with a FH of 1.0. Calculated FH values for the templated peptides with $n = 3–5$ fall in the range of 0.81 to 0.85, and thus Equation (2) grossly underestimates the experimental $-\theta_{222}$ values. Dividing $-\theta_{222}$ for $n = 5$ by its FH yields 61 000 as an approximate value for $-\theta_{H,222}$ in this helical length range ($k > 20$). The corresponding value for the first templated peptide ($n = 1, k \leq 7$) is 30 000. Redefined values for $-\theta_{H,222}$ must clearly double within the peptide size range of 7 to 20 residues, and improved models for calculating $-\theta_{222}$ must assign substantially different $[\theta_{H,222}]$ values to conformations containing large and small helical regions.

A simple two-state model accommodates the data of Figure 2. FH can be written as $FH_{\text{small}} + FH_{\text{large}}$ and distinct $[\theta_{H,222}]$ values can be assigned to each FH term [Eq. (3)].

$$[\theta_{H,222}]_{\text{calculated}} = [\theta_{H,222}]_{\text{small}} FH_{\text{small}} + [\theta_{H,222}]_{\text{large}} FH_{\text{large}} \quad (3)$$

We define FH_{small} to include conformations with $k \leq 11$ helical residues and set $[\theta_{H,222}]_{\text{small}}$ equal to Equation (2), $N = 11$, which provides a good fit for $[\theta]_{222}$ in this size range.^[7] We define FH_{large} to include conformations with $k > 11$ and set $[\theta_{H,222}]_{\text{large}}$ equal to $-61\,000$. Values for the AcHel N-capping template parameter, the initiation parameter, and helical propensities for Ala and Lys have all been reported previously.^[2, 5c] As seen in Figure 2, with these parameters, Equation (3) allows an excellent fit to the experimental $[\theta]_{222}$ value for the templated series AcHel-(Ala₄Lys)_{*n*}Ala₂-NH₂. The $[\theta]_{222}$ value for the nontemplated peptides of Figure 2 are also accommodated by Equation (3), provided that the average helical propensity is reduced by 9% to compensate for the helix-destabilizing effect of the N-terminal positive charge. This agreement, achieved for a limited data set using a simple two-state model, represents a first step toward a more rigorous relationship between $[\theta]_{222}$ and FH. A larger data base exploring length dependence will be needed to refine the model.

Is the 222 nm minimum appropriate for correlation of $[\theta]$ with FH? Though universally used, experimentally convenient, and relatively insensitive to the presence of nonhelical conformations, $[\theta]_{222}$ may be less reliable than $[\theta]_{208}$, which is cited in the older literature.^[8] The two short wavelength helical CD extrema at 195 nm and 208 nm correspond to perpendicular and parallel $\pi-\pi^*$ electronic transitions and have been modeled satisfactorily by theory, but the 222 nm $n-\pi^*$ band has not.^[9] Moreover, large variations in the ratio of $[\theta]_{208}$ to $[\theta]_{222}$ have been reported for helical peptides and these are evident in the data of Figure 1. Although this ratio has been correlated with the relative proportions of α and 3_{10} helical conformations within the helical manifold, the assignment of the limiting CD spectra for pure α and 3_{10} states remains controversial.^[10] It is unclear which $[\theta]$ value provides the better correlation with the overall FH, $FH(\alpha) + FH(3_{10})$.

Within our study two questions remain. The large length dependence of $-\left[\theta_{\text{H}}\right]_{222}$ in the range of 7 to 20 residues has been demonstrated by an independent method,^[11] but its cause is unknown. The preliminary two-state model outlined above appears to be inconsistent with experimental $\left[\theta\right]_{222}$ values reported for peptides with $N > 30$. Experiments to explore these issues are in progress, but their outcomes should not change our most important conclusion. For the class of alanine-rich, lysine-containing peptides with $15 < N < 27$, the literature equations relating FH to experimental $\left[\theta\right]_{222}$ values underestimate $\left[\theta_{\text{H}}\right]_{222}$,^[12] and the corresponding FH values are overestimated by 25–50%.

Experimental Section

Peptides were prepared as previously described^[5d] and purified by repeated reverse-phase high-performance liquid chromatography (RP-HPLC), and characterized by MALDI-MS (matrix-assisted laser desorption/ionization mass spectrometry) and amino acid analysis. The purity of all compounds is estimated to be higher than 95%. Solutions were maintained at pH 1–2 for consistency with NMR measurements, and NaClO_4 buffers were used for consistency with literature measurements. CD spectra were taken on a thermostated Aviv 62DS circular dichroism spectrometer calibrated according to literature procedures.^[13] Concentrations of CD solutions were determined within 5% error both by a quantitative ninhydrin assay^[7b] and by amino acid analysis.^[14] Solutions of AcHel-(Ala₄Lys)Ala₂-NH₂ ($n = 4, 5$) and selected nontemplated analogues were shown to be monomeric both by CD dilution studies and analytical ultracentrifugation.

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[M₃V₁₈O₄₂(H₂O)₁₂(XO₄)] · 24H₂O (M = Fe, Co; X = V, S): Metal Oxide Based Framework Materials Composed of Polyoxovanadate Clusters**

M. Ishaque Khan,* Elizabeth Yohannes, and Robert J. Doedens

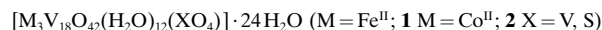
The early transition metal oxide clusters with M=O functionalities constitute a fast emerging class of compounds. Their properties are of both intrinsic and applied interest and envelop such diverse fields as analytical chemistry, biochemical and geochemical processes, catalysis, materials science, and medicine.^[1] Metal oxide clusters with molecular weights at par with proteins have been prepared and characterized.^[1e] The structure and bonding patterns in these molecular aggregates remarkably resemble the complex transition metal oxide surfaces^[1a, 2] employed as catalysts for organic transformations.^[2d, 3] Many such catalysts are poorly understood because of their inaccessibility to conventional physicochemical techniques and therefore are not amenable to improvements in their performances. With their proven role in catalysis^[4] and in the development of new oxide-supported transition metal catalysts,^[4f] metal oxide clusters offer attractive building units with well-defined properties^[5, 1b] for the preparation of catalysts and novel surfaces based on metal (and mixed-metal) oxides whose performance could possibly be rationalized in terms of their constituents at the molecular level.^[4a]

However, the technique of bringing suitable metal oxide building units together to generate true metal oxide surfaces and framework materials without the incorporation of additional conventional ligands is still in its infancy and has been limited to the synthesis of mainly one-dimensional chains.^[6] Here, we report the synthesis and characterization of two

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novel three-dimensional framework materials, **1** and **2**, composed of well-defined vanadium oxide clusters.



Black prism-shaped crystals of **1** and **2** were isolated in 50–60% yield from the dark colored solution obtained from the reaction of V_2O_5 with $LiOH \cdot H_2O$, hydrazinium sulfate, and $FeCl_2 \cdot 4H_2O$ or $CoSO_4 \cdot 6H_2O$ in water at 84–86 °C. The compounds were characterized by elemental analysis, manometric titration of the reduced V^{IV} sites, FT-IR spectroscopy, thermal methods, and X-ray single-crystal structure analysis. The IR spectra of these compounds exhibit absorption bands attributable to H_2O , $\nu(SO_4)$, $\nu(VO_4)$, $\nu(V-O_{term})$, and $\nu(V-(\mu_3-O))$ groups.

X-ray structure analysis^[7] of single crystals of **1** and **2** revealed highly symmetrical isomorphous structures (Figure 1). In each case, the structure consists of $[V_{18}O_{42}]$ cages^[8]

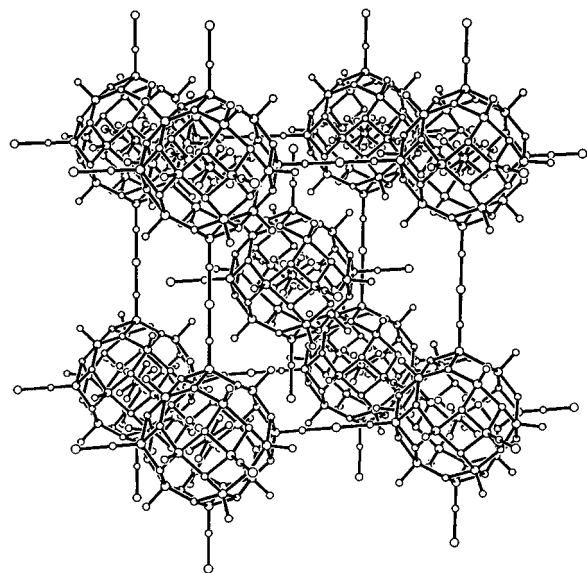


Figure 1. View of the unit cell to show the extended structure of **1** which contains arrays of $[V_{18}O_{42}(XO_4)]$ interconnected through $[Fe(H_2O)_4]$ bridging units. Water molecules have been omitted for clarity.

with crystallographic $m\bar{3}m$ (O_h) symmetry^[7b] linked by bridging $[M(H_2O)_4]$ groups into two interpenetrating three-dimensional networks; a consequence of body-centered symmetry.^[7c] Each cage hosts a twofold disordered tetrahedral $\{XO_4\}$ group. Based on crystallographic, chemical, and spectroscopic evidence,^[9] the final refinement model assumed a disordered distribution of both the VO_4^{3-} and SO_4^{2-} anions for the $\{XO_4\}$ group.

The host $[V_{18}O_{42}]$ shell, constructed from 18 $\{VO_5\}$ square pyramids sharing edges through 24 μ_3 -oxygen atoms, is known to incorporate VO_4^{3-} and SO_4^{2-} ions^[8a] and act as a container for other anions and molecules.^[8b] It exists with different electronic populations in two closely related structural forms with different symmetries (T_d and D_{4d}) that are influenced by the stereochemical needs^[5a, 10] and the extent of interaction of the encapsulated moiety with the V centers of the shell.^[8a-c] For example, in the $Na_6[H_7V^{IV}_{16}V^{V}_2O_{42}(VO_4)] \cdot 21H_2O$ clus-

ter,^[8a-b] the $\{VO_4^{3-}\}$ group ($V-O$ 1.71 Å) interacts through four μ_4 -O atoms with the twelve V centers of the shell to form covalent $(V-\mu_4-O)-V_{shell}$ (μ_4-O-V_{shell} 2.39 Å) bonds and confers tetrahedral symmetry to the anion. The cages in **1** and **2** (with O_h symmetry) represent, interestingly, somewhat different situations and illustrate further the structural flexibility of the $[V_{18}O_{42}]$ motif.

A view of the $[V_{18}O_{42}(XO_4)]$ cluster in **1** is shown in Figure 2. All bond lengths in the cluster are within normal ranges. The geometry around each V1 atom in twelve of the

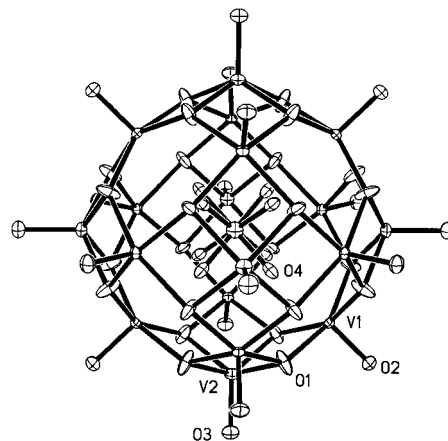


Figure 2. The $[V_{18}O_{42}(XO_4)]$ cluster in the crystal structure of **1**, with the atom labeling scheme in the asymmetric unit. Displacement ellipsoids are drawn at the 50% probability level. The unlabeled central atom, bonded to O4 atoms, represents X. Selected bond lengths [Å] and angles [°]: **1**: V1–O1 1.948(2), V1–O2 1.587(6), V2–O1 1.942(4), V2–O3 1.636(9), Fe1–O3 2.105(9); V1–O1–V2 98.16(14), V1–O1–V1 138.2(3); **2**: V1–O1 1.923(3), V1–O2 1.590(9), V2–O1 1.954(7), V2–O3 1.652(15), Co1–O3 2.118(14); V1–O1–V2 97.3(2), V1–O1–V1 138.7(5).

$\{VO_5\}$ groups is defined by a terminal oxo group (O2) and four μ_3 -oxygen atoms (O1). The geometry around V2 in the remaining six $\{VO_5\}$ units is defined by four μ_3 -oxo (O1) groups and an apical μ_2 -oxygen atom (O3) which is also bonded to the M^{II} center of one of the six $[M(H_2O)_4]$ bridges that interlink the $[V_{18}O_{42}(XO_4)]$ clusters. The coordination sphere of M^{II} is completed by four oxygen atoms (O6) from the aqua ligands ($M-O(H_2)$ 2.089 Å in **1** and 2.047 Å in **2**), each one exhibiting twofold disorder, and two *trans* μ_2 -(O3) groups. The elongated ellipsoid for O1, present in **1** and **2**, is probably a consequence of steric interactions that arise from a short O...O contact (2.545(9) Å in **1** and 2.521(13) Å in **2**) between O1 and an O4 site that is only 50% occupied.

The M1–O6 distance and the bond valence sum (BVS)^[11a] value (0.39) of the O6 group identify it as H_2O . This conclusion and the result of the manometric titration of V^{IV} sites ($\approx 9.5 V^{IV}$ per formula unit)^[11b] correspond to the stated formulation of **1** and **2**. The packing in **1** and **2** (Figure 1) generates rectangular tunnels occupied by water of solvation that is readily removed upon heating at about 70 °C.

The synthesis and characterization of **1** and **2** represent a step in the direction of the preparation of transition metal oxide based materials composed of suitable metal oxide motifs with controllable properties. In view of the rapidly expanding pool of well-characterized metal oxide clusters, this

has potential to provide access to a variety of synthetic materials. The heterometallic centers, such as Fe^{II} and Co^{II} , located in the walls of the tunnels in **1** and **2**, may be incorporated in new solids to fashion materials that may also exhibit properties, such as catalytic activity, which are associated with the heterometallic sites. We are currently evaluating the catalytic and sorptive properties of **1** and **2** and studying other metal oxide based materials containing different heterometallic centers.

Experimental Section

1: An aqueous solution of $\text{LiOH} \cdot \text{H}_2\text{O}$ (3 mL, 5 mmol) was added to a stirred slurry of V_2O_5 (2.5 mmol) in water (10 mL) maintained at $84-86^\circ\text{C}$. After the resulting solution was treated with hydrazinium sulfate (2.5 mmol), the reaction mixture was heated for another 10 min. The dark colored solution was diluted to 25 mL (pH = 4.6) and subsequently treated with $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (1.25 mmol) and heated for 3–7 h. The resultant solution was allowed to stand at room temperature in a stoppered flask for 12 h. Dark prism-shaped crystals were filtered from the mother liquor, washed with cold water to remove amorphous impurity, and dried in air at room temperature to give 0.38 g (56% yield based on vanadium) of **1**. FT-IR (KBr; $1200-400\text{ cm}^{-1}$): $\tilde{\nu} = 1131$ (m, SO_4), 990 (s, $\text{V}-\text{O}_{\text{term}}$), 807 (m, VO_4), 689 (m, $\text{V}-(\mu_3\text{-O})$), 631 (m, $\text{V}-(\mu_3\text{-O})$) cm^{-1} . Compound **2** was synthesized in an analogous manner with the equivalent amount of $\text{CoSO}_4 \cdot 6\text{H}_2\text{O}$. FT-IR (KBr): $\tilde{\nu} = 1131$ (m, SO_4), 990 (s, $\text{V}-\text{O}_{\text{term}}$), 801 (m, VO_4), 680 (m, $\text{V}-(\mu_3\text{-O})$), 630 (m, $\text{V}-(\mu_3\text{-O})$) cm^{-1} .

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- [7] a) Crystal structure analysis: The data were collected at 183 K on a Bruker SMART-CCD diffractometer (graphite monochromated $\text{Mo-K}\alpha$ radiation; $\lambda = 0.71073\text{ \AA}$) and phi scan frames. The structures were solved by direct methods (SHELXTL Version 5.10) and refined by full-matrix least squares on F^2 . Crystal data for $\text{H}_{72}\text{O}_{82}\text{S}_{0.5}\text{Fe}_3\text{V}_{18.5}$ (**1**): crystal dimensions $0.30 \times 0.23 \times 0.15\text{ mm}$, cubic, space group $Im\bar{3}m$ (no. 229), $a = 15.4679(4)\text{ \AA}$, $V = 3700.79(17)\text{ \AA}^3$, $Z = 2$, $\rho_{\text{calcd}} = 2.253\text{ g cm}^{-3}$, $\mu(\text{Mo-K}\alpha) = 2.926\text{ mm}^{-1}$. Of the 11888 reflections measured ($1.86^\circ \leq \theta \leq 27.43^\circ$), 458 independent reflections were used to solve the structure. Based on all these data and 40 refined parameters, final $R1 = 0.0445$ (all data), $wR2 = 0.1276$, and the goodness-of-fit on F^2 is 1.169. Crystal data for $\text{H}_{72}\text{O}_{82}\text{S}_{0.5}\text{Co}_3\text{V}_{18.5}$ (**2**): crystal dimensions $0.17 \times 0.13 \times 0.13\text{ mm}$, dark green prism, cubic, space group $Im\bar{3}m$ (no. 229), $a = 15.4536(5)\text{ \AA}$, $V = 3690.5(2)\text{ \AA}^3$, $Z = 2$, $\rho_{\text{calcd}} = 2.268\text{ g cm}^{-3}$, $\mu(\text{Mo-K}\alpha) = 3.019\text{ mm}^{-1}$. Of the 10552 reflections measured ($1.86^\circ \leq \theta \leq 26.10^\circ$), 403 symmetry-independent reflections were used to solve the structure. Based on all these data and 39 refined parameters, $R1 = 0.0664$ (all data), $wR2 = 0.1870$, and the goodness-of-fit on F^2 is 1.213. 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-410238 (**1**) and CSD-410239 (**2**). b) The O_h symmetry refers to the average of the two disordered components. The symmetry, for example, of a $\{\text{V}_{19}\text{O}_{46}\}$ species composed of a $\{\text{V}_{18}\text{O}_{42}\}$ cage encapsulating an ordered $\{\text{VO}_4\}$ group will be no higher than T_d ; c) S. R. Batten, R. Robson, *Angew. Chem.* **1998**, *110*, 1558; *Angew. Chem. Int. Ed.* **1998**, *37*, 1460.
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- [9] a) The X–O distances ($1.538(15)\text{ \AA}$ in **1** and $1.51(2)\text{ \AA}$ in **2**) are intermediate between the expected values for SO_4^{2-} and VO_4^{3-} . In the case of **1**, (for which the structural results are most precise), the O displacement ellipsoid is elongated along the X–O bond. The displacement parameter of X was higher than expected when it was refined as 100% V and unrealistically low when only S was included. In the final refinement, the displacement parameter of X was fixed at $0.015\text{ \AA}^2$ and the relative proportions of V and S were allowed to vary. This model converged to approximately equal proportions of V and S (fraction of V: 0.49(5) for **1** and 0.49(8) for **2**). Different displacement parameters would change these proportions, and so the uncertainties are therefore considerably greater than implied by the listed esds; b) IR spectra of **1** and **2** exhibit bands attributable to SO_4^{2-} and VO_4^{3-} ions. Elemental analysis (% S) corresponds to the stated formulation of **1** and **2**. All attempts to prepare **1** and **2** without sulfate were unsuccessful.
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- [11] a) I. D. Brown in *Structure and Bonding in Crystals, Vol. II* (Eds.: M. O'Keefe, A. Navrotsky), Academic Press, New York, **1981**, p. 1. b) Crystals of **1** oxidize in air. Consequently, the number of reduced sites decreases with time.

Structure and Magnetic Properties of a Dodecanuclear Twisted-Ring Iron(III) Cluster**

Andrea Caneschi, Andrea Cornia, Antonio C. Fabretti, and Dante Gatteschi*

Supramolecular approaches have provided a new tool for the design of large magnetic clusters.^[1, 2] Anion^[2b, 3] and cation^[4–6] recognition in particular provide the possibility of controlling the shape and the size of clusters. A clear example is the possibility to address the synthesis of molecular rings by exploiting host–guest interactions with alkali metal cations, because alkali metal cations of different ionic radius are hosted by rings of different size. Hexairon(III)^[4a, b, 5] and hexamanganese(III)^[4c] clusters with cyclic M_6O_{12} cores can easily accommodate Li^+ and Na^+ cations, both in the solid state and in solution, whereas Cs^+ ions require larger rings, such as M_8O_{16} .^[5] Small complex cations (NH_4^+),^[7a] anions (ClO_4^-),^[7b] or neutral molecules such as $(CH_3)_2CO$ ^[8a] and Et_2NH ^[8b] have been found in the cavity of eight-membered iron(III), cobalt(III), or chromium(III) cycles in the solid state and may play a role in the formation of the host frameworks. However, several molecular rings exhibit hollow cores and apparently “unsupported” structures. In these cases, the cyclic structure is a result of the subtle balance between the binding constraints of the ligands and the coordination requirements of the metal.^[2, 9, 10]

In an attempt to increase the size of the hexairon(III) coronates $[M'Fe_6(OCH_3)_{12}(dbm)_6]^+$ where $M' = Li$ or Na and $Hdbm$ = dibenzoylmethane,^[4a, b] we used larger alkali metal ions, such as K^+ or Cs^+ , and we found that a ring made up of twelve iron(III) ions, in $[Fe(OCH_3)_2(dbm)]_{12}$ (**1**), was obtained which did not host an alkali metal ion.

Compound **1** was obtained by reaction of equimolar amounts of $FeCl_3$ and $Hdbm$ in the presence of either potassium or cesium methylate (4 equiv) in anhydrous methanol. The composition of the crystalline compound, $1 \cdot 6CHCl_3$, was established by elemental analysis and single-crystal X-ray diffraction analysis at 173 K. The molecular structure of **1** viewed perpendicular to the average plane through the metal centers is displayed in Figure 1. Each iron(III) ion in **1** has a distorted octahedral environment with six oxygen donors from methoxide and dbm ligands. Fe–O bond lengths are in the range 1.94–2.07 Å, while bond angles at the bridging oxygen atoms vary from 102.5 to 106.8° (average: 104.7°). The ring is not planar and has crystallographic C_1 point group symmetry. However, two idealized C_2 axes bisect four edges of the metal dodecagon (Fe1–Fe2 and Fe7–Fe8; Fe4–Fe5 and Fe10–Fe11, respectively), and a third lies perpendicular to the plane of Figure 1 which leads to idealized D_2 symmetry. The Fe–Fe distances are in a narrow

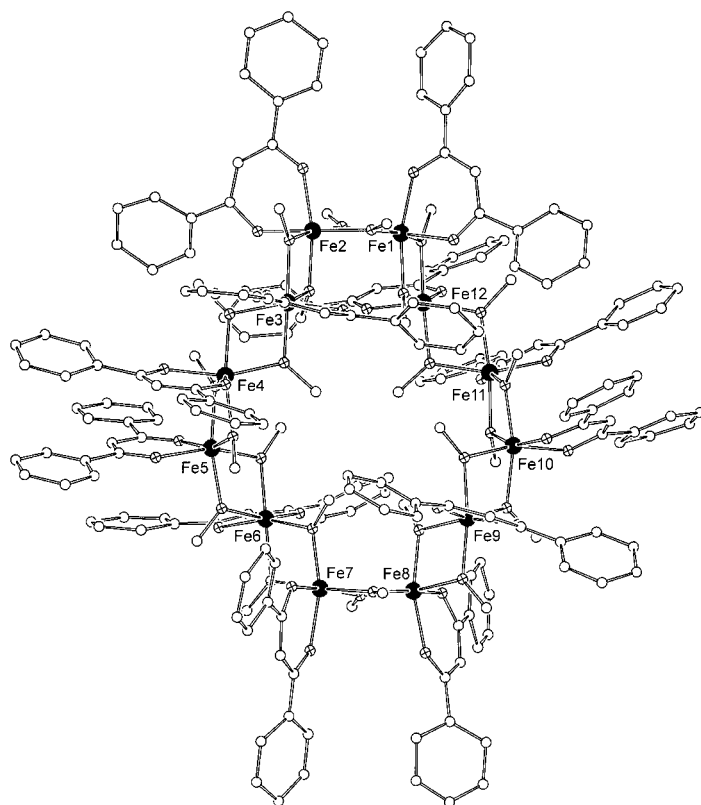


Figure 1. Ball-and-stick representation of **1**, viewed perpendicular to the average plane through the metals. Iron atoms are drawn as large black spheres, oxygen and carbon atoms are depicted as small cross-hatched and open circles, respectively. For clarity, only iron atoms are labeled and hydrogen atoms are omitted. Selected nonbonded distances [Å] and angles [°]: Fe1–Fe2 3.115(4), Fe2–Fe3 3.133(5), Fe3–Fe4 3.138(5), Fe4–Fe5 3.156(5), Fe5–Fe6 3.130(5), Fe6–Fe7 3.155(5), Fe7–Fe8 3.113(5), Fe8–Fe9 3.137(5), Fe9–Fe10 3.123(5), Fe10–Fe11 3.156(5), Fe11–Fe12 3.130(5), Fe12–Fe1 3.144(5); Fe2–Fe1–Fe12 118.1(1), Fe1–Fe2–Fe3 117.3(1), Fe2–Fe3–Fe4 132.4(2), Fe3–Fe4–Fe5 135.9(1), Fe4–Fe5–Fe6 135.5(1), Fe5–Fe6–Fe7 131.9(2), Fe6–Fe7–Fe8 119.3(1), Fe7–Fe8–Fe9 119.0(1), Fe8–Fe9–Fe10 134.2(1), Fe9–Fe10–Fe11 136.2(1), Fe10–Fe11–Fe12 135.7(1), Fe11–Fe12–Fe1 131.4(2).

range (3.113–3.156 Å), whereas the Fe–Fe–Fe angles (α) vary considerably from about 117.3 to 136.2° following a well-defined, smooth pattern around the ring. Ring closure considerations for a planar twelve-membered ring require $\alpha = 150^\circ$, while in edge-sharing octahedra the optimal angle is 120°, as observed in the Fe_6 species (av 119.8°).^[4a, b] The α angles around Fe1, Fe2, Fe7, and Fe8 are very close to this limit (av 118.4°), while the remaining α angles do not exceed 137°, with maxima at Fe4, Fe5, Fe10, and Fe11. Bis(μ -OR) units can thus easily support large cycles if the geometrical restraints of a planar ring are relaxed. The resulting molecular geometry is unprecedented and closely resembles a non-planar, twisted “ribbon”.

The dbm moieties follow the smooth oscillations of the dodecairon backbone and are positioned on all sides of the cluster to provide a hydrophobic shell which conveys a fairly large solubility in organic solvents to **1**. The ^1H NMR spectrum recorded at 200 MHz in CD_2Cl_2/CD_3OD (3/1) shows the paramagnetically shifted peaks of *m*-Ph and *p*-Ph protons of dbm at $\delta = 10.0$ and 6.9, respectively, in the

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appropriate ratio of 2:1 (Figure 2a). Addition of three equivalents of either NaBPh₄ or LiBPh₄ to the bright orange solution leads to major changes in the NMR spectrum. Although the signal from *p*-Ph protons is masked by the strong resonance of tetraphenylborate anion, the peak from the *m*-Ph protons is progressively replaced by resonances at

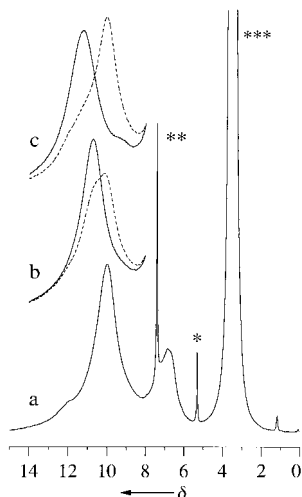
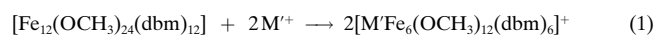


Figure 2. a) ¹H NMR spectrum of **1** recorded at 300 K in CD₂Cl₂/CD₃OD (3/1). Spectra recorded 10 min (---) and 360 min (—) after the addition of NaBPh₄ (b) or LiBPh₄ (c). Solvent peaks: * = CH₂Cl₂, ** = CHCl₃, *** = CH₃OD.

$\delta = 10.7$ and 11.3 upon the addition of the sodium and lithium salt, respectively. The spectra are time-dependent, which indicates that a slow equilibrium occurs (Figure 2b and c). The final spectra, which are observed after about six hours, are identical to those recorded for the NaCFe₆ and LiCFe₆ coronates.^[4a] Crystals of the two compounds were recovered by slow concentration of the solutions in the NMR tubes. We conclude that **1** can be quantitatively converted to hexa-iron(III) coronates by reaction with sodium or lithium salts in solution [Eq. (1)].



The origin of the slow equilibrium must be associated with the need to break the large rings into at least two fragments.

Although the largest known ring-shaped iron(III) compound is an octadecanuclear wheel described by Lippard et al.,^[9] compound **1** is the largest cyclic ferric cluster yet reported with chemically equivalent bridging units.^[10a,b] Therefore, it was of particular interest to investigate its magnetic properties in the solid state. The magnetic susceptibility measured in the range 2.3–254 K with an applied field of 1 T is indicative of antiferromagnetic interactions between the high-spin iron(III) ions ($S = 5/2$). The broad maximum at 155 K in the curve of χ_M as a function of T (Figure 3) can be nicely fit by a Heisenberg $S = 5/2$ quantum chain model with $J = 22.2 \text{ cm}^{-1}$ and $g = 2.00$ ($\mathbf{H} = J\sum_i \mathbf{S}_i \cdot \mathbf{S}_{i+1}$).^[11a] The departures from the calculated behavior at low temperature must arise from finite-size effects and the discreteness of the energy levels in **1**. For a ring of N ions, an energy gap $\Delta E \approx 4J/N = 7.4 \text{ cm}^{-1}$ is

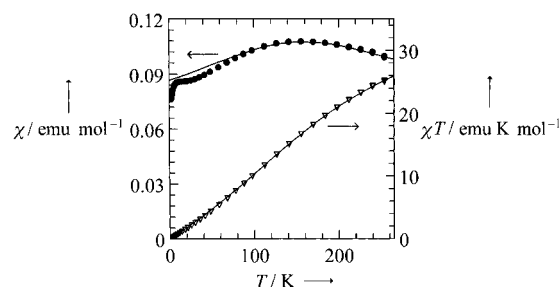


Figure 3. Molar magnetic susceptibility of a microcrystalline sample of **1** measured at 1 T as a function of temperature. The solid line represents the best-fit curve, as described in the text.

predicted between the ground $S = 0$ and the first excited $S = 1$ state.^[4b, 10a, 11b] χ_M should then drop to zero for $T \ll \Delta E$, while it extrapolates to a nonzero value in an infinite Heisenberg chain of half-integer spins.^[11a] The calculated J value is close to that found in dialkoxo-bridged iron(III) complexes with similar Fe–O–Fe angles, such as NaCFe₆ coronates ($\approx 20 \text{ cm}^{-1}$).^[4a,b, 12]

The present study shows that host–guest interactions and template effects may indeed be useful to address the synthesis of specific clusters and to determine the shape of the aggregate. However, in some cases, they may not be able to favor the largest possible clusters.

Experimental Section

The synthesis was performed under an argon atmosphere. Methanol was distilled over Mg(OCH₃)₂ shortly before use. Hdbm (Aldrich) and FeCl₃ (Carlo Erba) were used without further purification. Potassium methylate was used as a 25 % solution in methanol (Fluka), whereas cesium methylate was prepared by careful addition of Cs metal to anhydrous methanol. A solution of Hdbm (5 mmol) and potassium methylate (or cesium methylate) (20 mmol) in anhydrous methanol (60 mL) was added dropwise to iron(III) chloride (5 mmol) dissolved in anhydrous methanol (20 mL). After the addition was completed, the mixture was stirred for a further 30 min and the resulting yellow-orange precipitate was filtered off and dried with an argon flux. The dry precipitate was dissolved in CHCl₃/CH₃OH (1/1) and a small amount of insoluble material was filtered off. Slow diffusion of methanol vapors into the clear solution afforded yellow rodlike crystals in a few days. The crystalline material was separated by filtration, dried under vacuum and analyzed. Elemental analysis: calcd for [Fe(OCH₃)₂(dbm)]₁₂ · 0.67 CHCl₃, Fe₁₂C_{204.66}Cl₂H_{156.66}O₄₈ (%): C 58.88, H 4.95, Fe 16.09; found: C 58.88, H 4.83, Fe 16.01. Crystal structure data for **1**: C₂₁₀H₂₁₀Fe₁₂Cl₁₈O₄₈, $M_r = 4810.08$, crystal dimensions $0.2 \times 0.1 \times 0.1 \text{ mm}^3$, monoclinic, space group $P2_1/c$, $a = 30.530(5)$, $b = 22.780(5)$, $c = 32.050(5) \text{ \AA}$, $\beta = 93.400(5)^\circ$, $V = 22251(7) \text{ \AA}^3$, $Z = 4$, $\rho_{\text{calcd}} = 1.436 \text{ g cm}^{-3}$, $2\theta_{\text{max}} = 36^\circ$, MoK α radiation, $\lambda = 0.71069 \text{ \AA}$, $\omega - 2\theta$ scans, $T = 173(2) \text{ K}$. Of the 13875 reflections collected, 13343 were independent and were used in the structure refinement (Lorentz and polarization but no absorption correction applied). The structure was solved by direct methods (SIR-97)^[13a] and refined on F_o^2 by use of the SHELX-97 program package.^[13b] Disordered chloroform molecules were located in the lattice and refined with fractional occupancies to give a total of six CHCl₃ molecules per cluster. All Fe and most Cl atoms were refined anisotropically, while hydrogen atoms were added in idealized positions and treated isotropically. Restraints were applied to the anisotropic thermal parameters of Cl atoms and to the geometry of a few phenyl rings. The current values of R_1 and wR_2 are 0.0967 [$I > 2\sigma(I)$] and 0.3174 (all data) for 1370 parameters, which mainly reflect the bad X-ray quality of all the crystals examined. The maximum and minimum residual electron density is 1.135 and $-0.636 \text{ e \AA}^{-3}$. 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-110011. 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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Covalent Surface Functionalization and Self-Organization of Silica Nanoparticles**

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The large-scale manufacture of nanometer-scale particles with definite size and shape is a great challenge in materials chemistry.^[1] Perhaps even more exciting is the development of versatile methodologies that focus on the covalent functionalization of these particles,^[2] and which have an outstanding impact on fundamental research and on chemical and biochemical engineering: functionalized particles play an important role, for example, in pharmaceutical drug delivery systems,^[3] in the production of dispersion paints,^[4] in the optimization of catalysts,^[5,6] and in processes involving adhesives, varnishes, and lubricants.^[7] Silica particles might be particularly useful for applications where toxicity must be excluded. They can conveniently be prepared by the classical method of Stöber et al.^[8] Without surface modification these particles tend to coagulate and eventually transform, by polycondensation of their surface OH groups, into (nano-)porous glass or ceramics.^[9] Many applications, however, require strictly nonaggregated nanoparticles.

Most of the recent literature concerning surface functionalization is based on the weak adsorption of surfactants or polyelectrolytes.^[10] Up to now covalent coating of the hydrophilic SiO₂ surface has mostly been accomplished with silanes that contain lipophilic ligands. This leads to stable dispersions in several organic solvents;^[11,12] for instance, the surface modification of colloidal CdSe clusters by means of organoselenides has been reported.^[13] The covalent coating of nanostructured metals with thiol molecules is also well known.^[14] All these techniques prevent aggregation by appropriate surface modification. Philipse and Vrij have coated silica spheres with a dense layer of (3-methacryloxypropyltrimethoxysilane and these particles can be dispersed in several organic solvents.^[15] Hard sphere behavior was obtained by coating the surface with a monolayer of octadecyl alcohol.^[16] These coatings make the particles chemically inert, which prohibits agglomeration. Concomitantly, however, further functionalization is hardly possible.^[17]

Herein we present a covalent surface functionalization of silica nanoparticles that simultaneously excludes aggregation. Nearly monodisperse silica particles prepared by a modified Stöber synthesis are coated with a silane that contains two carboxylic acid groups. Particles with more than 10000 negative surface charges are generated upon dissociation in polar solvents. On the one hand these strictly nonagglomerated particles, as we demonstrate, exhibit novel colloidal

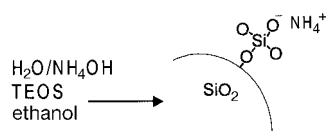
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properties that are a result of their stability, monodispersity, low refractive index, and their function as highly charged heterosupramolecular entities. On the other hand these particles can be considered, particular in view of the lack of toxicity, as the basis for biologically or pharmaceutically functionalized systems, for instance, for immunodiagnostics or drug delivery systems.^[18, 19]

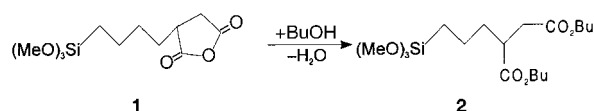
The starting materials of our synthesis are tetraethoxysilane (TEOS), 1-butanol, NaOH, NH₄OH, and, as a coating agent, (3-trimethoxysilyl)propylsuccinic anhydride (**1**; Scheme 1).



Scheme 1. Stöber synthesis of monodisperse silica spheres.

An alcosol is prepared in a mixture of 1200 mL ethanol and 60 mL ammonia (25%). TEOS (42 mL) is added, and the mixture is stirred at room temperature for one day. The resulting silica particles exhibit a diameter of 90 nm as determined by dynamic light scattering. The dispersion contains 7.7 g silica L⁻¹. Particle sizes of 21, 90, 286, and 440 nm are achieved with other TEOS concentrations. These particles, however, are only stable in the presence of a large surplus of NH₄OH, otherwise they irreversibly aggregate by polycondensation of their surface hydroxyl groups.

The coating agent **1** has to be chemically modified before use; the bifunctional methoxysilyl substituted succinic anhydride moiety would hydrolyze in the alcosol medium and the resulting carboxylic acid groups on the one end and the methoxysilyl groups on the other end of the molecules would react with the silica particles to form a macromolecular network. So we apply an esterification with butanol (Scheme 2). For that purpose 100 mL of the silane were

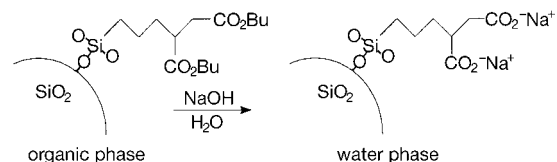


Scheme 2. Protection of the silane precursor.

mixed with 20 mL butanol in a second vessel and heated to 50 °C for two hours at reduced pressure (50 mbar) to remove the water from the reaction.

In the first vessel gaseous NH₃ is removed by evaporation and in this way the pH of the alcosol is reduced to pH = 7–8. The protected silane coupling agent **2** is then added. At this neutral pH the diester is stable and only the methoxy groups react with the OH surface groups of the silica particles in a condensation reaction with elimination of methanol. Methanol, ethanol, and water are distilled off at 50 °C and reduced pressure (20 mbar) after stirring the reaction mixture for 12 hours. Under these conditions butanol is not volatile, so the result is a milky dispersion of silica particles with lipophilic

surface groups in 1-butanol. After this procedure the mixture is placed in a separating funnel with an equal amount of water. The coated silica particles remain in the (milky) butanol phase, but on addition of solid NaOH and shaking, the esters are hydrolyzed to carboxylic acids by the increase of the pH value, that is, the protection caps are removed, and the now highly charged silica particles disperse in the aqueous phase (Scheme 3). The butanol phase then becomes transparent and



Scheme 3. Removal of the protection caps after coating of the silica spheres.

the water phase weakly turbid. Complete dispersion is a consequence of the mutual Coulombic repulsion of the nanoparticles (charge stabilization).

The phases are separated in the funnel, and the water phase containing the functionalized silica particles is dialyzed in a dialyzing tube against deionized water for three days to remove residual silane and NaOH. FT-IR spectra of the dried particles show bands at 1600 and 3450 cm⁻¹, which clearly prove the existence of carboxylic acid groups in the surface shell of the nanoparticles.

Particle sizes and size distributions of our products have been determined by dynamic light scattering using an ALV photon correlation spectrometer equipped with an ALV-5000E fast correlator. The results for our four samples are shown in Figure 1. The size distributions resulting from the

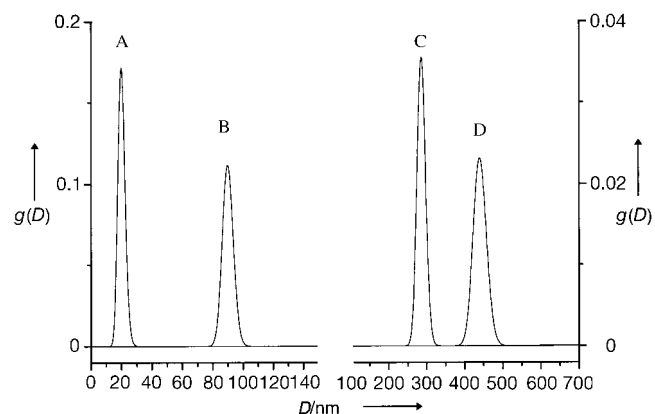


Figure 1. Particle size distribution of four selected batches of silica nanoparticles with average diameters of 21, 90, 286, and 440 nm.

measured autocorrelation functions are analyzed in terms of the log-normal distribution function [Eq. (1)],^[20] where the parameters μ and σ describe the median and the

$$g(D) = \frac{1}{\sqrt{2\pi D \ln \sigma}} \exp\left(-\frac{(\ln D - \ln \mu)^2}{2(\ln \sigma)^2}\right) \quad (1)$$

geometric standard deviation of the distribution, respectively; these quantities are listed in Table 1. In addition, we have

Table 1. Parameters of the size distribution of the silica nanoparticles (results obtained from the dynamic light scattering experiments).

Sample	μ [nm]	σ
sample A	21	1.10
sample B	90	1.03
sample C	286	1.04
sample D	440	1.04

looked at our particles by means of a transmission electron microscope (JEOL 200CX). Two typical micrographs are shown in Figure 2 and confirm the absence of aggregates and the monodispersity of our products.

The surface charges were characterized by measuring the electrophoretic mobilities with a Zetasizer III (Malvern Instruments) at pH 7 and a temperature of 25 °C. From the electrophoretic mobility the zeta potential is calculated. The resulting reduced inverse Debye–Hückel screening length, taken together with the particle radius, allows the determination of the effective, electrophoretic surface charge z_{eff} . We obtain $z_{\text{eff}} = 28530$ for the particles with $\mu = 286$ nm and $z_{\text{eff}} = 10230$ for those with $\mu = 90$ nm. These values even exceed those of highly charged polymer based colloids.^[21]

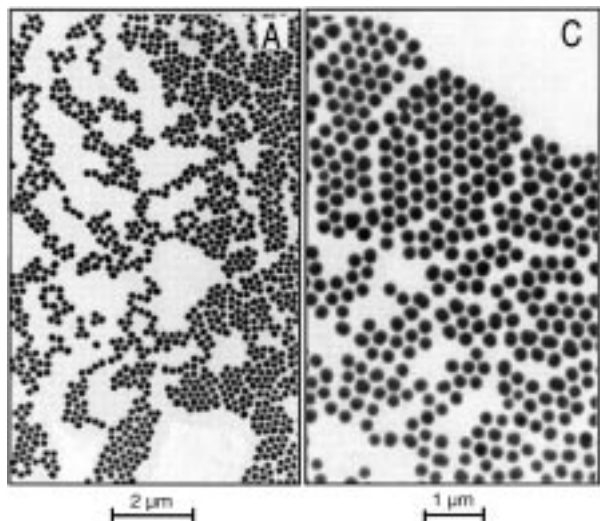


Figure 2. Transmission electron micrographs of samples A and C of Figure 1.

By means of a mixed-bed ion exchanger (Dowex 50W, Fluka) we completely removed the excess ions and thus reduced the ionic strength such that the conductivity reaches values as low as 13 μS . Under these conditions the Coulomb potential is hardly shielded anymore, and the repulsive interaction of the functionalized particles becomes long ranged. This leads to the formation of colloidal supercrystals in a polycrystalline form. The long-range ordering in the respective supercrystallites of sample B (with $\mu = 90$ nm) is evident from the opalescence phenomena, as demonstrated in Figure 3, which originate from Laue-backscattering of white visible light. Thereby the human eye acts as an energy-dispersive detector. From the well-known Bragg equation $n\lambda = 2d\sin\theta$ under backscattering conditions ($\sin\theta = 1$), with the index of refraction of water $n = 1.33$, and with the wave

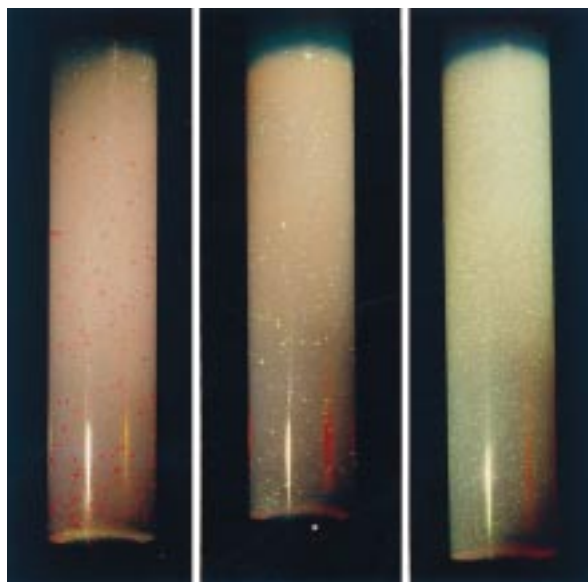


Figure 3. Colloidal supercrystals (in polycrystalline form) of 90 nm diameter silica nanoparticles with covalent surface functionalization; the red, yellow, and green speckles arise from Laue-backscattering of white visible light on suspensions of the same particles at different concentrations.

lengths $\lambda_{\text{red}} = 700$ nm, $\lambda_{\text{yellow}} = 590$, and $\lambda_{\text{green}} = 520$ nm, we estimate the following interplanar distances:

left tube, red reflections: 480 nm
middle tube, yellow reflections: 390 nm
right tube, green reflections: 330 nm.

For demonstration purposes the volume fractions had been chosen such that interference effects occur just in the visible range of electromagnetic radiation; the reciprocal third roots of the particle number densities yield the interparticle distances: left tube 550 nm, middle tube 403 nm, right tube 325 nm, which are in satisfactory agreement with the above estimation done just by eye.

In summary, we have prepared highly charged inorganic/organic colloidal core/shell particles. They are, for all practical purposes, monodisperse and perfectly dispersed. On the one hand these coated particles can be considered as a basis for a variety of applications since by an appropriate chemical reaction with their surface carboxylic acid groups further more complex functionalization can easily be accomplished. On the other hand, optical matching is easily possible as a consequence of the particles' low refractive index. This enables fundamental light-scattering studies of different phenomena, for instance of order–disorder phase transitions or hydrodynamic interactions at large concentrations without the problem of multiple scattering; investigations of this kind are in progress now in our laboratory.^[22]

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Real-Time Characterization of Ribozymes by Fluorescence Resonance Energy Transfer (FRET)**

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Ribozymes are increasingly applied to the inhibition of gene expression at the level of protein-encoding mRNAs.^[1, 2] The possibility of transferring ribozymes endo- or exogenous-

ly into living cells opens up a broad potential for application in gene therapy,^[1, 3-5] functional genomics,^[6] and biotechnology.^[7] The ribozyme most commonly used for these purposes is the hammerhead ribozyme (HHR), a small catalytic RNA that is able to cleave other RNA molecules in an intermolecular fashion.^[8] The specificity of this cleavage process is determined by substrate binding sites that are variable in sequence and length. As a result, the cleavage activity can be directed against almost any mRNA sequence. Despite their potential for universal application there are a number of factors that have to be taken into account when developing therapeutic ribozymes, for example, the accessibility of ribozyme binding sites on the target RNA in vivo,^[9] the selectivity of substrate recognition,^[1] or the cleavage efficiency in an intracellular compartment.^[10, 11] Although computer-assisted predictions can be made to address some of these points^[12] they still have to be verified experimentally by characterizing the respective HHR-constructs under various conditions. Therefore, there is currently a high demand for technologies that allow a high-throughput screening of ribozymes for a rapid qualitative evaluation of their kinetic parameters, especially as current conventional methods hardly provide sufficient solutions to this problem.

Here we report a novel approach based on FRET-oligonucleotides^[13] (FRET = fluorescence resonance energy transfer^[14]), which allows the real-time kinetic analysis of ribozymes within hours. The FRET-principle has previously been applied to various problems such as determination of phosphodiesterase activities,^[15] structural elucidations of RNA molecules,^[16, 17] and for quantification of PCR reactions.^[18] Intermolecular FRET-measurements with single-labeled substrates and hammerhead ribozymes have also been applied to measure the association and dissociation of substrate and product.^[19, 20]

In our FRET-substrate the fluorescence of a fluorophore (for example, 6-carboxyfluorescein (FAM)) is intramolecularly quenched because of the close spatial proximity of a fluorescence-quenching molecule (for example, 6-carboxy-*tert*-methylrhodamine (TAMRA); Figure 1a, b). Upon cleavage of such substrates by the ribozyme a fluorescence-signal is generated which can be quantified by an appropriate read-out system in real time (Figure 1c). As the increase in fluorescence is directly correlated with the rate of cleavage, this system is well suited for the sensitive, nonradioactive, rapid analysis of ribozyme activities.

Based on a published HHR/substrate complex (Figure 1a) we have constructed a FRET substrate (SL1), which was used for real-time determination of the activity of the ribozyme HHR1. An inactive HHR-mutant (HHR1_{mut}) with substrate binding sites identical to those in HHR1 was used as a reference and negative control. The Michaelis–Menten parameters of HHR1 could be obtained in a single experimental setup by measuring the increase in fluorescence as a result of cleavage at different substrate concentrations. The result of this experiment is shown in Figure 2b in the form of an Eadie–Hofstee plot for the determination of the k_{cat}/K_M values. To investigate whether the FAM/TAMRA label affects the cleavage efficiency we determined the kinetic parameters by conventional methods^[22] with a ³²P-labeled

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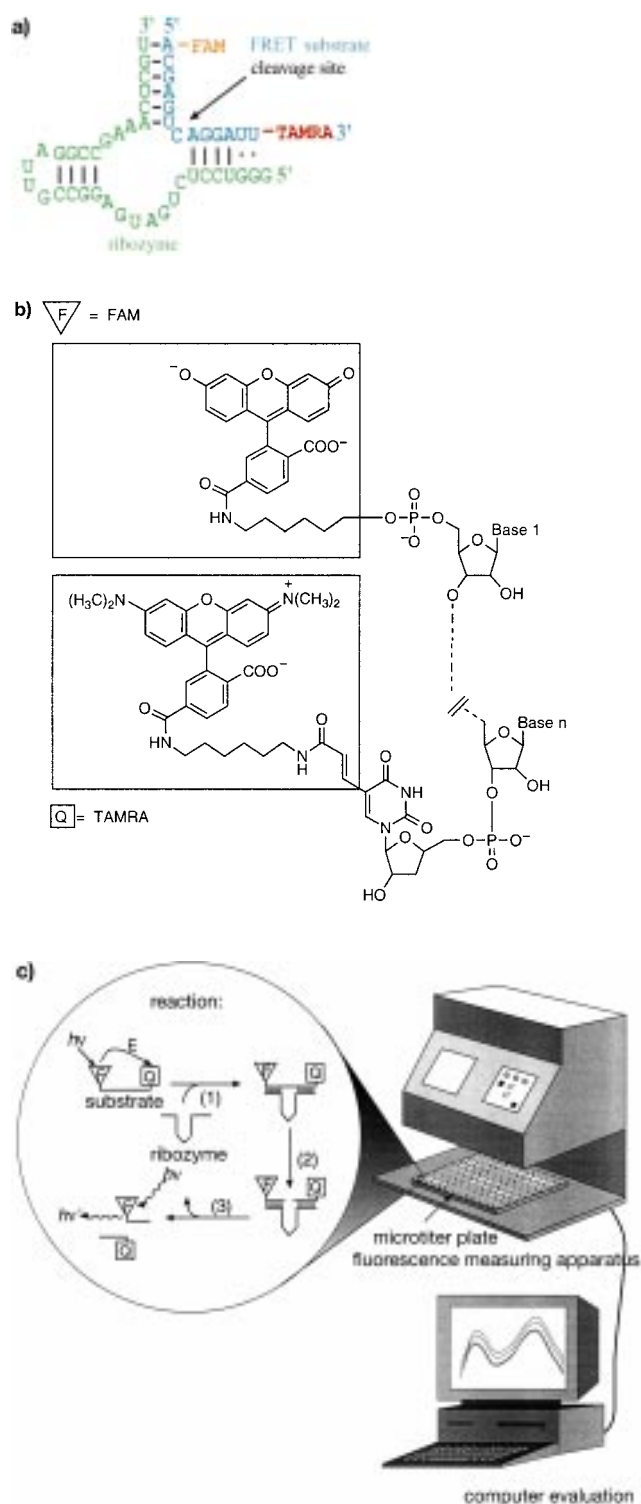


Figure 1. Schematic representation of the assay and automated analysis. a) Secondary structure of ribozyme and substrate. b) The fluorophore (FAM) and quencher molecule (TAMARA) c) Up to 96 different reactions can be monitored on a microtiter dish in real time and the data analyzed with an appropriate read-out system. The reaction is shown schematically on the left. 1) Binding of the doubly labeled RNA substrate (e.g. 5'-[F] and 3'-TAMRA [Q]-labeled RNA) to the ribozyme results in the formation of the catalytic complex. The fluorescence of the donor ($\lambda = 535$ nm) is intramolecularly quenched in the uncleaved substrate. 2) Cleavage of the doubly labelled substrate. 3) Dissociation of the reaction products. The ribozyme is now available for the next turnover. Neutralization of the quench-effect results in the increase of a fluorescence signal $h\nu'$ as a function of the cleavage of the substrate by the ribozyme.

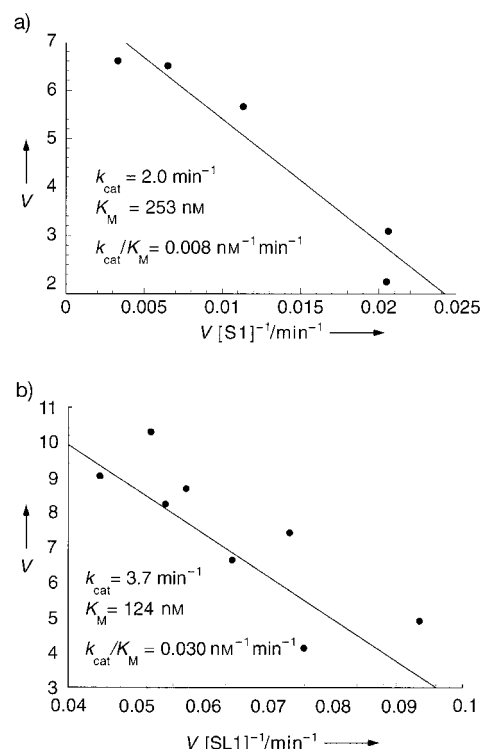


Figure 2. Determination of the kinetic parameters of HHR1 ($c = 4 \text{ nM}$) at 37°C . Eadie-Hofstee plot and kinetic parameters of cleavage of 5'- ^{32}P -labeled substrate, which carries no FRET labels S1, by HHR1 a) and b) of the FRET-substrate (SL1) by HHR1.

substrate (S1) without the FRET labels (Figure 2a). The conventional method involves separation of the cleavage products by electrophoresis on polyacrylamide gel with subsequent quantification of the bands by autoradiography.^[22] Figure 2 shows the data for both substrates (S1 and SL1). Both the k_{cat} and the K_M values are of the same order of magnitude, which indicates that the FRET substrate is very well suited for our objective.

To test whether the method can also be applied to the rapid characterization of ribozymes we measured cleavage activities in parallel experiments in which various physicochemical parameters were altered. Figure 3a shows the time-course of the cleavage reaction of an experiment under standard conditions. In Figures 3b–d the reaction rate is shown as a function of pH, temperature, and Mg^{2+} concentration. Under the conditions applied here, we obtained an optimum pH value of 8 and an optimal Mg^{2+} concentration of 8 mM. The optimum temperature of 30°C is rather low and probably reflects the relatively weak binding of the substrate to the ribozyme. This might be a consequence of the short hybridizing arms and the low Mg^{2+} concentration (4.0 mM).^[23] Finally, we determined the effect of the aminoglycoside neomycin B, a HHR inhibitor, on substrate cleavage (data not shown). The cleavage activity dropped to a third of the noninhibited reaction rate in the presence of $100 \mu\text{M}$ neomycin, which is in exact accordance with the data obtained by conventional methods in a previous study.^[24]

The data points were corrected with the ribozyme-independent negative-control values obtained with the inactive

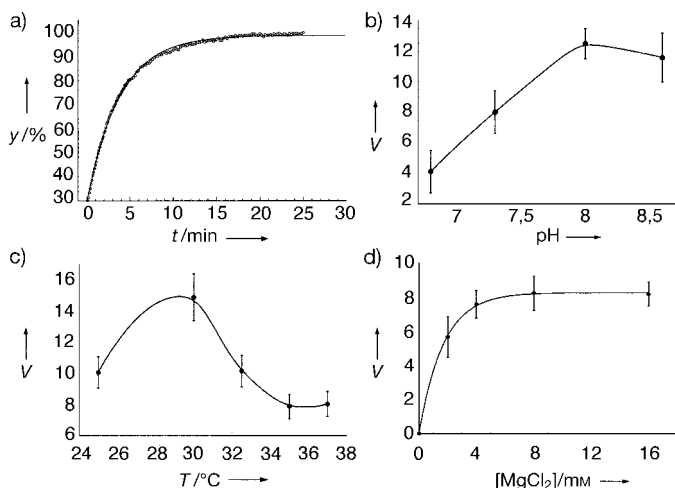


Figure 3. Characterization of the cleavage of 200 nm and 300 nm SL1 with 4 nm HHR1. a) Time course of cleavage with the reciprocal of the exponential regression curve (only 1 % of all data points are shown). y = amount of cleavage. b–d) Dependence of the reaction rate V of HHR1 on b) the pH value, c) temperature, d) the concentration of Mg^{2+} . The values of k_{cat}/K_M at 25 °C (0.0134 nm⁻¹ min⁻¹) and 30 °C (0.138 nm⁻¹ min⁻¹) were also determined.

mutant HHR1_{mut} to eliminate possible intrinsic instrumental inaccuracies or unspecific fluorescence effects (for example, fluctuation of the signal as a result of deviations in temperature). In addition to that, this method of adjustment enabled the determination of the activity of ribozymes generated in situ by T7 RNA polymerase dependent transcription of ribozyme-encoding DNA-templates in the presence of HeLa-nuclear extracts (Figure 4). As a control we carried out

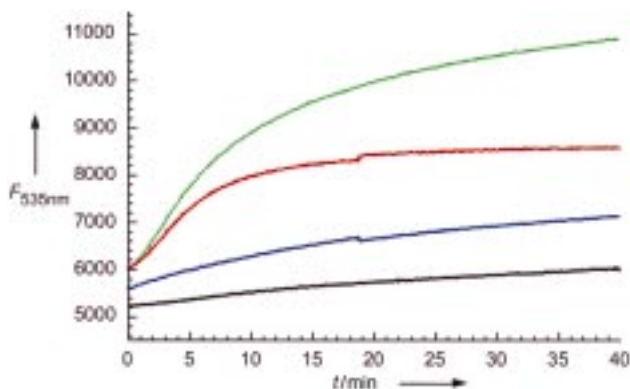


Figure 4. Real-time monitoring of the increase of fluorescence as a result of the activity of HHR1 generated in situ in the presence of nuclear cell extract. HHR1_{mut}-DNA + T7 RNA polymerase (black), HHR1-DNA without T7-RNA polymerase (blue), HHR1-DNA + T7-RNA polymerase (green). The red curve refers to the same measurement but corrected for the unspecific background signal (see experimental section).

parallel experiments in the absence of T7 RNA polymerase (blue) and with the inactive HHR-mutant (black). Both curves show a slight increase in the fluorescence signal, which might result from the unspecific degradation of the FRET substrate by RNases in the nuclear extract. The green curve shows the absolute fluorescence that was obtained with HHR1 generated in situ. The same data, corrected for the

superimposed background reaction (see experimental section), are represented by the red curve. As expected, the corrected curve reveals a sigmoidal shape that reflects the continuous increase of ribozyme concentration during transcription. These results show that it should in principle be possible to use the ribozyme as a reporter for measuring transcription rates in vitro and in vivo. Such a reporter system could be part of a high-throughput screening assay to search combinatorial compound libraries for inhibitors of transcription factors since it can be used fully automated without the need of additional pipetting steps.

In conclusion, we have shown that doubly labeled FRET substrates allow the characterization of ribozymes under various conditions in only a fraction of the time estimated for conventional methods currently available. In particular the very simple automatization and real-time analysis of many parallel reactions and the avoidance of radioactive labeling perfectly suits our system for the high-throughput screening of ribozyme activities and transcription rates.

Experimental Section

HHR1: the sense-DNA-strand of the ribozyme with the 5'-terminal T7-promotor sequence was synthesized on an Expedite oligonucleotide synthesizer (Millipore, USA): 5'-TCTAATACGACTCACTATAGGG TCC TCT GAT GAG GCC GTTAGG CCG AAA CTC GT-3' (HHR1-DNA; the primer binding sites are shown in *italics*). The synthesis of the antisense strand as well as the amplification of the double-stranded template was done by polymerase chain reaction (PCR) with primers 5'-TCTAATACGACTCACTATA-3' (5'-primer) and 3'-GGCAATCCGGCTTTGAGCA-5' (3'-primer). A standard 100 μ L PCR reaction contained 10 mM Tris-HCl, pH 8.9, 100 mM KCl, 1.5 mM MgCl₂, 50 mg mL⁻¹ BSA, 0.05 % Tween20 (v/v), 200 μ M dNTP, 2 μ M 5'- and 3'-primer, approximately 200 nm single-stranded DNA-template, and 2.5 Uth DNA polymerase. Amplification was performed according to the manufacturers protocol (Roche Mannheim) in four to five PCR cycles (95 °C, 55 s; 55 °C, 1 min; 72 °C, 1 min). Amplified DNA was isolated by standard methods^[25] and excess primers were removed by agarose gel electrophoresis.

The inactive ribozyme mutant HHR1mut was generated accordingly using the synthetic DNA-template 5'-TCT AAT ACG ACT CAC TAT A GGG TCC TCT TAG GAG GCC GTTAGG CCA GAA CTC GT-3' (HHR1mut; primer binding sites are shown in *italics*, four mutations are shown in **bold**) and the primers 5'-TCT AAT ACG ACT CAC TAT A-3' (5'-primer), 3'-GGCAA TCC GGT CTT GAG CA-5' (3'-primer). The 5'-FAM- and 3'-TAMRA-labeled substrate RNA was synthesized and purified by HPLC and gel electrophoresis at Eurogentec (Belgium). Conclusive experiments were found to strongly depend on the purity of the FRET substrate. The ratio of fluorophore and quencher dye, which is a measure of the purity of the sample, was calculated as a function of fluorescence as described.^[13, 26]

A standard 50 μ L reaction for the generation of ribozyme by in vitro transcription contained 40 mM Tris-HCl, pH 8.0, 50 mM NaCl, 2 mM spermidine, 5 mM dithiothreitol, 8 mM MgCl₂, 0.2–2 μ M HHR1-DNA or HHR1mut-DNA, respectively, 4 mM A/C/G/UTP, 40 U RNasin (Promega, Madison, WI), and 50 U T7 RNA polymerase (Stratagene, Heidelberg). The RNA was purified on a 16 % denaturing polyacrylamide gel (37.5:1). The sample also contained SL1 and 10 U HeLa-nuclear extract (HeLa Cell Extract Transcription System, Promega, Madison, WI) for measuring ribozyme activities of HHR1 generated in situ. In vitro transcription was started by the addition of polymerase to the nuclear extract and incubated at 37 °C during the course of one measurement.

Real-time measurements of the ribozyme dependent increase of fluorescence were done in 50 μ L volumes. The signal obtained reflects the chemical-cleavage step in addition to the dissociation of the cleavage products. Fluorescence quantifications in the transcription-controlled reactions were done in an ABI Prism 770 spectrometer (Applied Bio-

systems, USA). The emission spectra of the two dyes were recorded at intervals of 0.5–0.7 s (FAM: $\lambda_{\text{max}} = 535$ nm, TAMRA: $\lambda_{\text{max}} = 582$ nm, excitation wavelength: 488 nm). All experiments were done in triplicate. By plotting the relative fluorescence of the fluorophore (ΔR_n value) at 535 nm against time, the proportion of cleaved RNA substrate versus negative control (identical experiment with inactive HHR1mut-DNA instead of HHR1-DNA) was determined. The raw data were imported into Microsoft Excel, computed, and further processed.

To eliminate unspecific fluorescence effects it had to be taken into account that the absolute, but not the percental, deviation in fluorescence increases with the increase in fluorescence during the catalytic cleavage. The time course of the cleavage was corrected by the percental deviation of the curve obtained for the negative control experiments from the value at $t=0$ according to Equation (1) ($F_{\text{corr}}(t)$: corrected fluorescence at a given time point t ; $F_{\text{neg}}(t)$: fluorescence of the negative control at a given time point t ; $F_{\text{neg}}(t_0)$: fluorescence of the negative control at $t=0$; $F_{\text{HHR}}(t)$: increase in fluorescence in the presence of transcribed HHR at a given time point t).

$$F_{\text{corr}}(t) = \frac{F_{\text{neg}}(t)}{F_{\text{neg}}(t_0)} F_{\text{HHR}}(t) \quad (1)$$

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Exploiting Incommensurate Symmetry Numbers: Rational Design and Assembly of $\text{M}_2\text{M}'\text{L}_6$ Supramolecular Clusters with C_{3h} Symmetry**

Xiankai Sun, Darren W. Johnson, Dana L. Caulder, Ryan E. Powers, Kenneth N. Raymond,* and Edward H. Wong*

There are many elegant oligomeric or polymeric structures based on metal–ligand interactions that lead to well-organized supramolecular architectures. Examples include extended arrays^[1–7] in two or three dimensions and discrete clusters of various types.^[8–30] However, relatively few such clusters have been the result of predictive design. We have developed a rational design for the synthesis of high-symmetry clusters using coordination number incommensurate interactions. Resultant examples include M_2L_3 helicates^[15, 24, 31, 32] and their meso-counterparts (mesocates),^[32] and M_4L_6 ^[12, 33–35] and M_4L_4 ^[36] tetrahedra. In these clusters, three bidentate chelators coordinate a tri- or tetravalent metal ion in a pseudo-octahedral fashion to generate a threefold axis, and a symmetric, multi(bidentate) ligand generates the other symmetry element (twofold,^[15, 24, 31, 32] threefold,^[36] or mirror plane^[32]).

Here we present the rational design of a mixed-metal cluster in which, rather than deriving one symmetry element

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from a symmetric ligand, two different metals are used to generate the two incommensurate symmetry elements (Figure 1). This forms the basis for coordination number incommensurate cluster formation.^[37] In principle, the ligand forms part of an asymmetric unit of the cluster and must have two different interaction sites (e.g., one hard and one soft donor) that can each preferentially interact with one of the metal ions over the other.

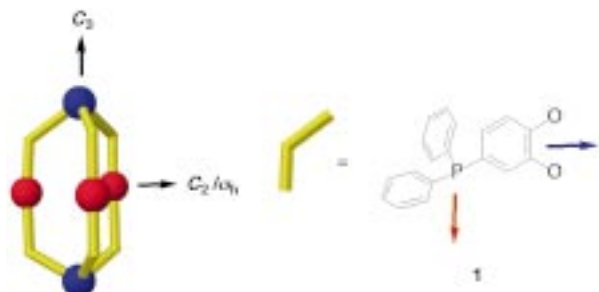


Figure 1. Cartoon showing the 90° offset, incommensurate interaction of a threefold symmetry element and a C_2 axis/mirror plane to generate a $M_2M_3L_6$ cluster. Blue spheres represent trivalent or tetravalent metals coordinated to the catechol functionality of **1**, red spheres are $PdBr_2$ fragments coordinated to the phosphane of **1**.

A chiral triple-helicate has idealized D_3 symmetry, while an achiral triple-mesocate has C_{3h} symmetry. To synthesize a mixed-metal helicate (or mesocate) of stoichiometry $M_2M_3L_6$, a threefold interaction site and an orthogonal twofold (mirror plane) interaction site must be generated (Figure 1).^[38]

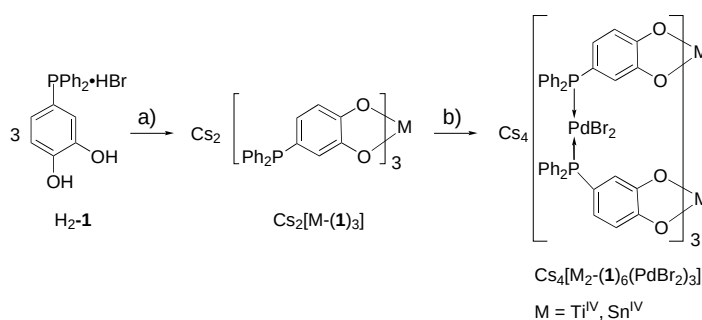
Catechol ligands are relatively hard donors and generate a C_3 axis when forming a tris-chelate with hard, tri- or tetravalent metals (e.g., Al^{III} , Ga^{III} , Fe^{III} , Sn^{IV} , Ti^{IV}).^[39, 40] Phosphane ligands, on the other hand, are soft donors and can generate a twofold axis or mirror plane when coordinated in a *trans* fashion to metals with square-planar geometry (e.g., Pd^{II} or Pt^{II}).^[41, 42] A hybrid ligand containing both these functionalities arranged in the proper geometry can, if properly designed, generate an $M_2M_3L_6$ cluster, because it is the smallest discrete species that would simultaneously fulfill the two 90° offset, incommensurate symmetry requirements.

While the phosphanyl and quinonic functional groups would be mutually incompatible,^[43–45] several reports of 2-phosphanyl-substituted *p*-dihydroquinones have appeared in the literature.^[46–48] These were designed to optimize metal chelation by the phosphane/phenolate donor set. For our purposes, a 4-phosphanylcatechol ligand was suggested by molecular modeling^[49] as having the ideal geometry for isolating the two types of coordination sites and permitting cluster assembly. To determine the type of cluster (mesocate or helicate) that would form, molecular modeling^[49] studies were performed on $Cs_4[Ti_2-(1)_6(PdBr_2)_3]$. The results indicated that the mesocate has a larger $Ti \cdots Ti$ separation (6.5 Å compared to 6.1 Å for the helicate) and a substantially lower energy by 26 kcal mol^{−1}.

The readily-prepared hybrid ligand 4- PPh_2 -catechol (H_2-1)^[50] was employed to synthesize the tris-(4- PPh_2 -catecholate) complexes $[M-(1)_3]^{2-}$ ($M = Ti, Sn$).^[51] In each case, a single set of resonances was observed in the ^{31}P , ^1H , and ^{13}C NMR

spectra even when the sample was cooled to $-60^\circ C$. This is consistent with the selective formation in solution^[52] of only the *fac* isomer with C_3 symmetry rather than the C_1 -symmetric, *mer* isomer, which should be statistically favored by a 3:1 ratio.

Reaction of two equivalents of $Cs_2[Ti-(1)_3]$ and three of $PdBr_2 \cdot 2PhCN$ in DMF gives an orange solution from which an orange-red solid can be isolated in good yield (95 %, Scheme 1). Solution ^{31}P , ^1H and ^{13}C NMR spectra reveal the formation of a single product with high symmetry.



Scheme 1. Synthesis of the C_{3h} -symmetric, mixed-metal mesocates. a) Cs_2CO_3 , $[Ti(OMe)_4]$ or $SnCl_4$ /methanol; b) 1.5 equiv $PdBr_2 \cdot 2PhCN$ /DMF.

Further, the observation of virtual $^{13}C\{^1H\}$ triplet resonances for both catecholate and phenyl ring carbon atoms is consistent with a *trans*-phosphane coordination of a Pd^{II} center. This *trans* coordination also results in strong $^2J(P,Pd,P)$ couplings.^[53] The FAB^+ mass spectrum indicates formation of a $[Ti_2-(1)_6Pd_3]^{2+}$ ion associated with varying numbers of cesium cations and bromine anions.

A single-crystal X-ray diffraction study confirms the structure of this heterometallic assembly.^[54] The cluster has crystallographically imposed C_{3h} symmetry (Figure 2). The



Figure 2. A stereographic representation (side view) of $[Ti_2-(1)_6-(PdBr_2)_3]^{4-}$. The Cs counterions, hydrogen atoms, and solvent molecules are omitted for clarity. The pink spheres represent Pd atoms, brown spheres are Br atoms, and orange spheres are Ti atoms. The wireframe represents the ligand with carbon atoms shown in gray, phosphorus atoms in yellow, and oxygen atoms in red.

cluster, therefore, is an achiral mesocate with one titanium atom having Δ and the other having Λ configuration. The $Ti \cdots Ti$ separation is 6.76 Å. The three Pd centers have distorted square-planar geometries and *trans*-coordinated phosphane ligands. Of particular interest is the location of the Cs^+ ions: three of them are embedded in the three clefts of the cluster and are coordinated by four *endo*-catecholate oxygen atoms in a rectangular array and two *exo*-molecules of THF solvent (Figure 3). The depth of these clefts is such that the THF

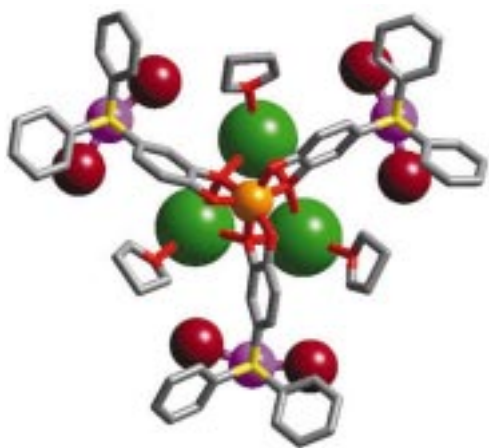


Figure 3. A view of $\text{Cs}_4[\text{Ti}_2-(\mathbf{1})_6(\text{PdBr}_2)_3]$ down the crystallographic three-fold axis of the cluster. One disordered Cs atom and all disordered solvent molecules and hydrogen atoms were omitted for clarity. The color scheme is the same as that in Figure 2, with Cs atoms represented by green spheres.

molecules can also be described as being buried. The low coordination number (6) of the cesium cations can be explained by the position of the peripheral bromine atoms: while not in van der Waals contact with the cesium cations, the Cs atoms are shielded from additional coordination by the Br atoms (Figure 3).

The heterometallic cluster can also be prepared as the Sn^{IV} analogue. Reaction of two equivalents of $\text{Cs}_2[\text{Sn}-(\mathbf{1})_3]$ and three of $\text{PdBr}_2 \cdot 2\text{PhCN}$ in DMF gives a single red product, $\text{Cs}_4[\text{Sn}_2-(\mathbf{1})_6(\text{PdBr}_2)_3]$. The crystal structure of this compound has also been determined (Figure 4).^[55] Although not crystal-



Figure 4. Stereographic representation (side view) of $[\text{Sn}_2-(\mathbf{1})_6(\text{PdBr}_2)_3]^{4-}$. The Cs counterions, hydrogen atoms, and solvent molecules are omitted for clarity. The pink spheres represent Pd atoms, brown spheres are Br atoms, and blue spheres are Sn atoms. The wireframe represents the ligand with carbon atoms shown in gray, phosphorus atoms in yellow, and oxygen atoms in red.

lographically imposed, the molecule has idealized C_{3h} symmetry similar to the Ti^{IV} complex described above. Three of the cesium cations are also located in the cluster clefts, but one of the bromine atoms from each of the three palladium centers is deflected in towards the cluster cavity and coordinates a cesium atom (Figure 5). The second bromine atom on each of the three palladium centers is deflected out from the cluster interior. The $\text{Sn} \cdots \text{Sn}$ separation is 6.88 Å, 0.12 Å larger than that for the Ti structure (vide supra). This increased separation, due to the larger ionic radius of Sn^{IV} compared to Ti^{IV} (0.830 Å and 0.745 Å, respectively),^[56] may allow the observed $\text{Cs} \cdots \text{Br}$ interactions to occur within the clefts of this cluster but not in the Ti analogue.

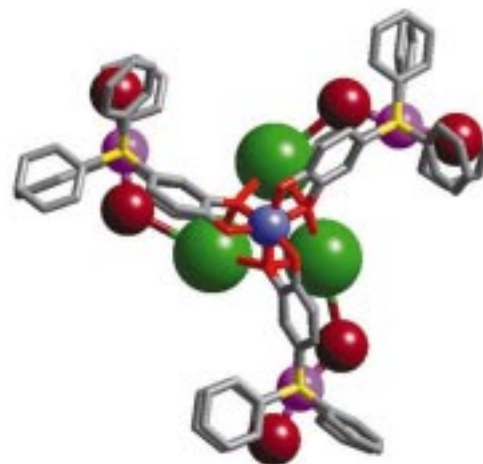


Figure 5. A view of $\text{Cs}_4[\text{Sn}_2-(\mathbf{1})_6(\text{PdBr}_2)_3]$ down the pseudo threefold axis of the cluster. All disordered solvent molecules, all hydrogen atoms, and one of the disordered Cs atoms are omitted for clarity. The color scheme is the same as that in Figure 4, with Cs atoms represented by green spheres.

Computer modeling studies of both clusters have demonstrated that the $\text{M}^{\text{IV}} \cdots \text{M}^{\text{IV}}$ distances are shorter in the helicate than in the mesocate (vide supra). In addition, only three *endo*-catecholate oxygen atoms are available for Cs^+ coordination at each of the three molecular clefts of the helicate compared to four for the mesocate. Thus, the mesocate provides a better coordination environment for the large Cs counterion. Therefore, the use of a smaller alkali metal cation may induce helix formation. There is already some indication that there is indeed a cation effect in cluster formation: the use of triethylammonium as the cation in the synthesis of the Ti cluster leads to the presence of two different species which can be converted back to the C_{3h} mesocate upon addition of CsOTf ($\text{Tf} = \text{F}_3\text{CSO}_2$). These cation effects are currently being investigated.

Our successful realization of this example of symmetry driven cluster formation augurs well for the future use of hybrid ligands and disparate metal coordination preferences in the generation of a variety of novel, mixed-metallic, supramolecular assemblies.

Experimental Section

All NMR spectra were measured with a Bruker 360 MHz or a JEOL FX-90Q spectrometer. Chemical shifts of ^1H and ^{13}C spectra are reported as δ downfield from TMS. The ligand $\text{H}_2-\mathbf{1} \cdot \text{HBr}$ has been fully characterized by NMR spectroscopy and elemental analysis. Reactions were performed under an atmosphere of purified nitrogen using standard Schlenk techniques.

$\text{Cs}_4[\text{Ti}_2-(\mathbf{1})_6(\text{PdBr}_2)_3]$: A solution of $\text{Cs}_2[\text{Ti}-(\mathbf{1})_3]$ (0.42 g, 0.35 mmol) and $\text{PdBr}_2 \cdot 2\text{PhCN}$ (0.25 g, 0.53 mmol) in DMF (20 mL) was stirred at room temperature for 2 h. Initial turbidity readily disappeared to give a clear, orange solution. Addition of THF precipitated an orange-yellow solid which was isolated in 95 % yield. The solid was redissolved in DMF/THF (1:10) and slow diffusion of diethyl ether into this solution gave well-formed, orange-red crystals suitable for single-crystal X-ray diffraction studies. Elemental analysis: calcd for $\text{C}_{108}\text{H}_{78}\text{Br}_6\text{Cs}_4\text{O}_{12}\text{Pd}_3\text{Ti}_2 \cdot 2.5\text{DMF} \cdot 3.5\text{H}_2\text{O}$ (%): C 40.55, H 3.05, N 1.12; found: C 40.92, H 3.00, N 0.76; ^1H NMR (360 MHz, $[\text{D}_7]\text{DMF}$, TMS): δ = 7.89 (t, J = 6.4 Hz, 1H, catechol), 7.63 (m, br d, 4H, phenyl), 7.37 (m, br d, 6H, phenyl), 6.40 (br d, 1H, catechol), 6.25 (d, J = 7.9 Hz, 1H, catechol); $^{13}\text{C}\{^1\text{H}\}$ NMR (89.9 MHz, $[\text{D}_7]\text{DMF}$, TMS): δ = 163.8 (s, catechol), 159.8 (t, $J(\text{PC})$ = 10.3 Hz, catechol), 134.7 (t, $J(\text{PC})$ = 5.6 Hz, phenyl), 134.4 (t, $J(\text{PC})$ = 20.2 Hz, phenyl),

129.7 (s, phenyl), 127.6 (t, $J(\text{P,C}) = 5.0$ Hz, phenyl), 124.9 (s, catechol), 121.9 (t, $J(\text{P,C}) = 13.9$ Hz, catechol), 115.7 (t, $J(\text{P,C}) = 28.2$ Hz, catechol), 111.0 (t, $J(\text{P,C}) = 6.0$ Hz, catechol); $^{31}\text{P}\{^1\text{H}\}$ NMR (36.3 MHz, $[\text{D}_7]\text{DMF}$, 85% H_3PO_4): $\delta = 22.11(\text{s})$; positive ion FAB-MS: m/z (nitrobenzyl alcohol, $\text{C}_6\text{H}_5\text{NO}_2$): m/z (m/z_{calcd}): 3311 (3312.6) [$\text{C}_6\text{H}_5\text{NO}_2 + 6\text{Br}^- + 5\text{Cs}^+$], 3179 (3180.7) [$\text{C}_6\text{H}_5\text{NO}_2 + 6\text{Br}^- + 4\text{Cs}^+ + 1\text{H}^+$], 3101 (3099.8) [$\text{C}_6\text{H}_5\text{NO}_2 + 5\text{Br}^- + 4\text{Cs}^+$], 3049 (3048.8) [$\text{C}_6\text{H}_5\text{NO}_2 + 6\text{Br}^- + 3\text{Cs}^+ + 2\text{H}^+$], 2968 (2967.9) [$\text{C}_6\text{H}_5\text{NO}_2 + 5\text{Br}^- + 3\text{Cs}^+ + 1\text{H}^+$], 2886 (2887.0) [$\text{C}_6\text{H}_5\text{NO}_2 + 4\text{Br}^- + 3\text{Cs}^+$], 2835 (2836.0) [$\text{C}_6\text{H}_5\text{NO}_2 + 5\text{Br}^- + 2\text{Cs}^+ + 2\text{H}^+$], 2807 (2807.0) [$\text{C}_6\text{H}_5\text{NO}_2 + 6\text{Br}^- + 1\text{Cs}^+ + 3\text{H}^+ + 1\text{Na}^+$], 2755 (2755.1) [$\text{C}_6\text{H}_5\text{NO}_2 + 4\text{Br}^- + 2\text{Cs}^+ + 1\text{H}^+$], 2675 (2675.1) [$\text{C}_6\text{H}_5\text{NO}_2 + 6\text{Br}^- + 4\text{H}^+ + 1\text{Na}^+$].

$\text{Cs}_4[\text{Sn}_2-(\text{I})_6(\text{PdBBr}_2)_3]$: Similarly prepared from $\text{Cs}_2[\text{Sn}-(\text{I})_3]$ in 90% yield. Slow diffusion of diethyl ether into a DMF/THF (1:1) solution of this complex gave well-formed, X-ray diffraction quality, orange-red single crystals. Elemental analyses: for $\text{C}_{126}\text{H}_{120}\text{Br}_6\text{N}_6\text{O}_{18}\text{P}_6\text{Pd}_3\text{Sn}_2$ (%): C 40.25, H 3.22, N 2.24; found: C 40.11, H 3.11, N 2.21; ^1H NMR (360 MHz, $[\text{D}_7]\text{DMF}$, TMS): $\delta = 8.16$ (td, $J = 7.3, 1.7$ Hz, 1H, catechol), 7.66 (m, 4H, phenyl), 7.38 (m, 6H, phenyl), 6.58 (d, $J = 7.9$ Hz, 1H, catechol), 6.41 (br d, 1H, catechol); $^{13}\text{C}\{^1\text{H}\}$ NMR (89.9 MHz, $[\text{D}_7]\text{DMF}$, TMS): $\delta = 156.2$ (s, catechol), 151.5 (t, $J(\text{P,C}) = 10.0$ Hz, catechol), 134.9 (t, $J(\text{P,C}) = 5.6$ Hz, phenyl), 134.2 (t, $J(\text{P,C}) = 24.9$ Hz, phenyl), 129.8 (s, phenyl), 127.6 (t, $J(\text{P,C}) = 5.0$ Hz, phenyl), 123.8 (br d, catechol), 114.7 (t, $J(\text{P,C}) = 28.2$ Hz, catechol), 113.4 (t, $J(\text{P,C}) = 4.0$ Hz, catechol); $^{31}\text{P}\{^1\text{H}\}$ NMR (36.3 MHz, $[\text{D}_7]\text{DMF}$, 85% H_3PO_4): $\delta = 21.27$ (s).

X-ray crystallography:^[54, 55] Crystal data for both compounds were collected using a Siemens SMART^[57] diffractometer equipped with a CCD area detector using MoK_α ($\lambda = 0.71073$ Å) radiation. Data in the frames corresponding to an arbitrary hemisphere of data were integrated using SAINT.^[58] Data were corrected for Lorentz and polarization effects. An empirical absorption correction based on the measurement of redundant and equivalent reflections and an ellipsoidal model for the absorption surface was applied using XPREP^[59] (Sn^{IV} structure) or SADABS^[60] (Ti^{IV} structure). The structure solution and refinement for the Sn^{IV} structure was performed using the teXsan^[61] crystallographic software package (refining on F); for the Ti^{IV} structure the initial solution was performed using teXsan, but the final refinements were performed using SHELXTL^[59] (refining on F^2). Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-103117 ($\text{Cs}_4[\text{Sn}_2-(\text{I})_6(\text{PdBBr}_2)_3]$) and CCDC-103118 ($\text{Cs}_4[\text{Ti}_2-(\text{I})_6(\text{PdBBr}_2)_3]$). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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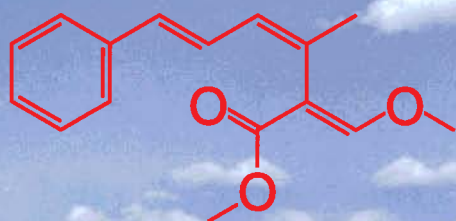
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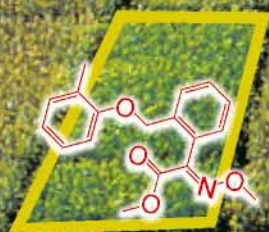
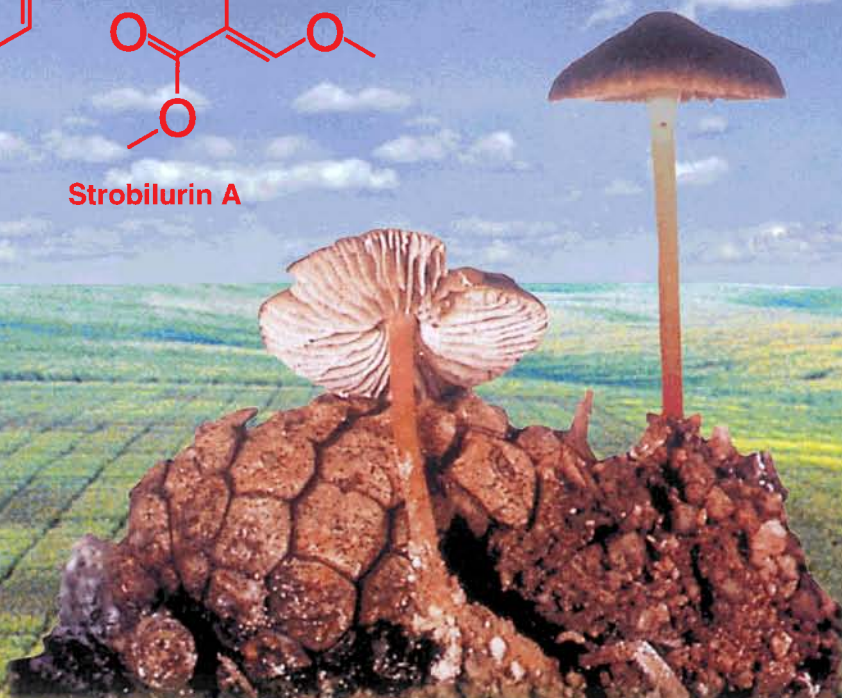
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- [54] $\text{Cs}_4[\text{Ti}_2(\text{1})_6(\text{PdBr}_2)_3] \cdot 9\text{THF} \cdot \text{H}_2\text{O} \cdot 3/2\text{Et}_2\text{O} \cdot 3/2\text{DMF}$: crystal size $0.38 \times 0.30 \times 0.30$ mm; $T = -122^\circ\text{C}$; hexagonal space group $P6_3/m$ (no. 176), $a = 20.4769(1)$, $b = 20.4769(1)$, $c = 21.9982(1)$ Å, $V = 7988.15(7)$ Å³, $Z = 2$, $\rho_{\text{calcd}} = 1.711$ g cm⁻³, $\mu = 2.953$ mm⁻¹, $F(000) = 4082$, $2\theta_{\text{max}} = 52.28^\circ$. Of the 38263 reflections which were collected, 5129 were unique ($R_{\text{int}} = 0.044$); equivalent reflections were merged. Empirical absorption correction: $T_{\text{max}} = 0.429$, $T_{\text{min}} = 0.341$. One of the Cs counterions, one of the bromine atoms, and much of the solvent were disordered; details can be found in the supporting information. The non-hydrogen atoms (excluding disordered solvent molecules and minor components of the disordered bromine) were refined anisotropically. Hydrogen atoms were included on all but the solvent molecules but not refined. Final $R1 = \sum(|F_o| - |F_c|)/\sum|F_o| = 0.0579$ for 3950 for $I > 2\sigma(I)$ (307 parameters, 1 restraint); for all 5129 data, $wR2 = [\sum w(F_o^2 - F_c^2)^2/\sum w(F_o^2)^2]^{1/2} = 0.1300$. Weighting scheme: $w = 1/[\sigma^2(F_o^2) + (0.0356P)^2 + 62.28P]$, where $P = (\max(F_o^2, 0) + 2F_c^2)/3$.
- [55] $\text{Cs}_4[\text{Sn}_2(\text{1})_6(\text{PdBr}_2)_3] \cdot 5\text{DMF} \cdot 2\text{H}_2\text{O} \cdot \text{THF} \cdot x\text{solvent}$: crystal size $0.10 \times 0.11 \times 0.15$ mm; $T = -110^\circ\text{C}$; triclinic space group $P\bar{1}$ (no. 2), $a = 18.0134(3)$, $b = 18.5299(3)$, $c = 28.6388(1)$ Å, $\alpha = 99.494(1)$, $\beta = 98.315(1)$, $\gamma = 119.077(1)$, $V = 7954.9(2)$ Å³, $Z = 2$, $\rho_{\text{calcd}} = 1.59$ g cm⁻³, $\mu = 3.157$ mm⁻¹, $F(000) = 3160$, $2\theta_{\text{max}} = 46.5^\circ$. Of the 36575 reflections which were collected, 22133 were unique ($R_{\text{int}} = 0.068$); equivalent reflections were merged. Empirical absorption correction: $T_{\text{max}} = 0.776$, $T_{\text{min}} = 0.521$. One of the Cs counterions and much of the solvent were disordered; details can be found in the supporting information. The non-hydrogen atoms (excluding the disordered Cs atom and disordered solvent molecules) were refined anisotropically. Hydrogen atoms were included on all but the solvent molecules but not refined. Additionally, all phenyl rings were refined as rigid groups. Final $R = \sum(|F_o| - |F_c|)/\sum|F_o| = 0.090$ for 12552 data for $I > 3\sigma(I)$ (822 parameters, 18 restraints), $Rw = [\sum w(|F_o| - |F_c|)^2/\sum wF_o^2]^{1/2} = 0.107$. Weighting scheme: $w = 1/\sigma^2(F_o) = 4F_o^2/\sigma^2(F_o^2)$.
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Strobilurin A

**Strobilurins—new
fungicides for
crop protection**



Kresoxim-methyl

Strobilurins: Evolution of a New Class of Active Substances

Hubert Sauter,* Wolfgang Steglich, and Timm Anke

Dedicated to Professor Hans-Jürgen Quadbeck-Seeger on the occasion of his 60th birthday

Nature provides active substances, as the result of special biosynthetic pathways, with the most diverse biological activities. Many of these substances perform functions that are important to the survival of the organism in which they are formed. For example, certain fungi produce antifungal strobilurins and oudemansins, agents to which the producers themselves are not sensitive, but which ward off other fungal species that grow on the same substrates. The period that followed the early breakthroughs in the 1970s—the discovery of the natural strobilurins and oude-

mansins, the identification of the β -methoxyacrylate group as their common structural element, and the elucidation of their mode of action—saw the focus of research activities shift away from the universities, ushering in an era of intense competition between rival industrial fungicide research teams. Their goal was, and remains, the development of fungicides with practical applications in agricultural crop protection. This review illustrates methodological approaches in the optimization of lead structures and the importance of understanding struc-

ture–activity relationships. The variation of natural product lead structures and the selection of suitable candidates for development led to an across-the-board evolution of the strobilurins as a new class of active substance. Several commercial products resulting from this effort are now already on the market, and more are expected to follow: a most intriguing success story.

Keywords: active substance research • fungicides • natural products • strobilurins • structure–activity relationships

1. Introduction

Since time immemorial, fungi have played an important part in the history of mankind, for example in the production of foodstuffs such as cheese, bread dough, and soy sauce or in alcoholic fermentation processes. In some Asian and American cultures fungi have been used by cults as the source of hallucinogens. Also when it comes to delicacies, the fruiting bodies of fungi (to the layman the actual, that is, visible, manifestations of fungi) have been valued since olden times, none more so than the truffles prized by gourmets. However, the importance of fungi as medicines was traditionally rather low compared with that of plants. This situation remained basically unchanged until the groundbreaking discovery of

penicillin by Alexander Fleming in 1928 and its development as the first highly active antibiotic obtained from a mold. Since then the importance of fungi as sources of antibiotics and other pharmacologically active natural products has become ever more evident.^[1]

2. The Natural Strobilurins and Oudemansins

2.1. Background

Strobilurins were first identified within the framework of a program begun in late 1976 aimed at discovering new antibiotic agents from basidiomycetes. This class of higher fungi, which includes many familiar species such as fly agaric and edible boletus, was in those days spurned by natural product researchers. The often sluggish growth of mycelial cultures of basidiomycetes meant they were regarded as being more difficult to handle than the extremely active substance-rich streptomycetes, which are classified as bacteria. Nevertheless, studies of the pigments and toxins of the fruiting bodies^[2] promised an original and diverse secondary metabolism in mycelial cultures too. In their work on the formation of polyacetylenes in basidiomycetes, Jones and Thaller had drawn parallels between the secondary metabolism of basidiomycetes and that of higher plants, notably *Apiaceae* and

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Asteraceae.^[3] This followed from the pioneering work by the groups of Anchel, Kavanagh, and Hervey,^[4] who in the 1940s and 1950s had screened hundreds of extracts of fruiting bodies, and in some cases of corresponding fungal mycelial cultures too, for the formation of antibiotics. A pivotal discovery to emerge from their studies was pleuromutilin, a new antibacterial antibiotic with an interesting site of action,^[4b] which was launched some years later by Sandoz as the semisynthetic derivative tiamulin for use as a veterinary medicine.^[5] All of this, plus the congenial research environment offered by the Sonderforschungsbereich in Tübingen headed by Zähler "Chemical Biology of Microorganisms", was the spur for one of us (T.A.) to commence work on basidiomycetes. Among the first antibiotics discovered were strobilurins A and B, obtained from fermentations of *Strobilurus tenacellus* (a fungus that grows on pinecones).^[6] Up till then no natural products had been isolated from this fungus. Our first publication in 1977^[6] reported the physical and chemical data of strobilurins A and B as well as their powerful antibiotic activity against a range of fungal species. The high cytotoxic activity towards Ehrlich ascites tumor cells led Douros of the National Cancer Institute (USA) to ask us later the same year to provide samples of the strobilurins to screen for antitumor activity. The first 117 mg of strobilurin A were dispatched in 1978. Soon afterwards the exciting news came that strobilurin A, although exhibiting only very weak antitumor activity, showed no acute toxicity in the tumor-bearing mice.

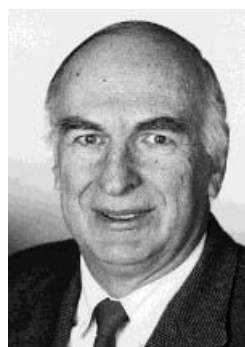
The structure elucidation by the research group of one of us (W.S.) showed the strobilurins to have an unusually simple

structure and apparently represent a new class of antifungal antibiotics whose abundance in nature was not confined to the genus *Strobilurus*.^[7] Strobilurin A has also been isolated from *Cyphellopsis* cultures and from two *Mycena* species. This compound was originally thought to have a side chain with an all-*trans* 9*E* configuration, but this was later (1984) revised to 9*Z*.^[8] Strobilurin A was found to show great similarity to mucidin, an antifungal antibiotic published in 1969^[9] and patented in 1970^[10] without a proposed structure; they had the same empirical formula, the same UV spectrum, and equal activity. However, the optical activity reported for mucidin (+33° (*c* = 10) at 546 nm) completely ruled out a common identity with the strobilurin A. Mucidin, which was isolated from *Oudemansiella mucida* (a fungus that grows on the trunks of beech trees), found use in Czechoslovakia as an antifungal ointment (Mucidermin "Spofa"). To be more certain that strobilurin A and mucidin were different compounds, samples of *O. mucida* were collected as starter material for mycelial cultures, which were then investigated for the formation of antifungal antibiotics. To our surprise we did not detect mucidin in these cultures, but instead a novel natural product, the crystalline, optically active oudemansin A, and the oily strobilurin A.^[11] Like strobilurins A and B, oudemansin A also exhibited powerful antifungal activity. The quest to establish which compound corresponded to "mucidin" proved to be highly protracted. Czech patents and publications yielded contradictory information. One patent,^[12] citing previous patents of the same origin,^[10] correctly described the structure as (9*Z*)-strobilurin A. No reference was made in this work to the optical rotation

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H. Sauter



W. Steglich



T. Anke

Wolfgang Steglich, Professor and Director at the Institute for Organic Chemistry at the Universität München, started studying chemistry in 1951 and received his PhD with F. Weygand in 1960. Later he carried out research with D. H. R. Barton in London and then he was Professor in Berlin, Bonn, and München. His interests lie in the field of natural product chemistry including structure determination and the development of new synthetic methods.

Timm Anke, who is a biochemist and microbiologist, is Professor of Biotechnology at the Universität Kaiserslautern. In 1964 he started studying biochemistry in Tübingen and gained his PhD in 1973 under the supervision of H. Dieckmann. After postdoctoral work with F. Lipman in New York, he completed his Habilitation in Tübingen, and in 1981 he was appointed as a Professor in Kaiserslautern. His work is predominantly involved with the discovery of biologically active natural products derived from basidiomycetes and the elucidation of their action mechanisms.

previously reported for mucidin.^[10] Then in 1981 with reference to the earlier patents,^[10,12] among them also the patent stating the correct 9*Z* configuration, Sedmera et al. confusingly reported the identity of mucidin as that of (9*E*)-strobilurin A.^[13] The optical rotation reported in the Czech patent^[10b] was referred to as a “printing error”. Final clarification was ultimately furnished in a 1986 paper,^[14] in which a direct spectroscopic comparison established the structures of (9*Z*)-strobilurin A and mucidin to be one and the same.

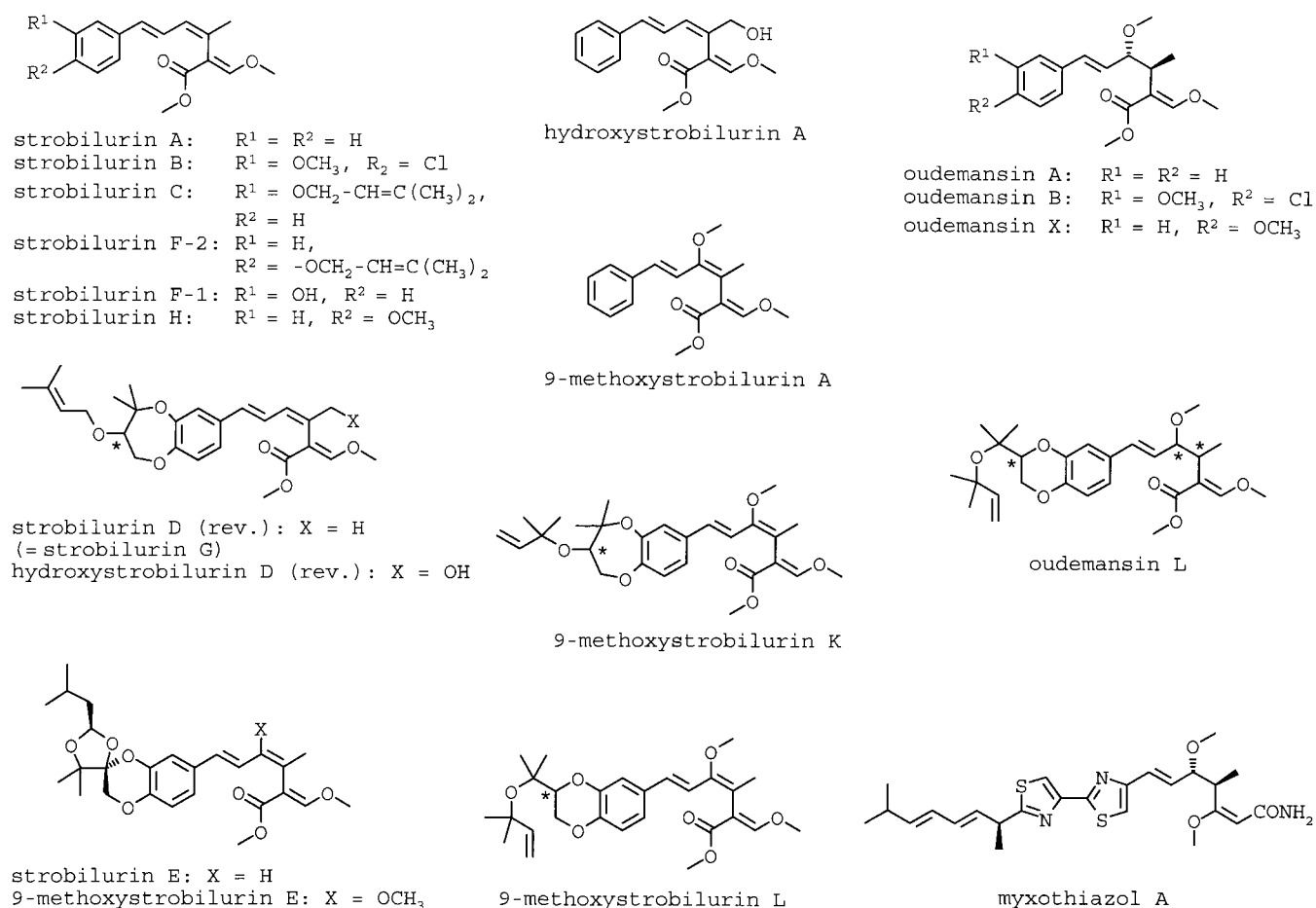
2.2. Abundance in Fungi

Fungi that produce strobilurins and oudemansins are found all over the world in all climate zones.^[17b] With one exception (*Bolinea lutea*, an ascomycete) all such species are basidiomycetes.^[15] Some of the structures (Scheme 1) are extremely complex, as in the case of strobilurin E, which is characterized by high antifungal activity and very high cytostatic activity.^[16] The structures of the 9-methoxystrobilurins K and L, strobilurin D, and hydroxystrobilurin D have since been revised.^[17] The antibacterial activity of 9-methoxystrobilurin L published by the company Xenova has, like the proposed structure, since been found to be erroneous.^[18]

2.3. Function of the Natural Products for the Producers

In the search for new active substances, in the group of one of us (T.A.) basidiomycetes are normally grown on complex, highly nutrient rich media to ensure good mycelial growth and high production of secondary metabolites. However, because these conditions in no way correspond to those of their natural substrates, such as soil, leaves, or (most commonly) wood, certain strobilurin producers were also grown on their natural, presterilized substrates and then investigated for the formation of antibiotics. Formation of both oudemansin A and strobilurins was observed in a *Pterula* species, a tropical *Favolaschia* species, and in *Mycena tintinnabulum*.^[19] In *Mycena tintinnabulum* strobilurin D was detected both in the fruiting bodies (caps) and in the mycelially colonized substrate (oak wood) in nature. The concentrations determined in the mycelially colonized oak wood were sufficient to inhibit the growth of other fungal species. This finding and the worldwide distribution of strobilurins and oudemansins leads us to conclude that the production of these compounds affords an advantage in terms of survival over other parasitic fungal species or fungi colonizing the same substrate.

But how do the fungi that produce strobilurins or oudemansins protect themselves against their own antifungal toxins, which are often produced in considerable quantities? We investigated this matter in collaboration with the group of



Scheme 1. The natural strobilurins and oudemansins^[17b, 66] and myxothiazole A.^[21a] The stereocenters whose configurations are unknown are marked with an asterisk.

Brand and von Jagow, and found that in *Strobilurus tenacellus* the site of action in the cytochrome b protein (see Section 2.4) is modified such that the binding of strobilurins and oudemansins becomes much more difficult. This is essentially due to the substitution of a small amino acid (alanine or threonine) in position 127 by a larger isoleucine residue. This resistance-inducing exchange occurs in a region that is highly preserved in the species sensitive to strobilurin-type toxins. In *S. tenacellus* an additional small amino acid in position 254 is replaced by glutamine, with the asparagine in position 261 being replaced by aspartic acid. All these amino acid residues are located in the binding envelope of the reduced coenzyme Q.^[20]

2.4. Mode of Action

Studies on the mode of action of strobilurins and oudemansins revealed very early on that these compounds inhibit the respiration of fungi. Investigations with Ehrlich ascites carcinoma cell cultures showed that the site of action lay exclusively in the respiratory chain (Figure 1). Other effects

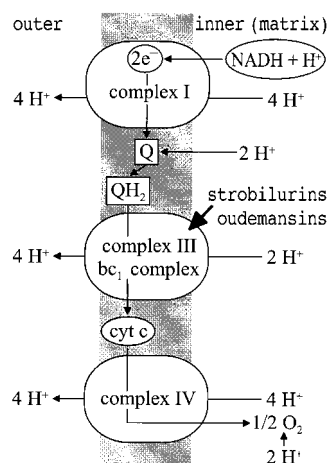


Figure 1. The site of action of strobilurins and oudemansins in the mitochondrial respiratory chain.

such as the complete inhibition of protein, RNA, and DNA synthesis were attributed by one of us (T.A.) to the resulting intracellular deficiency in ATP. The oxygen uptake and ATP synthesis of rat liver mitochondria were inhibited with both α -ketoglutarate and succinate as substrates; hence the site of action had to be in either complex III or IV of the respiratory chain.^[11] A fortunate turn of events led us to find in von Jagow a collaboration partner of the utmost competence, who succeeded in localizing fairly exactly the site of action of the strobilurins and oudemansins in the mitochondrial bc₁ complex. In our first joint publication we reported that strobilurins A and B, oudemansin A, and myxothiazole, a compound isolated by the groups of Reichenbach and Höfle at the Gesellschaft für Biotechnologische Forschung (Society for Biotechnological Research) in Braunschweig,^[21a] all bind at the same site of action.^[21b] A common structural element of these compounds that is clearly essential to their mode of

action is an (*E*)- β -methoxyacrylate subunit. Strobilurins, oudemansins, and myxothiazole bind reversibly to the ubihydroquinone oxidation (Qp) center of the bc₁ complex, a finding that has helped further our understanding of the structure and function of this part of the respiratory chain.^[22] Antimycin, an antifungal antibiotic commonly found in streptomycetes that is toxic to mammals, binds to the other center of the bc₁ complex, the Qn center. The crystal structure of the bc₁ complex from bovine heart tissue was recently solved by the Deisenhofer group, and the binding sites for myxothiazole and antimycin A were identified.^[23]

The first patent application for strobilurin A was submitted in collaboration with Hoechst^[26] and described the synthesis of strobilurin A, which seemed like it might find use as an antimycotic agent in human medicine. However, the compound compared poorly with other antimycotics on the market, and this patent was later relinquished. Several total syntheses of the natural strobilurins and oudemansins have been described and discussed in review articles.^[17b, 24]

3. Lead Structure Optimization in Rival Industrial Research Programs

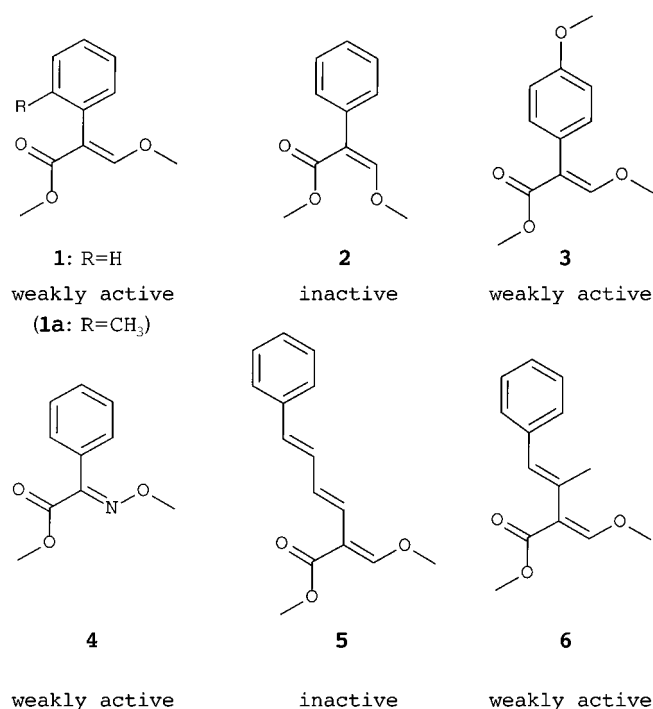
3.1. First Structural Variants

With their rare combination of favorable molecular and biological properties, the two natural products strobilurin A and oudemansin A were immediately seized upon as candidates for model compounds on which to base further synthetic modifications.^[24] A clear attraction was the simplicity of their structures, which is a rarity in bioactive natural products. Moreover, the presence of the β -methoxyacrylate group in both natural products gave us a clue that these were not merely solitary active substances in isolation, but were instead part of a group of active compounds. Secondly, their antifungal activity in laboratory tests proved to be attractively high, which together with their “novel” mode of action—inhibition of respiration combined with low toxicity towards mammalian cells—therefore gave us a powerful boost.

Consequently, syntheses of molecular variants of strobilurin A were underway as early as 1977 in the research group of one of us (W.S.). The goal was easily accessible analogues with antibiotic activity that was similar or enhanced as far as possible.^[25]

The β -methoxyacrylate group, whose heteroatoms are capable of intermolecular, polar interactions, was regarded as essential (i.e., as the pharmacophore) and was initially modified merely for the usual comparison purposes. The other regions of the molecule were judged to hold greater promise for structural variation. Such considerations gave rise not only to simple phenyl derivatives such as **1–3**, but also to norstrobilurin A (**5**) and the “shortened” analogue **6** (Scheme 2).^[24, 25]

Although such compounds showed a lower level of activity than the naturally occurring model compound in laboratory tests, some did show detectable, albeit weak, activity against *Penicillium notatum* as a representative fungal species both in



Scheme 2. Structure and activity of simple strobilurin analogues. Activities were determined on the basis of the inhibition of respiration and the agar diffusion test with *Penicillium notatum*.^[24, 25]

testing for inhibition of respiration and in the agar diffusion test.

From these results we were able to deduce some initial important structure–activity relationships.^[24, 25] The *E* configuration of the pharmacophore as in **1** is essential, as the *Z* isomer **2** was inactive. Substituents in the aryl ring such as in **3** appeared to be “allowed”; even a C/N exchange in the enol ether group, which provided the oxime ether **4**, did not result in any appreciable reduction in activity. A loss of activity was observed for the more linear (9*E*)-norstrobilurin A (**5**) and in the similarly shaped variant **6**; lacking in both compounds is the characteristic conformation of strobilurin A and oudemansin A, in which twisting of the central single bond results in an almost right-angled orientation of the β -methoxyacrylate pharmacophore relative to the phenyl-substituted side chain (Figure 2). In the variants **1–4** the side chain is missing completely.

In parallel to these variations of the lead structure, collaborations were sought with industrial partners with the aim of probing the usefulness of this class of active substances for the optimization of structure and activity under the viewpoint of potential applications as agricultural fungicides or clinical antimycotics. The response from industry to our overtures was, owing to other more pressing priorities, for the most part low-key. In 1982 Zeneca (then still part of ICI) requested a sample of oudemansin, which they obtained from one of us (T.A.). Interest shown by BASF in 1983 within the framework of a publicly funded joint project^[27] in collaboration with several university research groups led to a sample of strobilurin A being provided for in vivo greenhouse testing on plants infected with fungal pathogens. The test results were disappointing: The activity of strobilurin A was surprisingly

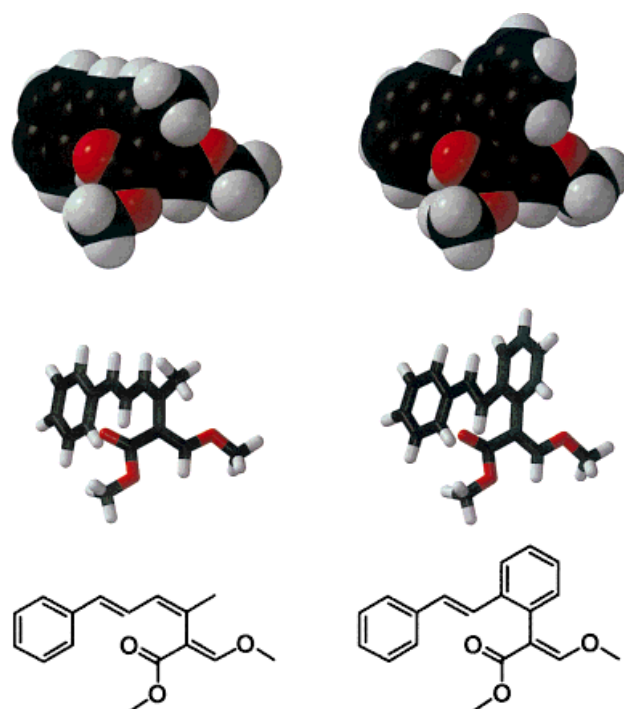
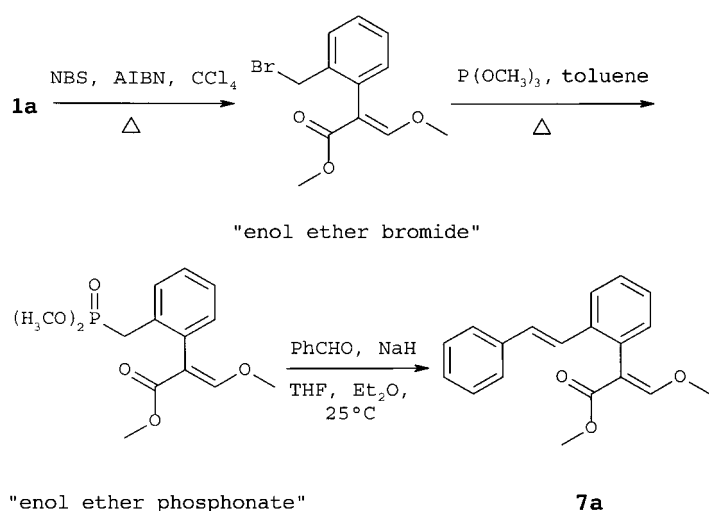


Figure 2. Computer-generated space-filling and stick models of strobilurin A (left) and the enol ether stilbene **7a** (right). Carbon: black, oxygen: red, hydrogen: white. (We are grateful to Dr. W. Altenhofen, BASF, for the calculations and the computer graphics.)

weak, being barely perceptible against only two out of six phytopathogenic fungal species at high application concentrations.^[28] This was contrary to expectations after the very good results in agar diffusion tests in the laboratory, and the question was posed as to what might be the possible causes.

Together with Schirmer, who at the time was responsible at BASF crop-protection research for collaboration with university research teams, we arrived at the hypothesis that the triene system of strobilurin A might have been the cause of a rapid photolytic and/or oxidative breakdown of the natural product, leading to a rapid loss of activity under the conditions of the greenhouse tests. The apparent plausibility of this hypothesis led us to dispense for the time being with verification through analytical experimentation. A different approach appeared more attractive: The idea we came up with was to stabilize the triene system with an aromatic bridge and, in the hope of improved in vivo activity, to synthesize and carry out tests on the “enol ether stilbene” **7a**. The synthesis was swiftly accomplished in the Steglich research group (Scheme 3).^[29a]

As it turned out, we hit the bull’s-eye with **7a**. Not only did it exhibit an activity against *P. notatum* in the inhibition of respiration test that was at least ten times higher than that of strobilurin A,^[29b,c] its potency and spectrum of activity in in vivo greenhouse tests in plants infected with fungal pathogens fulfilled our ambitious expectations. Good (<15% infection) to very good activities (<5% infection) at application concentrations of 63 ppm, and in some cases even at 16 ppm, were recorded for the following fungal diseases: powdery mildew of wheat (*Erysiphe graminis*), wheat brown rust (*Puccinia recondita*), rice blast disease (*Pyricularia*



Scheme 3. Synthesis of the enol ether stilbene **7a**.^[24, 29a] AIBN = 2,2'-azobisisobutyronitrile, NBS = *N*-bromosuccinimide.

oryzae), barley net blotch (*Drechslera teres*), grapevine downy mildew (*Plasmopara viticola*), and late blight (*Phytophthora infestans*).^[30]

The bridging by the central arene subunit in the enol ether stilbene **7a** did not result in a reduction of affinity for the mitochondrial target, it was if anything even increased, and clearly bestowed on the molecule—as hoped—the required stability. Thus, compared with strobilurin A, **7a** represented a modified, more potent, and overall more promising lead structure.

3.2. A Further Generation of Strobilurin Variants

Guided by this result, and with BASF broadening their involvement in the project, we turned our attention to the synthesis of some further variants of **7**, for example **8–12** (Scheme 4). In laboratory tests these compounds all showed activities comparable to that of strobilurin A, and exceeded this distinctly in the greenhouse tests. Some of the new derivatives exhibited even higher activity than the enol ether stilbene **7a**, depending on the test fungus.^[30]

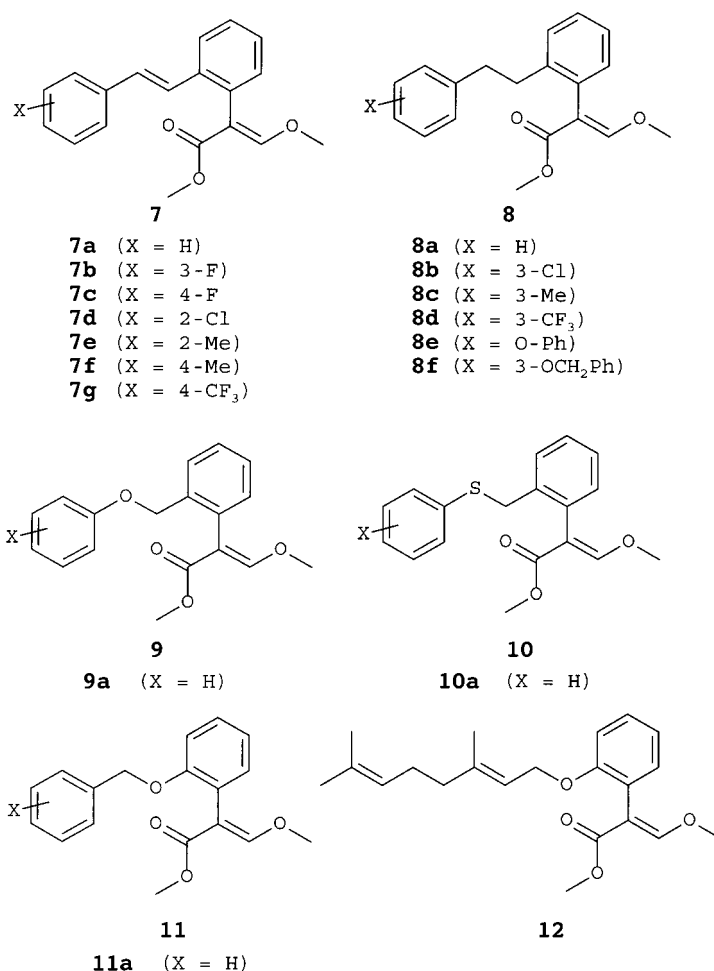
In the design of strobilurin variants a conceptual subdivision of the enol ether stilbene lead structure into three molecular regions proved useful: side chain, arene bridge, and pharmacophore (Scheme 5). Through variation of the side chain we succeeded in obtaining compounds of type **7**,^[31] **8**,^[32] **9** and **10**,^[33] and **11**,^[34] each the subject of separate patent applications.

3.3. Initial Field Trials at BASF

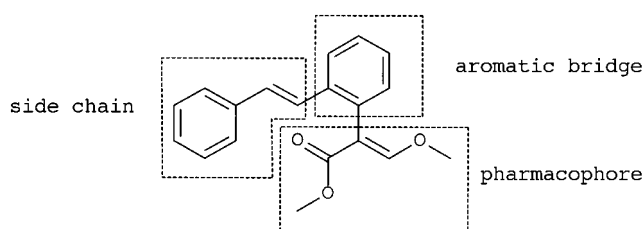
The next step was the biological evaluation of preselected candidates under outdoor conditions. In the course of optimization of a lead structure the transition from greenhouse tests to field trials demands particularly close attention. Three aspects are of prime importance here:

- The quantities of active substance required, and hence the outlay on their synthesis, rise dramatically. For in vitro

testing and other laboratory tests a few milligrams are normally sufficient. Even for an extensive series of dilutions in the greenhouse tests the amount of active material required is only about 100 mg in total. Thousands of individual structural variants can be tested per year as a matter of routine without undue difficulty. In contrast, with application of 100–1000 g ha⁻¹, preliminary field trials—normally in small plots of one square meter—require between 1 and 10 g per indication depending on the plant species, fungal pathogen, and number of treatments, that is, up to 100 g of each active substance must be supplied. Additional amounts of active substance are also required for the development of formulations suitable for application under outdoor conditions, for example as emulsion concentrates, suspension concentrates, or water-dispersible powders or granules.



Scheme 4. Strobilurin variants based on the enol ether stilbene **7a**.^[24, 31–34]



Scheme 5. Structurally modified regions of the enol ether stilbene lead structure.

- For these and other practical reasons the number of compounds that can be tested under outdoor conditions is restricted to barely more than one hundred per year. This means that compounds that fail to make it to the field trial stage are essentially dropped as individual candidates in the optimization process.

- Years of experience have shown the model tests in the greenhouse to be of only limited value as a predictor of the all-important activities in field trials under real-life conditions. Clearly discrepancies^[35a] are possible in the sense of false positive and false negative greenhouse results,^[35b] particularly when an attempt is made to assess the order of potency within individual families of active compounds.^[35c, 36] This special situation is evident from the values shown in the bottom line of Table 1. Here, the rank correlation coefficients^[39] between the field trial and greenhouse test results range from usable values (0.74 for powdery mildew of wheat, 0.6 for late blight) to values of little prognostic usefulness (for example –0.3 for net blotch disease).

In this respect the selection of the “right” candidates for the field trials, which due to seasonal growing cycles are only possible at certain times of the year,^[37] becomes the critical rate-determining step, and hence also the decisive step for the overall success^[36] of the lead structure optimization process (see Figure 4, Section 4.4).

Of the roughly 100 strobilurin derivatives synthesized up to December 1985 at BASF, a first batch of 15 compounds was selected for small plot trials in the 1986 season and tested in various indications according to the results obtained in the earlier greenhouse tests. Table 1 summarizes these compounds and the results obtained.^[38]

In field trials against powdery mildew of wheat a clear disparity is evident between the good results with the four dihydrostilbenes **8** and the lack of activity of the stilbenes **7**. This seems plausible in view of the known photolability of stilbenes, which can undergo *E*→*Z* isomerization and subsequent facile photocyclization to dihydrophenanthrenes followed by further reactions such as oxidative aromatization.^[40] Overall the rank correlation with the greenhouse test results of the family of compounds tested is good ($r_s = 0.74$), though the inferiority of the stilbenes **7** in the greenhouse tests is not so pronounced as under the more severe photochemical conditions in the field trials. Particularly noteworthy was the activity of **8d** against powdery mildew of wheat in field trials. The activity of **8d** at maximum infection pressure (100% infection in the untreated plots), albeit at higher rates of application (1000 g), exceeded that of the standard reference substance, Corbel—a most encouraging result.

In tests against net blotch disease even the three stilbenes **7** tested here proved to have some activity at a lower infection pressure (35%). By contrast, the sole dihydrostilbene tested, **8d**, again showed distinctly higher activity, exceeding—albeit at double the rate of application—the level of the standard Desmel.

In tests against glume blotch on wheat **8d**, the front runner against powdery mildew of wheat, again showed high activity, this time, however, closely followed by stilbene **7e**. The tests against wheat brown rust were not especially fruitful, owing to the particularly low infection pressure (18%) and the

relatively weak activity of the five dihydrostilbenes tested. In tests against late blight at a high infection pressure, the dihydrostilbene **8a** proved to be the best, although two of the stilbenes also showed measurable activity. In the case of activity against apple scab stilbene **7a** was in first place and on the level of the standard. In summary, from a structure–activity viewpoint the photolabile stilbenes were distinctly inferior to the dihydrostilbenes under outdoor conditions. The former did, however, show some activity in tests in which the interval between the application of the active substance and the evaluation of the test was relatively brief (applications against glume blotch, late blight, and apple scab), that is 8 days compared with about 30 days in the other tests. For the structure types **9** and **10** only a single example of each underwent field trials, hence a full, experimentally-based assessment of these structure types was not possible at that time.

The first results from these small-plot trials under outdoor conditions, which were of decisive importance to the assessment of the overall structure type, were obtained in May 1986. Even at this early stage some results were highly encouraging, for example the first indication that the activities of **8a**, **8b**, and **8d** were very good and of the same order as that of the standard reference material Corbel. In addition, initial results from tests against net blotch disease in barley, for example after treatment with **8b**, gave rise to high expectations and much euphoria. Not only had our good fortune seemingly seen us through the birth of this new class of fungicidal active substances, we were able to look ahead to the further development of the baby full of hope and confidence.

In the midst of our elation at this breakthrough we came face to face with a most unpleasant surprise. At the end of April 1986 a comprehensive patent application had been published by ICI (now Zeneca),^[41] which was brought to our attention a short time later. The first priority date was October 19, 1984.^[42] The basic formula **I** of what was an extremely broad and general claim is shown in Scheme 6. R^1 and R^2 represent a wide variety of substituents ranging from hydrogen to “optionally substituted” alkyl, aryl, cycloalkyl-alkyl, and other groups.^[43] Even richer in variants were the definitions for X, Y, and Z. On the other hand, the actual core element of these structures was defined in subclaim 7^[41] (formula **XII** in Scheme 6). Additional subclaims were submitted for structures of type **13**. As individual compounds “our” enol ether stilbene **7a** and the hydrogenated variant **8a** that had already attracted attention in our field trials, had been the subjects of specific claims. What was striking were the parallels with our ideas and concepts that lay at the heart of this application. All the BASF applications for the enol ether strobilurins^[31–34] were hit by this patent—a disaster that set us back all the more at the time, as our field trial results had led us to the brink of verifying the great potential of the strobilurins, not to mention their potential practical applications.

Back in the laboratory we had some hard questions to ask ourselves. How could such a thing have happened? Had we been caught napping? Had indiscretions, espionage even, played a part? The answer was soon in coming and was plain to see: All this had taken place in the context of a fair contest between rival research groups.

Table 1. Test results and order of activity of strobilurins against fungal diseases in 1986 field trials^[38] and greenhouse tests.^[30]

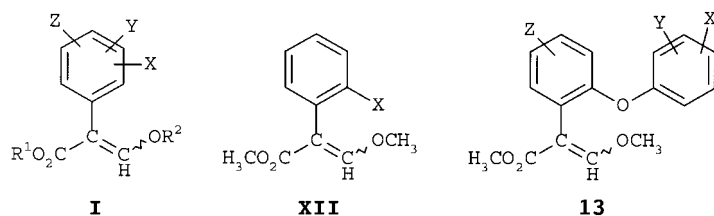
Compound	Powdery mildew of wheat ^[a]			Net blotch ^[f]			Wheat leaf spot ^[i]		
	infection ^[b] [%]	R_F ^[c]	R_G ^[d,e]	infection ^[g] [%]	R_F ^[c]	R_G ^[h]	infection ^[i] [%]	R_F ^[c]	R_G ^[k]
7a				28	5	2	30	6	1
7b	100	7	9	25	4	3			
7c	100	7	7						
7d				22	3	1			
7e	100	7	3				10	2	4.5
7f	100	7	8						
7g	100	7	6				61	7	8
8a	22	2.5	1				18	5	4.5
8b	22	2.5	5	4	1	4	13	4	4.5
8c	31	4	4				12	3	4.5
8d	15	1	2				8	1	4.5
8e							68	8	4.5
8f									
9a									
10a				18	2	5			
untreated	100			35			71		
standard	18			5			5		
r_s ^[u]		0.74			− 0.30			0.23	
Compound	Wheat brown rust ^[e]			Late blight ^[o]			Apple scab ^[r]		
	infection ^[m] [%]	R_F ^[c]	R_G ^[n]	infection ^[p] [%]	R_F ^[c]	R_G ^[q]	infection ^[s] [%]	R_F ^[c]	R_G ^[t]
7a				68	3	1	5	1	3
7b									
7c									
7d									
7e									
7f				61	2	2			
7g									
8a	18	5	4	33	1	3			
8b	10	1.5	2						
8c	10	1.5	4						
8d	12	3	4						
8e				84	5	5	8	2	2
8f				77	4	4	15	3	1
9a	15	4	1						
10a									
untreated	18			84			77		
standard	9			14			6		
r_s ^[u]		0.13			0.6			− 1.0	

[a] *Erysiphe graminis* f. sp. *tritici*. [b] Small plot trials in winter wheat; spontaneous infection; treatment after 2.5 % preinfection, in each case with 750 g ha^{−1} of active substance; evaluation 31 days after treatment; standard reference substance: Corbel, 750 g ha^{−1}. [c] R_F = ranking in field trials according to the definition in ref. [39] within the tested family of compounds (vertical columns). [d] R_G = ranking in greenhouse tests, in each case determined from a number of tests after treatment with various spray concentrations. [e] Determination of R_G based on greenhouse tests with spray concentrations of 16, 8, 4, 2, and 1 ppm. The ranking changes in the corresponding curative tests in the greenhouse; as might be expected, the results of these tests give a better rank correlation with the results of the (in some cases curative) field trials: r_s = 0.87. [f] *Pyrenophora teres* f. sp. *hordei* (barley net blotch). [g] Small-plot trial in winter barley; infection carried out twice 5 and 2 days before treatment with 1000 g ha^{−1} of active substance; evaluation 36 days after treatment; standard: Desmel 500 g ha^{−1}. [h] Determined from protective greenhouse tests after treatment with spray concentrations of 250, 125, 63, and 31 ppm. [i] *Leptosphaeria nodorum* (glume blotch) on wheat. [j] Small-plot trial in winter wheat. Artificial infection 3 days after treatment with 1000 g ha^{−1} of active substance; standard: Desmel 250 g ha^{−1}. [k] Determined from protective greenhouse tests after treatment with spray concentrations of 125, 63, and 31 ppm. [l] *Puccinia recondita* (wheat brown rust). [m] Small-plot trial in winter wheat; spontaneous infection; treatment without preinfection with 750 g ha^{−1} of active substance; evaluation 32 days after treatment; standard: Impact 125 g ha^{−1}. [n] Determined from curative greenhouse tests after treatment with spray concentrations of 250, 63, and 16 ppm. [o] *Phytophthora infestans* [p] Small-plot trial in potatoes; spontaneous infection; treatment carried out three times at weekly intervals with 1000 g ha^{−1} of active substance without preinfection; evaluation 30 days after last treatment; standard: Polyram-Combi (80 %) 500 g ha^{−1}. [q] Determined from protective greenhouse tests in tomato plants after treatment with spray concentrations of 63, 31, and 16 ppm. [r] *Venturia inaequalis*. [s] Tests an apple trees, strain Golden Delicious; spontaneous infection; treatment carried out 18 time at weekly intervals with 210 g ha^{−1} of active substance without preinfection; evaluation 7 days after last treatment; only the infection on the leaves present at the start of treatment was evaluated; standard: Baycor 140 g ha^{−1}. [t] Determined from curative greenhouse tests on apple seedlings after treatment with spray concentrations of 75 and 31 ppm. [u] r_s is the Spearman rank correlation coefficient.^[39] Only the rankings of rows R_G and R_F are correlated against one another.

3.4. The Route of the Zeneca Research Team to Azoxystrobin^[44, 45]

At the beginning of the 1980s Zeneca had embarked on a systematic and intensified research program looking into natural products as possible lead structures for fungicides. In

the course of these activities Baldwin, a biochemist working in the Zeneca fungicides team, had become aware of the work already described above^[21b] on the common structural elements and common mode of action of oudemansin, strobilurins A and B, and myxothiazole. Zeneca subsequently asked one of us (T.A.) to provide them with a sample of oudeman-

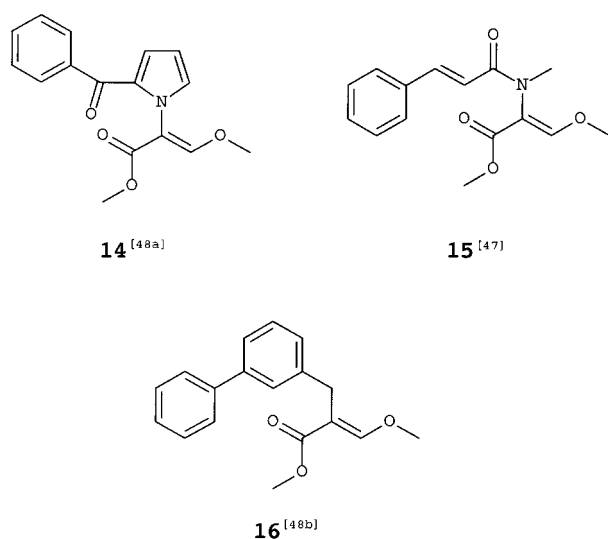


Scheme 6. Formulae from the claims in the ICI patent application for enol ether strobilurins^[41]. See text for details.

sin A for greenhouse testing, and in June 1982 had been sent 4 mg of the crystalline substance. Zeneca's greenhouse test results^[46] against eight different pathogens were highly impressive. Unlike strobilurin A, oudemansin A is sufficiently stable under greenhouse conditions and consequently shows very high fungicidal activities and a strikingly broad spectrum of activity even at the low application concentration of 33 ppm. This activity proved reproducible at Zeneca, and, spurred on by their impressive test results, the Zeneca team headed by Clough swiftly took to the topic of strobilurins—then still classified under the banner of β -methoxyacrylates—on a wide front. Their endeavors led among other things to the design and realization of stereoselective syntheses for both (9*E*)- and (9*Z*)-strobilurin A,^[45c] thereby providing independent confirmation once again of the configuration we had correctly assigned to the natural product strobilurin A. Subsequent analytic investigations revealed the inadequate activity of the synthetic (9*Z*)-strobilurin A in the greenhouse tests to be due, in addition to its relatively high volatility, to its extremely high photolability.^[45a, b]

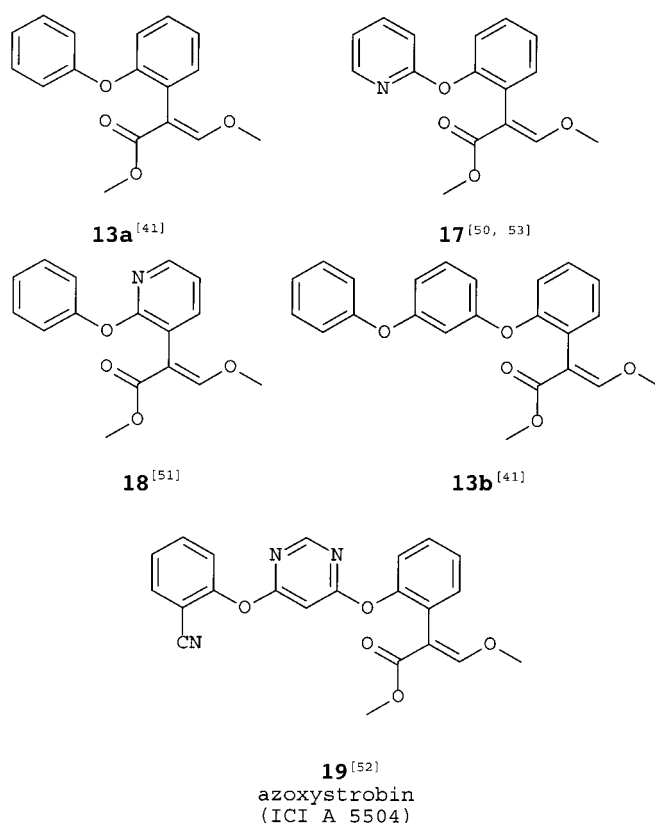
Considerations similar to ours likewise soon led the Zeneca team to the enol ether stilbene **7a**. In parallel to this, however, a range of other active, easily accessible, stabilized variants had also been synthesized, for example **14–16** (Scheme 7).

In the progress made at Zeneca a decisive role was played by the diphenyl ether **13a** (Scheme 8). Its photostability was even higher than that of the enol ether stilbene **7a**, it showed



Scheme 7. Stabilized, active strobilurin variants from the early phase of the lead structure optimization at Zeneca.^[45c]

high activity in greenhouse and field trials, and it exhibited a broad spectrum of activity.^[45c] It also possessed a molecular property that was a specific focus of Zeneca's biological screening program: the systemic transport of the active substance through the xylem and occasionally the phloem of the treated plants. This means that once applied, instead of being merely confined to the sprayed surface of the plant, a systemic active substance will penetrate the plant, thus allowing it to undergo transportation in solubilized form.^[49] Systemic transport of a fungicide ultimately means that its activity will not just remain localized, but, as a consequence of the distribution process, can extend to the unsprayed parts of the plant too. Zeneca concluded that although the systemic distribution of the diphenyl ether **13a** was the reason for the good performance as a fungicide in field trials, it was also responsible for the slight phytotoxic damage sustained by some sensitive plant species. Further optimization work was consequently pursued with the aim of keeping the lipophilicity of the new structural variants sufficiently low to permit systemic transport,^[49c] but without any accompanying phytotoxicity. The introduction of heteroatoms as in compounds **17** and **18** proved advantageous in this respect. Compound **13b** represented a further step forward from another viewpoint: Its activity in greenhouse tests was superior to that of **13a**, pointing to more efficient docking at the mitochondrial bc₁ complex. However, the additional phenoxy substituents enhanced the lipophilicity of the compound to such a degree it was no longer able to undergo systemic transportation.^[49c, 54] With the introduction of hydrophilic structural elements into



Scheme 8. Strobilurin analogues with variations in the side chain and aromatic bridge on the Zeneca route to azoxystrobin.^[45c]

the lipophilic side chain of **13b**, the lead structure optimization finally bore fruit in the form of compound **19**. This compound, initially developed under the code number ICIA 5504, possessed the desired physicochemical and biological properties, and was subsequently christened with the common name azoxystrobin before being launched onto the German market in February 1996 under the trade name Amistar.^[45e] Details of its biological action profile in comparison with that of other strobilurins are given in Section 5.3.

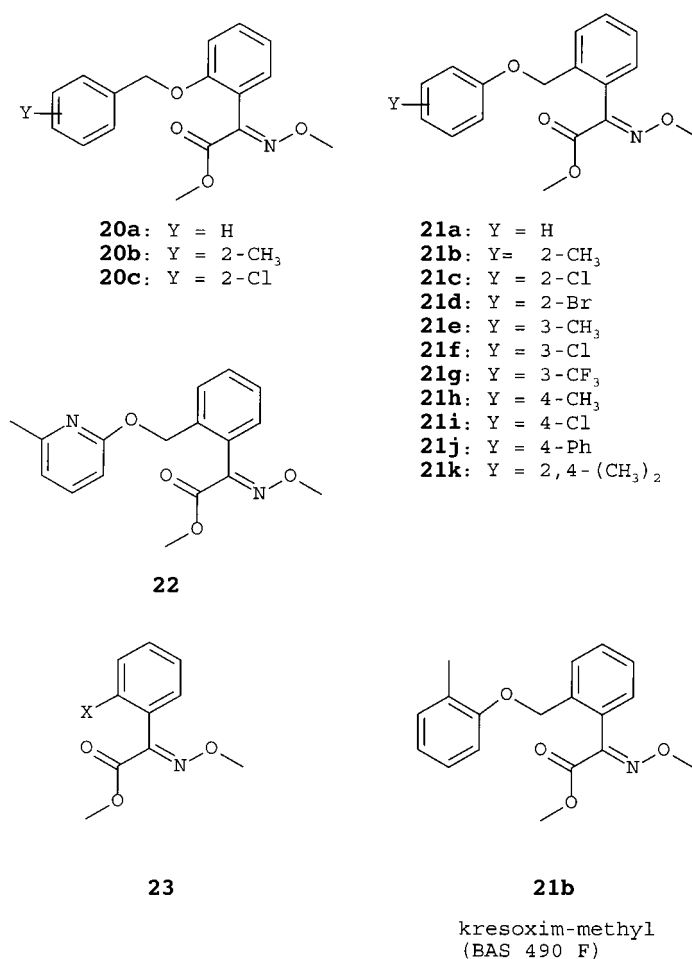
3.5. The Second Phase of the Lead Structure Optimization at BASF: The Route to Kresoxim-methyl^[28]

With the submission of the enol ether basic application, and especially after its publication in April 1986,^[41] the Zeneca team was in a relatively comfortable position from a patent point of view and could turn its attention to the optimization of the side chain while retaining the enol ether pharmacophore. In contrast, the BASF fungicide team was faced with a very different situation in May 1986. The claims published by Zeneca appeared watertight, the only real gaps that were immediately perceptible being in the arene bridge. There was a very real danger^[55] that all our efforts to date had been in vain, for us at least. Nevertheless, the recent, immensely promising BASF field trial results (see Table 1) left us no alternative: A way out had to be found. The biological potential of the new class of active substances seemed too tempting, as did the wide latitude in the variation of the arene bridge and side chain discernible *inter alia* from the Zeneca patent claims.^[41, 47] The BASF team consequently elected to go for the sole realistic loophole that still remained and to modify the pharmacophore as a way of getting around the tight confines of the Zeneca patent claims.^[56]

Variation of the pharmacophore in a class of active substance always carries a very high risk of a drastic loss of activity. This assumes that the pharmacophore is, by definition, the common functional group which through intermolecular interactions gives the class of active substances a decisive energetic boost for docking at the target. In practice this nearly always means the involvement of heteroatoms with their possibilities for polar interactions and unique vectorial-spatial arrangements relative to the rest of the active substance molecule. The enol ether pharmacophore contains three such heteroatoms, each of them oxygen. Nothing was known at the time about their function in the binding process at the bc₁ complex.

In the BASF team Wenderoth and Rentzea had already embarked on the task of substituting the enol ether group of the pharmacophore by an oxime ether unit. From a pharmacophore analysis viewpoint all three original heteroatoms were still retained, as were, in all probability, their vectorial orientation *possibilities*; this was evident even without the benefit of computer models. The sole requirement was preservation of the *E* configuration of the double bond, which was known to be essential (see Scheme 2, **1** versus **2**). Only the sp²-hybridized nitrogen atom was new. The first representatives (**20a** and **21a**) of structure types **20** and **21** (Scheme 9) had already been synthesized and subjected to

greenhouse tests. From the good results obtained *in vitro* and with initial greenhouse tests we were confident of the high biological potential of these oxime ether structures. In response to the situation with our known competitors at Zeneca, a corresponding patent application^[57] was very



Scheme 9. The first strobilurins (**20**–**22**) with an oxime ether pharmacophore of general structure **23** synthesized at BASF on the way to kresoxim-methyl.

quickly submitted in July 1986—a stroke of luck for us, as we later discovered. What we did not know at the time, but must have feared all too well, was that the Zeneca group had already been pursuing the same idea and had themselves been in the process of submitting a patent application for the oxime ethers.^[58] We then had to get through the usual nerve-racking 18-month waiting period following the submission of our patent.^[42] Finally in January 1988 it emerged that this time we had got there first with our submission, by a mere two days—it looked like round two of the contest may well have gone decidedly in our favor.

In the meantime further progress had been made at BASF with the optimization of the oxime ethers. For the 1988 field-trials season two derivatives of **20** (**20b** and **20c**) had been penciled in for small-plot trials on the basis of their good activity against powdery mildew of wheat in the greenhouse tests. This activity was confirmed, and was comparable to that

of the standard Corbel and thus approached the level required for a viable competing product.

A decisive factor in our continuing efforts towards optimization of the lead structure was that from June 1987 onwards a biochemical test was available at BASF^[59] which compared with the established greenhouse tests, or the field trials for that matter, was able to provide much more direct answers to the question of how effectively the strobilurin variants bind to the mitochondrial bc_1 complex. This test utilized submitochondrial preparations of yeast cells as model systems for the respiratory chain of fungi. The end result quantifies the inhibitory activity of the test substance towards the bc_1 complex in the form of an I_{50} value, which is the concentration of the active substance necessary to achieve 50% inhibition: the lower the I_{50} value, the more active the compound.^[60]

With the aid of this test, we were soon able to deduce some very clear structure–activity relationships (see also Section 4.1). A series of comparisons between pairs of enol ethers and oxime ethers with identical side chains—the arene bridge was a phenylene moiety in all cases—showed the two pharmacophore types to have equal affinities on the target level, giving rise to no appreciable differences in activity. The two pharmacophores thus bind equally strongly—a pivotal finding. Something else that emerged quite clearly, again from a series of pairwise comparisons, was that derivatives of type **21** were on average about ten times more active than the corresponding regioisomers **20**. This result could not have been deduced so clearly, if at all, from greenhouse tests in which the striking differences were blurred by the effects of various factors arising from the more complex study designs, thus being hidden so to speak by background noise.^[61]

These results were of crucial importance to our future studies. First and foremost, they swung things in favor of the oxime ethers **21** with a phenoxymethyl side chain for development within our synthetic program. Secondly, they led us to focus on variants of the more active structure type **21** in the selection of the candidates for field trials during the 1989 season. Ten variants (**21b**–**21k**) plus the heterocyclic derivative **22** were subjected to field trials. Expectations were high, as remarkable results had already been obtained in the 1988 field-trials season with two derivatives of type **20** that had shown lower activity on the target level. These expectations were borne out in full: All variants of type **21** proved active to a lesser or greater degree in the field trials, particularly towards cereal mildew, but also towards other cereal infections such as wheat leaf spot and leaf blotch. Comparisons with **20b** and **20c**, the two compounds of the previous generation with a benzyloxy side chain, demonstrated clearly the superiority of the new type **21** over **20** under outdoor conditions. Furthermore, in the tests against powdery mildew of wheat the activity of the oxime ethers **21** was clearly greater than that of some of the enol ethers employed for comparison purposes.

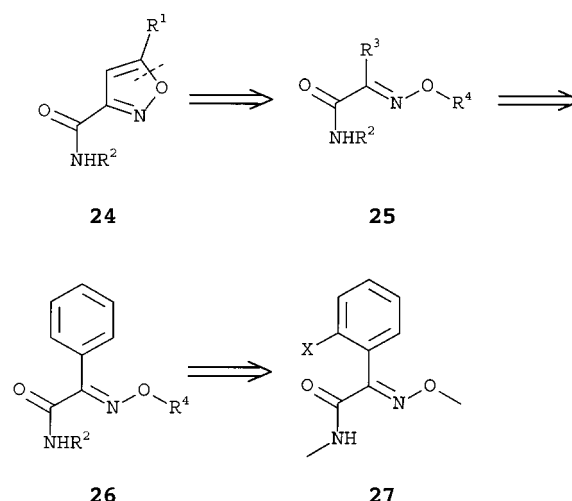
Of the outstandingly effective active substances of type **21**, it was **21b** (later named kresoxim-methyl) that for economic and other reasons was selected as the candidate for development. This compound represented an optimal combination of high fungicidal performance and a favorable outlook for a

cost-effective commercial synthesis, the sole synthon required for the construction of the side chain being the cheap bulk chemical *o*-cresol.^[62]

Compound **21b**, initially developed under the code number BAS490F, was later given the common name kresoxim-methyl and, again in competition with Zeneca, was subsequently registered by the German authorities and launched onto the market in February 1996 a few days before azoxystrobin. Details of its biological properties and resultant market positioning are presented in Section 5.3.

3.6. The Entry of the Shionogi Team into Strobilurin Chemistry^[63]

Rather by chance, and from another direction and different route entirely, fungicide researchers at Shionogi focused on the strobilurins. In their case it was not the natural products strobilurin A and oudemansin A with their extremely promising biological activities that had been the starting point, but a totally different, “in-house” class of compounds, the carbamoylisoxazoles **24** (Scheme 10).^[63] These compounds



Scheme 10. Steps in Shionogi's sequence of thoughts for the synthesis of oxime ether amides **27**.^[63]

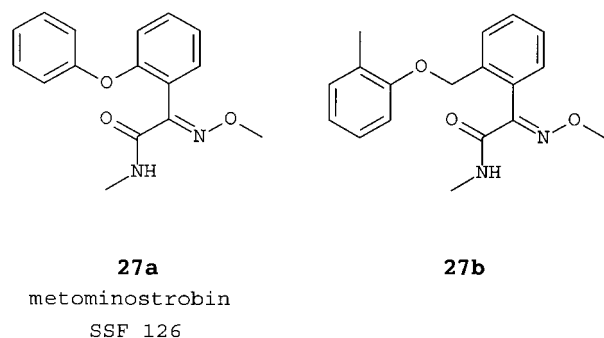
had shown fungicidal activity against rice diseases, but there was no apparent connection with strobilurins. Compounds of type **24** lack two of the properties typical of strobilurins: their broad fungicidal spectrum of activity and their characteristic mode of action in the inhibition of respiration. In the variation of structures of type **24**, trains of thought set in motion largely by purely chemical structural considerations and the efficient deployment of in-house synthetic know-how led to new structures and biological testing in the Shionogi team, culminating in the oxime ether amides of type **26** by the route outlined in Scheme 10.^[63] The structure of these compounds already resembled that of the oxime ether strobilurins very closely (see, for example, **4**, **20**–**23**), hence it is no surprise that systematic variation by the Shionogi team ultimately led to compounds of type **27** and to the recognition of their biological potential and the connection with the BASF

and Zeneca strobilurins. In the knowledge^[63b] of the oxime ether patents published in early 1988^[57, 58] and of the other strobilurin patents published at that time, the Shionogi group submitted their own patent application,^[64] based in essence on the general structure **27**. With **27** a new type of pharmacophore, in the shape of the oxime ether methyl amide, had been discovered that was isosteric with the oxime ether methyl esters.

In the course of their own studies on lead structure optimization, the BASF team had, in modifying the oxime ether pharmacophore, likewise turned their attention to the corresponding amides. However, they arrived on the scene late, as the optimization of the side chain in the development of BAS490F had been the top priority.

As the first and initially solitary variant of type **27**, compound **27b**, with the side chain that in the case of **21b** had proved optimal in the oxime ether series, was specifically selected and synthesized in late 1989 by BASF. The greenhouse test results were good, but not outstanding, and in the yeast test the I_{50} value of **27b** was more than ten times lower than that of **21b**. Because of this, and in the face of more attractive alternatives, it seemed unnecessary at the time to attach particular priority to this type of compound—a colossal error. It was only after further derivatives with better activities had come to light, though before the publication of the Shionogi claim,^[64] that a corresponding patent application was filed by BASF in late 1990.^[65] This led once more to patent interferences in the strobilurin field.

The efforts of the Shionogi team ultimately led to a development product with the code number SSF126^[66] (Scheme 11). This compound, subsequently received the common name metominostrobin, was launched onto the Japanese market in 1998 under the trade name oribright as a fungicide active against rice diseases.



Scheme 11. The Shionogi strobilurin **27a** and the first variant synthesized at BASF with an oxime ether amide pharmacophore (**27b**).

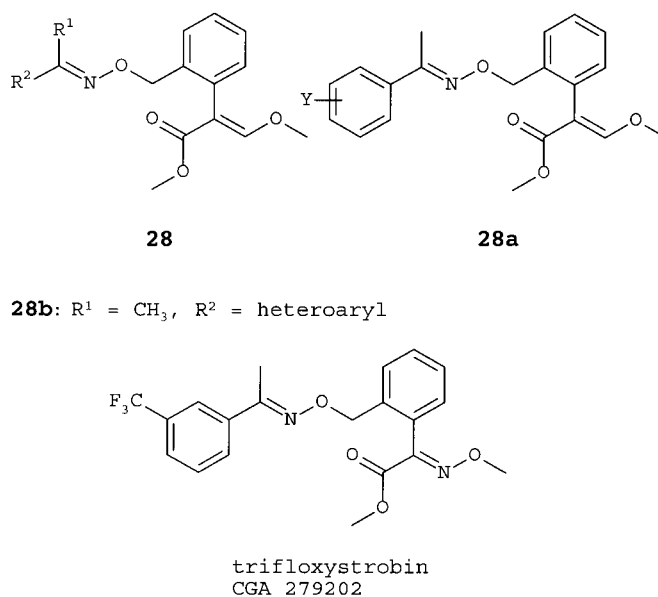
3.7. The Route of Novartis to Their Own Strobilurins

Four completely different aspects of this route merit particular attention:

- In the course of studies aimed at the identification of new, biologically active natural products, scientists at Ciba-Geigy had already isolated strobilurins in the 1980s and identified their antifungal activity,^[66] but at the time had elected not to follow them up any further.

- Quite independently, and clearly soon after the publication of the Zeneca basic application on the enol ethers,^[41] a fungicide synthesis group at Maag, at the time still a subsidiary of Hoffmann-La Roche, had discovered a gap in the Zeneca claims, and after charging through this gap with their own program of research, had submitted their own application at the turn of 1988/1989.^[67b] Company acquisitions then saw the Maag synthesis group along with the patents they held transferred first in 1990 to Ciba-Geigy and then, following the merger with Sandoz in 1996, to Novartis: a happy homecoming of the strobilurins to the former Ciba-Geigy?^[68]

- The core of the Maag application^[67b] is illustrated by structures **28–28b** (Scheme 12), and comprises the introduction of a new side chain into the enol ether—classic “me too”



Scheme 12. Strobilurins with the new oxime ether side chain. Structures **28–28b** illustrate the core of the two conflicting patent applications submitted by Zeneca^[67a] and Maag^[67b] only five weeks apart.

chemistry.^[69] Although it is disputable whether the side chain in **28** sidesteps the term “optionally substituted alkyl” used in the Zeneca basic application^[41] and is therefore safe from legal challenge, it is undeniably a substituted methyl group. What is most striking about the strobilurins with an oxime ether side chain is their somewhat higher biological activity,^[70] which, particularly in the case of derivatives of type **28a**, compares well even with the earlier, previously optimized phenoxy and phoxymethyl side chains (for example **27a, b**, Scheme 11).

- From a patent point of view, the period that followed saw a curious state of confusion develop around this oxime ether side chain. The Zeneca group had in the meanwhile also identified the high potential of this side chain, and had submitted a corresponding patent application^[67a]—based on the enol ether pharmacophore—some five weeks *ahead* of the Maag team. Further competitors joined in this almost grotesque competition, all completely in the dark as to the timing of the rival applications.^[42] All of a sudden the whole world seemed to have discovered the oxime ether side chain.

Within barely more two years eight patent applications had appeared from five different companies (Table 2).^[67] What was clear was that the development of the new pharmacophore variants had, with the usual time lag, in each case led to

Table 2. The first patents for strobilurin with an oxime ether side chain (see structures **28–28b** in Scheme 12).

First priority date	Patent holder (applicant)	Pharmacophore	Ref.
Nov. 21, 1988	Zeneca (ICI)	enol ether ^[a]	[67a]
Dec. 29, 1988	Novartis (Maag)	enol ether	[67b]
Aug. 22, 1989	Nihon Nohyaku	enol ether	[67c]
Nov. 2, 1989	Ube	enol ether	[67d]
June 5, 1990	Novartis (Maag)	oxime ether ester ^[b]	
		thioenol ether ^[c]	[67e]
June 27, 1990	BASF	oxime ether ester ^[d]	
		oxime ether amide ^[e]	[67f]
Aug. 22, 1990	Zeneca (ICI)	oxime ether ester	[67g]
Jan. 30, 1991	Zeneca (ICI)	oxime ether amide	[67h]

[a] See structure **XII** in Scheme 6. [b] See structure **23** in Scheme 9. [c] The pharmacophore has the *E*-configured formula $\text{—C(COOCH}_3\text{)=CHSCH}_3$. [d] At the priority date the Zeneca enol ether application^[67a] had already been published (date of publication May 1990). The claims for the enol ether initially included in the BASF application were for the most part withdrawn during the patent examination process. [e] See structure **27** in Scheme 10.

the submission of corresponding patent applications for the oxime ether side chain. An important point for Novartis was that the Maag team had managed to submit their application^[67e] for the combination of an oxime ether ester pharmacophore and an oxime ether side chain three weeks ahead of BASF^[67f] and eleven weeks ahead of Zeneca.^[67g] The question of the Novartis compounds being dependent on the first, in the general claims very broadly defined, Zeneca patent on the strobilurins with an oxime ether pharmacophore^[58] was settled in favor of Novartis, as the Zeneca patent^[58] had recently been revoked by the Board of Appeal of the European Patent Office. An active substance from this group that is in the advanced stages of development at Novartis under the code number CGA279202^[71] has been given the name trifloxystrobin (Scheme 12) and is currently in the process of registration in the USA.

4. Structure–Activity Relationships as a Driving Force in the Lead Structure Optimization

4.1. Molecular Characteristics and Activity Variables

The activity of a fungicide under real-life conditions in a complex system of the fungal pathogen, the plant, and the environment is determined by a multitude of quite different influencing factors.^[72] In the optimization of the lead structure it is useful to separate the complex of such influencing factors and the associated molecular properties in two parts.^[73] A decisive factor on the one hand is the affinity of the respective active substance for its molecular target in the fungus. In the case of the strobilurins this is primarily determined by the binding affinity of the substance for the bc_1 complex of the respiratory chain. On the other hand, just as important for the

in vivo activity under real-life conditions is how much of the active substance actually succeeds in reaching its target. Of critical importance here is the biokinetic behavior of the active substance, that is, the particular characteristics of the molecule that govern its absorption, transportation, breakdown, and, where appropriate, excretion.

Changes to the lead structure affect not only the target affinity of the active substance, but also, in a variety of ways, the biokinetic properties of the molecule. This ultimately influences the activity towards fungal pathogens in vivo, and has consequences for both the selectivity towards nontarget organisms (for example plants and beneficial insects) and for the environmental behavior (for example degradation and translocation in the soil and volatilization).

For a rationally conceived lead structure optimization it is important to consciously separate all these influencing factors, and wherever possible to experimentally establish at an early stage the associated molecular characteristics and their effects on target activity and the individual biokinetic parameters, and to exploit them in the optimization of the active substance structures with respect to their activity and environmental tolerability. Of particular importance for structure–activity analyses are the following, easily measurable molecular properties:

- The melting point to assess the rate of dispersion and bioavailability.
- The vapor pressure as a measure of the volatilization profile and quasisystemic mobility (see below) on the surface of the leaf.
- Lipophilicity and water solubility to determine the absorption and transportation behavior.
- The photostability to establish the lifetime of the active substance, especially under outdoor conditions.

With regard to the observable biological effects the following individual and complex parameters are of particular interest:^[74]

- The activity on the level of the molecular target. For the strobilurins this means the binding constant for the bc_1 complex or—a more easily measurable option—the active substance concentration necessary for 50 % inhibition of an appropriate enzyme preparation (the I_{50} value, for example from the test on yeast mitochondria used as a model system;^[60] see Section 3.5).
- The activity on the cellular level of the target organisms, for example the inhibition of respiration or of the growth of fungal cultures on artificial nutrient media in the laboratory.
- The rate of metabolic degradation (half-life) of the active substance in plant or fungal cell culture models or in whole plants.
- The proportions of the active substance on the surface and in the interior of the treated leaf and in untreated leaves of the plant after application onto a particular leaf as a function of time.
- The activity in greenhouse tests on plants infected with fungal pathogens.
- The fungicidal activity under outdoor conditions.
- The effects on aquatic and terrestrial nontarget organisms in laboratory tests.

- Degradation rates and translocation behavior in the soil (laboratory model).

This complex interplay between a multitude of variables means that particular caution must be exercised in the interpretation of test results. Structure–activity trends can often be discerned or guessed at only with great difficulty. For practical reasons it is hardly feasible to investigate all new test substances^[75] from every possible angle. At BASF only the yeast mitochondria test and the greenhouse tests are carried out routinely for all strobilurins. Of paramount importance therefore is the reasoned and systematic selection of candidate active substances for screening in additional, more elaborate test models.

Several examples based on different molecular variables have been published of structure–activity relationships for strobilurins in some of the tests mentioned.^[28, 45a–c, 76]

4.2. Variation of the Pharmacophore

In the identification of new pharmacophore variants the yeast mitochondria test has proved to be an extremely useful tool. To ensure the individual results were comparable, all test series were screened using a reference standard, the enol ether stilbene **7a**, against which the I_{50} value obtained for a test substance was referred:

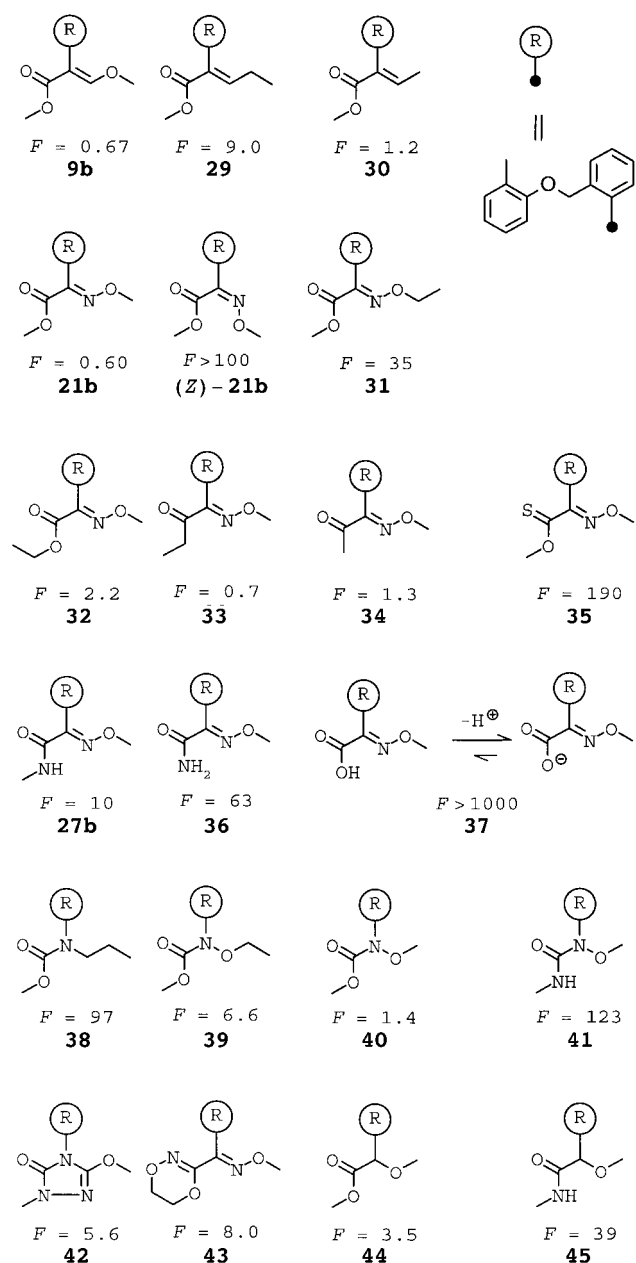
$$F = \frac{I_{50} \text{ (test substance)}}{I_{50} \text{ (7a)}}$$

By definition, $F=1$ for **7a**, and hence the smaller the F value, the higher the activity. Scheme 13 shows a small selection from the wealth of conceivable pharmacophore variants and should give some impression of the structure–activity relationships which are discussed here with respect to just a few, though important, aspects.^[77]

- What is most astonishing is the latitude with which the structures can be varied without incurring any fundamental loss of target activity. On a similar level of activity as the “classical” enol ether **9b** and oxime ether **21b** are the crotonic ester **30**,^[78] the ketones **33** and **34**,^[79] and the *N*-methoxycarbamate **40**.^[80a] The variants **27b**,^[64, 65] **41**,^[80] **42**,^[81] and **43**^[82] as well as the mandelic acid derivatives **44** and **45**^[83] also show remarkable activity.

- A drastic loss of activity is observed to a lesser or greater degree when the bulk of the substituents on either side of the periphery of the pharmacophore is increased (cf. **31/32** versus **21b**). The optimal size is clearly shown by truncated variants such as **30** (cf. **29**) and **40** (cf. **39**). The *E* configuration of the central double bond cannot be changed without suffering a catastrophic loss of activity, as shown by a comparison of (*E*)-**21b** and (*Z*)-**21b**.

- As is the case with the side chain variants (see Section 4.3, Figure 3), the activity of the pharmacophore variants is distinctly dependent on the lipophilicity of the molecule. Up to an optimum of $\lg P_{ow} > 4$, the activity increases with increasing lipophilicity, as exemplified by the isosteric ester/amide pairs **21b/27b**, **40/41**, and **44/45**. In each of these cases the ester is 0.7 $\lg P_{ow}$ units more lipophilic and shows correspondingly higher activity. In an assessment of the



Scheme 13. Strobilurins with the kresoxim-methyl side chain and variations on the pharmacophore. Their relative activities on the target level are given below the formula; F is inversely proportional to the activity (see text).

activity of the pharmacophore variants, the lipophilicity of the entire molecule must therefore be taken into account.

- The only essential heteroatom in the pharmacophore is the carbonyl oxygen atom, or an equivalent which also functions as a hydrogen-bond acceptor at the target. This is illustrated particularly clearly by the distinct loss of activity observed on going from $C=O$ to $C=S$ (cf. **21b** and **35**) and $C=CH_2$.^[77]

- The question of whether the $C=O$ bond points to the “northwest” (*s-trans* conformation) relative to the second double bond or to the “south” (*s-cis* conformation) is settled unequivocally in favor of the *s-trans* conformation, as evidenced by the high activity shown by the cyclic, fixed-geometry variant **42**.^[81a]

With regard to the biophysical and biokinetic properties of the individual pharmacophores, some important trends can likewise be discerned, that have consequences for the respective biological activities.

- When the remainder of the molecule is kept constant (®, see Scheme 13), the lipophilicity of the pharmacophore variants increases in the following order: cyclic variants (such as **42**, $\lg P_{ow} = 2.3$) < oxime ether amide (**27b**, $\lg P_{ow} = 2.8$) < oxime ether ester (**21b**, $\lg P_{ow} = 3.5$) < *N*-methoxycarbamate (**40**, $\lg P_{ow} = 3.7$) $\approx$ enol ether (**9b**, $\lg P_{ow} = 3.7$) < methyl ketone (**34**, $\lg P_{ow} = 4.0$) < crotonic ester (**30**, $\lg P_{ow} = 4.4$). The overall lipophilicity of the molecule not only influences the activity on the mitochondrial target level, but also has a considerable influence on absorption and transportation; this had to be taken into consideration in the design of new variants. Thus, in order to be in a lipophilicity area where systemic transport in plants is possible ($\lg P_{ow} < 4^{[49]}$), the lipophilic crotonic esters require side chains that are more hydrophilic than the intrinsically more hydrophilic oxime ether amides.

- The vapor pressure of the compounds is also determined to a considerable degree by the pharmacophore type. For example, in the case of the strongly polar oxime ether amides the vapor pressure is more than an order of magnitude lower than that of the corresponding oxime ether esters (**21b**: 2.3×10^{-8} hPa; **27b**: 7.5×10^{-10} hPa).^[28b] Because the vapor pressure correlates with the quasisystemic mobility of the active substance on the surface of the leaf,^[28b] it has a decisive influence on the distribution of strobilurins on the plants requiring protection. Consequently, a key practical consideration—particularly after protective application of a fungicide^[84d]—is to achieve a protective film that is as even as possible. Furthermore, for a larger number of strobilurins a correlation has also been demonstrated between vapor pressure and activity against powdery mildew of wheat^[28b] in more curatively oriented^[84d] field trials. Since powdery mildew of wheat is a fungal pathogen that grows largely on the surface of the leaf, this is understandable. Also understandable is the failure to observe—at least in curatively oriented studies—any corresponding correlation in the case of wheat brown rust, which prefers to grow inside the leaf.

- Likewise, for the rates of degradation of strobilurins in the soil and in plant and fungal cell cultures some general trends emerge in relation to the pharmacophore. The degradation rates tend to increase in the following order: cyclic variant (type **42**) $\leq$ oxime ether amide $\ll$ enol ether $\leq$ methoxycarbamate $\leq$ crotonic ester $\ll$ oxime ether ester. A consequence of this is that oxime ether amides that show extreme metabolic stability are characterized by a long duration of action under outdoor conditions. However, this is accompanied by a correspondingly high persistence in the soil—the reverse side of the coin. This long duration of action in the treated crops is ideal for systemic transport. Conversely, although the rapid metabolization of the oxime ether ester to the practically inactive carboxylic acid (cf. **37**, Scheme 13) prevents any marked systemic effects, it guarantees a particularly rapid degradation in the soil that is highly desirable, with half-lives ranging from hours to a few days.^[84a]

4.3. Variation of the Side Chain and the Arene Bridge

On the level of the target, clear, quantitative structure–activity relationships have been established in accordance with a bilinear equation (Figure 3) for a series of oxime ethers of type **21**.^[28a, 85] Similar correlations have been deduced for

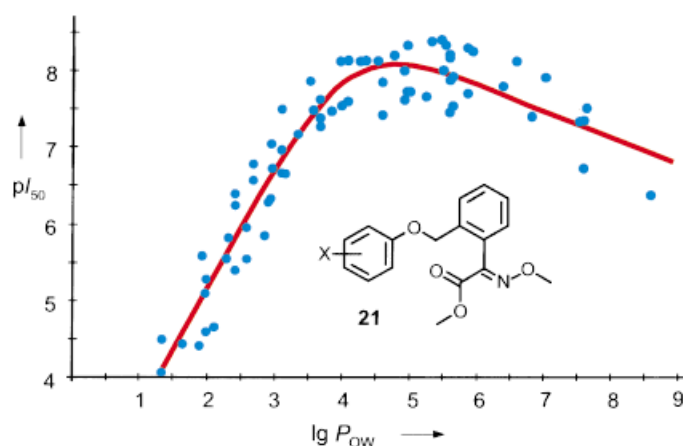


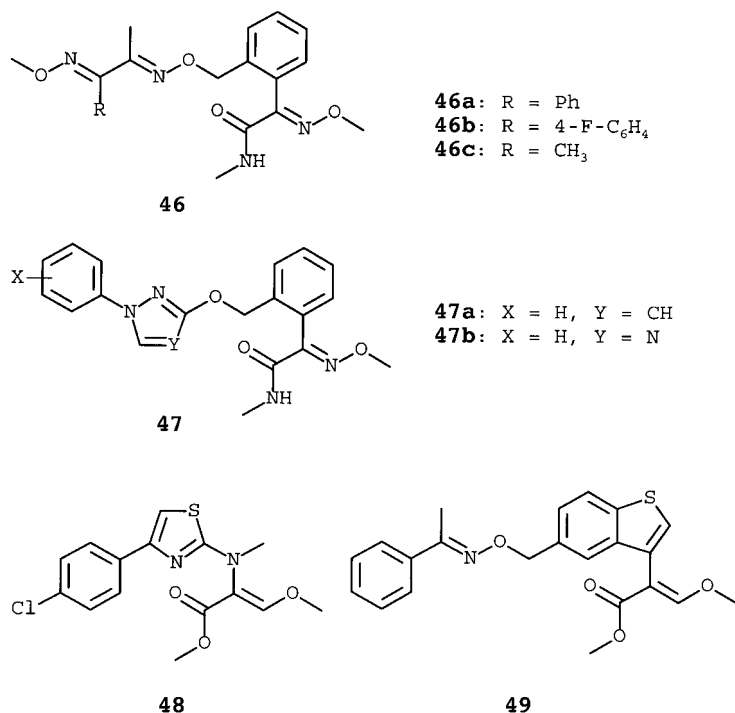
Figure 3. Target-level activity of a series of strobilurin oxime ethers **21** as a function of the lipophilicity of the substituents X. pI_{50} is the negative logarithm of the I_{50} value from the yeast mitochondria test. Each point represents a variant of structure **21**.^[28]

enol ethers of type **9**, oxime ether amides, and crotonic esters and methoxycarbamates.^[86] Thus, it is clearly the overall lipophilicity of the molecule—modified by the substituents X in the side chain—that is the critical influencing factor. Significant “underperformers” with respect to the curve shown in Figure 3 arise if, on account of certain substituents or substitution patterns X, the steric bulk of the side chain no longer permits optimal docking at the target.^[28a]

The influence of the arene bridge and the side chain on the biokinetic properties is illustrated, for example, by the route outlined in Section 3.4 followed by the Zeneca team in the optimization of the diphenyl ether **13a** to azoxystrobin.^[45a, c] A key element of their approach was the introduction of heteroatoms to specifically lower the lipophilicity of the molecule enough to allow systemic transportation, while keeping the attendant reduction in activity on the target level as low as possible.

A similar role is played by the heteroatoms in the oxime ether side chains already mentioned in Section 3.7, for example in compounds of type **28a**. Here, the side chain is two atoms longer than the phenoxymethyl side chains in **9**, without an accompanying drastic increase in lipophilicity:^[87] $\lg P_{ow}$ is 3.2 for **9a** and is only slightly higher at 3.6 in the case of **28a** (with Y = H). The extension of this principle to strobilurins with “double oxime ether” side chains such as in **46** led to derivatives with comparatively low lipophilicity ($\lg P_{ow} = 3.2$ for **46a**) and very good activities in field trials.^[88, 89] Even more hydrophilic side chains are present in **47a**^[90] ($\lg P_{ow} = 2.6$) and **47b**^[90] ($\lg P_{ow} = 1.5$).

The diverse range of variants that is possible without any fundamental loss of activity is illustrated by **48**^[91] and **49**,^[92] chosen more or less arbitrarily from the wealth of derivatives so far known (Scheme 14).



Scheme 14. Examples of strobilurins with variations of the side chain and aromatic bridge.

4.4. Variation of the Lead Structure and Selection of Candidates

In the evolution of a new class of active substance—as here with the strobilurins—an analogy can be drawn between the variations on the lead structure and the mutations that occur in the course of evolution in nature. The selection of individual compounds as candidates for the development of one or a handful of individual active substances to the point of marketability thus parallels the “survival of the fittest” (Figure 4). In the variation of the lead structure as well as in the selection of development candidates, optimal compromises must be found with regard to all the properties of the active substance. In both cases data obtained from studies of structure–activity relationships represent an important element of feedback control.

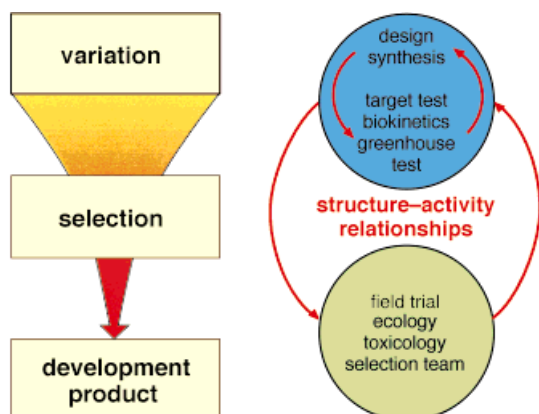


Figure 4. Schematic representation of the evolution process in the course of a lead structure optimization.

Here it is necessary to analyze and, particularly with respect to the net effects under real-life conditions, develop an understanding of the impact that structural variations within the various regions of the molecule have on the individual observables at various levels (e.g. target level, biokinetics). This is primarily a challenge for the chemist planning his synthetic program. No less critical, however, is the consideration of such structure–activity relationships in the subsequent selection process. Here, as before, the complex network of interrelationships means that it is often less a question of clearly derivable, conclusive inferences, but of provisional decisions based on “soft” data or mere estimates, or even outright speculation. Empirical social research has shown that in such situations the assessments and decisions of a team are fundamentally superior to those of even the most apt individual.^[93] Therefore, in this selection process during the optimization of the lead structure, it makes sense to exploit this “team advantage”^[93] to the full through the assembly of interdisciplinary teams.

5. Biological Properties of the Strobilurins

5.1. Importance of the Mode of Action

For the biological action profile of strobilurins, their mode of action (the inhibition of respiration) is of decisive importance from a number of perspectives. In all eukaryotes the bc₁ complex forms part of the mitochondrial respiratory chain. This poses the question as to the extent to which the structure of the bc₁ complexes of different species affect the binding affinities of individual strobilurin variants and hence result in selective toxicity on the target level. The submolecular intricacies of this question can be expected from knowledge of bc₁ complexes through crystal structure determinations at increasingly higher resolution.^[23, 94] The relevant amino acid sequences known so far for various species show a high degree of preservation in the region of the binding envelope.^[20, 95] With submitochondrial enzyme preparations of various species (fungi, housefly, rat, and maize), tests carried out on 14 strobilurins plus myxothiazole showed that *no* appreciable contributions to species selectivity can be expected at the target level.^[28, 60] On the other hand, the natural abundance of fungi species resistant to bc₁ inhibitors clearly derives from mutations of the bc₁ complex that prevent the docking of strobilurins in the binding envelope.^[20]

Consequently, the appearance of resistant plant pathogens in which similar mutations are present cannot be ruled out. The question of whether, and to what extent, this will be of importance in the development of serious resistance towards strobilurins under the selection pressure of continued strobilurin treatments in agricultural practice will only be answered through experience in the field over the coming years.^[96]

5.2. Physiological Consequences of the Mode of Action

On the biochemical level the mode of action for the inhibition of respiration results in the arrest of ATP forma-

tion, which is coupled to the respiratory chain. The consequence on the physiological level is a particularly pronounced inhibition of growth processes that are strongly energy-, and hence, respiration-dependent. These include not only the growth of tumor cell lines, but in particular the germination of fungal spores. The strobilurin BAS490F has been shown in several fungal species to completely inhibit spore germination at considerably lower active substance concentrations (factors of between 10 and 10000) than are required for 50% inhibition of mycelial growth in the same species.^[28b] Since among eukaryotes spore germination occurs almost exclusively in fungi,^[97] this accounts on the physiological level for the selectivity that is so important for practical applications.

5.3. Action Profiles and Practical Applications of Strobilurins

Because of the high vulnerability of the fungal spore germination to strobilurins, these compounds generally show a remarkably broad action potential against virtually all phytopathogens, particularly when applied early as a protective measure. Nevertheless, the maximum activities achievable in practical use as an agricultural fungicide and the associated ecological profile will depend to a considerable degree on the biophysical and biokinetic characteristics of the active substance in question.

- Kresoxim-methyl stands out in this respect on account of two properties. The still measurable vapor pressure (2.3×10^{-8} hPa) and relatively high lipophilicity ($\lg P_{ow} = 3.5$) of the active substance allow distribution processes between the lipophilic waxy cuticle of the plant and the gas-phase “boundary layers” immediately above. Although the biokinetic properties of kresoxim-methyl prevent it from undergoing systemic transport *within* the plant (see Section 4.2), diffusion into the gas phase is sufficient for migration and distribution processes to take place *on* the surface of the plant, thereby imbuing it with quasi-systemic mobility.^[28b] The second outstanding property of kresoxim-methyl is its rapid metabolic breakdown in nontarget organisms to the virtually inactive carboxylic acid. Thus, the inability to undergo systemic transportation within the plant is offset by high selectivity and a favorable ecological profile.^[84]

In its practical applications kresoxim-methyl is characterized—particularly if applied protectively—by a broad spectrum of activity against a wide range of phytopathogens.^[98] Peak activities—even for curative application after an infection—are achieved against cereal mildew, which grows on the surface of the leaf, and against apple scab at application rates of below 125 g ha^{-1} . Its key practical advantage is the increase in yields achievable particularly in cereal cultivation, which are distinctly higher than might be expected on the basis of its fungicidal effects alone.^[99]

Kresoxim-methyl finds use in commercial products such as Discus (marketed as Stroby in Japan) as the sole active ingredient particularly in fruit production, in Brio and Mentor as a mixture with the BASF amine fungicide fenpropimorph for the control of cereal mildew, and in a three-component mixture with Fenpropimorph and the BASF triazole fungicide

epoxiconazole in the broad-spectrum cereal fungicide Juwel Top. The use of kresoxim-methyl in mixtures serves both to expand the activity profile of the product and to help prevent the potential development of resistance.

- Azoxystrobin possesses distinctly different biophysical and biokinetic properties and a different, to a certain degree complementary, spectrum of activity.^[45e, 100] Its vapor pressure is considerably lower (1.1×10^{-12} hPa at 25°C), and consequently its activity, against cereal mildew, is only moderate. A slower rate of metabolic breakdown in the plant and the relatively low lipophilicity ($\lg P_{ow} = 2.5$) allows systemic transport in the treated plants. Marketed under trade names such as Amistar, Bankit, and Heritage, azoxystrobin is used to combat a wide range of phytopathogens in numerous agricultural crops.

- Metominostrobin possesses an even lower lipophilicity ($\lg P_{ow} = 2.3$) than azoxystrobin, and is about 20 times more soluble in water.^[20e, 101] Its oxime ether amide group endows the pharmacophore with particularly high metabolic stability. After application onto the water surface of rice paddies or onto the soil of seedling boxes it is absorbed through the roots into the rice plants, where, following systemic transportation, it is able to fight off fungal pathogens from within.^[101] It is used in Japan under the trade name Oribright particularly against blast disease *Pyricularia oryzae* and other diseases of rice.

- Like kresoxim-methyl, CGA 27 92 02 (trifloxystrobin) has an oxime ether ester pharmacophore. Compared with kresoxim-methyl it has higher lipophilicity ($\lg P_{ow} = 4.5$), a similar vapor pressure (3.4×10^{-8} hPa), and similar transport and distribution properties. It is used under the trade name FLINT against a range of fungal diseases in various agricultural crops.^[71]

6. Industrial Strobilurin Syntheses

A summarizing account describes the known processes for the construction of enol ether, oxime ether, and oxime ether amide pharmacophores as well as palladium-catalyzed couplings of entire pharmacophores to the bridging arenes *ortho* to preexisting side chains.^[45e] Key steps in an elegant synthesis of kresoxim-methyl are the irreversible lactone ring opening of phthalide with cresolate under alkaline conditions and the Pinner reaction of an acyl cyanide during the subsequent construction of the pharmacophore.^[28a, 102] In an interesting synthetic variant yielding compounds of the trifloxystrobin type, the coupling of the side chain and pharmacophore is achieved simultaneously with the construction of the arene bridge by means of a Diels–Alder reaction.^[103]

7. Economic Importance, Summary, and Outlook

What is already quite clear is that the strobilurin class of active substances represents one of the most significant product innovations in crop protection. A consequence of this is that virtually all the important crop-protection companies are now engaged in research in this field. Over 500 international patent applications have been published to date

from more than 20 companies and research institutes (Figure 5 and reference [45e]). Worldwide, industrial chemists have probably synthesized, as a conservative estimate, well over 30000 strobilurin analogues so far.

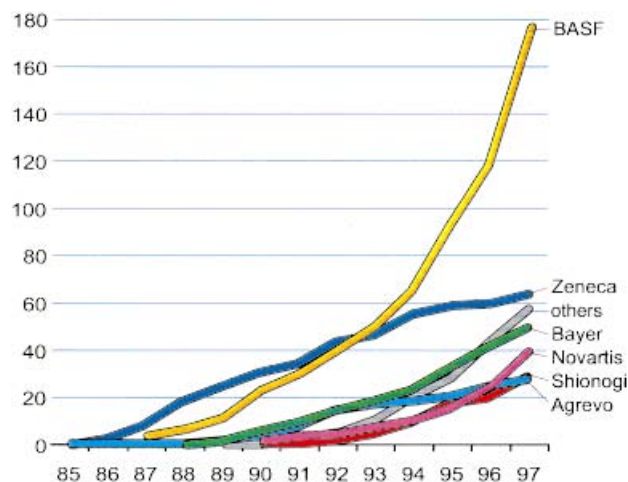


Figure 5. Number of published international strobilurin patent applications from 1986 to 1997.

The mode of action, which is novel for a fungicide, is based on inhibition of the cellular respiration of the fungal pathogen and thus a valuable asset in the management of resistance to established fungicides. It is also the reason for the particularly high vulnerability of the strongly respiration dependent spore germination of the fungus towards these active substances; this helps account for the selectivity of their action against fungi as the target organisms, a decisive factor for the generally very good environmental tolerability of strobilurins.

With their broad spectrum of activity, long duration of action, high activity at low rates of application, and outstanding environmental tolerability, the strobilurins have set new standards in the control of fungal diseases, thereby making a key contribution to integrated crop-protection strategies. Agricultural enterprises have taken this innovation on board with enthusiasm.^[104] Estimates of its future market potential are correspondingly high. By 2006—that is, ten years after the market launch of the first two strobilurins kresoxim-methyl and azoxystrobin—annual sales worldwide may well total over two billion German marks, if we include all the future products expected to arise from the international competition. This figure represents approximately a 20% share of the world fungicide market. Thus, provided there is no unexpected counterproductive development of resistance, the strobilurins might in the near future assume the number one position in the market, which is currently still held by the triazoles as the largest class of fungicidal active substances.

The story of strobilurin research is in many respects also a tale of applied chemistry. It describes the evolution of a new class of active substance, and is both exciting and stimulating. It is full of impulses and results, as the researcher might wish, but it is also packed with wrong turns, false dawns, disappointments, and questions that remain unanswered. These challenges as well as the provision of natural product lead structures of the strobilurin type should be regarded as a gift of nature.

In the spirit of the concluding words of this review, nature deserves our sincere gratitude. Our special thanks must, however, be reserved above all for those scientifically inspired colleagues and co-workers of our research groups mentioned in the text as well as in the notes and literature citations.

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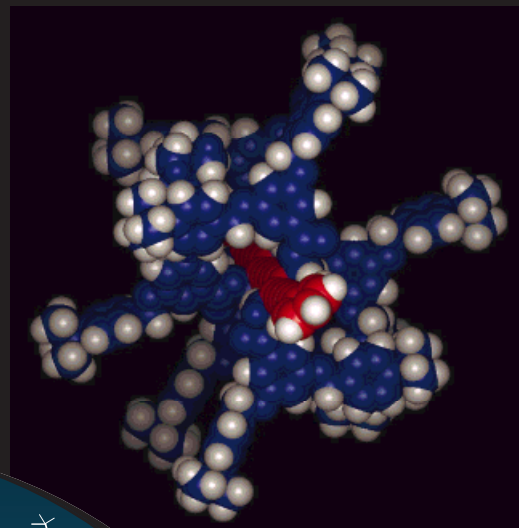
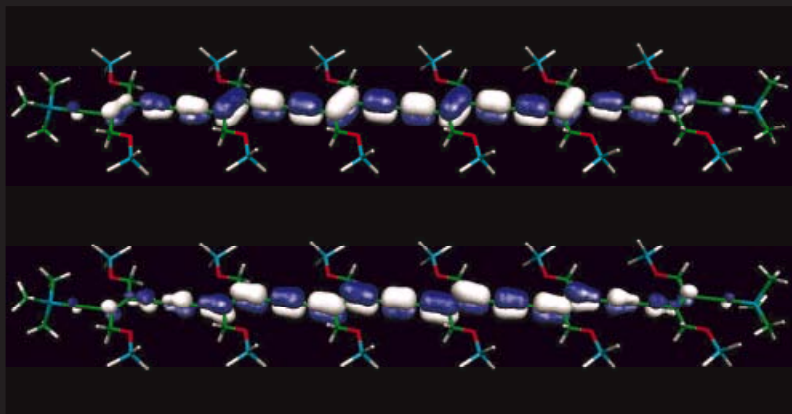
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- [36] This seemingly very serious difficulty faced by fungicide researchers increasingly became an issue during the lead structure optimization with the strobilurins, and led to an extensive reevaluation at BASF of the selection procedures adopted in going from greenhouse tests to field trials. H. Sauter is grateful to his many collaborators in this project for numerous interdisciplinary discussions on this matter, in particular the head of biological fungicide research at BASF, G. Lorenz, for her helpful support and insightful contributions.
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- For the definition of R_F and R_G , see Table 1; n is the number of compounds whose test results correlate with one another. In cases with equal rankings within columns, r_s was corrected in accordance with equation 9.4 on p. 207 of the book cited above.
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- [42] For a European patent application, there is an 18-month time lag between submission (first priority date) and publication. Following the priority date of his own application the submitter must therefore wait eighteen months before he can be certain there are no competing patents in the pipeline ahead of him.
- [43] A colleague jokingly remarked that the additional “optionally substituted” may well have been forgotten in the case of hydrogen.^[41]
- [44] From this point onwards reference is made in the text only to Zeneca, even though “ICI” may be technically more correct in the chronological sequence of events. b) In 1992/1993 ICI was split into two separate companies. The chemicals business continue to trade under the name ICI, and the life sciences enterprises were spun off and have thereafter operated under the name Zeneca.
- [45] The Zeneca team has published a number of excellent reviews describing their work on the strobilurins. Reference [45e] also gives an overall account of the historical course of strobilurin research. a) “Fungicidal β -methoxyacrylates: From natural products to novel synthetic agricultural fungicides”: K. Beauteament, J. M. Clough, P. J. de Fraine, C. R. A. Godfrey, *Pestic. Sci.* **1991**, *31*, 499–519. b) “Fungicidal β -methoxyacrylates: From natural products to novel synthetic agricultural fungicides”: J. M. Clough, P. J. de Fraine, T. E. M. Fraser, C. R. A. Godfrey, *ACS Symp. Ser.* **1992**, *504*, 372–383; c) “Role of Natural Products in Pesticide Discovery. The β -Methoxyacrylates”: J. M. Clough, A. D. Evans, P. J. de Fraine, T. E. M. Fraser, C. R. A. Godfrey, D. Youle, *ACS Symp. Ser.* **1994**, *551*, 37–53; d) “Fungicidal β -Methoxyacrylates. N-Linked Pyrroles”: K. Beauteament, J. M. Clough, P. J. de Fraine, C. R. A. Godfrey, *ACS Symp. Ser.* **1995**, *584*, 326–342; e) “The Strobilurin Fungicides”: J. M. Clough, C. R. A. Godfrey in *Fungicidal Activity* (Eds.: D. H. Hutson, J. Miyamoto), Wiley, Chichester, **1998**, pp. 109–148.
- [46] One of us (T. A.) received an ICI Plant Protection Division report (*Evaluation of Oudemansin for antifungal and anti-bacterial activity, January 1984*) from the German subsidiary of ICI.
- [47] J. M. Clough, I. T. Kay (ICI), EP-A 178 808, **1984**, (priority dates October 18, 1984, May 23, 1985).
- [48] a) P. DeFraine, B. K. Snell, J. M. Clough, V. M. Anthony, K. Beauteament (ICI), EP-A 206 523, **1985** (priority dates August 18, 1985, April 17, 1986); b) J. M. Clough, C. R. A. Godfrey (ICI), EP-A 212 859, **1985** (priority date August 22, 1985).
- [49] a) The transport of dissolved substances in higher plants essentially takes place within two separate transport systems. In the normally fast-flowing transpiration stream of the xylem (a system of vascular

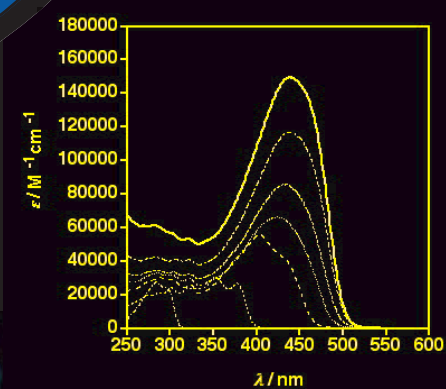
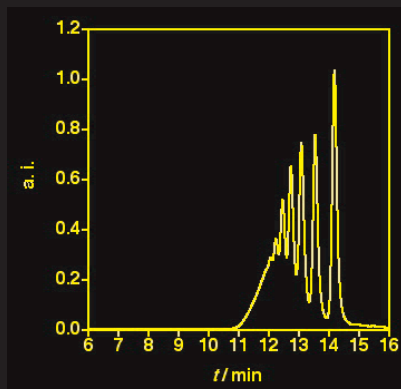
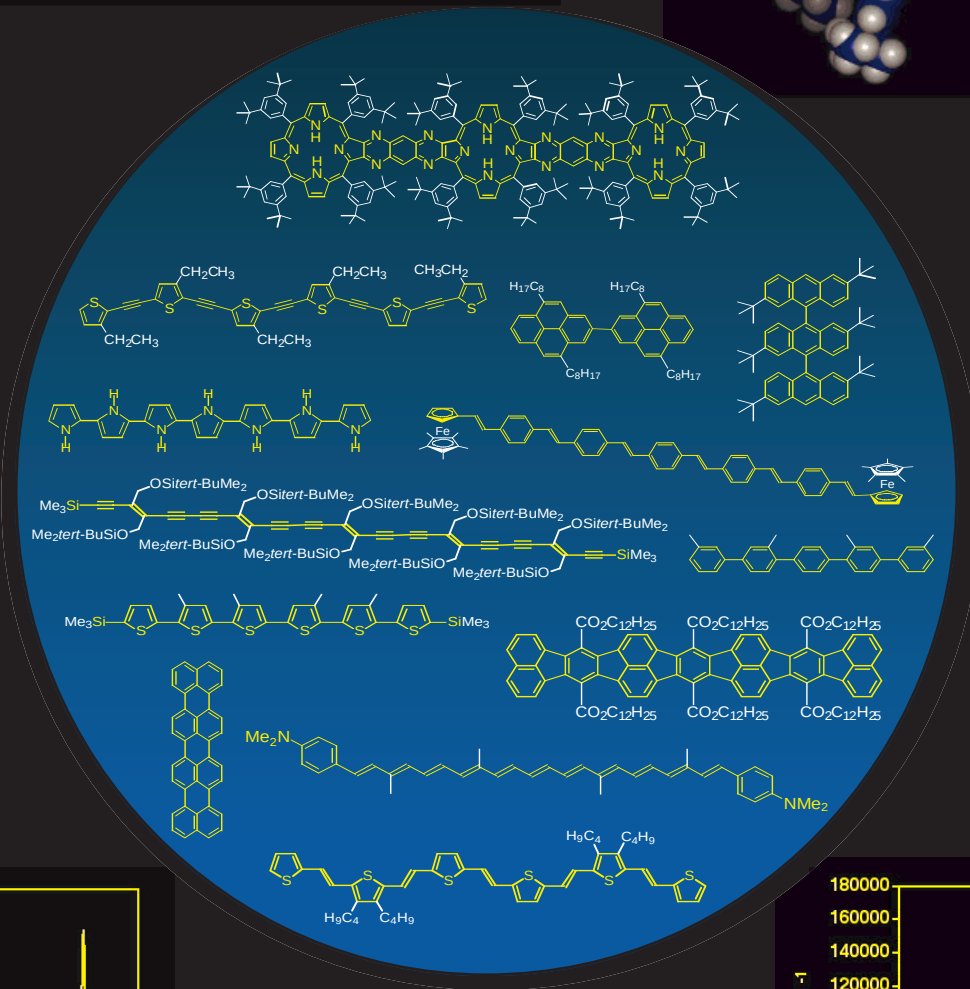
- bundles extending from the root to the tips of the leaves) minerals absorbed from the roots area are transported in an upwards direction (acropetally) towards the tips of the leaves. Transport in the other direction (basipetally) is possible only within the phloem system of the plant (sieve tubes). This is essential for example for the transport of assimilates from the place of their biosynthesis in the leaves in a downwards direction towards the leaf sheath and stem; see H. Mohr, P. Schopfer, *Pflanzenphysiologie*, Springer, Berlin, **1992**, pp. 487–517. b) For the systemic distribution of xenobiotics (e.g. fungicides) after application onto the leaf, the first step is generally *penetration* from the leaf surface through the waxy layer of the cuticle into the interior of the leaf, and thence into the aqueous phases of the xylem and phloem. This biophysical process corresponds in essence to a distribution process between three phases: an external aqueous or gaseous phase, the lipophilic cuticle, and the internal aqueous sap of the plant. For such processes there is consequently an optimal range for the lipid–water partition coefficients of the active substance in question, which corresponds approximately to their octanol–water partition coefficients (P_{ow}). For fungicides applied through the leaves, $\lg P_{ow}$ values of about 2–4 can be regarded as optimal; see “Principles of uptake and systemic transport of fungicides within the plant”: F. Jacob, S. Neumann in *Modern Selective Fungicides* (Ed.: H. Lyr), Longman Scientific & Technical, Essex, **1987**, pp. 13–30. c) A prerequisite for efficient transport of xenobiotics in the xylem of the plant is adequate solubility in water, which should generally be greater than 10 mg L^{-1} (correlating with $\lg P_{ow}$ values below 4). However, for individual active substances with extremely high activities, lower aqueous solubilities can sometimes be sufficient for the macroscopic observation of systemic effects, even though the amount of active substance undergoing transportation may be very low. d) For efficient basipetal transport of xenobiotics in the phloem, the water solubility of the active substance must, in comparison, be distinctly higher still. Since this is a process with a low rate of transportation, the substance must remain present in the phloem for a sufficiently long time. This will be the case if an active substance itself has particularly high water solubility (preferably greater than 10 g L^{-1} , correlating with $\lg P_{ow}$ values below 0) and/or is acidic (preferably with a $\text{p}K_s$ below 5) and is therefore present mainly as the anion in the slightly alkaline environment of the phloem (pH 7.4–8.7); see ref. [49b] and D. A. Kleier, *Pestic. Sci.* **1994**, *42*, 1–11. e) The somewhat opposing physical prerequisites on the one hand for penetration of the cuticle and on the other for transport in the aqueous systems of the plant means that highly lipophilic active substances ($\lg P_{ow} > 4$) will stand virtually no chance of systemic transportation. Conversely, active substances with very low lipophilicity ($\lg P_{ow} < 0$) are no longer easily able to penetrate the cuticle, and will require other routes of entry into the interior of the plant and/or special additives to the formulation; see P. J. G. Stevens, E. A. Baker, N. H. Anderson, *Pestic. Sci.* **1988**, *24*, 31–53; E. A. Baker, A. L. Hayes, R. C. Butler, *Pestic. Sci.* **1992**, *34*, 167–182. f) Systemic transport does, of course, mean that the active substance must have adequately high metabolic stability in the plant system to ensure its lifetime under these conditions is sufficiently long.
- [50] V. M. Anthony, J. M. Clough, P. DeFraine, C. R. A. Godfrey (ICI), EP-A 242 070, **1986** (priority dates April 17, 1986, January 22, 1987).
- [51] V. M. Anthony, J. M. Clough, P. DeFraine, C. R. A. Godfrey, P. J. Crowley, K. Anderton (ICI), EP-A 243 012, **1986** (priority dates April 17, 1986, January 26, 1987).
- [52] C. R. A. Godfrey, I. Streeting, R. Cheetham (ICI), EP-A 382 375, **1989** (priority date February 10, 1989).
- [53] V. M. Anthony, J. M. Clough, P. DeFraine, C. R. A. Godfrey, I. Ferguson, P. Crowley, M. G. Hutchings (Zeneca), EP-A 242 081, **1986** (priority dates April 17, 1986, December 23, 1986).
- [54] The value of $\lg P_{ow}$ is 3.3 for **13a**,^[45b] 5.1 for **13b**,^[45c] and 2.5 for **19**.^[45e]
- [55] However, as one knows, there where an emergency emerges, a source of rescue emerges as well (adapted from Friedrich Hölderlin, Patmos).
- [56] “Now it is no longer done with grumbling and cursing. (...) A despairing illness wants a daring medicine.” (translated from Friedrich Schiller in *Die Verschwörung des Fiesco zu Genua*, act 4, scene 6).
- [57] B. Wenderoth, C. Rentzea, E. Ammermann, E. H. Pommer, W. Steglich, T. Anke (BASF AG), EP-A 253 213, **1986** (priority date July 16, 1986).
- [58] V. M. Anthony, J. M. Clough, C. R. A. Godfrey, T. E. Wiggins (ICI), EP-A 254 426, **1986** (priority date July 18, 1986).
- [59] H. Sauter is indebted to F. Röhl both for the establishment and automation of this test, the reliable and expert testing of thousands of strobilurin variants, and the swift dispatch of the test results and their assessments.
- [60] For details of the test system, see a) F. Röhl, H. Sauter, *Biochem. Soc. Trans.* **1994**, *22*, 63S; b) ref. [28].
- [61] For the fundamental importance of biochemical test models in the discovery and optimization of lead structures, see for example J. Dancer, A. Schulz, T. Peine, K. Wright, *Pestic. Outlook* **1998**, *9*(3), 36–41, and references therein
- [62] For the synthesis see Section 6.
- [63] a) “Phenoxyphenyl alkoxyiminoacetamides. New broad spectrum fungicides”: Y. Hayase, T. Kataoka, M. Masuko, M. Niikawa, M. Ichinari, H. Takenaka, T. Takahashi, Y. Hayashi, R. Takeda, *ACS Symp. Ser.* **1995**, *584*, 343–353; b) M. Masuko, *Annu. Rep. Shionogi Res. Lab.* **1997**, *47*, 33–57.
- [64] Y. Hayase, T. Kataoka, H. Takenaka, M. Ichinari, M. Masuko, T. Takahashi, N. Tanimoto (Shionogi), EP-A 398 692, **1989** (priority dates May 17, 1989, December 29, 1989).
- [65] S. Brand, E. Ammermann, G. Lorenz, H. Sauter, K. Oberdorf, U. Kardorff, C. Künast (BASF AG), EP-A 477 631, **1990** (priority date September 22, 1990).
- [66] a) A. Fredenhagen, A. Kühn, H. H. Peter, V. Cuomo, U. Giuliano, *J. Antibiot.* **1990**, *43*, 655–660; b) A. Fredenhagen, P. Hug, H. H. Peter, *J. Antibiot.* **1990**, *43*, 661–667.
- [67] a) P. J. de Fraine, A. Martin (Zeneca), EP-A 370 629, **1988** (priority dates November 21, 1988, March 9, 1989); b) H. P. Isenring, S. Trah, B. Weiss (Hoffmann-La Roche), WO-A 90/07 493, **1988** (priority date December 29, 1988); c) K. Tsubata, N. Nijno, E. Endo, Y. Yamamoto, H. Kanno (Nihon Nohyaku), EP-A 414 153, **1989** (priority date August 22, 1989); d) M. Watanabe, T. Tanaka, H. Kobayashi, S. Yokoyama (Ube), EP-A 426 460, **1989** (priority dates November 2, 1989, February 27, 1990); e) H. P. Isenring, B. Weiss (Ciba-Geigy), EP-A 460 575, **1990** (priority dates June 5, 1990, April 23, 1991); f) S. Brand, U. Kardorff, R. Kirstgen, B. Müller, K. Oberdorf, H. Sauter, G. Lorenz, E. Ammermann, C. Künast, A. Harreus (BASF AG), EP-A 463 488, **1990** (priority date June 27, 1990); g) J. M. Clough, C. R. A. Godfrey, P. J. de Fraine (Zeneca), EP-A 472 300, **1990** (priority date August 22, 1990); h) J. M. Clough, C. R. A. Godfrey, P. J. de Fraine, I. R. Matthews (Zeneca), WO-A 92/13 830, **1991** (priority dates January 30, 1991, August 14, 1991).
- [68] BASF had also pursued a discreet interest in the acquisition of Maag, to no avail. At the forefront, however, of the interest shown by BASF was not the strobilurins, but the already existing fungicide business of Maag (e.g. Corbel).
- [69] This term is unfortunately still used too often in a pejorative sense. This is usually unjustified since the art of finding a usable gap in a tightly formulated patent claim demands rare creativity, and secondly because such “me too” variants could always lead to unusual and unexpected increases in activity, as was the case here (see text).
- [70] Compare with ref. [20e] and P. J. de Fraine, J. M. Clough, *Pestic. Sci.* **1995**, *44*, 77–79.
- [71] a) “CGA 279 202. A new Plant Health Product for Protection Against Diseases of Agronomic Crops, Vegetables, Tree Crops and Turf”: *Novartis Technical Bulletin* **1998**; b) “CGA 279 202: A new broad-spectrum strobilurin fungicide”: P. Margot, F. Huggenberger, J. Amrein, B. Weiss, *Abstr. Pap. Brighton Crop Prot. Conf. Pests and Diseases* (Farnham, UK) **1998**, pp. 275–382.
- [72] For an excellent introduction to this subject, see L. G. Copping, H. G. Hewitt, *Chemistry and Mode of Action of Crop Protection Agents*, The Royal Society of Chemistry, Cambridge, **1998**.
- [73] See for example “Optimization of Physicochemical and Biophysical Properties of Pesticides”: I. J. Graham-Bryce, *ACS Symp. Ser.* **1994**, *225*, 185–207.
- [74] H. Sauter is grateful to H. Köhle for numerous discussions and ideas in this context.

- [75] Over 15000 strobilurin variants have been synthesized at BASF to date.
- [76] a) H. Köhle, R. E. Gold, E. Ammermann, H. Sauter, *Biochem. Soc. Trans.* **1994**, 22, 65S.
- [77] A more detailed discussion is given in ref. [28a].
- [78] B. Wenderoth, H. Sauter, E. Ammermann, E. H. Pommer (BASF), EP-A 280 185, **1987** (priority date February 20, 1987).
- [79] R. Benoit, H. Sauter, R. Kirstgen, G. Lorenz, E. Ammermann (BASF), EP-A 498 188, **1991** (priority date February 7, 1991).
- [80] a) B. Müller, H. Sauter, F. Röhl, R. Dötzer, G. Lorenz, E. Ammermann (BASF), WO-A 93/15046, **1992** (priority dates January 29, 1992, June 26, 1992, October 5, 1992). b) Here there was further competition between rival patents. Ishihara's more weakly active *N*-alkylcarbamates were submitted first: T. Komyoji, I. Shigehava, N. Matsuo, H. Shimoharada, T. Ohshima, T. Akagi, S. Mitani (Ishihara), EP-A 498 396, **1991** (priority dates February 7, 1991, October 23, 1991). This was followed by our application.^[80a] Finally, Nihon Nohyaku also submitted an application for the *N*-methoxycarbamates: c) H. Ohnishi, S. Tajima, Y. Yamamoto, H. Kanno (Nihon Nohyaku), EP-A 619 301, **1993** (priority date April 4, 1993).
- [81] a) R. J. Brown, K.-M. Sun, D. A. Frasier (DuPont), WO-A 95/14009, **1993** (priority date November 19, 1993). b) Shortly after the publication of this application in May 1995 Novartis had submitted patent applications for strobilurins with similar cyclic pharmacophores and specific side chains: H. Ziegler, R. Zurfliuh, A. C. O'Sullivan (Novartis), WO-A 97/02255, **1995** (priority dates July 4, 1995, May 24, 1996).
- [82] B. W. Krüger, L. Assmann, H. Gayer, P. Gerdes, U. Heinemann, D. Kuhnt, U. Philipp, T. Seitz, J. Stetter, R. Tiemann, H. W. Dehne, S. Dutzmann, G. Häussler (Bayer AG), WO-A 95/04728, **1993** (priority dates August 11, 1993, March 10, 1994).
- [83] a) T. Ohtsuka, T. Murashi, S. Suzuki, M. Masuko, H. Takenaka (Shionogi), WO-A 95/27693, **1994** (priority dates April 6, 1994, July 7, 1994, November 4, 1994, January 27, 1995); b) K. Oberdorf, H. Sauter, H. König, A. Harreus, B. Müller, R. Kirstgen, W. Grammenos, H. Bayer, F. Röhl, G. Lorenz, E. Ammermann, V. Harries (BASF AG), WO-A 96/07633, **1994** (priority date September 10, 1994).
- [84] a) "The synthetic strobilurin BAS490F: profile of a modern fungicide": R. E. Gold, E. Ammermann, H. Köhle, G. M. E. Leinhos, G. Lorenz, J. B. Speakman, M. Stark-Urnau, H. Sauter in *Modern Fungicides and Antifungal Compounds* (Eds.: H. Lyr, P. E. Russell, H. D. Sisler), Intercept, Andover, **1996**, pp. 79–92; b) R. E. Gold, G. M. E. Leinhos, *Pestic. Sci.* **1995**, 43, 250–253. c) *Kresoxim-methyl*, active substance brochure of BASF, Ludwigshafen, **1993**. d) The terms "protective" and "curative" refer to the timing of the fungicide treatment, that is, whether it is before or after a fungal infection.
- [85] H. Sauter is indebted to H. Kubinyi for these and other QSAR calculations and for numerous other informative discussions.
- [86] W. Grammenos, H. Kubinyi, H. Sauter, unpublished results.
- [87] Furthermore, extending the bridging subunit linking the two aromatic rings of the phenoxy side chain in **13a** to the phenoxy-methyl side chain of **9a** increases the value of $\lg P_{ow}$ only by about 0.3.
- [88] Here too there was—at least in hindsight—a fascinating patent competition: a) H. Ziegler, S. Trah, S. Farooq, R. Zurfliuh (Ciba-Geigy), WO-A 95/18789, **1994** (priority dates January 5, 1994, July 1, 1994); b) H. Bayer, H. Sauter, R. Müller, W. Grammenos, A. Harreus, R. Kirstgen, F. Röhl, E. Ammermann, G. Lorenz (BASF AG), WO-A 95/21153, **1994** (priority dates February 4, 1994, June 17, 1994); c) H. Bayer, H. Sauter, R. Müller, W. Grammenos, A. Harreus, R. Kirstgen, F. Röhl, E. Ammermann, G. Lorenz (BASF AG), WO-A 95/21154, **1994** (Priority dates: 4.2.1994, 17.6.1994); d) H. Bayer, H. Sauter, R. Müller, W. Grammenos, A. Harreus, R. Kirstgen, F. Röhl, E. Ammermann, G. Lorenz (BASF AG), WO-A 95/21156, **1994** (priority dates February 4, 1994, June 17, 1994). e) See also ref. [81b], in which Novartis claims the special "double oxime ether" side chain with a "new" cyclic pharmacophore.
- [89] Despite ceding priority, BASF submitted its own patent applications for active substance mixtures with some of these strobilurins with a "double oxime ether" side chain, for example B. Schwalge, R. Müller, H. Bayer, H. Sauter, R. Saur, K. Schelberger, E. Ammermann, G. Lorenz, S. Strathmann (BASF AG), WO-A 97/06678, **1995** (priority date August 17, 1995).
- [90] R. Kirstgen, W. Grammenos, H. Bayer, R. Doetzer, H. Koenig, K. Oberdorf, H. Sauter, H. Wingert, G. Lorenz, E. Ammermann, V. Harries (BASF AG), WO-A 94/19331, **1993** (priority date February 23, 1993).
- [91] G. Kleefeld, A. Klausener, W. Krämer, W. Brandes, S. Dutzmann, G. Häussler (Bayer AG), EP-A 331966, **1988** (priority date March 5, 1988).
- [92] S. Trah, F. Gantz (Maag AG), EP-A 433233, **1989** (priority date December 14, 1989).
- [93] a) P. R. Hofstätter, *Gruppendynamik*, Rowohlt, Hamburg, **1957**, pp. 27–63; b) "Teamarbeit und Einzelarbeit aus Sicht der Arbeitspsychologie": B. Biäsch, *Ind. Org.* **1962**, 31, 139–142.
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- [96] a) "Fungicide Resistance": S. J. Kendall, D. W. Hollomon in *Fungicidal Activity* (Eds.: D. Hutson, J. Miyamoto), Wiley, Chichester, **1998**, pp. 7–108. b) Modern strategies for preventive resistance management include the use of mixtures and alternating treatments with fungicides of other active substance classes as well as pan-industrial cooperation and the agreement of application guidelines in FRAC (Fungicide Resistance Action Committee); compare with ref. [72], p. 111.
- [97] Besides fungi, only algae and mosses depend on spore germination.
- [98] "BAS490F – a broad-spectrum fungicide with a new mode of action": E. Ammermann, G. Lorenz, K. Schelberger, B. Wenderoth, H. Sauter, C. Rentzea, *Abstr. Pap. Brighton Crop Prot. Conf. Pests and Diseases, Vol. 1* (Farnham, UK) **1992**, pp. 403–410.
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Linear monodisperse π -conjugated oligomers



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Structure – property relationships

Linear Monodisperse π -Conjugated Oligomers: Model Compounds for Polymers and More

Rainer E. Martin and François Diederich*

Dedicated to Professor Hans-Jürgen Quadbeck-Seeger on the occasion of his 60th birthday

Research on monodisperse π -conjugated oligomers has dramatically expanded during the past decade. It has been fueled by advances in synthetic methodology, such as the development of metal-catalyzed cross-coupling reactions, and by advances in analytical techniques that now allow an accurate characterization of linear π -conjugated molecular rods with lengths exceeding 10 nm. An initial target of this research was the development of structure–property relationships to rationalize the properties of known high molecular weight π -conjugated polymers,

which are often poorly soluble and difficult to process, and to predict those of yet unrealized ones. However, in the meantime the study of well-defined monodisperse oligomers has advanced into a strong field of its own right, because of the promising electronic and optical properties of these compounds. Multinanometer-long π -conjugated oligomers are today seen as potential components in future molecular scale electronic devices, and their development has generated some spectacular molecular architecture. In this review, the recent progress achieved in

the synthesis and characterization of monodisperse π -conjugated oligomers is illustrated by featuring some of the most predominant systems. The manifold contributions of oligomer systems to today's understanding of polymer properties are discussed with a particular focus on how molecular properties of oligomers converge with increasing chain length to yield those of the infinite polymers.

Keywords: conjugation • molecular wires • oligomers • polymers • structure–property relationships

1. Introduction

During the past decade the number of π -conjugated polymers investigated as advanced materials for electronic and photonic applications has developed rapidly, and has caused an ever increasing interest from both academic and industrial research laboratories. The inherent synthetic flexibility, potential ease of processing, and the possibility of tailoring characteristic properties to accomplish a desired function makes them promising candidates for manifold applications in materials science. Thus, they are used as laser dyes,^[1] scintillators,^[1] light-emitting diodes,^[2] piezoelectric and pyroelectric materials,^[3] photoconductors,^[4] and are investigated for optical data storage,^[5] optical switching and signal processing,^[6] as well as in nonlinear optical applications.^[7–9]

The direct analytical characterization and physical study of extended π -chain polymers with high molecular weight is often hampered by low solubility. Structural defects also represent a common obstacle towards obtaining sound physical data. Soluble, monodisperse (that is, with the same number of monomer units) oligomers as finite model systems offer the possibility to attain, by extrapolation, specific information concerning the electronic, photonic, thermal, and morphological properties of their corresponding polydisperse high molecular weight analogues.^[10, 11] Furthermore, the systematic study of oligomers with precisely defined length, constitution, and conformation allows direct correlation of physical properties with chemical structures, and enables the generation of useful and predictive structure–property relationships. More recently, monodisperse oligomers have also been shown to be useful as model compounds for elucidating the folding properties of polymers.^[12] However, limitations to this “oligomeric approach”,^[13] also exist and not all physical or chemical properties of high molecular weight polymers can be modeled with the corresponding smaller oligomers. For instance, macromolecules differ from smaller analogues both in their solution (for example, high viscosity, diffusion behavior) and solid-state properties (for

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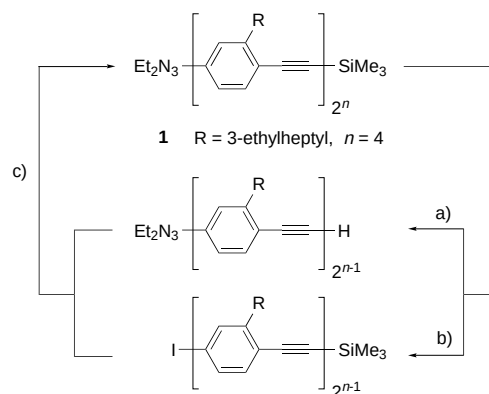
example, amorphous or semicrystalline structures), and such bulk effects are not likely to be reproduced by low molecular counterparts.^[14]

A second interest in monodisperse π -conjugated oligomers of defined length and constitution arises from their potential to act as molecular wires in molecular scale electronics^[15] and nanotechnological devices. A variety of spectacular molecular architecture has resulted from the efforts aimed at the construction of such wires. With the progress in synthetic organic methodology and the increasing availability of advanced analytical methods for the purification and characterization of very large molecules, monodisperse linear π -conjugated oligomers have recently reached the 10 nm length mark,^[16] which is about the current resolution limit for microstructure manufacturing by state-of-the-art lithographic techniques.^[17, 18] This achievement in organic synthesis has greatly stimulated the interest in the experimental^[19–22] and theoretical^[23] study of molecular scale mesoscopic devices. Although the fascinating concept of constructing a molecular computer based purely on organic molecules as an ultimate system^[24] in terms of information storage density and speed still remains an impetuously discussed and exceptionally controversial item,^[10] it is widely accepted that future nano-electronic or nanophotonic processes will likely require π -conjugated molecular rods of defined length as key components for ultradense and ultrafast information transportation, processing, and storage.^[10, 11, 16]

The emphasis of this review will be to provide an overview of the recent progress made in the synthesis and characterization of well-defined π -conjugated oligomers by looking at some of the most predominant recent systems. It should also illustrate the numerous contributions of monodisperse oligomers to the current understanding of the properties of polymers by relating molecular structures to macroscopic properties. For a more detailed treatment, the reader is referred to an excellent recent multi-author monograph^[13] and specialized reviews.^[10, 11, 14, 24–27]

2. General Strategies for the Synthesis of Monodisperse Oligomers

Despite much methodological progress, the synthesis and purification of monodisperse oligomers often remains quite tedious. New approaches to the preparation of well-defined π -conjugated oligomers have emerged in recent years.^[10, 11] The most elegant and efficient way to prepare long-chain oligomers uses an iterative divergent/convergent binomial strategy,^[28] which is illustrated in Scheme 1 for the synthesis of the



Scheme 1. Iterative binomial synthesis of the 12.8 nm long hexadecameric oligo(*p*-phenyleneethynylene) **1**.^[29, 30] a) K_2CO_3 , MeOH or $n\text{Bu}_4\text{NF}$, THF. b) MeI, 120 °C. c) $[\text{Pd}(\text{dba})_2]$, CuI, PPh_3 , $\text{HN}(\text{iPr})_2/\text{THF}$, (dba = dibenzylideneacetone).

12.8 nm long hexadecameric oligo(*p*-phenyleneethynylene) rod **1**, reported by Tour and co-workers (Scheme 1).^[29, 30] Three attractive features are combined in this approach: 1) two orthogonal protecting groups (SiMe_3 and Et_2N_3) allow the selective deprotection ($\text{C}\equiv\text{C}-\text{SiMe}_3 \rightarrow \text{C}\equiv\text{C}-\text{H}$) or activation ($\text{Ar}-\text{Et}_2\text{N}_3 \rightarrow \text{Ar}-\text{I}$) in very high yield (steps a and b); 2) the Pd-catalyzed cross-coupling reaction (step c)^[31] is selective and high yielding; and 3) the product in this chain elongation step is generally readily separable from by-

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F. Diederich

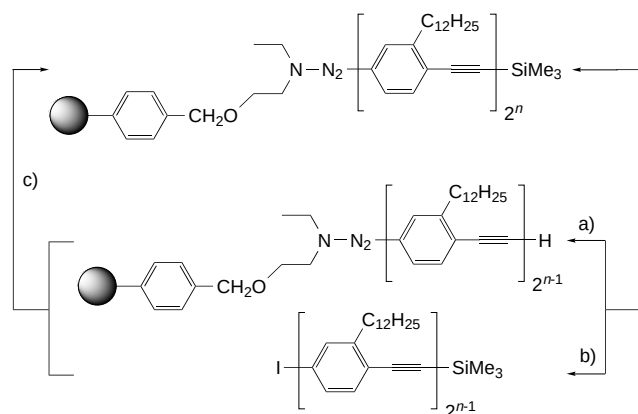


R. Martin

Rainer Martin, born 1970 in Dornbirn, Austria, studied chemistry at the Eidgenössische Technische Hochschule in Zürich, from where he received his diploma in 1995. He then joined the group of Prof. F. Diederich to work on the synthesis and physical investigation of monodisperse poly(triacetylene) oligomers and obtained his PhD in August 1998. In September 1998, he moved to the Melville Laboratory for Polymer Synthesis, Cambridge UK, where he is currently doing postdoctoral research with Prof. A. Holmes on electroluminescent polymers, supported by a fellowship from the Swiss National Science Foundation.

products and starting materials as a result of large differences in molecular weight.^[28] The same iterative strategy was used for the preparation of oligo(α -thiopheneethynylene) derivatives up to a heptadecamer.^[16, 32]

The binomial strategy for oligomer preparation is also applicable to protocols for solid-phase synthesis.^[30, 33] For this purpose, the starting monomer or oligomer is anchored covalently to an insoluble polymer resin such as poly(chloromethylstyrene) (Merrifield's resin). After orthogonal deprotection/activation (Scheme 2, steps a and b) cross-coupling



Scheme 2. Binomial oligomer synthesis on a Merrifield resin.^[30] a) $n\text{Bu}_4\text{NF}$, THF. b) MeI, 120 °C. c) $[\text{Pd}(\text{dba})_2]$, CuI, PPh_3 , NEt_3 .

occurs (step c) to yield the extended resin-bound oligomer, which can again be reactivated for the next coupling step. In the last step, low-weight molecules are removed by filtration and washing the resin and the completed oligomers are then cleaved from the polymer support.

The second possible strategy is homo- or heterocoupling of a symmetrical or asymmetrical monomer and eventual addition of an end-capping agent to the oligomerization mixture that will irreversibly block the chain ends to prevent any further reaction towards long-chain polymers. This one-pot approach generally lacks control of oligo-selectivity, and such a statistical polymerization often yields very low amounts of a particular oligomer. Nevertheless, this reaction protocol can be useful if high yielding cross-coupling reactions, which are required in the iterative binomial synthesis, are simply not available or if rapid access to an entire series of monodisperse oligomers is desirable and subsequent chromatographic separations are applicable. Such a strategy has been used in our group and by others for the synthesis, for example, of monodisperse poly(triacetylene) oligomers (see Section 4.3),^[34–39] for the synthesis of oligo(pyrrole)s,^[40] or for the preparation of linear (1,3-diethynylcyclobutadiene)cyclopentadienylcobalt oligomers.^[41]

The practical limit of this method often lies in difficulties encountered during the chromatographic separation (such as by size-exclusion chromatography (SEC)) of oligomers with relatively low differences in molecular weight. This problem can be alleviated by submitting already higher oligomers (“macro-oligomers”) to the endcapping oligomerization reaction, to yield oligomers with larger molecular weight differences that are more readily separable.^[42]

A general limitation for the construction of larger, well-defined oligomers often arises from the insolubility of π systems with extended π -electron conjugation, which can prevent chromatographic purification and isolation as well as physical characterization. Therefore, the attachment of suitable, solubility-providing side-chains to the repeat units is an essential design criterion in any synthesis planning. Furthermore, appropriate side chains can provide extra stability or modulate the morphological^[43] or electronic properties of the targeted oligomers or polymers in a desirable way. A very good example of how side chains can influence the optical properties is provided by poly(alkylthiophene)s. Depending on the number and nature of side chains, the colors of emission in polymer light-emitting devices (LEDs) can be changed from blue to red.^[2]

3. The Effective Conjugation Length in π -Conjugated Systems

The knowledge of the number of repeat or monomer units in a π -conjugated oligomer required to furnish size-independent redox, optical, or other properties that correspond to those of the related infinite-chain polymer is of great practical and theoretical interest. For instance, if π -conjugated materials with saturated properties are targeted, intractable high molecular weight polymers could be substituted in specific applications by shorter oligomers that have reached convergence of their physical properties and display better processability. Initially, “conjugation and no end” was thought to be a characteristic property of linearly π -conjugated systems, but soon, however, it turned out that an effective conjugation length (ECL)^[44] (or confinement length (CL), delocalization length (DL)^[45]) exists even in π -conjugated chain or ladder polymers for which structural defects can be excluded. Although the ECL is a numerical quantity that is not directly accessible by physical measurements, it rapidly evolved into one of the central concepts in the understanding of many properties of π -conjugated polymers.^[46] The usefulness of homologous series of oligomers to estimate the ECL experimentally has been widely demonstrated.^[42, 46–49]

The fact that π -conjugated polymers exhibit a convergent limit for certain physical properties (such as the longest-wavelength electronic absorption maximum λ_{max} and the optical end absorption (the solution optical “HOMO–LUMO” gap), redox potentials, vibrational frequencies, first and second hyperpolarizabilities) may arise in solution from mutual distortions from planarity of the conjugated backbone, for instance by rotations around single bonds. This results in reduced π overlap, and thus the conjugation exhibits a limited extension. In systems containing aromatic rings as part of the linear π -conjugated backbone, resonance stabilization within these rings is another factor that reduces the mobility of the π electrons. Furthermore, electron–electron correlations, which are often negligible in short π systems, become relevant in molecules that contain large numbers of π electrons.

One of the simplest theoretical models used to describe changes of physical properties as a function of chain length follows the description of a “particle in a box”.^[50] Since this

model contains no correction for electron–electron correlation effects, its validity is restricted to molecules with a limited number of π electrons. However, one-particle models have proved to have significant predicting power at both the qualitative and quantitative levels, although one has to be aware of certain limitations.^[51]

A simple way to evaluate the ECL includes plotting a relevant physical property (such as the longest-wavelength absorption energy E_{max} or the solution optical gap E_g) against the inverse number of monomer units n^{-1} .^[44] Extrapolation to infinite chain length yields n_{ECL} , the number of repeat units in an oligomer at which saturation of a property occurs. Usually, such a linear correlation among a homologous series of smaller oligomers yields only reasonable results in cases where no obvious saturation is observed. In fact, this estimation of convergence values by extrapolation can often only be a rough approximation and sometimes even results in misleading conclusions.^[44] However, satisfying results can be obtained in cases where higher oligomers or polydisperse polymers are available that already show saturation of their physical properties. In such cases, the crossing point of the linear regression line obtained from the oligomer series with the horizontal saturation level line for the polymers allows a good estimation of the ECL.^[34]

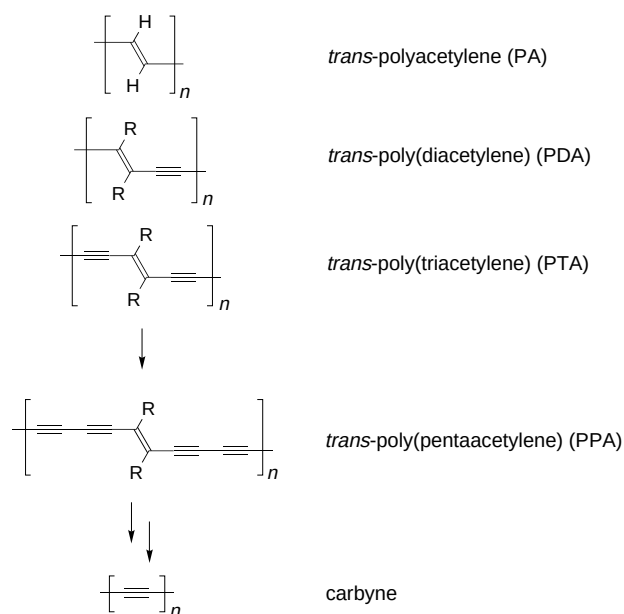
Wenz et al.^[52] deduced on the basis of the “free electron gas model” of Kuhn^[53] a linear relationship between the longest-wavelength optical absorption energy or the optical band gap E and N , the number of conjugated double and triple bonds per molecule, which was successfully applied to estimate the ECL in a series of poly(diacetylene)^[54] and poly(triacetylene)^[34] oligomers. Lewis and Calvin formulated a simple, linear relationship between the number of monomer units in the conjugated chain and the square of the wavelength of absorption.^[55] This equation, based on the harmonic oscillator model, was demonstrated to give excellent results in the case of polyenes.^[55] A modification of this model was later used by Hirayama to describe the position of the longest-wavelength absorption maximum as a function of oligomeric length in polyene derivatives and oligo(*p*-phenylene)s.^[56] In cases where physical data for only three oligomers are available, the approximation of Pade has found some significance to estimate the convergence value of the infinitely long chain.^[44, 57] Recently, Meier et al. presented an exponential equation, which accurately describes the dependence of electronic absorption and emission data on the number of repeating units n .^[44] Contrary to the algorithms presented so far, this approach allows the direct characterization of the overall effect of conjugation and the velocity of oligomeric growth, which serves as a measure of how fast the limit of convergence is actually attained.

4. Survey on Monodisperse π -Conjugated Oligomers

4.1. Oligoenes

Linear oligoenes may be viewed as structurally well-defined model compounds for polyacetylene (PA), which is the

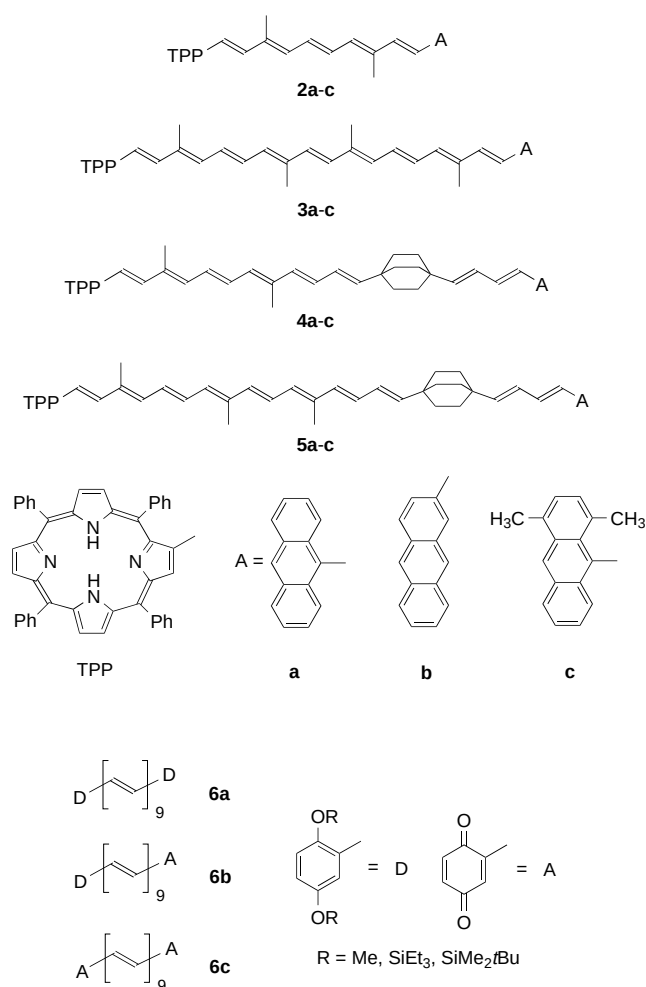
simplest π -conjugated polymer with an all-carbon backbone not composed of aromatic rings (Scheme 3). PA has been widely explored over the last years for its interesting material properties, in particular for its high electrical conductivity (up to 10^5 Scm^{-1}) upon doping.^[58–61]



Scheme 3. Progression of linear π -conjugated all-carbon backbones from *trans*-polyacetylene to carbyne.

Oligoene model compounds have played a dominant role in investigating the properties of PA.^[62] Unfortunately, unsubstituted PA is an intractable, insoluble, and infusible material and thus several attempts have been made to make PA more processable by using “precursor polymer routes”.^[26] However, substitution of the ethylenic hydrogen atoms by solubility-providing side chains leads to severe steric interactions and distortion of the π -conjugated backbone out of planarity, and results in a loss of conjugation and widening of the HOMO–LUMO gap. In oligoenes with a carotenoid backbone^[63] this effect, known as allylic 1,3-strain,^[64] also plays a significant role because of steric interactions between the methyl groups and the adjacent allylic hydrogen atoms.

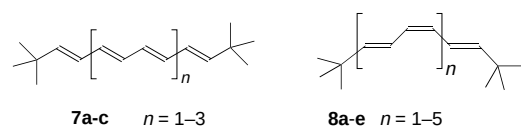
Various oligoenes with terminally functionalized donor (D), acceptor (A), or redox-active groups have been synthesized with the aim of investigating their intramolecular energy and electron transfer properties or exploring their use as molecular wires.^[10, 11, 24, 65–68] For instance, Lehn and co-workers demonstrated that α,ω -bispyridyl oligoenes incorporated into vesicle bilayer membranes function as molecular wires and mediate electron transfer from an external reducing phase to an internal oxidizing phase.^[67] Effenberger and Wolf synthesized the carotenoid polyenes **2–5** (Scheme 4) with different end groups to study intramolecular energy transfer processes. Interestingly, the interruption of the conjugation by a bicyclo[2.2.2]alkane unit in oligomers **4** and **5** resulted in a modified, but still observable energy transfer as evidenced by fluorescence quantum yield measurements.^[63] Duhamel and co-workers prepared the D–D, D–A, and A–A oligoenes **6a–c** that lack angular alkyl groups and, therefore, should



Scheme 4. Donor–acceptor substituted oligoenes for intramolecular energy and electron transfer studies.^[63, 68]

have a fully planar π -conjugated backbone.^[68] Such compounds could have the potential to function as molecular wires.

Müllen and co-workers applied the Stille cross-coupling reaction^[31] to the synthesis of the homologous series of stable oligoenes **7a–c** (Scheme 5) and studied their electron transfer



Scheme 5. All-*trans* oligoenes **7a–c** prepared by Stille cross-coupling^[62] and *trans(cis,trans)_n* oligoenes **8a–e** obtained by ring-opening metathesis polymerization.^[76]

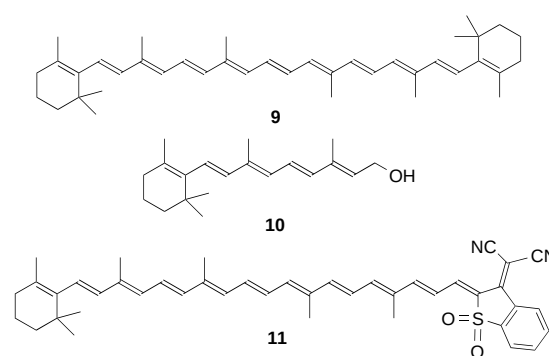
behavior by cyclic voltammetry (CV).^[62] The formation of $C(sp^2)–C(sp^2)$ single bonds in the cross-coupling reaction was found to provide a versatile alternative to the construction of oligoene backbones by formation of $C–C$ double bonds by the Wittig and analogous reactions.^[69] The oligomers **7a–c** are highly redox active, with **7c** displaying seven successive one-electron transfers, which lead from a tetraanion to a trication, that are reversible on the cyclic voltammetric time scale. From the plot of the electrochemical HOMO–LUMO gaps ΔE of **7a–c** against n^{-1} (n = number of double bonds), the ΔE value for PA was extrapolated to 1.7 eV, which is in agreement with

the band gap determined experimentally and theoretically for PA itself.^[70] A single crystal X-ray structure analysis of hexaene **7b** revealed a packing structure formed by layers of oligoenes with each linear chain being surrounded by six neighboring *tert*-butyl groups which occupy spaces between the layers. The oligomeric arrangement is similar to that of *trans*-PA as determined by X-ray diffraction and lattice packing calculations, which demonstrates the potential of crystalline **7b** to act as a model for bulk *trans*-PA.^[71] Furthermore, **7b** and **7c** can serve as models for doped PA, since oligoenes with six to ten or more $C–C$ double bonds are comparable in size to the regions of the PA chain that become charged upon oxidation or reduction.^[72]

The nature of the electrical conductivity in PA has been the subject of a tremendous amount of experimental and theoretical research and has also been addressed in several publications on oligoene model systems.^[73–75] Valence effective Hamiltonian (VEH) calculations on oligomers have been used to compute ionization potentials, optical transition energies, and electron affinities in PA.^[48] These have been found to be in remarkably good accordance with experimentally observed values.

An elegant way to synthesize oligoenes with odd and even numbers of $C–C$ double bonds was reported by Knoll and Schrock.^[76] Controlled ring-opening metathesis polymerization (ROMP) gave access to polydisperse mixtures of different oligomers endcapped by *tert*-butyl groups, and those having as many as 13 $C–C$ double bonds were subsequently separated by flash chromatography on silica gel under nitrogen at -40°C . The *trans(cis,trans)_n* series **8a–e** (Scheme 5) as well as odd and even series of all-*trans* oligomers containing up to nine $C–C$ double bonds were analyzed in more detail by UV/Vis, and ^1H and ^{13}C NMR spectroscopy. Oligoenes beyond the 17-mer were found to be rather unstable under the reaction and isolation conditions. Extrapolation by plotting UV/Vis absorption data versus n^{-1} revealed a HOMO–LUMO gap in solution of 1.79–1.80 eV for an infinite all-*trans* PA chain. In a different study, the same oligomers containing 3–13 conjugated double bonds were subjected to ^{60}Co γ -irradiation in Freon matrices. The generated radical cations were then used as model compounds to study the doping process in PA by means of UV/Vis spectroscopy.^[73]

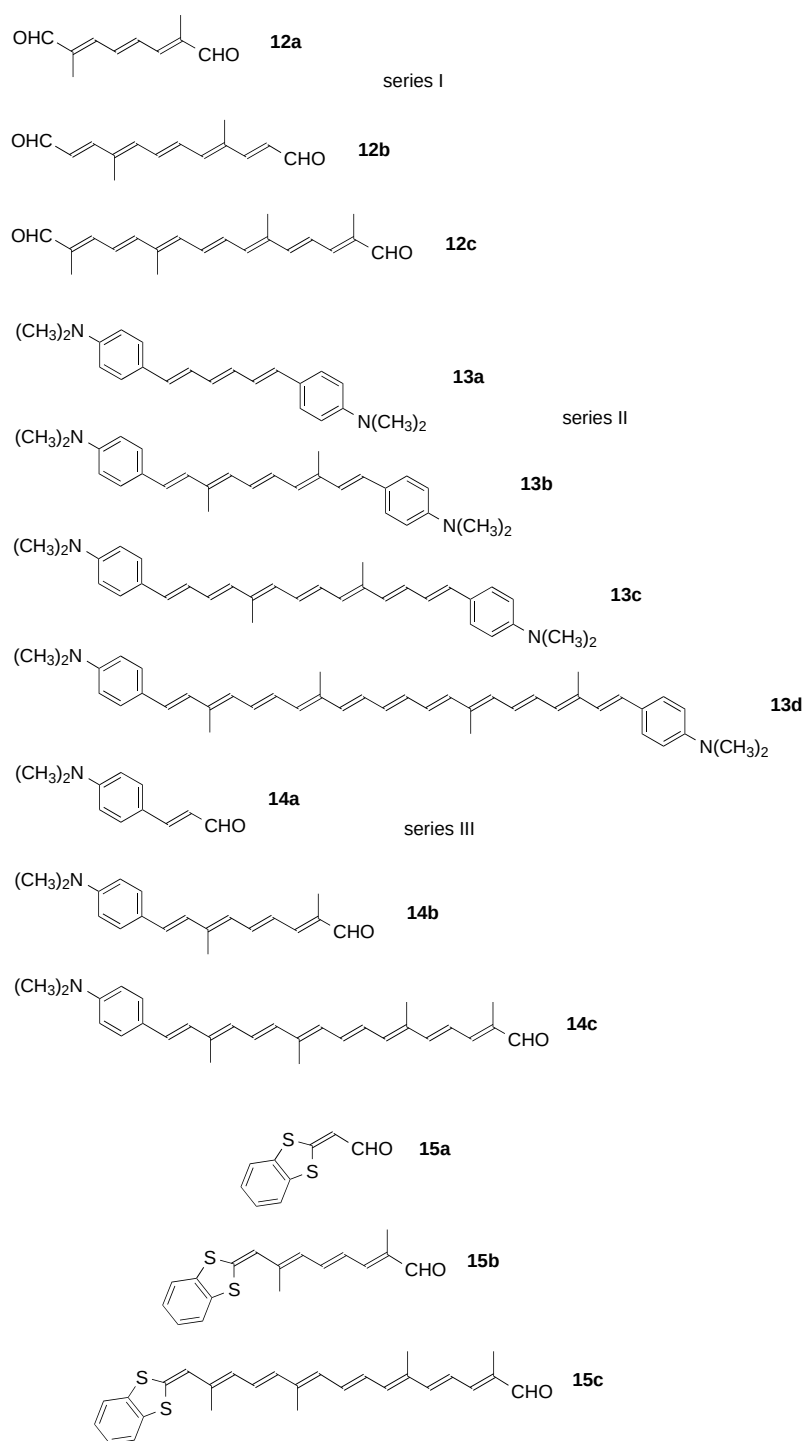
β -Carotene (**9**) and vitamin A (**10**) are naturally occurring polyene oligomers (Scheme 6) and thus were early targets for



Scheme 6. The natural polyenes β -carotene (**9**), vitamin A (**10**), and a carotene derivative (**11**) were investigated for third-order nonlinear optical properties.^[77, 78]

studying first (β) and second (γ) hyperpolarizabilities, which characterize the efficiency of molecular second- and third-order nonlinear optical effects. Large molecular third-order optical nonlinearities were measured in polarized carotenoid derivatives such as **11**, which displays a 35-fold enhancement of the second hyperpolarizability (measured at the three-photon resonance) relative to the symmetric parent β -carotene (**9**).^[77, 78] The dramatic improvement over β -carotene, which itself has one of the largest third-order nonlinearities known, has been attributed to symmetry breaking and to the large difference in dipole moments between the ground and excited states introduced by the strong terminal acceptor functionality in **11**.

Oligoenes serve as textbook examples for the correlation between various propounded theoretical models (such as the “particle in a box” model) and experimentally observed parameters. A variety of combined experimental and theoretical studies have been undertaken to establish structure–property relationships and design criteria for conjugated organic molecules with enhanced nonlinear optical responses.^[79–82] The chain length dependence of the third-order polarizability in three different series of α,ω -oligoenes (Scheme 7) bearing electron-withdrawing (series I: **12a–c**), electron-donating (series II: **13a–d**), or both donor and acceptor end groups (series III: **14a–c**) was studied systematically by Pucetti et al. and revealed the following trends:^[83] 1) In each series, the lengthening of the conjugated chain induces hyperchromic and bathochromic shifts in the UV/Vis absorptions and each series obeyed the previously observed^[55] linear dependence of the longest-wavelength absorption maximum λ_{max} from the square root of the number of C–C double bonds. 2) Measurements of the first hyperpolarizability β by EFISH (electric field induced second harmonic generation) using a laser excitation wavelength of 1.34 μm and the second hyperpolarizability γ by third harmonic generation (THG) at 1.91 μm showed a sharp increase in the γ values in all three series of compounds with increasing chain length. A nonlinear fit according to the power law relationship $\gamma = kn^a$ of the γ values against the number of monomer units n revealed an exponent of $a = 2.3$ for the bis-acceptor series I (**12a–c**) and 3.0 for the bis-donor series II (**13a–d**). 3) Exceptionally large γ values were observed for the bis-donor-substituted oligoenes **13a–d** and for the D–A series III (**14a–d**). Saturation of the nonlinear optical properties was not observed in any of the three series of compounds.

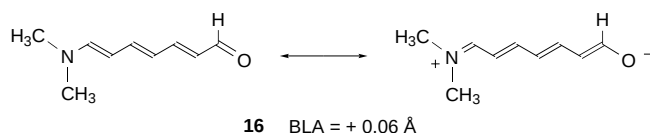


Scheme 7. Three series of α,ω -substituted oligoenes investigated for nonlinear optical properties.^[83, 84]

In a similar study, the asymmetrically substituted α,ω -polyenes **15a–c** (Scheme 7) were investigated using THG and EFISH techniques, and displayed an increase in γ by about two orders of magnitude for **15c** relative to the γ value for **15a**.^[84] For a small number of double bonds n , a power law dependence $\gamma = kn^a$ again is suitable to approximate the experimental results, with an exponent a between 3 and 6. For larger values of n , γ becomes linear in n and γn^{-1} approaches a constant value. The saturation of γn^{-1} in long-chain polyenes

with very narrow polydispersities obtained by living polymerization techniques has been found by THG measurements (1.9 μm) to occur at approximately 120 C–C double bonds^[85] and thus at considerably longer chain lengths than theoretically predicted.^[86] Based on comparisons with all other linearly π -conjugated polymers (see below), the accuracy of this exceptionally high ECL for polyenes must be seriously questioned and re-investigation is strongly suggested.

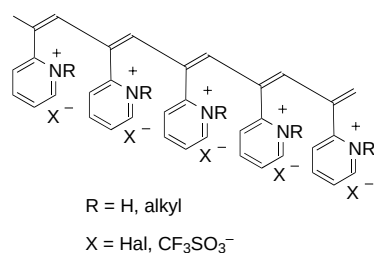
Utilizing D–A substituted oligoenes, specifically polymethine dyes such as **16** (Scheme 8), Marder and co-workers developed a bond length alternation model (BLA, defined as



Scheme 8. Bond length alternation (BLA) in the polymethine dye **16**.^[87]

the difference between the average lengths of carbon–carbon single and double bonds in a polymethine chain) from both experimental and theoretical results to relate the magnitude of the second hyperpolarizability γ to the extent of changes in molecular geometry.^[87] The γ value could be optimized in either a positive or negative sense for polymethine dyes of a given π -conjugation length by tuning the BLA by chromophoric structure, solvation effects, or an external electric field.^[88] These same studies, and others,^[89] suggested a strong correlation between linear and nonlinear optical properties (α , β , γ) of conjugated organic molecules and predicted that, as the first hyperpolarizability β is maximized for a given chromophore, the second hyperpolarizability γ should approach zero, which suggests there is a limit to the ability of D–A substitution to increase γ values. More recently, Shu et al. synthesized push–pull oligoenes up to a tetramer in which the π -conjugated backbone is rigidified through incorporation into fused ring structures. This configurational and conformational locking furnished NLO chromophores that possessed significantly enhanced thermal stability.^[90] A more detailed overview of the recent experimental and theoretical progress in the field of nonlinear optics dealing with oligomeric and polymeric chromophores can be found in references [8, 91–95].

Recently, Blumstein and co-workers reported some interesting developments that potentially promise to eliminate some of the disadvantages associated with classical PA and its doped derivatives.^[96] They described a way to make PA more soluble and processable by introducing one or two ionic 2-pyridinium side chains into each repeat unit (Scheme 9).



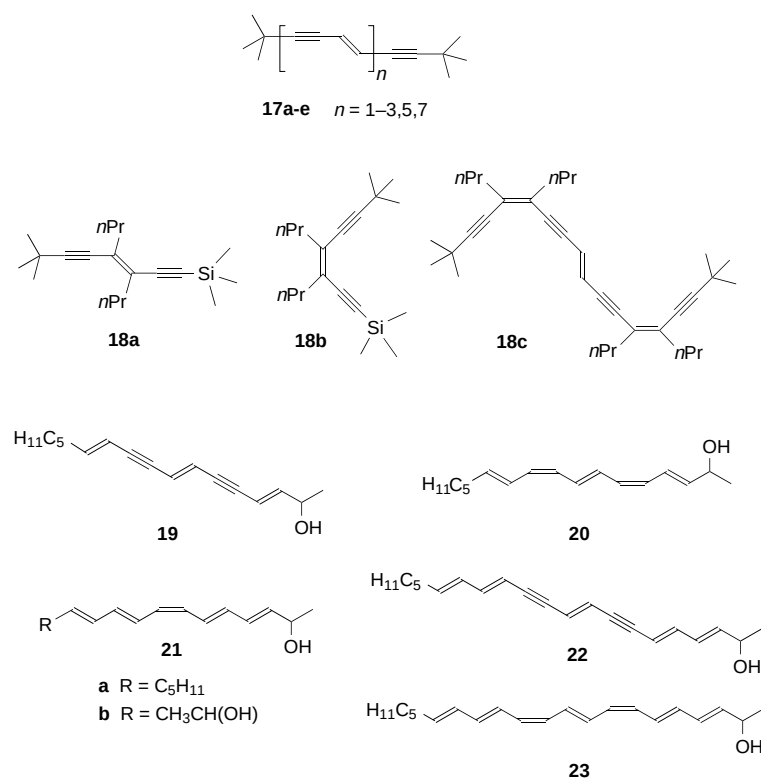
Scheme 9. Schematic representation of polyacetylenes with solubilizing ionic side chains.^[96]

The electrostatic repulsion between adjacent monomers apparently favors planarization of the PA backbone and results in significantly extended linear π conjugation relative to uncharged substituted PAs. By using different charged side chains, a wide range of solubilities could be achieved, extending from water to nonpolar solvents. Furthermore, variation in R enabled formation of amphiphilic or mesogenic systems, composites, and blends. The high film-forming potential of the amphiphilic structures makes them excellent candidates for LB (Langmuir–Blodgett) or electrostatic layer-by-layer deposition,^[97] and offers considerable potential to process PAs into thin-film architectures for device fabrication. It can be expected that monodisperse oligomeric model systems for these novel PA materials will also be developed in future work.

4.2. Oligoenynes

The second representatives in the progression of linearly π -conjugated nonaromatic all-carbon backbones are poly(di-acetylene)s (PDAs, Scheme 3). They represent a unique class of conjugated polymers insofar as PDAs can be obtained as perfect macroscopic single crystals by topochemical solid-state polymerization of suitably pre-arranged and substituted buta-1,3-dienes,^[52, 98] a requirement, however, which severely limits their accessibility. PDAs are not conducting upon doping but show large third-order nonlinear coefficients^[8, 99] and are considerably more stable than PAs. Oligoenynes have been much less investigated as model compounds for PDA than oligoenes (as models for PA), presumably as a result of the requirement for a topochemically controlled synthesis of the π -conjugated backbone. The first systematic series of *trans*-enynes oligomers dates back to 1986 and was published by Wudl and Bitler.^[100] They reported the synthesis of *tert*-butyl endcapped oligomers **17a–e** (Scheme 10) which showed, with increasing chain length, a color change from white (**17a, b**) to deep yellow (**17e**), as well as a rapidly decreasing solubility in *n*-hexane or benzene. The *tert*-butyl endcaps were found to be essential for high thermal stability, and the oligomers displayed no particular sensitivity to air and light as it is generally observed in the case of oligoenes. Interestingly, **17d** did not react with iodine over a period of greater than 24 h, which strongly contrasts the behavior of oligoenes with identical chain length. For example, β -carotene (**9**) can be easily doped with I_2 to give IR and Raman spectra similar to those obtained for I_2 -doped PA. Additionally, attempts to dope a film of **17d** with sodium naphthalenide and [18]crown-6 in Et_2O were unsuccessful.^[100]

In an in-depth study Giesa and Schulz used the oligoenynes **17a–d** and the substituted derivatives **18a–c** (Scheme 10) as model compounds for PDA and investigated their physical properties by UV/Vis, Raman, ^1H NMR, and ^{13}C NMR spectroscopy as a function of chain length and geometry.^[54] The ECL in PDAs was calculated to be around $n_{\text{ECL}} = 10$ monomer units, with the longest-wavelength electronic absorption energy at infinite chain length being extrapolated as 2.25 eV ($\lambda_{\text{max}} = 551 \text{ nm}$).^[54] The construction of the stereo-defined oligomers **19–23** (Scheme 10) was reported by

Scheme 10. Oligoenynes as models for poly(diacetylene)s (PDAs).^[54, 100, 101]

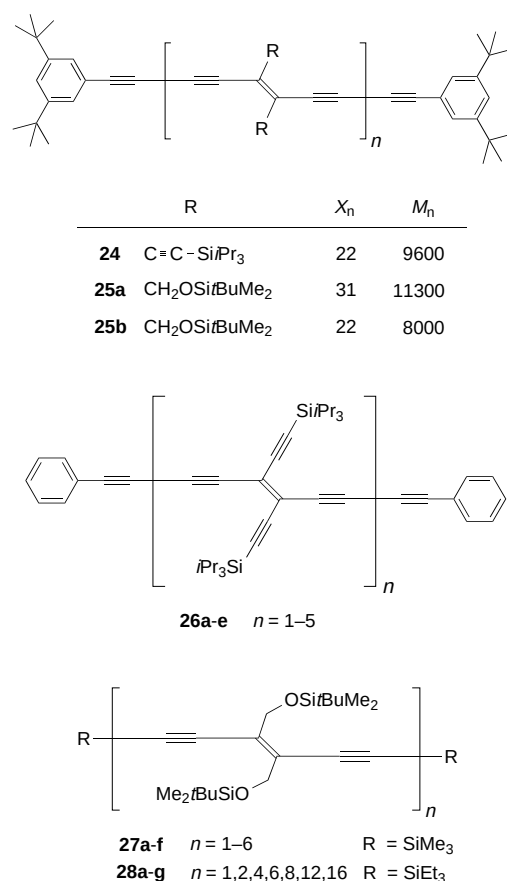
Crousse et al., who studied the influence of geometry (*Z/E*) or bond type (C–C double or triple bond) on their physical properties.^[101]

With the enhanced synthetic repertoire to form C(sp)–C(sp²) bonds provided by modern metal-catalyzed cross-coupling reactions^[31] we expect a renewed interest in the formation and physical study of oligoenynes as model systems for PDAs in future years.

4.3. Oligoenediynes

The third linear π -conjugated polymer without aromatic repeat units in the series that starts with PA and PDA is poly(triacetylene) (PTA, Scheme 3). Whereas the chemistry and physical properties of PA and PDA oligomers and polymers had been exhaustively investigated for about two decades, much less is known about PTAs, which were reported for the first time in 1994.^[36, 102] Despite this late discovery the synthetic accessibility of PTAs and functional derivatives by simple oxidative acetylenic coupling is more versatile than that of PAs and PDAs. Furthermore, the additional acetylenic moiety in each repeat unit of PTAs increases the spacing between laterally appended side chains, which, as a consequence, do not easily undergo intramolecular steric repulsions that cause distortion of the linear π -conjugated backbone from planarity. Similar to PDAs, PTA polymers such as **24** and **25** (Scheme 11) were found not to become conductive upon doping.^[102]

The first series of monodisperse PTA oligomers (oligoenediynes) prepared were the phenylacetylene endcapped com-

Scheme 11. Poly(triacetylene)s (PTAs) **24** and **25**^[102] and oligoenediyne model compounds **26–28**.^[34, 38, 42]

pounds **26a–e** (Scheme 11) that contained tetraethynylethene (TEE, 3,4-diethynylhex-3-ene-1,5-diyne) repeat units.^[36, 38, 102, 103] The highly colored compounds **26a–e** extend in length up to about 5 nm and are amazingly kinetically stable, high-melting materials that remain unchanged for months when exposed to air at ambient temperature. The X-ray crystal structure of dimeric **26b** displayed a perfectly planar π -conjugated backbone including the two terminal phenyl rings. The solution optical gap for the series **26a–e** extrapolated to infinite chain length was estimated to be $E_g = 2.3$ eV (536 nm), which is comparable to that of many PDAs ($E_g = 2.1$ eV).^[36] Electrochemical analysis of these π -conjugated rods revealed interesting redox properties. While none of the oligomers **26a–e** could be oxidized below +1.0 V in THF (versus ferrocene/ferrocenium (Fc/Fc⁺)), which helps to explain their amazingly high stability in air, they were all readily reduced with the number of reversible, one-electron reduction steps corresponding to the number of TEE moieties in each oligomer. Furthermore, the first electron transfer was strongly shifted to less negative potentials with increasing number of monomer units; thus, the first reduction of **26a** occurs at $E^\circ = -1.57$ V (versus Fc/Fc⁺, in THF +0.1 M Bu₄NPF₆) whereas the first reduction of **26e** is much facilitated and occurs at -1.07 V.^[36]

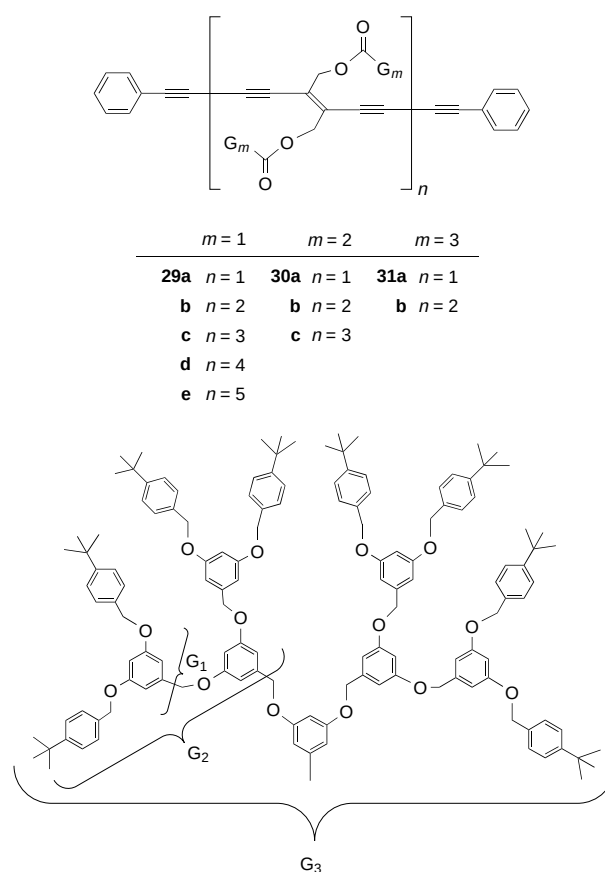
The Me₃Si endcapped oligoenediynes **27a–f** (Scheme 11) with *trans*-1,2-diethynylethene (DEE, (*E*)-hex-3-en-1,5-diyne) repeat units displayed significantly enhanced solubility and processability relative to the tetraethynylethene oligo-

mers **26a–e** as a consequence of the more flexible Me₂tBu-SiOCH₂ side-chains.^[34] These compounds ranged from a 0.96 nm long monomeric to a 4.61 nm long hexameric rod and allowed for a comprehensive investigation of structure–property relationships in PTAs. By extrapolative evaluation of linear optical and nonresonant third-order nonlinear optical data the ECL of PTAs was predicted to lie in the range of seven to ten monomer units, which corresponds to 21 or 30 conjugated double and triple bonds, respectively.^[34] Furthermore, a plot of γn^{-1} versus n for the PTA samples **27a–f** revealed an exponential correlation for γ with a fitted exponent $a = 2.5 \pm 0.1$.

For a direct determination of the ECL the Et₃Si-endcapped, monodisperse oligomers **28a–g** (Scheme 11) containing $n = 1, 2, 4, 6, 8, 12, 16$ monomeric units were prepared by a rapid and efficient statistical deprotection–oxidative oligomerization protocol.^[42] Longer-chain monomers were used as starting materials for the preparation of the higher oligomers ($n = 8, 12, 16$) in order to reduce separation problems by SEC (see Section 2). The gap between oligomer and polymer analysis was closed with the completion of this series of PTA oligomers. All compounds up to hexadecamer **28g** featured good solubility in apolar solvents, which allowed a direct determination of the ECL by means of UV/Vis spectroscopy. This study revealed the previously predicted value of $n_{\text{ECL}} = 10$. Raman scattering studies of oligoenynes **28a–g** in CHCl₃ solutions at 20 °C showed an exponential decrease in the frequencies of the C–C triple and double bond stretches $\tilde{\nu}_{(\text{C}=\text{C})}$ and $\tilde{\nu}_{(\text{C}=\text{C})}$, respectively, with lengthening of the π -conjugated backbone, and plots of $\tilde{\nu}_{(\text{C}=\text{C})}$ as a function of n also revealed an ECL of ten monomer units. The hexadecameric PTA rod **28g** with its 16 C–C double and 32 C–C triple bonds and a length of 11.9 nm between the terminal Si atoms is currently the longest linearly fully π -conjugated molecular wire that does not contain aromatic repeat units.

PTA monomers (TEEs and DEEs) and dimers substituted at the terminal alkynes with donor (D; *p*-(dimethylamino)-phenyl) and/or acceptor (A; *p*-nitrophenyl) groups were found to display very high second hyperpolarizabilities γ , which were determined by THG measurements.^[92, 104a, b] The second-order nonlinear optical properties of these compounds are also quite appealing.^[104c] Also, the photochemical *trans* → *cis* isomerization of such compounds was investigated comprehensively.^[105] Their photochemical behavior differs substantially from that of similarly substituted stilbenes and azobenzenes since, in contrast to the latter, both *cis* and *trans* isomers of the D/A-substituted TEEs display fully planar conjugated π chromophores.

PTA oligomers with backbones as in **27a–f** but with lateral dendritic side chains of generations one to three were prepared as insulated molecular wires.^[35] Interestingly, the insulating layer created by the dendritic, Fréchet-type wedges^[106] protects and stabilizes the central conjugated backbone of the tubular macromolecules **29–31** (Scheme 12) by preventing intermolecular reactions, but does not alter its electronic characteristics as evidenced by UV/Vis measurements. An estimation of the longest-wavelength absorption maximum E_{max} for an infinitely long polymer chain revealed exactly the same limiting value of 2.57 eV for all three



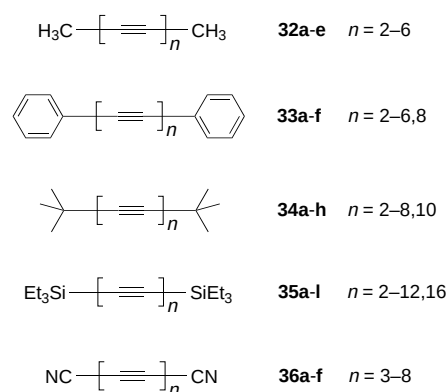
Scheme 12. Molecular oligoenediyne wires with lateral dendritic side chains.^[35]

oligomeric series **29–31**.^[35] It must be concluded from this result that there is no loss of π conjugation along the PTA backbone in the higher generation compounds despite distortion from planarity as a consequence of steric compression of the bulky dendritic side chains.^[107, 108]

4.4. Oligoenynes

A true challenge in contemporary chemistry is the preparation of infinite one-dimensional carbon allotropes, built exclusively from C≡C units.^[103, 109] Such polymers, which have been named as carbyne C_∞ (Scheme 3), have been intensively discussed in the literature^[110, 111] yet they remain highly controversial.^[112] The inability to attach side chains to carbyne or oligoyne model compounds to provide solubility, stability, or for fine-tuning physical properties is a significant drawback and severely limits the exploration of this class of compounds. Nevertheless, several series of endcapped monodisperse oligoenynes such as **32a–e**,^[113] **33a–f**,^[114] **34a–h**,^[111, 115] and **35a–l**^[116] (Scheme 13) have been prepared and investigated over the years in order to extrapolate properties of the infinite carbyne polymer.

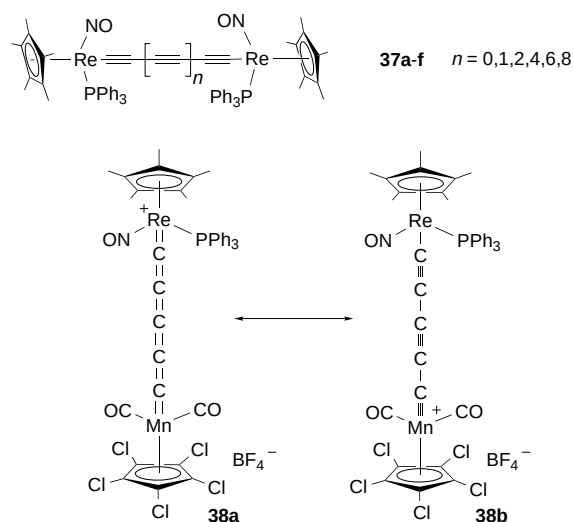
Hirsch and co-workers reported an extensive investigation of the properties of the rod-shaped α,ω -dicyano oligoenynes **36a–f** (Scheme 13) by spectroscopy, electrochemistry, and theoretical calculations.^[117] Together with the already known smaller representatives NC–CN, NC–C≡C–CN, and



Scheme 13. Oligoynes as model compounds for the carbon allotrope carbyne (see Scheme 3).^[113–117]

$\text{NC}-\text{C}\equiv\text{C}-\text{C}\equiv\text{C}-\text{CN}$,^[118] oligomers **36a–f** form a continuous homologous series of oligoynes. The preparation of compounds **36a–f** occurred in a truly spectacular way when graphite was vaporized under He in the presence of dicyanogen ($(\text{CN})_2$) under the conditions of the Krätschmer–Huffman fullerene synthesis. Dicyanogen completely quenches the linear oligoynes intermediates formed at the initial stages of the fullerene synthesis, and the molecular rods **36a–f** are isolated instead of the carbon spheres. Oligoynes with cyano end groups such as HC_nN ($n = 1, 3, 5, 7$) had previously been detected in interstellar space, and the search for such compounds paved the way for the first experimental observation of buckminsterfullerene C_{60} .^[119]

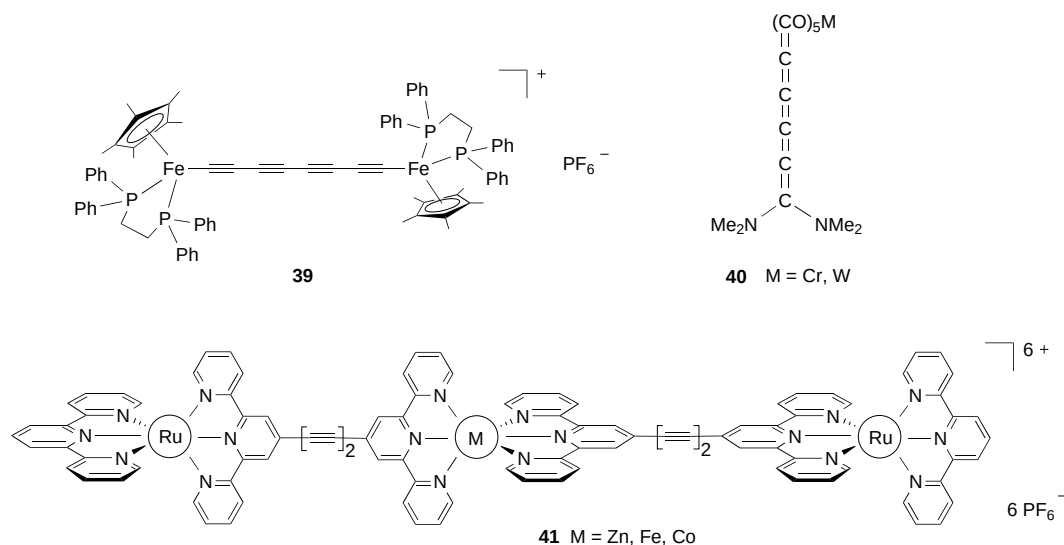
A major advance in the formation of stable long carbyne fragments containing up to ten conjugated $\text{C}\equiv\text{C}$ moieties was achieved by endcapping the carbon rods as transition metal complexes. Thus, Gladysz and co-workers prepared the dirhenium μ -oligoynediyl complexes **37a–f** (Scheme 14) by a step-growth approach by employing acetylenic homo- and cross-coupling reactions.^[120] The redox-active Re^{I} end groups undergo reversible oxidations to the Re^{II} state, with a potential separation of the two distinct oxidation events in **37a** of 0.53 V. As the oligoynes spacer between the metal centers, and thus the “resistance” of the bridge increases, the



Scheme 14. Long oligoynes stabilized by transition metal endcapping groups.^[120, 121]

two oxidations start to behave independently and merge to give a single, presumably two-electron, oxidation for **37f**, which represents with its C_{20} chain the longest stable oligoynes reported so far. The oxidation of $\text{Re}^{\text{I}} \rightarrow \text{Re}^{\text{II}}$ becomes thermodynamically less favorable with the lengthening of the C-atom chain in the series **37a–f**, which could reflect the strong electron-withdrawing effect of a chain of sp-hybridized C atoms. The same researchers also reported on the asymmetrically substituted oligomer **38** (Scheme 14) with an odd number of C atoms between the two metal centers.^[121] IR measurements on this compound revealed that the cumulenyl resonance form **38a** dominates over the alternative acetylenic one (**38b**).

Other monodisperse oligoynes endcapped with metal centers, such as **39**,^[122] **40**,^[123] and **41**^[124] (Scheme 15) have been investigated for the study of intramolecular electron-transfer processes between the metal centers across the bridging carbon fragment. In the thermally stable mixed-valence complex $[\text{Fe}^{\text{II}}-\text{C}_8-\text{Fe}^{\text{III}}]$ (**39**), the indistinguishable redox states of the two Fe centers suggest that the electron-transfer rate between the termini is very high.^[122]

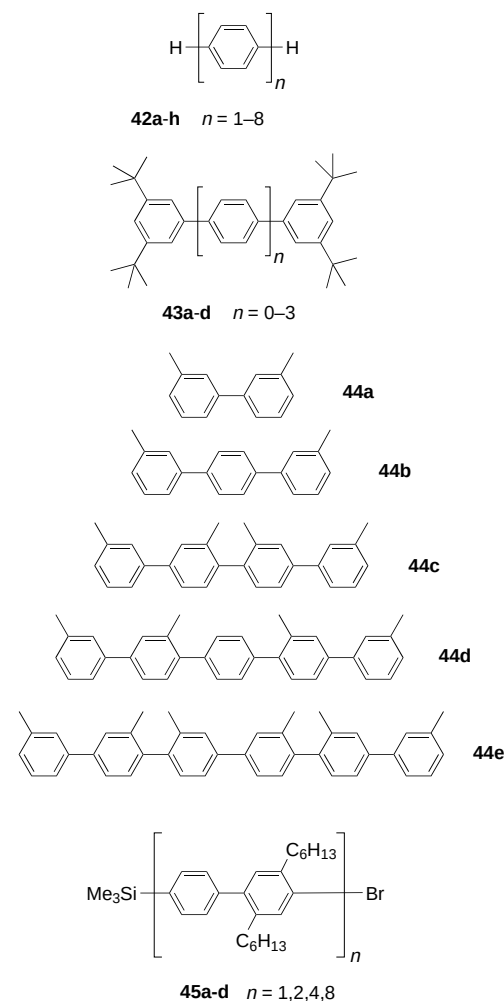


Scheme 15. Oligoynes investigated for intramolecular electron transfer processes.^[122–124]

4.5. Oligo(*p*-phenylene)s

Poly(*p*-phenylene) (PPP) has found considerable interest over the last years since it acts as an excellent organic conductor upon doping. Whereas in the neutral form PPP is an insulator and displays conductivities down to $10^{-12} \text{ Scm}^{-1}$, doping with I_2 or AsF_5 raises its conductivity into the metallic region with values around 500 Scm^{-1} .^[125, 126] It is therefore not surprising that PPPs have become some of the most widely investigated polymers as documented by several review articles covering all aspects from synthesis to physical properties.^[26, 61, 125–128] A second major interest arises from the fact that polymers with a PPP backbone can be used as the active component in blue light-emitting diodes (LEDs).^[2, 129–131] In parent oligo- or poly(*p*-phenylene)s, steric interactions between *ortho*-H atoms cause the planes of neighboring aryl rings to adopt dihedral angles up to 23° , which significantly reduces the π -orbital overlap and thus prevents extended π conjugation.^[125, 132, 133] The solubility properties of PPP-derived oligomers or polymers are readily enhanced by addition of substituents along the conjugated backbone. However as a severe drawback, replacing the H atoms by any larger group causes even greater dihedral angles between the planes of neighboring rings and results in a disturbance of the π -orbital overlap along the PPP backbone and a reduction in the ECL. Such distortions from planarity of the linear backbone are evidenced by hypsochromic shifts in the UV/Vis spectra.^[133] Recently, Schlüter and co-workers described the preparation of PPPs with Fréchet-type dendrons of generations one to three attached to each phenyl group.^[107] The resulting cylindrical macromolecules display very high solubilities.

Although unsubstituted oligo- and poly(*p*-phenylene)s often exhibit remarkable thermal stabilities, which makes them attractive candidates for numerous applications that require thermally robust π -conjugated organic materials, their low solubility and intractability has so far precluded any commercial development of this polymer class.^[125] In the oligomeric series **42a–h** (Scheme 16), solubility rapidly decreases with increasing chain length,^[134, 135] but solubility properties can be slightly enhanced by addition of *tert*-butyl groups to the terminal aryl rings as in the oligomeric series **43a–d** (Scheme 16).^[136–138] As expected, the ECLs determined by UV/Vis spectroscopy for both series **42a–h** and **43a–d** were found to be in good agreement and yield $n_{\text{ECL}} = 9$ and $n_{\text{ECL}} = 11$, respectively.^[44] The ECL determined from specific refractive index increments of dilute oligo(*p*-phenylene) solutions was found to be around $n_{\text{ECL}} = 6$ phenylene units and thus not too different from UV/Vis derived values.^[135] Oligomers of type **42** were also the subject of third-order nonlinear optical investigations by time-resolved degenerate four-wave mixing (DFWM), and revealed $\chi^{(3)}$ values of less than $1.6 \times 10^{-13} \text{ esu}$ for both quaterphenyl (**42d**) and quinquephenyl (**42e**), and an increase up to $1.7 \times 10^{-12} \text{ esu}$ for octiphenyl (**42h**).^[8, 139] Semiempirical calculations involving both singly and doubly excited configurations showed D–A functionalized oligo(*p*-phenylene)s to be much less efficient second and third harmonic generation chromophores than oligoenes or oligoynes of comparable length and substitution.^[80] This can be readily rationalized by the higher



Scheme 16. Oligo(*p*-phenylene)s investigated as model systems for PPP.^[134–138, 142, 143, 145]

degree of electron localization in the individual aryl rings of the PPP backbone.

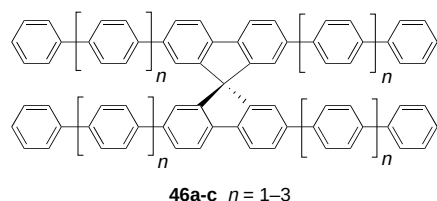
The evolution of the electronic structure in a series of oligo(*p*-phenylene)s, in comparison with oligoynes and oligo(*p*-phenylenevinylene)s, has been studied by ultraviolet photoelectron spectroscopy and quantum chemical calculations, based upon the valence effective Hamiltonian (VEH) method of Lögdlund et al.^[140] The authors found excellent agreement between theory and experiment, which allowed a detailed description of the evolution of the electronic structure in these types of polymers.

Oligo(*p*-phenylene)s have played a dominant role as model compounds for PPP in the study of physical mechanisms related to intra- and interchain charge transport or distribution and stabilization of charges and spins on π -conjugated chains. These mechanisms are of special interest in regard to the potential application of PPP and related conjugated polymers in rechargeable batteries.^[128, 136, 141] Oligomers **42c–d**, **43b–d**, and **44b–e** (Scheme 16) were investigated by cyclic voltammetry and the corresponding diamagnetic dianions, obtained by reaction with alkali metals, by NMR spectroscopy.^[136, 142] Electrochemical studies of **43b–d** showed an increasingly facilitated first reduction step and a decreasing difference between the first and second reduction potential

with increasing chain length. All compounds containing up to four benzene units underwent a two-electron reduction, whereas longer chains are capable of accepting more electrons. Centers of highest charge density are usually the *para* positions, especially on the inner rings. It became evident from NMR experiments that rotation about the C(aryl)–C(aryl) single bonds is significantly retarded in the reduced species. The radical monoanions in both series **43b–d** and **44a–e** were also studied by electronic absorption spectroscopy in THF solutions,^[143] and revealed interesting differences between the two oligomer series. In **44a–e** (Scheme 16), the methyl substituents significantly perturb the π conjugation by twisting the phenylene rings out of planarity. This results in the tendency of the radical anions to delocalize the charge in smaller sub-units. Thus, **44c** and **44e** resemble biphenyl radical anions, and **44d** a terphenyl radical anion rather than higher oligo(*p*-phenylene) oligomers with extended π conjugation. The first reduction step in oligo(*p*-phenylene)s causes a geometrical reorientation towards a more planar system, best described by quinoidal resonance structures, to stabilize the monoradical anion. The second electron is then able to delocalize over the entire π system. The ionization potentials, optical transition energies, and electron affinities for **42a–c** and PPP have been calculated by Brédas et al.^[48] Oligomers **42c–f** were also shown to be suitable model compounds for PPP in homogeneous photocatalysis processes.^[144]

Schlüter and co-workers prepared rigid rods **45a–d** (Scheme 16) in a repetitive sequence with up to 16 phenylene rings and orthogonal functional groups at both termini.^[145] Compound **45d** currently represents the longest monodisperse oligo(*p*-phenylene) rod reported so far. The C_6 alkyl chains attached to every second aromatic unit provided solubility in common organic solvents, thereby allowing full spectroscopic characterization of oligomers **45a–d**.

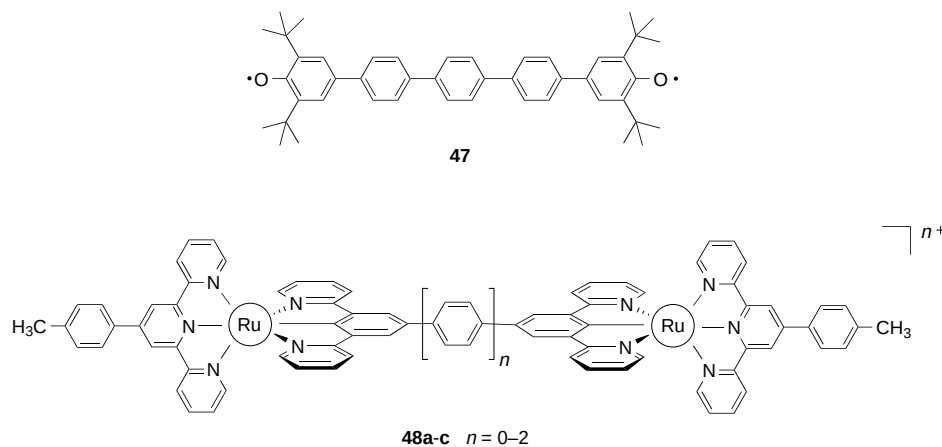
Spiro-linked oligo(*p*-phenylene)s such as **46a–c** (Scheme 17) have been used as blue emitters in electroluminescent applications. They are soluble in common solvents, show glass transition temperatures up to 250 °C, and with their high photoluminescence quantum efficiencies



Scheme 17. The spiro-linked oligo(*p*-phenylene)s **46a–c** can be used as blue emitters for electroluminescent applications.^[146]

in the solid state, represent an alternative to high molecular weight PPP polymers for the construction of LEDs.^[146]

For the diradical **47** (Scheme 18), in which two phenoxyl radicals are connected by a terphenyl spacer, antiferromagnetic



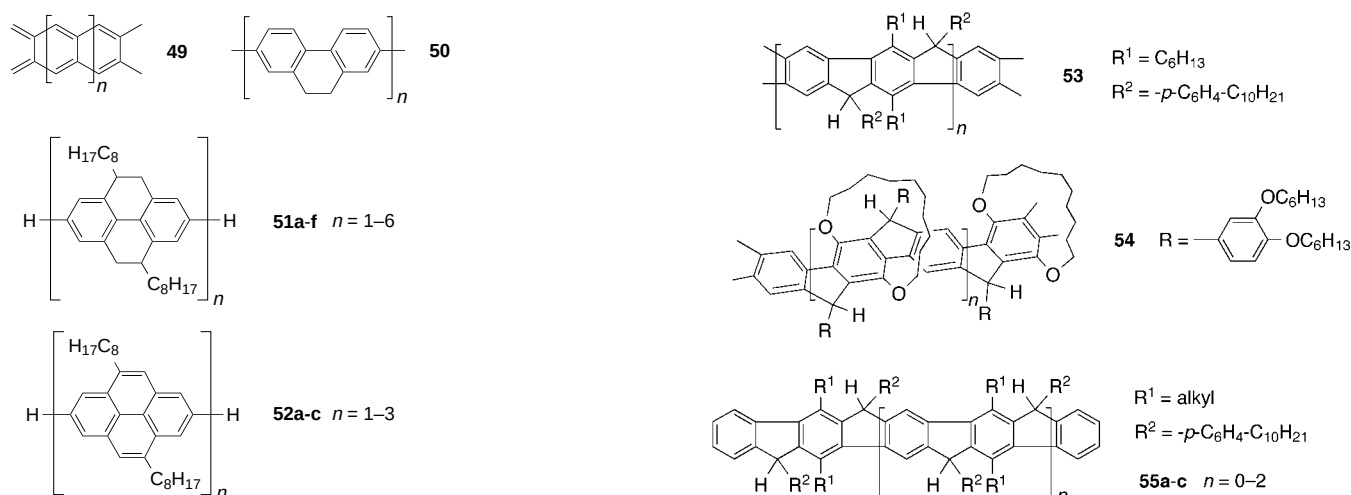
Scheme 18. The molecular rods **47** and **48a–c** were prepared to investigate electronic coupling mediated by oligo(*p*-phenylene) spacers.^[147, 148]

coupling between the radical termini is observed.^[147] Presumably a quinoidal singlet state is effectively prevented as a consequence of the twisted aryl backbone,.

The molecular rods **48a–c** (Scheme 18), which exhibit terminal cyclometalating bipyridylbenzene ligands (for N,C,N coordination) rigidly bridged by short oligo(*p*-phenylene) linkers, display pronounced electronic coupling over distances up to 2 nm in their mixed-valence forms. The much higher electron donor character of the anionic phenyl bridging ligands and the short Ru–C(phenyl) bonds lead to substantially increased metal–metal interactions relative to the analogous systems in which *meta*-terpyridine moieties are chosen for metal ion coordination.^[148]

4.6. Strategies to Planarize Oligo- and Poly(*p*-phenylene)s

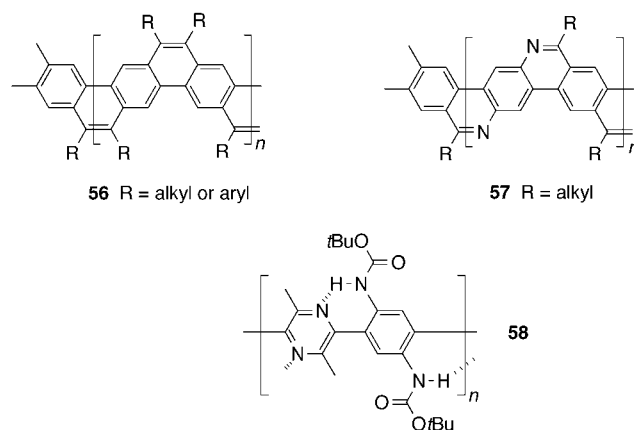
The planarization of oligo- and poly(*p*-phenylene)s to maximize the extent of conjugation by enhancing π -orbital overlap has been of notable interest for a number of years. One possible approach in pursuing this objective is the connection of adjacent aryl groups by 1,2-annellation rather than linear 1,4-connection as in the case of classical PPP. The resulting linear [*n*]acene oligomers **49** (Scheme 19) differ strikingly in their electronic character from PPPs, as a result of the very different connections between individual six-membered rings in the two classes of compounds. Thus, they feature low band gap energies and are inherently sensitive towards oxidation and dimerization.^[149] Nevertheless, they represent an interesting class of compounds from both theoretical and experimental viewpoints.^[150] Examples for planarized derivatives, in which the electronic characteristics of the PPP π -electron system are better retained, are oligomers and polymers with 5,6-dihydrophenanthrene-3,8-diyl repeat units (poly(phenanthrene)s, PPT, **50**)^[151] or with 4,5,9,10-tetrahydropyrene-2,7-diyl sub-structures (poly(tetra-

Scheme 19. Linear acenes **49**^[149] and planarized PPP models **50–52**.^[151–154]

hydropyrene)s, PTHP **51a–f** (Scheme 19).^[151–153] These systems with their ethane-1,2-diyl bridges between adjacent phenylene rings maintain inherent flexibility and are not fully planarized. Nevertheless, the investigation of the UV/Vis convergence behavior for the series **51a–f** revealed an ECL of $n_{\text{ECL}} = 19$ (n = number of phenylene rings in the linear backbone), which is nearly twice as high as those of the parent oligo(*p*-phenylene)s **42a–h** (n_{ECL} at $n \approx 7$) and **43a–d** (n_{ECL} at $n \approx 11$), respectively.^[44]

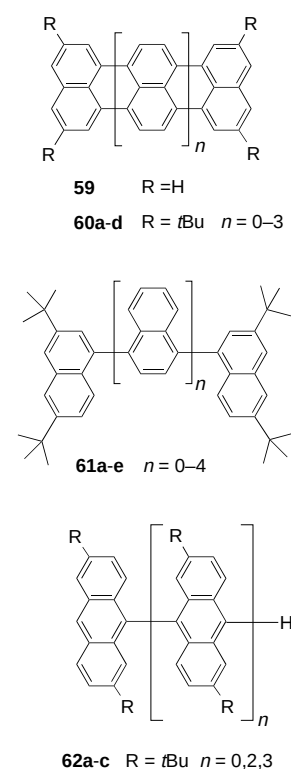
The oligo(pyrene)s **52a–c** (Scheme 19) were investigated by Müllen and co-workers as model oligomers for the corresponding infinite polymer and displayed several interesting features.^[154] First, the longest-wavelength electronic absorption maxima of **52a–c** displayed remarkably close values (λ_{max} [nm] = 344 (**52a**), 350 (**52b**), and 353 (**52c**)) which significantly contrasts with the optical behavior of other oligo(arylene)s. Second, cyclic voltammetry showed that the first reduction potentials E_1 are virtually independent of the number of pyrene units in the rods (**52b**: $E_1 = -2.24$ eV, **52c**: $E_1 = -2.27$ eV). Third, the generation of the dianion **52b**^{2–} did not lead to a diamagnetic state as was observed in the dianions of biphenyl or 1,1'-binaphthyl. All these results provide convincing evidence for the fact that the pyrene moieties are electronically decoupled from each other to a high extent in oligo- and poly(pyrene)s.

The planarization of a classical PPP backbone by the introduction of methylene bridges between the *ortho* positions of adjacent phenylene rings gave raise to the low-band gap polymers **53**^[151, 155] and **54** (Scheme 20).^[156a] Both polymers exist as mixtures of stereoisomers as a result of the stereogenic bridging centers; additionally, **54** features repeating elements of planar chirality, which increases the number of stereoisomers.^[157] These ladder polymers exhibit attractive optoelectronic properties such as extremely small Stoke shifts and are highly electroluminescent when used as the emitter material in light-emitting diodes.^[155, 156a, 158] Estimations of the ECL for model compounds **55a–c** (Scheme 20) revealed a significantly lower value of $n_{\text{ECL}} = 11-12$ (n = number of phenylene rings in the linear backbone) than that determined for the poly(tetrahydropyrene)s **51a–f** ($n_{\text{ECL}} = 19$).^[47] The

Scheme 20. Planarization of PPP analogues **53**,^[151, 155] **54**,^[156a] **55a–c**,^[47] **56**,^[159] and **57**^[132, 160] by bridging all *ortho*-positions of adjacent phenylene rings in the conjugated backbone. In **58** planarization of the backbone is achieved by intramolecular hydrogen bonding.^[161]

decreased ECL value for oligomers **55a–c** relative to **51a–f** is surprising and contrasts the expectation that planarization of the π -conjugated backbone should lead to a substantial increase in the ECL as a result of considerably facilitated π -electron delocalization. A series of well-defined poly(fluorene) oligomers up to the decamer was described by Klaerner and Miller.^[156b] The UV/Vis-based ECL for this partially rigidified polymer backbone was found to comprise about ten fluorene units. Other examples of fully planarized polymers with a PPP-type backbone are vinylene bridged **56**,^[159] imine-bridged **57**,^[132, 160] or **58** (Scheme 20), described by Meijer and co-workers, which form ladder-type structures as a result of strong intramolecular hydrogen bonding between the pyrazine-N acceptor centers and the amide N–H donor centers on neighboring rings.^[161]

The conjugated system **59** (Scheme 21), formally derived by connecting two parallel poly(*p*-phenylene) and *cis*-polyacetylene strands, provides another example of a fully planarized π -conjugated backbone.^[162–164] The synthesis of these oligo- and poly(*peri*-naphthalene)s was a long-targeted objective, since theoretical calculations predicted a low-lying HOMO–LUMO band gap, which would give rise to intrinsic conductivity.^[165] Various efforts towards their preparation were not



Scheme 21. Conjugation along the planar conjugated backbone of the oligorylenes **60a–d**^[162–164] is much more efficient than along the backbone of nonplanar **61a–e**^[163] or **62a–c**^[166]

(300 nm), and to **61c** (302 nm). This result reflects the significant steric interactions between adjacent naphthalene units that lead to a ground-state configuration in which neighboring naphthalene moieties adopt a nearly orthogonal orientation.^[163] A similar situation was found in the solubilized oligo(9,10-anthrylene)s **62a–c** (Scheme 21), which exhibit a longest-wavelength absorption maximum $\lambda_{\max}$ (CH_2Cl_2) of 402 nm for trimer **62b** and 408 nm for tetramer **62c**.^[166]

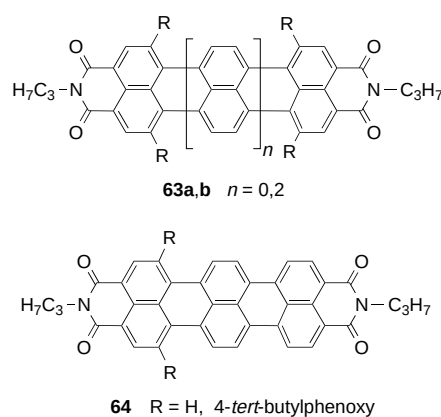
Further proof for the high rigidity of the conjugated backbones in **60a–d** was obtained by fluorescence spectroscopy in 1,4-dioxane: first by the very small Stoke shifts of 7 nm (**60a**: $\lambda_{\text{em}} = 446$ nm), 13 nm (**60b**: $\lambda_{\text{em}} = 573$ nm), and 18 nm (**60c**: $\lambda_{\text{em}} = 678$ nm) and second by the high fluorescence quantum yields Φ_{F} of 0.94 for **60a** and 0.70 for **60b**. However, the fluorescence of **60c** was found to be unexpectedly small, with $\Phi_{\text{F}} \approx 0.05$.^[162] The optical band gaps ΔE derived from UV/Vis and NIR measurements of the three successive oligomers **60b–60d** were used to extrapolate the band gap of the infinite poly(*peri*-naphthylene) polymer ΔE_{∞} to yield 0.98 eV in solution and 0.92 eV in the solid, non-crystalline state. The high redox activity of the oligorylenes **60a–d** is remarkable, and allows in the case of **60d** seven successive fully reversible redox steps (CV) that lead from a tetracation to a trianion. Oligomers **60a–d** were also used to determine the spin-density distribution in oligorylenes as a function of chain length.^[167] Study of the radical anions by electron paramagnetic resonance (EPR) and electron-nuclear double resonance (ENDOR) spectroscopy revealed a picture

successful as a result of substantial synthetic challenges and their expected extreme insolubility in organic solvents. Some of the solubility problems could be overcome by attaching *tert*-butyl groups and, thus, oligomers **60a–d** (Scheme 21), which have been named oligorylenes, provided a first opportunity for extrapolating experimentally some of the properties of poly(*peri*-naphthylene)s.^[162–164] The longest-wavelength electronic absorption maximum $\lambda_{\max}$ in 1,4-dioxane was found to undergo a large increase from 439 (**60a**) to 560 (**60b**) and to 660 nm (**60c**) as a result of the extension of the planar π chromophore.^[162]

In comparison, the related homologous oligo(1,4-naphthylene) series **61a–e** (Scheme 21) displayed hardly any change in $\lambda_{\max}$ values (CH_2Cl_2) on going from **61a** (293 nm), to **61b**

where the positions of the highest spin density are located at the outermost naphthalene units, which is in accordance with theoretical calculations (HMO/McLachlan and Pariser-Parr-Pople (PPP) methods). In contrast, radical anions of other linear π -conjugated systems, such as oligo(*p*-phenylenevinylene)s, show their highest spin densities in the central segments of the conjugated backbone already at small chain length.

The extended planar π systems of oligo(*peri*-naphthylene)s **60a–d** makes them attractive candidates for third-order NLO chromophores. The $\chi^{(3)}$ values from THG measurements at 1.064 μm of **60a–d** increased systematically with the reciprocal of the $S_0 \rightarrow S_1$ excitation energy, the position of the longest-wavelength absorption maximum $\lambda_{\max}$, and the chain length.^[8, 168, 169] The nonresonant $\chi^{(3)}$ value of **60d** was measured to be 80×10^{-12} esu, and the entire oligorylene series **60a–d** afforded a steep power law relationship with an exponent $a = 5.6$. Derivatization of oligorylene oligomers with terminal imide groups yielded the oligorylenimide compounds **63a, b** and **64** (Scheme 22), which represent a

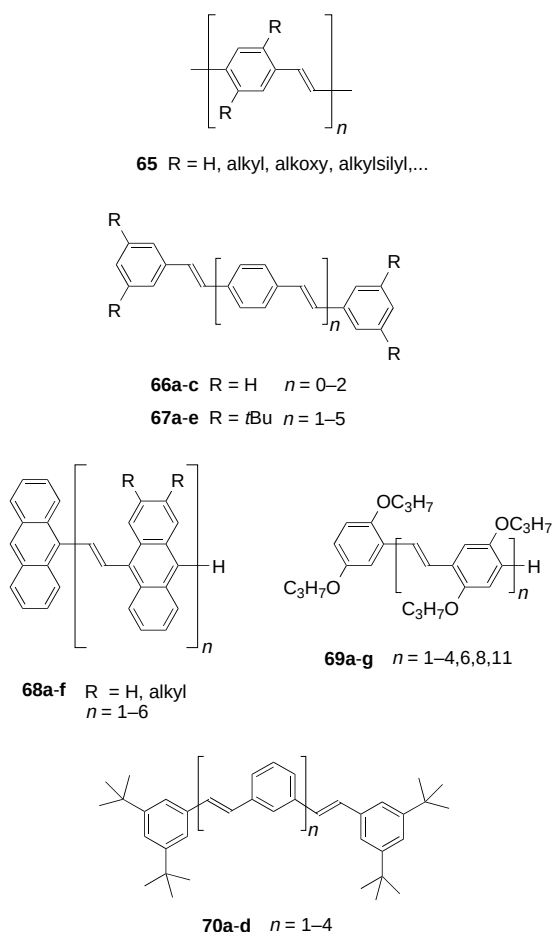


Scheme 22. Oligorylenimides **63a, b** and **64** represent a promising new class of NIR fluorescence dyes.^[170]

promising new class of NIR fluorescence dyes, as they exhibit high molar extinction coefficients, high fluorescence quantum yields, and excellent thermal, chemical, and photochemical stabilities.^[170]

4.7. Oligo(*p*-phenylenevinylene)s

The report of Friend, Holmes, and co-workers^[171] in 1990 that light-emitting devices (LEDs) can be constructed from poly(*p*-phenylenevinylene) (PPV, **65**, Scheme 23) films has provided a highly stimulating impetus to the field of π -conjugated organic molecules, and polymers with the PPV backbone are now among the most extensively studied ones.^[2, 129] The physical and electronic properties of PPV and its various, differently substituted derivatives have attracted much attention both from experimental and theoretical scientists.^[172] Synthetic chemistry has allowed the controlled manipulation of the HOMO–LUMO gap energy in PPV-based polymers, copolymers, and blends, and the manufacture of electroluminescent materials for the construction of

Scheme 23. PPVs^[172] and oligomeric model compounds **66–70**.^[184, 185, 187, 188]

LEDs,^[130, 173] with light emissions covering the whole visible spectrum, and light-emitting electrochemical cells (LECs) has come to reality.^[174] Even difficult to achieve blue light emitters can be made from PPV polymers with regions of limited conjugation length or from polymers containing short oligomers of the PPV type as side-chain chromophores.^[175] In this respect, oligo(*p*-phenylenevinylene) series such as **66a–c** or **67a–e** (Scheme 23) have helped a great deal in the drive to achieve a basic understanding of the optical properties of PPV polymers. The use of PPVs as emitter materials could also be successfully applied to the fabrication of organic-based lasers.^[176–178] More recently, Bazan and co-workers described the synthesis, spectroscopy, and morphology of tetra(stilbenoid)methanes. These compounds combine the advantages of small molecules (for example, high purity and volatility) with those of polymers (for example, amorphous morphology) and may be useful in the design of suitable materials for application in optoelectronics.^[177] An orthogonal synthetic approach, using Heck and Horner-Wadsworth-Emmons reactions, was applied by Maddux et al. to the preparation of a series of PPV oligomers, which were designed for the production of novel diblock copolymers.^[178] In this elegant, stepwise synthesis, the need for protecting group chemistry was eliminated.

Several groups have studied the photoexcited state behavior of PPVs by utilizing poly(*p*-phenylenevinylene) oligomers

of defined length and constitution to obtain structure–property relationships^[179] and to gain information about their decay behavior.^[180] Recently, Yu and co-workers evaluated in an elegant study the LED performance as a function of conjugation length for partially alkylated hexameric and decameric oligo(*p*-phenylenevinylene)s, which formed uniform films by spin-casting.^[181] Whereas UV/Vis, photoluminescence, and electroluminescence spectra showed small shifts for both oligomers, the LED device constructed from the 10-mer displayed a lower turn-on voltage, a smaller operation current, and greater stability than that made from the 6-mer. Furthermore, it has been demonstrated that oligo(*p*-phenylenevinylene)s can be vacuum evaporated to yield high purity thin films that have been studied to gain insight into solid-state structural aspects of π -conjugated materials.^[182] Correlated quantum-chemical calculations were of significance in clarifying the effects of intermolecular interactions on the electronic and optical properties of π -conjugated chains.^[183]

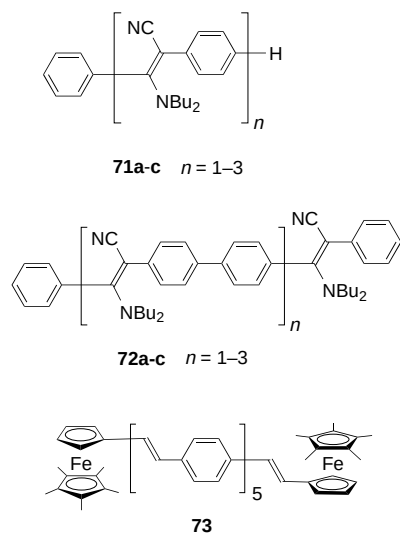
The ECL for the series **67a–e** was estimated from the chain length dependence of the longest-wavelength absorption maximum $\lambda_{\max}$ to be $n_{\text{ECL}} = 10$ monomeric units. The good accordance with the value obtained for the oligo(*p*-phenylene)s **43a–d** ($n_{\text{ECL}} = 11$) is surprising, considering the difference in length of PPV and PPP oligomers with identical numbers of repeat units and the reduced steric hindrance in the PPV relative to the PPP oligomers.^[184] Interestingly, the structurally related oligo(9,10-anthrylenevinylene)s **68a–f** (Scheme 23),^[184] which have an even lower tendency to planarize, revealed exactly the same ECL of $n_{\text{ECL}} = 10$ monomer units, but with a much less pronounced convergence behavior than observed for series **67a–e**.^[44] In the series **69a–g** (Scheme 23), with dialkoxy substituted repeat units, saturation of $\lambda_{\max}$ was attained for the longest oligomer **69g** ($n = 11$), which nicely conformed with the experimentally estimated ECL value of about $n_{\text{ECL}} = 10$ monomer units found for the unsubstituted series **67a–e**.^[44, 185] Oligomers similar to **69** were also subjected to a systematic investigation of the photoreactivity and photoconductivity in PPVs with dialkoxy substituted repeat units.^[186]

A comparison of the linearly π -conjugated oligomers **67a–e** with their *meta*-phenylenes-containing counterparts **70a–d** (Scheme 23) revealed several interesting differences. Whereas a bathochromic shift of the $\lambda_{\max}$ values with increasing number of monomer units is observed for the *para*-linked series **67a–e**, no chain length dependence of $\lambda_{\max}$ was observed for the constitutional isomers **70a–d** or their radical anions obtained upon doping with potassium.^[11, 187] The spectra of the monoanions in the *meta*-linked series are fully in accordance with the spectrum of the stilbene monoanion in each case. Furthermore, EPR/ENDOR spectroscopic investigations of **70b–d** left no doubt that the spin density in the radical anions is localized in a central stilbene unit.^[187, 188]

Optical measurements on oligomers **67a–e** provided evidence that a polaronic state within a PPV backbone affects at least about nine to ten styryl units.^[143, 189] Detailed studies concerning 1) the redox capacity of **67a–e** and several related model PPV oligomers, 2) the charge distribution in the corresponding monoanions, and 3) the structural changes

induced by electron transfer were performed by Müllen and co-workers.^[45, 57, 141, 190] For instance, all oligomers **67a–e** were found to be effective electron acceptors and the highest negative charge density was localized at the central olefinic bond. While in their neutral state, oligo(*p*-phenylenevinylene)s exhibit rapid rotation about all formal C–C single bonds, ion formation significantly enhances the corresponding rotational barriers and gives rise to detectable stereoisomers.^[57, 191]

The donor–acceptor substituted oligo(*p*-phenylenevinylene)s **71a–c** and the expanded analogues **72a–c** (Scheme 24) were prepared by an elegant cation–anion



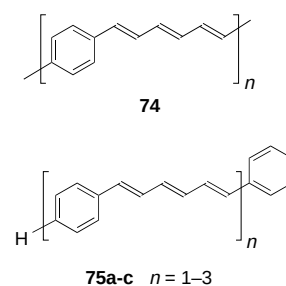
Scheme 24. Functionalized PPV oligomers **71–73**.^[192, 193]

coupling sequence, and targeted NLO chromophores with enhanced hyperpolarizabilities that resulted from large lateral dipole moments.^[192] However, the occurrence of configurational and conformational isomerism and Coulombic repulsion between the individual dipoles of each vinylene-bridged donor–acceptor pair prevented their additivity. Therefore, only a modest increase of the molecular dipole (**72a**: $\mu = 6.12$ D; **72c**: $\mu = 9.35$ D) with chain length was measured. Whereas unsubstituted PPV possesses an ECL of about $n = 10$ monomer units, convergence of the longest wavelength absorption maximum $\lambda_{\max}$ was found to occur in **72a–c** after five to six repeat units. Actually, by taking into consideration that a repeat unit in PPV counts three linearly conjugated olefinic and aromatic C–C double bonds and that the corresponding unit in **72a–c** counts five such bonds, convergence of linear optical properties in both systems occurs at around 30 C–C double bonds.

In other work, the intramolecular electron transfer along the conjugated π chain of PPV oligomers was investigated. Examples of compounds prepared for such studies are pentameric **73** (Scheme 24) with terminal pentamethylated ferrocene units,^[193] or a hexameric PPV oligomer terminated with porphyrin groups.^[194]

An expanded version of PPV with the poly(1,4-phenylenehexa-1,3,5-trienylene) (PPHT) backbone **74** (Scheme 25)

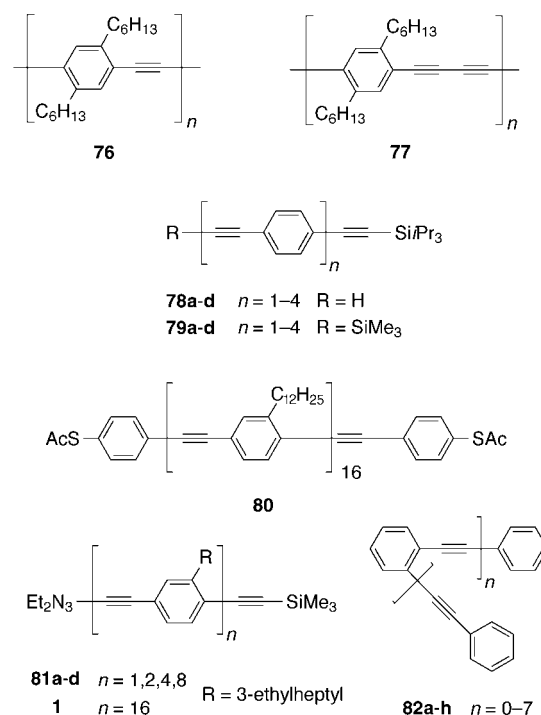
was proposed by Sonoda and Kaeriyama.^[195] Preliminary investigations of the model oligomers **75a–c** showed that PPHT readily undergoes thermally induced *cis*–*trans* isomerizations under iodine catalysis and displays a significant bathochromic shift of its longest-wavelength absorption maximum relative to PPV.



Scheme 25. Expanded PPVs with the poly(1,4-phenylenehexa-1,3,5-trienylene) (PPHT) backbone.^[195]

4.8. Oligo(*p*-phenyleneethynylene)s

The tremendous advances in the chemistry and physics of PPV over recent years have stimulated further interest in related types of structures, such as the poly(*p*-phenyleneethynylene) (PPE) polymers **76**, or the poly(*p*-phenylenebutadiynylene)s **77** (Scheme 26).^[196] PPEs exhibit large photoluminescence efficiencies both in solution and in the solid



Scheme 26. Poly(*p*-phenyleneethynylene)s (PPEs, **76**), poly(*p*-phenylenebutadiynylene)s **77**,^[196] and oligomeric model compounds **78–82** of PPEs.^[29, 201, 202, 206, 209]

state as a consequence of their high degree of rigidity, and their extremely stiff, linear backbone enables maximum orientation,^[30, 43, 197–202] which is an important feature for the preparation of oriented films or blends. Their potential as photoluminescence materials in organic polymer-based devices^[200] or as fluorescent chemosensors^[203] has already been demonstrated. Additionally, various oligo(*p*-phenyleneethynylene) spacers for the modular construction of organo-

metallic nonlinear optical chromophores^[204] or as building blocks for rigid-rod and hyperbranched, dendritic materials have been designed.^[205] Furthermore, Moore and co-workers recently demonstrated that poly(*m*-phenyleneethynylene) oligomers spontaneously can adopt a stable helical conformation with a large cavity and thus are valuable tools for studying folding processes in corresponding polymer systems.^[12] Oligo(*p*-phenyleneethynylene) series such as **78a–d** and **79a–d** (Scheme 26) should become useful to establish structure–property relationships.^[206]

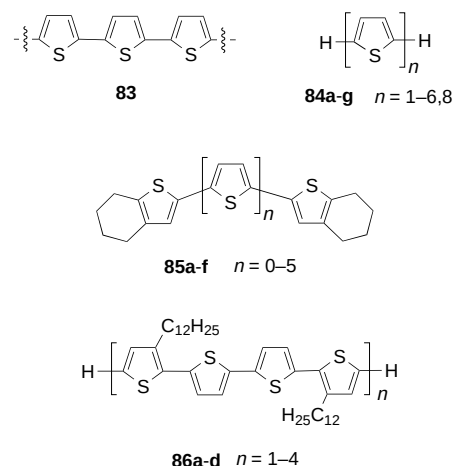
In an attempt to span the lithographically-derived probe gaps between two gold electrodes with a single molecule or with small packets of molecules, Tour and co-workers synthesized the 128 Å long oligo(*p*-phenyleneethynylene) rod **1** (see Section 2).^[29, 201] Thioacetyl groups were introduced into **80** (Scheme 26)^[201] to generate sites for adhesion to the gold surfaces, which undergo cleavage to the desired thiol end groups^[30] under mildly basic conditions.^[202] Saturation of the longest-wavelength absorption maximum λ_{max} in the oligomeric series **81a–d** and **1** (Scheme 26) was observed experimentally in the range of $n=10$ monomer units.^[29] An iterative convergent/divergent synthesis for hexyl- and isopentoxy-substituted PEEs was presented by Ziener and Godt.^[198] This efficient strategy, which afforded gram quantities of octamer, takes advantage of the selectivity of the Pd-catalyzed alkyne–aryl cross-coupling for aryl iodides over bromides and the possibility for converting an aryl bromide into an iodide by a halogen–metal exchange.

Allara, Tour, Weiss, and co-workers self-assembled a small number of short poly(*p*-phenyleneethynylene) oligomers into an insulating monolayer film of nonconjugated alkylthiols on a gold surface, from which the molecular “wires” protruded by about 7 Å.^[207] By searching the surface of the layer with a scanning tunneling microscopy (STM) probe tip, a significantly enhanced current flow was found for the conjugated molecules relative to the insulating monolayer. In a different experimental set-up using the mechanically controllable break junction, Tour and co-workers measured the conductance of a junction containing a single benzene-1,4-dithiol molecule.^[208]

A set of conjugated *ortho*-linked oligo(*o*-phenyleneethynylene)s **82a–h** (Scheme 26) were synthesized by Grubbs and Kratz, which showed no tendency for reduction under CV conditions but rather underwent irreversible oxidations in CH_2Cl_2 .^[209] Single X-ray crystal structure studies of **82c** and **82d** indicated that the compounds adopt a helical structure in the solid state.

4.9. Oligo(α -thiophene)s

Unquestionably, poly- and oligo(α -thiophene)s belong to one of the most carefully studied types of π -conjugated polymers and have received a great deal of attention for both fundamental and practical reasons. Poly(α -thiophene)s (PT, **83**) (Scheme 27) can generally be viewed as a *cis*-PA chain in which the structure is stabilized by the bridging sulfur atom.^[210] In comparison to PA, this provides several interesting features such as 1) higher environmental stability, 2) struc-



Scheme 27. Poly(α -thiophene) (**83**) and oligomeric model compounds **84–86**.^[228, 229, 233]

tural versatility that allows for the modulation of electronic and solubility properties by attaching appropriate side groups to the thiophene monomers, and 3) a nondegenerate ground state related to the nonenergetic equivalence of the two limiting mesomeric forms (aromatic and quinoid) of PT. Poly(α -thiophene)s have excellent semiconducting properties when doped,^[60, 61, 75] and oligomers exhibit many of the desirable electronic characteristics that have been evident in the long-chain polymers.^[211, 212] In addition, it has been shown that poly(α -thiophene)s can be chemically grafted onto glass surfaces to form thin conductive films,^[213] are suitable materials for Langmuir–Blodgett LEDs,^[214] and, if appropriately functionalized, act as nucleobase-responsive materials.^[215]

New developments involve the synthesis of PT co-oligomers and polymers with alternating donor–acceptor repeat units in order to limit the band gap widening that often occurs in fully substituted conjugated polymers.^[216] Using such a strategy Zhang and Tour developed new synthetic pathways to low optical band gap poly(α -thiophene)s^[217] and to imine-bridged, nearly planar, yet soluble PTs.^[218] Oligo(α -thiophenes) in tetrathiafulvalene hybrid systems have also been found to exhibit potent electron donor characteristics,^[219] to be attractive building blocks for nonlinear optical materials,^[8, 220] and to serve as active components in optical and redox switchable devices.^[221] Furthermore, monodisperse oligo(α -thiophene)s and fused derivatives thereof have found growing interest in device applications such as field-effect transistors (FETs),^[222] LEDs,^[223] photovoltaic cells,^[224] and light modulators.^[225] Poly- and oligo(α -thiophenes) can be prepared either by chemical^[26, 210] or electrochemical^[226] methodologies. Recently, the first solid-phase synthesis of oligo(α -thiophene)s on a chloromethylated macroporous resin, by using an alternating sequence of bromination and Stille cross-coupling reactions, was developed by Malenfant and Fréchet.^[227]

The second hyperpolarizability γ of the oligo(α -thiophene)s **84a–g** (Scheme 27) was studied as a function of increasing chain length.^[228] From these early measurements, the authors concluded that about $n=9–10$ repeating thiophene units are needed to achieve a respectable $\chi^{(3)}$ value of 10^{-9} esu. The UV/Vis related ECL of oligomers **84a–f** was

reported by Meier and co-workers to be around $n_{\text{ECL}} = 17$ monomer units.^[44]

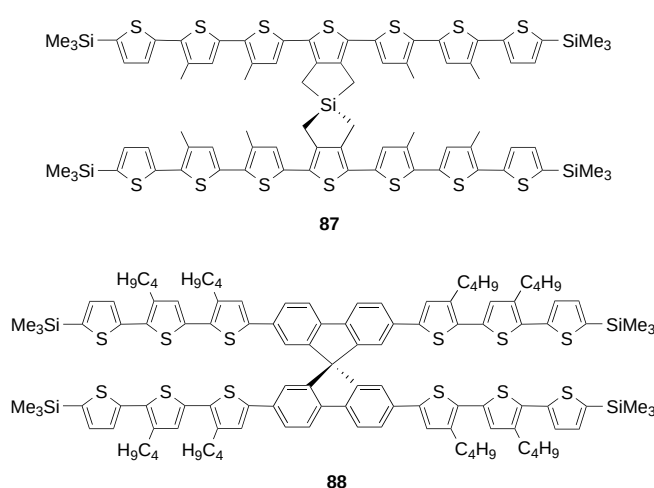
The redox characteristics of oligo(α -thiophene)s **85a–f** (Scheme 27) fused to cycloalkane endcaps to block the reactive α and β positions in the terminal thiophene rings have been studied thoroughly by Bäuerle et al.^[229] The first and second oxidation potentials were found to decrease with increasing chain length and to scale linearly with the inverse number of monomer units. This study also showed that the binding energy of the dimer, which is readily formed by oligo(α -thiophene) radical cations, increases with the oligomer chain length. The electronic and optical properties of similar oligo(α -thiophene)s were subject to careful and extensive investigations by several other research groups, who addressed, in particular, questions concerning polaron and bipolaron formation during the charging process.^[48, 49, 230–232] A series of oligo(α -thiophene)s bearing Me groups in the β positions of some of the monomeric repeat units and extending to the stage of an octamer was reported by Tour and Wu.^[211] From controlled oxidation of these compounds with FeCl_3 and subsequent spectroscopic studies, they estimated the delocalization length in PTs for the radical cation (polaron) to be around $n = 12$ monomer units and for the dication (bipolaron) around $n = 10$ monomer units.^[49, 231]

The ECL deduced from UV/Vis measurements for the homologous thiophene oligomer series **86a–d** (Scheme 27) was found to be slightly higher ($n_{\text{ECL}} = 20$ monomeric units) than for the oligo(α -thiophene)s **84a–f** ($n_{\text{ECL}} = 17$).^[44] Hexadecamers such as **86d** exhibit an end-to-end distance of 64 Å in their extended linear conformation.^[233] The longest monodisperse and fully characterized oligo(α -thiophene)s to date are the icosamer (20-mer) and heptacosamer (27-mer) recently reported by Otsubo and co-workers.^[234]

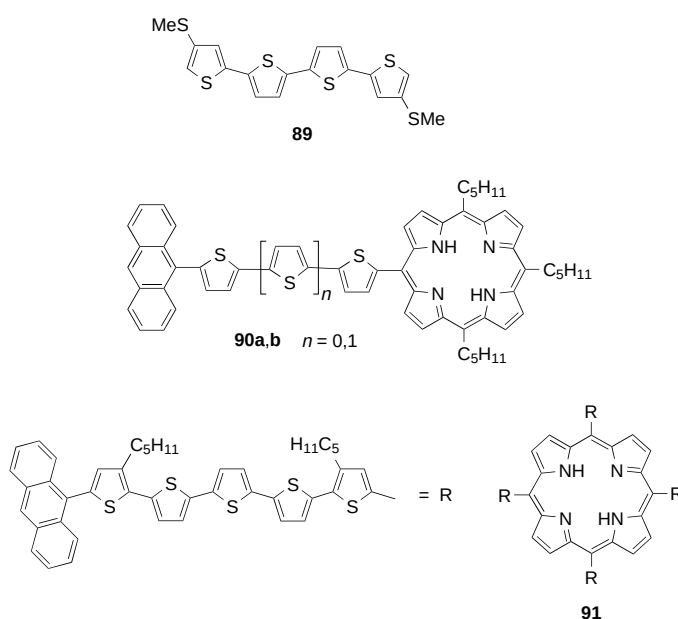
Using temperature dependent ^1H NMR techniques, LeGoff and co-workers found a barrier to atropisomerization in the ground state in the order of about 8 kcal mol^{−1} for a family of substituted poly(α -thiophene) oligomers, with no significant influence of the length of the oligomer.^[235] The barrier to isomerization in the first electronic excited state was determined from fluorescence lifetime measurements to be slightly reduced, with a value of about 5 kcal mol^{−1}. A theoretical study to evaluate the influence of chain length and derivatization on the lowest singlet and triplet states in oligo(α -thiophene)s was performed by Brédas and co-workers.^[236]

In an approach to orthogonally fused conducting polymers for molecular electronics, Tour and co-workers prepared the spiro-fused oligo(α -thiophene)s **87** and **88** (Scheme 28).^[237] The electronic communication between the two orthogonally arranged heptamer radical cations or the dications in **87** was found to be quite weak.^[238] Later, it was shown that the positively charged center (polaron) migration in **87** along the π -conjugated chain has an activation energy barrier of less than 100 cm^{−1}.^[239]

Among functionalized oligo(α -thiophene)s^[240] and corresponding (*S,S*)-dioxides,^[241] methylthio derivatives such as **89** (Scheme 29) are of particular interest, since they exhibit high phototoxic activities against nematodes, larvae and eggs of insects, bacteriae, algae, and fungi as a result of their ability to photosensitize the formation of singlet oxygen ($^1\text{O}_2$).^[242]



Scheme 28. Spiro-fused dimeric oligo(α -thiophene)s **87** and **88**.^[237]

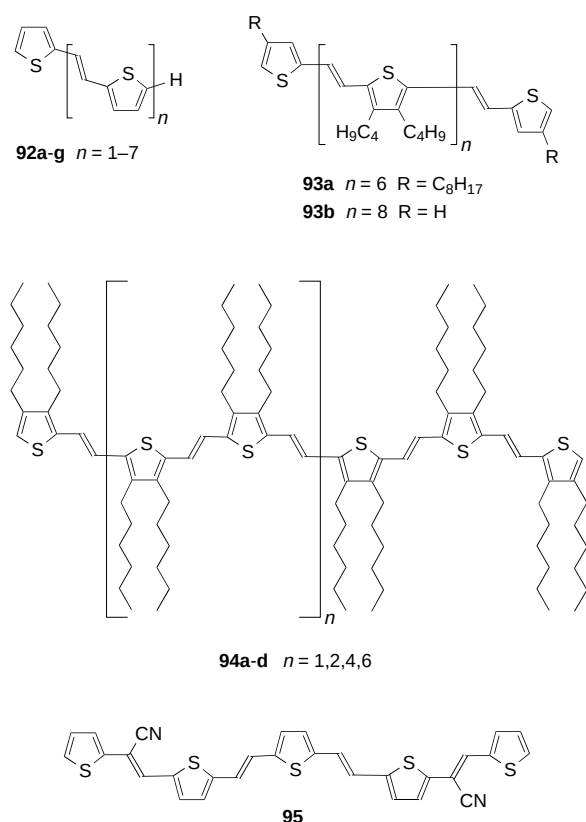


Scheme 29. Functionalized oligo(α -thiophene)s with phototoxic (**89**)^[242] and electron transfer activity (**90, 91**).^[243, 244]

Oligo(α -thiophene)s have also been used as conjugated linkers between donor–acceptor systems to study intramolecular energy transfer properties, and exhibit several intriguing advantages, when compared to oligoene spacers, such as improved thermal and chemical stabilities, higher fluorescence quantum yields, large degree of π -electron delocalization along the conjugated chain, and the inability to *cis–trans* isomerize.^[243] Examples are the anthraceneoligo(thienyl)porphyrin systems **90a, b** (Scheme 29) and the fourfold anthryl quinque(thienyl) substituted porphyrin **91** with its light-harvesting molecular antenna-type architecture.^[244]

4.10. Oligo(α -thiophenevinylene)s

Monodisperse oligo(α -thiophenevinylene)s such as the series **92a–g** (Scheme 30) have been synthesized utilizing controlled Wittig–Horner coupling reactions and low-valent

Scheme 30. Monodisperse oligo(α -thiophenevinylene)s **92–95**.^[245, 247–251]

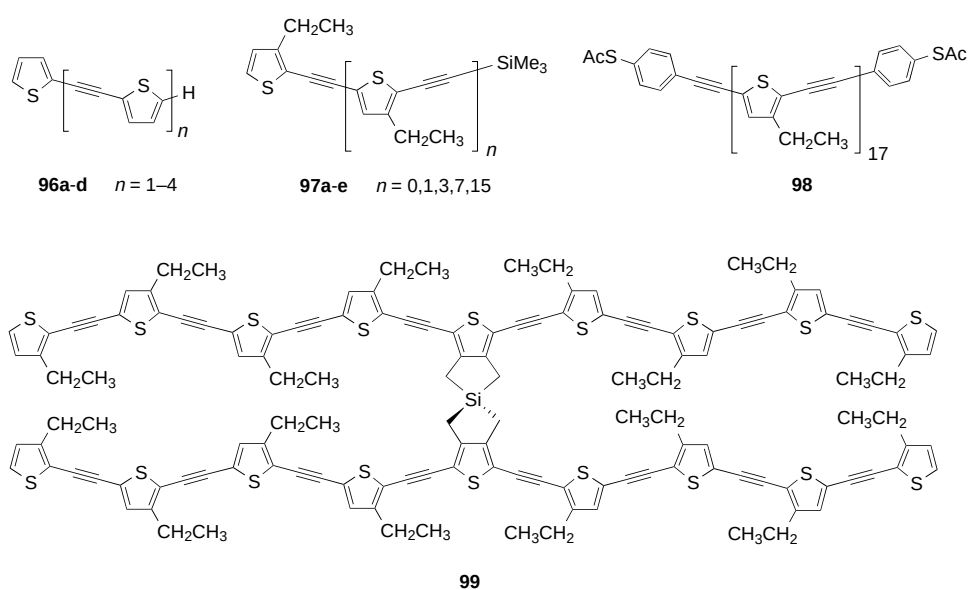
titanium reagents (McMurry reaction).^[245] THG measurements at the fundamental wavelength of 1.064 μm for thin films of **92b** and **92c** revealed values up to $\gamma = 1.65 \times 10^{-33}$ esu, which are larger than theoretically expected because of resonance enhancement.^[246] Adding double or triple bonds between the thiophene groups has only a modest effect on the second hyperpolarizability, which has a similar magnitude in oligo(α -thiophene)s and oligo(α -thiophenevinylene)s of comparable chain length.

The estimation of the ECL for the series **92a–f** from UV/Vis data revealed convergence at $n_{\text{ECL}} = 14$ repeat units. This is three monomeric units lower than the value reported for the oligo(α -thiophene)s compounds **84a–f**, which could be expected given the larger number of conjugated C–C double bonds in the repeat units of oligo(α -thiophenevinylene)s.^[44] The poly(α -thienylenevinylene) oligomers **93a, b** (Scheme 30) as well as corresponding shorter chain derivatives were prepared by Roncali and co-workers.^[247–250] The electrochemical analysis of octamers such

as **93b** provided conclusive evidence that dications in such systems are formed directly through a single-step two-electron transfer.^[248] The same research group also studied the influence of progressive steric shielding of the π -conjugated backbone on the electrochemical properties by attaching Fréchet-type dendrons to both termini of a thienylene vinylene tetramer.^[249] The hexadecamer **94d** of the oligo(α -thienylenevinylene) series **94a–d** (Scheme 30) is the longest monodisperse oligo(α -thiophenevinylene) published, and saturation of the effective conjugation was still not observed by UV/Vis spectroscopy.^[250] From a comparison of the longest-wavelength absorption maximum of oligomer **94d** with those of hexadecamers containing the same number of carbon atoms or similar dimensions such as the oligo(p -phenyleneethynylene) **1** (128 Å, $\lambda_{\text{max}} = 376$ nm in CH_2Cl_2) or a hexadeca(α -thiophene ethynylene) (100 Å, $\lambda_{\text{max}} = 442$ nm in CH_2Cl_2 ; see Section 4.11), it is apparent that the oligo(α -thiophenevinylene) **94d** (95 Å, $\lambda_{\text{max}} = 601$ nm in CH_2Cl_2) exhibits the largest extended π conjugation. An example of a thiophene-based oligomer with a high electron affinity is the cyano substituted compound **95**.^[251]

4.11. Oligo(α -thiopheneethynylene)s

The study of the third-order nonlinear optical properties of the poly(α -thiopheneethynylene) oligomers **96a–d** (Scheme 31)^[252] by THG measurements revealed γ values up to 2.30×10^{-33} esu for **96d**, and showed no dramatic increase compared to oligo(α -thiophene)s or oligo(α -thiophenevinylene)s of similar length.^[246] A preliminary value for the ECL deduced from UV/Vis measurements yielded $n_{\text{ECL}} = 12$ monomer units, which needs re-investigation because of a lack of data points.^[44] In the propagation starting with oligo(α -thiophene)s **84a–f** and oligo(α -thiophenevinylene)s **92a–f**, the oligo(α -thiopheneethynylene)s **96a–d** displayed the highest “velocity of convergence” as characterized by the increasing value of the parameter b_i according to Meier et al. (**84a–f**: $b_i = 0.352$; **92a–f**: $b_i = 0.425$; **96a–d**: $b_i = 0.446$).^[44]

Scheme 31. Monodisperse oligo(α -thiopheneethynylene)s **96–98** and spiro compound **99**.^[16, 32, 252–254]

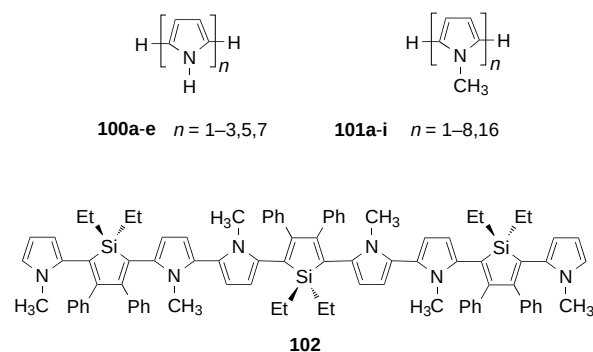
The synthesis of the soluble oligomers **97a–e** (Scheme 31) was described by Tour and co-workers.^[16, 32] Recently, the same group also presented the synthesis of the poly(α -thiopheneethynylene) heptadecamer **98** which is 128 Å long and contains acetylthiophenyl groups at the termini. After cleavage of the acetyl groups the resulting terminal thiol functions should allow attachment of the rod to proximal gold probes in order to study its use as a molecular wire in molecular scale electronic devices.^[16, 25, 253]

Investigations within the series **97a–e** disclosed important information about the scaling laws that describe the dependence of the second hyperpolarizability on chain length in such types of π -conjugated oligomers.^[254] THG measurements in THF solutions of **97a–e** showed a strong increase in γn^{-1} up to octamer **97d** but which tended to level off for hexadecamer **97e**, which demonstrated the effects of saturation. Tour and co-workers also prepared the orthogonally fused system **99** (Scheme 31) as a possible charge-transporting interconnection in molecular-scale electronics.^[254]

4.12. A Potpourri of π -Conjugated Oligomers

Substitution of C atoms in oligoenes by N atoms has considerable impact on the resonance energy and thus directly on the lowest-energy absorption maximum $\lambda_{\max}$ and other physical properties.^[255] Poly(methineimine) $-(CH=N)_n-$ oligomers,^[256] for instance, found attention from a theoretical point of view,^[257] but as a result of their limited stability they have been much less investigated than their all-carbon counterparts.

Despite the importance of poly(pyrrole) in the field of conducting polymers^[61] less experimental work has been devoted to the investigation of poly(pyrrole) oligomers. Although oligo(α -pyrrole)s are quite sensitive to heat and light, two series **100a–e**^[258] and the *N*-methyl derivatives **101a–i**^[259] (Scheme 32) allowed first estimations of the



Scheme 32. Oligo(pyrrole)s **100a–e** and **101a–i**^[258, 259] and (silole pyrrole) co-oligomer **102**.^[262]

properties of the corresponding polymers. Whereas for the first oligomer series the ECL was predicted to lie at $n_{\text{ECL}} \approx 13$, a value of $n_{\text{ECL}} = 7$ was found for **101a–g**, which reflects the increased steric interactions from the *N*-methyl groups in this second series.^[44] Recently, Meijer and co-workers presented the first oligo(pyrrole)s functionalized with terminal donor

(*p*-methoxyphenyl) and acceptor (*p*-nitrophenyl) groups in the range from monomer to tetramer.^[260] Their nonlinear optical properties were studied by hyper-Rayleigh scattering.

New developments led to the synthesis of low band gap zwitterionic and planar conjugated poly(pyrrole)s that possess a reversible pH-, solvent-, and ion-dependent absorption ranging from the UV to the NIR spectral region, which makes them attractive for polymer-derived sensor applications.^[261] First studies on novel poly(silolepyrrole) co-oligomers such as **102** (Scheme 32) revealed this π -conjugated backbone to be a promising candidate for low-band gap polymers.^[262]

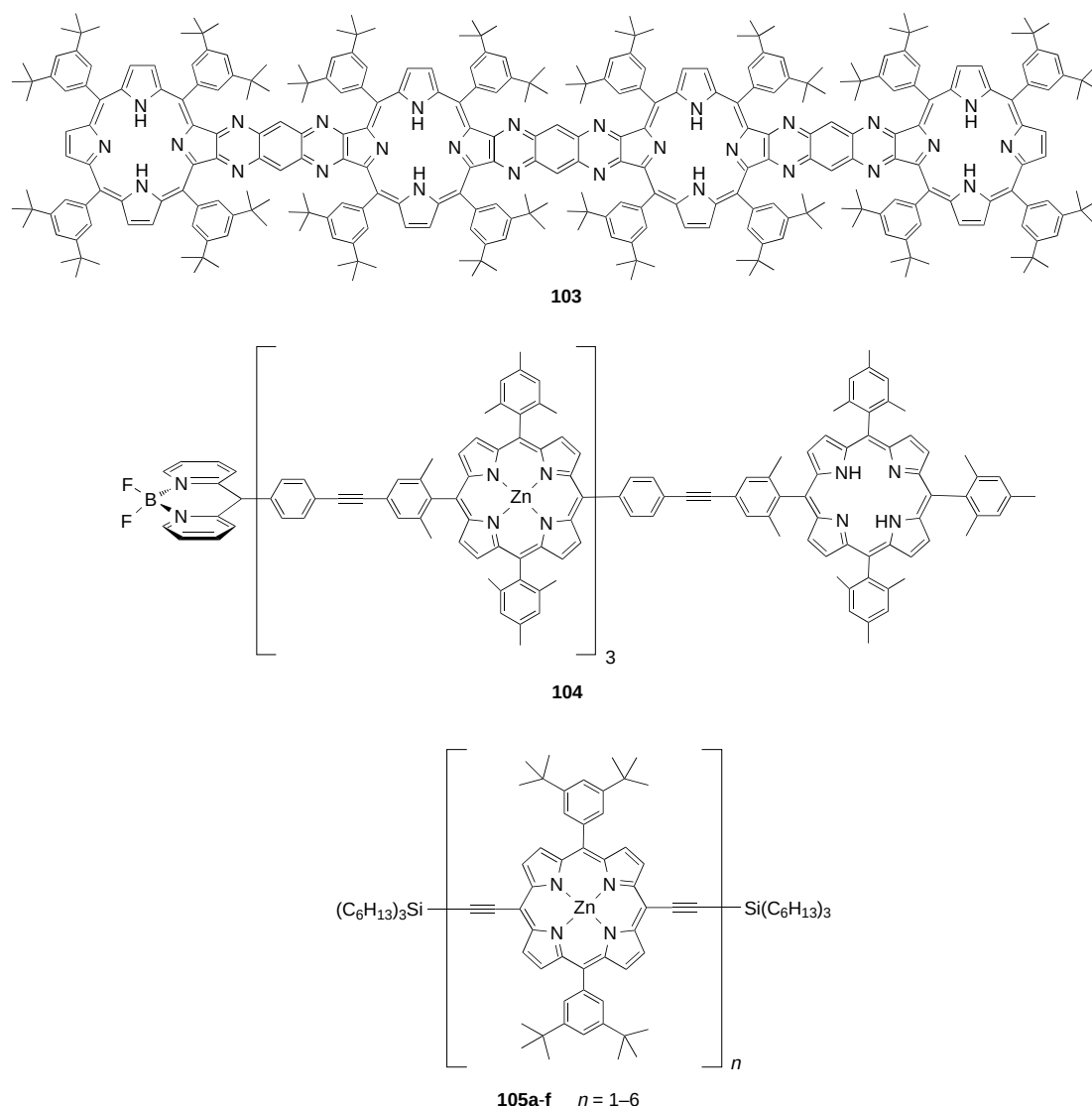
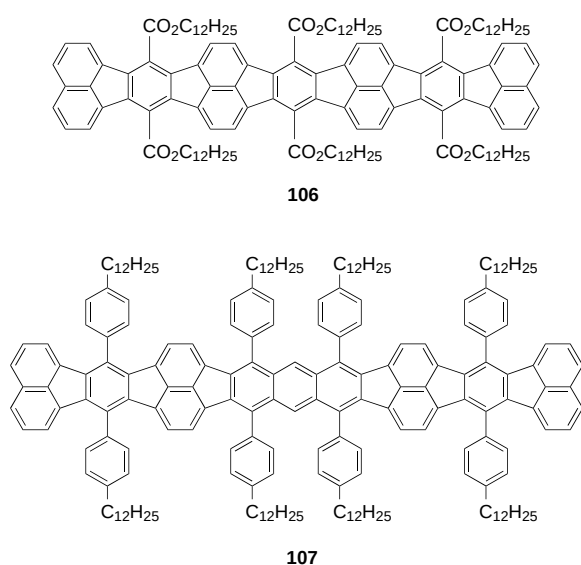
An approach to molecular wires based on linearly conjugated polyporphyrin systems was presented by Crossley and co-workers with the tetrakisporphyrin **103** (Scheme 33), which exhibits an edge-to-edge length of about 56 Å.^[263] Other examples are the multichromophoric photonic wire **104** (Scheme 33), studied by Wagner and Lindsey, which showed a remarkably high yield of energy transfer from the boron-dipyridylmethane terminus to the free base porphyrin at the other terminus,^[264] the one- and two-dimensional porphyrin oligomers and polymers containing oligothiophenyl molecular wires explored by Shimidzu et al.,^[265] the fascinating windmill-like porphyrin arrays from Osuka and co-workers, which are potent light-harvesting antenna structures,^[266] and the porphyrin-poly(triacetylene) co-oligomers studied by Wytko et al.^[267]

Recently, Anderson and co-workers presented a series of conjugated buta-1,3-diynediyl-linked porphyrin oligomers **105a–f** (Scheme 33) in the range from monomer to hexamer.^[268] From UV/Vis and fluorescence measurements, a HOMO–LUMO energy band gap for the infinitely long polymer of 1.55 eV (from absorption data) and 1.42 eV (from emission data) was extrapolated. The X-ray crystal structure of dimer **105b** revealed a remarkably planar conjugated backbone, which included the entire 56 atoms of the π system (excluding the *meso*-phenyl rings) with a maximum deviation from planarity of ± 0.391 Å. The dihedral angle between the two porphyrin planes was found to be near zero in the solid state, although rotation about the buta-1,3-diynediyl linker may freely occur in solution.

Examples of structurally perfect, fully π -conjugated double-stranded (ladder) oligomers are **106**^[269] and **107**^[270] synthesized by Schlüter and co-workers (Scheme 34). They have served as model compounds for analogous high molecular weight polymers, that showed conductivities in their doped state up to 0.01 Scm^{-1} ^[271] and are interesting materials for their optical nonlinearity, electroluminescence, and photovoltaic properties.^[272]

5. Outlook

The preparation and study of monodisperse oligomers to provide structure–property relationships is now well established and provides invaluable information on the properties of infinite, often poorly soluble and intractable polymers. However, monodisperse π -conjugated oligomers are more than just appropriate model compounds for the analogous high molecular weight polymers. Over the past years, they

Scheme 33. Molecular wires with porphyrin units.^[263, 264, 268]Scheme 34. Double-stranded (ladder) oligomers.^[269, 270]

have evolved into an independent class of their own, characterized by some spectacular molecular architecture and function, and proved their high potential in several technological applications such as in light-emitting diodes and organic transistors, or as laser dyes and nonlinear optical chromophores. One of the largest challenges fueling the field is certainly the proposed use of single monodisperse oligomers as molecular wires in molecular scale electronic devices. The synthesis of monodisperse π -conjugated oligomers with lengths exceeding 10 nm has brought this interesting prospect closer to reality. Ultimately, it should not be overlooked that the rapid progress in the field of large π -conjugated oligomers has been greatly stimulated by developments in other fields of science. Methods such as scanning tunneling microscopy (STM) or atomic force microscopy (AFM), which allow imaging on a submolecular level, provide entirely new information about the physical behavior of π -conjugated systems. In addition, analytical methods such as matrix-assisted laser-desorption-ionization time-of-flight mass spec-

trometry (MALDI-TOF MS) made the accurate characterization of large monodisperse oligomers possible for the first time. The developments described in this article have been particularly boosted by modern synthetic methodology, especially by the rich diversity of metal-catalyzed cross-coupling reactions that have been invented in recent years. A large fraction of the multianometer-scale oligomers described herein and in particular those currently considered for exploitation in molecular-scale electronic devices have been prepared by using cross-coupling reactions in the key steps. Resin-supported solid-phase preparations of oligomers are becoming increasingly efficient. It can also be expected that combinatorial techniques will in the future be applied to the construction of libraries of monodisperse co-oligomers with different repeat units. Efficient and convenient screening of such libraries for desired properties should greatly accelerate the search for technologically useful materials. It is clear that, once the principle of the utilization of π -conjugated oligomers in molecular scale electronic devices has been proved, the field described in this article will push ahead with further explosive growth.

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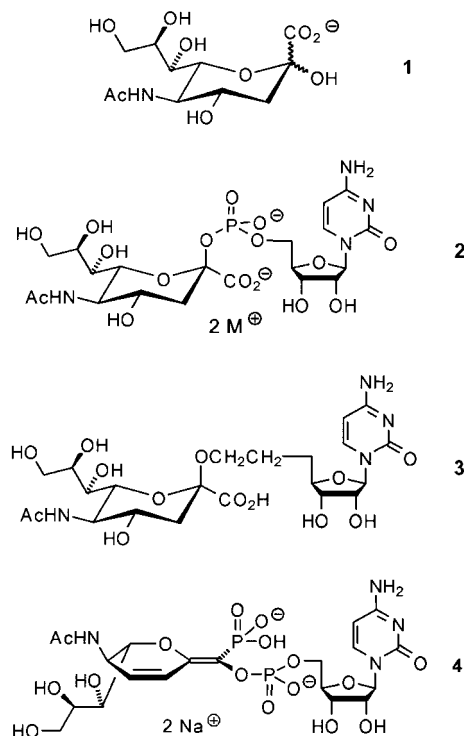
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From Substrate to Transition State Analogues: The First Potent Inhibitor of Sialyltransferases

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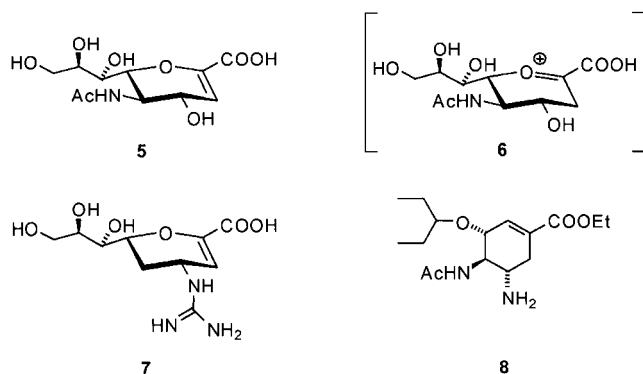
Sialic acid containing glycoconjugates are cell-type specific markers that are widely distributed throughout the organism. They play a vital role in a variety of fundamental physiologically and pathologically important processes.^[1–3] These include embryogenesis, organ development, immune defense, migration and homing of leukocytes, metastasis of neoplastic cells, as well as inflammation and the infection by a variety of pathogens. Up to now more than 40 different sialic acids have been discovered, of which *N*-acetylneuraminic acid (**1**) is the most abundant one. They are often attached to the exposed terminal position of the oligosaccharide moiety of glycoconjugates and determine many of the biological properties of these structures. The influenza infection (common flu) is a well-known example of a cell/virus interaction mediated by sialic acid. Both adhesion to the cell prior to infection as well as the release of newly formed viruses from the infected cells are accomplished by structures containing sialic acid. Thus, the virus-releasing enzyme influenza sialidase has been an early target for pharmaceutical research.^[3] Inhibitors of the biosynthesis of sialic acids on the other hand might prove to be useful as antiinflammatory, immunosuppressive, and anti-metastatic agents. Although the biosynthetic pathway that leads to *N*-acetylneuraminic acid was discovered some time ago,^[4] only a few such inhibitors have so far been developed.^[5]

Another issue of research is the inhibition of sialyltransferases, a class of enzymes that catalyze the transfer of sialic acid moieties to their specific acceptor molecules. Nineteen members of this enzyme class have been identified so far.^[6] Until a recent report by R. R. Schmidt et al., no potent and specific inhibitors of these enzymes had been described. The authors present the synthesis of compound **4**, which exhibits an inhibition constant of K_i of 40 nM to rat liver $\alpha(2-6)$ -sialyltransferase [EC2.4.99.1], and thus has a 1000-fold higher affinity than the natural substrate.^[7] Originally, the rational design of this inhibitor was based on cytidine monophosphate-*N*-acetylneuraminic acid (CMP-Neu5Ac, **2**), which is the common donor substrate of all known sialyltransferases. One example of such inhibitors of substrate analogues is the CMP-Neu5Ac-derived compound **3**, which reduces the transfer of



N-acetylneuraminic acid to lactosyl ceramide by 38 %, and to the ganglioside GM₃ by 63 %.^[8] The concept of transition state analogues gained more and more attention during the development of compound **4**. Back in 1946 Linus Pauling stated that inhibitors closely related structurally to the transition state of an enzyme-catalyzed transformation should bind more tightly to the active site of the enzyme than analogues of the substrate in its ground state.^[9] Kinetic studies indicated that for a one-substrate reaction the transition-state analogue is bound 10⁷ to 10¹⁵ times tighter than the substrate in its ground state.^[10] At the very beginning of the project, potent transition state analogues for the sialidases related to the replication of the influenza virus had already been developed. These compounds have been derived from the dehydroacetylneuraminic acid (Neu5Ac2en, **5**) and imitate the geometry of the proposed carboxonium intermediate **6** during the release of the *N*-acetylneuraminic acid moiety. Two of these drugs, derivative **7** with the guanidino substituent (Zanamivir, Glaxo Wellcome) and the cyclohexene derivative

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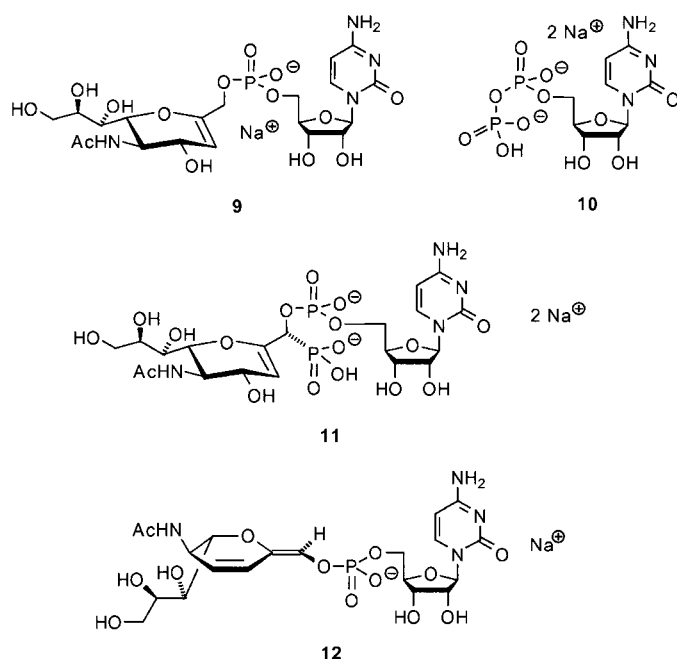
8 (GS4104, Hofmann-La Roche) successfully passed clinical testing phases I–III and are both believed to become approved by the Drug Administration authorities.^[3, 11] It was questionable whether this concept would be directly transferable to the development of sialyltransferase inhibitors because of its different mode of action: The transferase reaction proceeds under inversion at the anomeric carbon center while sialidases act under retention of configuration. Thus, it was not surprising that early attempts failed. Compound **9** has only a low affinity ($K_i = 2$ mM) to the $\alpha(2-6)$ -sialyltransferase from rat liver [EC 2.4.99.1],^[12] compared to the natural substrate, CMP-Neu5Ac, which has a Michaelis–Menten constant K_M of 46 μ M. On the other hand, cytidine

diphosphate (CDP, **10**) was known to inhibit this specific sialyltransferase with a K_i value of 10 μ M. The combination of the Neu5Ac2en moiety and the dianionic structure of CDP led to compound **11**, which is indeed a potent inhibitor with a K_i value of 350 nM.^[12] Replacement of the Neu5Ac2en moiety by **12**, an elimination by-product of the synthetic pathway that leads to **11**, even outperformed this result. Compound **4** is the most potent inhibitor of the $\alpha(2-6)$ -sialyltransferase known today. Subsequent studies revealed that it also strongly inhibits $\alpha(2-3)$ -sialyltransferases.

The work of Schmidt et al. is a convincing example of rational inhibitor design in the challenging area of glycosyl transferases. The transition state analogue **4** is an important tool that may serve to further elucidate the function of sialic acid containing glycoconjugates. To reach this goal it will nevertheless be necessary to develop cell-permeable analogues, for example, prodrugs.

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Keywords: enzyme inhibitors • neuraminic acids • rational design • sialyltransferases • transition state analogues



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Fullerene Pipes, Tube-in-Tube Membranes, and Carbon-Nanotube Tips: Adding New Dimensions to Molecular Technology

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Following the bonanza of fullerenes and endohedral metallofullerenes, other novel forms of carbon continue to spark great interest among chemists and physicists alike. These new varieties include single-wall carbon nanotubes (SWNTs) and multiwall carbon nanotubes (MWNTs) as well as bucky onions. Several large-scale routes that lead to carbon nanotubes (NTs) are now available.^[1] This article is intended to highlight recent advances in molecular technology involving carbon nanotubes.

In the focus of current interest are single-wall carbon nanotubes of molecular perfection ("fullerene nanotubes") as a result of their unique electronic and mechanical properties in combination with chemical stability.^[2] Promising applications of SWNTs include, for example, the storage of hydrogen and other gases,^[3] quantum wires,^[4] electronic devices,^[5] and catalyst supports.^[6] Until now there was a striking paucity of fullerene structures in the intermediate size range between typical fullerenes and long SWNTs, namely with a length of 10 to 300 nm. These would be of paramount importance as possible connectors and components for molecular electronic devices. A recent publication by Smalley et al. makes such "fullerene pipes" readily available for the first time and opens up a highly promising new branch of molecular technology.^[7]

The procedure that leads to fullerene pipes comprises several innovative steps. Crude SWNTs can be produced in a laser-oven in amounts of up to 20 g in two days by the use of a metal catalyst (Ni, Co, Fe).^[1c,d] However, this scaled-up method yields a material that contains substantial amounts of nanoscale impurities (for example, amorphous carbon, bucky onions, and spheroidal fullerenes; Figure 1 a). Thus an important first step is the purification of this SWNT material before cutting. It was found that refluxing the nanotubes in 2.6M nitric acid and resuspending them in water with surfactant at pH 10 followed by filtration with a cross-flow filtration system afforded purified fullerene fibers in 10–20% yield (by weight). A crude material of particularly low quality was chosen to demonstrate the efficiency of the purification procedure. According to scanning electron microscope (SEM) and transmission electron microscope (TEM) imaging the fullerene fibers form endless ropes that are highly tangled with one another. A unique free-standing mat of these tangled SWNT ropes ("bucky paper", Figure 1 b) could be made by passing a purified SWNT suspension through a polytetrafluoroethylene filter. Figure 1 c shows a torn edge of this bucky paper. It is clearly seen that tearing results in substantial alignment of the fullerene ropes.

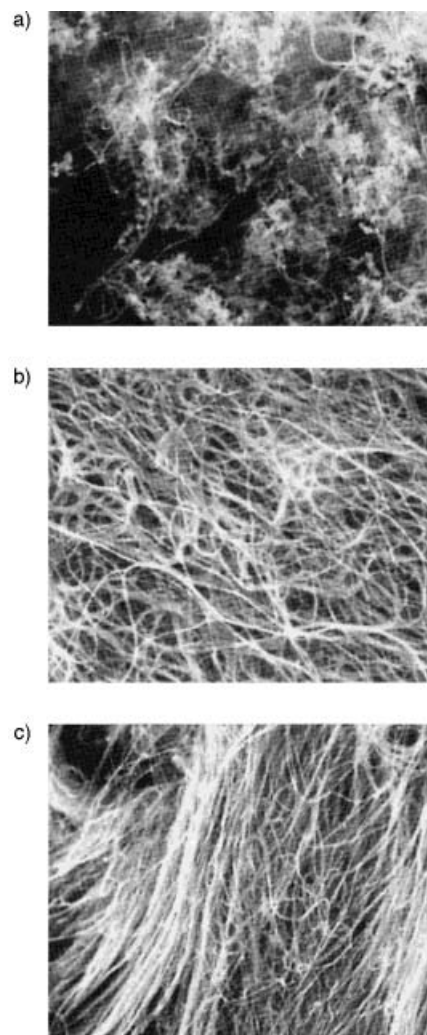


Figure 1. SEM images of a) raw SWNT material, b) purified bucky paper, and c) a torn edge of purified bucky paper (reprinted with permission from *Science*^[7]).

The following step, namely, the cutting of the nearly endless, tangled ropes can be performed either by simply cutting the bucky paper with a pair of scissors or by more sophisticated techniques such as bombardment with relativistic gold ions. However, the best and most effective method was found to be ultrasonication of purified SWNT ropes in a 3:1 mixture of concentrated $\text{H}_2\text{SO}_4:\text{HNO}_3$ at 40 °C (Houston room temperature). The effect of this particular treatment is twofold: First, ultrasonication creates open holes in the tube side. Subsequent attack of the oxidizing acid mixture then occurs at the points of damage, which leads to the efficient cutting of the SWNT ropes into thinned rope pieces and individual cut tubes (Figure 2).

It was found that stable colloidal suspensions of the resulting "fullerene pipes" could be obtained in the presence

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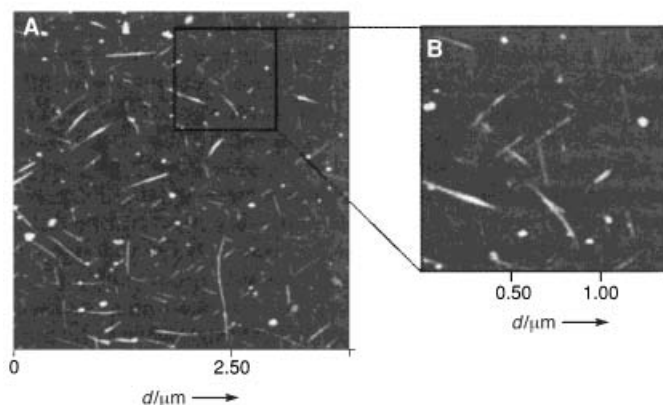


Figure 2. AFM images of fullerene pipes electrodeposited from a stable colloidal suspension onto HOPG (reprinted with permission from *Science*^[7]).

of anionic or nonionic surfactants. Imaging of the cut tubes was effected by a newly developed electrodeposition technique in which all suspended nanotubes are deposited onto the surface of highly oriented pyrolytic graphite (HOPG). Scanning the sample by atomic force microscopy (AFM) revealed the effective cutting of the original endless ropes into fullerene pipes (Figure 2). It is noteworthy that the cut tubes show a tendency to align 120° to one another. This phenomenon, which is observed only for chemically clean and molecularly perfect fullerene tubes, reflects the molecular geometry of the underlying graphite lattice. In subsequent experiments it was demonstrated that it is possible to “polish” the cut tubes and further manipulate their average length. This was achieved by further etching the cut nanotube pieces in acid without ultrasound treatment. Mixtures of concentrated sulfuric acid and either nitric acid or H_2O_2 were employed successfully to systematically shorten the length distribution of the tubes, with the amount of shortening being dependent on the exposure time to the acid. Shortening rates between roughly 130 and 200 nm h^{-1} were realized and varied with the reaction conditions. Whereas it was found that the fullerene pipes flocculate rapidly in pure water, their colloidal suspensions prepared with the aid of surfactants are easy to handle and even allow a separation by length. Figure 3 shows different fractions of cut fullerene nanotube pipes as obtained by field-flow fractionation and analyzed by AFM imaging.

In this context a recent publication by Duesberg et al. should be mentioned, which reports the successful separation of multiwall carbon nanotubes (MWNTs) by size-exclusion chromatography (SEC).^[8] Purified MWNTs of well-defined size distribution are of great potential interest for proposed applications such as field emission and electronic devices.^[5, 9] In this case the major goal was the development of a nondestructive method for purification and size separation. Previously reported methods include destructive methods such as oxidation of the crude nanotube material. These methods are effective in decomposing small impurity particles, but they have certain disadvantages in that a significant amount of material is lost and no size separation can be achieved.^[10] The main problem encountered with known nondestructive methods such as filtering is that the pores of the filters are easily blocked.^[11] It has now been discovered

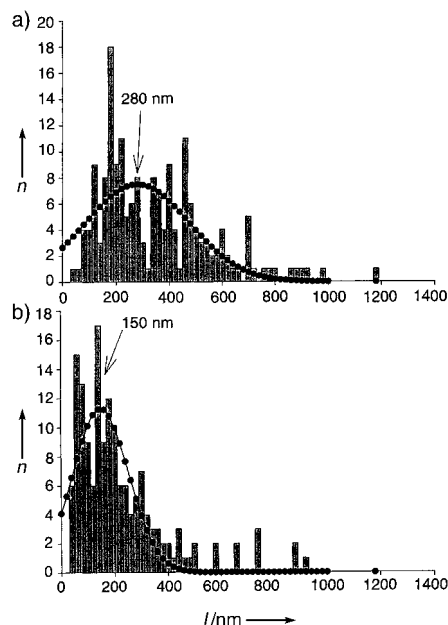


Figure 3. Histograms of cut nanotubes before (a) and after (b) stirring in a mixture of sulfuric and nitric acid (3:1) at 70°C for 1 h (reprinted with permission from *Science*^[7]).

that size-exclusion chromatography of crude nanotube material offers a highly efficient route to purified, chemically unmodified and size-separated MWNTs. SEC is known to be an excellent method for purifying, for example, biological macromolecules or virus particles. Fractionation of the MWNTs was achieved through the use of two successive columns packed with controlled pore glass (CPG) that had an average pore size of 140 and 300 nm, respectively. The CPG material used in this study is chemically inert and has the advantage of a narrow distribution of pore sizes. Characterization of the fractions was achieved by TEM and SEM imaging as well as through atomic force microscopy (AFM). Figure 4 demonstrates the absence of small spherical particles and hence the successful purification of the NTs. In addition, the method proved to be highly useful in achieving a separation of the nanotubes by size. A statistical evaluation of the size distribution in various fractions was carried out by SEM and AFM imaging of the samples after adsorption onto chemically modified Si wafers. As a result, the average MWNT length in different fractions of individual nanotubes varied between about 0.4 and $1\text{ }\mu\text{m}$. It was pointed out that the method can easily be scaled up and could even be further improved by using HPLC techniques.

More recently, Duesberg et al. have been able to demonstrate that the SEC purification method is also applicable to SWNTs.^[12] In this case oxidative methods cannot be applied because of the single-layer structure of the nanotubes, and known nondestructive methods such as filtration and/or flocculation are cumbersome as several filtration steps are necessary to achieve a reasonable purity. Once again SEC proved to be the method of choice for obtaining pure, chemically unmodified SWNTs and moreover allows some degree of size separation. The case of SWNTs is somewhat more complicated compared to the purification of multiwall nanotubes, because of the highly tangled nature of the nearly

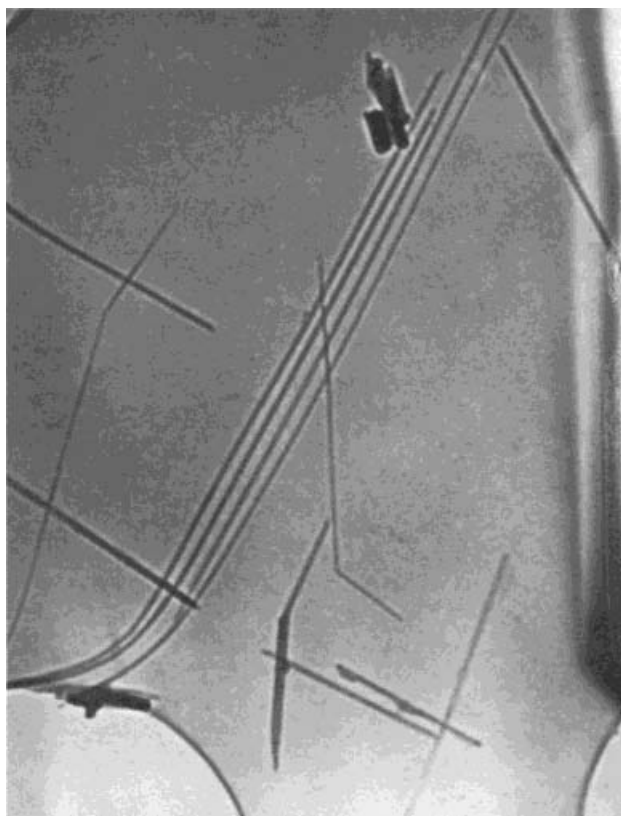


Figure 4. SEM image of purified MWNTs adsorbed on a chemically modified Si wafer (reprinted with permission from *Chem. Commun.*^[8]).

endless ropes and as a result of extensive crosslinking by catalyst particles that are encapsulated by a shell of amorphous carbon. Purification by SEC was achieved by the use of surfactant-stabilized nanotube dispersions made with the assistance of ultrasonication. The dispersed nanotube material was found to move completely (except for a few larger network particles) through a column of controlled pore glass with an average pore size of 300 nm. Characterization of individual fractions by AFM and TEM was carried out as described above. These investigations revealed that fractions containing almost exclusively SWNTs could be obtained and that the average nanotube lengths differed significantly between various fractions.

Coming back to the fullerene pipes, yet another interesting aspect of the work by Smalley et al. is the chemical derivatization of these cut tubes at their open ends.^[7] This experiment can be seen as an important first step in the direction towards the construction of molecular devices from fullerene tube assemblies. Following the assumption that the acid treatment leaves many carboxylic acid end groups at the open ends of the tubes, the fullerene pipes were treated with thionyl chloride to convert the end groups to the corresponding acid chloride functions. Further treatment with $\text{H}_2\text{N}(\text{CH}_2)_{11}\text{SH}$ in toluene afforded nanotubes with amide-linked alkanethiol end groups. The thiol end groups then allowed the functionalized tubes to be tethered to 10-nm gold particles. Figure 5 shows how most of the thiol-derivatized nanotubes have a single gold particle attached to one or both ends. In a control experiment it was verified that the

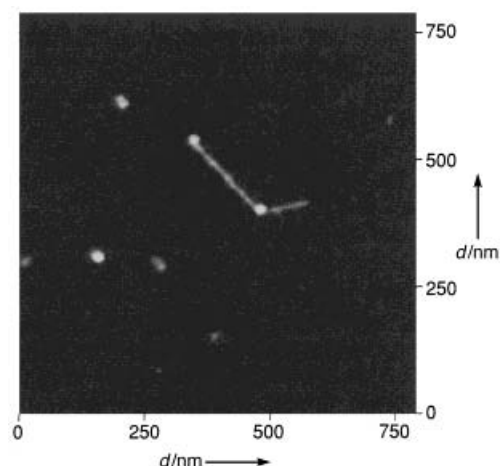


Figure 5. “Seven minutes to three”: AFM image of two derivatized fullerene pipes tethered to a 10 nm diameter gold sphere. The longer pipe (the minute hand) has another gold particle tethered to its other end (reprinted with permission from *Science*^[7]).

derivatization of the tube ends is a prerequisite for the cut SWNTs to be tethered to gold particles.

Arrays of functionalized fullerene pipes and metal particles are anticipated to be an important step towards the construction of molecular devices. The work by Smalley et al. also implies additional future applications with respect to a new area of molecular technology. For example, it was pointed out that the most interesting species, namely, small nanotubes shorter than 100 nm are lost during the newly developed filtration procedures. These would be of enormous interest as they approach the chemistry of molecular fullerenes. Future work in this field will undoubtedly be directed towards the isolation of such SWNTs with lengths less than 100 nm. Yet another exciting future option would be to close the open fullerene pipes at their open ends. This might simply be achieved by annealing at temperatures around 1000–1200 °C. The closing process should be guided by the same mechanisms as those that lead to the formation of fullerenes. According to Smalley, purified nanotubes will play an important role in the development of molecular electronics.^[13] Each tube behaves as a coherent quantum conductor, and it is not beyond the imagination that “buckycables” will eventually be constructed from new carbon polymers. A most recent paper by Dai et al. shows that innovative experimentation even leads to significant improvements in the preparation of individual SWNTs.^[13b] In a first step regularly patterned micrometer-scale catalytic islands were deposited on a Si wafer by covering the silicon surface with a polymethylmethacrylate (PMMA) film and creating square holes by electron beam lithography. The holes were then filled with a mixture of catalyst precursors ($\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, $[\text{MoO}_2(\text{acac})_2]$, Al_2O_3) followed by removal of the PMMA mask and heating the substrate to 1000 °C under an argon atmosphere. Chemical vapor deposition of methane on the catalyst-patterned substrate causes perfect, individual SWNTs with diameters of 0.8 to 3.0 nm and lengths of up to 20 μm to grow from the catalytic islands. Some SWNTs even bridge adjacent islands and thus create a macroscopic electrical network. These results will certainly have great impact on further research with individ-

ual SWNTs, as the nanotubes made by the new technique are easily located, manipulated, and characterized by SEM and AFM imaging.

Another promising way of manipulating carbon nanotubes is their alignment under formation of membranes,^[14] which was recently achieved by Martin et al.^[15a] These researchers used a chemical-vapor deposition (CVD) approach with ethylene as a precursor (no catalyst is required). When the CVD process is carried out within the pores (200 nm diameter) of a commercially available alumina template membrane, a carbon nanotube is formed within each pore in addition to a 20-nm thick carbon surface film covering both faces of the template. The underlying alumina template can then be dissolved away in 46% HF solution to leave a free-standing carbon nanotube membrane. The surface film appears to have an important function as it holds the tubes together in the aligned fashion after removal of the alumina template. Figure 6 shows that the carbon tubes that make up these membranes are vertically aligned, and that the ends of these nanotubes are open.

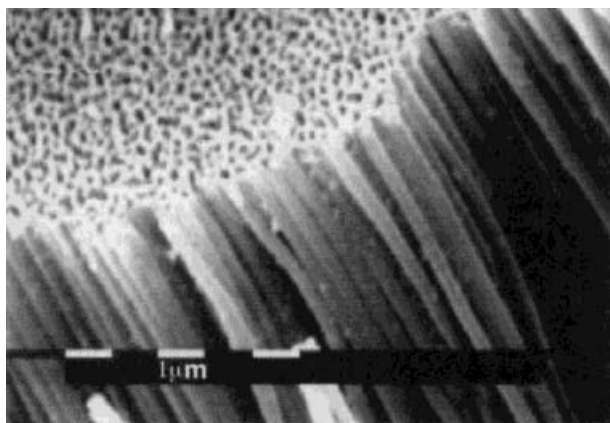


Figure 6. SEM image of a template-synthesized carbon nanotube membrane after dissolving the alumina template (reprinted with permission from *Nature*^[15a]).

In an extension of this work catalytic nanoparticles were prepared within the CVD template-synthesized carbon tubes. This was accomplished by immersing the carbon/alumina membrane in aqueous solutions containing the desired metal ions such as H_2PtCl_6 , $\text{H}_2\text{PtCl}_6/\text{RuCl}_3$, or $\text{Fe}(\text{NO}_3)_3$. After immersion, the membranes were dried in air, and the metal ions were reduced to the corresponding metal or alloy in a hydrogen stream at 580 °C. Dissolution of the alumina template was carried out as described above for the metal-free membranes. Figure 7 depicts a TEM image of a single tube that was removed from a membrane by ultrasonication, and in which the Pt/Ru nanoparticles can be clearly seen through the tube walls. It was demonstrated that such catalyst-loaded membranes can be employed to electrocatalyze O_2 reduction and methanol oxidation. Both reactions are of importance to fuel-cell technology.

It was further found that iron nanoparticles deposited inside the tubes can be used as catalysts for the CVD growth of narrower nanotubes within the template-synthesized tubes ("tube-in-tube membranes").^[15a] Figure 8 shows such a Fe-catalyzed CVD inner nanotube inside an outer tube. These

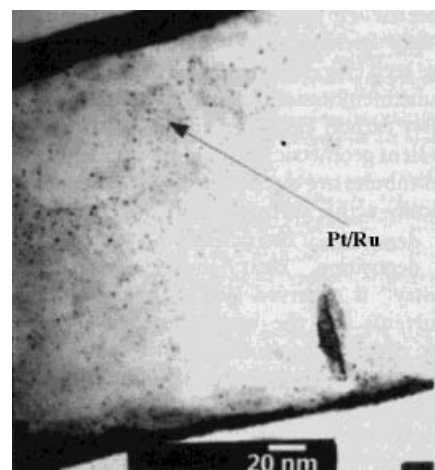


Figure 7. TEM micrograph of a tube filled with Pt/Ru nanoparticles (reprinted with permission from *Nature*^[15a]).

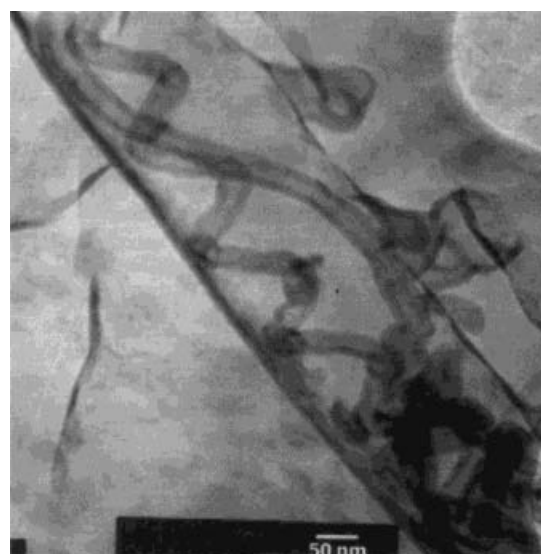


Figure 8. TEM micrograph of the inner carbon nanotubes prepared by Fe-catalyzed CVD inside the outer template-synthesized tubes (tube-in-tube membranes) (reprinted with permission from *Nature*^[15a]).

findings are expected to have an impact on the improvement of lithium-ion batteries as carbon is the anode material of choice in such batteries. It was indeed found that the nanotube membranes reversibly intercalate Li^+ ions. Moreover, the intercalation capacity was more than doubled when the tube-in-tube membranes were used. This is the first case where reversible lithium ion intercalation has been demonstrated for carbon nanotubes.

Carbon nanotubes are also known to be very good electron emitters. This is why since their discovery there has been a lot of speculation about the use of nanotubes in the construction of flat-panel devices. However, a prerequisite for a major break-through in this area would be their perfect alignment on a flat surface. Recently Ren and co-workers succeeded in growing large arrays of well-aligned carbon nanotubes on nickel-coated glass.^[15b] Their method involved plasma-enhanced chemical-vapor deposition using acetylene gas as the carbon source and ammonia gas both as catalyst and dilution gas. The use of ammonia allowed the reaction to proceed at

temperatures below 666 °C, which corresponds to the strain point of the best display glass. After removal from the underlying glass carrier SEM imaging showed that the nanotubes were uniformly aligned across the whole surface and each was capped with nickel. It was found that the diameters of the nanotubes, which varied from 20 to 400 nm, was controllable by changing the thickness of the nickel layer. These results can be considered a good step toward the construction of flat-panel displays with carbon nanotubes.

Yet another exciting field of innovative application for carbon nanotubes has recently been explored by Lieber et al. These researchers have investigated the use of carbon-nanotube tips and functionalized nanotubes as high-resolution probes in chemistry and biology. Based on findings by Smalley et al.^[16] their first paper reports an atomic force microscopy (AFM) imaging study with carbon-nanotube tips.^[17] The resolution of AFM is generally limited by the size and shape of the commercially available pyramid-shaped silicon probe tips. A significant improvement has now been achieved by attaching MWNTs or SWNTs to the ends of single-crystal silicon cantilever tip assemblies (Figure 9). Technically this can be achieved under the direct view of an optical microscope using an acrylic adhesive.

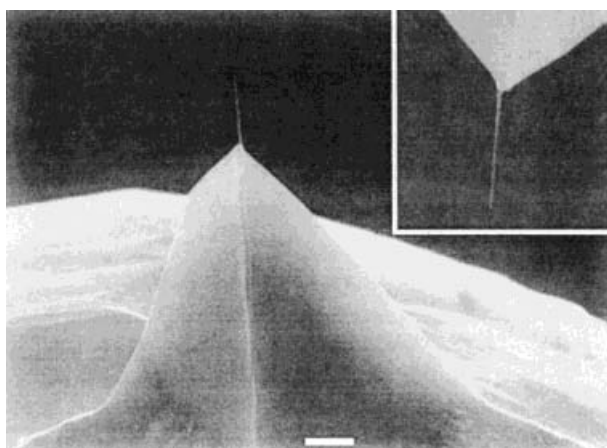


Figure 9. SEM image of a MWNT tip attached to a silicon cantilever tip assembly. The white bar corresponds to 1 μm (reprinted with permission from *J. Am. Chem. Soc.*^[17]).

Imaging by AFM revealed that the major portion of the nanotube tip corresponds to bundles of tightly packed tubes. The average MWNT tips exhibited radii of 9 nm as compared to 13–20 nm for Si probe tips. As a further advantage it was found that in the case of contamination or other deterioration the nanotube tips could be easily shortened and regenerated by a simple field evaporation technique. In AFM studies the MWNT tip assemblies have been demonstrated to exhibit a superior performance with respect to probing deep crevices and steep features (for example, in microelectronic devices) as well as delicate organic and biological samples. No damage of such biological objects is encountered as the nanotubes have been shown to buckle elastically above a critical force.^[18] The efficiency of carbon nanotube tips in the high-resolution imaging of biological samples was tested by investigating A β 40 fibrils, which play a role in Alzheimer's disease.^[19] Such A β fibrils had been investigated previously by atomic force

microscopy.^[20] Figure 10 shows a typical image of an A β fibril obtained by the MWNT tip technique. This image features a significantly higher resolution than previous AFM studies and even shows a detail not revealed by other methods, namely the decrease in size after the branching point. Further

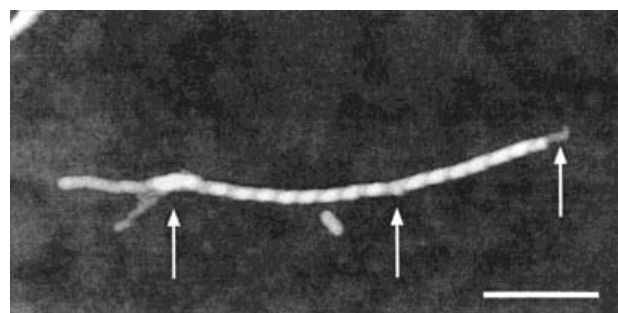


Figure 10. Typical AFM image of a A β 40 fibril acquired with a MWNT tip. The white bar corresponds to 250 nm (reprinted with permission from *J. Am. Chem. Soc.*^[17]).

improvements of the newly developed method are expected from the use of SWNT tips that have average radii of only 3 nm. Even true molecular resolution could eventually be approached when it becomes possible to expose individual nanotubes at the tip ends. Individual SWNTs have radii on the order of only 0.5 nm.^[21b]

Potential applications in various areas of chemistry and biology can be expected from molecular probes based on covalently functionalized nanotubes, which have also been described recently by Lieber et al.^[21] This work addresses the question as to whether carbon nanotubes could be chemically modified in such a way that they would sense and manipulate matter at the molecular level. The general approach is related to the functionalization of fullerene pipes as reported by Smalley et al.^[7] and makes use of the fact that oxidized nanotubes are terminated at their open ends by carboxyl groups. It was found that these carboxyl end groups can be "titrated" using the method of chemical force microscopy (CFM), that is, by measuring the adhesion force between the MWNT tips and a self-assembled monolayer (SAM) substrate terminating in hydroxyl groups (for example, 11-thioundecanol on gold-coated mica) at different pH values.^[21a] Such force titrations revealed a well-defined drop in the adhesion force around pH 4.5, which corresponds to the deprotonation of the carboxylic end groups. Chemical modification of the tube ends was achieved by coupling the carboxyl groups with either benzylamine or ethylenediamine in the presence of a suitable water-soluble carbodiimide (Figure 11). These experiments unambiguously verify the presence of carboxyl groups exposed at the ends of nanotube tips and provide probes modified with either non-ionizable hydrophobic (benzylamine) or ionizable (ethylenediamine) end groups.

It was then assumed that there should be significant differences in the adhesion force between, for example, the carboxyl-terminated nanotube tips and either COOH-terminated SAMs or CH₃-terminated SAMs.^[21a] Vice versa, the tips covalently modified with benzylamine should interact more strongly with the CH₃ than with the COOH regions of a SAM substrate. Indeed, using the functionalized nanotube probes it

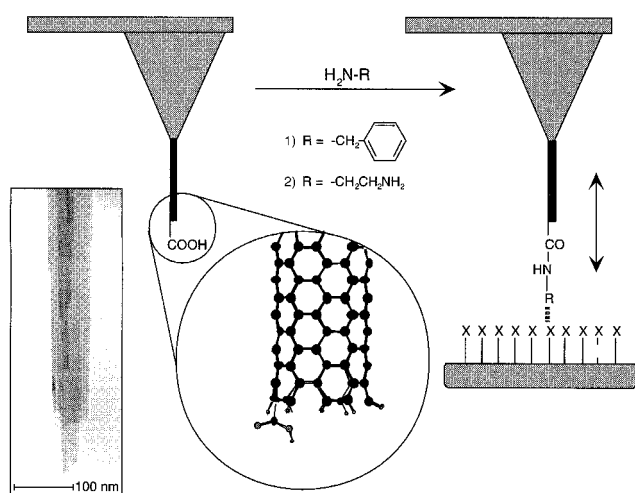


Figure 11. Diagram illustrating the chemical modification of a carboxyl-terminated nanotube tip by coupling with an amine, and the application of this probe to chemically sensitive imaging (X = functional groups of a substrate surface). The MWNT tips are attached to the pyramids of gold-coated Si cantilevers (reprinted with permission from *Nature*^[21a]).

was possible to visualize patterned substrates. Figure 12 shows the functionalized nanotube mapping of a substrate patterned with squares terminating in CH_3 groups and surrounded by areas terminated by COOH groups. The observed differences in phase between the two sample areas are a result of phase-lag differences, which can be related to differences in the adhesion forces. In other words, the method can be used to visualize differences in the chemical functionality even though there is no difference in height between the substrate areas.

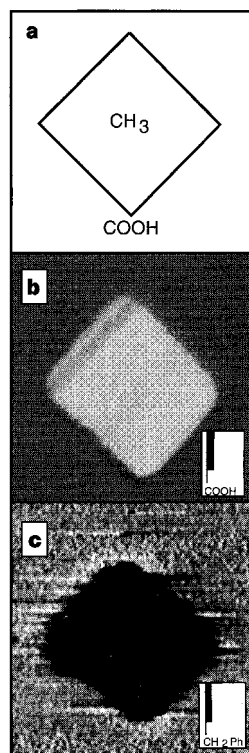
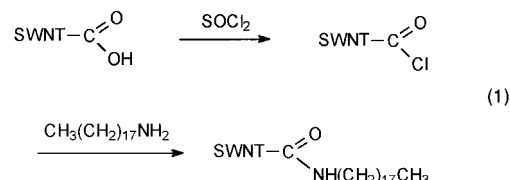


Figure 12. Chemically sensitive imaging of patterned substrates using functionalized nanotube tips. a) Schematic diagram of a patterned sample consisting of methyl-terminated squares surrounded by a COOH -terminated area; b) image of the patterned sample recorded with an unmodified (COOH -terminated) nanotube tip, and c) image recorded using a benzylamine-modified (phenyl-terminated) nanotube tip (reprinted with permission from *Nature*^[21a]).

Most recently the modification strategy has been successfully extended to SWNTs.^[21b] Selectively modified SWNT tips have been shown to produce images of chemically patterned sample with extraordinarily high lateral resolution. The chemical modification of the end groups has also been used to solubilize SWNTs for the first time in common organic solvents.^[22] Purified SWNTs were obtained following Small's procedure^[7] and converted into the COCl derivatives by treatment with thionylchloride. The key to the successful preparation of soluble SWNTs (*s*-SWNTs) was the reaction of SWNT-COCl with excess octadecylamine at elevated temperatures [Equation (1)]. During this process the volume of the



nanotubes increases several times, which indicates that the original bundles of SWNTs disintegrate into individual tubes. The resulting *s*-SWNTs give black-colored solutions in chlorinated and aromatic hydrocarbons, and in CS_2 . An initial reactivity study revealed that dichlorocarbene can be added to the walls of soluble SWNTs. Future investigations will show if the solution-phase wall chemistry of *s*-SWNTs will eventually rival that of the fullerenes. Further research in this area will certainly address the use of *s*-SWNTs as precursors for nanotube-based copolymers or as novel ligands in unusual transition metal complexes.

Another intriguing possible application is the use of covalently functionalized nanotube tips to probe biological systems on a nanometer scale. The ligand–receptor interaction of biotin–streptavidin^[23] was chosen to illustrate this approach. Nanotube tips covalently modified with biotin ligands were prepared by forming amide bonds between the carboxylic acid end groups and 5-(biotinamido)pentylamine. Subsequent force-displacement measurements carried out on mica surfaces containing immobilized streptavidin showed well defined binding force quanta of about 200 pN per biotin–streptavidin pair (Figure 13). No adhesion was regis-

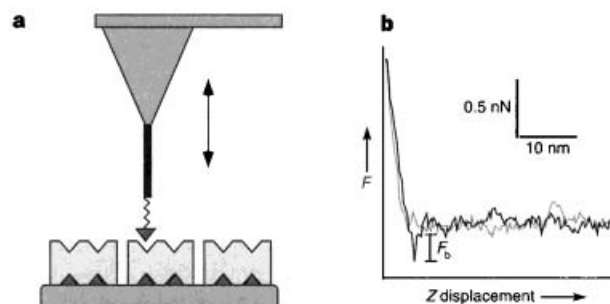


Figure 13. Ligand-modified nanotube tips as biological probes. a) Schematic diagram of a nanotube tip functionalized with a biotin ligand (black triangle) interacting with immobilized streptavidin protein receptors (light-grey blocks); b) force–displacement curve recorded with a biotin-modified nanotube tip on the streptavidin surface (F_b indicates the binding force) (reprinted with permission from *Nature*^[21a]).

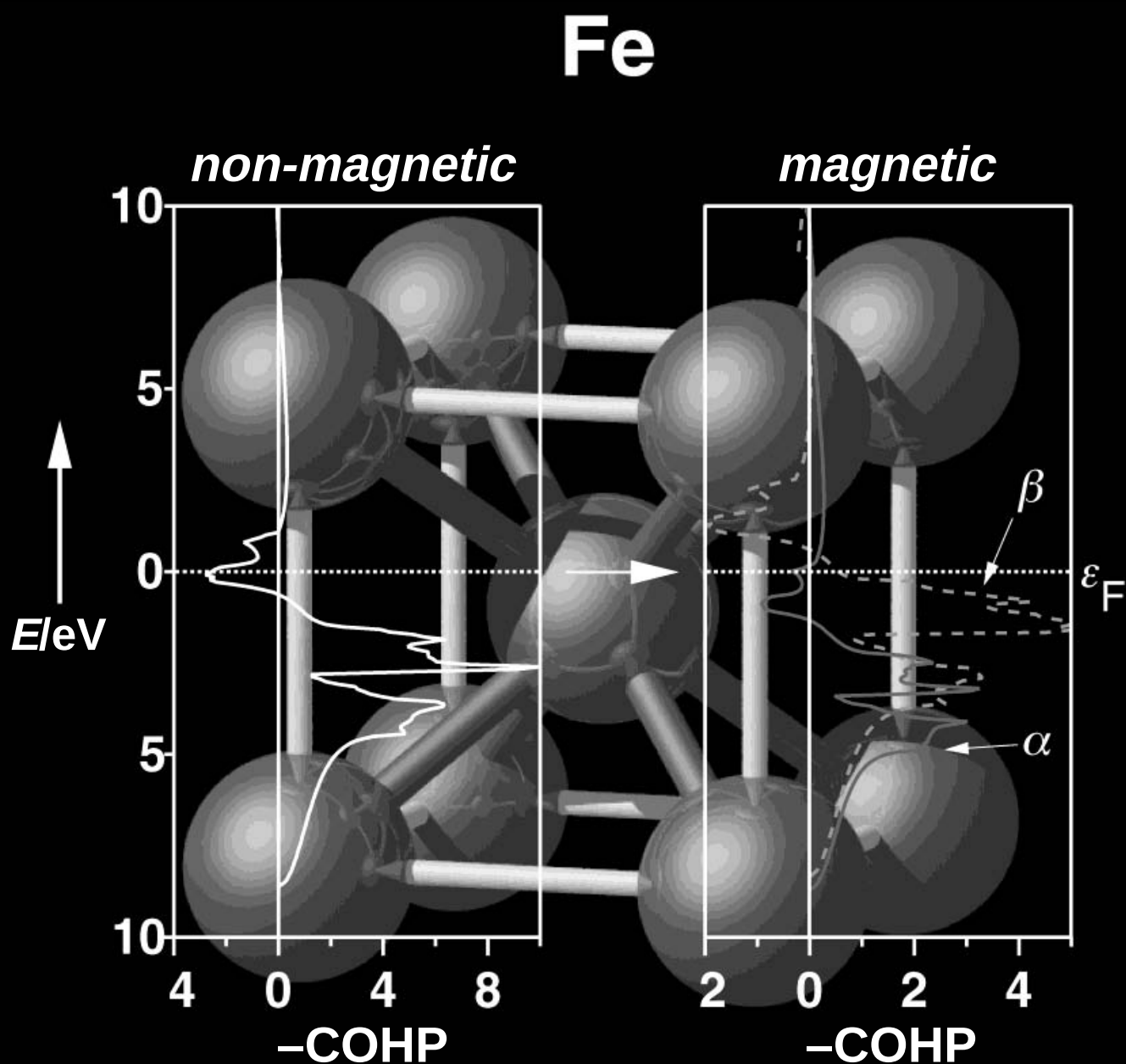
tered in control experiments using unmodified carbon nanotube tips.

According to these results it appears quite possible to attach individual active ligands, proteins or other macromolecules to the ends of carbon nanotube tips and to create high-resolution maps of binding domains on proteins or membranes by using such functionalized nanotube probes. Another area where these nanotube tips may be successfully employed is the imaging of self-assembled polymeric and biological materials. Exciting future application could become reality if one could attach metal catalysts to the carbon nanotube end. Future research will show if such “magic wands” can be used to modify or create structures at the molecular scale. In any event an entire new branch of molecular technology will emerge from the chemistry and physics of carbon nanotubes.

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The metallic ferromagnetism of Fe, Co, and Ni arises from a symmetry breakdown analogous to the Jahn–Teller effect—but electronic, not geometric—and therefore occurs only for a critical electron concentration.

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Ferromagnetism in Transition Metals: A Chemical Bonding Approach**

Gregory A. Landrum and Richard Dronskowski*

In memory of Jean Rouxel

While the microscopic factors underlying molecular magnetism are reasonably well understood from a chemical point of view, the same cannot be said of collective magnetic phenomena such as ferromagnetism and antiferromagnetism. Herein we present a simple and accurate explanation, extracted from the results of band structure calculations based on first principles, for the appearance of ferromagnetism in iron, cobalt, and nickel. The search for an understanding of ferromagnetism is truly historical: early explanatory attempts date back to the 1920s and 1930s,^[1] and the first reliable calculations, which reproduced the experimentally observed ferromagnetism but did not explain it, were performed in the 1960s.^[2]

One typically understands the paramagnetic properties of mononuclear transition metal complexes by examination of the ligand-field splitting at the metal center.^[3] After having constructed the ligand-field diagram for the central metal atom, the levels are filled from the bottom with the appropriate number of electrons, in a way similar to Hund's rules for the free atom, as illustrated for a d^6 octahedral complex in Figure 1 a.

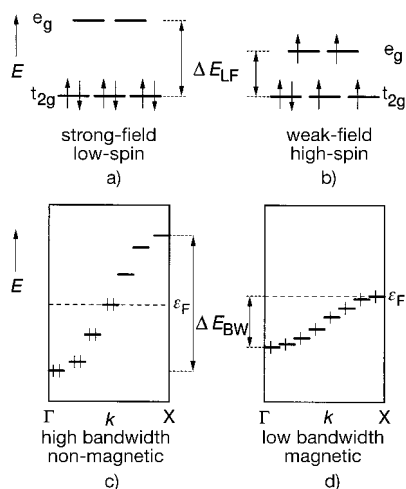


Figure 1. Schematic energy level diagrams and electron filling for a) a d^6 octahedral complex with strong-field ligands, b) the same complex with weak-field ligands, c) a one-dimensional chain with one orbital per unit cell when the bandwidth ΔE_{BW} is larger than the electron-pairing energy, d) the same chain when the bandwidth ΔE_{BW} is smaller than the electron-pairing energy. In (c) and (d) the horizontal dashed line indicates the position of the Fermi level ϵ_F .

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In the situation sketched here (strong field, large splitting) there are no unpaired electrons and the complex will be nonmagnetic.^[4] In the case of a small splitting (weak field) it may even be smaller than the energy required to pair electrons in an orbital (Figure 1 b). The levels are then filled as if they were degenerate, which gives rise to the four unpaired electrons in Figure 1 b. The presence of unpaired electrons leads to a paramagnetic complex. This treatment is, of course, a vast oversimplification of an approach that has been developed and refined over many years, but it conveys the essential features.

If we attempt to transfer this idea to the elemental transition metals we immediately run into difficulties. One might predict that many transition metals have unpaired electrons, so they should be magnetic. This is, of course, not the case. Only iron, cobalt, and nickel show ferromagnetic behavior (chromium and manganese exhibit antiferromagnetism, which is somewhat more complicated and will be examined separately). Metals in the second and third transition series, even those isoelectronic to the magnetic members of the first series, do not exhibit ferromagnetism. Indeed, the origins of ferromagnetism in Fe, Co, and Ni remain a matter of considerable interest (and debate) within the chemistry and physics communities.

Taking into account the paucity of magnetic metals, we find it useful to classify metals into two classes: those that are “normal” (nonferromagnetic), and those that are “anomalous” (ferromagnetic). We will first explain with this classification why practically all elemental metals are nonferromagnetic. Once that has been addressed we will explain why it is that Fe, Co, and Ni are ferromagnetic.

In the gas phase the ground-state electronic configuration of a first-row transition metal with n valence electrons is either $[\text{Ar}]4s^23d^{n-2}$ or $[\text{Ar}]4s^13d^{n-1}$. For example, in vanadium ($n=5$) the ground state is $[\text{Ar}]4s^23d^3$. Since the 3d atomic orbitals (AOs) are degenerate in the gas phase the isolated metal atom has unpaired electrons and exhibits paramagnetic behavior. When such a transition metal is embedded in a crystal, however, the 3d orbitals form bands, which simultaneously removes the fivefold degeneracy of the 3d levels and changes their occupations.

The electronic factors underlying the lack of magnetism in most elemental metal structures can be understood by using a simple model: a one-dimensional “crystal” that contains a single orbital per unit cell, namely, a chain of hydrogen atoms. If there is one electron per unit cell (or atom) there are two possible band fillings. If the band width is large—a situation analogous to the high-field ligand environment of Figure 1 a—then the electrons are found paired up at the bottom of the band (Figure 1 c). If, on the other hand, the band width is smaller than the energy required to pair electrons^[5]—analogous to Figure 1 b—then the crystal orbitals composing the band will all be half occupied (Figure 1 d).

Band width is proportional to the strength of the interatomic interactions in a crystal.^[6] Since these are quite strong in the elemental metals their bands are broad (ΔE_{BW} is large) and the electron filling is as shown in Figure 1 c. Therefore, electrons are paired in most metals and there is no permanent magnetism. Electron fillings similar to those in Figure 1 d are

found for the rare-earth metals. In these elements the Fermi level usually lies within the highly contracted 4f orbitals, which are typically not involved in substantial interatomic interactions and give rise to very narrow bands. This “localized electron magnetism” in the rare-earth metals, some of which exhibit very complicated magnetic structures,^[7] will not be discussed further here.

Elemental Fe, Co, and Ni exhibit “itinerant electron ferromagnetism”,^[7,8,9] that is, the conduction electrons, which are essentially delocalized, are responsible for the ferromagnetism, in contrast to the rare-earth metals (see above). Since the conduction electrons are located in bands with significant width, we can not use a picture such as that shown in Figure 1 d. It is not immediately straightforward to see exactly how unpaired electrons arise in a crystal with broad bands.

Figure 2a shows a qualitative view (according to Harrison^[10]) of the density of states (DOS) of a metal with significant band width. The shaded region indicates the occupied states, and its area is proportional to the total number of electrons. The DOS shown in Figure 2a includes

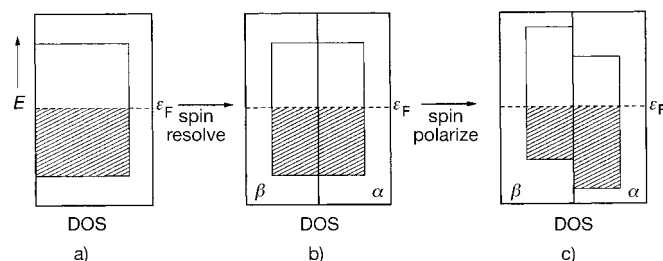


Figure 2. a) Qualitative density of states (DOS) for a metal. b) The same DOS, spin-resolved to show contributions from the α - and β -spin sub-lattices. c) The consequences of spin polarization upon the α - and β -spin sub-lattices. The horizontal dashed line indicates the position of the Fermi level ϵ_F ; shaded regions indicate electron filling.

contributions from both α and β spins of these electrons.^[11] If we “spin resolve” the DOS, to show the contributions from the α - and β -spin sub-lattices separately, we arrive at Figure 2b. Here the densities of states from the individual spin sub-lattices are identical; we have done nothing more than plot them individually. A magnetic moment arises when there is a difference between the occupations of the α - and β -spin sub-lattices. This difference, known as spin polarization, is illustrated in Figure 2c. Here the DOS for the β spins has moved up in energy relative to that of the α spins. Since the two densities of states must share a common Fermi level, more α than β states are populated, which gives rise to ferromagnetism.^[12]

In order to understand the electronic structure of α -Fe (the body-centered cubic (bcc) phase, which is stable under standard conditions) we start with its non-spin-polarized electronic structure. While this nonmagnetic form of Fe is unstable with respect to spin polarization (α -Fe wants to be magnetic), it provides a useful starting point for the analysis.

Each iron atom in α -Fe is coordinated by eight nearest neighbors in a cubic arrangement at a distance of 2.48 Å, and another six second-nearest neighbors octahedrally arranged at 2.87 Å. This environment splits the Fe 3d orbitals into two sets at high symmetry points of the Brillouin zone (BZ): e_g (d_{z^2} and $d_{x^2-y^2}$) and t_{2g} (d_{xy} , d_{xz} , and d_{yz}) (Figure 3).

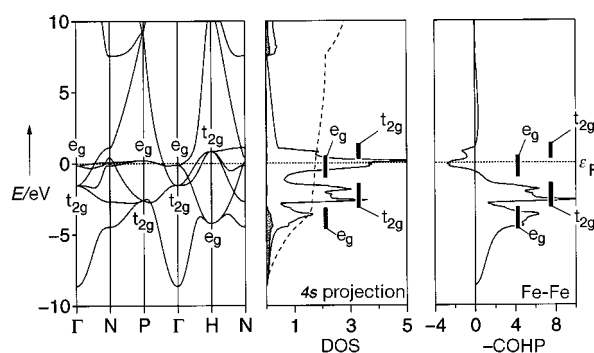


Figure 3. Non-spin-polarized band structure, DOS (shaded area: 4s projection), and Fe-Fe nearest neighbor crystal orbital Hamilton population (COHP) curve for bcc Fe. All curves are shifted so that the Fermi level ϵ_F , indicated with a horizontal dashed line, lies at 0 eV.

Bands at high-symmetry points in the BZ are labeled according to their symmetry, as are the major contributors to prominent peaks in the DOS and crystal orbital Hamilton population (COHP) curves (COHP is discussed in the computational methodology section below). The DOS of α -Fe exhibits a “three-peaked” shape that is typical for bcc transition metals. Surprisingly, the Fermi level ϵ_F lies in a region where the Fe-Fe interactions are *antibonding* (as determined from the COHP curve). The presence of antibonding levels at ϵ_F typically indicates structural instability; we expect these structures to undergo some form of distortion to remove or lessen the antibonding. α -Fe is no exception to this rule of thumb. However, unlike the structural distortions that are typically observed, the changes in α -Fe appear in its *electronic* structure: a realistic spin-polarized calculation results in a spin magnetic moment (difference between the occupations of the α and β spin sub-lattices) of 2.27 electrons, which is in excellent agreement with the experimental value of 2.21.^[7] The process of spin polarization also lowers the total energy of the system by 0.426 eV. Figure 4 shows the DOS and COHP curves for spin-polarized α -Fe.

The shapes of the α DOS and the β DOS are similar and they still resemble the non-spin-polarized DOS of Figure 3, though the β spins are shifted to higher energy. Within the α -spin sub-lattice ϵ_F appears almost at the top of the primarily 3d

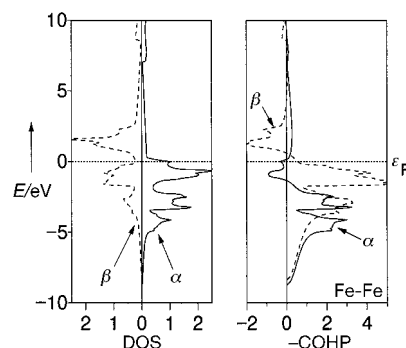


Figure 4. Spin-polarized DOS and Fe-Fe nearest neighbor COHP curves for ferromagnetic bcc Fe. In both plots the solid line corresponds to the α spins and the dashed line corresponds to the β spins. All curves are shifted so that the Fermi level ϵ_F , indicated with a horizontal dashed line, lies at 0 eV.

states. The β spins are shifted up enough to keep the number of electrons constant. The α and β COHP curves (Figure 4) are also similar in shape (and again similar to the one in Figure 3), but their relative sizes are quite different. The α sub-lattice states that lie just below ε_F are considerably less Fe–Fe antibonding than they were before (these are the states that appeared at ε_F in Figure 3). The equivalent states in the β sub-lattice lie more than 1 eV above ε_F . The β states just below ε_F (mostly t_{2g}) are quite strongly Fe–Fe bonding, more so than the equivalent α states. Based upon the integrations of the COHP values to ε_F (ICOHPs), the Fe–Fe interactions in the β sub-lattice (ICOHP = -0.975 eV/bond) are almost twice as bonding as those in the α sublattice (ICOHP = -0.552 eV/bond).^[13] The total Fe–Fe ICOHP (the sum of the α and β values) is -1.527 eV/bond, more than five percent larger than that in non-spin-polarized α -Fe (-1.448 eV/bond). *The process of spin-polarization has strengthened the Fe–Fe bonds.*

The spin-polarization process in α -Fe results in a redistribution of charge density, which is easily visualized by plotting the difference between the spin-polarized and non-spin-polarized electron densities. Figure 5 shows such contours

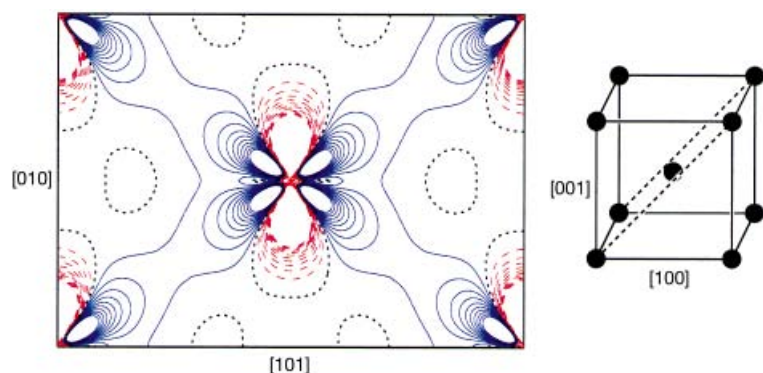


Figure 5. Contour plot of the quantity $\Delta\rho = \rho(\text{spin-polarized}) - \rho(\text{non-spin-polarized})$ evaluated in the plane delimited by the crystallographic [010] and [101] axes in α -Fe. Dashed red lines correspond to positive values, while solid blue lines correspond to negative values. Thick, dotted black lines indicate the zero contour. The location of the plane in the unit cell is illustrated schematically on the right.

evaluated in a plane through the center of the unit cell. There is a depletion of charge density in the regions of nearest-neighbor bonds (along the diagonals of the plot) and an increase in density between second-nearest-neighbor contacts along the [010] axis. Remember that in the non-spin-polarized electronic structure there are Fe–Fe antibonding states populated at ε_F . These states contribute to the electron density between nearest neighbors. Spin polarization lessens the antibonding interactions by shifting this “excess” electron density out of these regions.

We are left here with a question: Why is it that the Fe–Fe interactions in the β -spin sub-lattice are stronger than those in the α sub-lattice (Figure 4)? During spin-polarization the α states move down in energy while the β states move up. These energy shifts arise because the shielding of the nuclear charge between like spins (that is, α – α or β – β shielding) is less efficient than that between different spins (namely α – β shielding) as a consequence of the “exchange hole”.^[14] As spins move from the β - to the α -spin sub-lattice, the α spins

experience a higher effective nuclear charge from the depletion of β spins. The opposite is true of the β spins, which experience a lower nuclear charge (are better shielded) because of the “extra” α spins. Accompanying these changes in effective nuclear charge are changes in the spatial extents of the spin sub-lattices. While the α spins become more tightly bound to the nucleus (more spatially contracted), the β spins become less tightly bound (more spatially diffuse). This explains the changes in magnitudes of the Fe–Fe COHP curves upon spin polarization: the α -spin sub-lattice is more tightly bound, more contracted, and thus Fe–Fe interactions involving the α spins are weaker. The reverse is true of the β -spin sub-lattice: it is less tightly bound, more diffuse, so Fe–Fe interactions are stronger.

If bcc iron (α -Fe) were nonferromagnetic, the Fermi level would occur in a region of Fe–Fe antibonding interactions (see Figure 3). This gives rise to an electronic structural instability that leads to spin polarization and the onset of ferromagnetism. The spin polarization results in a contraction of the α -spin sub-lattice, which shifts down in energy and gains more electrons. At the same time, the β -spin sub-lattice shifts up in energy, loses electrons, and becomes more diffuse. The net effect of these changes in the electronic structure is to remove the Fe–Fe antibonding states from the vicinity of ε_F , and increase the strength of the Fe–Fe bonds by more than five percent. The results of calculations (not shown here) upon the other ferromagnetic transition metals (Co and Ni) are similar: ε_F appears in a region of M–M antibonding in the non-spin-polarized COHP curves. This antibonding is lessened by the spin-polarization process. In the early/late nonmagnetic first row transition metals (such as vanadium or copper) ε_F lies below/above the M–M antibonding states, so there is no tendency for spin polarization, and thus magnetism, to arise. A more detailed form of this model, including an examination of antiferromagnetism within the same framework, will be presented in a forthcoming paper.

Computational Methodology

Electronic structure calculations were performed using the Linear Muffin-Tin Orbital (LMTO) method^[15] within the local spin density approximation (LSDA).^[16] All calculations were checked for convergence of energies, orbital moments, integrated COHP values, and magnetic moments with respect to the number of k points used in the reciprocal space integrations. The program used was TB-LMTO 4.7.^[17]

While the LSDA is known to have problems with metallic Fe—and predicts a nonmagnetic face-centered cubic structure to be more stable than the magnetic bcc form^[18]—it reproduces the experimental magnetic moments of Fe, Co, and Ni (2.21, 1.74, and 0.62 unpaired electrons per atom respectively^[7]) quite closely (2.27, 1.60, and 0.62). The spin-polarized DOS of magnetic bcc Fe generated with a gradient correction scheme within the framework of a linear augmented plane wave (LAPW) calculation^[19] is essentially identical to that generated from the LSDA within the LMTO method. Since we are not interested here in energy differences between particular structures (using instead experimental structures and lattice constants), we believe that the LSDA is accurate enough for our purposes.

Crystal orbital Hamilton population (COHP) analysis^[20] is a partitioning scheme for the band structure energy (sum of the energies of the Kohn–Sham orbitals) in terms of orbital-pair contributions. COHP analysis, while in many ways analogous to the familiar crystal orbital

overlap population (COOP) analysis used in extended Hückel calculations,^[6, 21] provides a quantitative measure of bond strengths and is probably more appropriate for a calculation from first principles. All COHP curves are presented here in a format similar to COOP curves: positive values are bonding, and negative antibonding; this means we are plotting $-COHP$ instead of COHP.

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Keywords: band structure calculation • electronic structure • magnetic properties • transition metals

Efficient Intermolecular Charge Transport in Self-Assembled Fibers of Mono- and Bithiophene Bisurea Compounds**

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Conjugated oligomers and polymers of thiophenes are among the most promising organic semiconducting molecular materials for application as thin film transistors^[1] (TFT) and light-emitting diodes^[2] (LED) in (opto)electronic devices. The basis for this is their highly favorable electronic properties, such as a small band gap, high charge-carrier mobility (although low compared to the mobility in some aromatic molecular crystals),^[3] and high quantum yield for fluorescence.^[4] The properties and performance of these materials, however, do not only depend on the electronic structure of the molecules themselves, but also critically on supramolecular features such as the molecular packing of the thiophene moieties and the morphology of thin films and crystals of those compounds.^[5] Although full control of the molecular packing and morphology is still a far-fetched goal, progress has been made in directing the spacing and the orientation of π -stacked aromatic groups by making use of hydrogen bonding motifs.^[6, 7] A class of compounds that is particularly well suited for the spatial organization of functional entities are (bis)urea compounds.^[8] We showed recently that bisurea compounds self-assemble into ribbons, which stack into sheets or lamella.^[9] The bisurea compounds have an extended conformation in the ribbons and are amenable to form multiple hydrogen bonds with neighboring molecules, and thereby provide a framework for the organization of other functional entities in ribbons and lamella (Figure 1). The

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- [12] The local magnetic moments of Figure 1d also arise as a consequence of spin polarization. In this case the narrow bands give rise to sharp peaks in the DOS. In the spin-polarized DOS of this system ϵ_F occurs at the top of the α DOS and the entire β DOS is found above ϵ_F , that is, there is no occupation of the β -spin sub-lattice.
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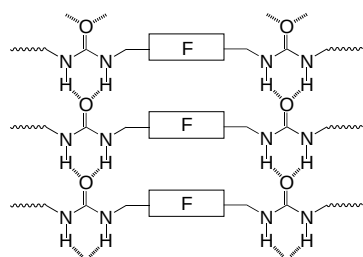
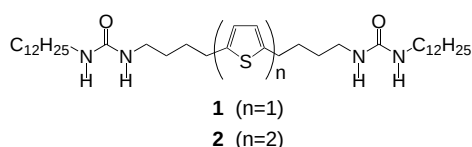


Figure 1. Ribbon of a bisurea compound used for the spatial organization of functional entities (F).

bisurea compounds **1** and **2** with a thiophene or bithiophene moiety incorporated in the spacer have been synthesized on the basis of this organization principle. The molecular packing and morphology of aggregates of these compounds, as well as the remarkably efficient charge transport in these materials, are presented here.



The bisurea thiophenes **1** and **2** were synthesized in several steps starting from thiophene and 2,2'-bithiophene, respectively (see experimental section). Compounds **1** and **2** are insoluble in organic solvents at room temperature, but upon heating to 50–100 °C they gradually dissolve. Upon cooling the solution to room temperature **1** and **2** precipitate in solvents such as ethanol, butyl acetate, and cyclohexane, but in tetralin and 1,2-dichloroethane organogels are formed.^[9, 10] The highly efficient aggregation of these compounds is caused by the formation of hydrogen bonds between urea groups. Infrared spectroscopic analysis of the gels **1** and **2** indeed showed a strong NH band at 3330 cm⁻¹ and amide I and II bands at 1620 cm⁻¹ and 1570 cm⁻¹, respectively, which correspond well with the value for the solids and are characteristic for the presence of hydrogen-bonded urea groups.^[11]

Light microscopic analysis of the gels and solids **1** and **2** revealed that these compounds self-assemble into elongated twisted fibers with a breadth of 2–10 μm and lengths of 20–100 μm. The fibers are strongly birefringent, which indicates a high degree of molecular ordering. Analysis by electron microscopy (Figure 2) and X-ray powder diffraction showed

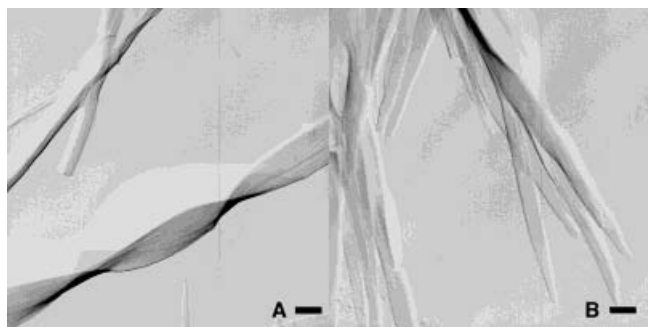


Figure 2. Electron micrographs of lamellar fibers of **1** (A) and of **2** (B). A platinum shadow has been applied with an angle of 10°. The bar corresponds to 500 nm.

that the fibers have a lamellar structure, with a spacing of 38.9 Å for **1** and 41.2 Å for **2**. These values are smaller than the molecular lengths of **1** and **2**, which amount to 51 and 53 Å, respectively. The bisurea compounds are most likely closely packed within the lamella with their long molecular axis parallel to each other and make a small angle with the normal of the lamella (Figure 3). A similar molecular

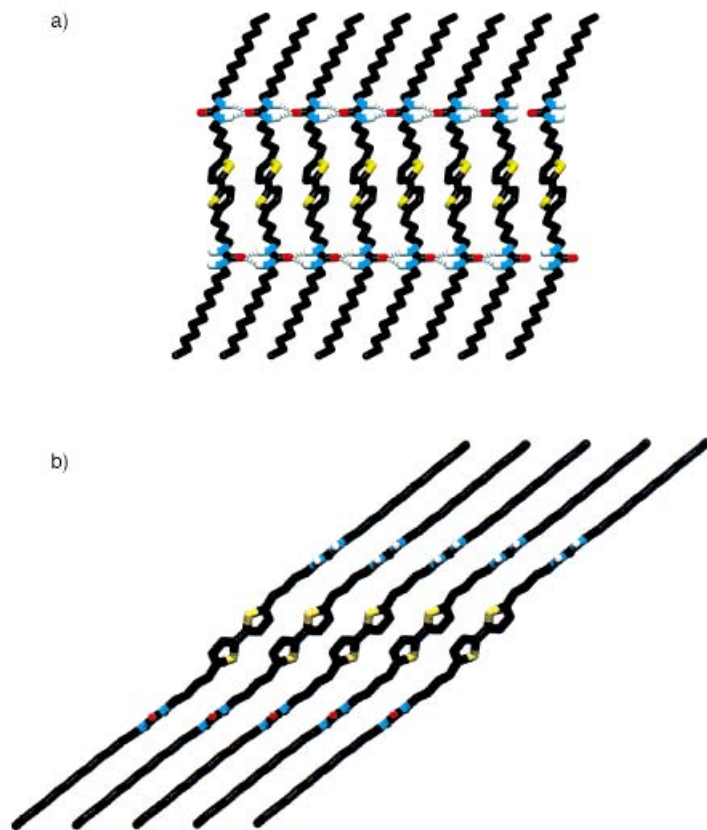
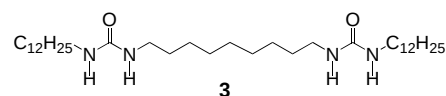


Figure 3. Possible arrangement of **2** in the lamella: a) view of a hydrogen-bonded ribbon, and b) a tilted stack of ribbons that form a lamella.

arrangement has been found for lamella formed by other linear bisurea compounds such as **3**.^[9]



Interestingly, UV/Vis spectroscopic analysis of both compounds showed that the formation of fibers (as in 1,2-dichloroethane gels) is accompanied by a broadening and decrease in intensity of the π - π^* absorption bands relative to homogeneous 1,2-dichloroethane solutions of **1** and **2**. These spectral changes can be attributed to a strong exciton coupling between the thiophene chromophores.^[12] Apparently, the thiophene moieties are present as π -stacked aggregates in fibers of **1** and **2**. A blue shift of the π - π^* absorption maximum occurred from 319 nm for a solution of the bithiophene compound **2** in 1,2-dichloroethane to 310 nm for a gel of **2**, which is characteristic of the formation of *H*-aggregates.^[12, 13] No shift of the π - π^* absorption maximum

has been observed in the case of compound **1** upon formation of the fibers. This points to an oblique arrangement of the monothiophene moieties in the fibers, but it should be noted that for monothiophenes the shifts of the absorption maxima as a result of exciton coupling are expected to be smaller because of the lower oscillator strength.^[14]

Molecular modeling studies were used to explore the possible arrangements of **1** and **2** in the fibers. A systematic search following the cluster approach of Gavezotti^[15, 16] yielded a number of stable lamellar structures within an energy range of 10 kcal mol⁻¹. These calculated polymorphs have a common structure in that the lamella have a sandwich structure, in which the central layer of each lamella is formed by the thiophene moieties and is flanked on both sides by layers that consist of the urea groups and the hydrocarbon chains of the compounds, respectively (Figure 3). Furthermore, it was found that both urea groups form hydrogen bonds with urea groups of neighboring molecules, and thereby participate in the formation of infinite chains of hydrogen-bonded urea moieties. The average repeat distance along the direction of the hydrogen-bonded chains is 4.5 Å, which is in good agreement with the average value of 4.6 Å found for crystal structures of urea compounds.^[8, 9] The clearest resemblance between the possible polymorphs is seen within the central aromatic layer where the bithiophene moieties adopt a closely packed arrangement with a face to face stacking of the thiophene moieties along the same direction as the infinite hydrogen-bonded chains. In Figure 3 one of the possible lamellar arrangements of **2** is shown, of which the thickness of 39 Å is very close to the experimentally determined spacing of 41.2 Å. In this arrangement the bithiophene moieties are cofacially stacked with an interplanar distance of 3 Å, and are displaced by 0.4 and 3.5 Å along the long and short molecular axis, respectively. Since the transition moment of the π - π^* transition for bithiophenes coincides with the long molecular axis,^[14] the structure shown in Figure 3 represents an *H*-aggregate, and is in excellent agreement with the experimental data presented. It should be stressed that other lamellar arrangements are also in reasonable agreement with the experimental data.

The presence of extended aggregates of π -stacked thiophene moieties in self-assembled fibers of **1** and **2** should provide an efficient path for charge-carrier transport within the fibers. The conductive properties of **1** and **2** were therefore studied with the pulse-radiolysis time-resolved microwave conductivity (PR-TRMC) technique.^[17] Solid-state powder samples (20–200 mg) of the materials were irradiated with 3-MeV electrons (2–20 ns pulses) from a Van de Graaff accelerator, which resulted in the creation of uniformly distributed pairs of electron-hole in low concentration in the sample. If the charge carriers formed are mobile, the conductivity of the sample will increase, which is monitored as a change in the microwave power that is reflected by the cell containing the sample. In this way, no electrode contacts are required, polarization of the sample is absent because of the ultra-high frequencies used (26.5–38 GHz), and the time scale of the observations is very short. This implies that effects arising from domain boundaries and impurities are minimized, since the charge carriers only move across short

distances within the materials while being probed. Thus the end-of-pulse conductivity can be considered to represent the maximum value obtainable with a charge-transport device with a monodomain between the electrodes. As a consequence of the fact that a fraction of the electron-hole pairs recombines during the ionization pulse and does not contribute to the observed conductivity, the PR-TRMC technique in fact provides a lower limit for this value. From frequency-dependent conductivity measurements in the range of 28 to 38 GHz the dose-normalized end-of-pulse conductivity $\Delta\sigma/D$ is obtained, and the lower limits of $\Sigma\mu_{\min}$ (the sum of the mobilities of the positive and negative charge carrier) are derived from the relationship $\Sigma\mu_{\min} = E_p(\Delta\sigma/D)$, where E_p is the average electron-hole pair formation energy.

Figure 4 shows the result of the PR-TRMC experiments on virgin samples of **1** and **2**. For comparison the results obtained for linear bisurea compound **3**, which contains only alkyl chains and urea groups, are also included. A very low

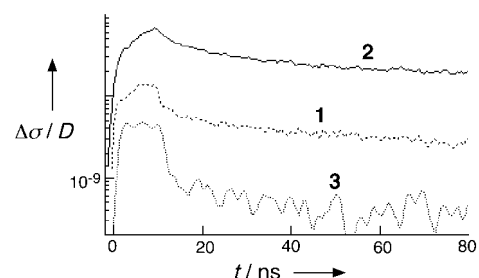


Figure 4. Radiation-induced conductivity transients of the three bisurea compounds **1**–**3**, as obtained with the PR-TRMC technique (10 ns pulse length, microwave frequency = 34 GHz). The dose-normalized conductivity ($\Delta\sigma/D$) is in Sm^2J^{-1} .

conductivity signal close to the detection limit of the system is observed for **3**, which indicates that the charge carriers have a very low mobility. Note that the initial part of the signal (during the 10 ns excitation pulse) is mainly a result of the presence of remnant air in the sample. The remaining transient observed after the pulse, which decays within 20 ns to the noise level, might be attributed to a conductivity change of $\Delta\sigma/D = 9 \times 10^{-10} \text{ Sm}^2\text{J}^{-1}$ at most, which corresponds to $\Sigma\mu_{\min} < 2 \times 10^{-4} \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ for **3**. In contrast, the conductivity signal of **1** ($\Delta\sigma/D = 8 \times 10^{-9} \text{ Sm}^2\text{J}^{-1}$) is dramatically higher than that of **3** and is relatively long-lived, which shows that the introduction of a thiophene unit in the bisurea compound leads to the occurrence of efficient charge-transport pathways ($\Sigma\mu_{\min} = (1 \pm 0.2) \times 10^{-3} \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ for **1**). Interestingly, the signal of **2** shows a substantial increase in the conductivity ($\Delta\sigma/D = 4 \times 10^{-8} \text{ Sm}^2\text{J}^{-1}$) relative to **1**, which might be attributed to the much better π overlap that is achieved between neighboring molecules as a consequence of the presence of two central thiophene units. The mobility value of **2** ($\Sigma\mu_{\min} = (5 \pm 0.2) \times 10^{-3} \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$) is in fact much higher than that of a cyclohexyl end-capped bithiophene oligomer ($\Sigma\mu_{\min} = 4 \times 10^{-4} \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$) studied before with the PR-TRMC technique^[18] and also higher than that of unsubstituted quaterthiophene mentioned in the same study ($\Sigma\mu_{\min} < 1 \times 10^{-3} \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$). Thus, the solid-state ordering achieved by

the bisurea moieties in **2** results in a clearly increased mobility relative to these two small oligothiophenes. Earlier PR-TRMC experiments on the conductivity of various alkyl-substituted polythiophenes^[9] yielded a mobility $\Sigma\mu_{\min} = 7 \times 10^{-3} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$, irrespective of the length of the alkyl side chain. Remarkably, the value of **2** is already close to that of the polymers, although the former contains only two conjugated thiophene units!

Fibers of linear bisurea compounds have a common feature in that they have excellent thermal stability, because of the presence of infinite chains of hydrogen bonded urea groups. The high thermal stability of fibers of **1** and **2** is reflected by their melting temperatures of 143 °C and 177 °C, respectively. Above these temperatures the compounds slowly decompose. Temperature-dependent conductivity measurements for both compounds showed that up to their melting points no large changes in conductivity occurred. Thus, the degree of organization achieved by the intermolecular hydrogen bonding is maintained over a large temperature range.

In conclusion, the mobilities measured for the bisurea thiophenes when compared to (oligo)thiophenes and especially the lower members, are remarkably high, especially when one takes into consideration that only a small fraction of the volume of the material consists of the aromatic thiophene part, with the larger volume fraction being occupied by insulating alkyl chains. For this reason, the high charge mobility in these materials must arise from the well-defined arrangement of the thiophene moieties in closely packed layers within the material. The molecules presented in this study are forced to adopt this arrangement as a result of the presence of the urea groups, which results in a close packing of the thiophenes and hence an efficient pathway for charge transport. Therefore, these thiophenes modified with bisurea units, with the capability for self-organization through hydrogen bonding, are promising candidates for inclusion in charge-transport devices based on organic semi-conducting layers. Research in this direction is in progress.

Experimental Section

The synthesis and properties of compound **3** can be found in ref. [9].

2,5-Di(4-chlorobutyl)thiophene (4): A solution of 2.5 M *n*-BuLi in hexanes (104 mL, 260 mmol) was added to a solution of thiophene (10.15 g, 121 mmol) in THF (150 mL), followed by the addition of 1-bromo-4-chlorobutane (42.8 g, 250 mmol). The initial rise in temperature of the reaction mixture was controlled by means of a cold water bath, and then the mixture was stirred overnight at 40–50 °C. After aqueous work-up and extraction of the product with diethyl ether, a dark brown residue was obtained, which was purified by column chromatography (basic Al₂O₃, activity I, pentane). A colorless liquid was obtained (30.5 g, 115 mmol, 95.0%). ¹H NMR: (200 MHz, CDCl₃): δ = 1.77–1.90 (m, 8H), 2.81 (t, ³J(H,H) = 6.8 Hz, 4H), 3.57 (t, ³J(H,H) = 3.7 Hz, 4H), 6.60 (s, 2H); ¹³C NMR (75.5 MHz, CDCl₃): δ = 28.7, 29.2, 31.7, 44.7, 123.8, 142.4; MS: m/z : 264 [M^+].

2,5-Di(4-azidobutyl)thiophene (5): Compound **4** (12.28 g, 46.3 mmol) was added to a suspension of NaN₃ (8.26 g, 127 mmol) in DMSO (100 mL) and then the temperature raised to 50 °C. The mixture was then stirred overnight. After aqueous work-up and extraction of the product with diethyl ether a yellowish liquid was obtained, which was purified by column chromatography (basic Al₂O₃, activity I, pentane/CH₂Cl₂ 9/1) to yield a colorless liquid (12.78 g, 45.9 mmol, 99%). ¹H NMR (300 MHz, CDCl₃): δ = 1.41–1.50 (m, 4H), 1.58–1.65 (m, 4H), 2.66 (t, ³J(H,H) = 7.0 Hz, 4H),

2.71 (t, ³J(H,H) = 7.5 Hz, 4H), 6.51 (s, 2H); ¹³C NMR (75.5 MHz, CDCl₃): δ = 28.8, 29.8, 33.0, 41.8, 123.3, 142.7; MS: m/z : 278 [M^+].

2,5-Di(4-aminobutyl)thiophene (6): 10% Pd/C (120 mg) was added to a solution of **5** (12.08 g, 43.4 mmol) in ethanol (150 mL). A balloon filled with hydrogen gas was mounted on top of the flask and the mixture was stirred vigorously at room temperature. Analysis of the mixture by ¹H NMR spectroscopy showed that the reaction was complete after almost 5 h. The catalyst was filtered off and the solvent removed in vacuo to yield a colorless liquid (9.82 g, 43.4 mmol, 100%). ¹H NMR (300 MHz, CDCl₃): δ = 1.41–1.50 (m, 4H), 1.58–1.65 (m, 4H), 2.66 (t, ³J(H,H) = 7.0 Hz, 4H), 2.71 (t, ³J(H,H) = 7.5 Hz, 4H), 6.51 (s, 2H); ¹³C NMR (75.5 MHz, CDCl₃): δ = 28.8, 29.8, 33.0, 41.8, 123.3, 142.7; MS: m/z : 226 [M^+].

2,5-Di(4-(3-dodecyl-ureido)butyl)thiophene (1): A solution of dodecylisocyanate (1.89 g, 8.95 mmol) in CH₂Cl₂ (10 mL) was added to a solution of **6** (0.98 g, 4.33 mmol) in CH₂Cl₂ (10 mL). The resulting mixture was stirred for three hours, during which period a white precipitate formed. The solvent was removed in vacuo to yield an off-white solid. This solid was dissolved in refluxing toluene/propanol (9/1), and then cooled down to room temperature and filtered under suction. This procedure was repeated three times, after which the product was obtained as a white powder (1.88 g, 2.90 mmol, 67%). M.p. 143 °C (decomp.); ¹H NMR (300 MHz, [D₆]DMSO): δ = 0.88 (t, ³J(H,H) = 6.6 Hz, 6H), 1.28 (brs, H), 1.37–1.51 (m, H), 1.57–1.66 (m, H), 2.73 (t, ³J(H,H) = 7.3 Hz, 4H), 3.01 (m, 8H), 5.45 (brs, 4H), 6.59 (s, 2H); elemental analysis: calcd: C 70.3, H 11.2, N 8.6, S 4.9%; found: C 70.0, H 11.1, N 8.6, S 4.9; IR (nujol): $\tilde{\nu}$ = 3322 (NH), 1620 (amide I), 1574 (amide II) cm⁻¹; UV/Vis (1,2-dichloroethane, 80 °C): $\lambda_{\max}$ (ϵ) = 242 (6850) nm.

5,5'-Di(4-chlorobutyl)-2,2'-bithiophene (7): This compound was synthesized as described for **4**, starting from 2,2'-bithiophene. After purification by column chromatography (basic Al₂O₃, activity I, pentane/CH₂Cl₂ 7/1) a slightly yellow solid was obtained in 93% yield; ¹H NMR (300 MHz, CDCl₃): δ = 1.81–1.90 (m, 8H), 2.84 (t, ³J(H,H) = 6.8 Hz, 4H), 3.56 (t, ³J(H,H) = 3.7 Hz, 4H), 6.68 (d, ³J(H,H) = 3.7 Hz, 2H), 6.92 (d, ³J(H,H) = 3.7 Hz, 2H); ¹³C NMR (75.5 MHz, CDCl₃): δ = 28.7, 29.3, 31.8, 44.7, 122.8, 125.0, 135.5, 143.5; MS: m/z : 346 [M^+].

5,5'-Di(4-azidobutyl)-2,2'-bithiophene (8): This compound was synthesized as described for **5**, starting from **7**. After purification by column chromatography (basic Al₂O₃, activity I, pentane/CH₂Cl₂ 7/1) a slightly yellow solid was obtained in 98% yield. ¹H NMR (300 MHz, CDCl₃): δ = 1.66–1.81 (m, 8H), 2.84 (t, ³J(H,H) = 7.1 Hz, 4H), 3.31 (t, ³J(H,H) = 6.6 Hz, 4H), 6.69 (d, ³J(H,H) = 3.7 Hz, 2H), 6.93 (d, ³J(H,H) = 3.7 Hz, 2H); ¹³C NMR (75.5 MHz, CDCl₃): δ = 28.1, 28.5, 29.5, 51.0, 122.7, 124.9, 135.4, 143.4; MS: m/z : 360 [M^+].

5,5'-Di(4-aminobutyl)-2,2'-bithiophene (9): This compound was synthesized as described for **6**, starting from **8**. Compound **9** was obtained as a colorless liquid that solidified on standing (yield 100%). ¹H NMR (300 MHz, CDCl₃): δ = 1.48–1.58 (m, 4H), 1.64–1.76 (m, 4H), 2.73 (t, ³J(H,H) = 7.0 Hz, 4H), 2.80 (t, ³J(H,H) = 7.3 Hz, 4H), 6.66 (d, ³J(H,H) = 3.6 Hz, 2H), 6.89 (d, ³J(H,H) = 3.6 Hz, 2H); ¹³C NMR (75.5 MHz, CDCl₃): δ = 28.7, 29.8, 33.1, 41.8, 122.7, 124.9, 135.4, 143.4; MS: m/z : 308 [M^+].

5,5'-Di(4-(3-dodecylureido)butyl)-2,2'-bithiophene (2): This compound was synthesized as described for **1**, starting from **9**. M.p. 177 °C (decomp.); ¹H NMR (300 MHz, [D₆]DMSO): δ = 0.88 (t, ³J(H,H) = 6.6 Hz, 6H), 1.28 (brs, H), 1.37–1.42 (m, 4H), 1.45–1.54 (m, 4H), 1.61–1.69 (m, 4H), 2.79 (t, ³J(H,H) = 7.3 Hz, 4H), 2.97–3.09 (m, 8H), 5.38 (brs, 2H), 5.44 (brs, 2H), 6.73 (d, ³J(H,H) = 3.6 Hz, 2H), 6.94 (d, ³J(H,H) = 3.6 Hz, 2H); elemental analysis: calcd: C 69.0, H 10.2, N 7.7, S 8.8; found: C 68.5, H 10.3, N 7.6, S 8.7; IR (nujol) $\tilde{\nu}$ = 3352, 3324 (NH), 1616 (amide I), 1572 (amide II) cm⁻¹; UV/Vis (1,2-dichloroethane, 80 °C): $\lambda_{\max}$ (ϵ) = 319 (12300) nm.

Gelation experiments: The gelation experiments and characterization of the gels and solid fibers by DSC, light and electron microscopy, and X-ray powder diffraction have been carried out as described before for other bisurea compounds.^[9] A gel of **1** (3 mm) or **2** (2.5 mm) in 1,2-dichloroethane was prepared in a 1 mm quartz cell for UV/Vis measurements. UV/Vis spectra were recorded at temperatures ranging from 20 °C to 80 °C at 10 °C intervals.

Molecular modeling: Calculations were done using the Charmm molecular mechanics package^[16] and QUANTA96, distributed by Molecular Simulations Incorporated. Preliminary calculations were done in the gas phase

with a dielectric constant of 1 and with a nonbonded cut-off range of 15 Å, with a switch function operating from 11 to 14 Å. For the final energy minimization the Ewald summation with a cut-off of 20 Å for nonbonding interactions was used. Electrostatic point charges were taken from templates provided by QUANTA. For the generation of a systematic search a charmm script was written, which is an implementation of the cluster search method of Gavezzotti^[15] but limited to translational symmetry operations. Each search started with a different conformation of the bisurea compound, and gave 5–10 trial structures. These trial structures were then energy minimized to an energy gradient of 0.00005.

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Fast Experiments for Charge-Density Determination: Topological Analysis and Electrostatic Potential of the Amino Acids L-Asn, DL-Glu, DL-Ser, and L-Thr**

Ralf Flaig, Tibor Koritsánszky, Jan Janczak, Hans-Georg Krane, Wolfgang Morgenroth, and Peter Luger*

X-ray diffraction experiments not only give atomic positions from which the geometry of a chemical structure can be determined, but the exact charge density distribution $\rho(\mathbf{r})$ can also be deduced,^[1] which is an observable in contrast to the wave-function in the Schrödinger equation. To accomplish this the experiment has to be carried out up to high resolution ($d \leq 0.5$ Å or $\sin\theta/\lambda \geq 1.0$ Å⁻¹) and at the lowest temperature possible. With conventional diffractometers and serial (scintillation) detection this required measurement periods of several weeks or even months, even for structures of moderate size (20–30 atoms), so that the method, although known since the 1960s,^[2] was hardly applied and led only to more qualitative results. In the meantime several developments have changed the situation drastically.

By means of Bader's theory of "atoms in molecules", which was developed on a quantum chemical basis, a topological analysis of $\rho(\mathbf{r})$ allows a well-defined partitioning of a chemical structure into sub-molecular regions (atoms or functional groups).^[3] This initiated the generation of appro-

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priate computer programs, among them XD,^[4] which is able to calculate all the properties that can be deduced from the charge density and display them as high quality graphics.

Almost at the same time experimental conditions have improved substantially, with intensive X-ray sources (especially synchrotron radiation) and low temperatures (down to 15 K) contributing to a better scattering from the sample. Thanks to the emergence of area detectors (imaging plates or CCDs) a decisive breakthrough has been accomplished and this has increased the number of reflections that could be measured per unit time by about two orders of magnitude. This reduced the measurement periods from several weeks to one or a few days.^[5]

Recently we started comparative charge density determinations in the group of the 20 naturally occurring amino acids to deduce, as far as possible, their topological and electrostatic properties, and in particular to study the reproducibility and transferability in the vicinity of the C_α-carbon atom. At the same time there is a chance to supplement Bader's theoretical works^[6] and prove experimentally the transferability of electronic properties of molecular and submolecular fragments from this class of compounds onto larger systems like oligopeptides.

Herein we describe the results for L-asparagine monohydrate, DL-glutamic acid monohydrate, DL-serine, and L-threonine, which are based mainly on fast diffraction experiments performed at HASYLAB/DESY with a combination of synchrotron radiation/CCD area detection^[7] (Table 1). A first experiment of this kind was reported recently.^[5] The synchrotron experiment with DL-serine,^[8] which was executed up to a resolution of $d=0.33 \text{ \AA}$ ($\sin\theta/\lambda=1.54 \text{ \AA}^{-1}$), is probably one of the most highly resolved diffraction experiments in charge density work. Further charge density studies based on conventional experiments that took several weeks are known for the amino acids L-alanine,^[9] glycine,^[10] DL-histidine,^[11] and DL-aspartic acid.^[12]

Table 1. Summary of the different measurements, experimental conditions, and figures of merit.

	Asn	Glu	Ser (Sy)	Ser (Mo)	Thr
radiation/beamline	Sy/F1	Sy/F1	Sy/D3	MoK _α /Smart	AgK _α /four circle
wavelength [Å]	0.5297	0.5297	0.4500	0.7107	0.5608
resolution ($d/\sin\theta/\lambda^{-1}$) [Å/Å ⁻¹]	0.34/1.46	0.39/1.30	0.33/1.54	0.41/1.22	0.37/1.34
temperature [K]	100	100	100	123	19
measurement time [d]			< 2		25
reflections					
total	58720	59716	49928	19567	12215
unique	11452	13940	11120	6333	5994
$F_o > 3\sigma(F_o)$	9972	9246	7127	5181	4487
$R(F)$	0.0333	0.0455	0.0362	0.0433	0.0204
$Rw(F)$	0.0266	0.0347	0.0309	0.0341	0.0167

Qualitative and quantitative results for the Laplacian function $\nabla^2\rho(\mathbf{r})$ ^[13] are compiled in Figure 1 and Table 2, respectively. Since $\nabla^2\rho(\mathbf{r})$ has a finer structure than $\rho(\mathbf{r})$ small changes of the charge density that arises from bond formation

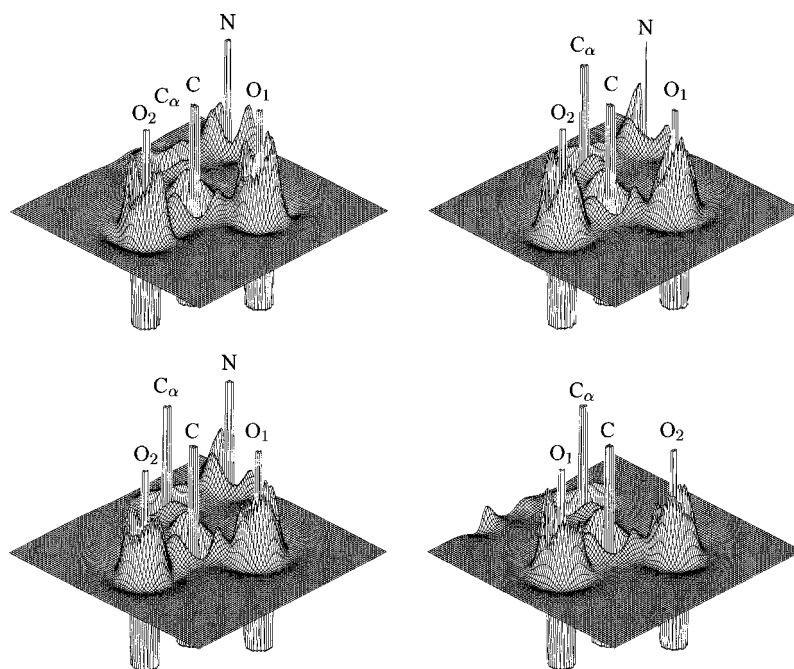


Figure 1. Relief representation of the negative Laplacian function in the plane of the carboxylate group: L-Asn (top left), DL-Glu (top right), DL-Ser (bottom left), and L-Thr (bottom right).

Table 2. Charge density [$\text{e \AA}^{-3}$] (first line) and Laplacian function [$\text{e \AA}^{-5}$] (second line) at the bond critical points. The two C–O bonds in the carboxylate group are distinguished by their different (partly to a small extent) bond lengths. C–O(1) corresponds to the longer and C–O(2) to the shorter bond.

bond	Asn	Glu	Ser (Sy)	Ser (Mo)	Thr	Pro ^[5]	Asp ^[12]	Ala ^[9]	Gly ^[10]	Average
O(1)–C	2.74(4) –34.3(2)	2.53(4) –27.3(2)	2.70(3) –36.1(1)	2.55(4) –32.0(3)	2.64(4) –30.7(3)	2.83(4) –39.3(3)	2.71(3) –37.6(2)	2.86 –29.6	2.67 –30.5	2.69(11) –33.0(40)
O(2)–C	2.90(4) –32.5(2)	2.77(4) –33.9(3)	2.74(3) –35.7(2)	2.67(4) –31.4(3)	2.78(4) –38.1(2)	2.84(4) –34.3(3)	2.87(3) –36.1(2)	3.02 –39.0	2.77 –32.8	2.82(10) –34.9(26)
N–C _α	1.74(3) –13.6(1)	1.72(3) –9.3(1)	1.69(2) –11.4(1)	1.72(3) –16.9(2)	1.72(3) –13.2(1)	1.68(2) –9.7(1)	1.69(2) –12.9(1)	1.70 –11.1	1.69 –11.9	1.71(2) –12.2(23)
C–C _α	1.67(3) –8.6(1)	1.86(2) –14.5(1)	1.77(2) –13.4(1)	1.86(3) –15.5(1)	1.80(3) –15.4(1)	1.88(2) –15.5(1)	1.69(2) –12.9(1)	1.76 –10.8	1.78 –15.6	1.79(7) –13.6(25)
C _β –C _β	1.83(3) –16.1(1)	1.68(2) –10.1(1)	1.79(2) –14.8(1)	1.77(3) –14.4(1)	1.80(3) –13.8(1)	1.66(2) –11.5(1)	1.61(2) –12.1(1)	1.67 –10.1	–	1.73(8) –12.9(22)

are easily detectable. As an example we show a relief plot of the negative Laplacian function in Figure 1 for the four amino acids. Its topology around the carboxylate group is in very good agreement for all amino acids. The distribution of $\nabla^2\rho(\mathbf{r})$ in the valence region, which corresponds to the Lewis electron pair model and the VSEPR model of Gillespie,^[3] allows an exact assignment of bonding and nonbonding electron pairs. All covalent bonds show the characteristic saddle-shaped bonding charge concentrations. The nonbonding charge concentrations of the oxygen atoms correspond to the lone pairs.

Quantitative values for $\rho(\mathbf{r})$ and $\nabla^2\rho(\mathbf{r})$ at the bond critical point, which give hints on the character and strength of a bond, are compiled in Table 2 for comparable bonds of the amino acids under investigation. The observed variations for a given bond in the range of 1–5 % for $\rho(\mathbf{r})$ and 7–19 % for $\nabla^2\rho(\mathbf{r})$ are even lower than those seen with Hartree–Fock calculations obtained from different basis sets.^[9, 12] This means that especially for $\rho(\mathbf{r})$ a high degree of reproducibility and transferability is observable in this group of the amino acids. The bigger variation for $\nabla^2\rho(\mathbf{r})$ is not too surprising since it is the second derivative of the charge density and therefore much more sensitive to the curvature at the critical point. The various C–O bonds in the amino acids allows them to be compared quantitatively. In the carboxylate group, common to all compounds, we find mean values for $\rho(\mathbf{r})$ of $2.69/2.82 \text{ e}\text{\AA}^{-3}$ at the bond critical points. For the C–O(H) bond of the hydroxyl group in Ser and Thr $\rho(\mathbf{r})$ is in the range of $1.81\text{--}1.85 \text{ e}\text{\AA}^{-3}$. In the carboxyl groups (Glu and Asp) we find values of 2.21 and $2.41 \text{ e}\text{\AA}^{-3}$ for $\rho(\mathbf{r})$ at the C–O(H) bond, whereas for the C=O bonds these values are 3.06 and $2.96 \text{ e}\text{\AA}^{-3}$. For the C=O bond in Asn we find $\rho(\mathbf{r}) = 2.89 \text{ e}\text{\AA}^{-3}$. The comparison of these values, which describe the bond strength quantitatively, with values from some oligopeptides we are currently working on, should show how far they change on forming a peptide bond and how the transferability to peptides is expressed.

The three-dimensional distribution of the Laplacian function, which we generated from the proline data measured earlier, contains important information about weak interactions. According to Bader's theory local charge accumulation corresponds to strongly negative regions in the Laplacian function. These are visible as holes in the green isosurface for $\nabla^2\rho = -12 \text{ e}\text{\AA}^{-5}$ in the environment of the two oxygen atoms displayed in Figure 2. If the oxygen atoms are connected to the H atoms of their intra- and intermolecular hydrogen bonding partners, it is interesting to note that each of these connecting vectors passes through one of these holes as can easily be seen in the stereo representation of Figure 2. Thus the Laplacian function also describes fine charge-density

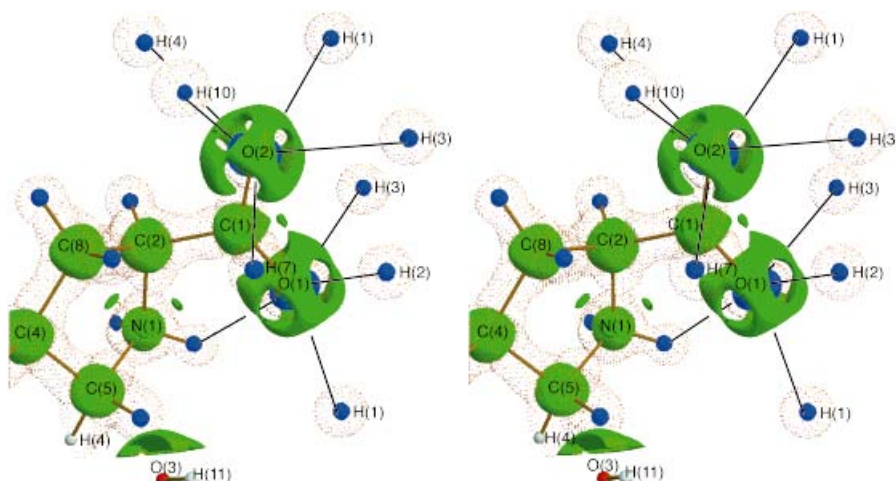


Figure 2. Stereo representation of three isosurfaces of the Laplacian function of DL-proline. Blue = $100 \text{ e}\text{\AA}^{-5}$, red dots = zero surface, green = $-12 \text{ e}\text{\AA}^{-5}$. In addition to the proline molecule all hydrogen atoms that are involved in intermolecular hydrogen bonds are drawn. All connecting vectors from the oxygen atoms to these hydrogen atoms pass through regions of local charge concentration, which can be seen as holes in the green isosurface.

reorganizations as a result of nonbonding interactions. Therefore, the directions where chemical interactions are favored can be observed from its topology.

The electrostatic potential (EP)^[14] displayed in Figure 3 considers an isolated molecule extracted from the crystal but which still bears the polarization effects induced by intermolecular interactions. Thus a three-dimensional distribution of the EP in the crystal is obtained. This chemical environment surely simulates better, for example, physiological conditions, than an EP extracted from an isolated molecule, which is the situation most often considered in quantum chemical calculations. The EP not only plays an important role in the treatment of chemical reactivity but also when considering molecular recognition (for example, drug receptor interaction). This is because electrostatic forces are long-range interactions and therefore determine the reaction path of two molecules.

The isosurface representations in Figure 3 all show a big kidney-shaped electronegative region as a common feature around the carboxylate group. This region is more extended relative to electrostatic potentials derived from theoretical calculations,^[5, 12] and we attribute this to polarization effects arising from intermolecular interactions in the crystal.

The following aspects seem important in regard to the above results: Thanks to fast experiments we were able to obtain comparable quantitative results on an electronic level for a number of amino acids. Similarity and molecular recognition play an important role in molecular modelling. The Laplacian function of a compound, which is now obtainable in a fast and reliable way with modern experimental methods, considers both aspects. The method used here yields the topology of the charge density in the crystalline state. In this state, in which molecular recognition is realized to a high degree, the derived properties of a system are surely closer to the “real” properties than those derived from the isolated stationary state. If it turns out that the functionality given by the Laplacian function is invariant with

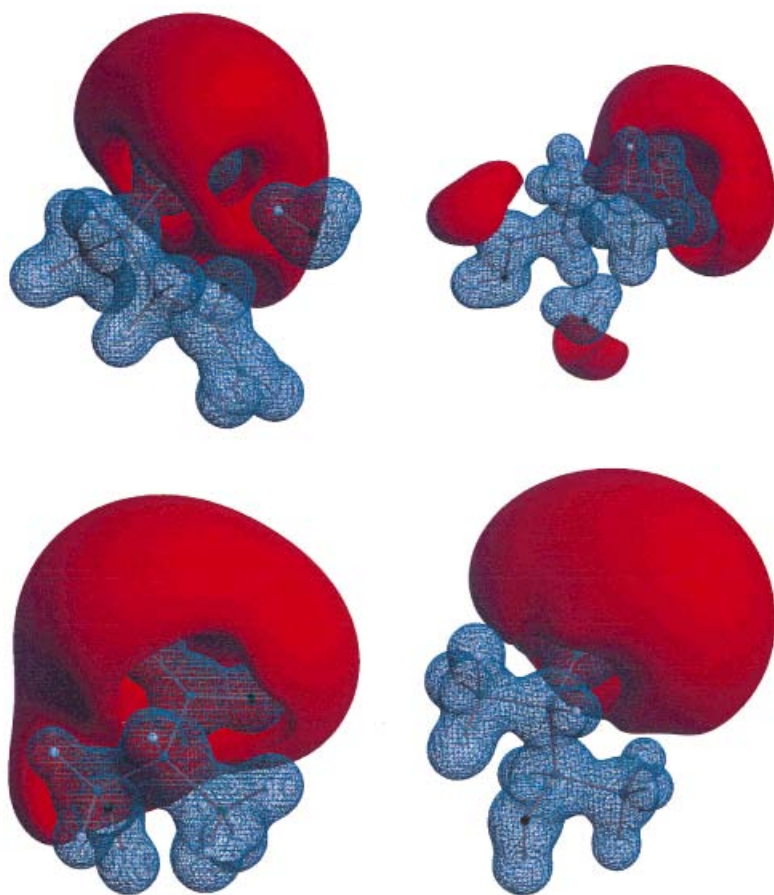


Figure 3. Isosurface representation of the experimental electrostatic potentials. Blue: positive potential, red: negative potential, L-Asn (top left, 0.5/−0.15), DL-Glu (top right, 0.5/−0.08), DL-Ser (bottom left, 0.5/−0.1), L-Thr (bottom right, 0.5/−0.1). Unit: e Å^{−1}.

respect to changing chemical environments (for example different crystal fields) then the behavior of a molecule in different chemical environments can be reliably predicted. Although atomic properties based on Bader's partitioning of the charge density were not determined, the similarity of the topology of chemically equivalent bonds found in this study yields an indirect proof for the transferability of atomic and group properties. The experimental determination of such properties will surely gain importance in the fields of molecular modelling and design as well as in crystal engineering.^[15]

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$$\nabla^2\rho(\mathbf{r}) = \frac{\partial^2\rho(\mathbf{r})}{\partial x^2} + \frac{\partial^2\rho(\mathbf{r})}{\partial y^2} + \frac{\partial^2\rho(\mathbf{r})}{\partial z^2} \quad (1)$$

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Zinc Silicates: Very Efficient Heterogeneous Catalysts for the Addition of Primary Alcohols to Alkynes and Allenes**

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Dedicated to Professor Hans-Jürgen Quadbeck-Seeger on the occasion of his 60th birthday

The addition of alcohols to alkynes is usually achieved in the liquid phase with mercury(II) or gold(I) catalysts under acidic conditions near room temperature. The products that are usually formed stem from a double addition (acetals).^[1] With the use of a strong base like KOH as catalyst at elevated temperature only the monoaddition products are obtained (vinyl ethers, products of Reppe vinylation).^[2]

Supported zinc and cadmium carboxylates, for example metal acetates supported on charcoal, have long been known as catalysts for the addition of carboxylic acids to acetylene in the gas phase.^[2] However, this reaction can also be accomplished in the liquid phase with the metal carboxylates as homogeneous catalysts. The addition of acetic acid to acetylene in the gas phase was the main process for the large-scale synthesis of vinyl acetate until the late 1960s. Vinyl propionate is still being produced nowadays with zinc propionate on active charcoal as the catalyst.^[3]

In contrast, the addition of alcohols to acetylenes using zinc catalysts has never been extensively investigated, although vinyl ethers are produced industrially on a large scale. In the literature, only the addition of methanol to propyne and allene in the gas phase above 250 °C with zinc oxide or zinc nitrate supported on active charcoal or silica gel as the catalyst has been described by Temkin and co-workers.^[4] However, no information regarding the catalyst preparation or the reactor used was given.

Propyne and allene are formed in large amounts as by-products in the process of steam cracking for ethylene synthesis, but up to now there have not been many attempts to use them.^[5] The methanol addition product 2-methoxypropene, which can be viewed as an activated acetone, is an interesting intermediate^[6] and led us to investigate this chemistry in detail.

In an attempt to reproduce the results reported by Temkin and co-workers,^[4a] we synthesized zinc oxide supported on commercially available, high-surface silica or active charcoal by thermal decomposition of previously deposited basic zinc

carbonate.^[7] However, the catalyst turned out to be inactive. In an attempt to reproduce some further results described by Temkin and co-workers,^[4b] catalysts consisting of zinc nitrate or zinc acetate supported on the carriers mentioned above were prepared. However, upon testing at 275 °C the fresh catalysts showed only a small activity. Interestingly, when they were tested at lower temperatures (ca. 185 °C), the catalysts containing zinc acetate or zinc nitrate supported on silica showed a dramatic change in activity during the first few hours on stream. As shown in Figure 1 the fresh catalysts turned out to be inactive, but both the conversion and the selectivity increased sharply in the first 200 minutes.

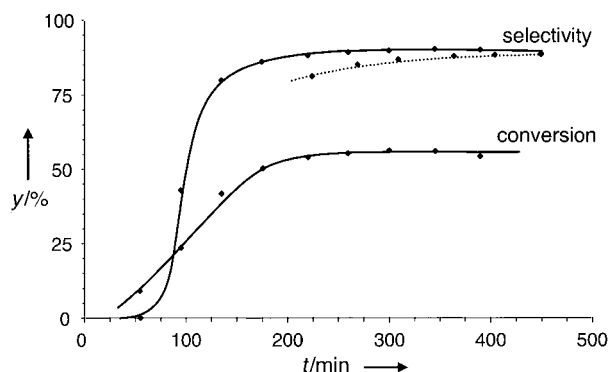


Figure 1. Variation in the conversion and selectivity (y) with time for the reaction of propyne/allene and methanol to 2-methoxypropene on stream using zinc acetate supported on silica gel as the catalyst precursor. The dotted selectivity curve was obtained by replacing propyne/propadiene by nitrogen during the first 200 minutes.

We considered this to be a clear indication that zinc acetate was not the actual catalyst, but instead the active species is formed in situ during the reaction. These preliminary results prompted us to have a closer look at both the catalyst and the mechanism of this reaction. Attempts to use other materials as supports—such as active charcoal, alumina, zirconia, titanium dioxide, silicon carbide, or silicon nitride—lead to completely inactive catalysts. We therefore concluded that silica was also involved in the formation of the active catalyst. The participation of methanol in the in situ formation of the active phase could be demonstrated by treating the freshly prepared catalyst with a propyne/propadiene mixture or with methanol alone (diluted with nitrogen) for 200 minutes at 175 °C and under normal pressure before adding the second reagent. While pretreatment with propyne/propadiene had no effect, treatment with methanol alone induced the formation of the catalytically active phase (dotted line in Figure 1).

The actual catalytically active phase was apparently being formed in situ by a solvothermal reaction between the zinc salt, methanol, and the silica in the solid state. Unfortunately, the true catalyst proved to be very difficult to characterize. The X-ray diffractogram showed only a diffuse band similar to that observed for the silica support, indicating that the material is more or less amorphous. The ²⁹Si CP-MAS NMR spectra of the formed catalysts showed only a broad signal at $\delta = -109$ with a small unresolved shoulder at $\delta = -99$, that is, very similar to the spectrum observed for the silica support after calcination at 900 °C (which removes all the adsorbed

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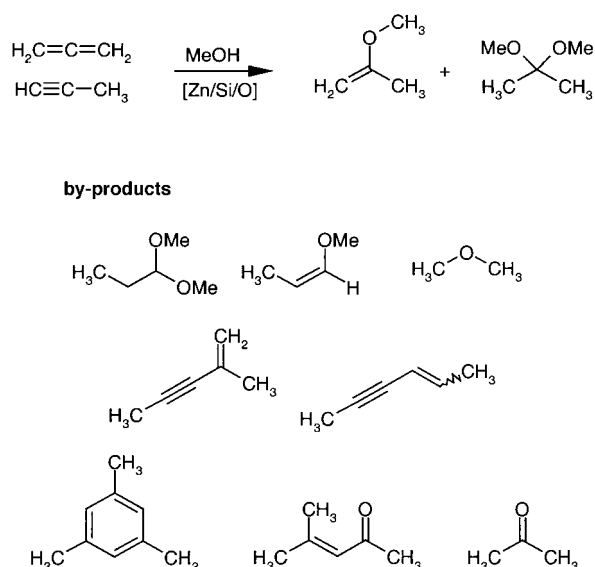
water and most of the surface SiOH groups). The spectrum shows no similarity to that of known zinc silicates.

An examination of the active catalyst with extended X-ray absorption fine structure (EXAFS) spectroscopy showed that the average coordination number of zinc was 3.9 oxygen atoms at a distance of about 1.97 Å ($\sigma^2 = 5.7 \times 10^{-3}$ Å²). However, the EXAFS spectra of all known zinc silicates mentioned below were very similar. Comparison of the diffuse reflectance IR (DRIFT) spectra of zinc acetate deposited on silica and of the active catalyst obtained from it clearly showed that in the process of building the active phase all the acetate is decomposed. As was shown by scanning electron microscopy combined with electron probe microanalysis (SEM/EPMA), zinc and silicon were very homogeneously distributed all over the catalyst pellet, and there were no regions with isolated zinc oxide. The catalyst is quite stable even after 800 hours on stream at 175 °C and at normal pressure. No further structure changes were observed by X-ray diffraction (XRD) and ²⁹Si CP-MAS NMR spectroscopy, indicating no further catalyst aging. However, heating the catalyst at temperatures above 350 °C leads to a loss of activity.

In an attempt to independently prepare the catalytically active phase, we allowed silica gel to react with zinc acetate in the presence of liquid methanol in an autoclave at 200 °C for 24 hours. The obtained white powder was crystalline, and the reflections observed by XRD could easily be assigned to willemite, also known as α -zinc orthosilicate (α -Zn₂SiO₄), which is the most stable phase in the Zn/Si/O phase diagram.^[8] Pure willemite, which was prepared according to a literature procedure,^[9] proved to be catalytically inactive. In the course of our work a number of other known zinc silicates were prepared and tested as catalysts. The β form of the zinc orthosilicate (β -Zn₂SiO₄)^[10] and sauconite (Zn₃Si₄O₁₀(OH)₂·4H₂O)^[11] both turned out to be inactive, but hemimorphite (Zn₄Si₂O₇(OH)₂·H₂O)^[12] was as active and selective as the amorphous zinc silicate obtained in situ from zinc acetate and silica.

Both hemimorphite and the amorphous zinc silicate are remarkable catalysts for the addition of methanol to propyne and/or allene. Catalytic activity is observed at temperatures as low as 120 °C at normal pressure. The reaction rate increases between 120 and 250 °C with an apparent activation energy of about 70 kJ mol⁻¹. In contrast to homogeneous Hg^{II} or Au^I catalysts, where propyne reacts about an order of magnitude faster than allene, with hemimorphite or amorphous zinc silicate as the catalyst allene reacts about 1.6 times faster than propyne. The addition of methanol to propyne and/or allene proceeds very selectively, the main products being 2-methoxypropene and 2,2-dimethoxypropane with a combined selectivity of about 96 % (Scheme 1).

The relative amounts of 2-methoxypropene and 2,2-dimethoxypropane formed depend strongly on the reaction conditions, because the zinc silicates also catalyze the reversible addition of methanol to 2-methoxypropene very effectively. Although the substrate spectrum has not yet been studied exhaustively, it is known that these silicates also catalyze the addition of water to propyne/allene to yield acetone as well as the reaction of ethanol, propanol, and



Scheme 1. Main and by-products of the zinc silicate catalyzed addition of methanol to propyne and/or allene.

1-butanol with acetylene to give mainly the corresponding enol ethers.

Since the addition of methanol to propyne or allene is a rather simple reaction and the structure of the catalytically active phase (hemimorphite) is known in detail,^[13] we tried to develop a plausible mechanism for this reaction using ab initio calculations. In view of the nature of the active metal (Zn²⁺ with a d¹⁰ closed shell) and of the small difference in reactivity between propyne and allene, an activation of the hydrocarbon seems improbable. On the other hand, cases where tetrahedrally coordinated zinc(II), as found in hemimorphite, is used to activate water or lower alcohols are known in homogeneous catalysis.^[14c] The best examples are probably the carbonic anhydrases, a family of ubiquitous enzymes which catalyze the reaction between water and CO₂ to form carbonic acid.^[14] The zinc-bound water molecule in a carbonic anhydrase is quite acidic, and it is the zinc hydroxide species formed after ionization that will react with CO₂. A network of water molecules provides for the necessary proton scrambling.^[14e, f, 15]

The question was which zinc atoms in hemimorphite could serve as active centers. Electron diffraction measurements with a transmission electron microscope on single crystals of hemimorphite obtained from our synthesis showed the preferred facet orientation in our crystals to be the (0,1,0) plane. Inspection of a model of the dehydrated hemimorphite (Figure 2) clearly shows that the lowest energy (0,1,0) facet is obtained by cutting the crystal along the [0,2,0] Miller plane.

The surface thus generated looks very promising from the view point of catalysis since it contains many tetrahedrally coordinated zinc atoms for which one of the coordination sites is occupied by water. Calculations showed that this water molecule is loosely bound and can easily be exchanged with methanol.

However, there are not enough properly coordinated zinc atoms to produce an active catalyst. Once again, the analogy with the carbonic anhydrase gave us the decisive hint: A key

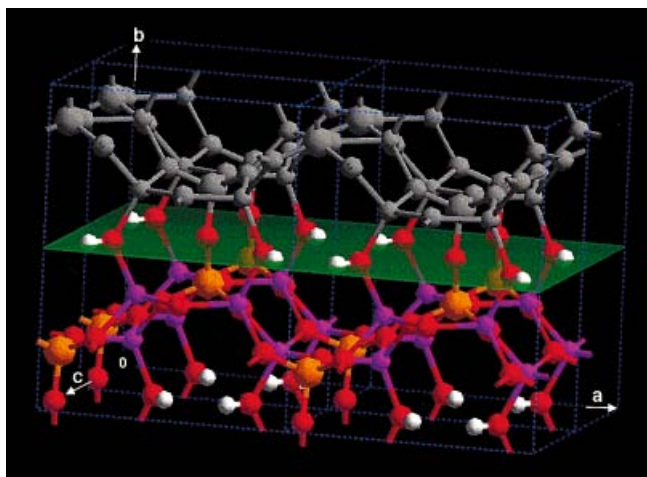


Figure 2. View of the expanded unit cell ($2a \times 2b \times c$) of hemimorphite showing the [0,2,0] Miller plane (green). On cleavage this plane gives the lowest energy (0,1,0) facet.

feature of these enzymes is the presence of a suitably located proton donor. Preliminary calculations on a simple model system^[16] indicated that the proton-donating atom should be located above the zinc methoxide. Such a geometry cannot be found within a single crystal plane. The next obvious place to search is at a step in the (0,1,0) surface, and indeed at this step a sterically unencumbered zinc and a silanol group, as the proton donor, are present in an adequate geometry (Figure 3).

The model system was created as follows: A crystal containing $3 \times 3 \times 7$ unit cells was built, and half of the top (0,1,0) layer removed. With this procedure, all of the surface Zn and Si atoms kept the oxygen atom to which they were bonded. The remaining free valences of the dangling oxygen atoms or hydroxyl groups were then saturated by adding protons to ensure overall electroneutrality. This produced terminal OH groups on the silicon atoms and both OH and H_2O moieties on the zinc atoms.^[17] To create the reactive site, one of the zinc-bound OH groups at the step was removed, producing a cationic zinc center. All the water molecules in the crystal channels were also removed since they are lost during catalysis, which has only a little effect on the crystal structure.^[13b]

The resulting model system (Figure 3) contains 2150 atoms. Since this is too large to be treated entirely with quantum-mechanical (QM) methods, we have defined a cluster around the reactive center which was treated using quantum mechanics, while the rest of the model is handled by simpler molecular mechanics (MM).

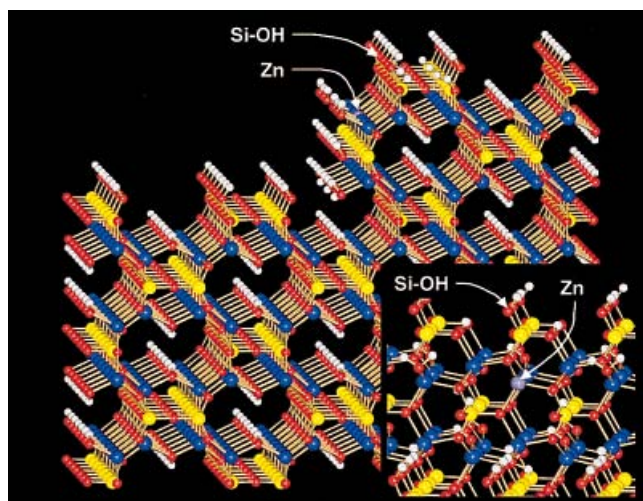
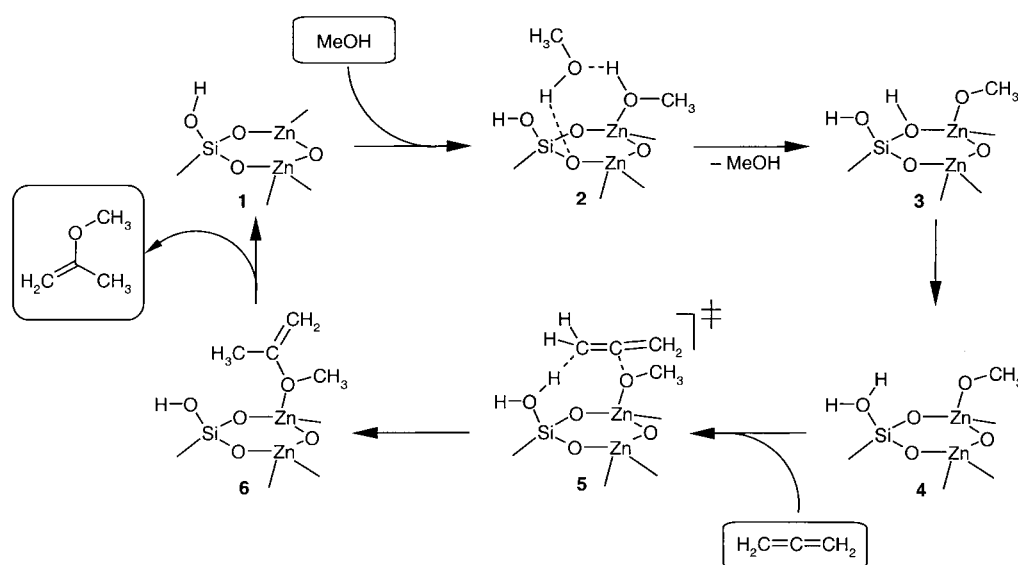


Figure 3. Structure of the catalyst model used in the calculations. Presented is a side view (along the c axis) of a step on the (0,1,0) surface showing the reactive zinc atom (purple) and proton-donating SiOH group. The inset is an enlarged front view (along the a axis) of the reactive center. Zn: blue, Si: yellow, O: red, H: white.

Within this QM/MM hybrid, or embedding approach, we have combined density functional theory (DFT)^[18] in the TURBOMOLE program suite^[19] for the QM part with the universal force field (UFF)^[20] in the Cerius² software package^[21] for the MM part.^[22] The QM cluster includes the reactive zinc atom and the first shell of zinc and silicon atoms surrounding it, which are connected and terminated by O atoms or OH groups (33 atoms in total, without reactants). In the QM calculations, the bonds of the QM cluster to the MM part of the crystal have been saturated by 14 hydrogen atoms.^[29] Only the QM atoms were relaxed within the structure optimizations.

The proposed reaction mechanism is shown in Scheme 2. The Langmuir–Rideal-type catalytic cycle starts at a zinc atom with a vacant coordination site (**1**). This zinc atom coordinates to a methanol molecule in an exothermic reaction



Scheme 2. Overview of the mechanism computed for the addition of methanol to allene. The species shown are all cationic.

(−171 kJ mol^{−1}). For geometrical reasons it is unlikely that the hydrogen atom of the methanol ligand is transferred directly to the silanol group. More likely the hydrogen transfer occurs by a hopping mechanism promoted by a second methanol molecule (2).^[30] The overall reaction of 1 to 2 is computed to be exothermic (−343 kJ mol^{−1}). A proton is first transferred to the bridging oxygen atom (3) and in a second step to the silanol group (4). The transformation of 2 into 4 is endothermic (+134 kJ mol^{−1}). The key intermediate 4 has the appropriate geometry to react with allene to produce 2-methoxypropene, which is coordinated to the zinc center (6). This reaction is exothermic (−117 kJ mol^{−1}) with no apparent activation barrier.^[31] Thus, the experimentally observed activation energy (70 kJ mol^{−1})^[32] is needed either for the desorption of the product, which is computed to be endothermal (+190 kJ mol^{−1}), or more likely for the dissociation of methanol. This result parallels the behavior of the enzyme carbonic anhydrase, where the rate-determining step is a proton transfer and not the attack of the zinc-bound hydroxide on CO₂.^[14d–f]

These calculations demonstrate the existence of one energetically viable pathway for the activation of methanol. The astonishing parallel between the catalytic pathways in hemimorphite and in the zinc enzyme carbonic anhydrase supports the suggested and calculated mechanism.

Experimental Section

Typical procedure for the addition of methanol to propyne/allene mixtures: The reported results were obtained using a C₃ fraction with the following approximate composition (vol %): propyne (32), allene (24), propene and propane (44), C₄ hydrocarbons (<2). The reaction was conducted in a stirred tank reactor designed for reactions in the gas phase on heterogeneous catalysts.^[33] The products were analyzed by on-line gas chromatography with cyclohexane as the internal standard. A typical experiment was conducted at 170 °C and 1.15 bar with hemimorphite (57.2 g; extrudate, pellet size 2 mm) as the catalyst. The feed contained propyne/propadiene (1.58 mmol min^{−1}), methanol (2.95 mmol min^{−1}), and cyclohexane (0.3 mmol min^{−1}; gas hourly space velocity 140 h^{−1}). The conversions of propyne and allene were 72 and 85 %, respectively. The selectivities were as follows: 2-methoxypropene (77 %), 2,2-dimethoxypropane (19 %), *cis*- and *trans*-1-methoxypropene (2 %), acetone (1 %), propyne/allene dimers and trimers (hex-2-en-4-yne, 2-methylpent-1-en-3-yne, mesitylene (1,3,5-trimethylbenzene); together 0.3 %), dimethyl ether (0.05 %), and 1,1-dimethoxypropane (0.1 %).

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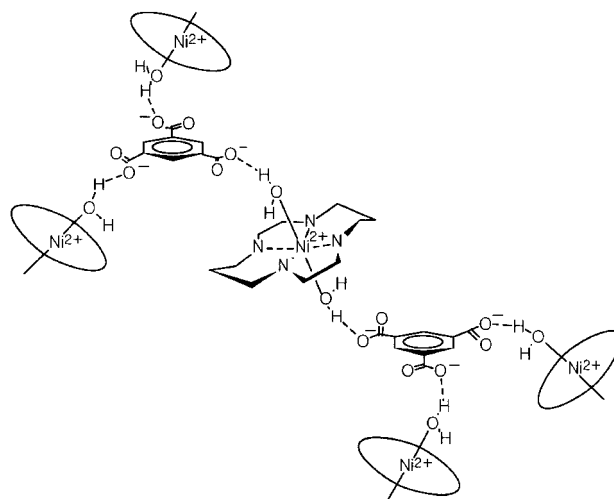
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Self-Assembly of a Molecular Floral Lace with One-Dimensional Channels and Inclusion of Glucose**

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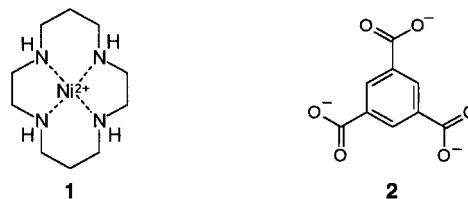
Supramolecular chemistry has advanced to a stage at which we can design and construct molecular solids with specific network topologies^[1] as well as with potentially interesting properties.^[2] Self-assembly of organic molecules and metal-ion building blocks may yield multidimensional networks containing channels or cavities of various sizes and shapes.^[3, 4] However, macrocyclic complexes have seldom been used in the construction of supramolecular networks although they could be used as the multifunctional building blocks.^[5]

Here we present a novel three-dimensional network containing one-dimensional channels, which is assembled by a nickel(II) macrocyclic complex. Our construction strategy (Scheme 1) was based on the ability of the nickel(II) complex



Scheme 1. Proposed formation of the network.

of cyclam (**1**) to bind water as the axial ligands^[6, 7] and hydrogen bond formation between the coordinated water molecules of the complex and the carboxylate oxygen atoms of 1,3,5-benzenetricarboxylate trianion (BTC^{3−}; **2**). Some



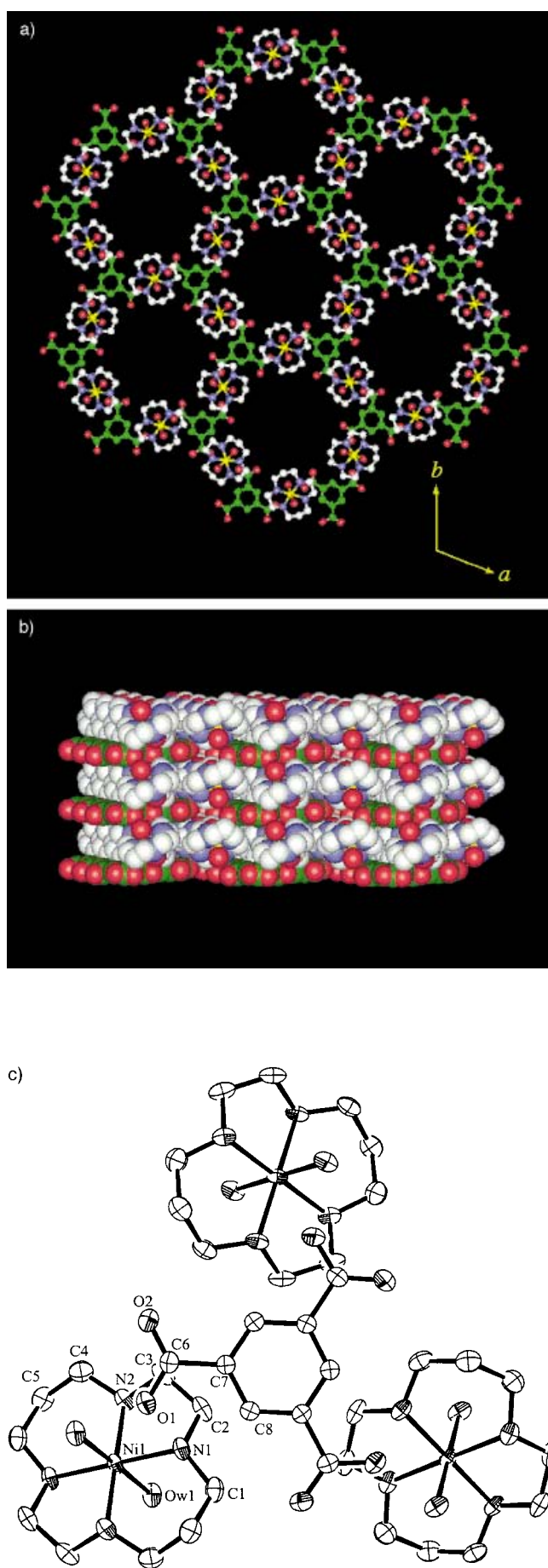
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nickel(II) macrocyclic complexes with a square-planar coordination geometry are in equilibrium in aqueous solution with the six-coordinated species that binds two water molecules at the *trans* position, with cyclam being the best ligand known to produce the six-coordinated species.^[6] In addition, **2** can be used as trigonal three-connectors in the construction of supramolecular assemblies.^[3b, 5, 8] Although $H_3\text{-2}$ itself forms two-dimensional infinite honeycomb grids, their pores are usually filled by interpenetration or catenation of other grids and thus extremely few uncatenated structures of $H_3\text{-2}$ are reported in which the cavities are occupied by some organic guests.^[9] Therefore, if **1** and **2** are self-assembled in water, each nickel(II) macrocycle will interact with two units of **2** up and down with respect to its macrocyclic plane and each unit of **2** will interact with three nickel(II) units since **2** contains three carboxylate groups. Based on this building scheme, the assembly will lead to a three-dimensional network.

A three-dimensional supramolecular network $[\text{Ni}(\text{cyclam})(\text{H}_2\text{O})_2]_3[\text{C}_6\text{H}_3(\text{COO})_3]_2 \cdot 24\text{H}_2\text{O}$ (**3**), which can be regarded as a molecular floral lace, has been assembled by **1** and **2** in water. The supramolecule **3** is extremely stable. It is insoluble in common organic solvents or in cold water and it maintains its crystal morphology even after the crystal is heated at 140°C. Single crystal X-ray analysis of **3**^[10, 11] revealed the structure shown in Figure 1. Viewed down the *c* axis, it is composed of rings, each of which consists of six nickel(II) macrocyclic complexes and six of the anions **2**, each being related with S_6 symmetry. Every nickel(II) macrocyclic complex is shared by two rings and every anion **2** is shared by three rings, which corresponds to the stoichiometry of the network $\text{Ni}^{II}/2 = 3/2$. The network generates prominent channels parallel to the *c* axis. In the three-dimensional network, nickel(II) macrocyclic complexes lie in a layer extending along the *ab* plane. The anions **2** lie on the other layer parallel to the *ab* plane. These positively charged inorganic and negatively charged organic layers are alternately packed. The distance between the centers of the positive and negative layers is 3.70 Å, so there is no open space on the *bc* or *ac* plane. Every nickel(II) complex of cyclam forms hydrogen bonds with two of the anions **2** located up and down with respect to its coordination plane with an inversion center at its nickel(II) ion.^[11] In addition, every anion **2** forms hydrogen bonds with three of the cyclam units. The macrocyclic coordination planes are tilted with respect to the benzene plane of the interacting anion **2** with a dihedral angle of 40.7°. The planes of the three macrocyclic rings linked to the anion have dihedral angles of 69.1° between them. The effective void of the channels is 10.3 Å in diameter, as measured by the distance between van der Waals surfaces of opposing macrocyclic walls. The channels are filled with guest water molecules, some of which are hydrogen bonded with oxygen atoms of **2**.^[12]

Figure 1. a) View of the *ab* plane in **3**. Water molecules included in the channels are omitted for clarity (yellow: nickel(II), red: oxygen, blue: nitrogen, white: carbon of macrocycle, green: carbon of **2**). b) CPK representation of the *ac*–*bc* plane of **3**. c) ORTEP drawing of the asymmetric unit of **3** showing the numbering scheme. The bond distances and angles are listed in ref. [11]. The atoms are represented by thermal ellipsoids at 30% probability. The center of symmetry is at the Ni^{II} atom.



Molecular-based materials that are stable even after removal of guests are important in view of the development of a new class of porous substances. Hydrogen-bonded organic networks are generally less robust than the inorganic analogues that are held together by covalent bonds. However, there are a number of open networks that are stable enough to sustain the frameworks upon partial or complete removal of guests.^[4b, 13] Thermal gravimetric analysis (TGA) data for the crystalline sample **3** indicate three stepwise losses of weight at 54, 85, and 135 °C, which correspond to the loss of 12 simple guests, 12 hydrogen-bonded water guests, and then two coordinated water molecules, respectively, per formula unit. The remaining compound can be heated to 210 °C without any additional weight loss. Differential scanning calorimetry (DSC) data show the phase changes at 95, 106, and 130 °C. The crystal morphology of **3** is maintained throughout the heating processes up to 140 °C.

X-ray diffraction (XRD) patterns of the dried solids are compared with those of **3** in Figure 2. The solid obtained by heating the crystals of **3** to 55 °C shows XRD patterns in which the positions and intensities of some lines are slightly changed

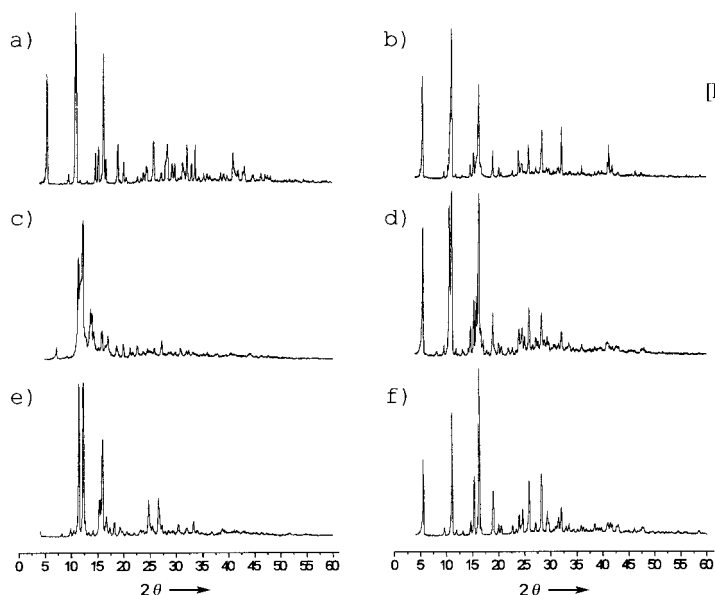


Figure 2. X-ray diffraction patterns for: a) the original crystal **3**, b) solid heated to 55 °C, c) solid heated to 90 °C for 2 h, d) solid obtained by immersing (c) in H₂O, e) solid heated to 135 °C, f) solid obtained by immersing (e) in H₂O.

relative to those of the original sample. When crystals of **3** are heated at 90 °C for 2 h, they show XRD patterns in which the lines are broadened and their positions are moved relative to those of **3**. However, when this solid is suspended in water the same XRD patterns as those of the original crystal are regenerated. Further heating of the dried solid to 135 °C results in sharp XRD lines whose positions are different from those of the original solid. The same XRD pattern as for **3** is, however, also regenerated when this solid is immersed in water for a few minutes.

D-Glucose and maltose, a dimer of glucose, were examined for inclusion into **3**.^[14] The binding experiments were carried

out in methanol containing 5 % water at 30 °C with the host dried at 90 °C for 2 h.^[15] D-Glucose was chosen as the guest molecule for **3** in view of its compatible molecular dimensions with the channel size and possibility to form hydrogen bonds with **2** in the channels. On the other hand, maltose is too large to be included in the channels.^[14]

The formation constant K_f for the complex formed between a guest molecule (G) and a binding site (BS) of an insoluble host can be defined as k_{ad}/k_{de} for Equation (1) by analogy with the Langmuir isotherm for adsorption of gas molecules on solid surfaces.^[16]



The plot of the concentration of G bound to BS ($[\text{BS} \cdot \text{G}]$) against total concentration of G ($[\text{G}]_0$) is illustrated in Figure 3 for complexation of glucose to **3**. Analysis of the

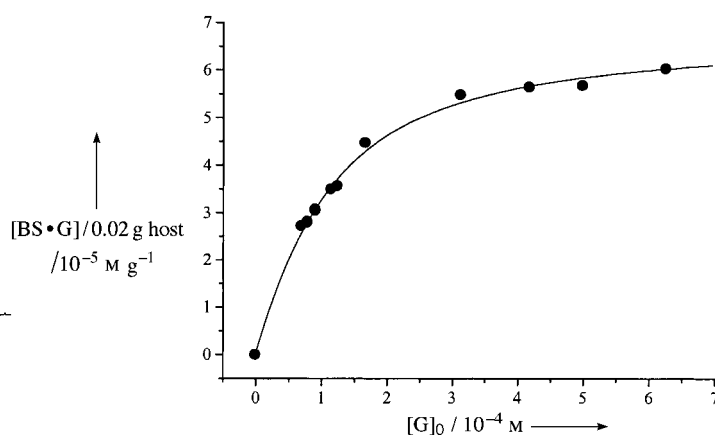


Figure 3. Binding of D-glucose to **3**.

data for glucose complexation according to a method described previously^[17] led to $K_f = (1.38 \pm 0.01) \times 10^4 \text{ M}^{-1}$.^[18, 19] The number of glucose binding sites occupied in **3** for the particular solids used in the binding experiment was estimated as $0.405 \pm 0.004 \text{ mol \%}$ relative to the formula unit of $[\text{Ni}(\text{cyclam})(\text{H}_2\text{O})_2]_3[\text{C}_6\text{H}_3(\text{COO})_3]_2$. The small number of the binding sites occupied suggests that inclusion of a glucose molecule effectively blocks succeeding inclusion of glucose molecules into the deeper side of the channels of **3**. In contrast to glucose, complexation of maltose to **3** was not detected, which suggests that the sugar binding is made through the inclusion into the channels rather than adsorption on the surface.^[15] The selective binding of **3** with glucose must be attributable to the size fit for glucose into the channels.

In conclusion, we have showed that we were able to create a molecular artwork by crystal engineering. We also demonstrated that a macrocyclic complex was utilized in the construction of hydrogen-bonded three-dimensional networks with one-dimensional channels. We are now extending this result by the preparation of some open frameworks with pore channels of various hole sizes through variation of the macrocycles and the bridging molecules.

Experimental Section

3: An aqueous solution (5 mL) of Na₃BTC (0.21 g) was added to a hot aqueous solution (15 mL) of [Ni(cyclam)](ClO₄)₂^[20] (0.52 g) and the solution allowed to stand at room temperature for several hours to give a good yield (ca. 75 %) of long rod-shaped pale-purple crystals.^[21, 22] Compound **3** is insoluble in common organic solvents or in cold water and maintains its crystal morphology except in alcohol where it turns into powder.

Binding experiments: Crystals of **3** were ground in a mortar until they became microcrystalline and then dried in an oven at 90 °C for 2 h.^[15] The transparent pale purple sample lost its transparency. The dried solid was preserved in a desiccator containing 4 Å molecular sieves. A stock solution of glucose was made in water and diluted with a 5 % solution of H₂O in MeOH. The glucose solution (1.00 mL) of various concentrations was added to each tube containing 0.020 g of dried host, and incubated at 25 °C in a shaker for 3 h. The amount of uncomplexed glucose was determined with glucoseoxidase.^[23, 24] The binding of maltose to **3** was studied under identical conditions. For determination of uncomplexed maltose, the maltose was converted into glucose with α -glucosidase and the resulting glucose was quantified by the aforementioned method.

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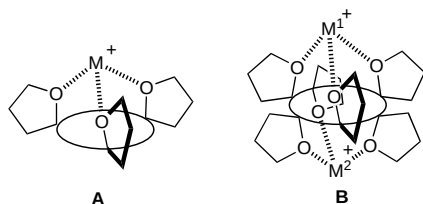
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- [11] Relevant bond lengths [Å] and angles [°]: Ni1–N2 2.057(3), Ni1–N1 2.070(3), Ni1–OW1 2.160(3), O1–C6 1.266(4), C6–O2 1.249(4), C7–C8 1.383(5); N1–Ni1–N2 85.50(12), N2–Ni1–OW1 87.66(12), N1–Ni1–OW1 91.39(11), O2–C6–O1 124.9(3), C8–C7–C6 120.6(3). Hydrogen bonding interaction: O1–OW1' 2.701 Å, O1–HW1A–OW1' 171°.
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- [14] The sizes of D-glucose and maltose are estimated as 9.4 × 8.2 × 6.7 and 11.5 × 10.4 × 8.2 Å³, respectively. The N₂ gas sorption on the dried solid (90 °C for 2 h) indicates that the diameter of the channels is 10.9 Å, which is similar to the value of 10.3 Å estimated from the X-ray structure. The crystalline sample had to be evacuated by heating under high vacuum overnight before we measured the gas sorption isotherm. Under this high vacuum condition the solid seemed to experience the deformation of two-dimensional layers and showed broad XRD patterns. Gas sorption data gave a poor pore surface area (21.2 m² g^{−1}) for this evacuated sample. The solid, however, also regenerated the XRD patterns of the original crystals when it was immersed in water.
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A Belted Monofacial Ionophore Featuring High Selectivity for Lithium Ion Complexation**

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The search for new ligands capable of exhibiting selectivity for binding to specific metal ions continues to grow at an unabated pace.^[1,2] The observed variations in complex stability have been rationalized principally in terms of the shape, cavity size, and conformational flexibility characteristics of a given macrocyclic ether.^[3] In select cases, interaction energies have been computed by molecular mechanics^[4] and molecular dynamics methods.^[5] Despite this, quantitative means for predicting the relationship of binding stability to the three-dimensional characteristics of a crown ether have only recently been reported.^[6]

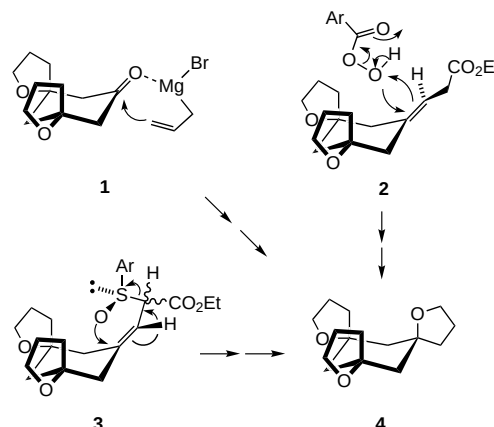
Several years ago, we suggested that the enhanced binding efficiency of tetrahydrofurans might be very suitably exploited by proper assimilation of these building blocks into belted spirocyclic networks.^[7] With level of substitution, locus of the oxygen atoms, and relative stereochemistry serving as the relevant principles of rational design, it was anticipated that heightened levels of structural preorganization could be achieved with resultant enhancement of selective metal ion binding. Moreover, such novel structures offer the unique potential of being specifically tailored to serve as monofacial (as in **A**)^[8] or bifacial ionophores (see **B**).^[9] The latter



compound class offers the added attractive option of incorporating two different metal ions (because of a cavity size dependency relating the two faces) and of elaborating ladder polymers of alternating ligand and M^+ components. We now report the synthesis and crystallographic characterization of *cis,cis*-1,8,14-trioxatrispiro[4.1.4.1]octadecane (**9**), define the extraordinary selectivity of its binding to alkali metal ions, and quantify computationally the structural requirements of each metal ion–donor interaction.

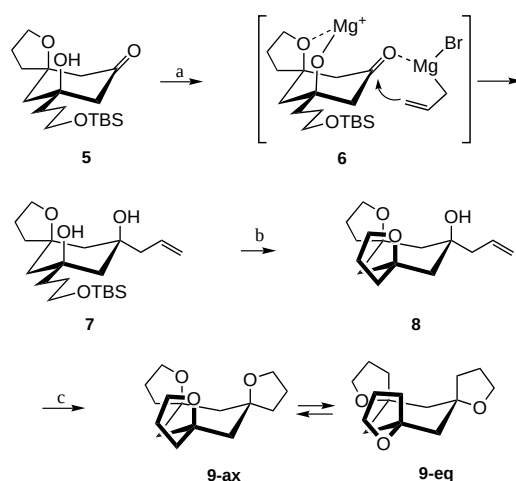
With the discovery that *cis*-dispirocyclohexanone **1**^[10] undergoes nucleophilic attack exclusively from the equatorial

direction, and that its *exo*-methylene derivatives **2** and **3** react cleanly from the opposite π surface (Scheme 1), came the realization that the formation of *cis,trans* trispiro product **4** had to be skirted by making recourse to a superior stereocontrol element. The silyl-protected 3-hydroxypropyl substituent in **5** proved well suited to this task. As expected, the



Scheme 1. π -Facial stereoselectivity of attack on *cis*-dispirocyclohexanone **1** and its *exo*-methylene congeners **2** and **3**.

bulkiness of this side chain guaranteed its equatorial occupancy and opened the way to rapid deprotonation with formation of chelate **6** upon treatment with Grignard reagents such as allylmagnesium bromide (see **6**, Scheme 2). The C–O



Scheme 2. Synthesis of the *syn, syn* trispiro ligand **9**: a) allylmagnesium bromide, Et_2O/THF (1/1), 20 °C, 15 h, 77 %; b) TBAF, THF, 20 °C, 4 h; $TsCl$, Et_3N , DMAP, CH_2Cl_2 , 20 °C, 12 h (88 % over 2 steps); c) BH_3 , THF, 0 °C, 1 h; $TsCl$, Et_3N , DMAP, CH_2Cl_2 , 20 °C for 6 h, then reflux for 15 h (81 % over 2 steps). TBAF = tetrabutylammonium fluoride, TBS = *tert*-butyldimethylsilyl, $TsCl$ = *p*-toluenesulfonyl chloride, DMAP = dimethylaminopyridine.

bonds, which are oriented axially in **6**, provide adequate steric shielding to deter *syn* approach to the carbonyl group. As a consequence, the triad array of oxygen atoms resident in **7** is fixed in an all-*syn* relationship. The C_{3v} symmetry of **9** (m.p. 111–113 °C) is quite apparent spectroscopically (five ^{13}C signals; see Table 1). The crystal structure (Figure 1)

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Table 1. Selected spectroscopic data for compounds **4**, **9**, and **10**.^[a]

4 : IR (film): $\tilde{\nu}$ = 1448, 1349, 1177, 1047 cm ⁻¹ ; ¹ H NMR (C ₆ D ₆): δ = 3.75–3.60 (m, 4H), 3.60–3.48 (m, 2H), 2.15–1.95 (m, 3H), 1.75–1.40 (series of m, 15H); ¹³ C NMR (C ₆ D ₆): δ = 82.9, 82.5, 66.7, 65.9, 48.0, 45.9, 38.9, 36.7, 26.4, 25.2; HR-MS: m/z calcd: 252.1725 [M^+]; found: 252.1730.
9 : IR (film): $\tilde{\nu}$ = 1454, 1222, 1044 cm ⁻¹ ; ¹ H NMR (CDCl ₃): δ = 3.80 (t, J = 6.9 Hz, 6H), 1.95–1.80 (m, 9H), 1.70–1.65 (m, 6H), 1.57 (d, J = 13.6 Hz, 3H); ¹³ C NMR (CDCl ₃): δ = 81.5, 66.0, 45.5, 37.5, 25.3; HR-MS: m/z calcd: 252.1725 [M^+]; found: 252.1712.
10 : ¹ H NMR (CDCl ₃): δ = 3.76 (t, J = 6.2 Hz, 6H), 1.95–1.70 (m, 15H), 1.55 (d, J = 14.0 Hz, 3H); ¹³ C NMR (CDCl ₃): δ = 81.6, 68.1, 45.3, 39.7, 25.0; positive-ion FAB-MS: m/z (%) calcd for 9 ·Li ⁺ : 259; found: 259.15 (100), m/z (%) calcd for (9) ₂ Li ⁺ : 511; found: 511.42 (13.5).

[a] NMR spectra recorded at 300 (¹H) and 75 MHz (¹³C). Correct elemental analyses were obtained for all compounds.

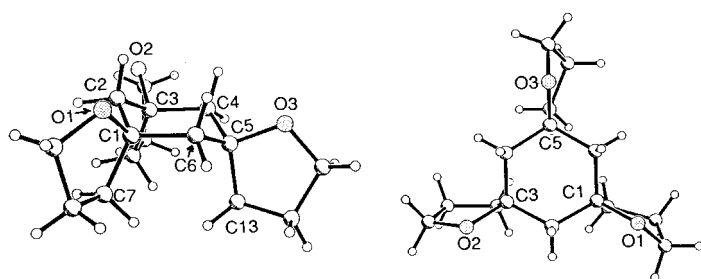


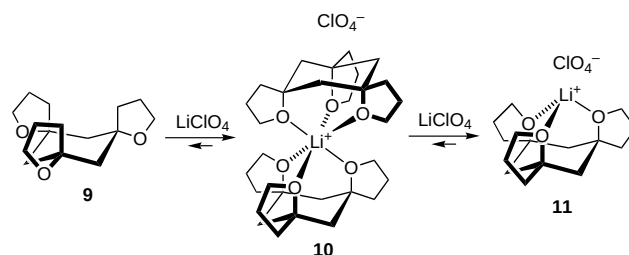
Figure 1. Side (left) and top views (right) of the molecular structure of **9** in the solid state. Selected bond lengths [Å] and angles [°]: O1–C1 1.465(4), O2–C3 1.459(4), O3–C5 1.453(4), C1–C6 1.520(5), C1–C2 1.527(4), C2–C3 1.522(4), C3–C4 1.514(4), C4–C5 1.534(5), C5–C6 1.527(4), C1–C2–C3 114.4(3), C2–C3–C4 109.7(3), C3–C4–C5 116.1(3), C4–C5–C6 109.7(3), C5–C6–C1 116.6(3), C6–C1–C2 110.3(2).

shows that the conformation with all oxygen atoms in equatorial positions is adopted in the solid state.^[11] This is rather remarkable in that MM3-based molecular mechanics (MacroModel 5.0) indicates **9-eq** to be 3.93 kcal mol⁻¹ less stable than **9-ax**. Although intermolecular stacking interactions may be held responsible here, this conformational preference appears to persist as well in solution (see below), a likely consequence of more favorable dipole–dipole interactions.

The association constants (K_a) for the complexation of **9** to Li⁺, Na⁺, and K⁺ picrates were determined in H₂O/CDCl₃.^[12] These data reveal **9** to exhibit high selectivity for the lithium ion ($K_a(\text{Li}^+)/K_a(\text{Na}^+) = 7.9 \times 10^7/2.5 \times 10^6 = 32$, $K_a(\text{Li}^+)/K_a(\text{K}^+) = 7.9 \times 10^7/3.3 \times 10^4 = 2400$) in a manner comparable to the behavior of highly preorganized spherands^[13] and smaller cryptands.^[14] The corresponding values for [15]crown-5 are $K_a(\text{Li}^+)/K_a(\text{Na}^+) = 0.94 \times 10^5/6.3 \times 10^6 = 0.015$ and $K_a(\text{Li}^+)/K_a(\text{K}^+) = 0.94 \times 10^5/1.1 \times 10^6 = 0.085$. Although the consequences of belting as in **9** are clearly quite special, there exists a strict requirement that placement of the three heteroatoms be precisely defined. This point is reflected in the inability of **4** to extract any of the picrate salts at measurable levels.

The independent preparation of 2:1 sandwich complexes of **9** to LiBF₄ and NaBF₄ provided compounds of type **10** (see Scheme 3), for which crystals amenable to X-ray analysis were obtained.^[15] The major difference in the two structures is the increased distance of Na⁺ (1.554 Å) relative to Li⁺ (1.279 Å)

above the plane defined by the three oxygen centers. Molecular mechanics calculations^[16] indicate the level of distortion experienced by **9** in accommodating Na⁺ to be approximately 3.4 kcal mol⁻¹ more than for Li⁺. Upon going from Na⁺ to K⁺, the energy increment rises by an additional 11 kcal mol⁻¹. Consequently, the concordance with size–match selectivity is excellent. ¹³C NMR titration experiments involving **9** and LiClO₄ in CH₃CN/C₆D₆ (4/1) clearly defined the generation of the 2:1 complex **10** (up to 0.50 equiv of salt). The latter is cleanly transformed into the 1:1 complex **11** as this amount of additive is exceeded (Scheme 3, Figure 2). The



Scheme 3. Formation of **10** upon addition of 0.5 equivalents of LiClO₄ to **9**, and its transformation to **11** upon addition of 1.00 equivalent of LiClO₄.

replacement of the set of signals due to **10** by those attributable to **11** is very clearly demarcated, thereby requiring slow exchange between the free ligand, **10**, and **11** under the conditions of measurement. The differences in the chemical shifts observed for the three species suggest that a conformational change is likely occurring upon metal ion complexation.^[16] If so, the present data suggest that the **9-ax** ⇌ **9-eq** equilibrium is defined by a barrier high enough to show a single conformation (**9-eq**) on the NMR time scale, but of sufficiently low energy to allow for facile complexation to the smaller alkali metal ions via **9-ax**.

Significantly, the readiness with which **9** forms 2:1 complexes such as **10** with Li⁺ and Na⁺ may well foreshadow the highly desirable capacity of its bifacial homologues to engage in unprecedented ladder complex formation. It is clear from the behavior of **9** that the presence of a belt provides positive inducement for selective ion complexation, most notably when the stereochemical and spatial arrangements satisfy those structural requirements conducive to strong coordination.

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[1] The Sci-Finder program of the Chemical Abstracts Service currently (September 20, 1998) lists 38 709 references to papers that contain the word “ionophore”, 12 215 reference citations for “crown ether”, and 1778 references to papers dealing with “cryptands”. Specific attention is called to studies involving the conformational control of cation binding by the research groups of W. C. Still (tetrahydropyrans; M. T. Burger, A. Armstrong, G. Guarneri, D. Q. McDonald, W. C. Still *J. Am. Chem. Soc.* **1994**, *116*, 3593) and U. Koert (tetrahydrofurans; U. Koert, M. Stein, H. Wagner, *Chem. Eur. J.* **1997**, *3*, 1170).

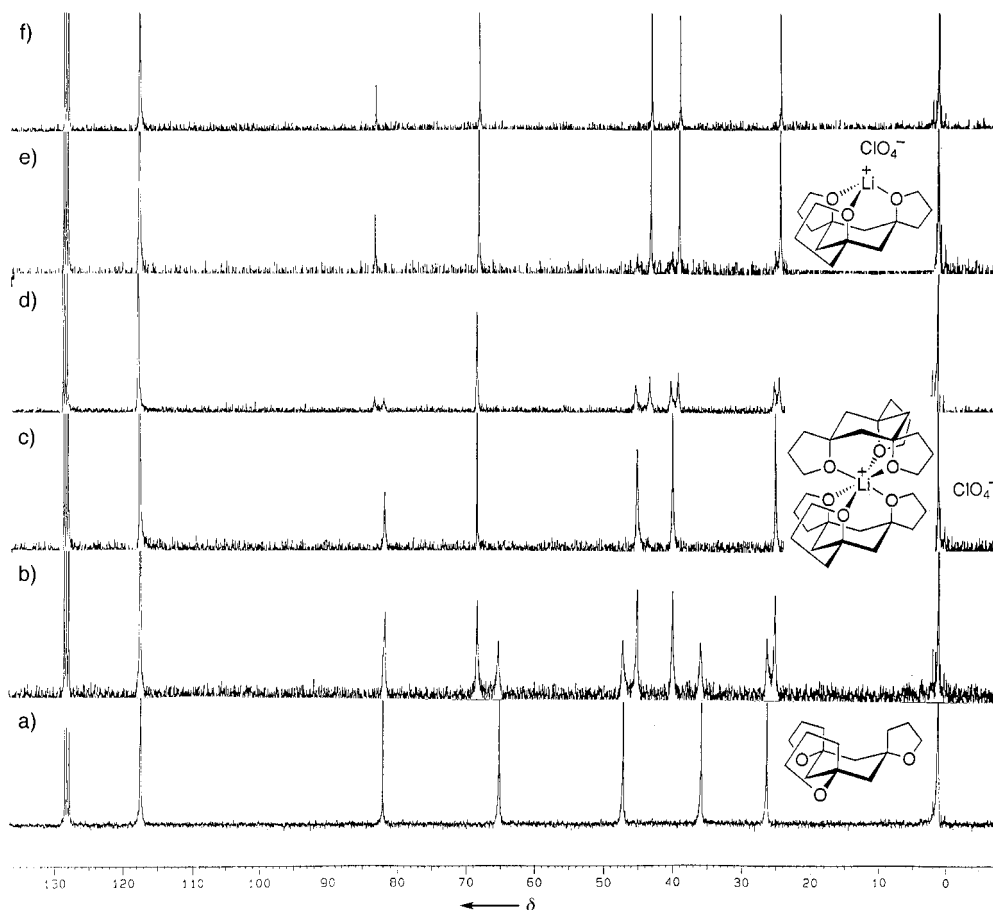


Figure 2. ^{13}C NMR spectra (75 MHz) for the incremental titration of **9** with LiClO_4 in $\text{CH}_3\text{CN}/\text{C}_6\text{D}_6$ (4/1). Equivalents of LiClO_4 added: a) 0.00, b) 0.25, c) 0.50, d) 0.75, e) 1.00, f) 1.50.

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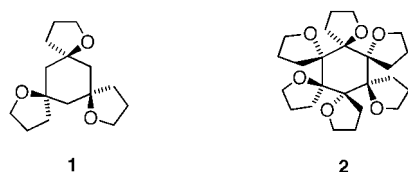
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- [11] Crystal data for **9**: $\text{C}_{15}\text{H}_{24}\text{O}_3$, $M_r = 252.34$, monoclinic, space group $P2_1/n$, $a = 5.9063(6)$, $b = 16.716(2)$, $c = 13.8555(14)$ Å, $\beta = 96.378(2)^\circ$, $V = 1359.5(2)$ Å³, $Z = 4$, $\rho_{\text{calcd}} = 1.233 \text{ Mg m}^{-3}$, $F(000) = 552$, $\mu(\text{Mo K}\alpha) = 0.084 \text{ mm}^{-1}$, $T = 173(2) \text{ K}$. A total of 8504 reflections were collected of which 3517 were unique ($R(\text{int}) = 0.056$) and 1623 were observed [$I > 2\sigma(I)$]. The refinement converged to $R1 = 0.092$, $wR2 = 0.214$, and $S = 1.045$. Crystallographic data (excluding structure factors) for this structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-111932. 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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Synthesis and Conformational Properties of the Sterically Crowded D_{3d} -Symmetric all-*trans* Hexa(spirotetrahydrofuran)cyclohexane System**

Leo A. Paquette,* Jinsung Tae, Bruce M. Branan, Shawn W. E. Eisenberg, and John E. Hofferberth

In the preceding paper,^[1] we introduced the belted trispiro ether **1** as a ligand capable of forming supramolecular complexes with alkali metal ions. This ionophore exhibits a distinct selectivity for Li^+ , which is well accommodated by the



conformer with oxygen atoms in the *syn*-axial positions. Very comparable spatial characteristics are resident in **2**, with the added feature that this functionalized molecular array in its O-axial conformation should be able to bind a metal ion on both of its electron-rich surfaces. Bifacial crown ethers are virtually unknown.^[2]

MM3-based calculations indicate the global energy minimum of this rather crowded molecule to be **2-eq**, with **2-ax** comparatively destabilized by $4.6 \text{ kcal mol}^{-1}$ (Figure 1 a). With the 1,3,5-oxygen triads present above and below the chairlike cyclohexane core in **2-ax**, the existing spatial characteristics are conducive to bifacial complexation. The possibility of gaining access to ladder polymers consisting of **2-ax** and alkali metal ions had given preliminary indication of being a realistic goal. The hypothetical $(\mathbf{2})_3 \cdot 4\text{Li}^+$ complex is shown as **3** in Figure 1 b.

On the basis of a significant number of experiments, it had become clear that the functionalized cyclohexanone **4**^[3, 4] enters into 1,2-addition reactions with organometallic reagents from the direction *syn* to the methylene carbon atoms. Equatorial attack on the all-equatorial oxygen conformation of **4** is therefore overwhelmingly favored kinetically^[5] (Scheme 1). As a consequence, the oxygen atom at this center must be introduced last. Therefore, tertiary carbinol **5b** was dehydrated and exposed to fluoride ion in order to generate homoallylic alcohol **6**. Following epoxidation of **6**, the readily separated oxirane **7** was oxidized to the aldehyde with tetra-*n*-propylammonium perruthenate^[6] and treated with triethylamine to effect β elimination. The conversion of **9** into diol **10** was accomplished by sequential hydrogenation and hydride

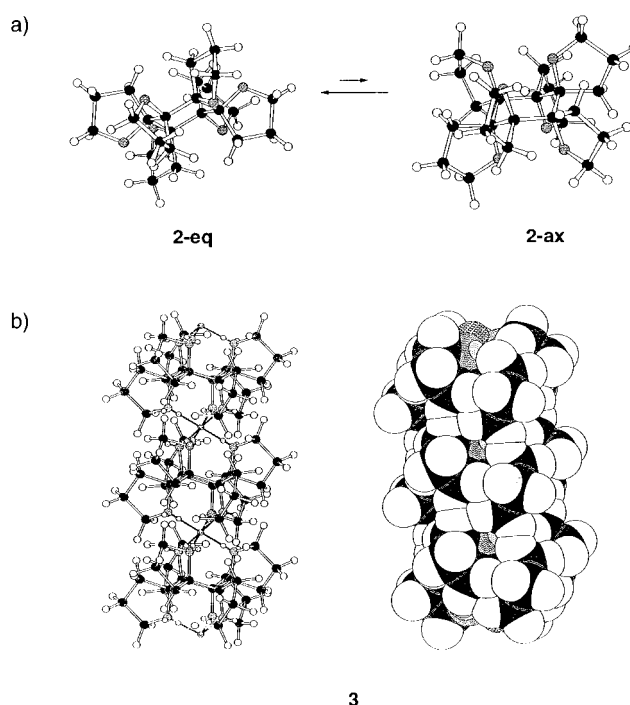
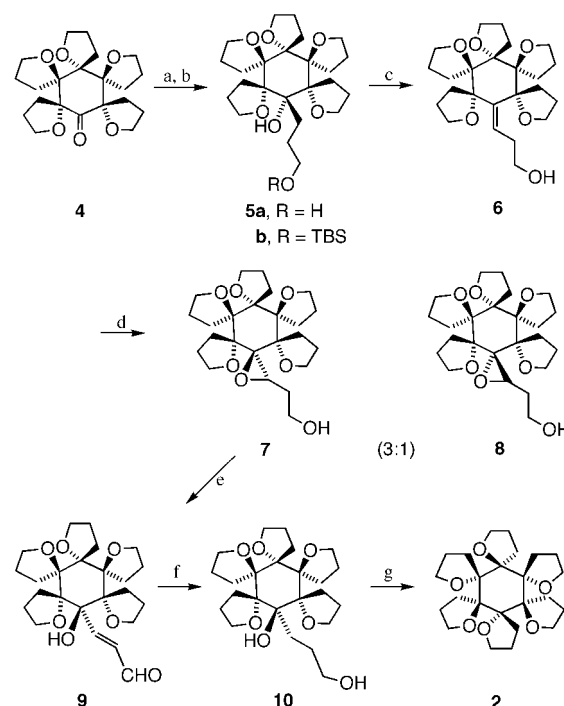


Figure 1. a) Ball-and-stick model of **2-eq** and **2-ax**, and their interconversion. b) Ball-and-stick (left) and space-filling models (right) of **3**, the hypothetical $(\mathbf{2})_3 \cdot 4\text{Li}^+$ complex.



Scheme 1. Synthesis of **2**: a) $\text{CClMg}(\text{CH}_2)_3\text{OMgCl}$, THF, -78°C , 10 min, 91%; b) TBSCl, Et_3N , DMAP, CH_2Cl_2 , 20°C , 18 h, 92%; c) SOCl_2 , pyridine, 0°C , 40 min; TBAF, THF, 20°C , 15 h, 90% over 2 steps; d) MCPBA, NaHCO_3 , CH_2Cl_2 , 20°C , 2 h, 97%; e) TPAP, NMO, $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN}$ (9:1), 20°C , 1 h, Et_3N , 30 min; f) 5% Pd/C, 1 atm of H_2 , $\text{C}_2\text{H}_5\text{OH}$, 10°C , 1 h; LiAlH_4 , THF, 20°C , 3 h; g) TsCl, Et_3N , DMAP, CH_2Cl_2 , 10 h at 20°C , then 13 h at reflux, 42% over 5 steps. DMAP = 4-dimethylaminopyridine, MCPBA = *meta*-chloroperoxybenzoic acid, NMO = *N*-methylmorpholine *N*-oxide, TBAF = tetrabutylammonium fluoride, TBSCl = *tert*-butyldimethylsilyl chloride, TPAP = tetrapropylammonium perruthenate, TsCl = *para*-toluenesulfonyl chloride.

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reduction. Transient formation of the primary monotosylate was followed by in situ cyclization^[7] to deliver **2** as a colorless, crystalline solid (m.p. 249–251 °C, decomp.). X-Ray structural analysis of **2** confirmed its adoption of a somewhat flattened cyclohexane core and the equatorial disposition of all six C–O bonds, much as in the MM3-derived **2-eq** model. Figure 2

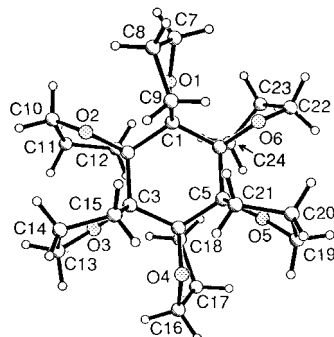


Figure 2. Molecular structure of **2** in the solid state. Selected bond lengths [Å] and angles [°]: O1–C1 1.455(2), O2–C2 1.447(2), O3–C3 1.446(3), O4–C4 1.449(2), O5–C5 1.446(2), O6–C6 1.448(3), C1–C2 1.571(3), O1–C7 1.431(3), C1–O1–C7 110.41(15), O1–C7–C8 104.85(8), C7–C8–C9 100.78(18), C8–C9–C1 105.53(7), C9–C1–C2 113.55(17), C6–C1–C2 112.51 (16).

shows a top view of the polyether which allows visual inspection of the conformations of the pendant heterocyclic rings.^[8] The greatly simplified ¹H and ¹³C NMR spectra (Table 1) testify to the inherently high symmetry of the compound.

Compound **2** is a fully substituted cyclohexane and is therefore a member of a class of compounds renown for their anomalous topological properties and unusually high barriers to conformational isomerization.^[9] With respect to **2**, the barriers for the chair-to-chair interconversions for **11**–**13**^[9, 10] seemed most meaningful. These data suggest that the **2-ax** ⇌ **2-eq** equilibrium should be characterized by a barrier

Table 1. Selected spectroscopic data for compounds **2**, **4**, **5a**, and **7**.^[a]

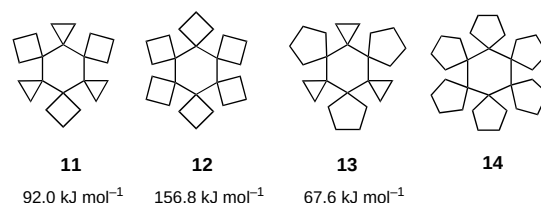
2: ¹H NMR (C₆D₆): δ = 3.58 (t, *J* = 6.7 Hz, 12H), 2.49 (t, *J* = 7.2 Hz, 12H), 1.88 (quint., *J* = 6.9 Hz, 12H); ¹³C NMR (C₆D₆): δ = 92.5, 67.7, 30.4, 29.3; HR-MS: *m/z* calcd: 420.2512 [*M*⁺]; found: 420.2485.

4: IR (film): ν̄ = 1715 cm^{−1}; ¹H NMR (C₆D₆): δ = 3.99 (ddd, *J* = 7.8, 7.8, 4.7 Hz, 1H), 3.82 (ddd, *J* = 7.8, 7.8, 2.3 Hz, 1H), 3.78–3.51 (m, 7H), 3.50–3.45 (m, 1H), 3.25 (ddd, *J* = 12.5, 8.1, 6.7 Hz, 1H), 2.78–2.71 (m, 1H), 2.70–2.60 (m, 2H), 2.55–2.39 (m, 3H), 2.31–2.15 (m, 1H), 2.11–1.91 (m, 3H), 1.77–1.45 (m, 8H), 1.12 (ddd, *J* = 12.6, 8.6, 2.8 Hz, 1H); ¹³C NMR (C₆D₆): δ = 207.4, 94.9, 93.2, 92.2, 90.5, 88.8, 69.6, 69.3, 68.9, 68.1, 67.3, 32.5, 31.0, 30.7, 30.5, 29.3, 28.9, 28.2, 27.1, 26.4, 25.4; HR-MS: *m/z* calcd: 378.2042 [*M*⁺]; found: 378.2030.

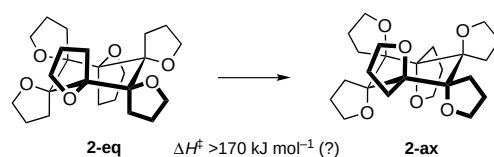
5a: ¹H NMR (C₆D₆): δ = 3.68–3.49 (m, 13H), 3.01–2.93 (m, 2H), 2.81–2.71 (m, 2H), 2.56–2.47 (m, 3H), 2.40–2.35 (t, *J* = 7.1 Hz, 2H), 1.95–1.82 (m, 8H), 1.80–1.63 (m, 4H), 1.60–1.48 (m, 4H); ¹³C NMR (C₆D₆): δ = 93.6, 92.4 (2 C), 81.6, 68.0, 67.4, 67.3, 64.0, 31.5, 30.7, 30.6, 30.5, 29.3, 28.2 (1 C unresolved); HR-MS: *m/z* calcd: 438.2618 [*M*⁺]; found: 438.2614.

7: ¹H NMR (C₆D₆): δ = 4.28 (s, 1H), 3.96 (dd, *J* = 9.0, 2.7 Hz, 1H), 3.73–3.46 (m, 12H), 2.69–2.52 (m, 2H), 2.50–2.35 (m, 6H), 2.14–1.90 (m, 10H), 1.89–1.73 (m, 4H); ¹³C NMR (C₆D₆): δ = 92.12, 92.08, 91.1, 89.3, 88.3, 68.9, 68.8, 67.9, 67.5, 67.4, 63.4, 62.3, 33.5, 32.8, 31.8, 30.6, 30.5, 30.4, 30.3, 29.4, 28.93, 28.86, 27.9, 27.4; HR-MS: *m/z* calcd: 436.2461 [*M*⁺]; found: 438.2494.

[a] NMR spectra recorded at 300 (¹H) and 75 MHz (¹³C). Correct elemental analyses have been obtained for most compounds.



significantly higher than that exhibited by **13** because of the constancy of the spiro substitution and the five-membered nature of its spirocyclic rings. In view of the fact that in going from **11** to **12** the barrier to inversion increases very significantly, the same should be true when proceeding from **13** to **14**, as a model for **2**. MM3 calculations on **12** and **14**^[11] clearly point in this direction. Consequently, it was by no means certain that an inversion of **2-eq** to give **2-ax** (Scheme 2) as a prerequisite for bifacial complexation would take place.



Scheme 2. Inversion of **2-eq** to give **2-ax**.

Indeed, a variety of measurements revealed that **2-eq** exhibits no measurable capability for complexing lithium ions, even under forcing conditions (LiBF₄ in benzene/acetonitrile (1/1) at 175 °C in a sealed reaction vessel). Consequently, dynamic NMR studies were undertaken to check the possibility for high-temperature equilibration with **2-ax**. Strikingly, however, the ¹H (500 MHz) and ¹³C NMR spectra (125 MHz) of **2-eq** remained unchanged upon heating up to 300 °C in a sealed NMR tube. Whether this is due to an unprecedented high barrier to the inversion of **2-eq** or whether inversion takes place but remains undetected because of an insufficient population of **2-ax** at the temperatures employed remains an open question. Until [6.5]- and [6.6]rotane are prepared, it is highly probable that **2-eq** has the highest inversion barrier recognized to the present time. The steric overcrowding in **2** has been shown to be antithetical to bifacial complexation.

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A New Approach to Glycopeptides**

Klaus Burger,* Michael Kluge, Susanna Fehn,
Beate Koks, Lothar Hennig, and Gerhard Müller*

The importance of carbohydrates for a living organism goes far beyond the function of serving as an energy reservoir.^[1] Carbohydrates are elementary building blocks of ribonucleic acids and glycoconjugates such as glycoproteins, -lipids, and -phospholipids,^[2] and in most cases the carbohydrate portion of a given molecule is the carrier of the biological information.^[3] As a component of the cell membrane, glycoconjugates fulfil important functions^[2-4] during cell-cell recognition, cell-matrix interaction, and cell growth regulation, and thus also in the development of tumors.^[5] Furthermore, they play a significant role in the interaction with biologically active factors such as enzymes, hormones, bacteriotoxins, and viruses.^[1, 4]

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The substructure of a carbohydrate, which undergoes permanent change^[6] during the life cycle of a cell, influences the biological properties of peptides and proteins.^[1] In general, it increases their proteolytic stability,^[7] improves their solubility and properties for transmembrane transport, and decreases their excretion rate^[8] thus enhancing their bioavailability. Since glycosylations can cause restrictions of the conformational flexibility of peptides, they play a significant role in peptide folding processes.^[9]

In many cases, the glycosylation of naturally occurring and non-natural peptides leads to a change in their activity profile. For instance, the analgesic activity of enkephalines^[10] could be increased by glycosylation which is attributed, among others, to an improved passage of the glycosylated form across the blood/brain barrier.^[11]

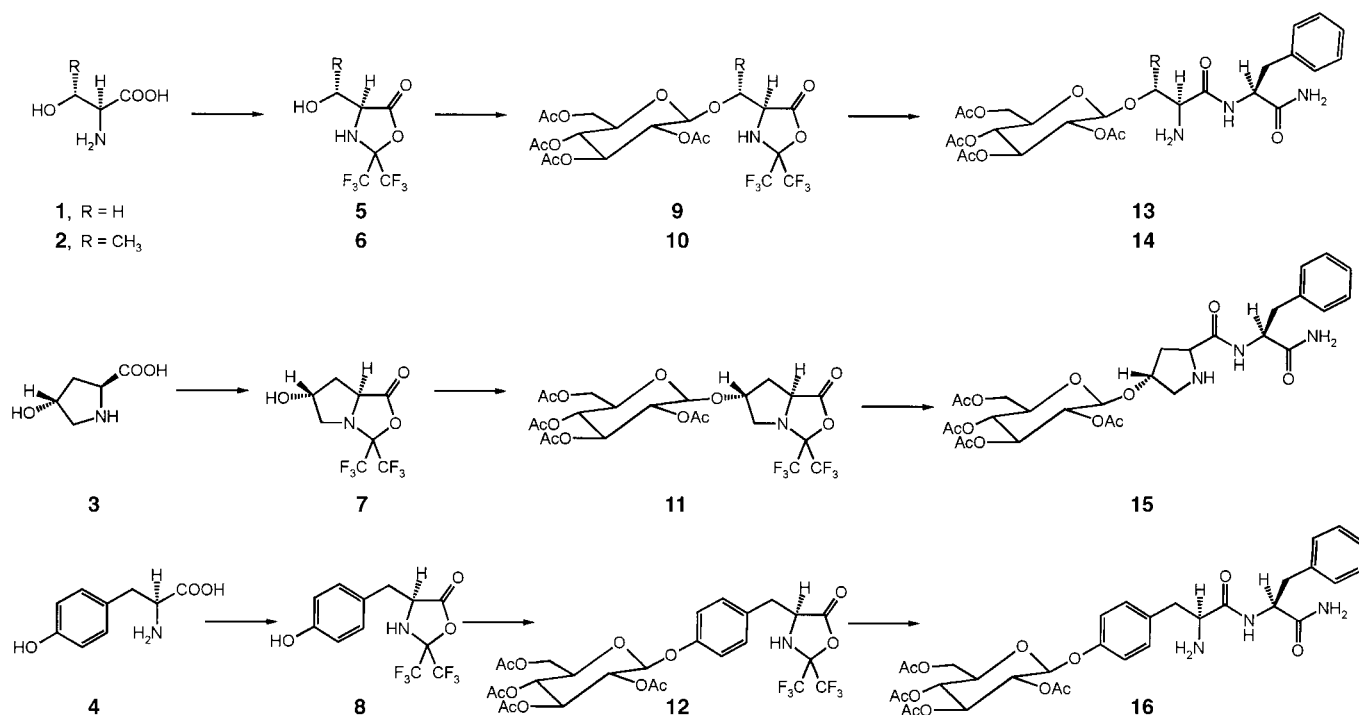
The convergent synthesis of *O*-glycosides by coupling reactions at the carbohydrate/peptide link is problematic because of the generally poor solubility of peptides under the glycosylation conditions and also because of regio- and stereochemical aspects.^[9a, 12] Therefore, the method of choice^[12] is an alternative synthetic strategy that involves the stepwise construction starting from *O*-glycosylated amino acid derivatives as well as small *O*-glycosylated peptide fragments, especially since further coupling reactions can be carried out enzymatically and in solid phase reactions.^[14]

The conventional synthesis of glycosyl amino acids and peptides requires the orthogonal protection of the α-amino and the α-carboxylic functionalities. After glycosylation of the free hydroxy group, the carboxylic acid group is deprotected, activated, and the C-terminus is coupled with an amino ester.

The new protecting group/activation strategy described herein offers significant advantages: The introduction and cleavage of the protecting group occurs under mild conditions. This resolves the issue of the acid lability of the *O*-glycoside bond as well as the tendency of the *O*-glycosylated serine and threonine derivatives to undergo β-elimination in the presence of strong bases. As a result, the new strategy offers the possibility of a shorter reaction sequence. Moreover, the reaction can be monitored easily and quickly by ¹⁹F NMR spectroscopy without any material loss.

Multifunctional α-amino acids such as serine, threonine, 4-hydroxyproline, and tyrosine react with hexafluoroacetone in very good yields to form 2,2-bis(trifluoromethyl)-1,3-oxazolidin-5-ones. The regioselective heterocyclization process in the case of serine and threonine not only allows the protection of the α-amino and the α-carboxylic groups in one step, but at the same time an additional activation of the carboxylic group. Since an excess of hexafluoroacetone is normally used, the hydroxy groups of serine, threonine, and 4-hydroxyproline in the crude product are partially present as hemiketals. The hexafluoroacetone can be cleaved by stirring a solution of the respective hemiketal in dichloromethane in the presence of silica gel at room temperature; the progress of the cleavage reaction can be monitored easily by ¹⁹F NMR spectroscopy. The 2,2-bis(trifluoromethyl)-1,3-oxazolidin-5-ones obtained in this manner can be stored for several months when kept refrigerated in a moisture-free environment.^[15]

The HFA-protected amino acids **5–8** (HFA = hexafluoroacetone) were allowed to react with 2,3,4,6-tetra-*O*-acetyl-α-



D-glucopyranosyl bromide/Hg(CN)₂ according to Helferich,^[16] with penta-*O*-acetyl-β-D-glucopyranose/BF₃ according to Paulsen,^[17] and with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl trichloroacetimidate according to Schmidt.^[18] The good yields of β-D-Glc-(HFA)-Xaa (Table 1) indicate that the new

Table 1. Glycosylation of HFA-protected amino acids according to various methods.

Compound	Method ^[a] /Yield [%]			m.p. [°C]
	A	B	C	
HFA-Ser(Glc)	57	61	78	173–174
HFA-Thr(Glc)	86	70	82	111–112
HFA-Hyp(Glc)	21	40 ^[b]	27	106–107
HFA-Tyr(Glc)	–	83	93	105–106

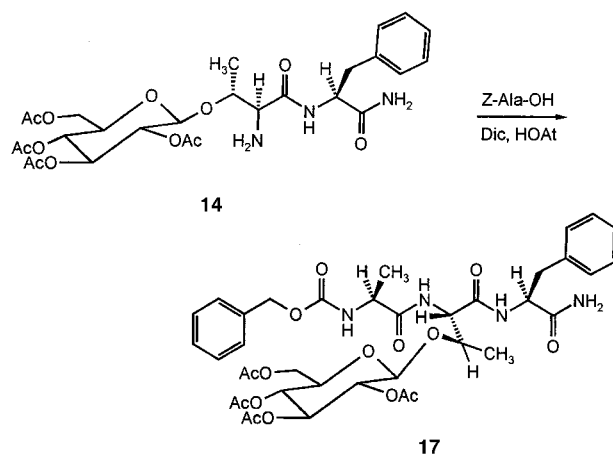
[a] Method A: Helferich variant of the Koenigs-Knorr reaction; method B: Procedure according to Paulsen; Method C: Procedure according to Schmidt. [b] Yield determined by ¹⁹F NMR spectroscopy.

protecting group strategy can be used successfully for all three glycosylation procedures. Compared to the glycosylation of HFA-Ser **5** and HFA-Thr **6**, the yield of HFA-Hyp (**7**→**11**) was surprisingly low (27%) even with the imidate method. The main product in this case (55%) was 4-Ac-(HFA)-Hyp which indicates that an orthoester is formed as an intermediate.^[12]

To date, only a small number of glycopeptides containing glycosyl-Tyr groups have been isolated from natural sources.^[12] β-D-Glycosyl-*O*-tyrosine itself was found as a tyrosine metabolite in *Lepidoptera*.^[19] The moderate yields (ca. 40%) in the glycosylation of 9-Fmoc-Tyr-OPfp (9-fluorenylmethoxycarbonyl-tyrosine-*O*-pentafluorophenyl) have been attributed the low nucleophilic character of the phenolic hydroxy group.^[20] The glycosylation of HFA-Tyr (**8**→**12**) by the imidate method gives a yield of 93%.

The *O*-glycosylated oxazolidin-5-ones **9**–**12** can be used directly as activated esters in peptide syntheses. When the lactone ring is opened with amino acid amides, glycosylated dipeptides are formed as solids in very good yields. With the new strategy, Tyr(β-D-Glc)-Phe-NH₂ was obtained from Tyr in only three steps with an overall yield of 70%. The deprotection of the amino group is coupled to the formation of the amide bond so that an extension of the peptide chain at the N-terminus can occur without any additional steps.

The synthesis of the glycosylated tripeptide Z-Ala-Thr(TAcGlc)-Phe-NH₂ **17** (Z = benzyloxycarbonyl) starting from **14** can be achieved with diisopropylcarbodiimide(Dic)/7-aza-1-hydroxy-1*H*-benzotriazole (HOAt) in 87% yield.



The protecting group/activation concept presented herein allows the construction of glycosylated dipeptide and tripeptide fragments starting from hydroxy amino acids in only three or four synthetic steps, and can therefore be considered superior to the pentafluorophenyl ester method.^[21]

Experimental Section

The experimental data of the threonine derivatives **10**, **14**, and **17** only are presented as examples. The NMR data have been assigned from ^1H , ^{13}C , APT, H,H-COSY, and HMQC spectra.

10: HFA-Thr **6**,^[15] (2 mmol, 534 mg), 2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl trichloroacetimidate (2 mmol, 984 mg) and $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (1 mmol, 142 mg, 125 μL) were stirred at room temperature in anhydrous CH_2Cl_2 (20 mL) for 1 h. H_2O (10 mL) and CH_2Cl_2 (20 mL) were added, and the resulting mixture was stirred vigorously for an additional 5 min. The organic layer was separated, dried (MgSO_4), and the solvent was removed in vacuo. The residue was purified by flash chromatography on silica gel (toluene/ethyl acetate, 5/1). Yield: 1 g (68 %), amorphous solid; m.p. 112–113 °C; IR (KBr): $\tilde{\nu}$ = 1830, 1750 cm^{-1} ; MS (70 eV): m/z : 597.42 [M^+]; ^1H NMR (CDCl_3): δ = 1.38 (d, J = 6.5 Hz, 3H), 2.01 (s, 3H), 2.02 (s, 3H), 2.06 (s, 3H), 2.07 (s, 3H), 3.66–3.76 (m, 3H), 4.06–4.13 (m, 2H), 4.22 (dd, J = 12.5 Hz, 4.2 Hz, 1H), 4.57 (d, J = 8.0 Hz, 1H), 4.95 (dd, J = 9.6 Hz, 8.0 Hz, 1H), 5.09 (dd, J = 9.7, 9.7 Hz, 1H), 5.20 (dd, J = 9.7, 9.6 Hz, 1H); ^{13}C NMR (CDCl_3): δ = 18.1, 20.4, 20.5, 20.8, 59.9, 61.3, 68.0, 71.2, 72.2, 72.6, 75.7, 89.3 (qq, J = 34, 34 Hz), 100.2, 120.2 (q, J = 286 Hz), 121.1 (q, J = 287 Hz), 169.2, 169.3, 169.4, 170.2, 170.7; ^{19}F NMR (CDCl_3): δ = –0.94 (q, J = 8.6 Hz, 3F), –2.89 (q, J = 8.6 Hz, 3F).

14: HFA-Thr(TacGlc) **10** (1 mmol, 597 mg) and L-phenylalanine amide (1 mmol, 164 mg) were stirred at room temperature in anhydrous ethyl acetate (20 mL) for 3 d (reaction control by ^{19}F NMR spectroscopy). The solvent was removed in vacuo and the residue was purified by flash chromatography on silica gel ($\text{CHCl}_3/\text{MeOH}$, 8/1). Yield: 403 mg (68 %), amorphous solid; m.p. 75–78 °C; M_{calcd} = 595.56, MS (MALDI-TOF): m/z : 618.19 [M^+ +Na], 635.25 [M^+ +K]; ^1H NMR (CD_3OD): δ = 1.12 (d, J = 6.5 Hz, 3H), 1.96 (s, 3H), 1.97 (s, 3H), 1.99 (s, 3H), 2.02 (s, 3H), 3.00 (dd_{ABX}, J = 14, 7.6 Hz, 1H), 3.13 (dd_{ABX}, J = 14, 5.8 Hz, 1H), 3.28 (d, J = 6.5 Hz, 1H), 3.86 (m, 1H), 3.99 (dq, J = 5.0, 6.5 Hz, 1H), 4.09 (dd_{ABX}, J = 12.3, 4.8 Hz, 1H), 4.28 (dd_{ABX}, J = 12.3, 2.2 Hz, 1H), 4.61 (dd, J = 6.0, 8.0 Hz, 1H), 4.73 (d, J = 8.0 Hz, 1H), 4.84 (dd, J = 8.0, 9.6 Hz, 1H), 4.98 (dd, J = 9.6, 9.9 Hz, 1H), 5.26 (dd, J = 9.6, 9.6 Hz, 1H), 7.21–7.33 (m, 5H); ^{13}C NMR (CD_3OD): δ = 16.81, 20.52, 20.53, 20.63, 20.65, 38.93, 55.47, 60.25, 63.01, 69.80, 72.83, 72.95, 74.11, 78.91, 100.57, 127.94, 129.54, 130.55, 138.20, 171.17, 171.25, 171.59, 172.37, 174.19, 175.72.

17: HOAt (0.34 mmol, 46.8 mg) and DIC (0.34 mmol, 53.3 μL) were added subsequently to a solution of Z-Ala-OH (0.34 mmol, 76.7 mg) in CH_2Cl_2 while stirring at room temperature. A solution of *O*-(2,3,4,5-tetra-*O*-acetyl- β -D-glucopyranosyl)-L-threonyl-L-phenylalanine amide **14** (0.17 mmol, 100 mg) in DMF (1 mL) was then added to the reaction mixture. After 2 h, the reaction mixture was concentrated, the residue was dissolved in ethyl acetate and then washed three times each with citric acid, saturated NaHCO_3 solution, and water. The organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified by flash chromatography on silica gel (eluent: $\text{CHCl}_3/\text{CH}_3\text{OH}$, 10/1). Yield: 101 mg (73 %), crystalline solid; m.p. 190 °C, M_{calcd} = 800.81, MS (MALDI-TOF): m/z : 823.23 [M^+ +Na], 839.12 [M^+ +K]; ^1H NMR (CD_3OD): δ = 1.12 (d, J = 6.2 Hz, 3H), 1.30 (d, J = 7.2 Hz, 3H), 1.93 (s, 3H), 1.98 (s, 3H), 2.00 (s, 3H), 2.03 (s, 3H), 3.04 (dd_{ABX}, J = 14, 7.7 Hz, 1H), 3.17 (dd_{ABX}, J = 14, 5.5 Hz, 1H), 3.90 (m, 1H), 4.07 (dd_{ABX}, J = 12.3, 5.3 Hz, 1H), 4.14 (m, 1H), 4.16 (m, 1H), 4.28 (dd_{ABX}, J = 12.3, 5.3 Hz, 1H), 4.45 (d, J = 6.2 Hz, 1H), 4.60 (m, 1H), 4.77 (d, J = 8.0 Hz, 1H), 4.85 (dd, J = 9.6, 9.6 Hz, 1H), 5.01 (dd, J = 9.6, 9.6 Hz, 1H), 5.05/5.10 (dd, J = 12.5, 12.5 Hz, 2H), 5.27 (dd, J = 9.6, 9.6 Hz, 1H), 7.15–7.35 (m, 10H); ^{13}C NMR (CD_3OD): δ = 16.47, 17.82, 20.53, 20.55, 20.65, 20.70, 38.62, 52.28, 55.75, 58.58, 63.08, 67.81, 69.80, 72.83, 73.17, 74.06, 76.44, 100.84, 127.98, 128.89, 129.03, 129.50, 129.60, 130.49, 138.25, 138.27, 158.50, 171.26, 171.40, 171.58, 172.42, 175.49, 175.87, 176.86.

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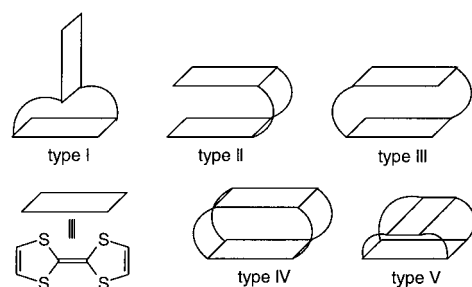
Keywords: glycopeptides • glycosylations

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Tetrathiafulvalene Belts with Large Cavities**

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The ability of the organic chemist to tailor host molecules with specific properties has had a tremendous impact on the development of supramolecular chemistry, which in turn has broadened the understanding of host–guest interactions.^[1] In this context, cyclophanes represent an important class of compounds.^[2] Owing to their rigid framework, primarily defined by the aromatic units, these molecules exist as conformers with very large cavities that are ready to accommodate charged or neutral guest molecules.^[3] The incorporation of redox-active components into these molecules would serve a dual purpose, namely, to increase the host–guest interaction with a complementary electroactive guest and to electrochemically signal the complexation event, making these molecules attractive components for sensor technology.^[4] The π -electron donor tetrathiafulvalene (TTF)^[5] is a good candidate as a building block for the preparation of electrochemically active macrocyclic systems with specific physical and chemical properties.^[6, 7] Bis-tetrathiafulvalenophanes (TTF-phanes) are of particular interest because the two TTF units can adopt a sandwich structure, which enables intermolecular interactions between the two redox units and increases the likelihood of strong noncovalent inter- and intramolecular interactions.^[7b] Double-bridged TTFs can exist as one of three stereoisomers (types I, II, or III; Scheme 1).^[8–10]



Scheme 1. Possible isomers of the dimeric TTF systems. Types I and III are depicted in the *trans* configuration only.

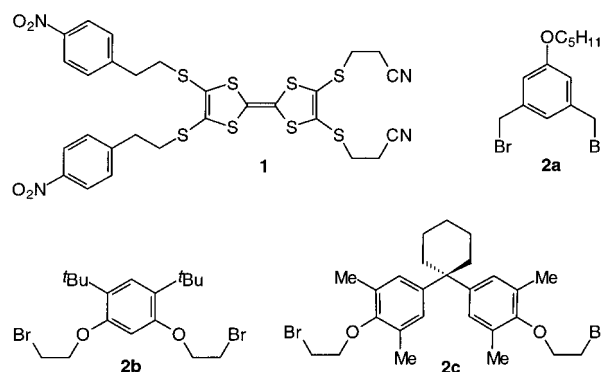
Two types of quadruple-bridged TTF-phanes can exist: the bis-TTF-belts,^[11] where the two TTF units are connected in a parallel fashion (type IV, Scheme 1), and the criss-cross overlaid TTF-phanes, where the two TTF units are orthogonal to each other (type V).^[12] Because of the restricted number of

available conformers, types IV and V present the best entry to TTF-phanes with large cavities.

The syntheses of nearly all the bis-TTFs known prior to 1995, including the only two known TTF-belts, are based on coupling of a bis-1,3-dithiole precursor^[7b] to give the TTF core in the final step of the synthesis (the preparation of one of the TTF-belts also involves an unusual metathesis-like dimerization step).^[11a] We have developed a new deprotection–realkylation protocol for the preparation of functionalized TTF derivatives,^[13] and this strategy has been used extensively by us^[14] and other groups^[15] for the incorporation of preformed TTFs into macrocyclic and supramolecular compounds.

Here we present a new stepwise synthesis of TTF-belts with well-defined large cavities. Our earlier attempts at preparing TTF-belts using a TTF precursor containing only one type of protecting group were unsuccessful, presumably because a sufficient level of selectivity could not be achieved in this system. However, the study of model systems indicated that the sequential stepwise functionalization of the TTF could be performed under complete control using a differentially protected TTF (in which the thiolates of the 2,3-positions are protected by a cyanoethyl group, whereas the thiolates of the 6,7-positions are protected by a 4-nitrophenylethyl group).^[16]

2,3-(2-Cyanoethylthio)-6,7-bis[2-(4-nitrophenyl)ethylthio]-tetrathiafulvalene (**1**), the key compound for the assembly of the TTF-belts, was prepared by standard cross-coupling.^[13c] The bis-alkylating reagents **2a–c**, chosen to confer rigidity as well as solubility to the belt molecule, were prepared in three steps from 5-hydroxyisophthalic acid, resorcinol, and 2,6-dimethylphenol, respectively.^[17]

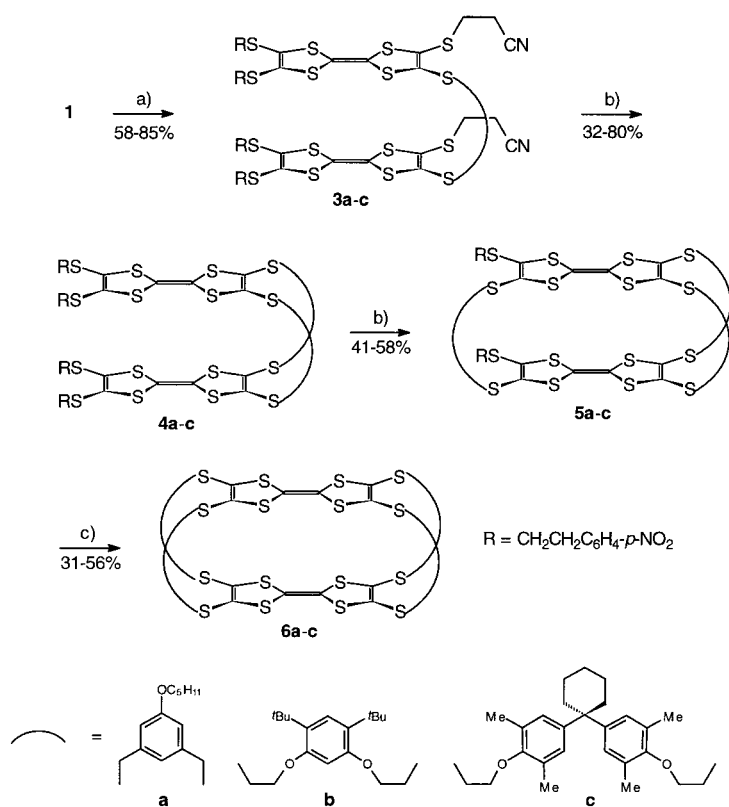


The TTF-belts **6a–c** were synthesized by a four-step strategy (Scheme 2).^[18, 19] Compound **1** was monodeprotected under standard conditions,^[13b,c] and the generated monothiolate was realkylated with the dibromides **2a–c** (0.5 equiv). The resulting bis-TTFs **3a–c** were purified by chromatography, isolated, and then treated with cesium hydroxide (2 equiv) in *N,N*-dimethylformamide to remove the two remaining cyanoethyl protecting groups. The resulting dithiolate was realkylated with **2a–c** (1 equiv) under high-dilution conditions. Purification by chromatography gave the macrocyclic bis-TTFs **4a–c** as the only isolable products. The structure of **4a–c** was confirmed on the basis of ¹H and ¹³C NMR spectroscopy, plasma desorption (PD) mass spectrometry, and elemental analysis. Next, the two TTF units of

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Scheme 2. Synthesis of the TTF-belts **6a–c**. a) 1. CsOH·H₂O (1.05 equiv), MeOH, DMF; 2. **2a–c** (0.5 equiv); b) 1. CsOH·H₂O (2.1 equiv), MeOH, DMF; 2. **2a–c** (1.0 equiv), DMF, high dilution; c) 1. CsOH·H₂O (3 equiv), MeOH, DMF; 2. **2a–c** (1.0 equiv), DMF, high dilution. All reactions were carried out under N₂ at room temperature.

4a–c were monodeprotected, and the resulting dithiolate (one thiolate group on each TTF) was realkylated with **2a–c** (1 equiv) under high-dilution conditions. The triple-bridged bis-TTFs **5a–c** were isolated as inseparable mixtures of two diastereoisomers differing in connectivity: In one isomer the TTFs are connected through the 6,6'-positions, whereas in the other they are connected through the 6,7'-positions.^[20] In the final step of the reaction, the remaining two protecting groups were cleaved with an excess of base (3 equiv). Realkylation with **2a–c** under high-dilution conditions gave **6a–c** in 32–56% yield after chromatography on silica gel.

The positive-ion PD mass spectra of **6a–c** revealed peaks at *m/z* 1418.6, 1762.9, and 2171.2 for the parent ions of **6a–c**, respectively; no oligomeric products were detected. NMR spectroscopy confirmed these observations.

The belt structure and large cavity of **6c** can be clearly seen in the X-ray crystal structure (Figure 1).^[21] The unit cell contains one centrosymmetric belt molecule, four chloroform molecules, and one methanol molecule (centrosymmetric on average, but disordered). Two of the chloroform molecules (C59–Cl1–Cl2–Cl3 and C59A–Cl1A–Cl2A–Cl3A) reside inside the spacious cavity of **6c**, while the other two (C60–Cl4–Cl5–Cl6 and C60A–Cl4A–Cl5A–Cl6A) are positioned at the rim of

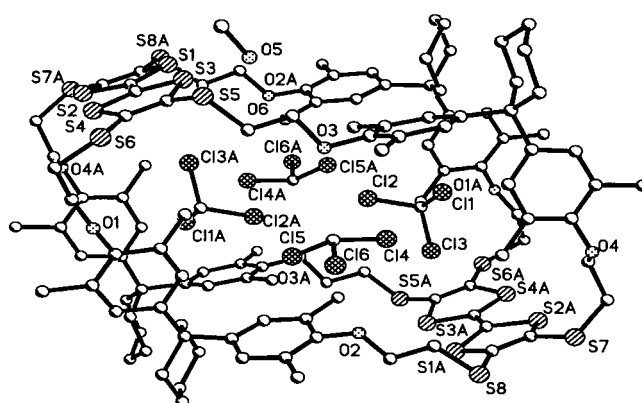


Figure 1. View of the molecular structure of **6c** including solvent molecules. For clarity the hydrogen atoms have been omitted, and atomic labels have been omitted for the carbon atoms.

the macrocycle. None of the chloroform molecules appears to be involved in any strong interactions with the TTF-belt itself. As seen in the packing diagram of **6c** (Figure 2), the packing is defined by intermolecular stacking between one TTF unit of one belt and the inversion-symmetric TTF unit in a neighboring belt molecule. The TTF units are parallel, but shifted somewhat sidewise. The distance between these planes is 3.36 Å, and there are two identical and somewhat short nonbonding S···S contacts of 3.624 Å between S2 and S3 (1 – *x*, – *y*, 1 – *z*) and between S3 and S2 (1 – *x*, – *y*, 1 – *z*).

Here we have described a new strategy for the preparation of belt-type bis-TTFs starting from a new protected TTF, and

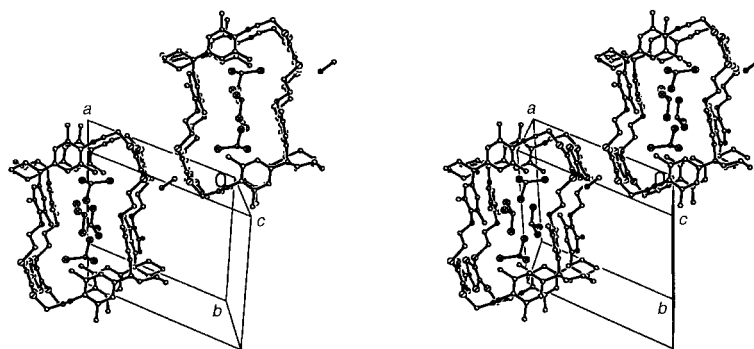


Figure 2. Stereodrawing of the packing diagram of **6c**.

demonstrated the synthetic utility of this method by synthesizing three TTF-belts. The TTF-belts **6a–c** are stable and isolable compounds that show some promise as host molecules, as indicated by the solid-state structure of **6c**, which crystallizes with two molecules of chloroform inside its spacious cavity. Preliminary complexation studies (¹H NMR) with 7,7,8,8-tetracyano-*p*-quinodimethane (TCNQ) and **6a** indicate that there is an interaction; when **6a** (1 equiv) was added to a solution of TCNQ in CDCl₃, the signal from TCNQ disappeared and the signals from the belt experienced a broadening. However, these results do not provide any

information regarding whether the guest (TCNQ) is complexed inside the cavity of the host molecule (**6a**) or if the interaction is on the outside of the TTF-belt; a solid-state structure determination of the complex is required.

Experimental Section

The synthesis of **6c** represents a general procedure for the deprotection–realkylation reactions.

6c: Compound **5c** (180 mg, 0.09 mmol) in dry DMF (23.0 mL) was degassed with N₂ for 15 min and then treated dropwise with a solution of CsOH·H₂O (46 mg, 0.27 mmol) in dry MeOH (2.0 mL) over a period of 30 min and left to stir for 30 min. This solution and a degassed solution of **2c** (48 mg, 0.09 mmol) in dry DMF (25.0 mL) were added simultaneously to 30 mL of dry degassed DMF under N₂ in a 250-mL three-necked flask under high-dilution conditions (3.0 mL h^{−1}) by means of a perfusor pump (Perfuser, Secura, Braun AG). After complete addition the reaction mixture was stirred for 4 h, the solvent was removed in vacuo, and the resulting orange solid was subjected to column chromatography (silica gel, CH₂Cl₂/cyclohexane 3/1, R_f = 0.5) to give **6c** as a pale orange crystalline powder upon removal of solvent. Recrystallization from CHCl₃/MeOH gave **6c** as orange crystals (0.060 g, 32%). M.p. > 250 °C (decomp.); ¹H NMR (250 MHz, CDCl₃): δ = 6.83–6.80 (2 × s, 12H; ArH), 4.40–3.70 (m, 12H, OCH₂CH₂S), 3.30–2.95 (m, 12H, OCH₂CH₂S), 2.17 (brs, 24H, CH₃), (brs, 16H, CH₂CCCH₂), 1.43 (brs, 24H, CH₂CH₂CH₂); PD-MS: m/z: 1085 [M⁺], 2172 [M⁺]; Elemental analysis calcd for C₁₁₆H₁₃₆O₈S₁₆·3CHCl₃ (2171.3): C 55.50, H 5.54; found: C 55.90, H 5.42; CV (in CH₂Cl₂, vs. Ag/AgCl): E_{1/2} = 0.58, 0.91 V.

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- [16] The 2-cyanoethyl group is cleaved in preference to the 2-(4-nitrophenyl)ethyl group upon exposure to cesium hydroxide. Therefore a stepwise cleavage can be performed by slow addition of the base in four subsequent steps.
- [17] Compound **1** was prepared by standard cross-coupling methodology^[13] by heating a 1:1 mixture of 4,5-bis(2-cyanoethylthio)-1,3-dithiole-2-one and 4,5-bis[2-(4-nitrophenyl)ethylthio]-1,3-dithiole-2-thione in freshly distilled triethylphosphite. Experimental details of the synthesis of **1** and **2a–c** will be reported elsewhere.
- [18] Compounds **3–6** were purified by chromatography on silica gel to give analytically pure products upon evaporation of solvent. All new compounds gave satisfactory spectroscopic data (¹H and ¹³C NMR), spectrometric data (PD-MS), cyclic voltammetries, and elemental analyses.
- [19] The intermediate compounds **3–5** as well as the final TTF-belts **6** can be manipulated in air without any precautions; this is in contrast to the case for a number of other bis-TTF sandwiches. This is probably a consequence of the rigidity of the belts, which prevents intramolecular interactions between the two redox-active units, which is known to lower the redox potential.
- [20] The latter isomer is chiral, but no attempt to separate the two enantiomers was carried out.
- [21] Deep orange crystals, suitable for X-ray crystallography, were prepared by allowing a hot solution of **6c** in CHCl₃/MeOH to cool slowly to room temperature in a Dewar flask. Crystal data for of **6c**·4CHCl₃·CH₃OH (C₁₂₁H₁₄₄Cl₁₂O₉S₁₆): M_r = 2680.72, triclinic, space group P $\bar{1}$, a = 16.002(3), b = 16.203(3), c = 16.415(3) Å, α = 114.68(3), β = 117.82(3), γ = 93.25(3)°, V = 3248.4(11) Å³, Z = 1, ρ_{calcd} = 1.370 g cm^{−3}, F(000) = 1402, graphite-monochromated MoK α radiation, λ = 0.71073 Å, T = 120(2) K; crystal size: 0.30 × 0.13 × 0.10 mm. μ = 0.567 mm^{−1}, min./max. transmission: 0.85/0.95. The intensities of 26379 reflections were measured on a Siemens SMART CCD diffractometer covering 99.7% of a complete hemisphere with θ_{max} = 23.26°; R_{int} = 0.0664. Structure solution and refinement and production of the crystallographic illustrations was carried out with the Siemens SHELXTL package (G. M. Sheldrick, *SHELXTL Users Manual*, Version 5.0, Siemens Analytical X-ray Instruments Inc., Madison, WI, USA, **1994**) and SHELX-97 (G. M. Sheldrick, *SHELX-97*, Universität Göttingen, Germany, **1997**). The refinement of 706

parameters with all 9299 unique reflections converged at $R_1 = 0.0824$ (for $F_o > 4\sigma(F_o)$). Hydrogen atoms were included in calculated positions; max./min. residual electron density 1.06/−0.89 e Å^{−3}. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-115498. 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).

A Novel Layered Silicate with a Helical Morphology

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Some layered silicate materials such as kanemite and magadiite (a mineral clay) are formed from silica under hydrothermal conditions.^[1] Various silicate phases can be formed only by calcination.^[2] When small amounts of aluminum hydroxide and tetramethylammonium hydroxide (TMA-OH) are added to a silica source and treated under hydrothermal conditions, high-silica zeolites such as Nu-1^[3], Fu-1,^[4] (Na, TMA)-E,^[5] and sodalite^[6] are formed. Furthermore, it was reported recently that the TMA ion is a template for the formation of the zeolite precursor RUB-15,^[7, 8] in which hemihedral cavities constitute a layered structure; however, the precise role of the TMA ions is still obscure. We considered that comparing products obtained with and without TMA or an aluminum component in organic or aqueous solution under hydrothermal conditions might give insights into this subject, and also provide an opportunity to elucidate the difference between the transformation of amorphous silica into layered and microporous structures. In the course of our investigations, under aluminum-free conditions, we found a novel silicate phase, the scanning electron micrograph of which (Figure 1) showed a helical morphology consisting of stacked layers. This morphology differs strongly from those of traditional layered silicates such as kanemite and magadiite^[1, 2] and from those of more recently reported layered silicates.^[7, 8]

Table 1 lists the reaction conditions under which the helix layered silicate (HLS) was formed. As indicated in runs 1–3 and 7–9, besides silica, NaOH, TMA-OH, and 1,4-dioxane are essential for the formation of HLS, which is also favored

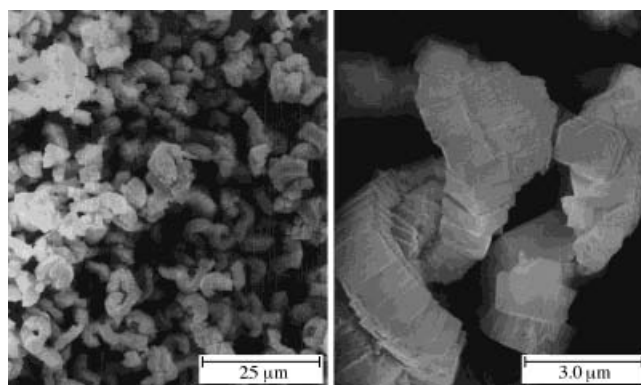


Figure 1. Two scanning electron micrographs of the novel silicate material obtained from run 1 (Table 1).

Table 1. Products obtained under different reaction conditions.^[a]

Run	Composition of the reaction mixture				Product ^[c]
	SiO ₂ [10 ^{−2} mol]	TMA-OH [10 ^{−3} mol]	NaOH [10 ^{−3} mol]	H ₂ O:1,4-dioxane ^[b]	
1	3.60	4.80	3.00	2.20	HLS
2	3.60	4.80	3.00	3.77	HLS
3	3.60	4.80	3.00	4.46	HLS
4	3.60	4.80	3.00	6.61	Amo
5	3.60	4.80	3.00	9.71	Amo
6	3.60	4.80	3.00	34.3	Amo
7	3.60	4.80	3.00	∞	Amo
8	3.60	0	3.00	2.45	Mag
9	3.60	4.80	0	34.3	Amo

[a] The reaction was carried out at 150 °C for 10 d. [b] In all runs, the total amount of 1,4-dioxane and H₂O was 18.0 g. [c] HLS = helix layered silicate, Amo = amorphous silica, Mag = magadiite. These phases were evaluated by means of XRD patterns.

by low H₂O:1,4-dioxane ratios. Under the conditions investigated here, TMA-OH did not transform silica into a layered silicate, whereas NaOH transformed silica into a magadiite phase. Consequently, it can be concluded that silica sol is transformed into the HLS by the cooperative action of both TMA-OH and NaOH. The elemental analysis of the HLS gave a TMA content of 13.2 wt %, consistent with the value obtained by thermogravimetric differential thermal analysis (TG-DTA), and the water content as determined by TG-DTA was 10.8 wt %. The silicon and sodium contents calculated from elemental analysis and energy dispersive analysis of X rays (EDAX) were 30.4 and 0.48 wt %, respectively. When a 0.1-g sample of the HLS was treated with 30 mL of 0.05 N HCl in aqueous solution, the EDAX peak of sodium disappeared completely, and the TMA content by elemental analysis decreased to 4.2 wt %. This indicates that both TMA and sodium ions in the HLS are easily replaced with protons.

We then investigated whether the HLS is a single phase or a mixture of different layered silicates. Figure 2 shows the ²⁹Si MAS NMR and ¹³C CP-MAS NMR spectra of the HLS. The ²⁹Si MAS NMR contains a strong signal at $\delta = -104.4$, which can be assigned to $-\text{Si}(\text{OSi})_3$ (Q³) units, and weak broad signals, probably due to unchanged amorphous silica. This Q³ connectivity is characteristic of layered silicates. The ¹³C CP-MAS NMR spectrum displays a strong signal for TMA at $\delta = 57.9$. When TMA is trapped in a confined space, its ¹³C NMR

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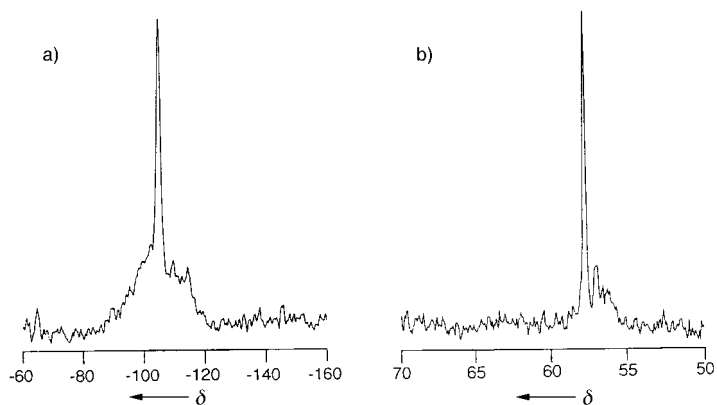


Figure 2. a) ^{29}Si MAS NMR and b) ^{13}C CP-MAS NMR spectra of the helix layered silicate shown in Figure 1.

signal of shifts from $\delta = 56.0$ (for free TMA) to 59.0 .^[9] In RUB-15^[7] TMA has a ^{13}C chemical shift of $\delta = 56.4$. Therefore, in the HLS prepared the TMA ions are located in a confined space, that is, between the anionic silicate sheets. There is no signal for 1,4-dioxane, and therefore it is not present in the HLS. This suggests that 1,4-dioxane may contribute to the formation of the sheet silicate, but is not a template for the helical layered structure. This is in contrast to silica sodalite, for which 1,3,5-trioxane and 1,3-dioxolane can act as templates.^[10]

Figure 3 shows the X-ray diffraction (XRD) pattern of the HLS after different reaction times. The diffraction pattern is characteristic of a layered structure and is the same for all four samples. However, the crystallinity increases with increasing

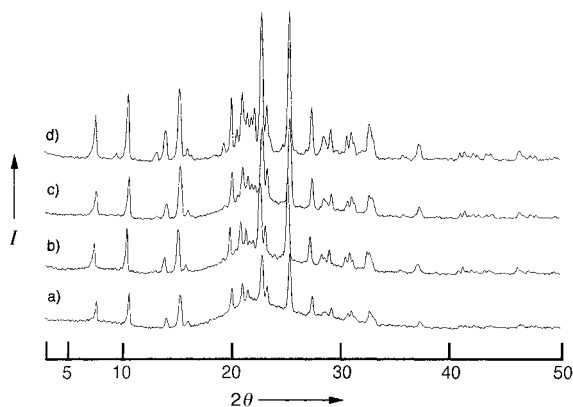


Figure 3. X-ray diffraction patterns of the helix layered silicate after a reaction time of a) 96, b) 119, c) 262, and d) 383 h. The reaction conditions are as for run 1 (Table 1).

reaction time, and the diffraction pattern differs from those of all silicate phases reported so far.^[2, 7, 8] When the HLS was heated to 200°C , the XRD pattern was changed drastically, but the original XRD pattern was completely restored when the product was left at room temperature in air (Figure 4). The HLS still contains TMA ions even at 200°C . A typical property of mineral clays is reversible adsorption and desorption of H_2O molecules between the layers,^[1, 11] and the restoration of the XRD pattern is due to the readsorption of H_2O molecules into the silicate layer structure. This reversible change in the XRD pattern suggests that the HLS

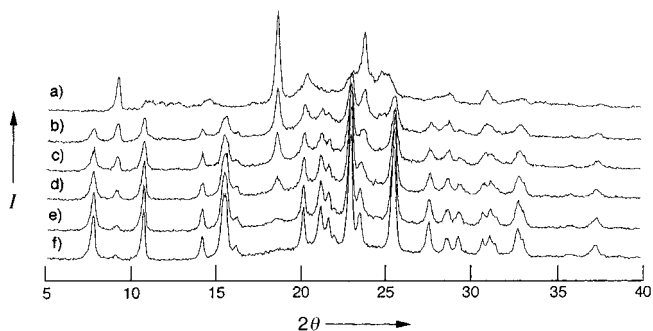


Figure 4. X-ray diffraction patterns of the HLS silicate a) 0, b) 20, c) 40, d) 60, e) 80, f) 100 min after calcination at 200°C . The sample from Figure 3c was used here.

is a single phase, because such a smooth and simple reversible transition is regarded as being difficult for a mixture of different layered structures. When the HLS was heated to above 300°C in air, the XRD pattern showed formation of an amorphous phase. At this temperature, the TMA ions were lost from the silicate (TG-DTA). Likewise, after removal of TMA and sodium ions by the treatment with 0.05N HCl , the HLS showed a similar change in the XRD pattern (drastic lowering of crystallinity). However, the morphology in the SEM images did not show so drastic a change as the XRD patterns.

The transformation of amorphous silica into layered crystalline helix silicate is believed to occur as follows: Firstly, NaOH plays a role in converting the silica sol into the layer structure (see run 8 in Table 1), and then TMA ions are incorporated and stabilize the layered structure. During formation of the the layered structure, 1,4-dioxane could induce a helical morphology, because a low H_2O :1,4-dioxane molar ratio or a high 1,4-dioxane concentration is an important factor for the formation of HLS. Since 1,4-dioxane molecules break hydrogen bonds between water molecules,^[12] they can also be expected to disrupt the hydration sphere around TMA ions. Hence, the hydration structure of TMA ions at low H_2O :1,4-dioxane ratios should be different from that of TMA ions in aqueous solution, and TMA ions in a solution with a low H_2O :1,4-dioxane ratio would interact with silicates in a different manner from those in aqueous solution and could induce the formation of HLS. This consideration may be supported by our finding that the helix silicate is also formed in H_2O /pyridine system at low H_2O :pyridine ratios.

Experimental Section

SiO_2 was synthesized by hydrolysis of tetraethyl orthosilicate (TEOS) at a $\text{TEOS}:\text{H}_2\text{O}:\text{ethanol}$ molar ratio of $0.3:20:1$ at 70°C for 24 h. The resulting gel was dried at 70°C under vacuum.

The novel silicate phase was obtained as follows: SiO_2 (3.60×10^{-2} mol), 1,4-dioxane (0.11 mol), TMA-OH (4.80×10^{-3} mol), NaOH (3.00×10^{-3} mol), and H_2O (0.37 mol) were mixed in a teflon-lined autoclave (Table 1, run 2). The mixture was heated to 150°C for 10 d. After the reaction was complete, the resulting powder was recovered by filtration, washed with acetone, and dried at 90°C for 1 d. When this hydrothermal synthesis was carried out at 170°C , the HLS was not obtained, and only amorphous silica powder was recovered.

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Light Harvesting and Energy Transfer in Novel Convergently Constructed Dendrimers**

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A characteristic of dendritic macromolecules is the presence of numerous peripheral chain ends that all surround a single core.^[1] If a long-range interaction could be introduced between these multiple chain ends and the focal point it would then be possible to influence the core of the macromolecule by events that initially occur with a higher probability on the dendrimer periphery.^[2] Following this rationale we report that it is possible to essentially create a light-harvesting antenna by functionalizing a dendrimer with suitable interacting chromophores.^[3] In these dye-labeled dendrimers light absorbed by the numerous peripheral chromophores is funneled to a central fluorescent core with a remarkably high efficiency and by a mechanism that is independent of the dendritic architecture. Hence, this new strategy is different from all the previous approaches to energy or electron transfer within

dendritic architectures,^[4] where the transfer was achieved by through-bond or “hopping” processes.

The array of terminal chromophores in the present macromolecules provides a large overall cross-section for collecting energy and plays the role of an antenna for light harvesting, while the focal dye (Figure 1) plays the role of a fluorescent

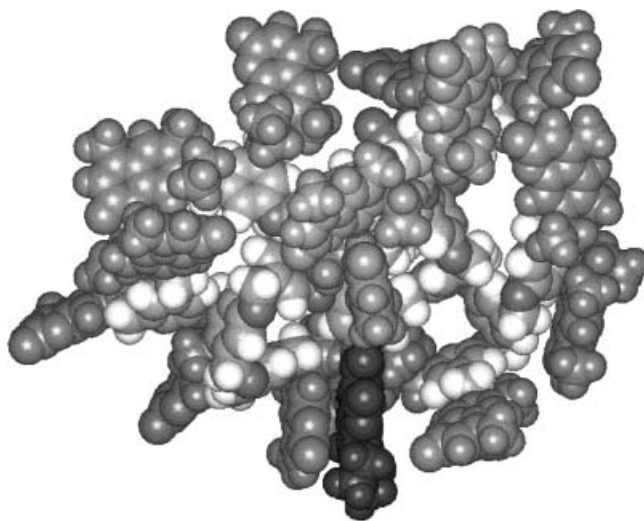


Figure 1. Molecular model (MM3) of the G-4 dendron showing peripheral donor chromophores surrounding the core acceptor (indicated in dark gray).

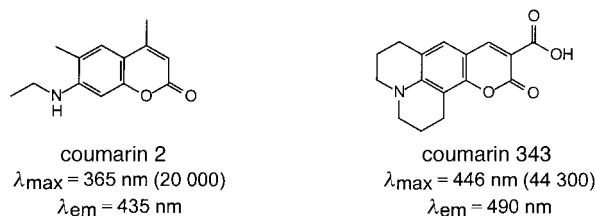
probe whose emission signals that energy is actually received at a single localized site. Furthermore, both the peripheral chromophores and the focal dye contribute to the absorption of the entire macromolecule. Since their individual absorptions each cover a different wavelength range, the absorption of the whole macromolecule is particularly broad and provides a larger spectral coverage than the focal dye alone. The energy transfer process converts this broad absorption into the narrow emission of the central laser dye. Therefore, such a dye-labeled dendrimer represents both a spatial and spectral light concentrator: the energy of any photon of any wavelength absorbed anywhere in this ensemble of chromophores is transferred to a single point and converted into a relatively narrow emission spectrum.

This scheme is indeed reminiscent of the primary event of photosynthesis, where solar energy absorbed anywhere in an array of hundreds of chlorophyll molecules is efficiently transferred to a single reaction center.^[5] The higher the dendrimer generation number, the higher the number of terminal groups that surround the core,^[1] and the larger the cross-section for energy collection. However, the average distance between the core and the peripheral groups also increases as the generation number $G-x$ ($x = 1-4$) increases. By considering the size of dendritic macromolecules,^[1] energy transfer from the periphery to the core would most likely take place through a dipole–dipole interaction. According to Förster theory^[6, 7] the efficiency of energy transfer decreases as the inverse sixth power of the interchromophoric distance. Clearly, it was necessary to determine whether this dipole–dipole interaction would be strong enough to achieve an efficient energy transfer, even at high generations.

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To realize this concept we reviewed carefully the properties of the chromophores currently available from commercial sources.^[8] We eventually chose the laser dye coumarin 2 as the donor terminal chromophore, and coumarin 343 as the acceptor focal dye (Scheme 1). A favorable intramolecular charge transfer takes place in the excited state of these



Scheme 1. Structures and spectral characteristics of the donor and acceptor dyes with extinction coefficients ($\text{L mol}^{-1} \text{cm}^{-1}$) given in parentheses.

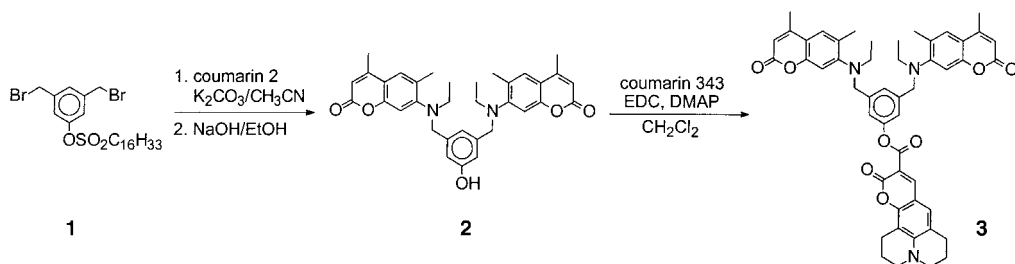
molecules, and accounts for their high extinction coefficients and high dipole moments,^[8] which are necessary for a strong dipole–dipole interaction. This pair of chromophores meets the basic requirement for energy transfer: the donor emission band strongly overlaps the acceptor absorption band (resonance condition). In addition, the emission of coumarin 2 displays a large Stokes shift, which lowers the probability of self-quenching between the numerous peripheral chromophores. Furthermore, because of their high fluorescence quantum yields and their solubility—a decisive advantage for building multichromophoric macromolecules—these coumarins are used extensively in dye lasers and are therefore commercially available in high purity (>99%).

Once the choice of chromophores was secured, a synthetic strategy had to be specifically developed to functionalize the chain ends of a poly(arylether) convergent dendritic backbone by the nucleophilic amino group of coumarin 2, and its focal point by the electrophilic acid functionality of coumarin 343. Since this reactivity polarity is reversed relative to the “classical” convergent synthesis of poly(arylether) dendrimers originally reported by Hawker and Fréchet,^[9] the distribution of reactive groups on the dendron, and hence the monomer, had to be inverted so that the same efficient coupling reactions could still be employed. Therefore, the AB_2 monomer used for the building of the “reversed” dendritic structure was a hexadecanesulfonate protected 3,5-bis(bromomethyl)phenol (**1**, Scheme 2),^[10] instead of the commonly employed 3,5-dihydroxybenzyl alcohol. The synthetic strategy for the preparation of the dye-labeled poly(arylether) dendrons^[11] of generation 1 to 4 began with the

nucleophilic attack of two coumarin 2 dyes on the benzyl bromide functionalities of the protected monomer. The resulting dye-labeled fragment was then deprotected with sodium hydroxide in refluxing absolute ethanol to yield the phenol **2**. This phenol could be coupled to coumarin 343 with *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide (EDC) and 4-dimethylaminopyridine (DMAP) in CH_2Cl_2 to produce the fully dye-labeled first generation dendron **3**. The G-1 phenol **2** was coupled to the AB_2 monomer by the convergent strategy of dendrimer synthesis^[9] to yield the second generation protected fragment, which now contained four coumarin 2 dyes. The deprotection and coupling steps were repeated to obtain the G-2 **4** and G-3 **5** dendrons functionalized with both coumarin 2 and coumarin 343 (Scheme 3). The fourth generation fragment was deprotected using sodium ethoxide in DMF. The coupling of the fourth generation phenol to coumarin 343 produced the fully dye-labeled G-4 macromolecule **6**. This synthetic strategy also gives access to model compounds that possess the same arylether backbone but lack either the acceptor dye at the focal point (**7**), or the donor chromophores at the periphery (**8**). The purity of all compounds was carefully monitored by NMR spectroscopy and by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) spectrometry (Figure 2), with special attention being paid to the complete functionalization of the dye-labeled dendrons.

The optical properties of each interactive component were investigated using the model compounds **7** and **8**.^[7] As expected the emission band of the donor moiety strongly overlaps the absorption band of the acceptor fragment, thus enabling a highly efficient energy transfer (Figure 3). Moreover, the high probability of heterotransfer over homotransfer can be visualized by comparing this large spectral overlap (gray) to the very weak overlap of the absorption and the emission bands of the donor moiety (black). Whereas the former represents energy transfer to the focal dye, the latter is associated with competitive self-quenching between the terminal chromophores.

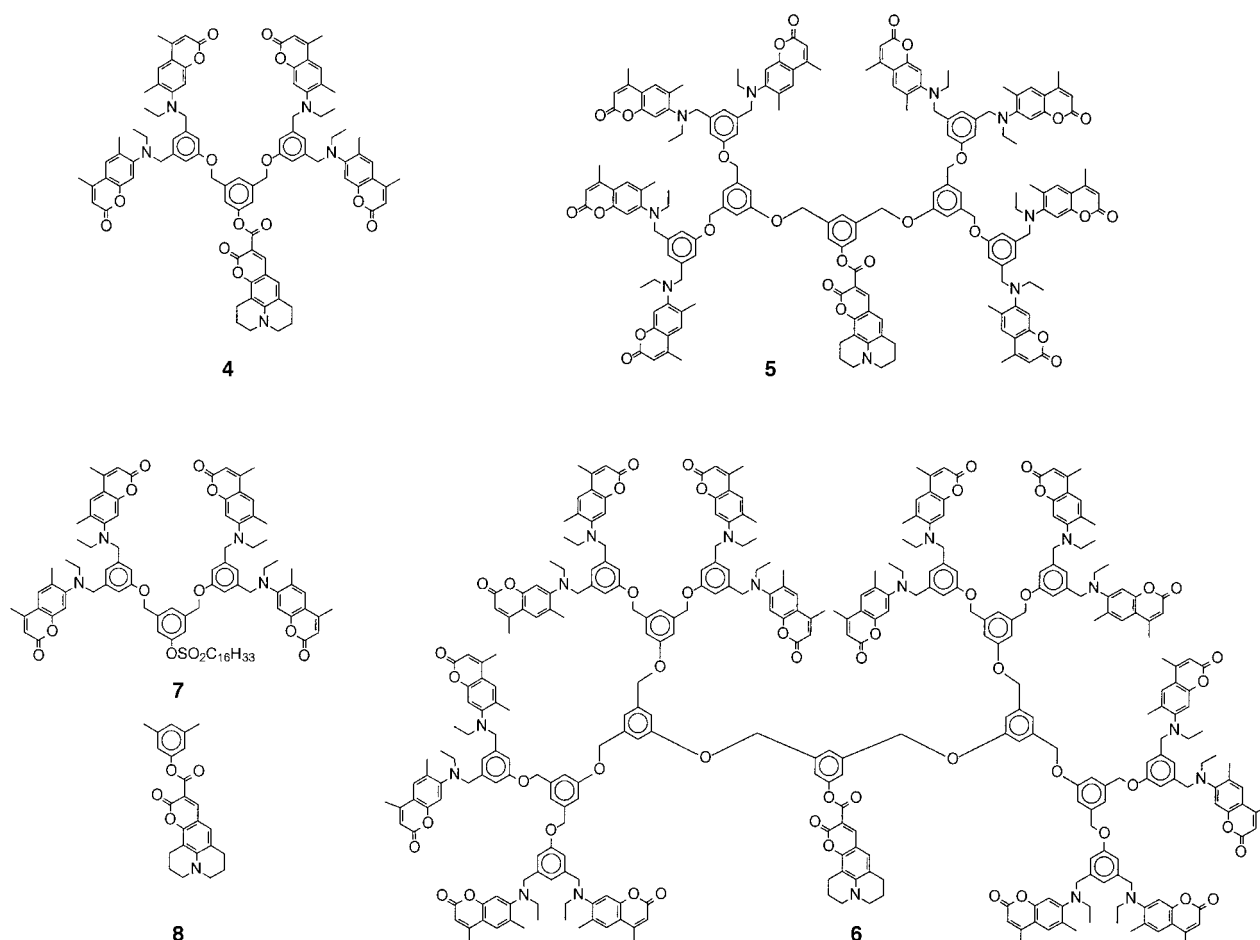
Figure 3 also shows that the absorption maximum of each donor or acceptor fragment corresponds exactly to a low minimum for the complementary moiety. It is therefore possible to selectively excite either the donor chromophores or the acceptor dye, which ensures the reliability of the energy transfer results and considerably simplifies analysis of the data. Most importantly, a transfer of energy through the dendritic backbone can be safely ruled out since the poly(arylether) structure itself does not absorb beyond 310 nm: indeed, there is no energy band gap within the internal



Scheme 2. Synthesis of the fully dye-labeled G-1 dendron.

dendritic framework that is small enough to match the energy of donor emission (418 nm) or the energy of the incoming photons under irradiation at wavelengths $\geq \lambda_{\text{max}}$ of the peripheral chromophores (343 nm).

The absorption of the whole macromolecule at each generation was found



Scheme 3. Structures of the G-2 **4**, G-3 **5**, and G-4 **6** dye-labeled dendrimers as well as the model donor **7** and model acceptor **8** compounds.

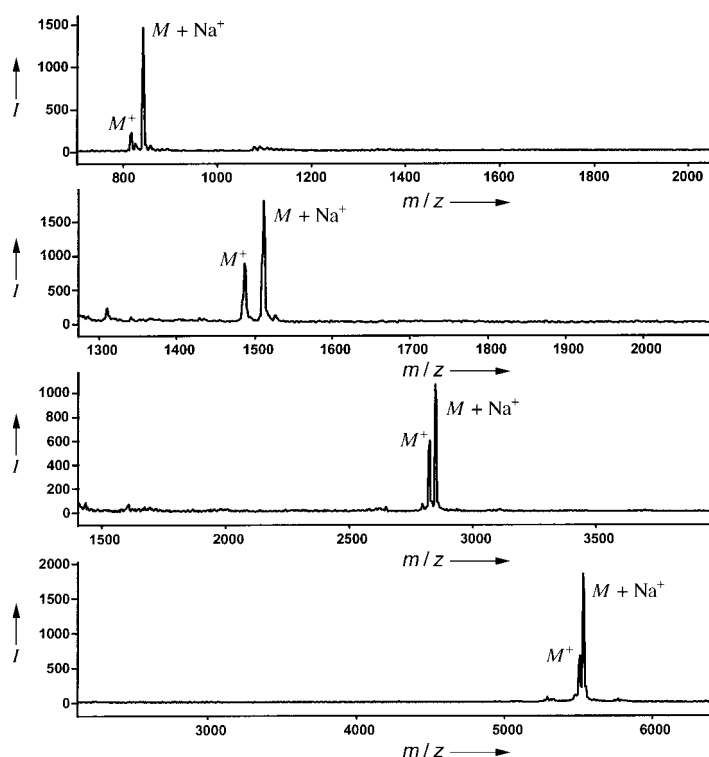


Figure 2. MALDI-TOF mass spectra of the G-1 to G-4 dye-labeled dendrimers **3–6** (from top to bottom, respectively).

to closely match the sum of the contributions of each of its individual fragments. This not only confirms the validity of the models, but also demonstrates that there is no interaction in the ground state between the two kinds of laser dye within the same dendron. Consequently, the absorption capacity of the dendrimer increases as a function of the number of terminal chromophores, and the light-harvesting antenna becomes more and more effective as the dendrimer generation increases.

The capacity to focus the energy absorbed by the peripheral antenna of the dendrimer to the central dye is demonstrated in Figure 4 for the G-3 dendron. Excitation of the terminal chromophores at 343 nm is followed by efficient energy transfer to the core and results mainly in the fluorescence at 470 nm (band b) of the focal dye alone. In contrast, excitation of the same peripheral chromophores in the G-3 model compound that lacks the acceptor focal dye results in their single emission at 418 nm (as illustrated in Figure 3 for the G-2 model donor compound). Therefore, the central coumarin functions as a “sink” or spatial energy concentrator that drains energy from the dendrimer periphery. The low intensity of fluorescence at the emission maximum λ_{em} of the peripheral dyes (Figure 4, band a) indicates that fluorescence quenching is remarkably efficient, whereas emission from the central chromophore (band b) demonstrates that the quenched energy is actually received on a localized site and

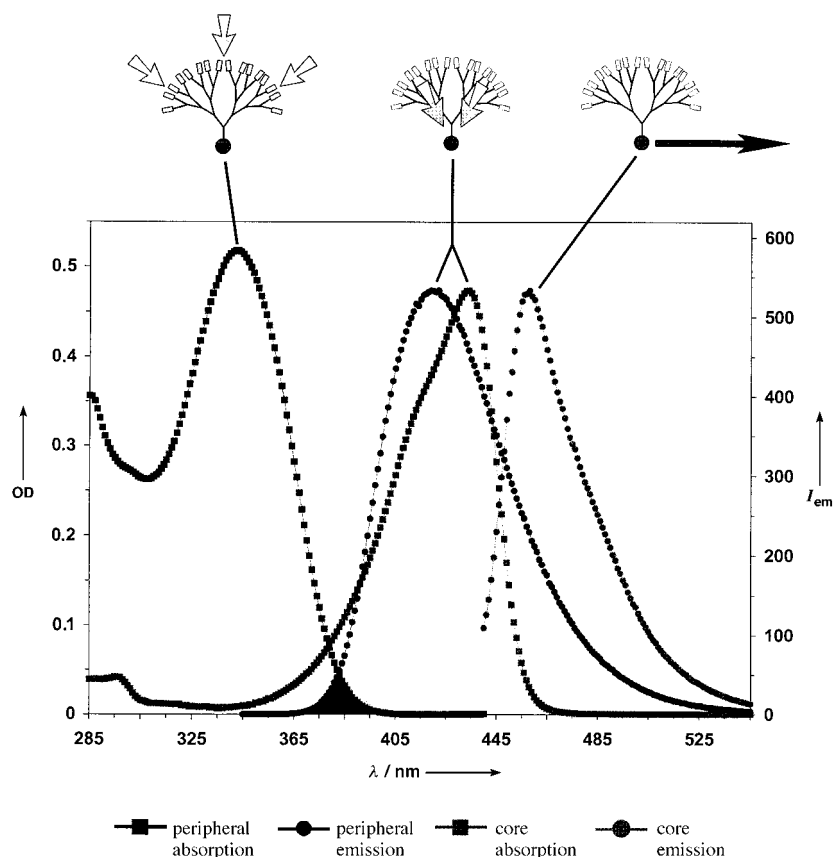


Figure 3. Absorption and emission spectra of the G-2 model donor and acceptor fragments in toluene. Spectral overlaps for heterotransfer and homotransfer are shaded in gray and black, respectively.

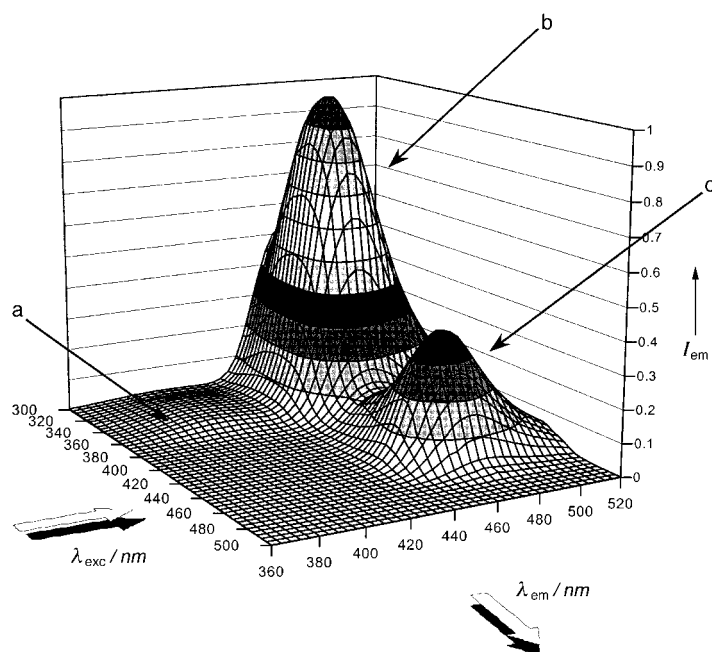


Figure 4. Total fluorescence of the G-3 dendron ($c = 1.17 \times 10^{-6} \text{ M}$). a) Spontaneous emission from the peripheral dyes; b) emission from the core dye after energy transfer from the peripheral chromophores; c) emission from the core dye upon direct excitation. All emission spectra have been corrected for slight variations in excitation intensity by dividing out the relative lamp output at each excitation wavelength.

used to excite the focal dye, instead of being dissipated nonradiatively.

The energy transfer efficiency for any given generation of the fully labeled dendrons can be determined in several ways.^[7a] One method involves comparing the absorption spectrum with the fluorescence excitation spectrum recorded at the λ_{em} of the focal chromophore. When these two spectra are normalized at the λ_{max} value of the central dye the decrease in intensity at the λ_{max} of the peripheral chromophores in the excitation spectrum relative to the absorption spectrum represents the energy lost in the transfer step.^[12] The similarity between the two normalized spectra for G-1 to G-3 is remarkable, and attests to a very high transfer efficiency (close to 100%). The efficiency starts to decrease in G-4 when the donors are excited at their λ_{max} values, as shown in the emission spectrum by some spontaneous fluorescence from these peripheral dyes.^[11] A more quantitative analysis of energy transfer efficiency involves the study of the fluorescence quenching of the donor dyes by the acceptor.^[7a] Indeed, the fluorescence intensity of the donor dyes in the presence of the acceptor was found to diminish by more than 40-fold in the first three generations, which indicates an energy transfer efficiency of greater than 97%. In the fourth generation the quenching was slightly less effective, and the energy transfer efficiency was found to decrease to 86%. Hence, the synthesized molecules display efficient energy transfer up to the fourth generation.

Figure 4 clearly demonstrates the concept of a spectral energy concentrator. Almost all of the absorbed energy is used to excite the focal dye regardless of the irradiation wavelength, and is eventually converted into its single emission spectrum. In addition, at high generations (G-3 and G-4) the spatial concentration of energy in these molecules results in a net fluorescence amplification when the direct to sensitized excitation of the focal dye are compared (see bands b and c in Figure 4). Since the fluorescence quantum yield of the central dye is large, the whole macromolecule appears to be an effective optical device with excellent absorption and emission features and very few energy losses.

We believe that our approach to energy transfer provides unprecedented flexibility since any dendritic architecture can, in principle, be used to build this type of photoactive device. Not only does this system take advantage of the specific properties of dendritic polymers (high solubility, processability, exact characterization, and well defined structure) in the design of multichromophoric arrays, but the ability to choose the dendritic framework provides versatility for the introduction of an additional key property, such as affinity towards a cell, a protein, or a drug, specific viscosity, specific glass transition, and electron/hole transport capacity. Moreover,

any pair of chromophores can be employed as long as they meet the requirements for mutual interaction. As a result, the emission wavelength of the device can in principle be set in the entire visible range, which is a key property for the design of light emitting materials. Therefore, such dye-labeled dendrimers are promising candidates for a variety of applications, which range, for example, from catalysis (cleavage and release of a photoactive drug or degradation of a photosensitive toxic substance) to amplification for display or mapping, low threshold stimulated emission,^[13] solar energy collection, spectral and spatial refocusing of light in fiber optics coatings, and organic optical media.

Experimental Section

Absorption spectra ($OD_{\max} < 0.1$) were recorded in toluene (unless otherwise noted) on a Uvicon 933 spectrophotometer. Extinction coefficients were calculated from the Beer–Lambert law, which is followed by all compounds up to an optical density of at least 0.6. The same samples and cells (1 cm) were used to record the emission and excitation spectra directly on an ISA/SPEX Fluorolog 3.22 equipped with a 450 W Xe lamp, double excitation and double emission monochromators, and a digital photon-counting photomultiplier. Slit widths were set to 1.3 nm bandpass on both excitation and emission. Correction for variations in lamp intensity over time and wavelength was achieved with a solid-state silicon photodiode as the reference. The spectra were further corrected for variations in photomultiplier response over wavelength and for the path difference between the sample and the reference by multiplication with emission and excitation correction curves generated on the instrument. The photostability of the dye-labeled dendrons was ascertained by monitoring the emission intensity of the central dye under irradiation at the $\lambda_{\max}$ of the peripheral chromophores: no photodegradation could be detected after 1 h of irradiation. Mass spectra were measured by MALDI-TOF on a Perseptive Biosystems Voyager-DE spectrometer in delayed extraction mode and with an acceleration voltage of 20 keV. Samples were prepared by using a 1:20 ratio of analyte (5 mg mL⁻¹ in THF) to matrix solution (*trans*-indoleacrylic acid, 10 mg mL⁻¹ in THF).

Synthesis of G-1 3: A mixture of **1** (0.590 g, 1.04×10^{-3} mol), coumarin 2 (0.674 g, 3.10×10^{-3} mol, 3 equiv), and K₂CO₃ (0.576 g, 5.43×10^{-3} mol, 5 equiv) was dissolved in dry acetonitrile (27 mL) and stirred at reflux under argon for 72 h. The mixture was then cooled, filtered, and evaporated to dryness in vacuo. The product was purified by chromatography on silica gel using hexanes:ethyl acetate (8:2) as the eluent (yield: 582 mg, 67%). Deprotection of this G-1 fragment (0.475 g, 5.65×10^{-4} mol) was accomplished by stirring it in refluxing ethanol (200 mL) containing 10 equiv of NaOH for 3 hours. The deprotected product, (C₂)₂-[G-1]-OH, was isolated by chromatography on silica gel using CH₂Cl₂:ethyl acetate (8:2) as the eluent (82%). The fully dye-labeled G-1^[11] molecule was synthesized by mixing the deprotected phenol **2** (0.100 g, 1.81×10^{-4} mol) with coumarin 343 (0.103 mg, 3.62×10^{-4} mol, 2 equiv), EDC (0.086 g, 4.53×10^{-4} mol, 2.5 equiv), and DMAP (0.005 g, 3.62×10^{-5} mol, 0.2 equiv) in CH₂Cl₂ and stirring the mixture under argon at room temperature overnight (93%).

The higher generation dendrimers^[12] were synthesized by subsequently coupling phenol **2** with the monomer **1**, then deprotecting, and coupling either to coumarin 343 (to obtain the fully labeled dendron) or to the monomer (to go to the next generation). Note that deprotection of G-4 was done in DMF rather than ethanol.

3: ¹H NMR (300 MHz, CDCl₃, TMS): δ = 0.99 (t, J = 7.0 Hz, 6H), 1.96 (m, 4H), 2.34 (s, 6H), 2.36 (d, J = 1.0 Hz, 6H), 2.75 (t, J = 6.1 Hz, 2H), 2.87 (t, J = 6.3 Hz, 2H), 2.99 (q, J = 7.0 Hz, 4H), 3.33 (q, J = 6.1 Hz, 4H), 4.14 (s, 4H), 6.10 (q, J = 1.0 Hz, 2H), 6.68 (s, 2H), 6.97 (s, 1H), 7.03 (s, 2H), 7.13 (s, 1H), 7.33 (s, 2H), 8.47 (s, 1H); ¹³C NMR (100 MHz, CDCl₃): δ = 11.82, 18.56, 18.72, 20.05, 21.11, 27.41, 46.23, 49.97, 50.36, 56.57, 105.75, 107.67, 109.10, 112.54, 114.81, 119.47, 120.46, 124.91, 126.60, 127.33, 129.51, 139.87, 149.11, 150.12, 151.22, 152.43, 152.65, 153.70, 158.50, 161.59, 162.80; MS (MALDI): m/z : 818.53 [M^+], 842.03 [$M+Na^+$], calcd: 819.95, 842.94; UV/Vis (toluene): $\lambda_{\max}$ (ϵ) = 343 (25 000), 437 nm (45 000).

4: ¹H NMR (300 MHz, CDCl₃, TMS): δ = 1.01 (t, J = 7.0 Hz, 12H), 1.99 (m, 4H), 2.37 (s, 24H), 2.78 (t, J = 6.1 Hz, 2H), 2.90 (t, J = 6.4 Hz, 2H), 3.00 (q, J = 7.0 Hz, 8H), 3.36 (q, J = 6.4 Hz, 4H), 4.13 (s, 8H), 3.84 (s, 4H), 6.10 (s, 4H), 6.80 (s, 4H), 6.86 (s, 2H), 6.88 (s, 4H), 6.98 (s, 1H), 7.26 (s, 2H), 7.31 (s, 1H), 7.35 (s, 4H), 8.48 (s, 1H); ¹³C NMR (100 MHz, CDCl₃): δ = 11.86, 14.10, 18.56, 18.71, 20.03, 21.07, 22.63, 25.26, 25.59, 27.39, 31.57, 34.64, 46.25, 49.97, 50.35, 56.62, 67.95, 69.29, 105.61, 105.74, 107.63, 108.99, 112.45, 113.16, 114.66, 119.49, 120.43, 126.55, 127.33, 129.36, 138.78, 139.94, 149.15, 150.08, 151.34, 152.42, 152.61, 153.74, 158.90, 161.56, 162.70; MS (MALDI): m/z : 1486.61 [M^+], 1510.88 [$M+Na^+$], calcd: 1490.75, 1513.74; UV/Vis (toluene): $\lambda_{\max}$ (ϵ) = 343 (45 000), 438 nm (40 000).

5: ¹H NMR (300 MHz, CDCl₃, TMS): δ = 0.99 (t, J = 7.0 Hz, 24H), 1.96 (m, 4H), 2.34 (s, 48H), 2.75 (t, J = 6.1 Hz, 2H), 2.87 (t, J = 6.3 Hz, 2H), 2.99 (q, J = 7.0 Hz, 16H), 3.33 (m, 4H), 4.12 (s, 16H), 4.95 (s, 8H), 5.06 (s, 4H), 6.08 (q, J = 1.2 Hz, 8H), 6.80 (s, 8H), 6.85 (s, 4H), 6.86 (s, 8H), 6.93 (s, 1H), 6.99 (s, 4H), 7.06 (s, 2H), 7.30 (s, 2H), 7.32 (s, 9H), 8.44 (s, 1H); ¹³C NMR (100 MHz, CDCl₃): δ = 11.84, 18.53, 18.67, 20.01, 20.04, 21.03, 25.57, 27.37, 35.38, 46.22, 49.92, 50.32, 56.59, 67.94, 69.35, 69.66, 105.42, 105.67, 107.53, 108.95, 112.42, 113.16, 113.30, 114.64, 118.97, 119.48, 120.37, 122.96, 126.53, 127.27, 129.30, 138.64, 138.90, 139.90, 149.16, 150.01, 151.40, 152.39, 152.58, 153.71, 158.34, 158.95, 159.09, 161.49, 162.73; MS (MALDI): m/z : 2828.37 [M^+], 2852.23 [$M+Na^+$], calcd: 2832.37, 2855.35; UV/Vis (toluene): $\lambda_{\max}$ (ϵ) = 343 (82 000), 441 nm (39 000).

6: ¹H NMR (500 MHz, CDCl₃, TMS): δ = 0.98 (t, J = 7.5 Hz, 48H), 1.93 (m, 4H), 2.32 (s, 96H), 2.70 (m, 2H), 2.81 (m, 2H), 2.98 (q, J = 7 Hz, 32H), 3.33 (m, 4H), 4.10 (s, 32H), 4.94 (s, 16H), 5.02 (s, 8H), 5.09 (s, 4H), 6.05 (s, 16H), 6.78 (s, 16H), 6.84 (s, 24H), 7.00 (s, 8H), 7.05 (s, 4H), 7.06 (s, 4H), 7.12 (s, 2H), 7.31 (s, 16H), 8.44 (s, 1H); ¹³C NMR (125 MHz, CDCl₃): δ = 11.86, 18.52, 18.68, 25.59, 46.24, 56.53, 67.96, 69.69, 108.91, 112.41, 113.15, 113.33, 114.62, 120.37, 126.54, 129.26, 138.73, 138.86, 139.89, 152.38, 152.57, 153.69, 158.95, 159.18, 161.45; MS (MALDI): m/z : 5515.05 [M^+], 5534.46 [$M+Na^+$], calcd: 5515.59, 5538.58; UV/Vis (toluene): $\lambda_{\max}$ (ϵ) = 344 (152 000), 442 nm (38 000).

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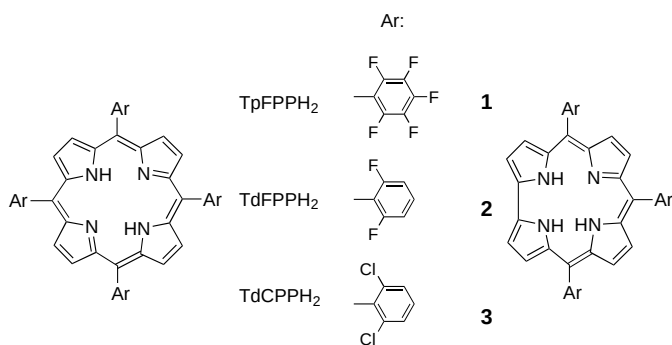
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The First Direct Synthesis of Corroles from Pyrrole**

Zeev Gross,* Nitsa Galili, and Irena Saltsman

Core-modified porphyrins have been receiving increased attention in recent years,^[1] mainly because of their superiority over porphyrins in many applications, most notably as agents for photodynamic therapy.^[2] Some of these macrocycles form complexes with transition metals, which due to the alteration of the ligand structure have very different properties than the analogous metalloporphyrins. The most interesting compounds in this regard are corroles, whose skeletons are contracted by one carbon atom with respect to porphyrins (Scheme 1).^[3] In contrast to the diprotonic porphyrins, corroles act as tetradentate trianionic ligands toward metal ions. The most remarkable feature of corroles is the stabilization of unusually high oxidation states, such as iron(IV), cobalt(IV), and cobalt(V).^[4] Surprisingly, however, there is no reported application in any field for either corroles or their



Scheme 1. Schematic representation of tetraarylporphyrins TpFPPH₂, TdFPPH₂, and TdCPPH₂ (left) and the corresponding triarylcorroles 1–3 (right).

metal complexes. The reason for that is almost certainly the lack of obvious procedures for their preparation. Thus, in spite of the significant progress in corrole synthesis, even the most simple procedures reported to date require starting materials that are not commercially available.^[5] Similarly, the first synthesis of a *meso*-substituted corrole—*meso*-aryl substitution is most likely a prerequisite for the utilization of metallocorroles in catalysis—appeared only in 1993.^[6]

Owing to our ongoing interest in the synthesis of porphyrins and their core-modified analogues,^[7] we explored a new approach, the solvent-free condensation of pyrrole and aldehydes. The research goal was the development of a simple synthetic methodology for the preparation of porphyrins, driven by the anticipation that the rather unusual reaction conditions might also lead to the formation of some porphyrin isomers. We have focused on two aldehydes: benzaldehyde, as the prototype of other *meso*-aryl-substituted porphyrins, and perfluorobenzaldehyde, because the metal complexes of the corresponding porphyrin (TpFPPH₂, Scheme 1) are among the most efficient oxygenation catalysts. The reaction conditions were very simple indeed, consisting of mixing pyrrole and the aldehyde in equimolar quantities on a solid support, heating to 100 °C for four hours, oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), and chromatographic separation. The reaction vessel was open to air, which not only simplifies the procedure but was also found to be required. With benzaldehyde as reactant, tetraphenylporphyrin (TPPH₂) was obtained in 5–8 % yield, depending on the solid support (see Experimental Section).

When the same reaction conditions (best with basic alumina as solid support) were applied for perfluorobenzaldehyde, very different results were observed. Only traces of the corresponding porphyrin (TpFPPH₂) were obtained, accompanied by a compound (**1**, isolated in 11 % yield) which showed a strong porphyrin-like fluorescence and an electronic spectrum similar to that of TpFPPH₂ (Figure 1). However, while the ¹H NMR spectrum of TpFPPH₂ consists of two singlets in a ratio of 8:2 at δ = 8.91 (sharp) and –2.93 (broad) for the β -pyrrole and the inner nitrogen hydrogen atoms, respectively, in the spectrum of **1** three doublets ($J \approx 4.5$ Hz) in the ratio of 1:1:2 were obtained in the pyrrole hydrogen region (δ = 9.10, 8.75, and 8.57) together with an extremely broad resonance at δ = –2.25 (Figure 1, inset). Similarly, the

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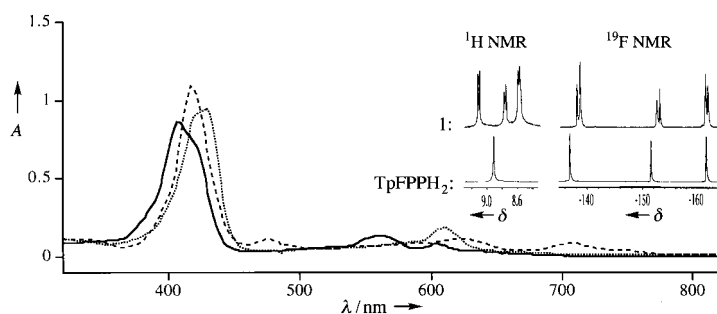


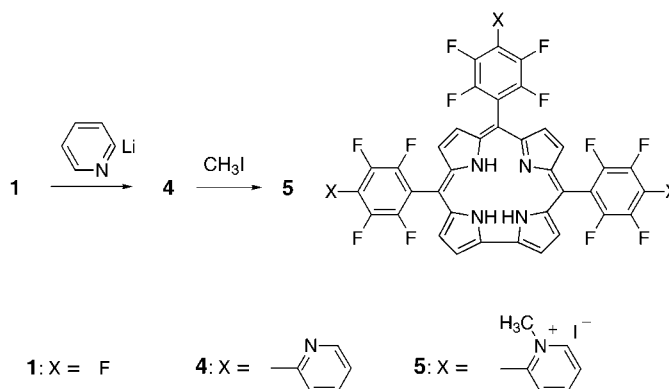
Figure 1. UV/Vis spectra of **1** (—), and its protonated (---); reaction with $\text{CF}_3\text{CO}_2\text{H}$ and deprotonated forms (....; reaction with Et_3N). The spectra were measured in CH_2Cl_2 at identical concentrations. Inset: The ^1H and ^{19}F NMR spectra of **1** and TpFPPH_2 in CDCl_3 .

signal for only one type of pentafluorophenyl group is present in the ^{19}F NMR of TpFPPH_2 , but two such groups in the ratio of 2:1 are evident for **1**. This data, together with the mass spectrum of the compound (m/z 796) allowed the identification of **1** as 5,10,15-tri(2,3,4,5,6-pentafluorophenyl)corrole, the first β -pyrrole-unsubstituted corrole.

Because of the difference in reactivity between benzaldehyde and pentafluorobenzaldehyde—formation of porphyrin and corrole, respectively—we turned our attention to other halogeno-substituted benzaldehydes, namely, the 3,5-dichloro, 2,6-dichloro, and 2,6-difluoro derivatives. Traces of porphyrin and 6% of 5,10,15-tri(2,6-difluorophenyl)corrole (**2**) were formed in the reaction of pyrrole and 2,6-difluorobenzaldehyde (equimolar amounts). However, no corrole was formed with the two chlorinated aldehydes, and only trace amounts of porphyrin appeared with 3,5-dichlorobenzaldehyde. The only isolated product (7%) from the reaction of 2,6-dichlorobenzaldehyde was the *meso*-aryl- α -benzylidipyrromethene.^[8,9] Reexamination of the reactions with pentafluoro- and 2,6-difluorobenzaldehyde revealed that analogous compounds were also obtained as minor components. As dipyrromethenes are the H_2O elimination products of the chain-growing polypyrroles which lead to both porphyrin and corrole,^[10] we anticipate that further optimization of the synthetic procedure will allow the successful preparation of additional triarylcorroles. Indeed, a simple change of the pyrrole:aldehyde ratio to 2:1 allowed the isolation of 5,10,15-tri(2,6-dichlorophenyl)corrole (**3**) in 1% yield.

The absence of electron-donating alkyl groups in the β -pyrrole positions and the presence of the electron-withdrawing pentafluorophenyl groups at the *meso*-carbon atoms in corrole **1** lead to some unique properties. For example, the known larger NH acidity of corroles relative to porphyrins is brought to quite an extreme in **1**. In ethanolic solutions the dominant species is the monoanion of **1**, and in organic solvents **1** is deprotonated by weak bases. This is demonstrated in Figure 1, which shows the changes in the electronic spectrum of **1** upon its deprotonation by triethylamine. Interestingly, the single imine-like nitrogen atom of **1** remains basic; that is, it is protonated by a sufficiently strong acid (Figure 1). These spectral changes are accompanied in both cases by a color change from violet to green. Finally, another peculiarity of **1** is that it may serve as the precursor of many other corroles, since its *para*-fluoro substituents can easily be

replaced by nucleophilic substitution. This is demonstrated by the reaction of **1** with *ortho*-pyridyllithium to provide 5,10,15-tris[4-(2-pyridyl)-2,3,5,6-tetrafluorophenyl]corrole (**4**; Scheme 2; see Experimental Section). The reaction of **4** with iodomethane leads to **5**, which to our knowledge is the first ever reported ionic corrole. The solubility of **5** in water is about 2 mg mL^{-1} , and at least in the range determinable by UV/Vis spectroscopy there is no sign of aggregation.



Scheme 2. Synthesis of **4** and water-soluble **5** from **1**.

To summarize, we report the first corrole synthesis to start with commercially available reactants (pyrrole and aldehyde), the first corroles without substituents at the β -pyrrole positions, and the first water-soluble corrole. We have already prepared the iron, cobalt, rhodium, and copper complexes of **1**, whose full characterization and examination in various applications is currently in progress. We also trust that the facile synthesis described herein will soon make corrole derivatives commercially available.^[11]

Experimental Section

General procedure described for the synthesis of TPPH_2 : The solid absorbant (florisil, silica, or alumina; 1 g) was mixed in a 50-mL flask with a solution of benzaldehyde (1.06 g, 10 mmol) and pyrrole (0.67 g, 10 mmol) in CH_2Cl_2 (2 mL), and the solvent was distilled at normal pressure. The condenser was removed, and the solid mixture was heated to 100°C , upon which the color changed to black in 5–10 min. After heating of the mixture for 4 h, the solid support was washed with CH_2Cl_2 (50 mL), DDO (1.13 mg, 5 mmol) was added, and the product was obtained by chromatography on basic alumina with hexane/ CH_2Cl_2 (2.5/1) as eluent. With florisil, silica, neutral alumina, and basic alumina as solid supports the isolated chemical yields of TPPH_2 were 5, 5, 7, and 8%, respectively. When the first step of the reaction was carried out under an inert atmosphere, neither porphyrins or corroles were obtained.

1: Under the same reaction conditions as for TPPH_2 , with the solid absorbant (0.5 g), pentafluorobenzaldehyde (0.49 g, 2.5 mmol), and pyrrole (0.17 g, 2.5 mmol), traces of TpFPPH_2 and significant amounts of **1** were obtained (R_f (hexane/ CH_2Cl_2 3/1) = 0.52 and 0.34, respectively). The yields of isolated **1** (after crystallization from CH_2Cl_2 /hexane) were 4%, traces, 11%, and 11%, with florisil, silica, neutral alumina, and basic alumina as solid support, respectively. Identical chemical yields were obtained in a reaction with half the amount of solid support; that is, 55 mg of **1** were isolated from the reaction supported by 0.25 g of basic alumina. UV/Vis (CH_2Cl_2): λ_{max} ($\epsilon \times 10^{-3}$) = 408 (114.0), 560 (17.6), 602 nm (9.3); ^1H NMR (CDCl_3): δ = 9.10 (d, J = 4.4 Hz, 2H), 8.75 (d, J = 4.4 Hz, 2H), 8.57 (d, J = 4.4 Hz, 4H), -2.25 (brs, 3H); ^{19}F NMR (CDCl_3): δ = -137.55 (dd, ^1J = 24.2, ^2J = 8.1 Hz, 2F), -138.14 (dd, ^1J = 23.03, ^2J = 6.9 Hz, 4F), -152.52 (t, J = 19.6 Hz, 2F), -153.10 (t, J = 20.7 Hz, 1F), -161.78 (dt, ^1J = 24.2, ^2J =

8.1 Hz, 4F), -162.35 (dt, $^1J=23.03$, $^2J=6.9$ Hz, 2F); HR-MS: m/z : 797.0854 (calcd for $C_{37}H_{12}N_4F_{15}$: 797.0822).

2: UV/Vis (CH_2Cl_2): λ_{max} ($\epsilon \times 10^{-3}$): 406 (118.6), 562 (20.3), 602 nm (11.9); ^1H NMR ($CDCl_3$): $\delta=8.99$ (d, $J=4.3$ Hz, 2H), 8.71 (d, $J=4.3$ Hz, 2H), 8.52 (t, $J=4.3$ Hz, 4H), 7.72 (m, 3H), 7.33 (m, 6H), -2.1 (brs, 3H); ^{19}F NMR ($CDCl_3$): $\delta=-109.32$ (t, $J=6.4$ Hz, 2F), -109.75 (t, $J=6.4$ Hz, 4F); HR-MS: m/z : 635.1660 (calcd for $C_{37}H_{21}N_4F_6$: 635.1670).

3: UV/Vis (CH_2Cl_2): λ_{max} ($\epsilon \times 10^{-3}$): 408 (106.1), 422 (86.6), 560 (16.7), 604 nm (9.3); ^1H NMR ($CDCl_3$): $\delta=8.91$ (d, $J=4.2$ Hz, 2H), 8.49 (d, $J=4.8$ Hz, 2H), 8.35 (d, $J=4.6$ Hz, 4H), 7.72 (m, 3H), 7.33 (m, 6H), -1.7 (brs, 3H); HR-MS: m/z : 729.9810 (calcd for $C_{37}H_{20}N_4Cl_6$: 729.9819).

4: A 1.6 M $nBuLi$ solution (0.42 mL, 0.7 mmol) was added to a stirred solution of 2-bromopyridine (0.054 mL, 0.56 mmol) in dry THF (6 mL) under an argon atmosphere at $-78^\circ C$, at such a rate that the temperature of the reaction mixture did not exceed $-70^\circ C$. After the addition was complete, the reaction mixture was stirred for 1 h at $-78^\circ C$, resulting in a clear yellow solution. Next, a solution of **1** (0.03 g, 0.038 mmol) in dry THF (6 mL) was added dropwise. The mixture was stirred for 1 h at $-78^\circ C$ and then hydrolyzed with saturated aqueous bicarbonate solution. The layers were separated, the aqueous layer was washed with diethyl ether, and the combined diethyl ether extracts were dried and evaporated to yield a solid residue. The product was purified by column chromatography on silica gel (EtOAc/hexane 1/1) and recrystallized from CH_2Cl_2 /hexane to provide 13 mg (35% yield) of pure **4** as a violet solid. UV/Vis (CH_2Cl_2): λ_{max} ($\epsilon \times 10^{-3}$): 414 (111.6), 564 (18.4), 606 nm (sh); ^1H NMR ($CDCl_3$): $\delta=9.12$ (d, $J=3.9$ Hz, 2H), 8.93 (m, 5H), 8.73 (d, $J=4.88$ Hz, 2H), 8.66 (d, $J=3.91$ Hz, 2H), 8.00 (dt, $^1J=7.81$, $^2J=1.95$ Hz, 3H), 7.84 (brd, $J=7.81$ Hz, 3H), 7.51 (dt, $^1J=6.84$, $^2J=1.95$ Hz, 3H), -2.02 (brs, 3H); ^{19}F NMR ($CDCl_3$): $\delta=-138.19$ (q, $J=23.79$ Hz, 2F), -138.81 (q, $J=23.79$ Hz, 4F), -144.11 (q, $J=23.79$ Hz, 4F), -144.57 (q, $J=23.79$ Hz, 2F); HR-MS: m/z : 973.1910 (calcd for $C_{52}H_{23}N_7F_{12}$: 973.1823).

5: A mixture of **4** (11 mg, 11 μ mol) and CH_3I (0.8 mL, 13 mmol) in freshly distilled DMF (2 mL) was heated to $70^\circ C$ for 3 h. After evaporation of the solvent, the product was recrystallized from MeOH/diethyl ether to provide 15.5 mg (98% yield) of **5** as a green solid. UV/Vis (MeOH): λ_{max} ($\epsilon \times 10^{-3}$): 430 (76.2), 576 (10.9), 622 nm (17.8); ^1H NMR ($[D_6]DMSO$): $\delta=9.49$ (d, $J=5.98$ Hz, 3H), 9.16 (brm, 8H), 9.00 (t, $J=8.54$ Hz, 3H), 8.75 (t, $J=7.68$ Hz, 3H), 8.51 (t, $J=7.68$ Hz, 3H), 4.68 (s, 3H), 4.65 (s, 6H); ^{19}F NMR ($[D_6]DMSO$): $\delta=-137.26$ (brm, 4F), -138.04 (brm, 6F), -138.60 (brm, 2F); electron spray MS: m/z : 339.9 ($[M^+ - 3I]/3$, 100%).

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Regioselective Reduction of NAD^+ Models with $[Cp^*Rh(bpy)H]^+$: Structure–Activity Relationships and Mechanistic Aspects in the Formation of the 1,4-NADH Derivatives**

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The interest in practical methods for the regeneration of the co-enzyme 1,4-NADH, the reduced form of nicotinamide adenine dinucleotide (NAD^+), has continued to be high in the field of biocatalysis, where enzymatic reduction reactions are important for the synthesis of chiral organic compounds.^[1a, b] Conversion of NAD^+ into 1,4-NADH by enzymatic, chemical, photochemical, and electrochemical methods has been studied extensively in order to increase the rate of the regeneration, while maintaining the necessary high regioselectivity. The regeneration is frequently the limiting step in the eventual use of 1,4-NADH in enzymatic synthesis, particularly for higher volume and more energy intensive processes.^[1a, b]

In the search for higher rates and a more economical regeneration process various transition metal hydrides have been studied as catalysts for the regioselective reduction of NAD^+ and NAD^+ models to their corresponding 1,4-NADH derivatives.^[2a–g] In the most successful example, Steckhan and co-workers have described the use of $[Cp^*Rh(bpy)(H)]^+$ (Cp^* = pentamethylcyclopentadienyl, bpy = 2,2'-bipyridyl), generated in situ, for the regiospecific reduction of NAD^+ to 1,4-NADH,^[2b] and then demonstrated the cofactor regeneration process in enzymatic, chiral reduction reactions.^[3, 4]

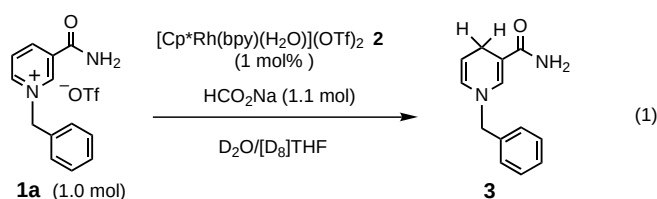
While the above mentioned reduction of NAD^+ by $[Cp^*Rh(bpy)H]^+$ was shown to be regiospecific for 1,4-NADH,^[2b, 5a] the full mechanistic details of this important

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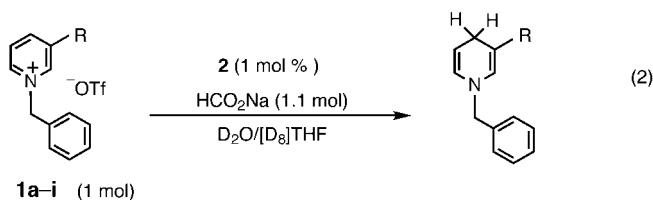
cofactor conversion and the role of the substituents on the nicotinamide moiety have not been elucidated. Thus, we have initiated a program to determine the source of this high regioselectivity and other mechanistic aspects with the model NAD^+ compound 1-benzylpyridinium triflate (**1a**)^[6] by using $[\text{Cp}^*\text{Rh}(\text{bpy})(\text{H}_2\text{O})](\text{OTf})_2$ (**2**) as the catalyst precursor and sodium formate as the hydride source.^[2b] Furthermore, a variety of 3-substituted derivatives of 1-benzylpyridinium triflate (**1b–i**) were synthesized and studied along with **1a** to ascertain how other substituents in position 3 would influence the regioselectivity. We were also interested in examining any electronic and steric effects that these substituents in the 3 position might impart on the rate of hydride transfer.

Initial ^1H NMR experiments in $\text{D}_2\text{O}/[\text{D}_8]\text{THF}$ of **1a** and **2** in the presence of the hydride source HCO_2Na showed that both 1-benzyl-1,4-dihydronicotinamide (**3**, >95%) and its 1,6-isomer (<5%) were formed [Eq. (1)]. However, the 1,4-dihydro product **3** was separately found to be converted,



under similar reaction conditions, into less than 5% (2 h) of the 1,6-dihydro isomer by a rearrangement catalyzed by complex **2**. Thus, as with NAD^+ itself, the model compound **1a** also provides high regioselectivity for the 1,4-dihydro product with $[\text{Cp}^*\text{Rh}(\text{bpy})\text{H}]^+$, but is mitigated, to a very minor extent, by a rearrangement catalyzed by complex **2** of the kinetic 1,4-dihydro product to the 1,6-dihydro isomer; the overall ratio of **3** to its 1,6-isomer in Equation (1) was found to be about 20:1. More importantly, when we used DCO_2Na (98% D) as the hydride source, the deuterium was found to be incorporated at the C4 position (>95%, ^1H NMR spectroscopy) rather than the C6 position. This observation, therefore, further defined the initial kinetic product as being the 1,4-isomer, **3**.

Table 1 shows the relative rates and turnover frequencies of **1a** and other *N*-benzylpyridinium compounds substituted in position 3 (**1b** and **1d–g**) that also provide regioselective 1,4-dihydro products under the same conditions [Eq. (2)]. Additionally, complex **2** also catalyzed the rearrangement of the



1,4-dihydro derivatives of **1b**, and **1d–g** to the corresponding 1,6-dihydro derivative (ca. 5–15%). It is interesting to note that the $\text{C}(\text{S})\text{NH}_2$ group in **1d** increased the relative rate by approximately 30% relative to the $\text{C}(\text{O})\text{NH}_2$ group in **1a**, and this suggests that the more polarizable, soft S atom, which is a

Table 1. Relative rates and turnover frequencies of the regioselective reductions of NAD^+ models [see Equation (2)].^[a]

Substrate	R	Relative reaction rate ^[b]	TOF [h] ^[c]
1a		1.0	8
1b		0.9	8
1c		0.0	0
1d		1.3	11
1e		1.1	9
1f		1.3	11
1g	CN	0.9	8
1h	CH ₃	0.0	0
1i	H	0.0	0

[a] The reactions were conducted in presilylated NMR tubes (J. Young) and were followed by ^1H NMR spectroscopy. [b] The relative reaction rates were determined by observing the disappearance of the pyridinium signals versus an internal standard, $[(\text{CH}_3)_4\text{N}]\text{OTf}$, during the first 2 h, with the relative rate of **1a** set to 1.0. [c] The turnover frequencies (TOFs) were calculated by the amount of product [mmol] per amount of the catalyst [mmol] per hour.

better σ donor, coordinates more readily to the Cp^*Rh metal center and, thereby, facilitates hydride transfer to C4. Moreover, the $\text{C}(\text{O})\text{OCH}_3$ group in the 3 position in **1f** also increased the relative rate by about 30%, presumably for reasons associated with both coordination and the favorable electron-withdrawing effect. In contrast, replacement of the substituent in position 3 with a noncoordinating, electron-donating CH_3 group (**1h**) or a hydrogen atom (**1i**) leads to decomposition of the $[\text{Cp}^*\text{Rh}(\text{bpy})\text{H}]^+$ complex^[5b] rather than formation of the corresponding 1,4-dihydro compound.

We observed no 1,4-dihydro product and only $[\text{Cp}^*\text{Rh}(\text{bpy})\text{H}]^+$ decomposition when the $\text{C}(\text{O})\text{NH}_2$ group in **1a** ($E_p = -1.23$ V versus Ag/AgCl) was replaced with a $-\text{C}(\text{O})\text{NEt}_2$ group (**1c**; $E_p = -1.26$ V versus Ag/AgCl), even though both substituents would be of equal σ -donating ability and equal overall electronic effect. A plausible explanation would entail that steric interactions between the methyl groups of the Cp^* ligand and the ethyl groups in position 3 (NEt_2) inhibit coordination of the substrate to the Cp^*Rh metal center, thereby limiting the hydride-transfer process. More interestingly, this result occurs even when the most electrophilic and sterically unencumbered C6 position of **1c** is readily available, which further strengthens the concept of the coordination of the carbonyl unit of the $\text{C}(\text{O})\text{NH}_2$ group in **1a**, as well as of those substituents in substrates **1b** and **1d–g**, to the Cp^*Rh metal center.^[7]

Several competitive substrate reduction experiments were performed to establish whether the σ -donating ability or the electron-withdrawing effect of the substituent in the 3 position was the more important parameter. We found that when we reduced **1a** in the presence of either **1e** (competitive rate = 0.9; $E_p = -1.08$ V versus Ag/AgCl) or **1g** (competitive rate = 0.8; $E_p = -0.97$ V versus Ag/AgCl) substrate **1a** with a $-\text{C}(\text{O})\text{NH}_2$ group was more reactive than either the

$-\text{C}(\text{O})\text{CH}_3$ or $-\text{CN}$ analogues (1.1 and 1.3 times faster, respectively), and if we reduced **1a** in the presence of **1d** or **1f** the $-\text{C}(\text{S})\text{NH}_2$ and $-\text{C}(\text{O})\text{OCH}_3$ derivatives were both found to be more reactive (1.3 and 1.2 times faster, respectively). Thus, we tentatively conclude that the σ -donating ability of the substituent in the 3 position (for example, in **1a**, **d**, and **f**) is a more important parameter than the electron-withdrawing effect of this group (for example, in **1e**, and **g**) in the overall reduction reaction.

We also examined the possible electronic effect of substituents bound to the nitrogen atom of the nicotinamide nucleus. Thus, we replaced the 1-benzyl group in **1a** with a 1-methyl group (**4**; $E_p = -1.31$ V versus Ag/AgCl), and found that this change in substituent reduced the relative rate to 0.5, and provided a ratio of the relative rate of the 1-benzyl/1-methyl derivatives of 2.0. The implication of this latter result is that the 1-benzyl group is a better electron-withdrawing substituent than the electron-donating 1-methyl group, and this further facilitates hydride attack at C4. The electron reorganization upon hydride transfer to C4 would benefit from a through-bond electron-withdrawing substituent on the nitrogen atom of the nicotinamide nucleus.

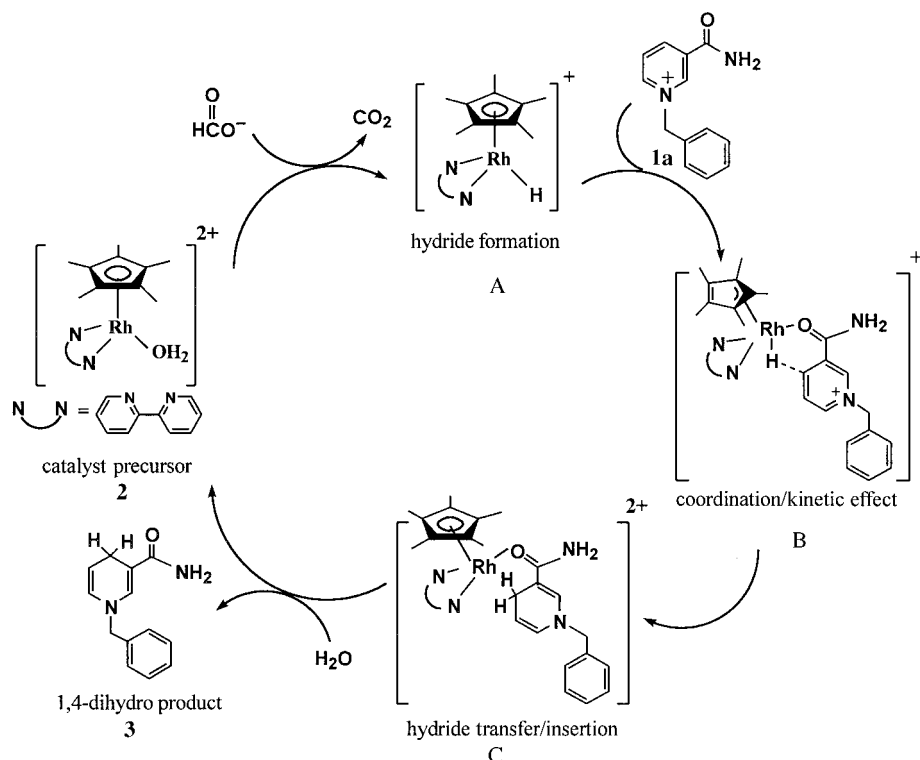
Therefore, our initial results allow us to propose a catalytic cycle for the regioselective reduction of the NAD^+ model compound **1a** with **2** as the catalyst precursor, and sodium formate as the hydride source (Scheme 1). The reaction of **2** with HCO_2Na provides the $[\text{Cp}^*\text{Rh}(\text{bpy})(\text{H})]^+$ complex through a β -hydrogen elimination reaction to produce CO_2 (A in Scheme 1).^[2b, 5a] The critical role of the amide functionality is to coordinate to the Cp^*Rh metal center through an open coordination site on the metal center. We suggest that this open site occurs by the well documented ring-slippage

mechanism^[8] of the Cp^* ring by a change in the coordination mode of this ligand from η^5 to η^3 . This mechanism includes a kinetically favorable six-membered ring transition state (B in Scheme 1), which further provides a driving force for regioselective hydride transfer at C4 (C in Scheme 1; this step also includes a reversion of the η^3 to η^5 coordination of the Cp^* ligand). During this coordination process, the induced electronic effect of the bound carbonyl group may also cause the C4 position to be a more electrophilic site towards hydride transfer. We also suggest that the other pyridinium NAD^+ models substituted at position 3 (**1b**, **d–g**) can coordinate to the Cp^*Rh metal center and exert an electron-withdrawing effect at C4 by a similar mechanism as shown for the NAD^+ model compound **1a** in Scheme 1.

It is also interesting to note that the reduced forms of both complexes $[\text{Rh}\{\text{tris}(2,2'\text{-bpy})\}]^{3+}$ and $[\text{Rh}\{\text{bis}(\text{terpy})\}]^{3+}$ (terpy = 2,2':6',2''-terpyridine) provide similar regioselectivities in NAD^+ reductions;^[2f–h] however, the rate of reduction of NAD^+ with **2** was reported to be 20 times faster^[4a] than that by $[\text{Rh}\{\text{tris}(2,2'\text{-bpy})\}]^{3+}$. Since this latter inorganic complex cannot undergo a ring-slippage mechanism this reaction rate data may provide further support for the participation of this process.

Thus, we have provided evidence, for the first time, that regioselective hydride transfer from $[\text{Cp}^*\text{Rh}(\text{bpy})\text{H}]^+$ to NAD^+ to give 1,4- NADH is a consequence of the crucial role of the ability of the amide to coordinate to the ring-slipped Cp^*Rh metal center. The overall rate of this regioselective hydride transfer process is further affected by the induced electron-withdrawing effect of the bound amide group on C4, as well as by the substituent in position 1; that is, the 1-ribose group of the nicotinamide moiety of NAD^+ . More important-

ly, the role of the amide carbonyl or other coordinating groups at the 3 position may also be prevalent in other regioselective transition metal hydride reactions^[2c] with NAD^+ models that provide the 1,4-dihydro isomer. It is worthwhile to note that a sterically encumbered porphyrin-Ru-H complex provided the 1,6-dihydro isomer regioselectively as the sole kinetic product with substrate **1c**, with no evidence for the 1,4-dihydro isomer.^[2d] To reiterate, we see no reduction product with **1c**, even though C6 is fully accessible and, therefore, coordination appears to be a mandatory step in the pathway to the 1,4-dihydro isomer with $[\text{Cp}^*\text{Rh}(\text{bpy})\text{H}]^+$. In addition, the non-coordinating, direct hydride reducing agent NaBH_4 only provides a mixture of 1,2- and 1,6-dihydro isomers with substrates **1f** and **1g**, with no 1,4-dihydro isomer evident.^[9] Thus, BH_4^- appears to attack the most electrophilic carbon



Scheme 1. Proposed mechanism for the regioselective, catalytic reduction of an NAD^+ model substrate in $\text{H}_2\text{O}/\text{THF}$ (1/1).

atoms, which again is exactly opposite to our findings.

We will continue these regioselective cofactor regeneration studies using electrochemical techniques to form the $[\text{Cp}^*\text{Rh}(\text{bpy})\text{H}]^+$ complex, attempt to synthesize the indenyl analogue^[8] of **2** for further evidence of the ring-slippage mechanism, and also synthesize a new, water soluble NAD^+ model to fully comprehend the effect of pH on the rates, the turnover frequency, and if enzymes recognize the 1,4-dihydro derivative in chiral reduction reactions.

Experimental Section

General procedure for the synthesis of NAD^+ model substrates: The chloride or bromide salts of the NAD^+ model substrates were prepared by related published methods;^[6] however, THF was used as the reaction solvent so as to simplify the purification process. Anion exchange was conducted either by utilizing AgOTf (1.0 equiv in MeOH) or NaOTf (1.05 equiv in acetone). After solvent removal from the filtrate the crude products were further purified by recrystallization from acetone/ CH_2Cl_2 (1/1) and Et_2O , followed by refrigeration at -15°C . The resulting crystals were collected on a glass-fritted funnel and washed with acetone (0°C), then dried in vacuo over P_2O_5 (yield 86–97%).

1-Benzyl-*N*-methylnicotinamide (triflate salt, **1b**): ^1H NMR (D_2O): $\delta = 9.20$ (s, 1H, H2 on Py), 8.96 (d, $J = 5.8$ Hz, 1H, H6 on Py), 8.75 (d, $J = 8.0$ Hz, 1H), 8.08 (app. t, $J = 7.2$ Hz, 1H), 7.43 (app. s, 5H), 5.81 (s, 2H, $-\text{CH}_2\text{Ph}$), 2.88 (s, 3H); elemental analysis calcd for $\text{C}_{15}\text{H}_{15}\text{F}_3\text{N}_2\text{O}_4\text{S}$ (376.39): C 47.86, H 4.03, N 7.44; found: C 47.66, H 4.35, N 7.08.

1-Benzylthionicotinamide (triflate salt, **1d**): ^1H NMR (D_2O): $\delta = 9.31$ (s, 1H, H2 on Py), 8.88 (d, $J = 6.4$ Hz, 1H, H6 on Py), 8.71 (d, $J = 8.4$ Hz, 1H), 8.00 (dd, $J = 6.0$, 8.1 Hz, 1H), 7.41 (app. s, 5H), 5.77 (s, 2H, $-\text{CH}_2\text{Ph}$); elemental analysis calcd for $\text{C}_{14}\text{H}_{13}\text{F}_3\text{N}_2\text{O}_3\text{S}_2$ (378.43): C 44.43, H 3.47, N 7.40; found: C 44.36, H 3.56, N 7.65.

1-Benzyl-3-acetylpyridinium triflate (**1e**): ^1H NMR (D_2O): $\delta = 9.35$ (s, 1H, H2 on Py), 8.97 (d, $J = 6.6$ Hz, 1H, H6 on Py), 8.93 (d, $J = 8.0$ Hz, 1H), 8.11 (app. t, $J = 7.2$ Hz, 1H), 7.40 (app. s, 5H), 5.81 (s, 2H, $-\text{CH}_2\text{Ph}$), 2.65 (s, 3H); elemental analysis calcd for $\text{C}_{15}\text{H}_{14}\text{F}_3\text{NO}_4\text{S}$ (361.37): C 49.85, H 3.91, N 3.88; found: C 50.12, H 3.57, N 3.89.

1-Benzyl-3-methylpyridinium triflate (**1h**): ^1H NMR (D_2O): $\delta = 8.62$ (s, 1H, H2 on Py), 8.61 (d, $J = 5.0$ Hz, 1H, H6 on Py), 8.25 (d, $J = 7.8$ Hz, 1H), 7.81 (app. t, $J = 7.2$ Hz, 1H), 7.37 (app. s, 5H), 5.64 (s, 2H, $-\text{CH}_2\text{Ph}$), 2.40 (s, 3H); elemental analysis calcd for $\text{C}_{14}\text{H}_{14}\text{F}_3\text{NO}_3\text{S}$ (333.36): C 50.44, H 4.24, N 4.20; found: C 50.01, H 4.56, N 4.64.

1-Benzylpyridinium triflate (**1i**): ^1H NMR (D_2O): $\delta = 8.82$ (d, $J = 5.9$ Hz, 2H), 8.48 (app. t, $J = 7.8$ Hz, 1H), 7.98 (t, $J = 6.5$ Hz, 2H), 7.42 (app. s, 5H), 5.73 (s, 2H, $-\text{CH}_2\text{Ph}$); elemental analysis calcd for $\text{C}_{13}\text{H}_{12}\text{F}_3\text{NO}_3\text{S}$ (319.33): C 48.89, H 3.80, N 4.39; found: C 49.01, H 3.78, N 4.55.

1-Methylnicotinamide (triflate salt, **4**): ^1H NMR (D_2O): $\delta = 9.19$ (s, 1H, H2 on Py), 8.88 (d, $J = 6.0$ Hz, 1H, H6 on Py), 8.80 (d, $J = 8.7$ Hz, 1H), 8.10 (app. t, $J = 7.1$ Hz, 1H), 4.39 (s, 3H, CH_3); elemental analysis calcd for $\text{C}_8\text{H}_9\text{F}_3\text{N}_2\text{O}_4\text{S}$ (286.26): C 33.56, H 3.18, N 9.79; found: C 33.80, H 3.23, N 10.02.

Electrochemical measurements: These were carried out by means of cyclic voltammetry using a standard three electrode cell with a glassy carbon electrode and a Ag/AgCl reference electrode. The solutions were generally a water/THF mixture with an appropriate buffer salt. All of the electrochemical responses of the compounds **1a–i** exhibited a chemically irreversible peak in the voltammetric experiments when a $20\text{--}50\text{ mV s}^{-1}$ sweep rate was used.

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Reversible Fixation of Ethylene on a Sm^{II} Calix-Pyrrole Complex**

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The chemistry of lanthanides has become a very important field of inorganic chemistry since the 1980s when a series of reports describing the unique richness and variety of reactivity of Sm^{II} ^[1] rejuvenated the interest in this field.^[2] Cyclopentadienyl (Cp) and related ligands have been used to develop the major part of the chemistry of Sm^{II} .^[3] Given the caliber of transformations afforded by $[\text{Cp}_2^*\text{Sm}]$ ($\text{Cp}^* = \text{C}_5\text{Me}_5$), it is not surprising that several attempts have been made to prepare complexes of divalent samarium in different ligand environ-

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ments.^[4] However, in spite of progress made in this direction,^[5] so far no other ligand system has been able to display the same variety of reactivity as discovered with decamethylsamarocene.

Attempts to isolate a Sm^{II} complex of a calix-pyrrole ligand^[6] led to the isolation of a compound in which dinitrogen was encapsulated into a Sm₂Li₄ cage.^[7] The rarity of this transformation and the considerable extent of reduction of N₂ indicated that this ligand system is perhaps a valid alternative to the Cp systems. Herein we report the isolation and characterization of Sm^{II} complexes of two calix-pyrrole ligands and their reaction with ethylene which provides the first example of reversible ethylene fixation in lanthanide chemistry.^[8]

Reduction of [(R₈-calix-pyrrole)ClSm{Li(thf)}₂{Li(thf)}₂-(μ₃-Cl)] (R = Et (**1a**), -(CH₂)₅- (**1b**))^[7] with lithium under argon in THF formed dark green solutions (Scheme 1). The result of the reduction depended on the aliphatic chains attached to the macrocycle. Two compounds formulated as [(R₈-calix-pyrrole)(L)Sm{Li(thf)}₂{Li(thf)}₂-(μ₃-X)(Et₂O)_{1.5}] (R = {-(CH₂)₅-}_{0.5}, X = Cl, L = thf (**2b**); R = Et, X = OCHCH₂, L = Et₂O (**3a**)) were isolated in good yield and characterized. In the case of the octaethyl derivative the enolate group, probably arising from a THF cleavage process,^[9] replaced the Cl⁻ ion. Reaction of the tetralithium salts of both ligands^[9, 10] with [SmI₂(thf)₂] afforded low yields of the two isomorphous enolate derivatives **3a** and **3b**. Both complexes are paramagnetic, and informative NMR spectra were obtained only in the case of **3a**.

The crystal structure of **3a** consists of a samarium atom surrounded by a calix-pyrrole ligand arranged in a basketlike conformation (Figure 1). The coordination geometry of the samarium atom is distorted pseudo-trigonal bipyramidal and is defined by two nitrogen atoms of two pyrrole rings (σ-bonded), two centroids of the other two pyrrole rings (π-bonded), and the oxygen atom of one molecule of coordinated

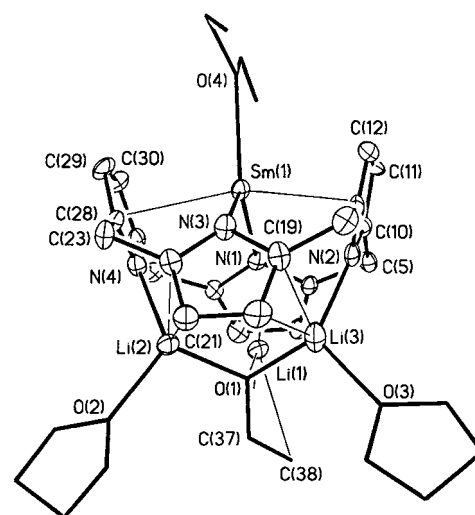
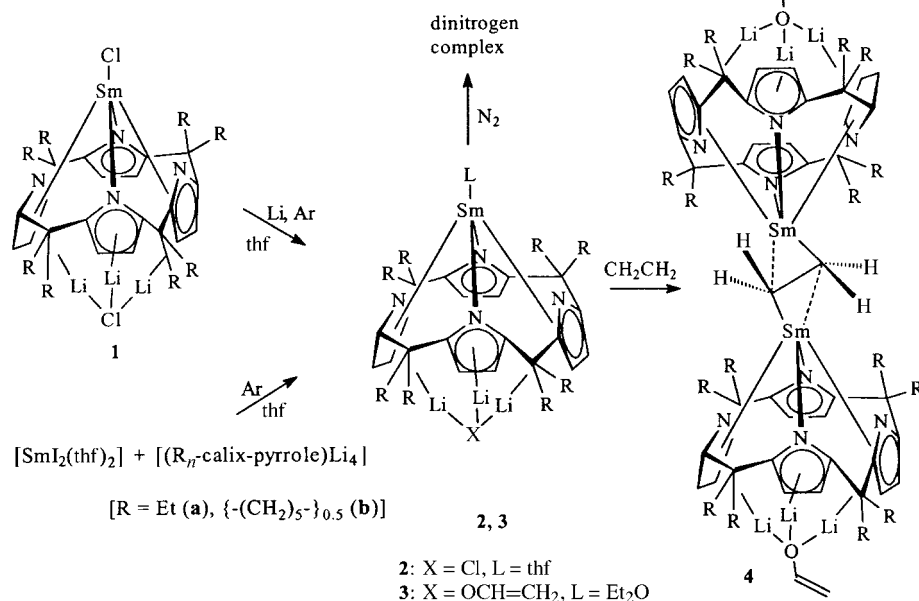


Figure 1. ORTEP drawing of **3a**. Thermal ellipsoids are drawn at the 30% probability level. Bond lengths [Å] and angles [°]: Sm(1)–N(1) 2.597(7), Sm(1)–N(3) 2.602(7), Sm(1)–N(2) 2.853(6), Sm1–C(10) 2.913(8), Sm(1)–C(11) 2.986(8), Sm(1)–C(12) 2.976(9), Sm(1)–C(13) 2.901(8), Li(2)–N(4) 2.09(2), Li(1)–N(1) 2.16(2), Li(1)–C(1) 2.18(2), Li(1)–C(2) 2.22(2), Li(1)–C(3) 2.24(2), Li(1)–C(4) 2.17(2), Li(1)–O(1) 1.84(2), Li(2)–O(1) 1.93(2), Li(3)–O(1) 1.90(2), O(1)–C(37) 1.330(11), C(37)–C(38) 1.303(13), Li(1)–C(37) 2.37(2), Li(1)–C(38) 2.67(2); N(1)–Sm(1)–N(3) 123.2(2), N(1)–Sm(1)–O(4) 114.6(3), N(3)–Sm(1)–O(4) 122.2(3), centroid–Sm(1)–centroid 161.6(3), O(1)–C(37)–C(38) 125.1(10), Li(1)–O(1)–Li(2) 104.5(7), Li(1)–O(1)–Li(3) 102.1(7), Li(2)–O(1)–Li(3) 110.0(8).

solvent (diethyl ether or THF). On the opposite side of the macrocycle, three lithium atoms are bonded to the pyrrole rings, and adopt either σ- or π-bonding modes and are also connected to the oxygen atom of the enolate group (or a chlorine atom as in the case of **2b**).^[11]

The enolate Sm^{II} derivatives **3a** and **3b** react reversibly with ethylene in a fast reaction, the extent of which depends on the nature of the R group. Upon exposure to ethylene, the initially dark green color of the solution persists in the case of **3a** but turns light green in the case of **3b**. Light green paramagnetic crystals of [(R₈-calix-pyrrole)[(CH₂=CHO)Li]-[Li(thf)₂Sm]₂(μ-CH₂CH₂)] (**4a, b**) may be isolated in good yield. The magnetic moment measured at room temperature for **4b** (μ_{eff} = 1.40 μ_B per metal center) supports the premise of complete oxidation to Sm^{III}. In each case crystals suitable for X-ray analysis were obtained.

The structure of **4a** consists of a dimer lying on an inversion center where each samarium atom is surrounded by the calix-pyrrole ligand (Figure 2). One C₂ unit is located between the two metal centers in a distorted side-on fashion (Sm–C(37) 2.427(5), Sm–C(37A) 2.711(5) Å), but still forms a planar Sm–C–C–Sm core. On the opposite side of the



Scheme 1.

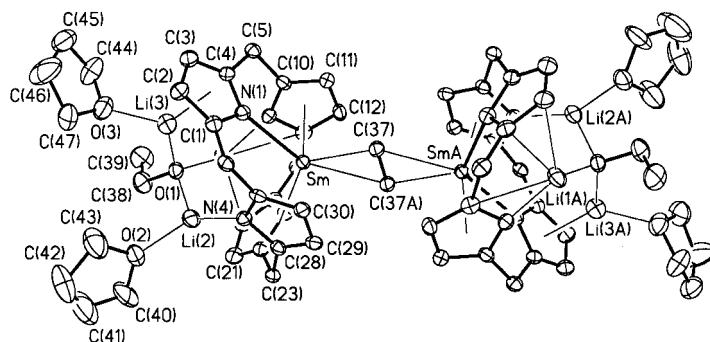


Figure 2. ORTEP drawing of **4a**. Thermal ellipsoids are drawn at the 30% probability level. Bond lengths [Å] and angles [°]: C(37)–C(37A) 1.493(15), Sm–C(37) 2.427(5), Sm–C(37A) 2.711(5), Sm–N(2) 2.738(4), Sm–N(4) 2.720(3), Sm–N(1) 2.549(4), Sm–C(10) 2.855(4), Sm–C(11) 2.899(9), Sm–C(12) 2.892(9), Sm–C(13) 2.853(4), N(2)–Li(1) 2.014(9), N(4)–Li(2) 2.024(9), Li(3)–N(1) 2.512(10), Li(3)–C(1) 2.434(10), Li(3)–C(2) 2.318(10), Li(3)–C(3) 2.312(10), Li(3)–C(4) 2.433(10), O(1)–Li(1) 1.860(9), O(1)–Li(2) 1.886(9), O(1)–Li(3) 1.859(9), O(1)–C(38) 1.399(6), C(38)–C(39) 1.427(8); C(37)–Sm–C(37A) 33.0(2), centroid–Sm–centroid 165.1(3), N(2)–Sm–N(4) 122.4(2), O(1)–C(38)–C(39) 114.6(5).

cavities defined by each of the calix-pyrroles, three lithium atoms are bonded to the pyrrolyl rings. The C–C bond length of the coordinated ethylene group is rather long (C(37)–C(37A) 1.487(10) Å) and indicative of the presence of a C–C single bond. Thus, in combination with the light color, this suggests the complete reduction of ethylene and oxidation of the two metal centers. The crystal structure was of sufficient quality to locate and refine the hydrogen atoms attached to the coordinated ethylene group. According to the positions of the hydrogen atoms, the ethylene molecule is perpendicular to the Sm₂ vector. The hydrogen atoms attached to each carbon atom deviate from the ethylene plane and point in the opposite direction with respect to the neighboring samarium atom, thus conferring to the carbon center a distorted tetrahedral geometry (Sm–C(37)–H(37A) 112.9°, Sm–C(37)–H(37B) 115.4°, H(37A)–C(37)–H(37B) 107.2°). This indicates that ethylene predominantly adopted a σ - rather than π -bonding mode which is also consistent with the remarkable elongation of the C–C bond and the zigzag conformation of the Sm₂C₂ core. In spite of the presence of significant Sm–C_{ethylene} σ -bond character, the fixation of ethylene is a reversible process as indicated by the behavior of solutions of both **4a** and **4b**. Addition of pale green crystals of **4** to C₆D₆ gave dark green solutions for which the peak for free ethylene was clearly visible at $\delta = 5.23$ in the ¹H NMR spectrum. In addition, the resonances characteristic of **2** were also clearly identified in the NMR spectrum, although these partly overlapped with the resonance of **4**. Conversely, subsequent saturation of the same sample in the NMR tube with ethylene conferred the characteristic pale green color to the solution in the case of **4a**, while the resonances of the enolate starting complex disappeared. In the case of **4b**, however, the solutions always remained dark green and the peaks of the starting **3b** were invariably present in the NMR spectra. The peaks for coordinated ethylene in complex **4a** were clearly identified by using C₂D₄ and are present as two resonances of equal intensity at $\delta = 19.08$ and 18.97 in the ²H NMR spectrum.

In spite of the dominant σ character of the Sm–C_{ethylene} bond, this complex does not insert further ethylene under normal reaction conditions and does not perform polymerization, which strikingly contrasts with the fact that decamethylsamarocene is an ethylene polymerization initiator.^[12]

Experimental Section

2b: A suspension of [SmCl₃(thf)₃] (3.20 g, 6.80 mmol) in THF (150 mL) was treated with colorless [(R₈-calix-pyrrole){Li(thf)}₄] (R = {–(CH₂)₅–}_{0.5}) (6.80 mmol). The resulting golden-yellow suspension was heated gently for about 10 min and then stirred at room temperature for 2 h. Lithium foil (70 mg, 10 mmol) was added to the reaction mixture under argon, and the color changed from yellow to deep green over 30 min. After stirring the mixture for 18 h, the solvent was removed in vacuo and the resulting deep green solid residue was extracted with diethyl ether (75 mL). The extract was concentrated to 50 mL and left to stand at –30 °C overnight, which afforded large dark green crystals of **2b** (75 %). Crystals suitable for X-ray structural analysis^[10] were grown at room temperature upon cooling a concentrated solution of **2b** in THF. IR (Nujol mull): $\tilde{\nu} = 3090(\text{w})$, 2730(w), 2662(w), 1550(w), 1355(m), 1315(w), 1286(s), 1270(s), 1256(s), 1191(s), 1140(m), 1074(s), 970(m), 845(m), 780(s) cm^{–1}; elemental analysis calcd for SmLi₃ClC₅₆H₈₀N₄ (%): C 62.28, H 7.47, N 5.19; found: C 63.22, H 8.31, N 5.88; $\mu_{\text{eff}} = 3.70 \mu_{\text{B}}$.

3a: The procedure was analogous to that for **2b**. Yield 79 %. IR (Nujol mull): $\tilde{\nu} = 3090(\text{w})$, 2723(w), 1625(m), 1585(m), 1322(s), 1260(s), 1195(s), 1156(w), 1104(w), 1046(s), 975(s), 925(w), 888(s), 799(s), 756(s) cm^{–1}; elemental analysis calcd for Sm₂Li₆C₁₀₄H₁₆₆N₈O₉ (%): C 62.06, H 8.21, N 5.56; found: C 61.94, H 7.91, N 5.57; $\mu_{\text{eff}} = 3.62 \mu_{\text{B}}$; ¹H NMR (500 MHz, C₆D₆, 23 °C): $\delta = 14.53$ (s, 4H, CH pyrrole), 14.22 (br. s, 4H, CH₂ ether), 8.73 (s, 4H, CH pyrrole), 5.35 (t, 6H, CH₃ ether), 3.85 (br. s, 8H, THF), 3.75 (s, 4H, CH₂ ethyl), 1.20 (s, 4H, CH₂ ethyl), 0.97 (s, 8H, THF), –2.98 (s, 12H, CH₃ ethyl), –4.67 (br. s, 1H, enolate), –5.01 (s, 12H, CH₃ ethyl), –6.95 (br. s, 1H, enolate), –7.03 (s, 8H, CH₂ ethyl), –15.62 (br. s, 1H, enolate).

4b: A stirred solution of [SmI₂(thf)₂] (2.0 g, 3.65 mmol) in THF (75 mL) was treated with [(R₈-calix-pyrrole)Li]₄ (2.23 g, 3.66 mmol). The resulting red-brown solution was refluxed for 2 h then stirred at room temperature overnight. The solvent was removed in vacuo to yield a deep green solid residue which was extracted with hexane (100 mL). Upon exposure of the deep green hexane solution to ethylene (1 atm), the color changed to a lighter green. After the solution had been left to stand at room temperature for two days, light green crystals separated (1.5 g, 0.78 mmol, 43 %). IR (Nujol mull): $\tilde{\nu} = 3088(\text{w})$, 2731(w), 2664(w), 1564(w), 1350(m), 1309(s), 1267(s), 1240(s), 1185(s), 1140(m), 1070(s), 1040(s), 978(m), 898(s), 877(s), 830(m), 777(s), 740(s), 690(m) cm^{–1}; elemental analysis calcd for Sm₂Li₆C₁₀₂H₁₃₈O₈N₈ (%): C 62.08, H 7.65, N 5.16; found: C 61.30, H 7.00, N 5.45; $\mu_{\text{eff}} = 1.40 \mu_{\text{B}}$. A similar procedure was followed for the preparation of **4a**. Yield (39 %). The extreme air-sensitivity and spontaneous loss of ethylene prevented satisfactory analytical data from being obtained. ¹H NMR (500 MHz, C₆D₆, 23 °C): $\delta = 19.08$ (br. s, 2H, ethylene), 18.97 (br. s, 2H, ethylene), 8.60 (br. s, 8H, THF), 7.17 (s, 16H, CH pyrrole), 4.01 (br. s, 8H, CH₂ ethyl), 3.47 (br. s, 8H, THF), 2.13 (br. s, 8H, CH₂ ethyl), 1.25 (br. s, 8H, CH₂ ethyl), 1.22 (br. s, 8H, CH₂ ethyl), –0.66 (br. s, 24H, CH₃ ethyl), –2.68 (br. s, 24H, CH₃ ethyl), –4.53 (br. s, 2H, enolate), –6.75 (br. s, 2H, enolate), –15.60 (br. s, 2H, enolate).

Crystal data **3a**: C₁₀₄H₁₆₆Li₆N₈O₉Sm₂, *M*_r = 2014.79, triclinic *P* $\bar{1}$, *a* = 13.6634(2), *b* = 15.7346(2), *c* = 27.7960(5) Å, $\alpha = 100.779(1)^\circ$, $\beta = 91.537(1)^\circ$, $\gamma = 91.893(1)^\circ$, *V* = 5863.9(2) Å³, *Z* = 2, $\rho_{\text{calcd}} = 1.141 \text{ Mg m}^{-3}$, absorption coefficient 10.42 cm^{–1}, *F*(000) = 2120, 38 577 reflections collected, 16 227 independent reflections, GOF = 1.011, *R* = 0.0715, *wR*₂ = 0.1560. All non-hydrogen atoms were located and refined anisotropically. Hydrogen atoms were introduced at their idealized positions and refined with a riding mode. Absorption corrections (SADABS) were applied to the data. Two crystallographically independent but chemically equivalent molecules and one molecule of diethyl ether were found in the unit cell.

4a: C₉₄H₁₃₈Li₆N₈O₈Sm₂, *M*_r = 1818.46, orthorhombic, *Pbca*, *a* = 20.926(2), *b* = 20.614(2), *c* = 21.577(2) Å, *V* = 9308(1) Å³, *Z* = 4, $\rho_{\text{calcd}} = 1.298 \text{ Mg m}^{-3}$, absorption coefficient 13.04 cm^{–1}, *F*(000) = 3792, 73 045 reflections collected, 6089 independent reflections, GOF = 1.024, *R* = 0.0318, *wR*₂ = 0.0869.

All non-hydrogen atoms were located and refined anisotropically. Hydrogen atoms were located from different Fourier maps and refined with a riding mode. 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-101917 and CCDC-101918. 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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Directed Metalation Linked to Transition Metal Catalyzed Cascade Reactions: Two Total Syntheses of Plicadin, the Alleged Coumestan from *Psoralea plicata**^{*}

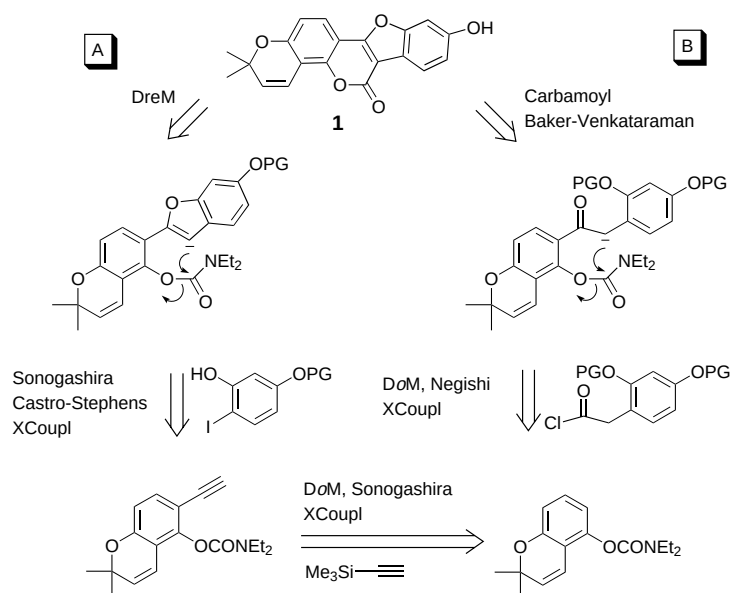
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Plicadin, isolated in 1991 from the herb *Psoralea plicata*^[1] and assigned structure **1** (see Scheme 1),^[2] has a compact, oxygen-rich heterocyclic structure that poses a synthetic challenge from the perspective of combined directed *ortho* metalation/directed remote metalation (DoM/DreM) and transition metal catalyzed coupling strategies, a current theme in our laboratories.^[3] Herein we report two syntheses of plicadin (**1**) which incorporate effective use of the following sequences: a) DoM/DreM, Sonogashira, and Castro–Stevens reactions (Scheme 1, retrosynthesis A), and b) DoM, Negishi, and carbamoyl Baker–Venkataraman reactions^[4] (retrosynthesis B). We also demonstrate inconsistencies between physical and spectral data of the synthetic material and those reported for the natural product isolated from *Psoralea*

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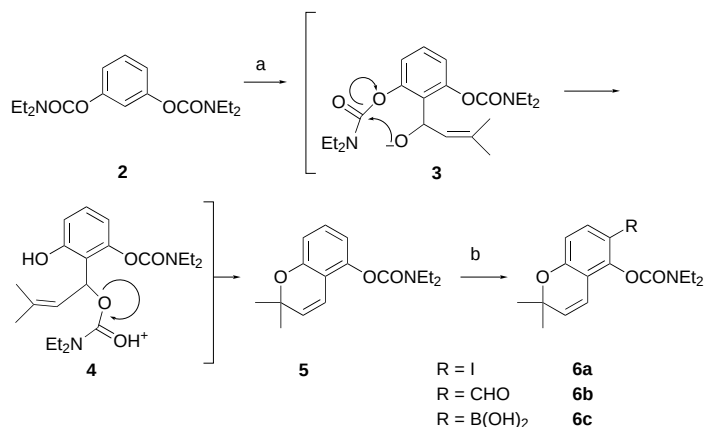
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Scheme 1. Retrosynthetic analysis and strategies for the total synthesis of plicadin (1). XCoupl = cross-coupling, PG = protecting group.

plicata and named plicadin. In addition to presenting short and didactic routes which diverge broadly from classical construction modes for coumestans,^[2] this work suggests new synthetic methodologies of potentially general value: a) a regioselective synthesis of chromenes not based on a Friedel–Crafts reaction (2→5); b) original carbamate metalation chemistry (5→6), and c) metalation, Sonogashira, and Castro–Stephens cascade reactions (Scheme 2).

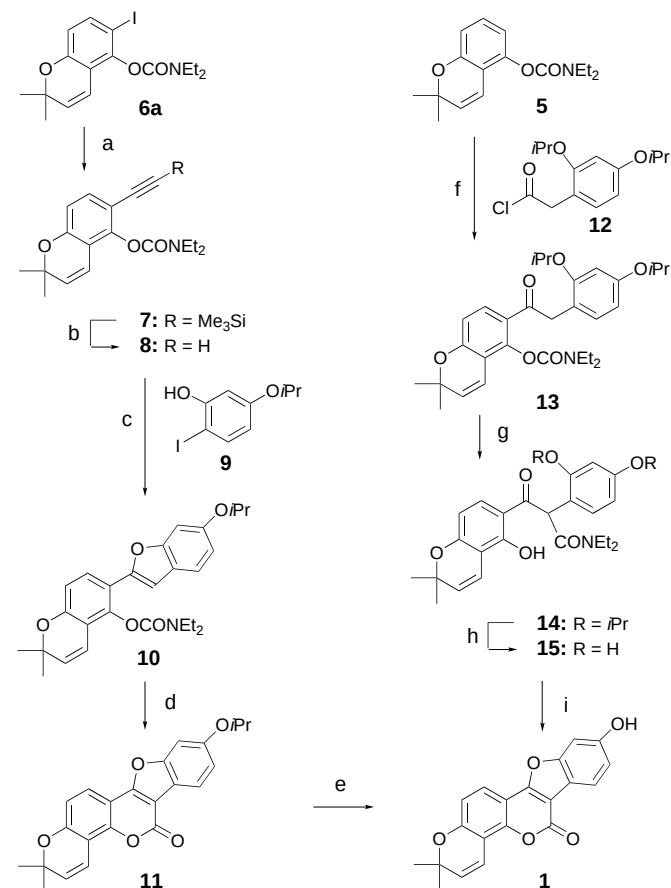


Scheme 2. Synthesis and metalation of chromene 5. a) *t*BuLi (1.1 equiv), THF (*c* = 0.5 M), −78 °C/30 min; then Me₂C=CHCHO (1.3 equiv), −78 °C/15 min → 25 °C/1 h; then HOAc (3 equiv), 0 → 25 °C/1 h, 58%; b) *s*BuLi (1.2 equiv), THF (*c* = 0.25–0.5 M), −78 °C/1 h; 6a: then I₂ (1.3 equiv), −25 °C/1 h, 82%; 6b: then DMF (4 equiv), −25 °C/1 h, 85%; 6c: then B(OMe)₃ (3 equiv), −25 °C/1 h, quantitative.

The chromene carbamate 5, which is common to both routes, was readily constructed in good yield from resorcinol dicarbamate 2 by a quench experiment with *t*BuLi and senecialdehyde followed by acetic acid catalyzed cyclization (Scheme 2). This one-pot sequence takes advantage of regioselective cooperative DoM^[3a] and intramolecular O-to-

O carbamoyl transfer (3) to facilitate formal S_N2' carbamate expulsion (4) and provide a substituted chromene (5), which cannot be obtained regioselectively by classical tactics based on electrophilic aromatic substitution.^[5]

A second DoM on 5 with *s*BuLi followed by an electrophilic quench furnished substituted derivatives 6a–c regioselectively and in high yields. Sonogashira coupling of 6a under conditions of [PdCl₂(PPh₃)₂]/CuI catalysis^[6] led to 7, which upon desilylation gave the acetylenic chromene 8 (Scheme 3).



Scheme 3. Syntheses of 1. a) Me₃SiC≡CH (2 equiv), [PdCl₂(PPh₃)₂] (0.05 equiv), CuI (0.05 equiv), NEt₃/MeCN (4/1), 50 °C, 2 h, 92%; b) K₂CO₃ (0.1 equiv), MeOH, 25 °C, 3 h, 86%; c) 9 (1.1 equiv), [Pd(OAc)₂(PPh₃)₂] (0.05 equiv), CuI (0.05 equiv), DMF/NEt₃ (1/1), 80 °C, 45 h, 44%; d) LDA (3.5 equiv), THF, 0 °C, 15 min, then HOAc, reflux, 10 min, 84%; e) BCl₃ (7.7 equiv), CH₂Cl₂ (*c* = 0.005 M), 25 °C, 10 h, 50%; f) *s*BuLi (1.2 equiv), THF, −78 °C, 1 h, then ZnCl₂ (1.2 equiv), −78 °C, 15 min, then 12 (1.4 equiv), [PdCl₂(PPh₃)₂] (0.05 equiv), DIBALH (0.1 equiv), toluene, 0 °C, 2 h, 25 °C, 1 h, 74% (85% based on recovered 5); g) NaH (2.5 equiv), toluene, reflux, 2 h, 88%; h) BCl₃ (5 equiv), CH₂Cl₂ (*c* = 0.05 M), −78 → −10 °C, 1 h, then MeOH quench at −78 °C, 25 °C, Rochell's salt workup, 99%; i) PTSA (2 equiv), MeOH, reflux, 6 h, 49%. LDA = lithium diisopropylamide, DIBALH = diisobutylaluminum hydride; PTSA = *p*-toluenesulfonic acid.

Reaction with the *o*-iodophenol 9^[7] under [Pd(OAc)₂(PPh₃)₂]/CuI catalysis resulted in a sequential Sonogashira/Castro–Stephens process^[8] to give the benzofuran 10 in acceptable overall yield. Delightfully, the sequence of DreM, carbamoyl migration, and acid-catalyzed cyclization^[3] proceeded in 84% yield to give the coumestan 11. Deprotection of 11 with BCl₃^[9]

concluded the synthesis of plicadin (**1**) according to retrosynthesis A in seven steps from **2** in 6.8% overall yield.

Retrosynthesis B (Scheme 1) was commenced by Negishi acylative cross-coupling of chromene carbamate **5** with protected acid chloride **12**, prepared by Willgerodt–Kindler reaction from the corresponding acetophenone,^[10] to give the ketone **13**. Carbamoyl Baker–Venkataraman reaction under NaH conditions afforded the ketoamide **14**,^[4] which was effectively deprotected with BCl₃ to yield triphenol **15**. Acid-catalyzed cyclization of **15** concluded the second synthesis of plicadin (**1**) in five steps from **2** in 20.5% overall yield.

Examination of the reported data for plicadin^[1] showed several discrepancies with those obtained for the synthetic material. A melting point of 117 °C (EtOH/benzene) was reported for plicadin, whereas sublimation at 290 °C (EtOH/benzene) was observed for the synthetic material. With the exception of the splitting patterns for three signals in the ¹H NMR spectrum (see Table 1), which may be a result of

In summary, the purported plicadin (**1**) has been obtained by two efficient and comparable syntheses. The syntheses are showcases for directed *ortho* and remote metalation and transition metal catalyzed reactions (Negishi, Sonogashira, and Castro–Stephens) and demonstrate the tactical advantages of tandem sequencing.

Experimental Section

5: To a cooled (–78 °C) solution of **2** (14.92 g, 48.2 mmol) in THF (100 mL, *c* = 0.5 M) was added *t*BuLi (34 mL, 57.5 mmol, *c* = 1.71 M in pentane) over 1 h; the internal temperature was kept below –75 °C. The reaction mixture was stirred for 15 min, 3-methyl-2-butenal (6.3 mL, 65 mmol) was added over 30 min (internal temperature below –74 °C), and the mixture was stirred for 1 h at –78 °C. The reaction mixture was allowed to warm to room temperature, stirred for 1 h, cooled to 0 °C, sequentially treated with acetic acid (7.2 mL, 125 mmol) and brine solution (100 mL), and stirred for 1 h at room temperature. The organic phase was separated, the aqueous phase was extracted with Et₂O (3 × 50 mL), and the combined organic extracts were evaporated under vacuum. Excess aldehyde was removed under high vacuum, the residue was dissolved in Et₂O (150 mL), and the solution was dried (Na₂SO₄), passed through a plug of silica gel, and distilled to provide **5** as a thick yellow oil (7.16 g, 54%); b.p. (bath temp) 120–140 °C (0.1–0.25 Torr). IR (neat): $\tilde{\nu}$ = 2977, 1722, 1638, 1611, 1460, 1418, 1277, 1225, 1156, 1116, 1060, 758 cm^{–1}; ¹H NMR (250 MHz, CDCl₃): δ = 7.06 (t, *J* = 8.1 Hz, 1H), 6.64 (d, *J* = 8.1 Hz, 1H), 6.63 (d, *J* = 8.1 Hz, 1H), 6.38 (d, *J* = 10.0 Hz, 1H), 5.62 (d, *J* = 10.0 Hz, 1H), 3.47–3.37 (m, 4H), 1.43 (s, 6H), 1.29–1.17 (m, 6H); ¹³C NMR (50.3 MHz, CDCl₃): δ = 153.8, 153.6, 147.1, 130.8, 128.5, 116.4, 114.7, 114.4, 113.3, 75.9, 42.2, 41.8, 27.8, 14.3, 13.3; MS (EI): *m/z* (%): 275 (26) [*M*⁺], 261 (18), 260 (46), 161 (22), 160 (25), 101 (15), 100 (100), 72 (40); HR-MS (EI): calcd for C₁₆H₂₁NO₃: 275.1521, found: 275.1523. (Carrying out the same reaction on a 20-mmol scale and at a concentration of 0.2 M gave **5** in 58% yield.)

6a: To a cooled (–78 °C) solution of **5** (3.79 g, 13.73 mmol) in THF (30 mL, *c* = 0.46 M) was added *s*BuLi (12.9 mL, 16.48 mmol, 1.28 M in cyclohexane) over 1 h; the internal temperature was kept below –75 °C. After stirring the mixture for 1 h at –78 °C, a solution of I₂ (4.53 g, 17.85 mmol) in THF (20 mL) was added and the reaction mixture was warmed to room temperature (6 h) and treated with saturated NH₄Cl solution (5 mL). THF was removed under vacuum, Et₂O (50 mL) and H₂O (20 mL) were added, and the organic layer was separated, dried (Na₂SO₄), and concentrated under vacuum. The residue was purified by flash column chromatography (EtOAc/hexanes 3/17) to afford **6a** as a yellow oil (4.50 g, 82%). IR (neat): $\tilde{\nu}$ = 2947, 1726, 1415, 1270, 1051 cm^{–1}; ¹H NMR (200 MHz, CDCl₃): δ = 1.17–1.35 (m, 6H), 1.40 (s, 6H), 3.36–3.49 (m, 4H), 5.62 (d, *J* = 10.0 Hz, 1H), 6.30 (d, *J* = 10.0 Hz, 1H), 6.47 (d, *J* = 8.6 Hz, 1H), 7.44 (d, *J* = 8.6 Hz, 1H); ¹³C NMR (62.9 MHz, CDCl₃): δ = 153.7, 152.1, 147.0, 137.2, 131.4, 116.6, 116.5, 115.7, 80.1, 76.1, 42.1, 41.9, 27.6, 14.3, 13.1; MS (EI): *m/z* (%): 401 (75) [*M*⁺], 386 (70), 274 (75), 100 (100), 72 (97); HR-MS (EI): calcd for C₁₆H₂₀INO₃: 401.0489, found: 401.0494.

1: A solution of triphenol **15** (350 mg, 0.82 mmol) and *p*-toluenesulfonic acid monohydrate (313 mg, 1.65 mmol) in MeOH (30 mL) was heated under reflux for 6 h and concentrated under vacuum to about 10 mL. The resulting precipitate was collected by filtration, washed with cold MeOH (2 × 5 mL), and dried under vacuum to provide **1** as a yellow-green solid (136 mg, 0.41 mmol, 49%). Recrystallization gave yellow crystals; m.p. 289–290 °C (sublim, EtOH); m.p. 290 °C (sublim, EtOH/benzene). IR (KBr): $\tilde{\nu}$ = 3379 (br), 1717, 1645, 1628, 1601 cm^{–1}; ¹³C NMR (62.9 MHz, [D₆]DMSO): δ = 159.0, 157.2, 156.9, 156.0, 155.1, 148.4, 131.8, 121.2, 120.6, 114.3, 114.2, 114.0, 113.5, 109.1, 105.5, 102.5, 98.6, 77.4, 27.6; MS (EI): *m/z* (%): 334 (29) [*M*⁺], 320 (22), 319 (100), 318 (7), 160 (8); HR-MS (EI): calcd for C₂₀H₁₄O₅: 334.0841, found: 334.0854.

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Table 1. ¹H NMR data observed for synthetic **1** and reported for plicadin.^[a]

δ (1) ^[b]	δ (plicadin) ^[c]
10.04 (s, 1H)	— ^[d]
7.71 (d, <i>J</i> = 8.5 Hz, 1H)	7.76 (d, <i>J</i> = 8.5 Hz, 1H)
7.68 (d, <i>J</i> = 8.4 Hz, 1H)	7.70 (d, <i>J</i> = 8.4 Hz, 1H)
7.14 (d, <i>J</i> = 2.0 Hz, 1H)	7.12 (d, <i>J</i> = 1.8 Hz, 1H)
6.93 (dd, <i>J</i> = 8.4, 2.0 Hz, 1H)*	7.00 (d, <i>J</i> = 10 Hz, 1H)***
6.87 (d, <i>J</i> = 8.5 Hz, 1H)**	6.88 (dd, <i>J</i> = 8.5, 1.8 Hz, 1H)*
6.78 (d, <i>J</i> = 10 Hz, 1H)***	6.80 (d, <i>J</i> = 8.5 Hz, 1H)**
5.94 (d, <i>J</i> = 10 Hz, 1H)	5.81 (d, <i>J</i> = 10 Hz, 1H)
1.44 (s, 6H)	1.49 (s, 6H)

[a] The asterisks indicate the shifts for which there are discrepancies between the data reported for plicadin and those obtained for the synthetic material. [b] Measured at 500 MHz in [D₆]DMSO. [c] Measured at 400 MHz in [D₆]DMSO. [d] Not reported.

inadvertent inversion in the assignment, all signals and coupling constants are in agreement within experimental error with those reported (Table 1).^[1] The structure of the synthetic material was fully confirmed by an X-ray crystallographic analysis (Figure 1).^[11]

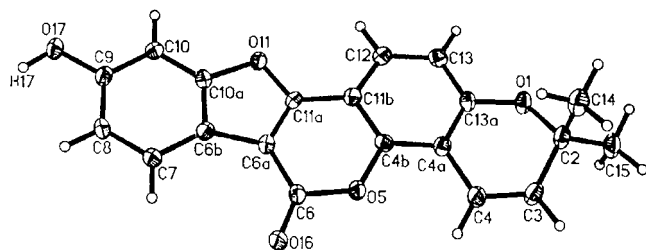


Figure 1. Crystal structure of **1**.

The discrepancy in the melting points cannot be explained and requires clarification by Amad, Malik, and co-workers. Additional comparison by ¹³C NMR spectroscopy was not feasible owing to the absence of this data in the original report.^[1, 12] Therefore, the structure of the compound isolated from *Psoralea plicata* and named plicadin remains uncertain.

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- [11] 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-101777. 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).
- [12] Despite numerous attempts, we were unable to obtain an authentic sample, a copy of the original ¹H NMR spectrum, and ¹³C NMR data from the authors.

Biodegradable Polymeric Materials—Not the Origin but the Chemical Structure Determines Biodegradability

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Dedicated to Professor Hans-Jürgen Quadbeck-Seeger on the occasion of his 60th birthday

Biodegradable polymeric materials (BPMs) are of ever increasing interest because of the many problems associated with solid wastes. As early as the end of the 1970s first attempts had been made to develop polymeric materials that combine good usage properties (such as thermoplastic processing ability for films, fibers and moulding) of conventional plastics and biodegradability by microorganisms.^[1] In the meantime, such “bio-plastics” have become commercially available or are in the pre-marketing phase.^[2] It is in principal possible to fall back on different sources of raw materials to produce such polymers. Thus, it is possible to distinguish between BPMs based on renewable or on petrochemical sources. Examples of such renewable sources are starch derivatives and starch blends, polyhydroxybutyrates, polylactic acid, cellophane, cellulose derivatives, caseine, and chitin polymers. BPMs produced from petrochemical resources are, for example, aliphatic polyesters such as polycaprolactone and polybutylene succinate, aromatic–aliphatic copolyesters, and polyester amides.

Whereas the biodegradability of BPMs based on renewable resources is plausible, there were doubts as to whether polymers based on petrochemical sources could also be mineralized by a natural process caused by microorganisms such as bacteria or fungi.

Aliphatic polyesters were extensively investigated as to their biodegradability.^[3–5] The primary cleavage of the usually insoluble polymer chain takes place outside of the microorganisms and is catalyzed by specific enzymes that are produced in the cell and excreted by microorganisms such as bacteria or fungi. The microorganisms are then able to take up the fragments in their cells and utilize them as food.

Examinations of the biodegradability of aliphatic–aromatic copolyesters have only been known for a few years.^[6–8] The chemical structure of such copolyesters examined in the literature is shown in Scheme 1. Such copolyesters are a very interesting class of substances in reference to the assessment of their biodegradation behavior because of their high aromatic component content. The introduction of the aromatic component is useful for improving physical and

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Copolyester	x	y	Abbreviation ^[a]
$\left[\text{I} - (\text{CH}_2)_y - \text{O} - \text{C}(=\text{O}) - (\text{CH}_2)_y - \text{C}(=\text{O}) - \text{O} - / m - (\text{CH}_2)_x - \text{C} - \text{C}(=\text{O}) - \text{C}_6\text{H}_4 - \text{C}(=\text{O}) - \text{C} \right]_n$			
poly[(ethylenehexandioate)-co-(ethyleneterephthalate)]	2	4	ETA
poly[(trimethylenehexandioate)-co-(trimethyleneterephthalate)]	3	4	PTA
poly[(trimethylenedecandioate)-co-(trimethyleneterephthalate)]	3	8	PTS
poly[(tetramethylenehexandioate)-co-(tetramethyleneterephthalate)]	4	4	BTA

Scheme 1. Formula, nomenclature, and abbreviations of the copolyesters. [a] E: 1,2-ethanediol, P: 1,3-propanediol, B: 1,4-butanediol, A: adipic acid, S: sebacic acid, T: terephthalic acid.

mechanical properties. The amount of terephthalic acid in these copolyesters was up to about 30 wt. % (ca. 25 mol %). The first investigations for proving biodegradability concentrated on the weight losses of specimens. Sheetting was usually buried in soil or compost and the weight losses were determined after exposure to the medium (Figures 1 and 2).^[9] According to Ottow both soil and compost are degradation matrices that provide a wide microbial spectrum.^[10] All

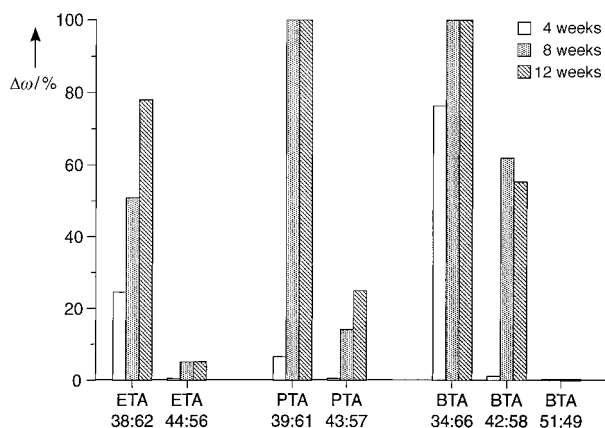


Figure 1. Weight loss (Δw) of the copolyesters during burial in soil.

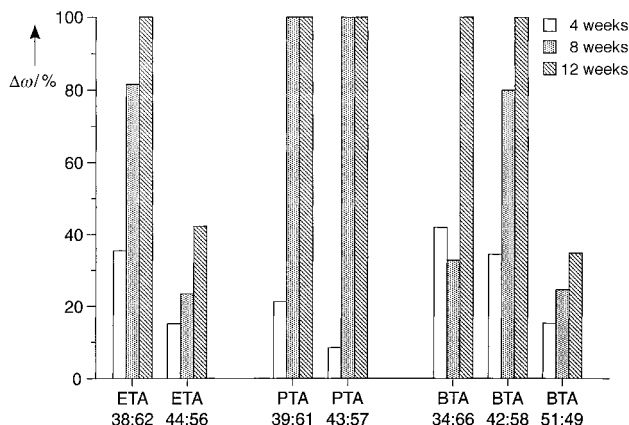


Figure 2. Weight loss (Δw) of the copolyesters during compost tests.

copolyesters investigated were attacked in soil and in compost within 12 weeks (except BTA 51:49 in soil). No significant influence of the diol components used on biodegradability of the copolyesters was observed. In all cases the content of terephthalic acid had a significant influence on the rate of degradation. Reliable statements on the mineralization of a polymer, that is, the formation of natural intermediates caused by metabolism of the microorganisms, cannot only be made by reference to weight losses.

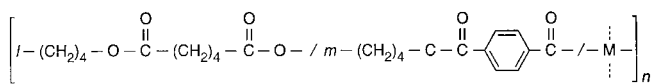
A technical standard, DIN V 54900, recently became available for the determination of the biodegradability of polymeric materials. The tests carried out in accordance with this standard show whether a polymeric material is biodegraded (mineralized) or disintegrated into compost components (Table 1) under the conditions of a controlled composting

Table 1. DIN V 54900—Investigation of compostability of polymeric materials.

Description
part 1 chemical test
part 2 evaluation of biodegradability of the polymeric materials in laboratory tests
part 3 testing of compostability under conditions of practical relevance including compost quality assessment

procedure. Different procedures are described in part 2 of the standard for the evaluation of complete biodegradability. According to the “controlled composting test” a defined quantity of polymer is mixed with compost and the amount of carbon dioxide generated by microbial conversion is measured. As a control the same amount of compost with cellulose instead of the polymeric material and a sample of the same compost without ingredients are also tested.

A type of copolyester marketed by BASF AG since early 1998 under the trademark Ecoflex[®] (Scheme 2) was examined according to the standard mentioned above.^[11] The results



Scheme 2. Formula of the copolyester Ecoflex[®]. M, for example, is a branching agent or a chain extender.

according to part 2 of the standard are shown in Figure 3. The degradation rates (amount of CO₂ produced relative to the theoretical amount), were measured in three parallel reactors. After 124 days the amounts of CO₂ were 93 % (PS1), 95 % (PS2), and 96 % (PS3). The mean value of the degradation curves is shown in Figure 3 and is 95 % at the end of the test. The result of the compostability test according to DIN V 54900, part 3 (disintegration of the material under practical composting conditions), is shown in Figure 4. The carbon of the polymer is not necessarily converted to 100 % carbon dioxide during composting, which can be explained as follows: In addition to the formation of carbon dioxide during mineralization of a substance, some parts of the carbon can be converted into biomass. Furthermore, remaining carbon could be caused by non or partial degradation of the polymer. A

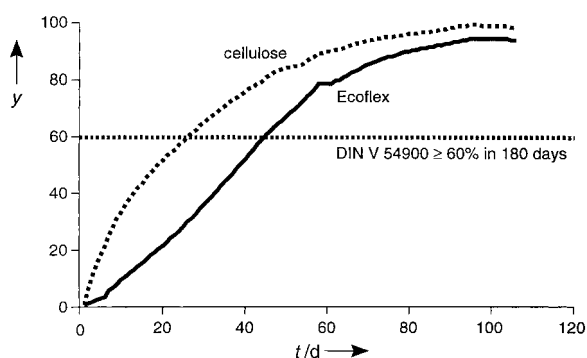


Figure 3. Biodegradability of Ecoflex according to DIN V 54900, part 2. Plastics such as Ecoflex have to be classified as fully biodegradable when at least 60 % of the total organic carbon of the polymer has been converted into carbon dioxide during a test interval of no more than 180 days. y : Amount of degradation [%] (fraction of the theoretical amount of CO_2 produced).

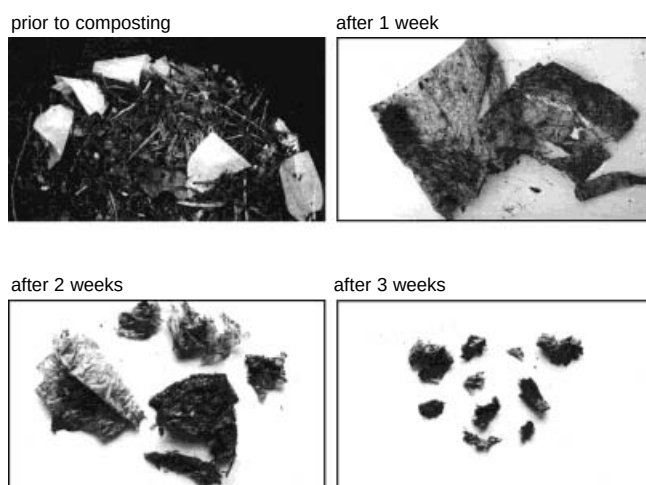


Figure 4. Investigation of the compostability of the copolyester Ecoflex® according to DIN V 54900, part 3.

sufficiently exact measurement of biomass in such a complex medium such as compost is not possible. By contrast, the measurement of residual polymer is different. The residual polymer can be quantified by gel permeation chromatography (GPC; Table 2) after taking aliquots from the compost and extracting with a suitable polymer solvent. After extraction the quantities of residual polymer in aliquots from the three parallel test reactors, with reference to the total amounts of inserted polymer, were 2.3, 1.3, and 3.4 %. The quantities of extracted polymer in the parallel control tests were between 0.00 and 0.02 per 100 g of polymer-compost mixture. Thus, these values were under the detection limit of identification of less than 0.1 g per 100 g. Altogether, in these tests (without

determination of biomass) the average rate of recovery was about 97 %.

The stoichiometric composition of the extracted residual polymer was analyzed by ^{13}C NMR spectroscopy. A significant increase in the amount of the aromatic acid would point to a slow or nondegradation of aromatic polymer components. Within the accuracy of the method employed, no change in the stoichiometric composition was observed at the end of the test. Inhomogeneities within the solid compost matrix, for example too wet, too dry or not well ventilated zones, or polymer particles that adhered to the wall of the reactor, are plausible explanations for the existence of residual polymer. The high rate of mineralization (about 95 % of the theoretical amount of CO_2 produced) shows that the material is biologically attacked and that most of it is degraded by combustion. However, no accurate conclusions can be drawn as to the degradation behavior of individual chain components.

For unequivocal proof of complete biodegradability the degradation behavior of the longer aromatic sequences in the copolyester must be proved selectively, since it is well known that high molecular weight pure polybutylene terephthalate (for example, Ultradur®) is not a biodegradable plastic. According to Cowie the following theoretical sequence distributions result from a random copolyester with an acid ratio of 50/50 (Figure 5).^[12] As shown in Figure 5, from 100 M_1 and 100 M_2 sequences of the 50/50 copolyester 50 single, 25 double, 12 triple, and only 6 quadruple sequences are in the chain. If the polymer chain is cleaved by biodegradation, longer aromatic sequences might remain as nondegradable or difficult to degrade residues (Scheme 3).

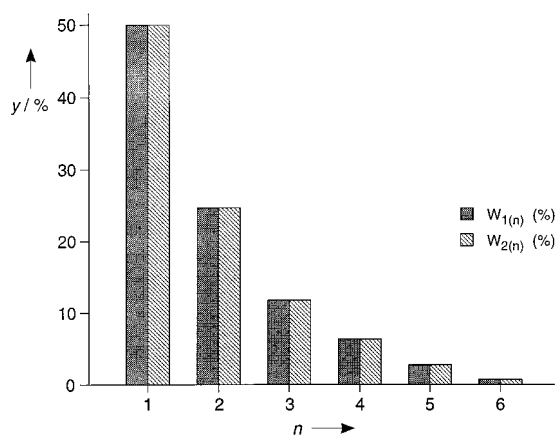
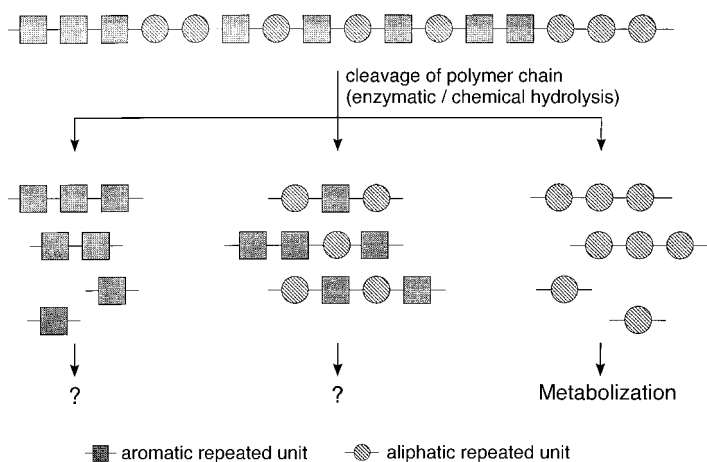


Figure 5. Sequence distributions of a copolymer. Ratio of monomers $[M_1]:[M_2] = 50:50$. $W_{1(n)}$ = Number [%] of the M_1 sequences as a function of the sequence length n ; $W_{2(n)}$ = number [%] of the M_2 sequences as a function of the sequence length n .

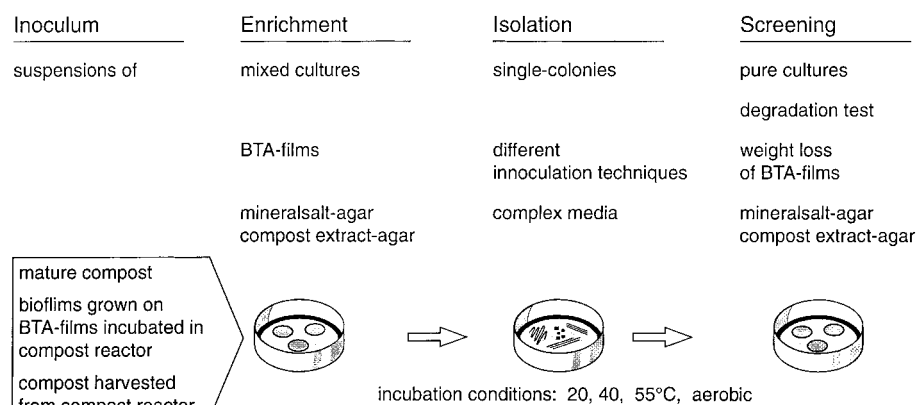
Table 2. Analysis of residual polymer from 100 g of a dried mixture of compost and polymer for the investigation of compostability of the copolyester Ecoflex according to DIN V 54900, part 3.

Sample	Initial amount of polymer [g/100 g mixture]	Residual polymer [g/100 g mixture]			Residual polymer [%] average
		measurement 1	measurement 2	average	
PS1	15	0.39	0.30	0.35	2.3
PS2	15	0.15	0.22	0.19	1.3
PS3	15	0.57	0.44	0.51	3.4



Scheme 3. Degradation of an aliphatic-aromatic copolyester with a random distribution of the monomers along the polymer chain.

To answer the question of complete biodegradability special microorganisms that are able to degrade the copolyester were isolated from compost (Scheme 4).^[13] It was possible to use these organisms in an aquatic test system,



Scheme 4. Scheme for screening and isolation of microorganisms that are able to degrade the copolyester.

which is an excellent medium for analyzing polymeric residues. The polymer chain of the copolyester Ecoflex was cleaved with these microorganisms within a few days, and at the end of the test only water soluble intermediates were found. Although the cleavage of the copolyester chain was very fast, the microorganisms were not able to mineralize the fragments. The intermediates therefore accumulated in the aqueous media. The tests were stopped at different times and the water-soluble fragments produced were characterized by GC/MS after derivatization (Table 3) to show the course of the degradation. It could be shown that the isolated microorganisms are able to degrade the polymer chain completely into the monomers. A subsequent complete metabolization of the monomers was possible by adding a mixed culture of microorganisms, which represented the whole spectrum of ubiquitous microorganisms, from the compost to the test solution (Table 3, Test 4). In these test solutions no intermediates were detected by GC analysis.

Table 3. Fragments of Ecoflex after degradation with isolated pure strain (test 1–3) and with pure and mixed cultures from compost (test 4). × = detected; – = not detected.

Test ^[a]	Monomers ^[b]			Aliph. oligomers ^[b]		Arom. oligomers ^[b]	
	B	A	T	BA	ABA	BT	BTB
1	×	×	×	×	×	×	×
2	×	×	×	×	×	–	–
3	×	×	×	–	–	–	–
4	–	–	–	–	–	–	–

[a] Test 1: 1750 mg polyester in 80 mL media. Intermediates from isolated pure culture after 21 days. Enzyme activity stopped by pH shift (in situ building of high amounts of acids during degradation). Test 2–4: 350 mg polyester in 80 mL media. Intermediates from isolated pure culture after 7 days (test 2) and after 21 days (test 3). Test 4: Residues after a 7-day inoculation by isolated pure culture and a 14-day inoculation with compost eluate. [b] A: Adipinic acid, B: 1,4 butanediol, T: terephthalic acid. Intermediates from the cleavage of M (see Scheme 4) are not considered. According to test 4 no intermediates were detectable by GC analysis.

The process in which individual organisms are able to perform only one degradation step, and other organisms the next, and that the microorganisms live off the metabolic products of others is called symbiosis (which means different

organisms living together to their mutual benefit). It has not been proved that a symbiosis really happened in this case. It is likely that the copolyester structure “accidentally” looks like the structure of natural substrates. This means that the polymer chain could be degraded by the excreted enzymes, but the microorganisms are not able to digest the soluble fragments in their cells.

It could be shown with the described degradation tests that microorganisms that are ubiquitously present in compost are able to degrade materials from petrochemical sources, for example, the aliphatic-aromatic copolyester Ecoflex®, into natural products. Consequently, what is deci-

sive for biodegradability is only the structure and not the origin of the raw material.

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Radical Dimerization of 5,5'-Diphenyl-3,3',4,4'-tetramethoxy-2,2'-bipyrrole: π Dimer in the Crystal, σ Dimer in Solution.**

Andreas Merz*, Jürgen Kronberger, Lothar Dunsch, Andreas Neudeck, Andreas Petr, and Laszlo Parkanyi

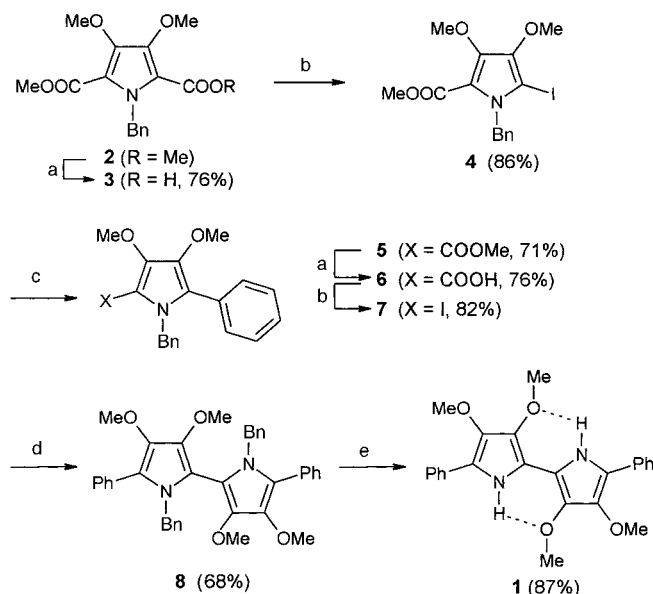
Dedicated to Professor Gottfried Märkl
on the occasion of his 70th birthday

The highly electron-rich dimethoxypyrrole^[1] (DMOP) can be electrochemically polymerized to give good conducting films with conductivities that exceed those of all other 3-, 3, 4-, or *N*-substituted pyrroles, and often even of polypyrrole itself.^[2] According to ESR and spectroelectrochemical studies the conductivity of poly-DMOP might involve a transport of the charge carriers not only along the polymer chain but also across neighboring chains by “interchain hopping”, similar to the situation in crystalline TTF derivatives and salts of arene cations where π stapling occurs.^[3]

Spectroelectrochemical experiments with end-capped pyrrole,^[4] thiophene,^[5, 6] or mixed thiophene–pyrrole^[7] oligomers provide evidence for equilibria between the corresponding radical cations and spinless π dimers. On the other hand a detailed investigation of certain diphenyl polyenes by cyclic voltammetry suggested that the corresponding radical cations dimerize to σ dimers and not to π dimers.^[8] In the cited work^[8]

the existence of π dimers of pyrrole and thiophene oligomers was questioned. Recently, the radical cation of ω,ω -diphenyl- α -terthiophene was shown by an X-ray structure to form endless π staples in which π dimers with shorter distances can be recognized.^[9] We now report on diphenyltetramethoxypyrrole **1** whose radical cation crystallizes as a spinless π dimer ($1^{+\cdot} \cdot \text{PF}_6^-$) but dimerizes as a σ dimer in solution at low temperature.

The bipyrrole **1** is obtained as shown in Scheme 1 from easily accessible pyrrole **3**.^[1c] The important steps are the partial hydrolysis of the of the ester **2**^[10] and the iododecarboxylation of carboxylic acids **3** and **6**.^[11] The introduction of



Scheme 1. a) *t*BuOK/H₂O (1/1), THF, 0 °C, 12 h; b) I₂/KI, Na₂CO₃, 12 h, RT, extraction with Et₂O; c) PhB(OH)₂, Pd[PPh₃]₄, 1,2-dimethoxyethane; d) Cu powder, melt, 200 °C; e) 1. Na, liquid NH₃, 2. EtOH.

the phenyl end group is achieved by a Suzuki coupling^[12] and an Ullmann coupling in the melt to give **8**.^[13] The benzyl group is an ideal protective group for the pyrrole nitrogen atom because a twist in the bond between the pyrrole units makes the intermediates less prone towards oxidation. The removal of the benzyl groups in **8** is achieved smoothly with Na in liquid NH₃ and quenching with aqueous NaHCO₃.^[14] Using analogous steps higher oligopyrrole homologues with or without end caps are obtained from **2**.^[15]

The bipyrrole **1** crystallizes from a solution in CH₃OH in two different polymorphic crystals. At 90 °C the tetragonal needles **1a** turn, without melting, into the monoclinic cuboids of **1b**, which melt at 171–172 °C. Both crystal structures (Figure 1)^[16] are dominated characteristically by hydrogen bonds between the NH protons of the pyrrole rings and the oxygen atoms of the inner methoxy groups. In **1b** the almost planar *D*_{2h} molecules are stabilized in an *anti* conformation of the pyrrole rings by H bonds. In the tetragonal modification **1a** the *syn* oriented bipyrroles are arranged with a 90° turn along a *C*₂ axes by intermolecular H bonds. The H bonds are also seen in the ¹H NMR spectra of **1** as a broad singlet at $\delta = 8$ ($\delta = 7$ for the corresponding signal in DMOP^[1a]).

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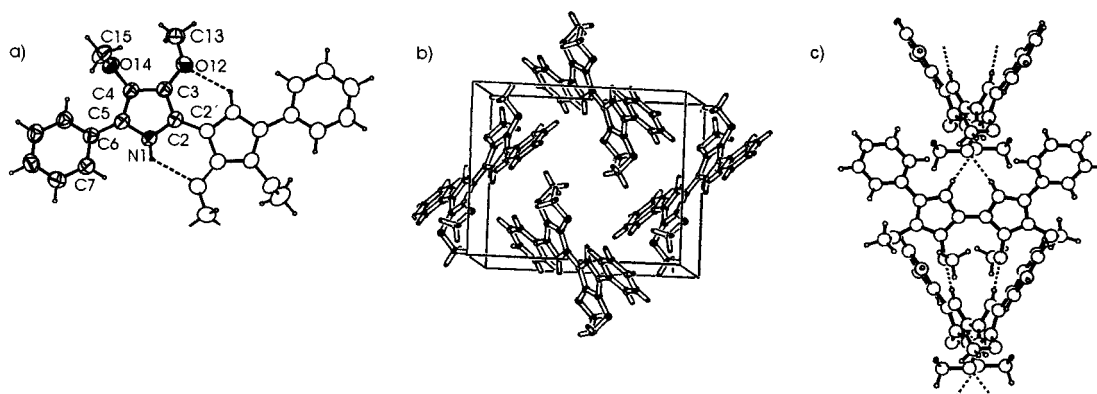


Figure 1. Structures of **1a** and **1b** in the crystal. a) Molecule of the monoclinic modification **1b**. b) Arrangement of **1b** in the unit cell. c) Arrangement of the molecules of the tetragonal modification **1a** along the crystallographic C_2 axis. For bonding parameters see Table 1.

The first redox potential of **1** is only +0.11 V (versus Ag/AgCl), hence **1** is easily oxidized to the radical cation $1^{+\bullet}$ by ferrocenium hexafluorophosphate ($E = +0.49$ V) in CH_3CN . Bluish black, electrically nonconducting needles crystallize from the green solution at -20°C , and an X-ray structure analysis of the crystals was resolved.^[16] The crystal (Figure 2)

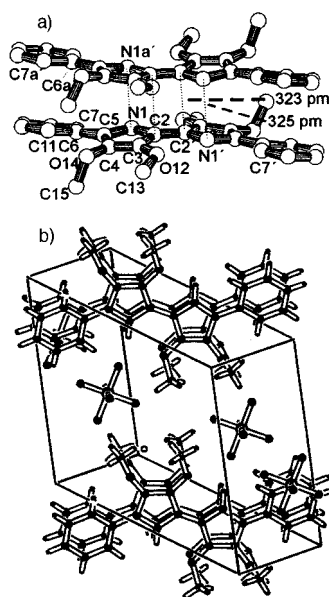


Figure 2. Structure of the π dimer $(1^{+\bullet} \cdot \text{PF}_6^-)_2$ in the crystal. a) View of the centrosymmetric π sandwich. b) Position of the π -dimeric units in the lattice. For bonding parameters see Table 1.

contains narrowly spaced, sandwich-type centric π dimers $(1^{+\bullet} \cdot \text{PF}_6^-)_2$. Contrary to many π -staple crystals of radical cations of aromatic compounds^[3, 17] or tetrafulvalenes,^[3, 18] which undergo substoichiometric oxidation to their cations, the two radical cations in $(1^{+\bullet} \cdot \text{PF}_6^-)_2$ are ordered in an almost congruent manner and only slightly laterally displaced. Such an arrangement has been postulated for some π dimers in solution.^[19–21] In the above mentioned terthiophene radical cation^[9] one of the molecules is rotated by 180° around its longer axis.

The closest contact of the two radical cations in $(1^{+\bullet} \cdot \text{PF}_6^-)_2$ is found at the central bipyrrrole bond with $\text{C2} - \text{C2}' = 323$, $\text{N1} - \text{N1}' = 326$, and a central distance of the five membered rings of 344 pm, which corresponds approximately to a van der Waals contact.^[22] The structure is widened towards the phenyl groups beginning with atoms C2 and C3 of the pyrrole ring (average distance 376 pm, shortest distance 364 pm). As in **1b**, the inner methoxy groups are coplanar with the corresponding pyrrole ring, as a consequence of hydrogen bond formation, but the outer methoxy groups are rotated out of the plane and in the same direction in both molecules of the π dimer. This is clearly the reason for the deviation from planarity in the outer region of the complex. In a detailed comparison of the structural parameters of **1a**, **1b**, and $(1^{+\bullet} \cdot \text{PF}_6^-)_2$ (see Table 1) the pyrrole rings of **1a** and **1b** have ordinary pyrrole rings, while in the noncongruent halves of the π dimer the distinctly longer bond distance of $\text{C2} - \text{C3}$ (ca. 30 pm) and $\text{C4} - \text{C5}$ (ca. 50 pm) as well as the shorter bonds of $\text{C3} - \text{C4}$ (ca. 30 pm) and $\text{C2} - \text{C2}'$ (ca. 60 pm) are remarkable. The dihedral angle $\text{N} - \text{C2} - \text{C2}' - \text{N}'$ in **1b** is exactly 180° , while in the dimer this angle is 178.7° and the $\text{C3} - \text{C2}$ and $\text{C2}' - \text{C3}'$ bonds are considerably twisted. The C3 methoxy groups in **1a** and **1b** are coplanar with the $\text{C3} - \text{C4}$ bond, while in the dimer a dihedral angle of 10° is observed.

The crystals of $(1^{+\bullet} \cdot \text{PF}_6^-)_2$ are diamagnetic. In the solid state only about one thousandth of the expected ESR value for a paramagnetic ESR species is measured, thus, the dimer is not simply a tight package of radical cations. When ether is added to the green CH_3CN solution a fine black precipitate is obtained, which has a much higher paramagnetism (75 % of the theoretical value). This result suggests the presence of radical ions $1^{+\bullet}$ in an amorphous structure. To clarify if the π dimer $(1^{+\bullet} \cdot \text{PF}_6^-)_2$ is also present in solution UV/Vis and ^1H NMR investigations were undertaken and completed by cyclovoltammetric studies with synchronous measurements of UV/Vis and ESR spectra.

The UV/Vis spectrum of $(1^{+\bullet} \cdot \text{PF}_6^-)_2$ in dry CH_2Cl_2 (2×10^{-5} M under Ar) shows a long-wave band at 739 nm at room temperature as expected for a radical cation, as well as an intense band at 463 nm and a shoulder at 389 nm. Upon cooling the solution down to 173 K a new band at 430 nm reversibly rises on account of the 463 and 739 nm bands with

Table 1. Selected bond lengths [pm], bond angles [°], and dihedral angles [°].

Bond length: ^[a]	N–C2	C2–C3	C3–C4	C4–C5	N–C5	C2–C2'
1a	137	139	141	138	138	144
1b	138	138	142	138	138	145
(1 ·PF ₆) ₂	137	141	139	143	136	139
(1 ·PF ₆) ₂ ^[b]	137	142	138	141	137	139
Bonding angle:	C2–N–C5	N–C2–C3	C2–C3–C4	C3–C4–C5	C4–C5–N	C3–C2–C2'
1a	111.0	106.7	107.9	108.2	106.2	131.1
1b	110.6	107.3	107.4	108.2	106.5	130.5
(1 ·PF ₆) ₂	110.3	106.7	107.7	107.5	107.8	128.1
(1 ·PF ₆) ₂ ^[b]	110.7	106.4	107.8	108.3	106.8	129.1
Dihedral angle:	N–C2–C2'–N'	C3–C2–C2'–C3	N–C5–C6–C11	C4–C5–C6–C7	C4–C3–O14–O15	C3–C4–O12–O13
1a	40.2	45.44	16.5	16.6	1.3	104.63
1b	180.0	180.0	18.8	23.5	0.9	86.1
(1 ·PF ₆) ₂	178.7	164.1	20.0	22.0	9.9	99.9
(1 ·PF ₆) ₂ ^[b]	178.7	164.1	3.71	4.71	10.4	90.9

[a] Hydrogen bonds (N1–H1–O12): **1a**: 223, **1b**: 228; (**1**·PF₆)₂: 230 (N1), and 223 (N1'). [b] Atom numbers with apostrophes.

an isobestic point (Figure 3a). The new band seems to be in accord with the theory of the formation of the π dimer.^[4–7] After the solution has stood for some time the color of the probe bleaches out ($\lambda_{\text{max}} = 380$ and a shoulder at 351 nm).

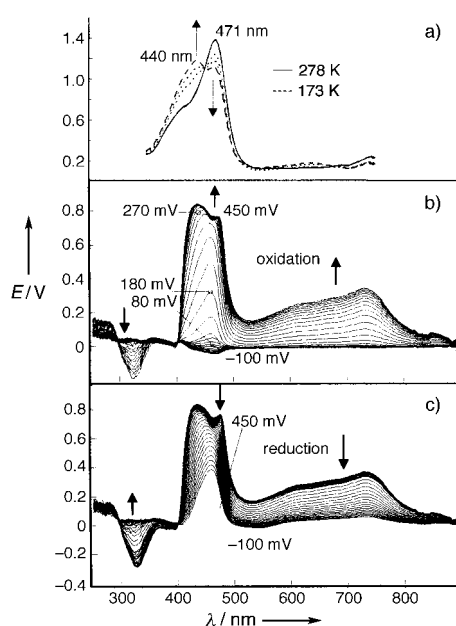


Figure 3. a) UV/Vis spectra of (**1**⁺·PF₆[−])₂ in CH₂Cl₂ at various temperatures. b) Dependence of the UV/Vis absorption on potential during the oxidation of **1** in the cyclovoltammogram between −100 and 450 mV versus Ag/AgCl. c) Corresponding trace for the reduction. In (a) and (b) the proportion of **1** initially present and of the products that are formed in a photochemical side reaction is subtracted.

Low temperature ¹H NMR spectra of (**1**⁺·PF₆[−])₂ in dry and Ar saturated CH₂Cl₂ were obtained in order to identify and characterize the equilibrium component found in the UV/Vis spectrum (Figure 4). At 298 K a spectrum with a very low intensity was found with NH and aromatic protons, as well as four methoxy signals ($\delta = 4.40, 4.15, 4.08, 3.71$) in the ratio 1:5:6 (see spectrum at 273 K). No signals of **1**⁺ and species in

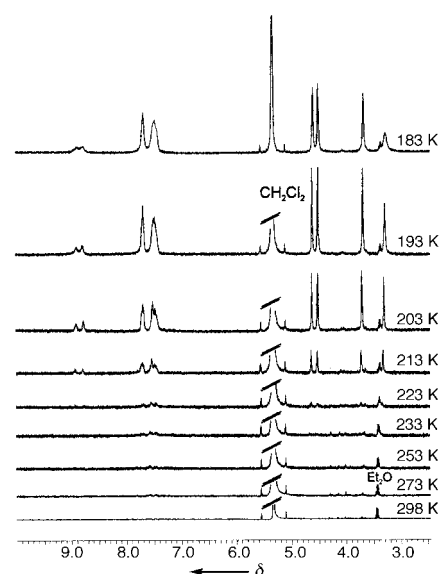


Figure 4. ¹H NMR spectra of a solution of (**1**⁺·PF₆[−])₂ in CD₂Cl₂ between 298 and 183 K.

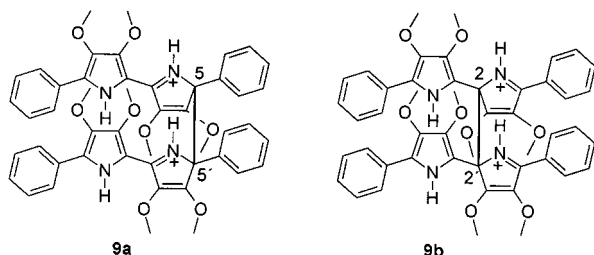
equilibrium with it can be observed because of paramagnetic broadening. A new set of proton signals starts to grow out of the background at 223 K and is intensified on further cooling (Figure 4). The intensity can be estimated from the signal corresponding to a trace of diethyl ether.

Surprisingly, the new low temperature spectrum again shows four methoxy signals together with two NH signals and two aromatic multiplets ($\delta = 4.61, 4.49, 3.68, 3.28, 7.69, 7.47, 8.90, 8.86$; relative intensities 3:3:3:3:2:1:1). Clearly this spectrum is not the one of paramagnetic **1**⁺. On the other hand, one would expect only two methoxy and one NH signals for the π dimer as a result of the free rotation of the methoxy groups in solution. A simultaneous broadening of the the singlets at $\delta = 3.28$ and 8.86 in the spectrum recorded at 183 K shows that such molecular motions just begin to freeze at very low temperature.

In a cyclovoltammetric study of a related thiophene derivative a σ dimerization has been postulated based on

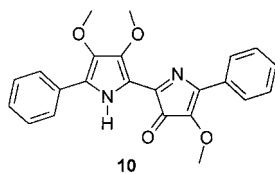
kinetic and thermodynamic data and a PM3 calculation of the σ dimer.^[8b]

Now, in the case of 1^{+} , a σ dimer **9a** or **9b** has for the first time been unequivocally observed by the symmetry of the spinless low temperature species. The π dimer ($1^{+} \cdot \text{PF}_6^{-}$)₂ is



not observed in the low temperature NMR spectrum, it may be present in a very low concentration or in rapid equilibrium with 1^{+} . The fact that only one of eight isomeric σ dimers (including three pairs of enantiomers) is formed may suggest that the C–C bond formation of the radical cations to the σ dimer is preceded by an association step via the π dimer. The observed UV/Vis spectrum (Figure 3a) at low temperature must thus be related to the σ dimer.

After prolonged standing of the solution or addition of traces of water to the probe an NMR spectrum is obtained similar to the 273 K spectrum accompanied by many small signals. The UV/Vis spectrum of this probe is identical to the final spectrum mentioned above. Follow-up reactions of the σ dimer may involve addition of water and partial deprotonation to give several isomers. In the FAB mass spectrum of the solution only the initial radical cation 1^{+} is seen. Attempts to isolate the modified σ dimer were unsuccessful, only a follow-up product with three methoxy groups was found (EI-MS: $m/z=390$), which could for example be **10**.



The electrochemical oxidation of **1** and the follow-up reactions of 1^{+} were studied by recording the UV/Vis spectroelectrochromograms and simultaneous ESR spectra with a specially designed cell^[23] with laminated gold electrodes^[24] (Figure 3b, c and 5). The cyclovoltammograms recorded in

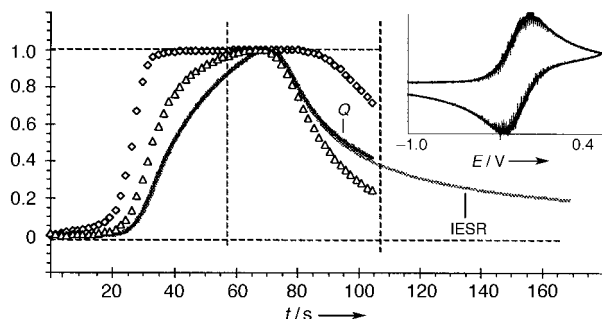


Figure 5. Dependence of the UV/Vis absorptions at 464 ($\diamond$) and 735 nm ($\triangle$), the charge flow Q (black line), and the ESR intensity (grey line) on the potential in the course of a cyclovoltammogram. All four units are normalized with respect to the maximal values. The corresponding CV is shown at the upper right corner.

situ, which were the same as those recorded with conventional disk electrodes, show two reversible waves, only the first of which is shown in Figure 5. The measured ESR intensity is synchronous with the charge flow (Figure 5). The spin concentration n_{ESR} calculated from the calibrated ESR signals shows the concentration of the cation radicals is too low by a factor of about 5 relative to the charge flow for the determined amount of reaction product. The ESR intensity and charge flow vary identically with time, and since a peak current ratio of unity is found in the cyclovoltammogram, the reduced radical concentration cannot arise from an irreversible chemical follow-up reaction. Consequently, there must be a subsequent equilibrium with a spinless dimer.

The calibrated UV/Vis absorption of 1^{+} (739 nm) follows the same intensity–time relation as the charge and ESR curves, with the absorption at 464 nm being more complex. In Figures 3a and 3b the formation of two new bands between 400 and 500 nm can be seen. Only at the beginning of the oxidative branch of the cyclovoltammogram is the 464 nm band of 1^{+} observed, afterwards it is superimposed by the blue-shifted band of the spinless dimer at 430 nm. From the reduction curve (Figure 4c) the chemical and electrochemical reversibility of the entire process is demonstrated.

Experimental Section

1: A solution of **8** (0.578 g, 1 mmol) in Et_2O (2 mL) was added to a solution of Na (0.184 g, 8 mmol) in NH_3 . After stirring the solution at -78°C for 3 h a small amount of aqueous NaHCO_3 was added. The NH_3 was removed by a N_2 stream and the residue washed with H_2O (3×1 mL). It was then dissolved in CH_2Cl_2 and filtered through Al_2O_3 III. The solvent was removed, and the residue recrystallized from MeOH (0.35 g, 87%) to give needle (**1a**) and cuboid crystals (**1b**, m.p. $171-172^\circ\text{C}$). IR (**1a**, KBr): $\tilde{\nu}=3340\text{ cm}^{-1}$ (s, NH); IR (**1b**, KBr): $\tilde{\nu}=3430\text{ cm}^{-1}$ (NH); UV/Vis (CH_3CN): $\lambda(\epsilon)=371$ (32200), 390 (27500) nm; ^1H NMR (250 MHz, CDCl_3 , basic Al_2O_3): $\delta=8.36$ (2H, brs, NH), 7.60 (4H, d, $J=7.6$ Hz, Ph(H2,H2')), 7.39 (4H, pseudo-t, $J=7.6$ Hz, Ph(H3,H3')), 7.18 (2H, pseudo-t, $J=7.2$ Hz, Ph(H4)), 4.04 (6H, brs⁺ 3,3'-OCH₃), 3.88 (6H, s, 4,4'-OCH₃); ^{13}C NMR (63 MHz, CDCl_3 , basic Al_2O_3): $\delta=137.14$ (C4,C4'), 135.05 (C3,C3'), 131.77 (Ph(C1)), 128.85 (Ph(C3,C3')), 125.51 (Ph(C4)), 123.61 (Ph(C2,C2')), 115.85 (C5,C5'), 111.39 (C2,C2'), 61.18 (3,3',4,4'-OCH₃); EI-MS (70 eV): m/z (%): 404 [M^{+}] (95), 389 [$M-\text{CH}_3$]⁺ (100); FAB-MS (glycerol): m/z (%): 809 [$2M+H$] (<1), 405 [$M+H$] (87), 404 [M^{+}] (100), 389 [$M-\text{CH}_3$]⁺ (33).

(**1**· PF_6)₂: A solution of ferrocenium hexafluorophosphate (33 mg, 0.1 mmol) in CH_3CN (1 mL) was added to **1** (40 mg, 0.1 mmol) in CH_3CN (1 mL) at RT. The resulting green solution was washed with hexanes (3×5 mL) to remove excess ferrocene and kept for 48 h at -20°C under exclusion of light and moisture. This produced (**1**· PF_6)₂ (40 mg, 72%) as bluish-black needles, which above 250°C decompose without melting. IR: $\tilde{\nu}=3430$ (NH), 2995, 2938, 2854, 1544, 1372, 1013, 830 (PF), 777, 688, 558 cm^{-1} ; MS (FAB, CH_2Cl_2 /nitrobenzyl alcohol matrix): m/z : 404 [1^{+}]

The preparation of precursors **1-8** is given in the supporting information.

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- [16] Crystal structures: **1a** (tetragonal isomorph): $C_{24}H_{24}N_2O_4$, $M_r = 404.47$, yellow crystals from MeOH, m.p. 171–172 °C; crystal dimensions: $0.50 \times 0.13 \times 0.13$ mm; tetragonal, space group $I4_1cd$ (No. 110), $a = 18.831(3)$, $b = 18.831(3)$, $c = 11.639(2)$ Å, $\alpha = 90^\circ$, $V = 4127.3(12)$ Å³, $Z = 8$, $\rho_{\text{calcd}} = 1.302$ g cm⁻³; $F(000) = 1712$; $\mu_{\text{Cu}} = 7.3$ cm⁻¹; $\theta_{\text{max}} = 74.97^\circ$; 4372 determined, 2058 independent, 1918 observed reflections with $(F_o^2 > 2\sigma F_o^2)$; $R1 = 0.0260$, $wR2 = 0.0714$, GOF (F^2) = 1.083 for 137 parameters, residual electron density 0.026/–0.092 e Å⁻³. **1b** (monoclinic isomorph): $C_{24}H_{24}N_2O_4$, $M_r = 404.47$, yellow crystals from MeOH, m.p. 171–172 °C; crystal dimensions: $0.20 \times 0.20 \times 0.09$ mm; monoclinic, space group $P2_1/n$ (No. 14), $a = 7.943(1)$, $b = 10.499(1)$, $c = 12.7930(1)$ Å, $\alpha = 90^\circ$, $\beta = 107.00(1)^\circ$, $V = 1020.24(19)$ Å³, $Z = 2$, $\rho_{\text{calcd}} = 1.317$ g cm⁻³; $F(000) = 428$; $\mu_{\text{Cu}} = 7.3$ cm⁻¹; $\theta_{\text{max}} = 75^\circ$; 2200 determined, 2017 independent, 1662 observed reflections ($F_o^2 > 2\sigma F_o^2$); $R1 = 0.0358$, $wR2 = 0.1183$, GOF (F^2) = 1.004 for 139 parameters, residual electron density 0.215/–0.132 e Å⁻³. **(1 · PF₆)₂**: $(C_{24}H_{24}N_2O_4F_6P)_2$, $M/2 = 549.43$, bluish-black needles, decomp. > 250 °C; crystal dimensions: $0.25 \times 0.08 \times 0.03$ mm; triclinic, space group $P\bar{1}$ (No. 2), $a = 8.36(1)$, $b = 11.220(1)$, $c = 13.067(1)$ Å, $\alpha = 111.78(1)$, $\beta = 96.23(1)$, $\gamma = 90.56(1)^\circ$; $V = 1129.8(8)$ Å³, $Z = 2$, $\rho_{\text{calcd}} = 1.444$ g cm⁻³; $F(000) = 510$; $\mu_{\text{Mo}} = 1.021$ cm⁻¹; $\theta_{\text{max}} = 59.99^\circ$; 3304 determined 3304 independent, 1813 observed reflections ($F_o^2 > 2\sigma F_o^2$); $R1 = 0.0496$, $wR2 = 0.1234$, GOF (F^2) = 0.908 for 339 parameters, residual electron density 0.297/–0.216 e Å⁻³. The crystallographic data (excluding structure factors for the structures reported in this paper have been deposited with the Crystallographic Data Centre as supplementary publication nos CCDC-102195 (**1a**), -102194 (**1b**), and -102196 (**1 · PF₆**)₂. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44)1223-336-033; e-mail deposit@ccdc.cam.ac.uk).
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Topological Links between Duplex DNA and a Circular DNA Single Strand**

Heiko Kuhn,* Vadim V. Demidov,* and Maxim D. Frank-Kamenetskii*

DNA is well known to adopt various topological (and pseudotopological) structures like knots, catenanes, Borromean rings, and pseudorotaxanes.^[1] It has long been recognized that DNA topology plays a crucial role in such fundamental biological phenomena as DNA supercoiling and topoisomerization.^[2] Another reason for the considerable interest in higher order DNA topology structures stems from the realization that DNA topological and pseudotopological forms may provide stable and sequence-specific targeting of DNA. Accordingly, highly localized DNA detection and precise spatial positioning of various ligands on the DNA scaffold become possible. This may lead to new applications in molecular biotechnology, in gene therapy, and in the emerging field of DNA nanotechnology.^[1d, 3]

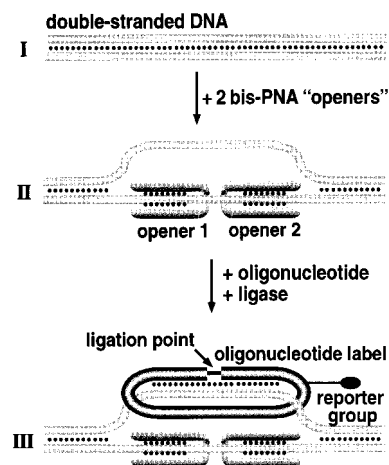
In this connection, one of the promising DNA pseudotopological constructions is the DNA padlock, consisting of a long single-stranded (ss) DNA molecule forming a pseudorotaxane with a short cyclic oligodeoxyribonucleotide (cODN).^[4] Another interesting pseudorotaxane-type structure, the sliding clamp, contains a short cODN threaded on double-stranded (ds) DNA.^[1c] Notwithstanding the value of the indicated pseudotopological structures for DNA labeling, note that in these constructions the cODN tag is allowed to slide along the target for considerable distances, compromising the precision of spatial positioning of the label.

We have assembled a new supramolecular structure, a linked DNA pseudorotaxane, in which part of a cODN appears to be threaded sequence specifically between complementary strands of dsDNA (see Scheme 1 and Figure 1 a). Our structure forms a true topological link as long as the

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participating dsDNA macromolecule retains its native duplex conformation. In this case, the cODN tag will remain correctly positioned along the double helix during various possible postassembly manipulations. Scheme 1 shows the successive steps of our assembly. First of all, we open dsDNA at two closely located sites using a pair of peptide nucleic acid (PNA) openers.^[5, 6] The PNA openers are bound to one of the two DNA strands, leaving the opposite strand displaced and thus accessible for hybridization with an ODN.^[5a]



Scheme 1. Major steps to assemble topologically linked pseudorotaxane and catenane (**III**) on dsDNA (**I**). First, the target sequence within dsDNA (linear or ccDNA) is opened at two closely located sites by strand invasion of a pair of PNA clamps. As a result, structure **II** forms, in which one of two dsDNA strands becomes accessible for hybridization with an ODN. The ODN may contain one or more reporter groups, and it is designed in such a way that its termini are complementary to the exposed DNA target and are in juxtaposition upon hybridization. After hybridization, the ODN is circularized by enzymatic ligation.

Like in the case of the padlock assembly,^[4a] we design a linear ODN which contains an unrelated sequence flanked by two stretches that are eight to ten nucleotides long and complementary to the displaced DNA strand (see the Experimental Section). After hybridization to the target, both ends of the linear ODN appear in juxtaposition and are covalently joined by DNA ligase. The resulting cODN forms almost two turns of a double helix with the displaced DNA strand and is therefore topologically linked with one of the two DNA strands. Consequently, the resulting cODN–dsDNA complex can only be separated by cleavage of the DNA backbone or by complete separation of the DNA complementary strands. Thus, a topological label resembling an earring was introduced sequence-specifically into dsDNA (see Figures 1a and 2a).

In our initial experiments we used a linear 340 base pair (bp) long dsDNA fragment carrying the target site, and analyzed the resulting complex by electrophoretic mobility shift assay (EMSA). Figure 1b (lane 3) demonstrates the formation of a specific product **III** (migrating as slow as a 1050 bp long DNA fragment) only in the presence of both T4 DNA ligase and the ODN.^[7] Our topological label carried a biotin molecule, and after streptavidin was added, specific extra retardation of **III** resulting in **III*** was observed due to the

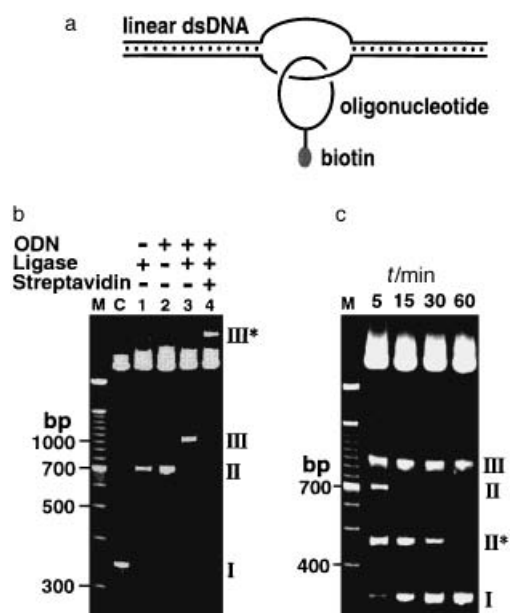


Figure 1. a) Schematic representation (not in scale) of the pseudorotaxane **III** obtained using a linear 340 bp long dsDNA target fragment. As a reporter group, a biotinylated segment was incorporated into the backbone of the oligonucleotide. b) Specific formation of structure **III** (see Scheme 1) as demonstrated by EMSA (lanes 3 and 4; see the experimental section for details). Lane C is for the fragment alone (**I**); here and below, lane M is for a 100 bp long marker (Gibco BRL). Lanes 1 and 2 correspond to control experiments. In lane 4, streptavidin was added to the resulting product before electrophoresis. Bands coinciding with 700 base pairs correspond to DNA complex **II** with both PNA openers. c) EMSA analysis of the stability of structure **III**. A mixture of **II** and **III** was heated in the ligation buffer at 65 °C for the indicated periods of time. While PNA openers gradually dissociate from **II** to yield intermediate complex **II*** (dsDNA target + one PNA opener) and the initial dsDNA target **I**, the amount of pseudorotaxane **III** remains virtually unchanged.

complexation of biotin to streptavidin (Figure 1b, lane 4). If **III** were a linked pseudorotaxane, we would expect it to be stable at elevated temperatures at which regular ODN–DNA duplexes are unstable. To check this, we heated **III** together with PNA–DNA complex **II** for one hour at 65 °C. During this period the PNA openers, which form very stable complexes with DNA,^[6] completely dissociated from DNA, while the amount of **III** remained unchanged (Figure 1c).

Only binding of all three specific ligands (two PNA openers and the ODN probe) to the dsDNA target resulted in product formation. Practically zero tolerance of the structure formation to mismatches between cODN and dsDNA target could be expected. Indeed, mismatches in the openers' binding sites can be readily discriminated kinetically at the PNA binding step.^[8] Moreover, such mismatches will affect the supramolecular assembly twice, during dsDNA opening and at the step of ODN hybridization. As a result, they have to be extremely unfavorable. One can expect a mismatch to be more tolerable when it is located between the binding sites of the PNA openers. In this case, however, the mismatch is located either directly at or very close to the ligation point, so that the weakening of the ODN–DNA complex together with the intrinsic fidelity of the ligation enzyme^[9] should prevent circularization of the ODN. In accordance with our expectation, we were unable to assemble a linked pseudorotaxane

on a dsDNA fragment carrying a single mismatch in the sequence between the two PNA binding sites (data not shown; for the mismatch position, see the Experimental Section).

We then assembled a DNA catenane with the sequence-specifically locked junction using closed-circular (cc) dsDNA plasmid as a target (Figure 2). Note that the sliding-clamp approach cannot be used for topological labeling of ccDNA

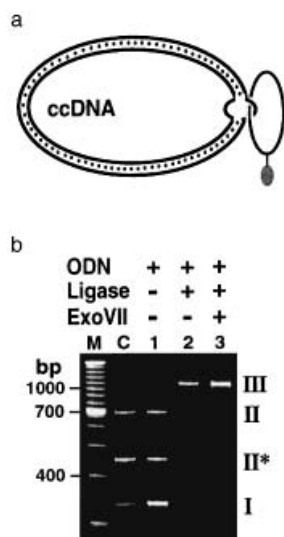


Figure 2. a) Schematic representation (not in scale) of the catenated structure obtained using the ccDNA target. b) After formation of the catenated complex with the ccDNA target, the sample was digested with *PvuII* endonuclease to perform the EMSA analysis. Complex **III** was obtained in virtually quantitative yield (lane 2). To prove circularization of the oligonucleotide, in this experiment the product was incubated with ten units of ExoVII for 1 h at 37 °C (lane 3).^[10] Lane C is a control in which the ODN was preincubated with ten units of ExoVII for 1 h at 37 °C before a ligation reaction, analogous to the reaction shown in lane 2 was performed. In this control as well as in case of control without ligase (lane 1), several bands corresponding to structures **I**, **II***, and **II** are observed due to partial dissociation of PNA oligomers from complex **II** during the enzymatic digestion.

since it requires accessible ends of the duplex DNA template.^[1c] To analyze the product by EMSA, it was digested with a restriction enzyme to generate a linear 340 bp long dsDNA fragment. Complex **III** with a characteristically reduced electrophoretic mobility similar to that in Figure 1 was observed (Figure 2b, lane 2). Compound **III** was insensitive to digestion by exonuclease VII (ExoVII, Figure 2b, lane 3).^[10] At the same time, noncircularized ODN was degraded by ExoVII (Figure 2b, lane C), providing convincing evidence for circularization of the ODN probe in this case.

Thus, all data are in agreement with the proposed formation of linked pseudorotaxane and catenane yielding sequence-specific earring labels immovably connected to the dsDNA target. The described DNA topological structures are substantially different from previously constructed pseudorotaxanes.^[1c, 4a] We have assembled a true topological link between dsDNA and cODN in such a manner that the precisely positioned label has to occupy a strictly fixed site along the

double helix. Together with remarkable sequence specificity of the structure formation, this feature should be useful for highly localized detection of various marker sequences within genomes. In addition, the PNA-directed assembly described here introduces PNA oligomers into the repertoire of DNA nanotechnological tools.

Experimental Section

By cloning of the appropriate oligonucleotide pairs into the *Bam*HI site of pUC19, target plasmid containing the sequence 5'-GATC₄T₂C-GA₂C₃T₂CT₂TGATC-3'/3'-GATCA₃GA₂G₂T₂CGA₂G₄ATC-5' (the ODN binding site is italicized, and the PNA binding sites are in boldface) was obtained. The single mismatched target contained an A–T instead of the underlined G–C base pair between the PNA binding sites. The following PNAs were used as openers: H-TC₄T₂C-eg1-(Lys)₂-eg1-JT₂J₄T-Lys-NH₂ and H-Lys₂-T₃JT₂J₂-(eg1)₃-C₂T₂CT₃-Lys-NH₂ (Lys denotes lysine, J is for pseudoisocytosine, and eg1 is for 8-amino-3,6-dioxaoctanoic acid as a linker unit). Both PNAs were synthesized as previously described,^[11] and purified by reverse-phase HPLC. Their identity was confirmed by MALDI-TOF mass spectrometry analysis. 5'-Phosphorylated ODN, 5'-GA₂G₄AT₃GT₃C-T₂AXT₂GT₃AT₃A₂GA₂G₂T₂C-3' (obtained from Operon; X denotes a biotinylated reporter segment (Ibotech) incorporated into the backbone) was used for circularization. The end stretches that are eight to ten nucleotide long and complementary to the DNA target are italicized.

To assemble linked pseudorotaxane **III**, plasmid DNA was first digested with *PvuII* endonuclease resulting in two fragments of 2367 and 340 base pairs in length, the latter being target fragment **I**. The digested DNA was then dephosphorylated by alkaline phosphatase (CIP, New England BioLabs). In a typical experiment, PNA–DNA complex **II** was obtained by addition of 10 µL of 0.1 M sodium phosphate buffer (pH 6.8), 63 µL of H₂O, and 10 µL each of 20 µM PNA solutions to 7 µL of digested, dephosphorylated plasmid DNA (7 µg, 4 pmol), and incubation for 4 h at 37 °C. Nonbound PNAs were then removed by gel filtration (Sephadex G-50, Sigma). To a 10-µL aliquot of sample containing **II** (≈0.7 µg total DNA) 2 µL of 10 × ligation buffer (Fermentas), 5 µL of H₂O, 2 µL of 4 µM ODN, and 1 µL of T4 DNA ligase (Fermentas, 30 units µL⁻¹) were added, and sequentially incubated for 2 h at 16 °C, 15 min at 45 °C, and 2 h at 16 °C. Treatment with exonucleaseVII (USB) was performed in 1 × ligation buffer by addition of 1 µL of exoVII (10 units), and incubation for 1 h at 37 °C. Before electrophoresis, samples were desalted by gel filtration, extracted with phenol/chloroform, precipitated with ethanol, and redissolved in 10 µL of TE buffer (10 mM tris(hydroxymethyl)aminomethane (Tris), 0.1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.4). Complex **III*** was obtained by incubation of 10 µL of **III**-containing sample with 2 µL of 1 mM streptavidin for 1 h at 37 °C.

To assemble the linked catenane, supercoiled plasmid DNA was used. The formation of catenated complex was performed analogous to that of **III**. In this case, the samples were cut with *PvuII* endonuclease after ligation reaction.

The EMSA analysis was performed in non-denaturing 10% polyacrylamide gels using TBE buffer (90 mM Tris, 90 mM boric acid, 2 mM EDTA, pH 8.0). The electrophoresis was run at 250 V and 20 mA for 4 h at room temperature. The gels were stained with ethidium bromide and scanned with a CCD camera using the IS-1000 digital imaging system (Alpha Innotech Corporation).

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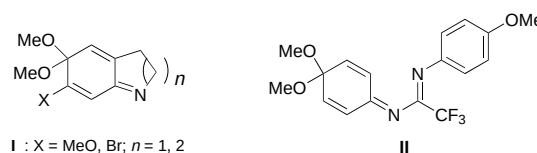
Keywords: catenanes • DNA structures • nanostructures • rotaxanes • supramolecular chemistry

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Direct Synthesis of *N*-Arylquinone Imine Acetals and Quinol Imines from Acetals**

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Although quinone imines and diimines have a long history in chemistry,^[1] their synthetic potential has not been extensively exploited.^[2, 3] The most general method for the preparation of these derivatives relies on the chemical oxidation of *p*-methoxyanilides or *p*-phenylenediamides^[3a, b] with cerium(IV) salts^[4] or Pb(AcO)₄.^[5] However, the presence of an imide (*N*-acyl or *N*-arylsulfonyl group) is required to prevent decomposition of the oxidation product. Although *N*-acylated quinone imine acetals^[6] are easily available by the anodic oxidation of substituted anilides,^[7] the electrochemical oxidation of *p*-aminophenols^[8] only allows for the in situ formation of simple quinone imines, which cannot be isolated in pure form due to their lack of stability under the oxidation conditions. The few examples of *N*-alkylquinone imines described to date refer to the labile derivatives of type **I**.^[9] Recently, compound **II**, a new type of *N*-protected quinone imine acetal, has been isolated by oxidation of the trifluoromethylamidine precursor.^[10]



In connection with a project directed towards the total synthesis of pyridoacridine alkaloids based on cycloaddition chemistry,^[11] we examined the condensation of quinone acetals with anilines. Surprisingly, we found that acetals react with anilines to yield the corresponding imines in the absence of any added catalyst.

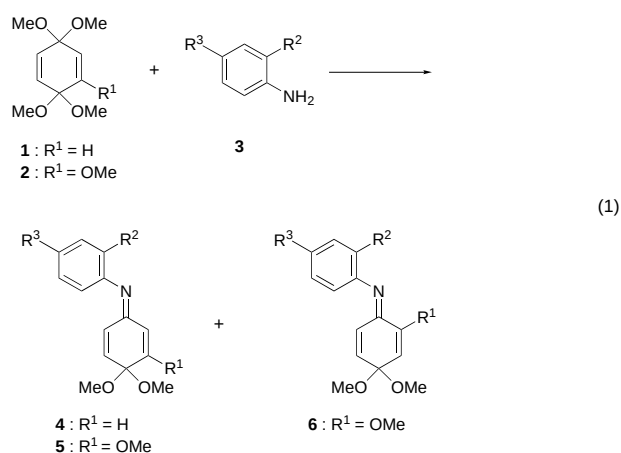
As shown in Table 1, bisacetal **1**^[12] reacted with anilines at 36–80 °C to give derivatives **4** [Eq. (1)]. The reactions were carried out without especial precautions in the presence of air. Interestingly, while *p*-methoxyaniline (**3b**) failed to react with **1** (entry 1), less nucleophilic *p*-nitroaniline (**3c**) reacted cleanly to give quinone imine monoacetal **4c** (entry 2). Reaction of **1** with *o*-aminocinnamaldehyde *N,N*-dimethylhydrazone^[13] was carried out in the absence of solvent to give **4g** (entry 3). Bisacetal **2**^[12] was more reactive than **1** and led

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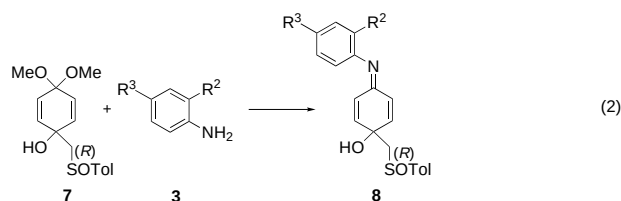
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regioselectively to compounds **5** as mixtures of *anti* and *syn* isomers in good to excellent yields (entries 4–13). In a few instances, minor amounts of regioisomers **6** were also detected in the crude reaction mixtures. Although the condensations were usually carried out in CH₂Cl₂ or CHCl₃, best results were obtained in the condensation between **2** and anilines **3f** or **3g** in methanol (entries 11 and 13). Quinol derivative **7**^[14] (Tol = tolyl = CH₃C₆H₄) reacted readily with the anilines at room temperature to afford *N*-aryl-*p*-quinol imines **8** [Eq. (2); Table 1, entries 14–17].^[15,16]



As expected, some of the condensations leading to the quinone imine acetals and quinol imines could also be catalyzed by TsOH (4-methylbenzenesulfonic acid) at room temperature. However, the reaction mixtures were less clean under these conditions, and decomposition of the imines was observed after a few hours. Significantly, one-electron oxidants such as (NH₄)₂[Ce(NO₃)₆] and AgBF₄ also catalyzed the reaction between **2** and **3b**. These results point to the formation of the aniline radical cation as the active acidic species in the catalysis.^[17, 18] Indeed, no reaction was observed between **2** and **3b** after the reagents were stirred in thoroughly deoxygenated CDCl₃. Brief exposure of the reaction mixture to air triggered the desired condensation to form **5b** (Table 1, entry 5). On the other hand, alkylamines do not condense with the acetals under these conditions, and more basic amines such as Et₃N inhibit the reaction.

Interestingly, *p*-nitroaniline (**3c**) was about five times more reactive towards diacetal **2** (room temperature, CDCl₃) than *p*-methoxyaniline (**3b**). The higher acidity of the radical cation of anilines bearing electron-withdrawing groups^[19] is in accord with the higher reactivity of otherwise less nucleophilic *p*-nitroaniline (**3c**) in the condensation reaction (compare entries 1, 2 and 5, 6). Therefore, the formation of the imines appears to be catalyzed by the aniline radical cation or the acid liberated upon its decomposition. In accordance with this proposal, in a competing experiment the more acidic radical cation of *p*-nitroaniline (**3c**) should be able to promote the formation of the imine of the more nucleophilic aniline. Indeed, when a 1:1:1 mixture of **3b**, **3c**, and **2** was allowed to react in CDCl₃ at room temperature, exclusive formation of imine **5b** was observed.

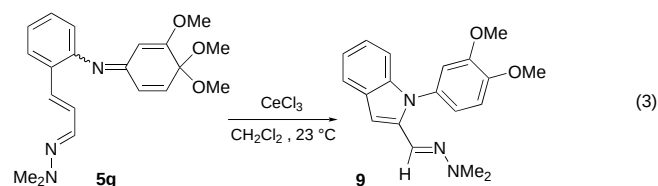
It is important to note that this procedure allows the isolation of acid-labile substrates. Thus, for example, the

Table 1. Reactions of acetals **1**, **2**, and **7** with anilines **3** [Eqs. (1) and (2)].

Entry	Acetal	Aniline	R ²	R ³	Solvent	T [°C]	Product (<i>anti</i> : <i>syn</i>)	Yield [%]
1	1	3b	H	OMe	CHCl ₃	65	— ^[a]	—
2	1	3c	H	NO ₂	CH ₂ Cl ₂	36	4c	60
3	1	3g		H	—	80	4g	35
4	2	3a	H	H	CH ₂ Cl ₂	36	5a (3:2)	68
5	2	3b	H	OMe	CHCl ₃	65	5b (2:1) ^[b]	95
6	2	3c	H	NO ₂	CHCl ₃	23	5c (3:1) ^[c]	77
7	2	3d	OMe	H	CH ₂ Cl ₂	23	5d (2:1) ^[d]	59
8	2	3d	OMe	H	CHCl ₃	65	5d (3:2)	83
9	2	3e	Br	H	CH ₂ Cl ₂	36	5e (3:2)	82
10	2	3f		H	CH ₂ Cl ₂	23	5f (3:2)	40
11	2	3f		H	MeOH	23	5f (3:2)	100
12	2	3g		H	CH ₂ Cl ₂	23	5g (2:1)	62
13	2	3g		H	MeOH	23	5g (2:1)	95
14	7	3a	H	H	CH ₂ Cl ₂	23	8a ^[e]	55
15	7	3b	H	OMe	CH ₂ Cl ₂	23	8b ^[e]	100
16	7	3c	H	NO ₂	CH ₂ Cl ₂	23	8c ^[e]	50 ^[f]
17	7	3g		H	CH ₂ Cl ₂	23	8g ^[e]	83

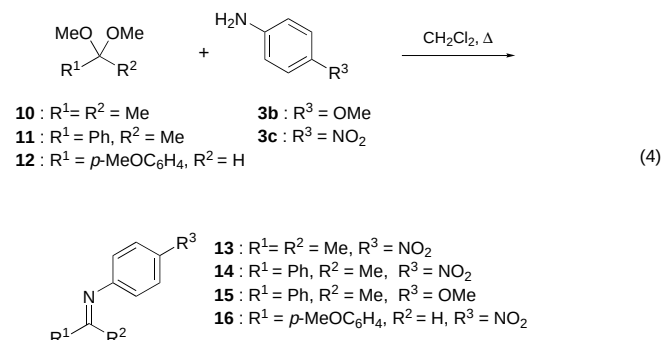
[a] No reaction was observed. [b] The minor regioisomer **6b** was also formed (10%). [c] The minor regioisomer **6c** was also formed (5%). [d] The minor regioisomer **6d** was also formed (20%). [e] A 1:1 mixture of *R,R*_{sulfur} and *S,S*_{sulfur} diastereomers. [f] Additionally, compounds **A** and **B** were obtained in 40% yield.^[16]

mixture of diastereomers **5g** (entry 13) reacted readily in the presence of protic acids to give indole **9** [Eq. (3)].^[20] After a



thorough search of acid catalysts, best results for this cyclization were obtained with CeCl_3 (15 mol %, CH_2Cl_2 , 23 °C, 64 h; 90 %). This rather remarkable transformation presumably involves the addition of an electrophilic nitrogen atom to C2 of the side chain to form a benzylic carbocation.

Simple ketone and aldehyde acetals **10–12** also react with anilines **3b** and **3c** in CH_2Cl_2 under reflux conditions to afford Schiff bases **13–16** in quantitative yields [Eq. (4)].^[21] In



accordance with the arguments advanced before, aniline **3c** was more reactive than **3b** in the condensations with acetophenone dimethyl acetal (**11**). However, when both **3b** and **3c** were allowed to react with **11** (CDCl_3 , room temperature), selective formation of **15** was observed.

In summary, we have uncovered the unprecedented reaction of acetals with arylamines to afford the corresponding imines in the absence of an added catalyst. The available evidence suggests that the reaction is catalyzed by the acidic radical cation formed in situ by the ready one-electron oxidation of the arylamine. This transformation is particularly valuable for the ready formation of acid-labile quinone imine acetals and quinol imines.

Experimental Section

General procedure for the reactions summarized in Table 1 [Eqs. (1), (2), and (4)]: A solution of acetals **1**, **2**, **7** (0.5 mmol), or **10–12** and the corresponding aniline **3** (0.5 mmol) was stirred in the corresponding solvent (5 mL) at the stated temperature for 24–48 h. When the reaction was completed (thin-layer chromatography), the solvent was evaporated and the residue was purified by flash-column chromatography (hexane/EtOAc; see the supporting information for spectroscopic data).

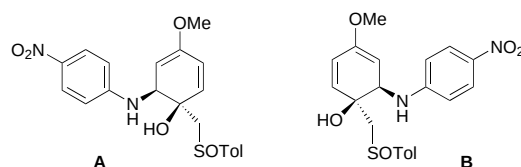
9: A mixture of **5g** (23 mg, 0.07 mmol) and CeCl_3 (4 mg, 0.01 mmol) in CH_2Cl_2 (5 mL) was stirred at 23 °C for 64 h. The mixture was partitioned between CH_2Cl_2 and water. After extractive workup and chromatography (hexane/EtOAc 7/3), indole **9** (19 mg, 90 %) was obtained as a colorless solid: m.p. 158–160 °C; ^1H NMR (300 MHz, CDCl_3): δ = 7.68–7.57 (m, 1H), 7.15–7.08 (m, 3H), 7.00–6.85 (m, 5H), 3.99 (s, 3H), 3.77 (s, 3H), 2.90 (s, 6H); ^{13}C NMR (75 MHz, CDCl_3): δ = 149.20, 148.47, 138.82, 137.64,

130.59, 128.16, 123.76, 121.67, 120.38 (2C), 111.63, 111.01, 110.00, 98.83, 56.01 (2C), 42.58 (2C) (one C signal was not observed); EI-MS: m/z (%): 323 (100, $[\text{M}^+]$), 279 (38), 265 (41); HR-MS calcd for $\text{C}_{19}\text{H}_{21}\text{N}_3\text{O}_2$; 323.1634, found: 323.1634.

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Keywords: acetals • quinones • radical ions • Schiff bases

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In accordance with the expected stereodirecting effect of the vicinal hydroxyl and bulky *p*-tolylsulfinylmethyl groups,^[16b] we tentatively assign a *cis* relationship between the hydroxyl and the aniline

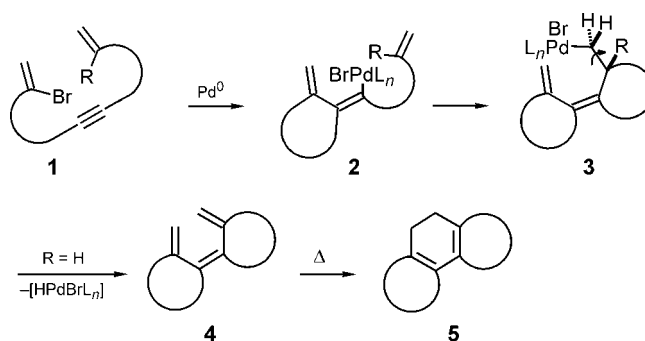
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Two New Modes of Pd-Catalyzed Domino-Tetracyclization of Bromodienynes—5-*exo-trig* Cyclization Wins over β -Hydride Elimination**

Stefan Schweizer, Zhi-Zhong Song, Frank E. Meyer, Philip J. Parsons, and Armin de Meijere*

Dedicated to Professor Heinz Georg Wagner on the occasion of his 70th birthday

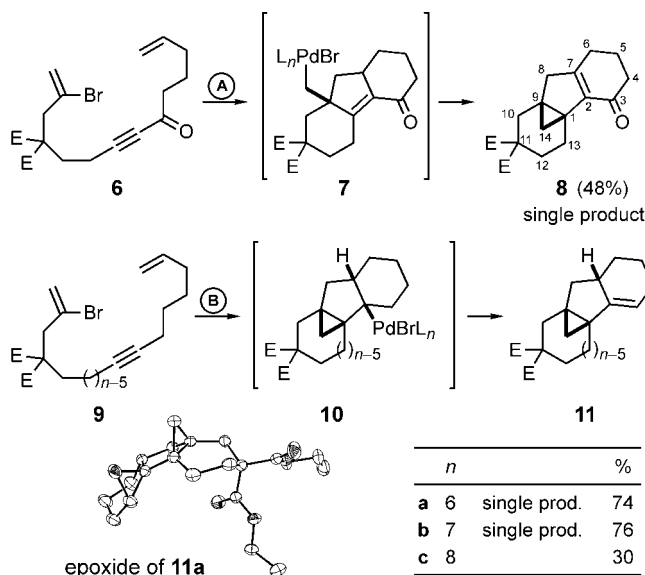
Multistep sequential transformations—domino^[1] or cascade^[2] reactions—which permit remarkable increases in molecular complexity in a single synthetic operation are gaining steadily increasing importance for the construction of complex organic molecules.^[1–3] Among them, a variety of transition metal and in particular palladium-catalyzed multi-step cascades are especially noteworthy in terms of atom economy, stereocontrol, and overall efficiency.^[4, 5] As we have previously demonstrated, 2-bromododeca-1,11-diene-6-yne and 2-bromotrideca-1,12-diene-7-yne, under palladium catalysis, cleanly undergo overall tricyclizations in a sequence of two consecutive intramolecular Heck-type couplings and subsequent 6 π electrocyclozation (Scheme 1).^[6, 7] Only when



Scheme 1.

the β -hydride elimination in the penultimate step is blocked by a substituent $R \neq H$ adjacent to the alkene terminus of the starting material **1**, does the intermediate **3** follow a different route to eventually yield a tetracyclic system with a bridging cyclopropane moiety between the A- and B-rings of its tricyclic skeleton.^[6a, 8] We now report that 2-bromotetradeca-1,13-diene-7-yne, which would have to give tricyclo[8.4.0.0^{2,7}]tetradeca-1(10),2(7)-dienes (1,2,3,4,5,6,7,8,9,10-decahydrophenanthrenes) by the usual Heck–Heck 6 π -electrocyclization sequence, in reality undergo two types of tetracyclization depending on the pattern and the nature of substitution.

When the bromodieneyne **6**^[9] was treated with palladium acetate, triphenylphosphane, and silver carbonate in acetonitrile at 80 °C, complete conversion was observed after three days, and the tetracyclo[7.4.1.0^{1,9}.0^{2,7}]tetradec-2(7)-en-3-one **8** was isolated in 45 % yield.^[10] Apparently, the alkylpalladium bromide intermediate of type **3** formed after two 6-*exo-trig* cyclizations undergoes a 5-*exo-trig* carbopalladation more rapidly than a β -hydride elimination, the neopentyl-type alkylpalladium bromide **7** then must continue to react by a 3-*exo-trig* carbopalladation before β -hydride elimination can occur (Scheme 2). The same type of tetracyclization occurred



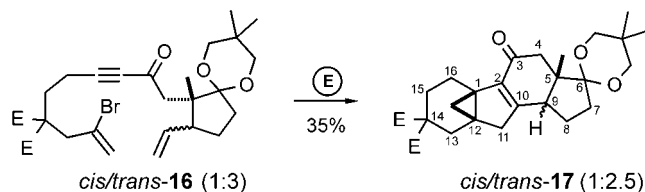
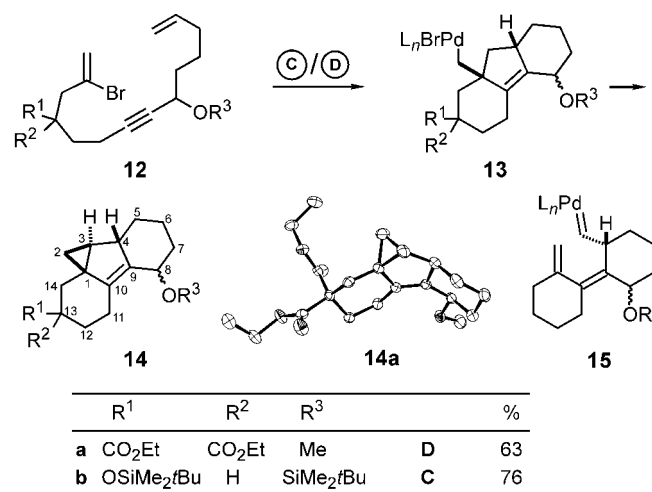
Scheme 2. A) Palladacycle^[11] (5 mol %), K₂CO₃ (2.5 equiv), *n*Bu₄NBr (0.5 equiv), LiCl (0.5 equiv), DMF, 110 °C, 2 d; B) Pd(OAc)₂ (10 mol %), PPh₃ (20 mol %), Ag₂CO₃ (3 equiv), MeCN, 80 °C, 3 d. Below: Structure of the epoxide obtained from **11a** with dimethyldioxirane in the crystal.^[13] E = CO₂Et.

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with the bromodieneyne **9a** as well as its homologues **9b** and **9c**,^[10] except that final β -hydride elimination in the intermediates **10a–c** removed one of the methylene hydrogen atoms in the six-membered C-ring rather than the angular hydrogen atom between the B- and C-ring in the corresponding intermediate leading to **8**. The yields of isolated **11a** and even **11b**, in which a seven-membered ring is formed in the initial Heck-type coupling, were around 75 %, and **11a** and **11b** were the only products. The tetracyclic enone **8** was also formed as a single product just as **11a, b**, but partial decomposition of **8** may have occurred upon silica gel chromatography due to its electrophilic enone moiety. The relative configuration of **11a** as depicted was proved by an X-ray crystal structure analysis of the epoxide formed from **11a** with dimethyldioxirane.^[13] It is particularly remarkable that even **9c**, in which an eight-membered ring is formed in the first step,^[12] underwent the same type of tetracyclization, not as cleanly though as **9a, b**, and thus the yield of isolated product was only 30 %.

Even more surprising is the fact that bromodieneyne **12a** which differs from **9a** only by the methoxy group in the 9-position, gave the completely different tetracyclo-[8.4.0.0^{1,3}.0^{4,9}]tetradec-9-ene derivative **14a** as a separable mixture of two diastereomers (1.2:1.0) in good yield (63 %). The structure of the major diastereomer was proved by X-ray crystal structure analysis^[13] to be that of the 8-*exo*-methoxy derivative *exo*-**14a**, while ¹H and ¹³C NMR spectra corroborated the minor product to be the 8-*endo* diastereomer *endo*-**14a** (Scheme 3). The 4,9-disilyloxy-2-bromotetradeca-1,13-diene-7-yne (**12b**) (1:1 mixture of two diastereomers) gave the tetracycle **14b** as a separable mixture of four diastereomers (ratio 1.2:1.0:1.7:2.0) in even better yield (76 %).



Scheme 3. C) Same as B) in Scheme 2, but 2 d; D) Pd(OAc)₂ (10 mol %), PPh₃ (20 mol %), Ag₂CO₃ (1.5 equiv), (iPr)₂NH (3 equiv), MeCN, 120 °C, 18 h; E) Same as D), but 2 d. In the center: Structure of **14a** in the crystal.^[13]

There is no experimental evidence as to how the skeleton of **14** evolves, but it might form via an alkylpalladium intermediate **13** which is analogous to the intermediate **7** en route to the other tetracyclic skeleton. An unprecedented γ -hydride elimination in **13** would then lead to **14**.^[14] Alternatively, an alkylpalladium intermediate of type **3** might undergo α -dehydrobromination (α with respect to the metal atom) to yield a palladium carbene complex **15**^[15] that would certainly intramolecularly cyclopropanate the opposite exomethylene group. It is not obvious, though, why and how the 9-oxy substituents in **12a, b** would cause the corresponding intermediates of type **3** or **13** to undergo α -dehydrobromination or γ -hydride elimination, respectively.

In an approach to a steroidal skeleton, the cyclopentanone derivative **16** (1:3 mixture of *cis*- and *trans*-diastereomers) resembling the acyclic tetradecadieneynone **6**, was assembled^[9] and subjected to typical Heck coupling conditions. This did indeed react in complete analogy to **6** and led to the pentacyclic compounds *cis*-/*trans*-**17** (ratio 1:2.5) which resemble a steroidal skeleton with a five-membered B-ring. While the major diastereomer was isolated as an oil, the minor one gave good crystals and was proved by X-ray structure analysis to have a *cis* C/D-ring junction with the angular methyl group on the same side as the bridging cyclopropane ring (Figure 1).^[13] By comparison of the ¹H and ¹³C NMR spectra, the major isomer could thus be assigned the *trans*-configured structure *trans*-**17**. Although the yield was only

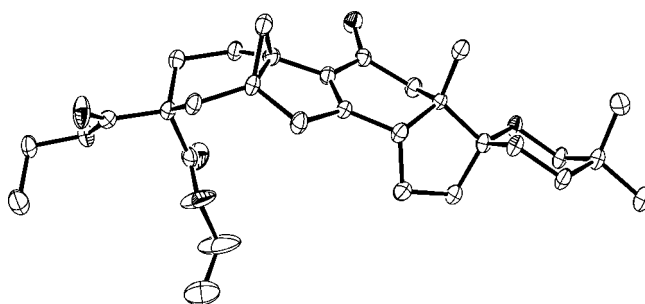


Figure 1. Structure of compound *cis*-**17** in the crystal.^[13]

moderate (35 %), this new domino cyclization offers an access to interesting steroid analogues. Pure *cis*-**16** under the same conditions gave *cis*-**17** in 40 % yield. It remains to be seen whether changes in the type and pattern of substituents will alter the mode of cyclization.

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Palladium-Catalyzed Synthesis of Substituted Hydantoins—A New Carbonylation Reaction for the Synthesis of Amino Acid Derivatives**

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Amino acids and their derivatives are unequivocally one of the most important classes of organic compounds. In addition to biochemical applications, amino acid derivatives are used as chemical feedstocks for industrial fine chemical synthesis.^[1] Despite the well known “classical methods” such as the Strecker synthesis^[2] and the highly selective procedures developed in the research groups of Schöllkopf,^[3] Seebach,^[4] Evans,^[5] Williams^[6] as well as recent developments,^[7] a need for new, more efficient protocols for the preparation of amino acid derivatives remains. In the past, researchers focused exclusively on the asymmetric synthesis of amino acid derivatives, and successful procedures were judged accordingly. However, other important factors also need to be addressed. For example, the procedures need to be improved in terms of their atom economy.^[8] This also applies to the asymmetric hydrogenation of acetamidoacrylates and acetamidocinnamates,^[9] since the hydrogenation precursors are often expensive and difficult to prepare.^[10]

Racemic imidazolidine-2,4-diones, generally called hydantoins,^[11] are important building blocks for enantioselective amino acid synthesis because enantiomerically pure amino acids can be prepared from these by dynamic kinetic racemic resolution.^[12] The practicability of this method was demonstrated on an industrial scale by Ajinomoto^[13] and Kanegafuchi^[14] for the production of D-*p*-hydroxyphenylglycine. Substituted hydantoins are also of pharmacological interest and are used, for example, for the treatment of epilepsy.^[15] Since only atom economic procedures for the production of N-unsubstituted hydantoins are known (Bucherer–Bergs reaction,^[16] amidoalkylation^[17]), we set out to examine to what extent substituted hydantoins can be made directly from simple, inexpensive starting materials. We describe here a new one-pot synthesis of 5-, 3,5-, and 1,3,5-substituted hydantoins that is based on the carbonylation of aldehydes in the presence of urea derivatives.

In the context of our work on the carbonylation of aldehydes with amides (amidocarbonylation)^[18] in the presence of a palladium catalyst,^[19] we examined to what extent sulfonamide, urethanes and urea derivatives can be used as amide components. The conversion of cyclohexanecarbalde-

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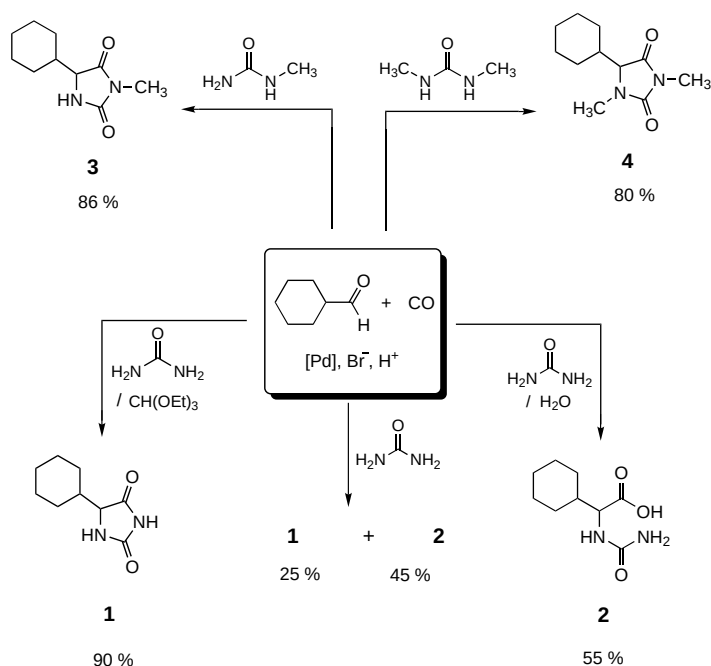
hyde with the respective amide component served as a model reaction. Although the reactions of sulfonamides and urethanes did not generate the desired product at 100 °C, the reaction with urea produced the 5-cyclohexylhydantoin **1** in 25 % yield and *N*-carbamoyl-cyclohexylglycine **2** in 45 % yield.^[20] A 1:1 mixture of the starting materials in *N*-methylpyrrolidinone (NMP) as solvent in the presence of 0.5 mol % palladium(II) bromide/1 mol % PPh₃ was placed into a Parr autoclave and heated at 100 °C. After 12 h, the reaction mixture was allowed to come to ambient temperature, the volatile components were removed, and the products were isolated by simple extraction from water and ethyl acetate.

In order to demonstrate the utility of this reaction, the amidocarbonylation of cyclohexanecarbaldehyde was examined with a variety of substituted urea derivatives (Scheme 1; Table 1). Indeed, free urea gave very good selectivities (90 %) for the hydantoin **1** when water-absorbing agents such as triethyl orthoformate or acetic anhydride were used. Interestingly,

Table 1. Palladium-catalyzed ureidocarbonylation with urea according to Equation (1).^[a]

Entry	R ¹	Additive [equiv]	Hydantoin product	yield [%] ^[b]	<i>N</i> -Carbamoyl acid product	yield [%] ^[b]
1		–	1	25	2	45
2		CH(OC ₂ H ₅) ₃ [1.0]	1	90	2	n.b.
3		(CH ₃ CO) ₂ O [1.0]	1	86	2	n.b.
4		H ₂ O [1.3]	1	20	2	55
5		–	5	5	6	35
6		CH(OC ₂ H ₅) ₃ [1.0]	5	64	6	n.b.

[a] Conditions: 25.0 mL solution (1.0 M in urea and aldehyde) in NMP, 0.25 mol % in situ prepared dibrombis(triphenylphosphane)palladium(II), 30 mol % LiBr, 1 mol % H₂SO₄, 60 bar carbon monoxide, 100 °C, 12 h. [b] Yield of isolated product; n.o. = not observed.



Scheme 1. Palladium-catalyzed carbonylation of cyclohexanecarbaldehyde with urea derivatives.

the free *N*-carbamoyl amino acid **2** can be prepared in good yields by simply combining one equivalent of water with the corresponding hydantoin.^[21] The conversion of cyclohexanecarbaldehyde with monosubstituted dimethylurea occurs selectively. For example, at 80 °C the 5-cyclohexyl-3-methyl

hydantoin **3** was obtained in very good yield (86 %). The reaction with symmetrical dimethylurea led to the 5-cyclohexyl-1,3-dimethylhydantoin **4** in comparably high yield (80 %). Prior to our work, 1,3,5-substituted hydantoins could only be prepared by multistep synthesis starting from the appropriate amino acids. In the first step, the nitrogen atom of the amino acid is monosubstituted by reductive alkylation. Acylation with isocyanate produces the appropriate carbamoyl, and subsequent base-promoted cyclization gives the hydantoin.^[22]

The high selectivity of the 3,5-substituted hydantoins permits us to postulate a mechanism for this new ureidocarbonylation reaction. In agreement with the expected basicity of the different nitrogen atoms of monosubstituted urea derivatives, we assume that in a preequilibrium step, selective nucleophilic attack of the free NH₂ group on the carbonyl group takes place. An α -halogencarbamoyl species generated by nucleophilic substitution subsequently undergoes oxidative addition to the palladium(0) species. After CO insertion, the acylpalladium complex undergoes either an intramolecular reaction to give the hydantoin directly, or first undergoes intermolecular attack of water to give the *N*-carbamoyl acid which subsequently cyclizes to the hydantoin.

The scope of this new multicomponent synthesis for hydantoins was demonstrated by the results summarized in Table 2. 3-Alkyl, 3-aryl or 3-benzyl substituted hydantoins were obtained in high selectivity from the corresponding monosubstituted urea derivatives (Eq. (2); Table 2, entries 1–5). When *N,N*-disubstituted urea derivatives were em-

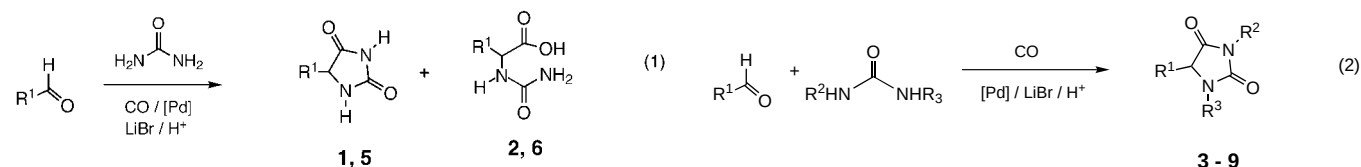


Table 2. Palladium-catalyzed ureidocarbonylation of aldehydes to substituted hydantoins according to Equation (2).^[a]

Entry	R ¹	R ²	R ³	Product	T [°C]	Yield [%] ^[b]	TON
1		CH ₃	H	3	80	86	344
2		CH ₃	CH ₃	4	80	80	320
3		C ₂ H ₅	H	7	100	51	204
4			H	8	100	64	256
5			H	9	100	50	200
6 ^[c]		CH ₃	CH ₃	10	100	80	80
7		C ₂ H ₅	C ₂ H ₅	11	100	89	356
8		CH ₃	CH ₃	12	120	61	244
9 ^[d]	H			13	130	93	372
10	H	CH ₃	CH ₃	14	100	73	292
11		CH ₃	CH ₃	15	100	85	340
12		CH ₃	CH ₃	16	100	79	316
13 ^[c]		CH ₃	CH ₃	17	100	70	70
14		CH ₃	CH ₃	18	100	44	176
15		CH ₃	CH ₃	19	100	39	156

[a] All conditions except the reaction temperature as in Table 1. [b] Yield of isolated product. [c] Catalyst: 1.0 mol % Pd/C. [d] Reaction time 48 h.

ployed, substitution at positions 1, 3, and 5 with both alkyl and aryl residues was achieved (entries 6–11). It should also be emphasized that 5-arylhydantoins **15**–**19** were produced in good yields, which also illustrates that a wide variety of functional groups are tolerated. In addition to the catalyst system PdBr₂/2 PPh₃, palladium on activated charcoal is also able to catalyze the carbonylation efficiently (Table 2, entries 6 and 13). The latter catalyst is particularly advantageous due to the very simple separation of the catalyst after the reaction. This is especially important for potential pharmaceutical preparations of hydantoins. The only limitation of this procedure that we have discovered thus far occurred in the reaction of aldehydes of the form RCH₂CHO with urea and methylurea. Here, we isolated the 2-oxotetrahydropyrimidine as the main product.^[23]

In conclusion, the palladium-catalyzed carbonylation of aldehydes with urea derivatives provides a remarkably simple, pharmacologically interesting method for the preparation of 5-, 3,5-, and 1,3,5-substituted hydantoins in good to very good yields. The excellent chemo- and regioselectivities are an

important advantage of this new one-pot multicomponent reaction over classical methods. Moreover, we have also demonstrated the practicability of this method. The preparation of 47 g of 5-cyclohexyl-1,3-dimethylhydantoin (**10**) described in the Experimental Section indicates that this carbonylation procedure is also suitable for the multi-gram production of hydantoins.

Experimental Section

Synthesis of **10**: Cyclohexanecarbaldehyde (29 g, 0.25 mol), *N,N'*-dimethylurea (22 g, 0.25 mol), palladium dibromide (0.16 g), triphenylphosphane (0.32 g), LiBr (4.5 g), sulfuric acid (0.25 g), and *N*-methylpyrrolidone (150 mL) were transferred into a 300 mL autoclave and allowed to react under 60 bar carbon monoxide at 100 °C for 24 h. The volatile components were removed in vacuo and the residue was taken up in ethyl acetate and washed with water. The organic phase was collected and the solvent was removed in vacuo to give **10** (47 g, 0.22 mol) in >97 % purity.

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Synthesis and Stereoselective Reactions of New Stable α -Ferrocenyllithium Derivatives. An Umpolung of the Ferrocene Reactivity**

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The design of new chiral ligands for asymmetric catalysis is an important synthetic goal. In particular, ferrocenyl ligands have attracted considerable attention and numerous synthetic methods for the preparation of new ferrocenyl derivatives have been developed.^[1] Many useful ferrocenyl ligands can be prepared by the nucleophilic substitution of readily available α -ferrocenylcarbinol derivatives of type **1** (Scheme 1).^[1, 2] According to Ugi^[2b] these substitutions proceed with retention of configuration via a chiral cationic intermediate **2** and lead to products of type **3**. Herein we report the umpolung of this reactivity. It was possible to generate α -ferrocenyllithium derivatives^[3] of type **4** and to react them stereoselectively with different electrophiles to give the new chiral ferrocenyl derivatives **5** with complete retention of configuration.

In preliminary experiments the ferrocenyl thioether **6a** (95% *ee*) was treated with lithium naphthalenide (3 equiv) at

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- [9] Removal of the TMS group in **7f** leads to the (*R*)-(α -benzylphenylmethyl)ferrocene (95 % *ee* determined by HPLC on a chiral phase (Chiracel OD column)). The same enantiomer was obtained by a nucleophilic substitution of the (*R*)-(α -acetoxyphenylmethyl)ferrocene with benzylzinc bromide,^[2d] which proved the retention of configuration in the former reaction.
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Nucleotide Platination Induced by Visible Light**

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Patrick J. Bednarski, and Peter J. Sadler*

There is increasing interest in the development of photoactive metal complexes as luminescent markers and photocleaving agents for DNA,^[1] and in the design of photosensitisers for photodynamic therapy.^[2,3] Photosensitisers catalyze the production of reactive singlet oxygen and therefore rely on the presence of oxygen in the target tissue. An advantage of photo-chemotherapeutic agents is that they can be activated selectively at the target site, thereby minimizing the side-effects of chemotherapy. We are investigating the design of oxygen-independent photoactivated metallodrugs, in particular photoactive analogues of the anticancer drug cisplatin, *cis*-[PtCl₂(NH₃)₂]. Iodo complexes of Pt^{IV} are promising in this respect because their cytotoxicity against tumor cells is potentiated by visible light.^[4] We show here that stereospecific reactions between a diiododiamine–Pt^{IV} complex and the nucleotide guanosine 5'-monophosphate (5'-GMP) can be induced by visible light, and that photoactivation can be controlled by the axial ligands, which will allow fine-tuning of the photoreactivity of this class of complexes. This appears to be the first report of nucleotide platination induced solely by visible light.

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Photoreactions of the ¹⁵N-labelled diiodo–Pt^{IV} complexes ¹⁵N-**1** and ¹⁵N-**2** in the presence of 5'-GMP were followed by 1D ¹H, 2D [¹H, ¹⁵N] HSQC and 2D [¹H, ¹⁵N] HSQC-TOCSY NMR spectroscopy. Photo-irradiation was carried out using an argon ion laser equipped with a fibre optic link designed to deliver light directly into the sample within the magnet of the NMR spectrometer. No reactions between these Pt complexes and 5'-GMP occurred within 30 h in the dark.

trans,cis-[Pt(OCOCH₃)₂I₂(¹⁵N-en)] ¹⁵N-**1**

trans,cis-[Pt(OH)₂I₂(¹⁵N-en)] ¹⁵N-**2**

The time-course of the photoreaction of the acetato complex **1** ($\epsilon_{457.9} = 327 \text{ M}^{-1} \text{ cm}^{-1}$) with 1 equivalent of 5'-GMP (200 μM in water, 298 K, pH 6.2)^[5] was observed by 1D ¹H NMR spectroscopy (Figure 1; for the structures and

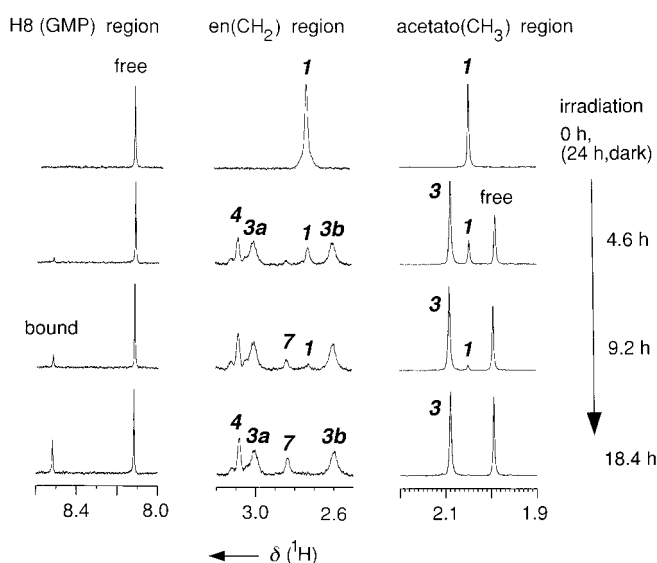


Figure 1. Selected regions of 500 MHz 1D ¹H NMR spectra acquired during the photoreaction ($\lambda_{\text{irr}} = 457.9 \text{ nm}$) of **1** in the presence of 5'-GMP at irradiation times of 0, 4.6, 9.2, and 18.4 h (and 24 h in the dark), pH 6.2, 298 K. Binding of 5'-GMP is preceded by initial light-induced ligand exchange of **1** to give **3**.

numbering of the complexes see Scheme 1A). After irradiation with low-power visible light ($\lambda_{\text{irr}} = 457.9 \text{ nm}$, $12.2 \times 10^{-3} \text{ J s}^{-1}$), the signals for **1** (CH_2 : $\delta = 2.7$; CH_3 : $\delta = 2.05$) decreased in intensity, and two new multiplets in the en(CH₂) region of the spectrum at $\delta = 3.01$ (**3a**) and $\delta = 2.60$ (**3b**), and a singlet at $\delta = 3.08$ (**4**) appeared. Two new singlets that could be assigned to coordinated acetate ($\delta = 2.09$; **3**) and to released acetate ($\delta = 1.99$) were detected in the methyl region. During later stages of the photoreaction a new singlet was observed at $\delta = 8.52$, which was assigned to H8 of Pt-bound 5'-GMP (free 5'-GMP: $\delta = 8.10$). A high frequency shift such as this for the H8 resonance of 5'-GMP is typical of metal coordination to N7 of 5'-GMP.^[6]

The pathway of the photoinduced reaction of ¹⁵N-**1** with 5'-GMP was further elucidated by 2D [¹H, ¹⁵N] HSQC and HSQC-TOCSY NMR spectroscopy (Figure 2). After 1 h irradiation (Figure 2A) the cross peak for **1** with a chemical

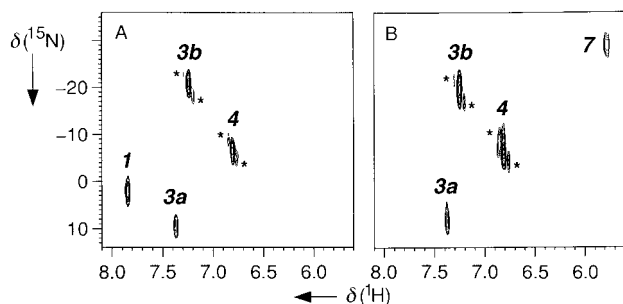
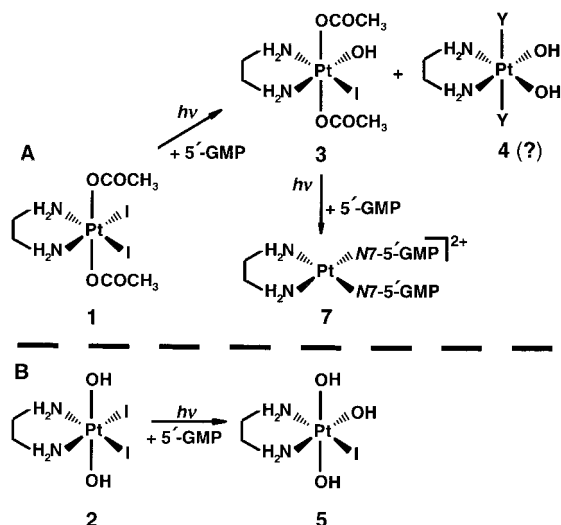


Figure 2. 2D $[^1\text{H}, ^{15}\text{N}]$ HSQC NMR spectra for the reaction of ^{15}N -**1** in the presence of 5'-GMP (molar ratio 1:1, 200 μM) at irradiation times of A) 1 h and B) 4.6 h, pH 6.2, 298 K. Asterisks indicate ^{195}Pt satellites.

shift of 7.84/1.98 ($^1\text{H}/^{15}\text{N}$) and three new cross peaks with chemical shifts of 7.30/9.54 (**3a**), 7.30/−20.9 (**3b**), and 6.81/−6.45 (**4**) were observed. The corresponding 2D $[^1\text{H}, ^{15}\text{N}]$ HSQC-TOCSY NMR spectrum showed that the two multiplets (**3a** and **3b**) are part of the same spin system and are correlated with the (en)NH₂ cross peaks at 7.30/9.54 (**3a**) and 7.30/−20.9 (**3b**), and the CH₂ singlet at $\delta = 3.08$ with the cross peak at 6.81/−6.45 (**4**). The inequivalence of the two CH₂ groups of the coordinated en ligand (**3a** and **3b**) and the observation of two $^1\text{H}/^{15}\text{N}$ cross-peaks, compatible with NH₂ of en *trans* to I (**3a**) and O (**3b**), suggest that *trans,cis*-[Pt(OCOCH₃)₂(OH)I(^{15}N -en)] ^{15}N -**3** is formed during the photoreaction (Scheme 1 A). The shifts of the peaks for the



Scheme 1. Photoreaction pathways for A) **1** and B) **2** in the presence of 5'-GMP. The structure assigned to **4** is tentative; Y may be I or OH.

Table 1. Chemical shifts (^1H , ^{15}N) and coupling constants $^1J(^{195}\text{Pt}, ^{15}\text{N})$ [Hz].

Pt complex		CH ₃ $\delta(^1\text{H})$	CH ₂ $\delta(^1\text{H})$	NH ₂ $\delta(^1\text{H})$ (<i>trans</i> to)	$\delta(^{15}\text{N})$ (<i>trans</i> to)	$^1J(^{195}\text{Pt}, ^{15}\text{N})$ (<i>trans</i> to)
<i>trans,cis</i> -[Pt(OCOCH ₃) ₂ I ₂ (en)]	1 ^[a]	2.05	2.72	7.84 (I)	1.98 (I)	
<i>trans,cis</i> -[Pt(OH) ₂ I ₂ (en)]	2 ^[a]		2.82	6.56 (I)	14.03 (I)	
<i>trans,cis</i> -[Pt(OCOCH ₃) ₂ (OH)I(en)]	3	2.09	3.01, 2.60	7.30 (I,O)	9.54 (I), −20.9 (O)	221 (O)
<i>trans,cis</i> -[PtY ₂ (OH) ₂ (en)] ^[a]	4		3.08	6.81	−6.45	213
<i>mer</i> -[Pt(OH) ₃ I(en)]	5		3.06, 2.70	6.33 (I,O)	16.32 (I), −20.07 (O)	268 (O)
[Pt(OH) ₄ (en)]	6		2.94	6.19 (O)	−16.77 (O)	292 (O)
[Pt(5'-GMP-N7) ₂ (en)] ²⁺	7		2.84	5.79, 5.72 (N)	−30.9 (N)	

[a] tentative assignment; Y = I or OH.

second, simultaneously formed, photoproduct **4** and the observation of only one $^1\text{H}/^{15}\text{N}$ cross-peak ($\delta(^1\text{H})/\delta(^{15}\text{N}) = 6.81/−6.45$, (en)CH₂: $\delta(^1\text{H}) = 3.08$), together with the appearance of a peak for free acetate, are consistent with a symmetrical Pt^{IV} complex (equivalent CH₂ groups of the coordinated en ligand), which has lost the two acetato ligands. A mixture of *trans,cis*-[Pt(OH)₂I₂(^{15}N -en)] ^{15}N -**2**, *mer*-[Pt(OH)₃I(^{15}N -en)] ^{15}N -**5**, and [Pt(OH)₄(^{15}N -en)] ^{15}N -**6** was studied by 2D $[^1\text{H}, ^{15}\text{N}]$ HSQC-TOCSY NMR experiments to aid identification of **4** (Figure 3). However, the differences

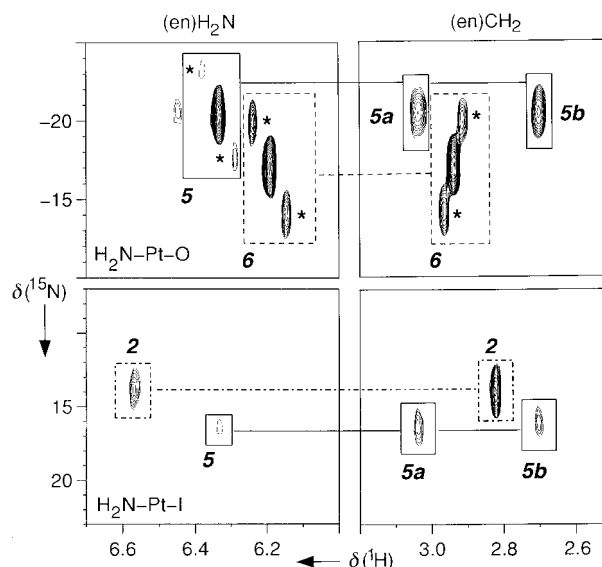


Figure 3. Characterization of a mixture of ^{15}N -labelled ^{15}N -**2**, ^{15}N -**5**, and ^{15}N -**6** at pH 6.2, 298 K by 2D $[^1\text{H}, ^{15}\text{N}]$ HSQC-TOCSY NMR spectroscopy. Asterisks indicate ^{195}Pt satellites (see the text for further information).

in chemical shifts (Table 1) rule out the formation of these complexes during the photoreaction of **1**. A possible assignment for **4** is to a dihydroxo-Pt^{IV}(en) complex with one or two axial iodo ligands.

After irradiation of the reaction mixture for 4.6 h (Figure 2 B), two close new $^1\text{H}/^{15}\text{N}$ cross peaks ($\delta = 5.79/−30.9$ and $5.72/−30.9$, **7**), assignable to nonequivalent NH protons of the en ligand, appeared in the 2D $[^1\text{H}, ^{15}\text{N}]$ HSQC NMR spectrum. $^1\text{H}/^{15}\text{N}$ chemical shifts in this range are characteristic of a Pt^{II} rather than a Pt^{IV} complex, and these values are the same as those reported previously for [Pt(5'-GMP-N7)₂(^{15}N -en)]²⁺ **7**.^[6, 7]

From the kinetic course of the reaction (Figure 4A, Scheme 1A), it is apparent that *trans,cis*-[Pt(OCOCH₃)₂-(OH)I(¹⁵N-en)] ¹⁵N-**3** is photoreactive towards the nucleotide to give the Pt^{II}-(5'-GMP)₂ adduct **7** directly, whereas the Pt^{IV} photoproduct **4** is stable.

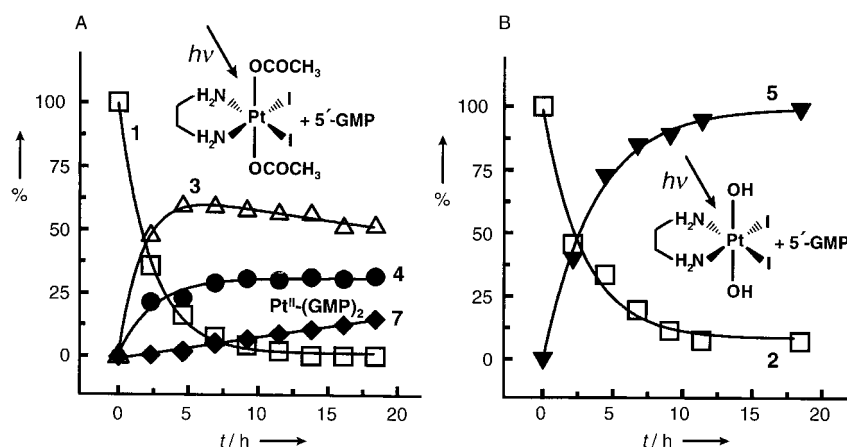


Figure 4. Kinetics of the photoreaction of A) **1** and B) **2**, in the presence of 1 equivalent of 5'-GMP; 200 μ M, pH 6.2, 298 K. A) $\square$ **1**, $\triangle$ **3**, $\bullet$ **4**, $\blacklozenge$ **7**; B) $\square$ **2**, $\blacktriangledown$ **5**. The kinetic data were determined by integration of (en)CH₂ ¹H NMR peaks; the total integral of the (en)CH₂ signals was constant during the reaction course.

Similar 1D ¹H, 2D [¹H, ¹⁵N] HSQC and 2D [¹H, ¹⁵N] HSQC-TOCSY NMR studies of photoreactions of the dihydroxo complex **2** ($\epsilon_{457.9} = 246 \text{ M}^{-1} \text{ cm}^{-1}$) in the presence of 5'-GMP (1:1, 200 μ M, in water, 298 K, pH 6.2)^[5] were carried out. In contrast to irradiation of **1**, only one photoproduct was observed with peaks assignable to *mer*-[Pt(OH)₃I(¹⁵N-en)] ¹⁵N-**5** (Figure 3, Table 1, Scheme 1B), that is, neither complex **2** nor the photoproduct **5** reacted with the nucleotide over the time period studied (30 h). The rate constant for the light-induced ligand exchange of **1** ($9.27 \times 10^{-5} \text{ s}^{-1}$, half-life 1.61 h, $\phi = 0.16$, Figure 4) is similar to that for **2** ($7.55 \times 10^{-5} \text{ s}^{-1}$; half-life 2.02 h, $\phi = 0.11$), but clearly the axial ligands play a major role in determining the subsequent course of the photo-reactions. It can be proposed that the photo-substitution reactions induced by irradiation into the ligand $\rightarrow$ metal charge transfer (LMCT) band arise from the transfer of a valence electron from I⁻ into the antibonding d orbitals of d₆-Pt^{IV} to give a transient Pt^{III} species. Reactions of Pt^{IV} with nucleotides in the dark are known to be very slow,^[8, 9] and in the present case no reactions of complexes **1** or **2** were observed within 30 h in the dark.

Previous reports of photoreactions of Pt^{IV} complexes have been concerned largely with UV irradiation.^[10] Light-induced ligand exchange reactions have been investigated, although there appear to be no previous examples of photo-aquation *trans* to am(m)ine ligands. Photoreduction of Pt^{IV} to Pt^{II} is known,^[10, 11] and photo-isomerization has been demonstrated for an isomer of the oral Pt^{IV} anticancer drug *cis,trans*-[PtCl₂(OAc)₂(NH₃)(*c*-C₆H₁₁NH₂)] (JM216).^[12] However, there appear to be few previous studies of sequential photo-substitution and photo-redox processes and of the influence of the axial ligands on the photoreaction pathways. UV irradiation can result in Pt^{II}-N7G bond cleavage for Pt^{II} complexes.^[13]

The photoactivation of Pt^{IV} complexes with visible light to give Pt^{II} bis-nucleotide adducts might provide a powerful method for preventing the repair of long-lived mono-Pt^{II}-nucleotide adducts in cancer cells.^[14] The success of photo-chemotherapeutic agents is highly dependent on their stability under physiological conditions, and diiodo-diamine-Pt^{IV} complexes are likely to be too reactive towards reducing agents, such as glutathione, to be candidates for photo-chemotherapy.^[15] However, there is evidence that by making small changes in the design of the Pt^{IV} complex, photosensitivity can be retained and chemical reactivity towards thiols greatly reduced.

Experimental Section

1 and **2** were synthesized as previously described.^[4] Compounds **5** and **6** were obtained by treatment of [PtI₂(en)] in water with 1 or 2 equivalents of AgNO₃, respectively (6 h, RT), removal of AgI by centrifugation, and reaction with 10 equivalents of H₂O₂ (24 h, RT). The Pt complexes were incubated with 5'-GMP (200 μ M, 1 equiv) in H₂O/D₂O (90/10) at 298 K, pH 6.2 in the presence and absence of light. The irradiation was carried out using an argon ion laser (Omnichrome 543-MAP) equipped with a fibre optic (FT-600-UMT, $\varnothing$ 600 μ m; Elliot Scientific Ltd.) to deliver light ($\lambda_{\text{irr}} = 457.9 \text{ nm}$) directly into the sample within the magnet of the NMR spectrometer. The laser output, after the fibre, was $12.2 \times 10^{-3} \text{ J s}^{-1}$ and linear in the range 2.1 to $12.2 \times 10^{-3} \text{ J s}^{-1}$, as measured by a LaserMate power meter (Coherent Ltd.). Quantum efficiencies for the decrease of **1** and **2** were determined at 457.9 nm using iron(III) oxalate as a chemical actinometer. 1D ¹H, 2D [¹H, ¹⁵N] HSQC, and 2D [¹H, ¹⁵N] HSQC-TOCSY NMR spectra (TOCSY: mixing time of 50 ms) were recorded on a Bruker DMX 500 NMR spectrometer (¹H: 500 MHz, ¹⁵N: 50.7 MHz).^[6] Dioxane was used as internal ¹H chemical shift standard ($\delta = 3.764$ relative to [D₄]3-trimethylsilyl)propionate (TSP)). All ¹⁵N chemical shifts were referenced externally to ¹⁵NH₄⁺ at $\delta = 0$. Values of pH were measured with a pH-meter (Corning 145) equipped with a microcombination electrode (Aldrich) calibrated with Aldrich standard buffers (pH 4, 7, and 10) and were adjusted with dilute solutions of HClO₄ and NaOH. Rate constants were obtained by fitting the kinetic data to appropriate differential equations by a nonlinear optimization procedure with the program SCIENTIST (version 2.01, MicroMath Inc.).

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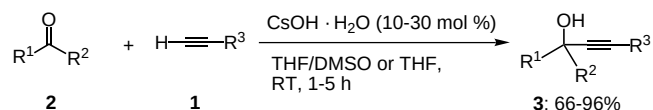
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Cesium Hydroxide: A Superior Base for the Catalytic Alkynylation of Aldehydes and Ketones and Catalytic Alkenylation of Nitriles**

Dimitrios Tzalis and Paul Knochel*

The metal-catalyzed formation of new carbon–carbon bonds is an important synthetic tool.^[1] Particularly attractive in this category of reactions are additions to multiple bonds that proceed without the formation of side products (atom economic reactions).^[2] Thus, the metal-catalyzed addition of alkynes of type **1** to carbonyl compounds of type **2** that lead to propargyl alcohols of type **3** has a considerable synthetic and industrial importance. Whereas the performance of these reactions that use a stoichiometric amount of a base such as an organolithium or organomagnesium reagent to generate an intermediate metal acetylide^[3] has been extensively described, only a few reports concerning the catalytic activation of an alkyne and subsequent addition to the carbonyl derivative have been reported.^[4,5] They are mostly restricted to acetylene itself^[4] or are applicable only to some carbonyl compounds such as cycloalkanones.^[5] Herein, we report the exceptional activity of cesium hydroxide^[6,7] for the catalytic generation of highly nucleophilic, stabilized organometallic species (Scheme 1). Thus, in the presence of a catalytic



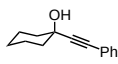
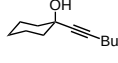
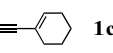
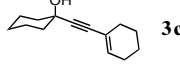
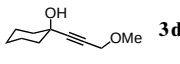
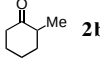
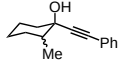
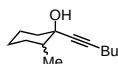
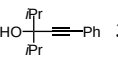
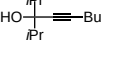
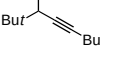
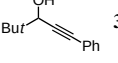
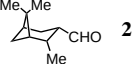
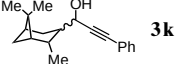
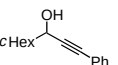
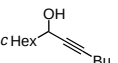
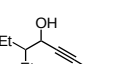
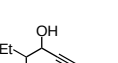
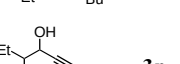
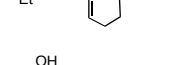
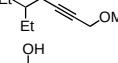
Scheme 1. CsOH-catalyzed alkynylation of aldehydes and ketones with terminal alkynes. R^1 , R^2 = alkyl or H, R^3 = alkyl, aryl, alkenyl.

amount of $\text{CsOH} \cdot \text{H}_2\text{O}$ (10 mol %) phenylacetylene (**1a**: 1.5 equiv) adds to cyclohexanone (**2a**) in THF within 1 h at RT. The desired propargyl alcohol (**3a**) is isolated after workup in 88% yield (entry 1 of Table 1). Under these conditions phenylacetylene adds to various aliphatic ketones^[8] or to aliphatic aldehydes in yields between 66 and 96%. In many cases, these reactions are complete within five minutes at RT. A 1:1 mixture of THF and DMSO was used as solvent together with $\text{CsOH} \cdot \text{H}_2\text{O}$ (30 mol %) for the addition of less acidic alkynes. Under these conditions most terminal alkynes undergo a smooth addition to secondary or tertiary aliphatic aldehydes or aliphatic ketones to give the corresponding propargyl alcohols of type **3** (Table 1). To avoid competitive aldol reactions a slow addition of the aldehyde with a syringe pump was performed (addition of 0.5 mL h^{-1}). The addition of alkynes to carbonyl compounds with diastereotopic faces such as 2-methylcyclohexanone (**2b**) or the

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Table 1. Preparation of propargyl alcohols of type **3** by CsOH-catalyzed addition of alkynes to aldehydes and ketones.

Entry	Electrophile	Nucleophile	Product	Method	Yield [%] ^[a]
1	 2a	HC≡CPh 1a	 3a	A	88
2	2a	HC≡CBu 1b	 3b	B	82 (81) ^[b]
3	2a	 1c	 3c	B	96
4	2a	HC≡CHCH ₂ OMe 1d	 3d	B	91
5	 2b	1a	 3e	A	72
6	2b	1b	 3f	B	74
7	<i>i</i> PrCO <i>i</i> Pr 2c	1a	 3g	A	91
8	2c	1b	 3h	B	59
9	<i>t</i> BuCHO 2d	1a	 3i	A	86
10	2d	1b	 3j	B	74
11	 2e	1a	 3k	A	96
12	<i>c</i> HexCHO 2f	1a	 3l	A	90
13	2f	1b	 3m	B	91
14	Et ₂ CHCHO 2g	1a	 3n	A	81
15	2g	1b	 3o	B	91
16	2g	1c	 3p	B	91
17	2g	1d	 3q	B	90
18	PrCHO 2h	1a	 3r	A	66 (72) ^[b]

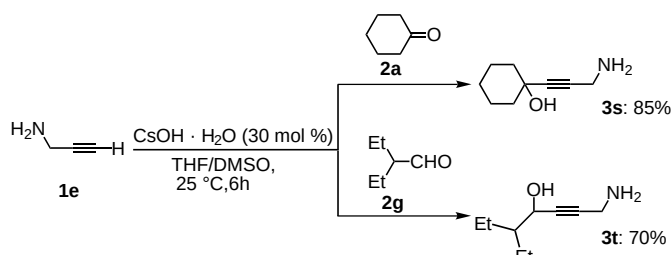
[a] Yield of isolated, analytically pure product. [b] The yield in parenthesis corresponds to a reaction performed in NMP.

aldehyde **2c** produces a 1:1 mixture of diastereomeric propargyl alcohols (entries 5, 6, and 11 of Table 1). It is possible to use *N*-methylpyrrolidinone (NMP) instead of DMSO with the same reaction time to get similar yields (entries 2 and 18 of Table 1).

Functionalized alkynes such as propargylamine (**1e**) have also been used with success. Thus, the addition of **1** to

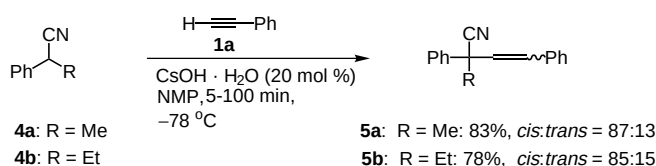
cyclohexanone and 2-ethylbutyraldehyde furnishes the desired 1,4-aminopropargyl alcohols **3s** and **3t** in 85 % and 70 % yield, respectively (Scheme 2).

In order to explore further the utility of CsOH · H₂O in the activation of acetylenic C–H bonds we have examined the reaction of benzylic nitriles with alkynes.^[9] A very fast addition of the phenylacetonitriles **4a,b** to phenylacetylene

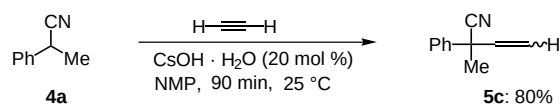


Scheme 2. CsOH-catalyzed alkylation of propargylamine.

occurs in the presence of catalytic amounts of CsOH·H₂O (20 mol %) in NMP (−78 °C, 5–100 min) to provide a *cis:trans* mixture of the addition products **5a** and **b** in 78–83 % yield (Scheme 3 and Method C). The use of acetylene allows a mild vinylation of phenylacetonitrile **4a** to give the unsaturated adduct **5c** in good yield (Scheme 4 and Method D).



Scheme 3. CsOH-catalyzed vinylation of phenylacetonitriles with phenylacetylene under mild reaction conditions.



Scheme 4. Efficient CsOH-catalyzed vinylation of phenylacetonitrile with acetylene.

In summary, we have shown that CsOH·H₂O allows a catalytic C–H activation of various alkynes that leads, in the presence of aliphatic aldehydes or ketones, to propargylic alcohols in good yields. It was also possible to perform the vinylation of phenylacetonitriles with CsOH·H₂O (20 mol %). We are currently investigating the scope of CsOH for the catalytic generation of other stabilized and unstabilized carbanions.^[10]

Experimental Section

Preparation of propargyl alcohols by CsOH catalysis:

Method A: Reaction with phenylacetylene (**1a**): Preparation of 4-ethyl-1-phenyl-1-hexyn-3-ol (**3n**): A 25-mL Schlenk flask was charged with CsOH·H₂O (68 mg, 0.41 mmol). THF (10 mL) and phenylacetylene (660 mg, 6.0 mmol) were added successively by syringe. The reaction mixture was vigorously stirred and 2-ethylbutyraldehyde (410 mg, 4.1 mmol) was added slowly and stirred at RT for 1 h. The reaction mixture was poured into diethyl ether (150 mL), and washed with H₂O (2 × 75 mL) and brine (75 mL). The combined ethereal layers were dried (MgSO₄). After evaporation of the solvent the residue was purified by flash chromatography (pentane/ethyl acetate, 9/1) to afford the desired product as a colorless oil (650 mg, 81 %).

Method B. Reactions with other alkynes: Preparation of 3-ethyl-5-decyn-1-ol (**3o**): A 25-mL Schlenk flask was charged with CsOH·H₂O (205 mg, 1.23 mmol). A 1:1 mixture of THF:DMSO (10 mL) and hexyne (670 mg, 8.2 mmol) were added successively by syringe. The reaction mixture was

vigorously stirred and a solution of 2-ethylbutyraldehyde (410 mg, 4.1 mmol) in a 1:1 mixture of THF and DMSO (2 mL) was added slowly by syringe pump (0.5 mL h^{−1}), and then stirred at RT for 3 h. After work-up as described in Method A the product was obtained as a colorless oil (670 mg, 90 %).

CsOH catalyzed vinylation.

Method C. Reaction with phenylacetylene (**1a**): Preparation of 2-methyl-2,4-diphenyl-3-butenenitrile (**5a**): A 10-mL Schlenk flask was charged with CsOH·H₂O (12 mg, 72 μmol). NMP (1 mL), 1-phenylethyl cyanide (**4a**) (50 μL, 0.38 mmol), and phenylacetylene (80 μL, 0.76 mmol) were added successively by syringe at −78 °C and the mixture stirred vigorously for 5 min. The reaction was poured into diethyl ether (30 mL) and washed with H₂O (2 × 20 mL) and brine (10 mL). The ethereal layer was dried (MgSO₄). After evaporation of the solvent the product was purified by flash chromatography (pentane/ethyl acetate, 9/1) to yield the desired product as a colorless oil (68 mg, 83 %).

Method D. Reaction with acetylene: Preparation of 2-methyl-2-phenyl-3-butenenitrile (**5c**): A 50-mL Schlenk flask was charged with CsOH·H₂O (254 mg, 1.5 mmol), NMP (20 mL), and 1-phenylethyl cyanide (**4a**) (1 g, 7.6 mmol). The reaction mixture was stirred vigorously and acetylene was slowly bubbled through the solution for 1.5 h. After work-up as described in Method C, the product was obtained as a colorless oil (955 mg, 80 %).

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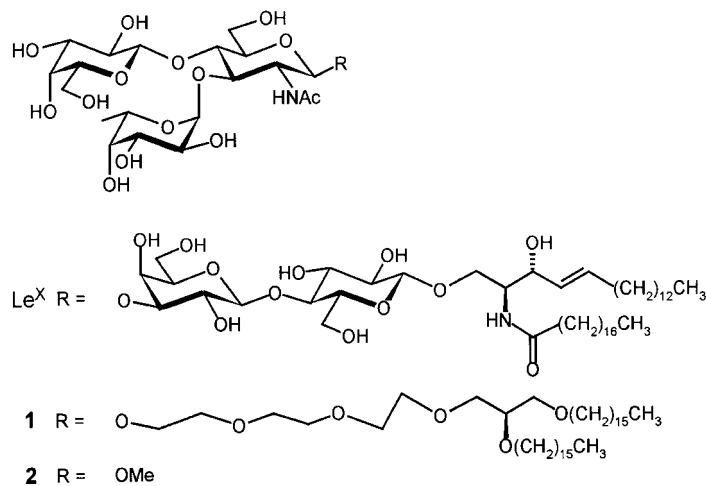
Carbohydrate–Carbohydrate Recognition between Lewis^X Glycoconjugates**

Armin Geyer,* Christian Gege, and Richard R. Schmidt

Dedicated to Professor Hans-Jürgen Quadbeck-Seeger on the occasion of his 60th birthday

Weak polyvalent interactions play a large part in biological systems.^[1] Whereas many examples are now known of protein–protein and protein–carbohydrate interactions, hardly any studies exist of carbohydrate–carbohydrate recognition. Polyvalent, intercellular carbohydrate–carbohydrate contacts add up to forces that are relevant for cell adhesion. So far it has not been established conclusively how far the glycosphingolipid (GSL) microdomains (lipid clusters) formed on the membrane from GSLs through lateral segregation play a part.^[2] Side-by-side contacts of the GSLs stabilize the microdomains, head-to-head contacts between GSLs from different membranes bring about cell adhesion.^[2b] The GSL microdomains can be identified indirectly from a count of aggregated cells^[3] or from the distribution of GSL antibodies on cell membranes.^[4] Recently, GSL domains with diameters of 20 nm were observed directly.^[5]

The aggregation of cells by means of homotypic head-to-head interactions was mainly investigated for the Lewis^X (Le^X) GSL, which bears the trisaccharide D-Galβ(1→4)[L-Fuca(1→3)]D-GlcNAcβ(1→OR) as its head group.^[2–4] It was also shown that Le^X oligosaccharides which are not anchored in the membrane are able to undo these cell–cell contacts.^[3] A direct NMR spectroscopic observation of Le^X–Le^X dimers is not possible as the dimers are mainly dissociated in solution owing to the weak adhesive force.^[6] The transient bond of a low molecular weight ligand to a high molecular weight receptor can, however, be detected with NMR spectroscopy by means of transfer NOEs.^[7] Carbohydrates exhibit high off-rates upon receptor binding, therefore they are very suitable for such measurements.^[8] However, the method can be also employed for the quantification of the membrane affinity of low molecular weight compounds.^[9] As the NOEs of an oligosaccharide bonded transiently to a receptor in the membrane are strongly negative, the very weak affinities of the carbohydrate–carbohydrate recognition should also be detectable. The native Le^X GSL was reduced to two model compounds which bear only the groups relevant for homotypic recognition (**1** and **2**). To avoid the physical separation of the membrane components favored by ceramide,^[10] 1,2-di-*O*-hexadecyl-*sn*-glycerol serves as a flexible membrane anchor



in **1**; in addition, lactose was replaced by a triethylene glycol spacer.

Compound **1** can insert into phosphatidylcholine membranes and serves as a high molecular weight receptor with which low molecular weight **2** interacts (Figure 1). Several model membranes in the fluid *L_α* phase were tested. Experi-

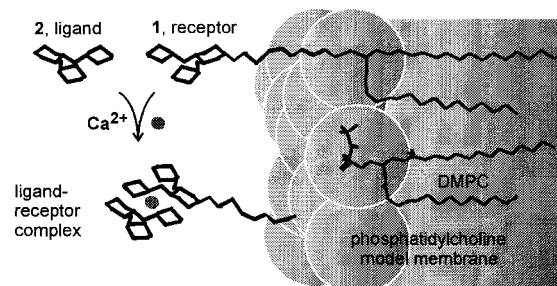


Figure 1. The low molecular weight ligand **2** coordinates transiently to the membrane-bound **1**, which functions as a receptor. The change in average rotational diffusion of trisaccharides with identical chemical shifts is quantified by NMR spectroscopy from NOESY spectra. DMPC = dimyristoylphosphatidylcholine.

ments with DMPC liposomes^[11] or with disk-shaped DMPC/DHPC bicells^[12] (DHPC = dihexanoylphosphatidylcholine), however, remained unsuccessful.^[13] At 300 K and therefore below the temperature of phase transition for the formation of bicells, the DMPC/DHPC mixture (3/1, molar) forms liposomes.^[14] Similar to cholesterol in native membranes, the short-chain membrane component DHPC increases the fluidity of the membrane. The surface curvature of the liposomes (diameter approximately 20 nm) and the membrane fluidity suppress side-to-side contacts of the GSLs and thus the formation of insoluble glycolipid aggregates. Therefore the addition of 6 mg of **1** to a solution of DMPC/DHPC liposomes (3/1, 300 K, 50 mg in 0.5 mL D₂O, 10 mM CaCl₂) does not lead to the formation of precipitate. Compound **1** inserts in the model membrane, and spin diffusion is observed for the positive intramolecular cross-signals in the NOESY spectrum^[15] (negative NOE). In the ¹H NMR spectrum **2** is equivalent in chemical shift to **1**; however, owing to its small relative molecular mass, **2** shows negative cross-signals in the NOESY spectrum (positive NOE). During the stepwise

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addition of **2** (3.6–36 mM) to a solution of **1** in DMPC/DHPC liposomes, the NOESY cross-signals of the two shift-equivalent trisaccharides add up. As a consequence of the transient binding of **2** to **1**, the measured intensities of the cross-signals, which directly reflect the average molecular mobility of **1** and **2**, are distinctly more positive than the sum of the cross-signals of separate solutions of **1** and **2** in DMPC/DHPC liposomes. From the difference, the slowing down of the mean rotational diffusion of **2** can be quantified.^[16] The very weak adhesive forces between **1** and **2** can only be detected because of the strong dependency of the NOE on the molecular mobility. Several intensive intramolecular NOEs served as experimental values. The calcium dependence found in cell assays was also observed, as the addition of EDTA reduces the NOE intensity to the value expected in the absence of $\text{Le}^x\text{-Le}^x$ contacts. Clouding or even formation of precipitate did not occur. The affinity constant between the Le^x trisaccharides was found to be $2\text{--}3\text{ M}^{-1}$ if one assumes the concentration of **1** to be equal to the receptor concentration.

The $\text{Le}^x\text{-Le}^x$ dimers can only be thermodynamically stabilized in water by means of hydrophobic contacts between pyranose rings and concomitant reorganization of the solvating envelope.^[17] Previous models^[18] assume either complete desolvation of the calcium ion or a coordination number below seven—both are very improbable for the weak bond of a neutral trisaccharide ligand to calcium ions.^[6, 19] The contact of the two Le^x trisaccharides can be stabilized by complementary hydrophobic surfaces. Thus, the rigid solution conformation of the Le^x trisaccharide^[20] allows an antiparallel dimerization with a hydrophobic contact between the α face of galactose and β face of *N*-acetyl glucosamine. The calcium ion can coordinate transiently twice to three oxygen atoms (Fuc-O2, Fuc-O3, Gal-O6) in the two Le^x trisaccharides and bridge them; the remaining coordination sites of the calcium ion can be saturated by water. A similar bridging, C_2 -symmetric coordination is known for calcium from a crystal structure analysis.^[21]

The experimental strategy introduced here reduces a complex biological recognition to a minimalized ligand–receptor system that allows the observation of the calcium-dependent head-to-head dimerization between Le^x glycoconjugates. This is only one example for the homotypic recognition between glycoconjugates. By variation of the dissolved component or of that anchored in the membrane, the affinities between other glycoconjugates are also quantifiable under standardizable experimental conditions.

Experimental Section

The glycoconjugates **1** and **2** were obtained by the reaction of the Le^x trisaccharide imide^[22] with the triethylene glycol spacer^[23] or with methanol and subsequent deprotection (see ref. [24]).

The mixture of DMPC and DHPC (3/1, molar) was prepared once and dissolved in portions of 50 mg in 0.5 mL D_2O for the experiments. The triethylene glycol membrane anchor in **1** showed no affinity towards calcium ions. This was examined for sialyl-Lewis^x which was joined to model membranes (sodium lauryl sulfate micelles, dodecylphosphocholine micelles and DMPC/DHPC bicells) with spacers of different lengths.^[25] Furthermore, in the concentration range between 5 and 15 mM no lipid clusters are formed from side-to-side contacts of **1**. This was established by independent titration experiments in which the systematic variation of the

concentration of **1** or CaCl_2 did not lead to a change of the line shape in the ^1H NMR spectrum of **1**.

All NMR experiments were carried with a Bruker DRX-600 spectrometer. The 2D spectra were recorded in the phase-sensitive mode with TPPI (time-proportional phase incrementation). The NOESY data matrices contained 2 K items in f_2 and 256 items in f_1 . For processing, the matrix elements of the indirect dimension were filled with zeros and an apodization was carried out with a square sinus function shifted by $\pi/2$ in both dimensions.

The calculation of the affinity constant for the carbohydrate-carbohydrate interaction ($K_a = [\mathbf{1}\cdot\mathbf{2}]/[\mathbf{1}][\mathbf{2}]$, where $[\mathbf{1}\cdot\mathbf{2}]$ is the concentration of the calcium-bridged $\text{Le}^x\text{-Le}^x$ complex) was made under the assumption, which was justified at this degree of dilution, that the complex is mainly present in its dissociated form: $[\mathbf{2}] = [\mathbf{2}]_{\text{total}}$. Calcium ions were present in surplus, and the ratio $[\mathbf{1}\cdot\mathbf{2}]/[\mathbf{2}]$ was calculated from the ratio of cross- to diagonal signal in the NOESY spectrum (cf. ref. [16]).

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Chelated Enolates of Amino Acid Esters—Efficient Nucleophiles in Palladium-Catalyzed Allylic Substitutions**

Uli Kazmaier* and Franz L. Zumpe

*Dedicated to Professor Ulrich Schmidt
on the occasion of his 75th birthday*

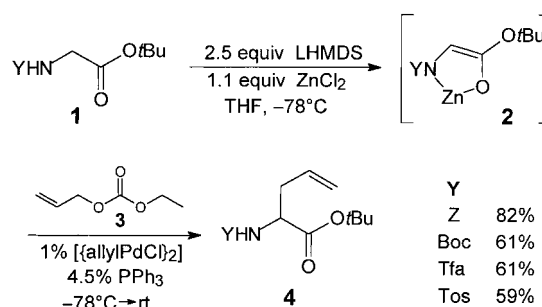
Because of the very mild reaction conditions required, transition metal catalyzed reactions are becoming more and more popular in organic synthesis.^[1] As many of these reactions tolerate a variety of functional groups, they are especially suited for the synthesis of complex molecules (natural products, etc.). Palladium, which can be used for cyclizations, cross-coupling reactions, or allylic substitutions,^[2]

holds a predominant position among the transition metals. Especially allylic substitutions are of particular interest, because asymmetric variations of these reactions are possible.^[3] In addition to heteronucleophiles mainly symmetrical stabilized carbanions like malonates are used as C-nucleophiles. It is very advantageous that during the C–C bond forming step only one stereogenic center is generated in the allyl moiety. Thus, its configuration can be controlled quite easily.

With the use of unsymmetrical C-nucleophiles like β -keto esters^[4] or imines of amino acid esters,^[5] mixtures of the diastereomers are usually obtained. This is due to the configurational lability of the allylated nucleophiles. Thus, considerably better results are obtained with alkylated derivatives.^[6] Trost et al. recently reported the use of substituted azlactones as nucleophiles, giving rise to α -alkylated γ,δ -unsaturated amino acids in a highly stereoselective fashion.^[7]

In contrast to the extremely well investigated reactions of stabilized “soft” carbanions, there are only a few reports concerning unstabilized enolates, for example those of ketones or esters, although the resulting products are often more interesting. The reactions of these enolates seem to be limited to only a few substrates. In the case of enolates of ketones, the best results are obtained with tin^[8] and boron enolates.^[9] These enolates attack the terminal positions of the allyl moiety *trans* to the palladium atom. In contrast, lithium enolates of esters preferentially attack the central position of the allyl moiety,^[10] giving rise to cyclopropane derivatives.^[11] The main problems with the conversion of these unstabilized enolates are probably due to coordination to the palladium atom, what might lead to inactive complexes.

This prompted us to investigate chelated enolates of amino acid esters **2** (Scheme 1) as nucleophiles in palladium-catalyzed allylic substitutions. Chelation causes a marked



Scheme 1. Synthesis of the unsaturated amino acid ester **4**. LHMDS = lithium bis(trimethylsilyl)amide, Z = benzyloxycarbonyl, Boc = *tert*-butoxycarbonyl, Tfa = trifluoroacetyl, Tos = toluene-4-sulfonyl.

enhancement of thermal stability without having any negative influence on the reactivity of these enolates. Due to the fixed geometry of the enolates, their conversions often proceed with a high degree of stereoselectivity. Therefore, we are investigating conversions of these enolates providing non-natural amino acids. We are especially interested in reactions which cannot be carried out with “normal” nonchelated enolates. Thus, chelated enolates of amino acid esters undergo

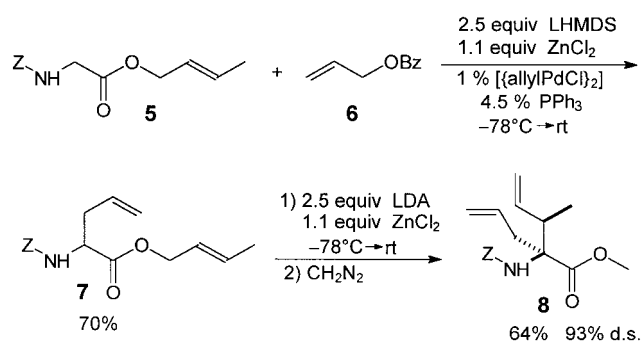
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a Claisen rearrangement upon being warmed to room temperature to provide γ,δ -unsaturated amino acids.^[12] With use of substituted *trans*-allyl esters the *syn*-configured products are obtained diastereoselectively via the chairlike transition state. The *anti* products, however, cannot be obtained from the corresponding *cis*-esters as easily. Therefore, we wanted to figure out if the *anti* products might be made accessible by allylic substitutions. Bearing in mind the problems with enolates mentioned above, we found this approach to be especially interesting.

We first investigated the reactions of different N-protected glycine esters with allyl carbonate **3** (Scheme 1).^[13] Deprotonation with excess LHMDS and addition of zinc chloride presumably leads to the formation of the chelated enolate **2**, which reacts with **3** in the presence of 2 mol % Pd(0) to provide the corresponding unsaturated amino acid ester **4**.^[14] The best results were obtained with the Z-protected derivative. As a result of the high reactivity of the chelated enolates, the allylation already takes place under very mild conditions at -78°C .

Consequently, this reaction can also be applied to substrates that give rise to by-products under more drastic reaction conditions. One example is crotyl ester **5** (Scheme 2). It is known that in the absence of a palladium catalyst the



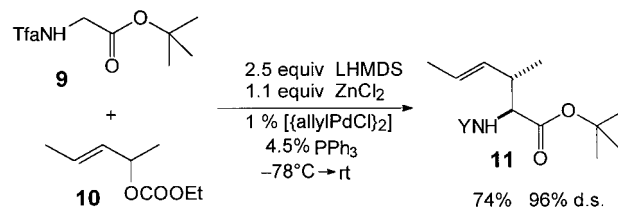
Scheme 2. Allylation of **5** and synthesis of **8**.

corresponding chelated enolate undergoes a Claisen rearrangement upon being warmed to -20°C to provide a γ,δ -unsaturated amino acid.^[15] Like other allyl esters or allyl carbonates this crotyl ester should be able to form a π -allylpalladium complex as well. Cleavage of the ester would be the consequence.

Nevertheless, reaction of **5** with **6**^[13] (after deprotonation with 2.5 equiv of LHMDS) yielded the desired allylated product **7**. No significant amounts of products resulting from cleavage or rearrangement could be detected. Even in the presence of a larger excess of LHMDS (3.5 equiv) and on warming of the reaction mixture to room temperature, Claisen rearrangement did not occur.^[16] Apparently, LHMDS is not able to deprotonate the allylated product **7**. Repeated generation of a chelated complex, which would be prone to the rearrangement, did not take place. Still, deprotonation of **7** is possible when lithium diisopropylamide (LDA)^[17] is applied. Thus, the allylic alkylation can be followed by a chelated enolate Claisen rearrangement resulting in the formation of the diallylated amino acid **8**. The two stereogenic

centers (*syn* configuration) are stereoselectively introduced by the Claisen rearrangement. By use of chiral allyl alcohols, access to optically active amino acids is possible.^[18]

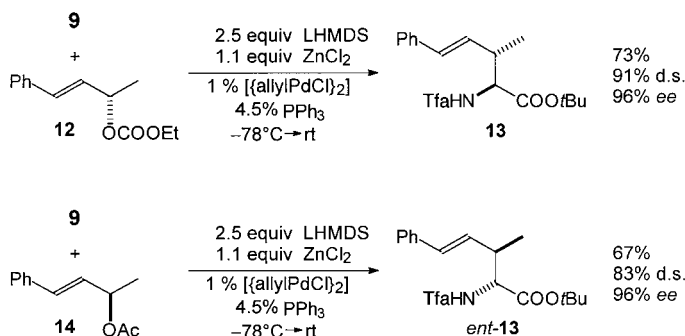
What about substituted allyl substrates? Is it possible to control the stereochemical outcome of the reactions as well? To answer this question, we investigated the reaction of 1,3-dimethylated allyl carbonate **10** with the Tfa-protected glycine ester **9** (Scheme 3). We decided to use **10**, because



Scheme 3. Diastereoselective allylation.

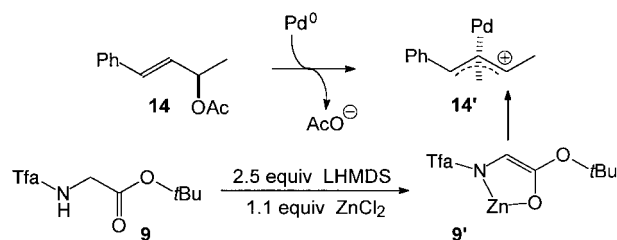
the intermediate π -allylpalladium complex is symmetrical. For that reason, no problem concerning regioselectivity arises in the nucleophilic attack. Ester **9** allows an easy determination of the diastereomeric ratio by gas chromatography. The desired allylated product **11** was obtained in good yield and with excellent diastereoselectivity. The diastereomerically pure product was accessible by simple recrystallization. Moreover, it was very pleasing to notice that the *anti* product was preferentially obtained from the palladium-catalyzed allylic alkylation. This allows us to synthesize both the *syn* diastereomer (by ester enolate Claisen rearrangement) and the *anti* diastereomer (by palladium-catalyzed allylic substitution). Both methods outstandingly complement one another.

Now we had to clarify if this method is also suited for the enantioselective synthesis of such unsaturated amino acids. For this purpose we used chiral allyl esters, which could easily be obtained by an enzymatic kinetic resolution of the corresponding racemic allyl alcohols.^[19] The reaction of carbonate **12** with glycine ester **9** yielded the desired allylated product **13** as a single regioisomer (Scheme 4). Again, the diastereoselectivity was very high, and moreover the transfer of chirality was excellent. With the corresponding acetate **14** the enantiomeric product *ent*-**13** was obtained with a comparable *ee* value, although the diastereoselectivity was clearly lower.



Scheme 4. Enantioselective allylation.

The stereochemical course of the reaction can be explained by the following model (Scheme 5): The π -allyl complex **14'** is formed by attack of palladium(0) on the allyl acetate **14** with inversion of the configuration. Subsequently **14'** reacts with



Scheme 5. Proposed mechanism.

the chelate enolate **9'** as shown. The attack of the nucleophile occurs in such a way that the resulting double bond is conjugated to the aromatic π system. Because of the staggered arrangement of the substituents, steric interactions in the transition state between the π -allyl complex and the nucleophile are minimized. The bulky *tert*-butyl group is located at the less hindered methyl-substituted side of the allyl complex, so no interactions should be expected here as well. This model is in accordance with the observation that no significant diastereoselectivity can be obtained if the corresponding methyl glycinate is used. The influence of the leaving group on selectivity as well as some applications of this reaction and the allylic substitution in the presence of chiral ligands are currently under investigation.

Experimental Section

The protected amino acid ester (1 mmol) was dissolved in THF (4 mL). At -78°C a freshly prepared solution of LHMDS (2.5 mmol) in THF (2 mL) was added. After 30 min at -78°C a solution of ZnCl_2 (1.1 mmol) in THF (5 mL) was added with stirring. After an additional 30 min a solution of $[\text{allylPdCl}]_2$ (1 mol %), PPh_3 (4.5 mol %), and the corresponding allyl ester (1.5 mmol) in THF (3 mL) was added. The solution was stirred and warmed to room temperature overnight. Subsequently, the solution was diluted with diethyl ether and hydrolyzed with 1N KHSO_4 solution. The aqueous phase was extracted twice with diethyl ether, and the combined organic phases were dried over anhydrous Na_2SO_4 . After evaporation of the solvent the crude product was purified by silica gel column chromatography (eluent: hexanes/ethyl acetate).

7: M.p. $62-63^{\circ}\text{C}$; ^1H NMR (300 MHz, CDCl_3): δ = 1.03 (d, J = 6.9 Hz, 3 H), 1.45 (s, 9 H), 1.65 (d, J = 6.3 Hz, 3 H), 2.75 (m, 1 H), 4.42 (dd, J = 8.7, 4.7 Hz, 1 H), 5.24 (dd, J = 15.3, 7.7 Hz, 1 H), 5.52 (dq, J = 15.3, 6.3, 1.0 Hz, 1 H), 6.65 (brs, 1 H); ^{13}C NMR (75 MHz, CDCl_3): δ = 16.36, 17.63, 27.75, 39.37, 56.96, 82.83, 115.56 (q, J = 288 Hz), 128.03, 129.30, 156.74 (q, J = 372 Hz), 168.83; elemental analysis calcd for $\text{C}_{13}\text{H}_{20}\text{F}_3\text{NO}_3$: C 52.88, H 6.83, N 4.74; found: C 52.85, H 6.66, N 4.71.

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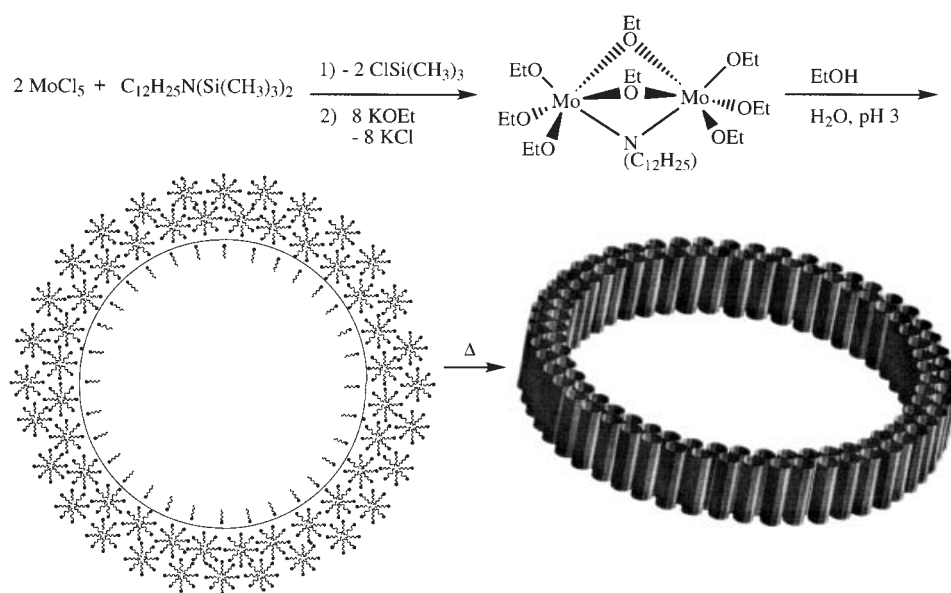
Phase Changes and Electronic Properties in Toroidal Mesoporous Molybdenum Oxides**

David M. Antonelli* and Michel Trudeau

Highly ordered porous transition metal oxides are predicted to be useful in a wide variety of areas where flexible redox activity and unique magnetic and electronic properties are necessary. Such areas include the synthesis of quantum wires and nanomagnetic devices, the design of hydrogen storage materials for batteries, the development of new catalysts for oxidation of organic substrates, and the fabrication of wave guides, membranes, and sensor components. By manipulating synthesis conditions it is possible to control the porosity of metal oxides on both the nanometer^[1–3] and the micron level.^[4,5] Hexagonally packed mesoporous silicas can be synthesized with a wide variety of shapes including toroid and discoid morphologies,^[6–8] demonstrating that not only can the meso- and macroscopic porosity of a material be controlled, but the overall shape and macroscopic design of the oxide array can be manipulated to synthesize materials with order on several levels of scale. Herein we describe the synthesis of semiconducting mesostructured mixed oxidation state molybdenum bronzes which selectively form toroid shapes with diameters of several hundred nanometers.

While d⁰ mesoporous transition metal oxides of Nb,^[3,9] Ta,^[10] Zr,^[11–13] Ti,^[14] and Hf^[15] have been synthesized, there are few examples of mesoporous oxides of metals with partially filled d shells. The presence of unpaired electrons in the walls of the mesostructure should lead to unusual magnetic and electronic properties not displayed by their d⁰ counterparts. Mixed oxidation state mesoporous Mn oxides were recently synthesized by a liquid crystal templating strategy.^[8] This material was the

first conducting mesoporous oxide. In order to exploit the inherent magnetic properties of unpaired electrons in metal oxide materials we aimed at synthesizing a d¹ mesoporous Mo^V oxide. It is known that self-assembly of W^{VI} and Mo^{VI} oxides in the presence of cationic surfactants leads to the formation of hexagonally packed surfactant salts of W and Mo oxo clusters which were not stable to surfactant removal.^[16] Mesoporous niobium and tantalum oxides can be synthesized by ligation of amine templates to M(OEt)₅ (M = Nb, Ta) followed by hydrolysis and template removal by acid treatment. By analogy, Mo(OEt)₅ was combined with dodecylamine in varying concentrations and subsequently hydrolyzed. Under these conditions only amorphous materials were formed. Under the assumption that the lower Lewis acidity of the d¹ Mo center as compared to the d⁰ Nb center was retarding dodecylamine binding, we synthesized a dimeric molybdenum ethoxide complex (Scheme 1) with a bridging dodecylimido group, thus establishing the required surfac-



Scheme 1. Synthetic scheme for formation of mesostructured molybdenum oxide (Mo-TMS1) toroids. Treatment of molybdenum pentachloride with bis(trimethylsilyl)dodecylamine in dichloromethane led to the formation of a molybdenum imido chloride complex, the dimeric structure of which was assigned on the basis of analogous imido complexes previously synthesized. Reaction of this complex with potassium ethoxide in toluene led to the formation of a viscous brown oil formulated as the imido octaethoxy dimer (shown top right). Dissolution of this complex in ethanol followed by treatment of the resulting brown solution with water at pH 3 gave a foamy brown solution. Heating this solution for two weeks at 90 °C gave toroidal Mo-TMS1. The formation of toroids is rationalized on the basis of microphase separation through microbubble (as shown in the center) or vesicle formation which templates the crystallization of micellar molybdenum oxide/surfactant aggregates. In this strategy the dodecylamine acts as a two-way templating agent, directing formation of both the micelles and the spherical macrostructure (shown schematically bottom right).

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tant–metal interaction prior to hydrolysis. The stoichiometry of this complex ensured that the 2:1 metal to surfactant ratio, optimal for Nb-TMS1 formation, was maintained. Addition of water to solutions of this complex in ethanol at pH values ranging from 3–7 led to formation of foamy brown solutions which yielded bronze mesostructured Mo oxide (Mo-TMS1) after aging at 90 °C for two weeks. The X-ray powder diffraction (XRD) pattern (Figure 1A) shows a high-intensity (100) peak at $d = 32 \text{ \AA}$ and less resolved lower angle peaks,

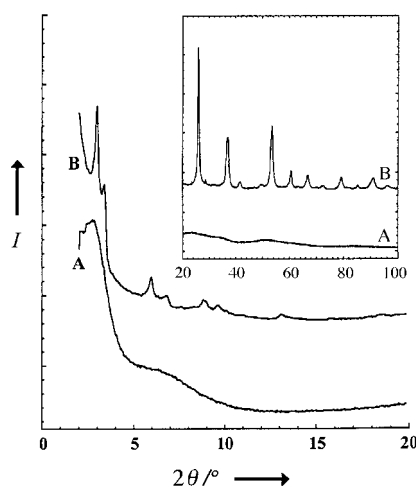


Figure 1. XRD patterns of as-synthesized a) hexagonal bronze and b) lamellar blue mesostructured molybdenum oxide obtained on a Siemens D500 θ - 2θ diffractometer with $\text{CuK}\alpha$ radiation ($\lambda = 1.5418 \text{ \AA}$). The phase change from the molybdenum bronze to the molybdenum blue is accompanied by the appearance of higher angle peaks as shown in the inset.

which confirm the mesostructured nature of this material. The high-angle diffraction pattern (insert) does not show any structure. This is indicative of the amorphous nature of the wall structure. The broadened reflections as compared to those of MCM-41 are typical of materials synthesized with neutral templates,^[17] and suggest a lower degree of mesoscopic order than those materials synthesized with charged surfactants. When ethanol was not added to the synthesis mixture or the hydrolysis was conducted at pH values less than 1, lamellar materials were formed. The elemental analysis of the hexagonal Mo-TMS1 gave values of C 19.83, H 4.04, and N 1.71 %, demonstrating that the surfactant had been retained in the structure. Figure 2 presents the X-ray photoelectron spectra (XPS) for the air-exposed surface of the powder taken using monochromatic $\text{AlK}\alpha$ radiation. The peak

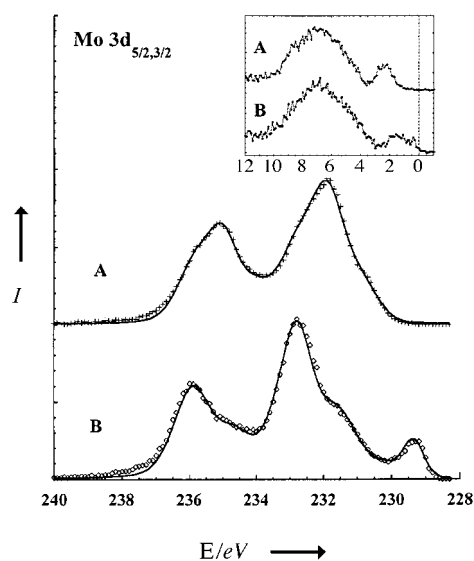


Figure 2. XPS 3d emission for the two Mo samples (A = bronze, B = blue) with an inset showing their corresponding valence spectra. The dashed line in this inset represents the Fermi level. The valence spectra values suggest that the bronze is semiconducting and the blue is metallic.

positions were referenced to the C-(C,H) carbon emission at 284.8 eV. Peak deconvolution revealed that the $3d_{5/2}$ emission is composed of three contributions: a major one at 231.9 eV and two smaller ones at 232.8 and 230.9 eV. Based on reference tables^[18] this would indicate that the molybdenum at the surface would be present mostly in the +6 state, indicating that a small degree of surface air oxidation has taken place during the synthesis procedure. On the other hand, the measured surface Mo:O ratio was found to be about 51 %, after removing the O atoms present in C–O groups, which would agree with the formation of MoO_2 . It should be mentioned that an emission at 232 eV has previously been attributed to MoO_2 .^[19] Ozin et al. report that 1 eV can be added for each increase in oxidation state from Mo metal (228 eV),^[20] and thus the peaks at 232–233 eV could also be due to Mo^V . The inset in Figure 2 presents the valence spectra for the bronze mesostructure and shows the semiconducting nature of the bronze mesostructure with a gap of about 1.3 eV to the Fermi level. This indicates that this material is a semiconductor, which may have numerous applications in the nanofabrication of materials with unique electronic properties. Conductivity measurements of this material gave a value of $2.5 \times 10^{-12} \Omega \text{ cm}^{-1}$, which is extremely low on the basis of the XPS data and can be explained by the discontinuous nature and low density of the Mo bronze which can lead to experimental densification and resistivity measurement problems. This was confirmed by the UV/Vis reflectance mode spectrum^[21] of this material which displayed an absorbance at 330 nm corresponding to a band gap of 3.75 eV. This is very similar to the value of 3.18 eV observed for mesoporous Mn oxide.

Low magnification transmission electron microscope (TEM) images of samples of Mo-TMS1 prepared by laying thin microtomed sections over carbon-coated grids revealed a fascinating array of ringlike structures (Figure 3a) approximately 300 nm in diameter. Some of the rings had smaller rings inside them, forming unique living cell-like structures. Although most of these structures were free-standing rings, some had fused together to form random structures similar to those observed for mesolamellar aluminophosphates.^[22, 23] This contrasts with emulsion-templated macroporous Ti, Zr, and Si oxide-based materials,^[5] which form sheets with long-range hexagonal packing of the individual macropores but have no well-ordered porous patterns on the mesoscopic level. Higher magnification of the toroidal face in samples of Mo-TMS1 revealed that these structures were constructed of mesotubules of roughly 50 nm in length and 3 nm in diameter (Figure 3b). This value is consistent with the position of the (100) peak at 32 Å in the XRD pattern. Unusually, clusters of smaller toroids reorder into larger toroids over a matter of seconds under the influence of a TEM electron beam. Energy dispersive X-ray spectroscopy (EDS) showed that this material was rich in molybdenum, confirming that the ringlets were indeed composed of mesostructured molybdenum oxide. Previous work has shown that toroidal mesoporous silicate forms can be generated by manipulation of the synthesis conditions to favor curvature in propagating silica–surfactant micelles.^[7] The mesotubes in these silica-based toroids run along the plane of the ringlets and not perpendicular to the

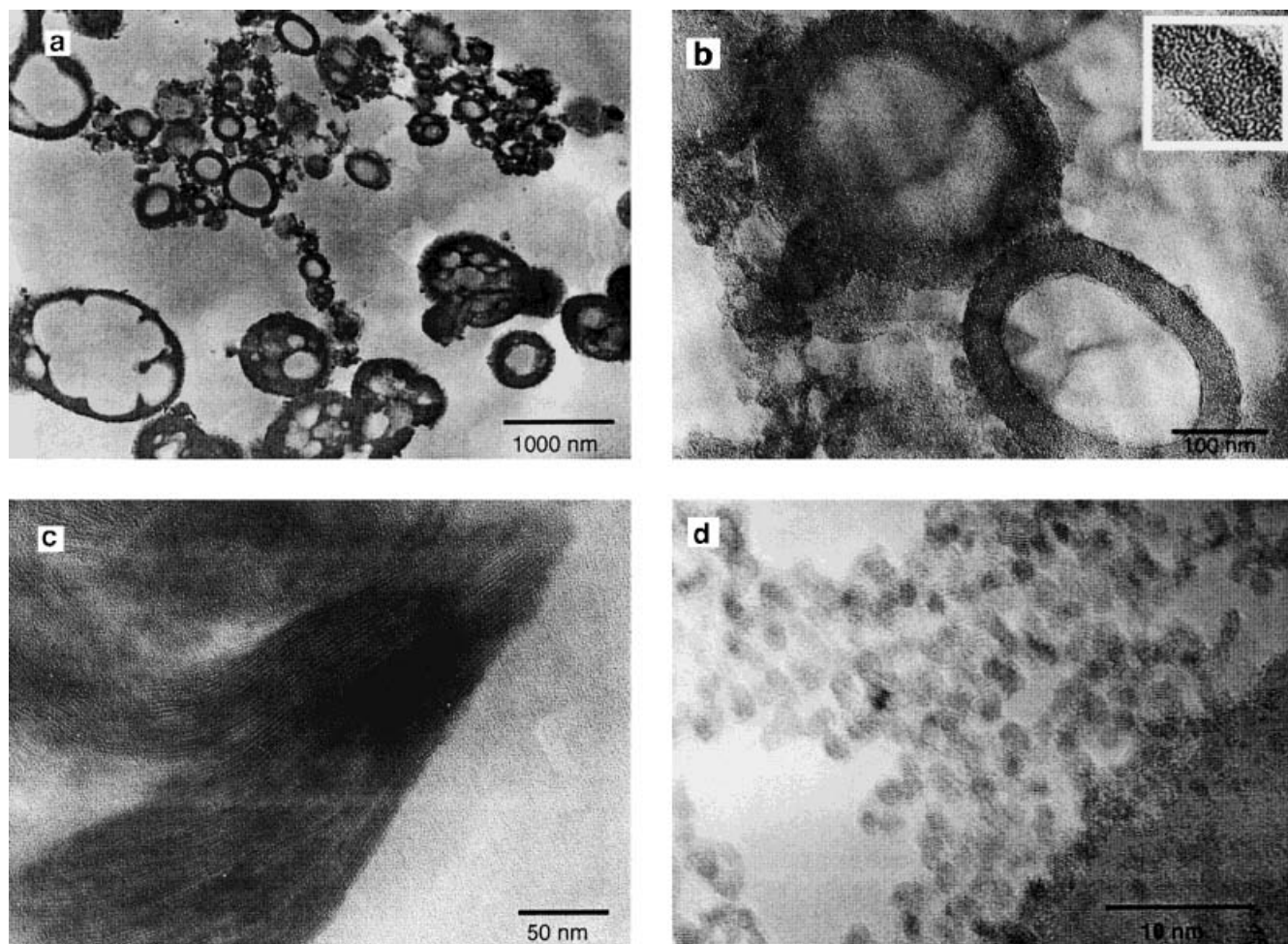


Figure 3. TEM images of molybdenum oxide bronzes: a) at low magnification revealing the toroidal structures (see text for details); b) at higher magnification. The further-magnified inset reveals the mesostructured nature of the walls of the toroid. Scanning electron microscopy (SEM) revealed no salient structural features or tubular forms, indicating that these toroids are relatively flat (ca. 50 nm). Other areas of sample exist in which there is no higher order to the mesostructured system, although the toroidal regions dominate the material; c) lamellar region of the mesostructured molybdenum oxide blue showing that certain regions of the bronze have reordered into a layered phase in the blue; d) close up of a region of the molybdenum blue showing where crystallization of the walls of the mesostructure into oxide grains about 1.5 nm in size has led to partial degradation of the occluded mesopores. Samples were prepared either by sonication in isopropyl alcohol or microtoming followed by deposition on a carbon-coated copper grid. Micrographs were recorded at 300 KV on a Hitachi H-9000 STEM.

plane as observed for the molybdenum bronze toroids. This suggests a mechanism in which the surfactant acts as a twofold templating agent, not only directing the formation of the individual mesotubes, but also forming either vesicles or microbubbles or by microphase separation which template the crystallization of the tubules into highly ordered ring structures (Scheme 1). This mechanism is likely related to that proposed for diatomaceous mesolamellar aluminophosphates,^[22, 23] with the exception that ordered pores rather than layers are formed on the mesoscopic level. The factors governing formation of these toroids were studied and it was found that the presence of KCl in the synthesis mixture clearly promotes toroid formation, as removing the KCl prior to hydrolysis leads to bulk mesostructured Mo-TMS1 with no macroscopic order. The influence of salts on the formation of mesoporous silicate macrotubules has been studied recently.^[24]

Attempts to remove surfactant either by acid treatment or ozone treatment led to formation of layered and amorphous

materials, respectively. In an attempt to improve the structural strength of the material so that it would retain its structure on template removal, we subjected the bronze Mo-TMS1 to hydrothermal treatment in a sealed tube at 150 °C for ten days. Surprisingly, both the material and the supernatant had turned deep blue in color, a common hue for mixed oxidation state Mo oxides. The XRD pattern of this new molybdenum blue (Figure 1 B) showed that the material had converted into a lamellar structure with major peak at about 30 and 26 Å. Lower angle peaks with *d* values ranging from 15 to 6 Å were also observed, indicative that the walls or lamellae of the material had crystallized. The high-angle diffraction pattern revealed a series of peaks which can be indexed to a single-phase MoO₂ (Tugarinovite) that has a monoclinic structure (space group *P*2₁/*c*; *a* = 5.608, *b* = 4.859, *c* = 5.537 Å, β = 119.37°). This is indicative of crystallization of the walls of the bronze mesostructure. Highly crystallized walls have only been observed once in a mesoporous material.^[8] The elemental analysis of this material showed that the stoichiometry of

the molybdenum blue had not changed from the bronze. The XPS $3d_{5/2,3/2}$ emission (Figure 2) indicates that the surface oxidation state was modified with a distinct emission around 229.4 eV indicative of the enhanced presence of lower bound states for this material. On the other hand, interestingly, the Mo:O ratio (after removing the contribution of oxygen atoms related to C–O groups) was found to be again around 52 %, in agreement with the presence of MoO_2 . This is consistent with past work on mixed oxidation state molybdenum oxide clusters in which XPS studies revealed a mixture of Mo^{IV} and Mo^{VI} .^[20] The valence spectra (Figure 2 inset) reveal that this mesostructure is a much better conductor than the bronze sample with practically no gap between the valence band and the Fermi level. This is comparable to that of MnO_2 , which is nearly metallic in nature. The conductivity of this sample was $6.3 \times 10^{-7} \Omega \text{cm}^{-1}$, which is similar to the values previously reported for mesoporous Mn oxide.^[8] Part of this conductivity, which should be even higher due to the position of the Fermi level in respect to the valence band, can be explained by the nanocrystalline nature of the material. Because the average grain size is on the order of 15 Å, a large part of the conductivity will be dominated by the grain boundary contribution, which can decrease the conductivity by at least one or two orders of magnitude as compared to the coarse grain counterparts.

TEM images at low magnification showed regions of lamellar structure with approximately 3 nm between the layers (Figure 3c) as well as some regions in which the toroidal structures were maintained. Further magnification at the fringes of the porous regions revealed that the mesopores had undergone a phase change into discontinuous nanoparticles of roughly 15 Å in size (Figure 3d). These regions can be compared to mesostructured tungsten-based materials in which the walls of the mesostructure are constructed of well-defined, but isolated tungsten oxo clusters.^[16] The presence of grains in the individual lamellae of the layered region could not be confirmed in this study. This suggests that there are two rivaling phase changes at work in this system. In the first, the walls of the hexagonal mesopores restructure into individual grains, hence imposing a new level of structure on the material while at the same time destroying the structural integrity of the walls. In the second, the mesopores undergo a collapse into a lamellar structure with no crystallinity in the oxide lamellae. Phase changes between lamellar, cubic, and hexagonal mesostructures are common. Further heating did little to alter the XRD pattern of the material. These materials provide insight into the thermal collapse of mesoporous metal oxides into bulk crystalline phases of the metal oxide, suggesting that crystallization of the walls in a mesostructure leads to grain formation and growth along with destruction of the porous matrix. This further suggests that in many cases it may not be possible to form mesoporous oxides in which the walls are crystalline, as in zeolites, since crystallization of the walls may lead to angstrom-level reordering of the material into a different structure.

In summary, the templating of molybdenum oxides under conditions favoring both rodlike micelle and vesicle or microbubble formation has led to mixed-valent conducting toroidal structures which display long- and short-range order

on many levels of scale. While emulsions and microbubbles can lead to macroporous structures with applications as waveguides and ultralightweight materials, liquid crystal micelle templating can lead to structures with order on the nanometer scale. By controlling nucleation conditions and growth, hierarchical structures with order on both the macro- and mesoscale can be achieved. Treatment of these toroids with a TEM electron beam leads to a unique phase evolution to form larger toroids, while heating to 150 °C leads to reordering of the mesostructure and formation of a new lamellar phase and nanocrystalline molybdenum oxide. These phase changes are accompanied by a color change from brown to blue and a dramatic narrowing of the gap to the Fermi level. We anticipate that these materials may open the doorway to electronically active macro-mesostructures with applications in electronic materials or catalysis.

Experimental Section

Molybdenum pentachloride (5.0 g, 18.3 mmol) was treated with bis(trimethylsilyl)dodecylamine (3.0 g, 9.2 mmol) in dry dichloromethane (50 mL) for 2 h at ambient temperature. The solvent was then removed in vacuo and potassium ethoxide (6.14 g, 73.2 mmol) in ethanol (50 mL) was added with cooling. Water (100 mL) was added to this mixture, and the resulting brown liquid was allowed to sit at ambient temperature for 24 h and then heated to 90 °C for two weeks. The deep bronze precipitate was collected by filtration and washed with three portions of ethanol and water and then dried at 100 °C overnight. This as-synthesized mesostructured molybdenum oxide material exhibited a peak in the XRD pattern at $d = 32$ Å and showed a large proportion of toroidal regions by TEM. Heat treatment for ten days at 150 °C in a sealed tube resulted in formation of a blue material with a lamellar XRD pattern as well as high-angle peaks attributable to Tugarinovite. This material had very few toroidal regions.

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Ni(tpt)(NO₃)₂—A Three-Dimensional Network with the Exceptional (12,3) Topology: A Self-Entangled Single Net**

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 and Richard Robson*

In Memory of Dr. George Winter

Extended framework solids are currently of considerable interest and importance because of the scope they offer for the generation by design of new materials with a range of potentially useful properties.^[1] A valuable conceptual approach to building and describing extended frameworks, in particular those involving interpenetration,^[2] is based on the idea of a net, a valuable catalogue of which has been provided by Wells.^[3]

Connecting ligands providing two or more bidentate metal-binding sites that are now starting to be explored,^[4] are particularly appealing as building blocks for coordination polymers because they promise robust networks with good electronic communication between metal centers. When an octahedral metal center takes on three ligands of this type the metal center itself adopts the role of 3-connecting node giving access to a range of still little known 3-connecting nets. We suspect that many examples of 3-connecting nets will appear in the near future. The symbol (*n*,3) can be used to characterize the topologies of nets in which all nodes are 3-connecting and where the shortest circuits involving all three different pairs of connections radiating from each node are *n*-gons. An interesting question that arises when one considers the set

of (*n*,3) nets is “what is the largest possible value for *n*?”—or, in other words “what is the three-dimensional (3D) 3-connected net whose shortest circuits are as large as possible?”. The (12,3) net is the one with the largest value of *n* considered by Wells.^[1,5] What we describe here is, to the best of our knowledge, the first real chemical example of a (12,3) net.

A representation of a (12,3) net with planar nodes is shown in Figure 1. The net, which is intrinsically chiral, can be visualized in terms of “double” sixfold helices, all of the same

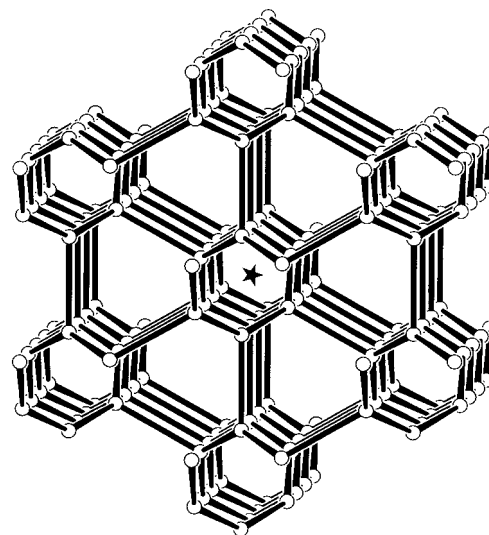
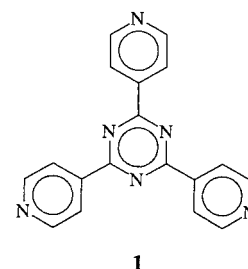


Figure 1. Representation of the (12,3) net in which all nodes are planar and equivalent. One sixfold double helix is highlighted with a star for ease of identification.

handedness and all parallel, connected together by links that are perpendicular to the helical axis. The extension of the (12,3) net in the direction of the helical axis is dictated by the angle at each node internal to the helix which can, in principle, take any value from 120° (regular trigonal nodes) to 180° (T-shaped nodes)—the larger this angle, the greater the pitch of the helices and the more extended in the direction of the helical axis does the structure become.

Whilst neither strictly trigonal nodes alone nor strictly T-shaped nodes alone can be used to generate the (12,3) net, it can be constructed in a completely strain-free manner from alternating T and trigonal nodes. This is precisely what we observe, as described below, in the structure of the crystalline coordination polymer^[6] obtained from nickel nitrate and the trigonal 3-connecting ligand tri-4-pyridyl-1,3,5-triazine (tpt; **1**). This geometrically simple ligand has already afforded a wide variety of unusual and symmetrical 3D coordination networks.^[7] The crystal consists of a well-defined coordination polymer framework with the (12,3) topology containing equal numbers of tpt and Ni²⁺ centers. Disordered solvent occupies the chiral channels running parallel with the helical axes. The essence of the connectivity of the network is



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[**] The authors gratefully acknowledge support from the Australian Research Council.

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Ni(tpt)(NO₃)₂—A Three-Dimensional Network with the Exceptional (12,3) Topology: A Self-Entangled Single Net**

Brendan F. Abrahams, Stuart R. Batten,
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 and Richard Robson*

In Memory of Dr. George Winter

Extended framework solids are currently of considerable interest and importance because of the scope they offer for the generation by design of new materials with a range of potentially useful properties.^[1] A valuable conceptual approach to building and describing extended frameworks, in particular those involving interpenetration,^[2] is based on the idea of a net, a valuable catalogue of which has been provided by Wells.^[3]

Connecting ligands providing two or more bidentate metal-binding sites that are now starting to be explored,^[4] are particularly appealing as building blocks for coordination polymers because they promise robust networks with good electronic communication between metal centers. When an octahedral metal center takes on three ligands of this type the metal center itself adopts the role of 3-connecting node giving access to a range of still little known 3-connecting nets. We suspect that many examples of 3-connecting nets will appear in the near future. The symbol (*n*,3) can be used to characterize the topologies of nets in which all nodes are 3-connecting and where the shortest circuits involving all three different pairs of connections radiating from each node are *n*-gons. An interesting question that arises when one considers the set

of (*n*,3) nets is “what is the largest possible value for *n*?”—or, in other words “what is the three-dimensional (3D) 3-connected net whose shortest circuits are as large as possible?”. The (12,3) net is the one with the largest value of *n* considered by Wells.^[1,5] What we describe here is, to the best of our knowledge, the first real chemical example of a (12,3) net.

A representation of a (12,3) net with planar nodes is shown in Figure 1. The net, which is intrinsically chiral, can be visualized in terms of “double” sixfold helices, all of the same

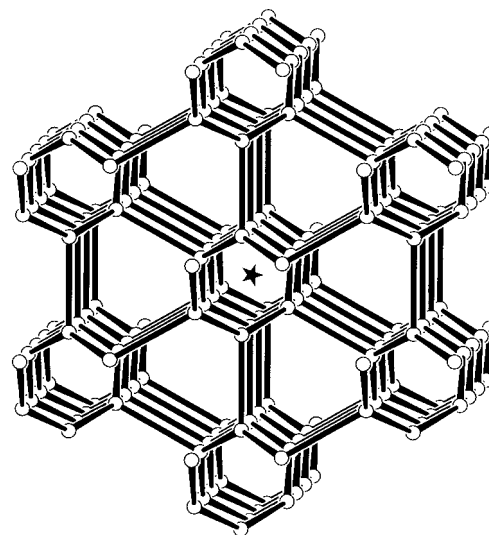
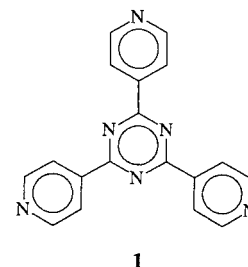


Figure 1. Representation of the (12,3) net in which all nodes are planar and equivalent. One sixfold double helix is highlighted with a star for ease of identification.

handedness and all parallel, connected together by links that are perpendicular to the helical axis. The extension of the (12,3) net in the direction of the helical axis is dictated by the angle at each node internal to the helix which can, in principle, take any value from 120° (regular trigonal nodes) to 180° (T-shaped nodes)—the larger this angle, the greater the pitch of the helices and the more extended in the direction of the helical axis does the structure become.

Whilst neither strictly trigonal nodes alone nor strictly T-shaped nodes alone can be used to generate the (12,3) net, it can be constructed in a completely strain-free manner from alternating T and trigonal nodes. This is precisely what we observe, as described below, in the structure of the crystalline coordination polymer^[6] obtained from nickel nitrate and the trigonal 3-connecting ligand tri-4-pyridyl-1,3,5-triazine (tpt; **1**). This geometrically simple ligand has already afforded a wide variety of unusual and symmetrical 3D coordination networks.^[7] The crystal consists of a well-defined coordination polymer framework with the (12,3) topology containing equal numbers of tpt and Ni²⁺ centers. Disordered solvent occupies the chiral channels running parallel with the helical axes. The essence of the connectivity of the network is



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represented in Figure 2. The particular crystal studied in detail had the absolute configuration shown in Figure 2 but a second crystal chosen at random was found to have the opposite handedness. All nickel centers (the T-nodes^[8] in Figure 2) are equivalent as are all tpt units (the trigonal nodes

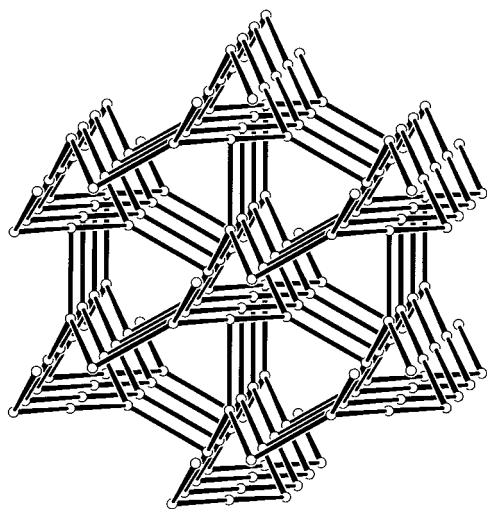


Figure 2. Representation of the (12,3) net consisting of alternating trigonal and T-shaped nodes as seen in the structure of solvated $\text{Ni}(\text{tpt})(\text{NO}_3)_2$. The trigonal nodes are located at the centers of the tpt triazine rings and the T-shaped nodes are located at the Ni centers. Node-to-node separation 7.64 Å.

in Figure 2 which are located at the midpoints of the tpt triazine rings). Each nickel center together with one of its coordinated pyridyl groups lies on a twofold axis, the other two pyridyl groups are *cis* to the first and related to each other by the twofold axis. The octahedral coordination of the nickel center is completed by oxygen atoms from monodentate and bidentate nitrate anions which are disordered around the twofold axis.

The presence of the two types of 3-connecting centers (trigonal and T) means that the double sixfold helices have now become double threefold helices with a triangular cross-section, as can be clearly seen in Figure 2. In the (12,3) net consisting of T-shaped nodes with 90° angles and regular trigonal nodes with 120° angles the orientation of the trigonal units to the hexagonal axes is “fixed” by the geometry and the proportions of the unit cell are predetermined ($a/c = 2/\sqrt{6} = 0.8165$). There is a very close correspondence between these proportions dictated by simple geometry and those observed in the real crystal ($a/c = 0.8159$).

A representation of the beautifully interwoven network, showing all the non-hydrogen atoms of the tpt units and the nickel centers in three adjacent double helices, is presented in Figure 3.

Within the series of uniform $(n,3)$ nets the (12,3) net is exceptional in showing self-entanglement such that some of its *shortest* circuits have *other shortest circuits* passing through them. Figure 4 provides an example of two 12-gons passing one through the other. It is true that if one were prepared to search for continuous circuits that are large enough, one could identify, in any extended network, pairs of such circuits that

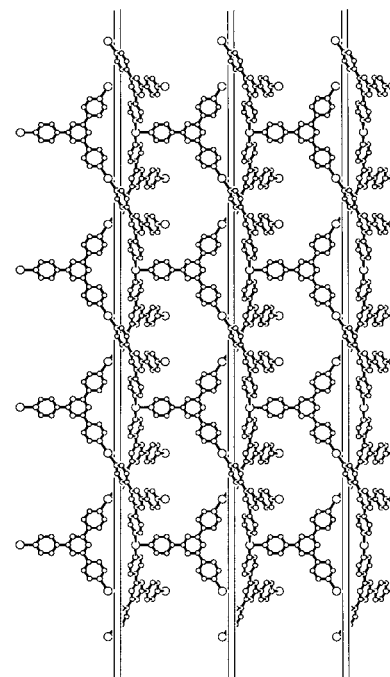


Figure 3. Three interconnected double helices showing the C, N, and Ni atoms. For ease of visualization the double helices are wrapped around imaginary “poles”.

pass one through the other, but generally they would be very much larger than the shortest circuits that constitute one of the fundamental characterizing features of the net topology. It does appear to us, therefore, that nets in which two *shortest* circuits pass one through the other constitute a special topological class which could properly be described as *self-penetrating* and/or *self-entangled*. It is interesting that as n increases in the series of $(n,3)$ nets a point is reached where the system, in a manner of speaking, “folds back on itself”, as occurs in (12,3) with the formation of double helices within what still remains a single net; the catenane-related self-penetration stems from this, as can be seen in Figure 4. If examples with $n > 12$ were to be capable of existence it seems likely this catenane-like self-entanglement would prove to be one of their inherent features.

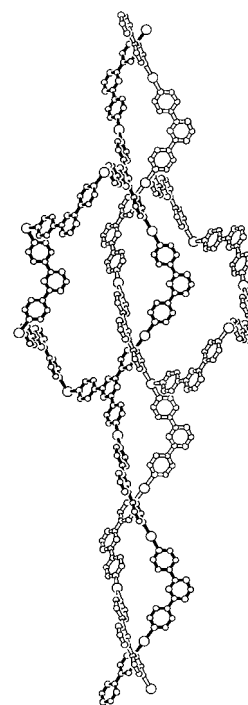


Figure 4. A double helix and one of the catenane-like pairs of 12-gons associated with it. One chain of the double helix and the 12-gon associated with it are here differentiated from the other chain/12-gon combination but all are part of a single 3D net.

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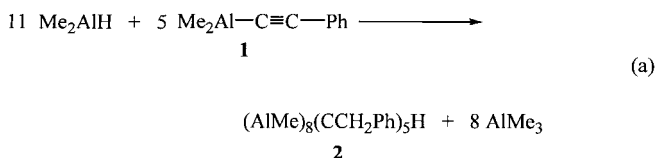
Pentacarba-*arachno*-tridecaalane (AlMe)₃(CCH₂Ph)₅H with an Al₈C₅ Skeleton—The First Polyhedral Carbaalane**

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- [5] We do not know whether $n = 12$ is a mathematically imposed upper limit or whether topologically uniform ($n,3$) nets with $n > 12$ "exist" and are awaiting discovery or whether such nets have already been considered by others unbeknown to us.
- [6] Crystals of Ni(tpt)(NO₃)₂·solvate were obtained by layering a solution of Ni(NO₃)₂·6H₂O (45 mg) in ethanol (10 mL) on top of a solution of tpt (25 mg) in 1,1,2,2-tetrachloroethane (10 mL); ethanol (5 mL) served as a "buffer layer" between the two solutions. Crystals grew over a period of days as the solutions slowly interdiffused. Crystal structure data: $0.45 \times 0.25 \times 0.25$ mm³, trigonal, $P3_121$, $a = 15.216(2)$ Å, $c = 18.650(6)$ Å, $V = 3740(1)$ Å³, $Z = 3$, $\rho_{\text{calcd}} = 1.554$ g cm⁻³, $2\theta_{\text{max}} = 55^\circ$, $\lambda(\text{MoK}\alpha) = 0.71069$ Å, $\omega/2\theta$ scan mode, $T = 130$ K, 7302 measured reflections, 5719 independent reflections ($R_{\text{int}} = 0.0216$). Corrections were applied for Lorentz and polarization effects. Absorption corrections applied (indexed crystal faces, SHELX-76^[9]), $\mu = 1.29$ mm⁻¹, min./max. transmission factors 0.5784, 0.7343. Structure solved by using Patterson methods (SHELXS-86^[10]). Full-matrix least squares refinement based on F^2 , 321 parameters (SHELXL-97^[11]), pyridyl hydrogen atoms placed at geometrically estimated positions, $R1 = 0.0839$, $wR2 = 0.2352$, max. residual electron density 1.049 e Å⁻³. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-108609. 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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Carbaboranes contain clusters formed by boron and carbon atoms. Owing to their unique structures and bonding they have been intensively investigated in the last few decades and have been included in inorganic chemistry textbooks for a long time.^[1] Up to now the corresponding carbaalanes with a skeleton containing only aluminum and carbon atoms were unknown; however, some compounds in which single aluminum atoms are inserted into borane or carbaborane clusters have been reported.^[2] A suitable method for the synthesis of carbaboranes is based on the hydroboration of alkynyl dialkylboranes with an excess of dialkylboron hydrides, but often the yields are very low.^[3] Recently, our group has begun systematic investigations into the related hydroalumination reaction, in particular with regard to the synthesis of polyaluminum compounds. The latter have been attracting growing interest as chelating Lewis acids in phase-transfer processes or for the recognition of anions.^[4] The hydroalumination of aluminum alkynides was described once before, however, the products were never isolated or characterized, and subsequent work up by hydrolysis generally yielded a mixture of hydrocarbons.^[5] Similar treatment was reported for the products of the hydroalumination of organic alkynes.^[6] The organoaluminum intermediates are, however, of great interest with regard to our investigations, and one of our aims is to isolate them in a pure form for a complete characterization. We report here on the remarkable reaction of dimethylaluminum hydride with dimethylaluminum phenylethyne (**1**).

When **1** was treated with dimethylaluminum hydride in *n*-pentane in a stoichiometric ratio, the color of the reaction mixture changed to red, but the ethynide was recovered in an almost quantitative yield. Compound **1** was only consumed completely when it was dissolved in an excess of the dimethylaluminum hydride and heated without a solvent to 80 °C over a period of two days. Monitoring the reaction by NMR spectroscopy indicated the formation of a large amount of trimethylaluminum, which was distilled off in vacuo at room temperature. Recrystallization of the reddish, solid residue from cyclopentane gave colorless crystals of the product **2** in 60 % yield [Eq. (a)].



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Compound **2** possesses a unique crystal structure (Figure 1)^[7] with a skeleton consisting of eight aluminum and five carbon atoms. The eight aluminum atoms form a slightly

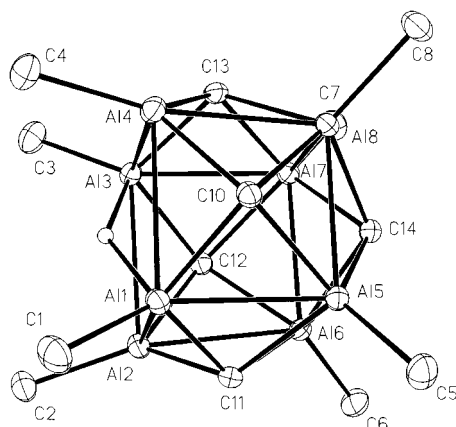


Figure 1. Molecular structure of **2**; the benzyl groups at C10 to C14 were omitted for clarity; bridging hydrogen atom H1 with an arbitrary radius. Selected bond lengths [pm]: Al1–Al2 279.61(9), Al1–Al4 279.19(9), Al1–Al5 259.76(9), Al2–Al3 279.66(9), Al2–Al6 259.95(9), Al3–Al4 280.22(9), Al3–Al7 259.00(9), Al4–Al8 260.98(9), Al5–Al6 262.20(8), Al5–Al8 261.55(8), Al6–Al7 259.96(8), Al7–Al8 260.56(8), Al1–H1 190(3), Al3–H1 191(3), Al1–C10 202.9(2), Al1–C11 202.6(2), Al2–C11 202.2(2), Al2–C12 201.8(2), Al3–C12 203.1(2), Al3–C13 204.0(2), Al4–C10 201.8(2), Al4–C13 202.0(2), Al5–C10 212.5(2), Al5–C11 214.1(2), Al5–C14 203.7(2), Al6–C11 209.3(2), Al6–C12 211.4(2), Al6–C14 208.1(2), Al7–C12 211.5(2), Al7–C13 212.1(2), Al7–C14 205.5(2), Al8–C10 209.4(2), Al8–C13 212.0(2), Al8–C14 208.1(2).

distorted cube, five faces of which are occupied by the carbon atoms. The remaining face is bridged by the hydrogen atom H1. Every Al atom binds to a terminal methyl group and every carbon atom of the cluster to a benzyl group. While long Al–Al distances (av 279.67 pm) are observed for the hydrogen-bridged face of the Al_8 cube, the remaining eight edges are shortened to 260.50 pm. They are similar to the Al–Al distances in $(\text{AlMe}_2)_2$ ^[8] (261 pm) or in organoelement compounds with Al–Al single bonds ($\text{R}_2\text{Al–AlR}_2$; 265–266 pm).^[9] The Al–C bond lengths to the methyl groups are in the expected range (195.0 pm), whereas the strongly differing Al–C distances of the cluster are significantly lengthened. The separations between the atoms C10 to C13 and the aluminum atoms Al1 to Al4 of the hydrogen-bridged face (202.6 pm), which are attached to only three carbon atoms, are shorter than those to the atoms Al5 to Al8 (211.5 pm), which all have four carbon neighbors. In contrast, the distances to C14 are in a narrow range between 204 and 208 pm. That face of the cube, which is bridged by the hydrogen atom H1, is distorted to a rhombus with trans-annular Al–Al contacts of 363.7 (Al1–Al3) and 424.9 pm (Al2–Al4). This distortion causes different distances of H1 to these aluminum atoms of 191 (Al1, Al3) and 221 pm (Al2, Al4); the Al–H–Al bridge is longer than usually observed in alkylaluminum hydrides.

Compound **2** fits well into the electronic concept that was developed originally for the description of borane and carbaborane clusters (Wade rules).^[10] Each aluminum atom contributes two electrons and each carbon atom three

electrons to the bonding in the cluster. If the bridging hydrogen atom is taken into consideration, 16 electron pairs result, which for the 13-atom cluster in **2** gives an *arachno* configuration. Ab initio calculations^[11] on the corresponding *closo* boron cluster ($[\text{B}_{15}\text{H}_{15}]^{2-}$) revealed an hexagonal antiprismatic structure in which one hexagon was occupied by one boron atom, the second one by two boron atoms. This motif can also be found in **2**, and is best recognized when the cluster is placed on one vertex, for example, Al8. This atom has six nearest neighbors in the cluster and bridges a chairlike six-membered ring (Al4, Al5, Al7, C10, C13, and C14). The next plane comprises only five atoms (Al1, Al3, Al6, C11, and C12), and one vertex of the prism remains unoccupied. This is nearly the position of H1. The atom Al2 is located above this plane. Thus, in comparison to the *closo*- B_{15} cluster, two positions are unoccupied, as expected for an *arachno* structure. However, **2** does not adopt an antiprismatic structure, but owing to the large differences between the Al–Al and Al–C distances the planes are in an almost eclipsed conformation. The polyborane $\text{B}_{13}\text{H}_{19}$ is isoelectronic to **2**;^[12] however, in accordance with the maximum number of twelve boron atoms for each cluster observed so far in boron chemistry not a large cluster, but a *conjuncto*-borane is formed.

In agreement with the crystal structure, two resonances of equal intensity were observed in the ^1H NMR spectrum in the expected range for methyl groups attached to aluminum atoms ($\delta = -0.68$ and -0.30). The two resonances for the CH_2 groups of the benzyl substituents in the intensity ratio of 1:4 at $\delta = 3.52$ and 3.07 , respectively, and in particular the resonance of the Al–H proton ($\delta = 5.23$) are shifted to considerably higher field. Apart from some small alterations of the chemical shifts and a small broadening of the resonances, the spectrum did not change up to -90°C , thus, as expected, the interaction between the four aluminum atoms Al1 to Al4 and the hydrogen atom H1 seems to be highly dynamic. The resonances of the methyl carbon atoms in the ^{13}C NMR spectrum were observed in an expected range at $\delta = -11.7$ and -12.7 . However, the carbon atoms of the cluster showed signals with an unusual chemical shift of $\delta = 30.0$ and 24.1 . Remarkably, similar ^{13}C NMR shifts were detected experimentally and by quantum chemical calculations for the *arachno*-carbaborane $\text{C}_6\text{B}_6\text{H}_6\text{Et}_6$, in which as in **2** the different cluster atoms are arranged in an alternating fashion.^[3]

The formation of **2** may be explained by the hydroalumination^[13] of dimethylaluminum phenylethyne, in which, as expected, the Al atom of the entering group attacks the negatively charged α -C atom of the alkynide.^[5, 6, 13] The twofold addition of R_2AlH results in the formation of a benzyl group. The elimination of trimethylaluminum from the hypothetical intermediate $(\text{R}_2\text{Al})_3\text{CCH}_2\text{Ph}$ may lead to a reactive, unsaturated species as the first building block of the cluster. The hydrogen atom H1 may be introduced by a hydroalumination step with MeAlH_2 , which is easily formed by the dismutation equilibrium of Me_2AlH . The synthesis of the first known carbaalane **2** by a facile high-yield method has opened the route to a new class of aluminum compounds, which in analogy to carbaboranes may lead to a wide field of activity for preparative and theoretical chemistry.

Experimental Section

All procedures were carried out under argon. *n*-Pentane and cyclopentane were dried over LiAlH₄.

1: *n*BuLi (11.4 mL of a 1.6 M solution in *n*-hexane) was added dropwise to a solution of phenylethyne (1.86 g, 2 mL, 18.2 mmol) in *n*-pentane (15 mL) at 0 °C. The suspension was stirred for 2 h at room temperature, cooled to 0 °C, and treated with Me₂AlCl (1.7 mL, 1.68 g, 18.2 mmol) dissolved in *n*-pentane (15 mL). The mixture was stirred for 16 h at room temperature, filtered, and evaporated. The residue was recrystallized from *n*-pentane (20/–30 °C). Yield: 1.64 g (57 %); colorless, extremely air-sensitive crystals. M.p. (sealed capillary): 76–80 °C. ¹H NMR (300 MHz, C₆D₆): δ = 7.32 (2H, pseudo-d, Ph), 6.89 (1H, pseudo-t, Ph), 6.78 (2H, pseudo-t, Ph), 0.03 (6H, Me); ¹³C NMR (75 MHz, C₆D₆): δ = 135.5, 133.8, 131.5, and 128.8 (Ph), 119.9 and 96.8 (C≡C), –6.8 (AlC); IR (Nujol): $\tilde{\nu}$ [cm^{–1}] = 2089 (ν_{C≡C}).

2: A mixture of **1** (628 mg, 3.97 mmol) and Me₂AlH^[14] (1.214 g, 20.9 mmol) was heated for 48 h to 80 °C without a solvent. After about 2 h, a solid started to precipitate from the red solution. All volatile components were distilled off in vacuo after cooling to room temperature. The residue was treated with cyclopentane, filtered, and recrystallized at –30 °C. The crystals of **2** included up to 0.9 molecules cyclopentane per formula unit after thorough evacuation. Yield: 445 mg (0.486 mmol, 61 %), colorless, slightly air-sensitive crystals. M.p. (sealed capillary): 175 °C (decomp.). ¹H NMR (300 MHz, C₆D₆): δ = 7.5–7.0 (m, Ph), 5.23 (1H, s, AlHAl), 3.52 (2H, s, CH₂ at C14), 3.07 (8H, s, CH₂), –0.30 and –0.68 (each 12H, s, AlMe); ¹³C NMR (75 MHz, C₆D₆): δ = 147.9 (*ipso*-C at C14), 147.5 (*ipso*-C of the other phenyl groups), 129.6, 129.0, 128.7, 126.1 and 125.7 (Ph), 35.5 (CH₂ at C14), 34.2 (CH₂ at C10 to C13), 30.0 (C10 to C13), 24.1 (C14), –11.7 and –12.7 (AlMe); assignment on the basis of *j*-modulated and ¹H–¹³C correlated NMR spectra; IR (Nujol, CsBr plates): $\tilde{\nu}$ [cm^{–1}] = 1599 m (Ph), 1462 vs, 1377 vs (Nujol), 1300 m (ν_{Al–H}), 1186 s, 1154 m, 1072 w, 1030 w (ν_{C–C}), 959 s, 930 m, 907 m, 824 m (Ph), 750 vs, 710 vs, 691 vs, 673 vs, 665 vs (δ_{Ph}, ν_{Al–C}), 581 m, 550 m, 525 m, 505 w, 428 m (ν_{Al–C}); UV/Vis (cyclopentane): λ_{max} [nm] (lg ε) = 250 (4.1).

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wR2 (all data) = 0.139, max./min. residual electron density: 0.96 (near the disordered cyclopentane molecule)/–0.53 × 10³⁰ e[–] m^{–3}. The position of the hydrogen atom H1 was taken from a difference Fourier map, and refined isotropically. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-113238. 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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Stereospecific Migration of P from N to C_{sp}²: Ring-Expansion Reaction of Chiral Diazaphospholidine Oxides

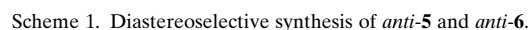
Olivier Legrand, Jean Michel Brunel, and Gérard Buono*

Many modern synthetic targets, in particular those of interest for pharmaceutical and agrochemical preparations, are either benzenoid or incorporate key aromatic or heteroatomic components.^[1, 2] In this context, *ortho*-lithiation has appeared as one of the most important methods for the regiospecific construction of polysubstituted aromatic compounds.^[3] When the *ortho*-lithiation directing group contains an electronegative atom attached to a π-unsaturated or coordinatively unsaturated group, the lithiated species may undergo 1,3-migration to the *ortho* position on the aromatic ring. Such metalation-induced 1,3-migration is very common in benzene systems and has been seen for the rearrangement of 1) arenesulfonamides of N-substituted anilines to N-substituted 2-aminoaryl aryl sulfones,^[4] 2) aryl *O*-carbamates to salicylamides,^[5] 3) *o*-bromophenyl esters to *o*-hydroxyaryl ketones,^[6] 4) arylphosphate esters to 2-hydroxyaryl phosphonates,^[7] and 5) (triarylsiloxy)benzenes to *o*-(triarylsilyl)phenols.^[8]

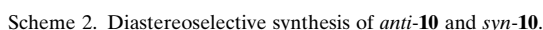
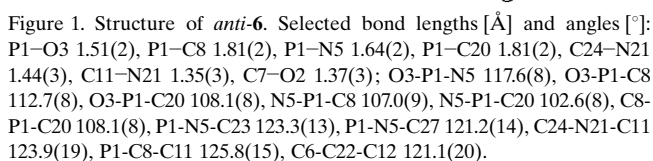
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- [7] Crystal structure of **2**: Single crystals of **2** were obtained from cyclopentane at 0 °C; C₄₈H₆₀Al₈·C₅H₁₀, triclinic, space group P $\bar{1}$, *a* = 1189.67(8), *b* = 1416.02(9), *c* = 1674.5(1) pm, α = 113.943(7), β = 91.812(8), γ = 94.609(8)°, *V* = 2563.4(3) Å³, *Z* = 2, ρ_{calcd} = 1.196 g cm^{–3}, crystal dimensions 0.75 × 0.66 × 0.34 mm, Stoe IPDS diffractometer, MoK_α radiation, 193 K, measurement range: 4.2 < 2θ < 51.9°, 238 exposures, Δφ = 1.3°, 9361 independent reflections, 7194 reflections with *I* > 4σ(*I*), μ = 0.194 mm^{–1}, programs SHELXTL PLUS REL 4.1 and SHELXL-93, 541 parameters; *R*₁ = 0.044 and

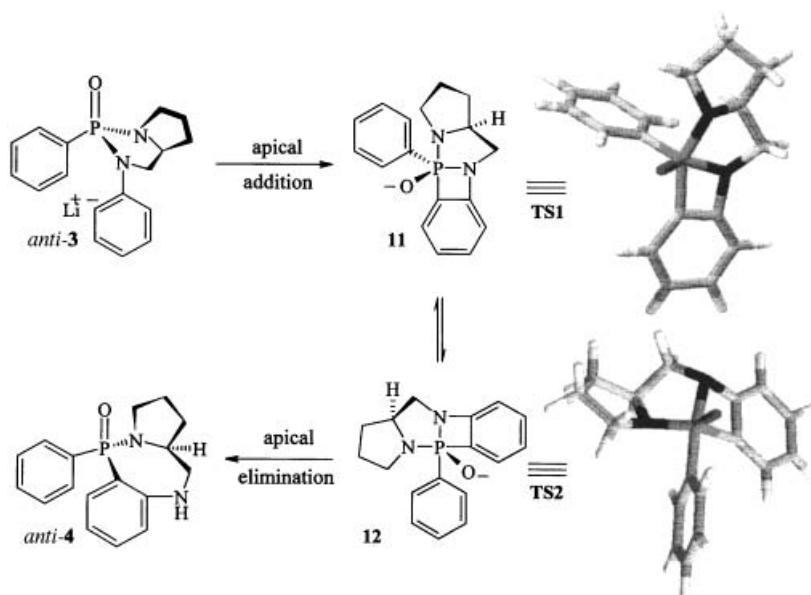
Reaction of pure diastereomer *anti*-**1** in presence of two equivalents of lithium diisopropylamide (LDA) at -78°C to room temperature led to the stereospecific formation of the hydroxyphenyldiazaphospholidine oxide *anti*-**3** in 94% yield.^[9] However, the new compound *anti*-**5** is formed in 89% yield when the reaction is performed with eight equivalents of LDA and with stirring at room temperature overnight (Scheme 1).



Extension of this reaction to phenyldiazaphospholidine oxides compounds as substrates led to remarkable results (Scheme 2). Precursors **9** were readily synthesized by a substitution reaction between phenylphosphonic dichloride



Since it was clearly established that the 1,3-phosphorus migration proceeds with retention of configuration at the phosphorus atom, a mechanism via a trigonal-bipyramidal (TBP) intermediate can be postulated (Scheme 3).^[17] Mislow has suggested that if the bond-making or bond-breaking step is rate determining, then apical addition and elimination will



Scheme 3. The rearrangement mechanism of the stereospecific migration of P from N to C_{sp}².

be more favorable than either equatorial addition and apical elimination or apical addition and equatorial elimination.^[18] Formation of a pentacoordinate intermediate with the entering nucleophile in an apical position of the trigonal bipyramid may occur by nucleophilic attack of the tetrahedral phosphorus center. Owing to the constrained diazaphospholidine ring, *ortho*-phenyl anion attack would lead to a TBP intermediate **11**, in which the four-membered azaphosphetane and the five-membered diazaphospholane rings adopt axial–equatorial positions^[19–22] and the electron-donating oxygen anion ligand adopts an equatorial position.^[23–25] Considering a low-energy Berry pseudorotation^[26] (**11** ⇌ **12**), the more apicophilic nitrogen atom of the azaphosphetane ring may adopt an apical position. At the same time, the exocyclic oxygen anion group tends to remain equatorial throughout the pseudorotation process by serving as a pivot.^[27] Moreover, the inversion of configuration at the phosphorus atom is energetically unfavorable since it involves the epimerization of the phosphorus(v) atom in the TBP intermediates, implying improbable intermediates in which either the azaphosphetane or diazaphospholane rings are forced to adopt a diequatorial position with the oxygen anion group in an apical position.^[28]

In conclusion, we have described the first totally stereospecific ring-expansion reaction of diazaphospholidine oxides by a 1,3-migration rearrangement. A mechanistic rationale involving an addition–pseudorotation–elimination pathway has been proposed in agreement with the experimental results.

Experimental Section

General procedure for the preparation of the new ring-expansion compounds: To a stirred solution of the corresponding precursor (1.0 mmol) in dry THF (15 mL) under argon atmosphere was slowly added at -78°C a solution of LDA (4 mmol (for *anti*-**10** and *syn*-**10**) or 8 mmol (for *anti*-**5**, *syn*-**5**, and *anti*-**6**), 2 M in THF). The mixture was allowed to warm to room temperature and stirred overnight. Then the reaction was quenched by addition of a saturated solution of NH₄Cl (20 mL). The

product was extracted with ethyl acetate ($3 \times 10\text{ mL}$). The combined organic phases were dried over MgSO₄, filtered, and evaporated under reduced pressure. The residue was purified by flash chromatography on a silica gel column.

anti-**5**: Purification by column chromatography (silica gel; ethyl acetate) afforded *anti*-**5** as a pale yellow solid (280 mg (89 %) from pure *anti*-**1**; 140 mg (81 % based on *anti*-**1**) from a 55/45 mixture of *anti*-**1** and *syn*-**1**). M.p. 202°C ; ¹H NMR (200 MHz): $\delta = 1.67\text{--}2.08$ (m, 4H), 3.01–3.05 (m, 1H), 3.48–3.69 (m, 4H), 3.40–4.20 (brs, 1H), 6.68 (d, $J = 8.8\text{ Hz}$, 1H), 6.71 (d, $J = 7.7\text{ Hz}$, 1H), 6.82 (td, $J = 7.5, 2.5\text{ Hz}$, 1H), 6.96 (dd, $J = 8.2, 5.3\text{ Hz}$, 1H), 7.17–7.31 (m, 3H), 7.38 (dd, $J = 8.3, 7.3\text{ Hz}$, 1H), 11.60 (s, 1H); ¹³C NMR (50 MHz): $\delta = 24.6$ (d, $J = 6.8\text{ Hz}$), 32.1 (d, $J = 5.8\text{ Hz}$), 48.5 (d, $J = 2.3\text{ Hz}$), 51.8 (d, $J = 2.3\text{ Hz}$), 62.9 (d, $J = 2.5\text{ Hz}$), 112.1 (d, $J = 130.4\text{ Hz}$), 116.7 (d, $J = 128.2\text{ Hz}$), 118.0 (d, $J = 8.9\text{ Hz}$), 118.5 (d, $J = 10.1\text{ Hz}$), 118.7 (d, $J = 14.3\text{ Hz}$), 119.1 (d, $J = 12.0\text{ Hz}$), 132.3 (d, $J = 7.9\text{ Hz}$), 132.5, 133.9 (d, $J = 11.6\text{ Hz}$), 134.2, 149.9 (d, $J = 4.2\text{ Hz}$), 163.8 (d, $J = 5.5\text{ Hz}$); ³¹P NMR (40.5 MHz) $\delta = 39.5$; elemental analysis calcd for C₁₇H₁₉N₂O₂P (314.3): C 65.0, H 6.1, N 8.9, P 9.9; found: C 65.2, H 6.0, N 9.0, P 9.9.

syn-**5**: Purification by column chromatography (silica gel; ethyl acetate) afforded *syn*-**5** as a pale yellow solid (105 mg (73 % based on *syn*-**1**) from a 55/45 mixture of *anti*-**1** and *syn*-**1**). M.p. 196°C ; ¹H NMR (200 MHz): $\delta = 1.68\text{--}2.14$ (m, 4H), 2.94–3.35 (m, 3H), 3.40–3.75 (very brs, 1H), 3.50 (dd, $J = 12.0, 4.0\text{ Hz}$, 1H), 3.85–3.95 (m, 1H), 6.75–6.84 (m, 2H), 6.88 (dd, $J = 8.3, 5.5\text{ Hz}$, 1H), 6.96 (tdd, $J = 7.6, 2.2, 1.0\text{ Hz}$, 1H), 7.24–7.36 (m, 2H), 7.59 (ddd, $J = 14.2, 7.7, 1.5\text{ Hz}$, 1H), 7.75 (ddd, $J = 14.4, 7.7, 1.6\text{ Hz}$, 1H), 11.58 (s, 1H); ¹³C NMR (50 MHz): $\delta = 25.3$ (d, $J = 8.7\text{ Hz}$), 30.6 (d, $J = 7.4\text{ Hz}$), 46.7 (d, $J = 2.9\text{ Hz}$), 54.0, 57.1 (d, $J = 4.3\text{ Hz}$), 114.0 (d, $J = 132.2\text{ Hz}$), 117.7 (d, $J = 9.0\text{ Hz}$), 118.9 (d, $J = 13.0\text{ Hz}$), 120.9 (d, $J = 9.9\text{ Hz}$), 121.5 (d, $J = 127.6\text{ Hz}$), 121.8 (d, $J = 13.0\text{ Hz}$), 133.0 (d, $J = 4.3\text{ Hz}$), 133.2, 134.1, 134.9 (d, $J = 10.1\text{ Hz}$), 149.4 (d, $J = 5.6\text{ Hz}$), 162.5 (d, $J = 5.6\text{ Hz}$); ³¹P NMR (40.5 MHz): $\delta = 31.3$; elemental analysis calcd for C₁₇H₁₉N₂O₂P (314.3): C 65.0, H 6.1, N 8.9, P 9.9; found: C 64.9, H 6.2, N 8.8, P 10.0.

anti-**6**: Purification by column chromatography (silica gel; ethyl acetate/methanol 80/20) afforded *anti*-**6** as a pale yellow solid (330 mg, 84 %). M.p. 208°C ; $[\alpha]_D^{20} = +168$ ($c = 0.44$, CH₂Cl₂); ¹H NMR (200 MHz): $\delta = 1.64\text{--}2.09$ (m, 4H), 3.03–3.17 (m, 1H), 3.50–3.75 (m, 4H), 4.55 (brs, 1H), 6.68–6.82 (m, 2H), 6.88 (td, $J = 7.6, 2.9\text{ Hz}$, 1H), 7.19–7.48 (m, 7H), 7.63–7.68 (m, 2H), 11.97 (s, 1H); ¹³C NMR (50 MHz): $\delta = 24.7$ (d, $J = 6.7\text{ Hz}$), 32.0 (d, $J = 6.0\text{ Hz}$), 48.4 (d, $J = 2.2\text{ Hz}$), 52.1, 62.7 (d, $J = 2.5\text{ Hz}$), 112.7 (d, $J = 129.4\text{ Hz}$), 117.2 (d, $J = 126.9\text{ Hz}$), 118.6 (d, $J = 9.9\text{ Hz}$), 119.0 (d, $J = 13.2\text{ Hz}$, 2C), 127.1, 128.1 (s, 2C), 129.5 (s, 2C), 130.4 (d, $J = 9.9\text{ Hz}$), 131.7 (d, $J = 8.5\text{ Hz}$), 132.6, 134.1 (d, $J = 11.5\text{ Hz}$), 135.0, 137.9, 149.9 (d, $J = 4.4\text{ Hz}$), 160.8 (d, $J = 5.6\text{ Hz}$); ³¹P NMR (40.5 MHz): $\delta = 39.7$; elemental analysis calcd for C₂₃H₂₃N₂O₂P (390.42): C 70.8, H 5.9, N 7.3, P 8.0.

anti-**10**: Purification by column chromatography (silica gel; ethyl acetate/methanol 80/20) afforded *anti*-**10** as a pale yellow solid (270 mg, 90 %). M.p. 174°C ; $[\alpha]_D^{20} = +113$ ($c = 0.40$, CH₂Cl₂); ¹H NMR (200 MHz): $\delta = 1.67\text{--}2.04$ (m, 4H), 3.10 (dd, $J = 14.4, 6.0\text{ Hz}$, 1H), 3.22–3.33 (m, 2H), 3.36–3.52 (m, 2H), 4.37 (brs, 1H), 6.72 (dd, $J = 8.0, 5.5\text{ Hz}$, 1H), 6.88 (tdd, $J = 7.5, 2.0, 1.0\text{ Hz}$, 1H), 7.26 (tt, $J = 7.7, 1.3\text{ Hz}$, 1H), 7.38–7.45 (m, 3H), 7.60–7.68 (m, 2H), 7.72 (ddd, $J = 13.3, 7.7, 1.5\text{ Hz}$, 1H); ¹³C NMR (50 MHz): $\delta = 24.9$ (d, $J = 8.6\text{ Hz}$), 30.6 (d, $J = 7.4\text{ Hz}$), 47.2, 51.3, 60.9 (d, $J = 3.8\text{ Hz}$), 115.9 (d, $J = 117.7\text{ Hz}$), 118.1 (d, $J = 9.3\text{ Hz}$), 118.4 (d, $J = 11.9\text{ Hz}$), 128.2 (d, $J = 12.9\text{ Hz}$, 2C), 131.0 (d, $J = 10.5\text{ Hz}$, 2C), 131.2, 132.0 (d, $J = 1.9\text{ Hz}$), 134.8 (d, $J = 7.4\text{ Hz}$), 134.8 (d, $J = 126.3\text{ Hz}$), 152.1 (d, $J = 5.8\text{ Hz}$); ³¹P NMR (40.5 MHz): $\delta = 29.0$; elemental analysis calcd for C₁₇H₁₉N₂O₂P 298.32: C 68.4, H 6.4, N 9.4, P 10.4; found: C 68.5, H 6.3, N 9.4, P 10.4.

syn-**10**: Purification by column chromatography (silica gel; ethyl acetate/methanol 80/20) afforded *syn*-**10** as a pale yellow solid (230 mg, 76 %). M.p. 186°C ; $[\alpha]_D^{20} = +20$ ($c = 0.40$, CH₂Cl₂); ¹H NMR (200 MHz): $\delta = 1.74\text{--}2.06$ (m, 4H), 2.93–3.10 (m, 2H), 3.29 (dd, $J = 13.2, 5.7\text{ Hz}$, 1H), 3.78 (dd,

$J = 13.2, 3.5$ Hz, 1 H), 3.89–3.94 (m, 1 H), 4.32 (brs, 1 H), 6.73 (ddd, $J = 8.0, 5.3, 0.8$ Hz, 1 H), 6.83 (tdd, $J = 7.7, 2.2, 1.1$ Hz, 1 H), 7.22 (tt, $J = 7.7, 1.4$ Hz, 1 H), 7.34–7.50 (m, 4 H), 7.81 (ddd, $J = 12.2, 7.7, 1.6$ Hz, 2 H); ^{13}C NMR (50 MHz): $\delta = 25.3$ (d, $J = 8.7$ Hz), 30.5 (d, $J = 7.5$ Hz), 48.2 (d, $J = 3.2$ Hz), 52.6, 58.8 (d, $J = 4.2$ Hz), 114.1 (d, $J = 174.0$ Hz), 119.1 (d, $J = 8.8$ Hz), 119.7 (d, $J = 13.0$ Hz), 119.8 (d, $J = 125.1$ Hz), 128.0 (d, $J = 12.9$ Hz, 2 C), 132.3 (2 C), 134.5 (d, $J = 10.1$ Hz), 151.8 (d, $J = 4.4$ Hz); ^{31}P NMR (40.5 MHz): $\delta = 26.0$; elemental analysis calcd for $\text{C}_{17}\text{H}_{19}\text{N}_2\text{OP}$ 298.32: C 68.4, H 6.4, N 9.4, P 10.4; found: C 68.4, H 6.5, N 9.3, P 10.4.

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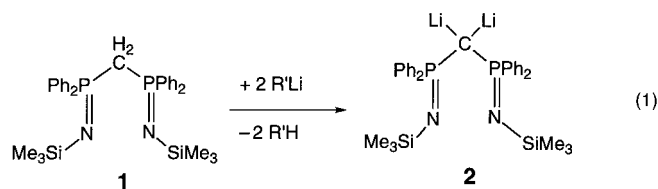
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[Ph₂P(NSiMe₃)₂]₂Li₂: A Dilithium Dianionic Methanide Salt with an Unusual Li₄C₂ Cluster Structure**

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Bis(phosphane) ligands and their oxidized derivatives of the form CH₂(R₂P=E)₂ (E = O, S, NR') have been widely used to form stable mono- and polymetallic complexes with both early and late transition metals.^[1, 2] Generally the ligand retains its neutral character, and the methylene bridge between the two phosphorus atoms is preserved. However, these backbone protons are moderately acidic, and the ligand may be deprotonated by strong bases such as LiN(SiMe₃)₂, NaH, and alkyllithium reagents to generate monoanionic species. Many metal compounds have been derived from these monoanionic ligand precursors, and typically M–C bonds stabilized by additional coordination of the ligand are formed.^[3–9] A few unexpected complexes have been reported in which double deprotonation of the P–CH₂–P backbone has been structurally established—namely, [[Pd(μ-Cl)₂Pt–C(PPh₂)₂]]_n,^[10] [Pt₂{C(Ph₂P=S)₂}(MeOcod)₂]^[11] (MeOcod = 8-methoxycyclooct-4-ene-1-yl), [(AlR){C(Ph₂P=O)₂}(AlR₂)₂] (R = Me,^[12] Et^[13]), and [[Al(C₄H₉)₂](Ph₂P(=S)CP(Ph)₂–(S)₂){Al(C₄H₉)₂}]^[14]—but to date there has been no rational synthesis of a species with the doubly deprotonated P–C–P methanide moiety. Herein we report the first synthesis and crystal structure of a complex containing such a ligand formed by the double deprotonation of CH₂(Ph₂P=NR')₂. This represents the first example of a structurally characterized Group 1 metal complex of the CH₂(R₂P=E)₂ (E = O, S, NR') ligand systems. In addition the structure is an unusual, highly symmetric dimer cluster of lithium and carbon.

Reaction of CH₂(Ph₂P=NSiMe₃)₂ (**1**) with two moles of PhLi or MeLi in toluene leads smoothly to the dilithium complex **2** as colorless air- and moisture-sensitive crystals [Eq. (1)].^[15]



The ³¹P NMR spectrum of the final reaction mixtures showed quantitative conversion of **1** into one single phosphorus-containing product. Complex **2** was isolated in only moderate yield due to its high solubility, and it was fully characterized by elemental analysis and spectroscopic methods. The ³¹P NMR spectrum of **2** consists of one sharp singlet, indicating that the two phosphorus nuclei are equivalent. The ³¹P resonance is shifted downfield by 19.1 ppm compared to that of the starting compound **1**. The ¹H NMR spectrum showed the absence of the methylene resonance of the P–CH₂–P backbone, indicating that the ligand has been doubly deprotonated. No ¹³C{¹H} NMR signal was observed for the quaternary P–C–P carbon atom despite trials with long acquisition periods, possibly because the signal is very broad due to coupling with the lithium nuclei. However, the lack of suitable protons proximate to the methine carbon atoms provides minimal ¹H → ¹³C magnetization transfer, so it is perhaps not too surprising that this signal is not observed.

The molecular structure of **2**,^[16] determined by X-ray diffraction, is unusual and interesting. Figure 1 shows an ORTEP^[17] plot of the central cluster structure. The complex is a dimer, with four lithium atoms forming a square plane which is capped above and below by carbon atoms

C(1) and C(2) of the two P–C–P frames. The bridging atoms C(1) and C(2) lie 1.67 Å above and below the Li₄ square plane to form a near perfect octahedron of atoms in the center of the cluster, which deviates only slightly from a regular octahedron because of the presence of the different axial atoms. The ligand N–P–C–P–N units are approximately planar, and there is only a small displacement (0.16–0.30 Å) of the Si atoms from the P–N–C planes. The N–P–C–P–N ligand planes are orthogonal to each other (the dihedral angle between the two planes is 88.12(11)°) and in turn

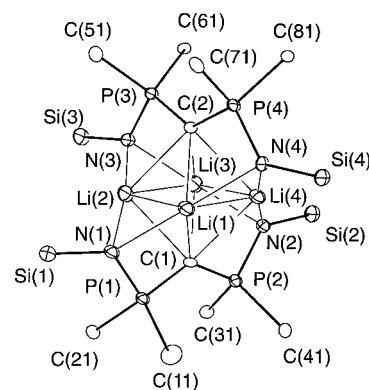


Figure 1. ORTEP^[17] view of the central core of **2** showing the atom labeling scheme. All of the hydrogen atoms, the methyl carbon atoms on the silicon atoms, and all phenyl-ring carbon atoms (except the *ipso*-carbon atoms) have been removed for clarity. The atoms are represented by Gaussian ellipsoids at the 20% probability level. The thin lines serve to emphasize the geometrical relationship between the carbon and lithium atoms and are not to be considered normal bonds. Selected interatomic distances [Å] and angles [°]: P(1)–N(1) 1.619(4), P(1)–C(1) 1.695(5), P(2)–C(1) 1.681(5), C(1)–Li 2.33(1)–2.42(1), C(2)–Li 2.312(9)–2.45(1), N(1)–Li(2) 2.128(9), N(2)–Li(4) 2.108(9); P(1)–C(1)–P(2) 132.6(3), N(1)–P(1)–C(1) 104.4(2), N(2)–P(2)–C(1) 103.9(2).

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orthogonal to the plane formed by the four lithium atoms (the dihedral angle is $89.81(19)^\circ$ between planes N(1)–P(1)–C(1)–P(2)–N(2) and Li(1)–Li(2)–Li(3)–Li(4), and $88.6(2)^\circ$ between planes N(3)–P(3)–C(2)–P(4)–N(4) and Li(1)–Li(2)–Li(3)–Li(4)).

There are no solvent molecules coordinated to the lithium atoms in **2**, rather the lithium atoms are intramolecularly solvated by the silylated imine groups. A similar internal solvation was observed for the lithiated monophosphane imines $[\text{LiCH}_2\text{P}(\text{Me})_2(\text{NSiMe}_3)_4]_4$ and $[\text{LiCMe}_2\text{P}(\text{iPr})_2(\text{N-SiMe}_3)_2]_2$.^[18] In the central core of **2** each lithium atom is close to two other lithium, two carbon, and two imine nitrogen atoms. The essence of the structure is the central cluster core, an octahedron of four lithium and two carbon atoms supported by four nitrogen atoms which bridge opposite pairs of lithium atoms. The Li–C distances (av $2.38(1)$ Å) are comparable to those in the oligomeric aggregates $[\text{PhLi}(\text{tmeda})_2]_2$ (tmeda = *N,N,N',N'*-tetramethylethylenediamine) and $[\text{PhLi} \cdot \text{OEt}_2]_4$.^[19] The Li–N distances for the N atoms bridging the Li atoms are in the range observed for lithiated monophosphanylimines ($2.052(10)$ – $2.151(9)$ Å)^[18, 20, 21] The Li–Li distances fall within a narrow range (2.393 – 2.410 Å) and shorter than those found in the alkyllithium aggregates $[(\text{LiR})_n]$ (R = Me, Et, Bu).^[22–24]

The phosphorus atoms in complex **2** also lie very close to the lithium atoms, but probably only as a result of the tight cluster structure. Surprisingly, however, these phosphorus–lithium distances fall within the range of a P–Li single bond (2.64 – 2.69 Å) and are comparable with those reported for $[\text{LiCH}(\text{PPh}_2)_2]$ and related compounds.^[8, 25, 26] The P=N bond lengths in **2** (av $1.621(4)$ Å) are slightly elongated relative to those found in $\text{CH}_3\text{CH}[\text{Ph}_2\text{P}=\text{N}(\text{Tol})]_2$ ^[27] and $\text{H}_2\text{C}(\text{Cy}_2\text{P}=\text{NSiMe}_3)_2$.^[28] The exocyclic P–C bonds are of normal length. The endocyclic P–C bond distances are, however, significantly shortened and the N–Li distances are also shorter than the C–Li distances, suggesting that there is a considerable delocalization of the π electron density over the N–P–C–P–N backbone. The P–C–P bond angles in **2** (av $132.4(3)^\circ$) are significantly expanded compared to the corresponding angles in $\text{CH}_3\text{CH}[\text{Ph}_2\text{P}=\text{N}(\text{Tol})]_2$ ($112.39(19)^\circ$)^[27] and $\text{H}_2\text{C}(\text{Cy}_2\text{P}=\text{NSiMe}_3)_2$ ($117.41(12)^\circ$),^[28] where the carbon has a more sp^3 geometry.

The successful synthesis of this doubly deprotonated dilithiated salt can be attributed to the enhanced acidity of the P–CH₂–P backbone protons in the bisimine system.^[29]

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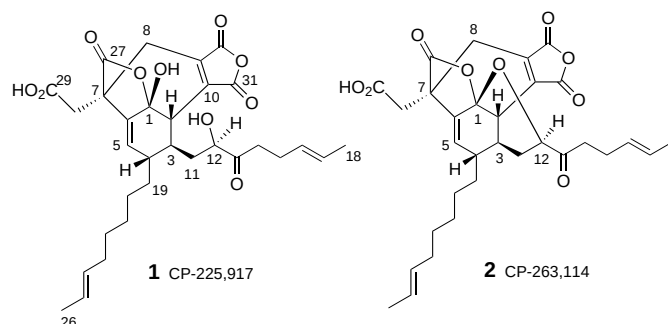
Stereospecific Sulfur-Mediated Cleavage of a Spirocyclobutanone: Synthesis of a Fully Functional Precursor to the CP Compounds**

Dongfang Meng and Samuel J. Danishefsky*

The screening of diverse sources of naturally occurring organic substances has many purposes. First, and no doubt foremost, is the hope that the activity profiles of some of the isolates may be of value for biomedical purposes. Compounds derived in this way may themselves become drugs, or may provide orienting leads for drug discovery. Indeed, some of the most important agents in the pharmaceutical industry began life in the context of natural products. Given major advances in separation sciences, structure determination by physical measurements, and the means for identifying specific cellular targets, the prospects for natural products as resources in drug discovery have never been better.^[1, 2]

On rare occasion, compounds which arise from screening exercises are sufficiently striking that they emerge as milestone targets for the science of chemical synthesis. A mastery at the level of chemical synthesis of such systems may well result in analogue candidates for drug development. However, given enough architectural novelty, the milestone classification is not necessarily tied to any likely pharmaceutical advantage to be gained from chemical synthesis. Rather, the structure may pose an implicit challenge to the capabilities of synthesis. The vistas of synthesis grow from such confrontations, and the capacity of synthesis to contribute to projects with more likely benefit margins grows correspondingly.

Two recent compounds which well warrant the "milestone" designation are CP-225,917 and CP-263,114 (**1** and **2**, respec-



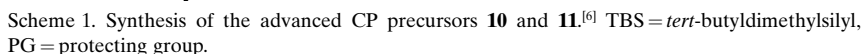
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tively) isolated at the Pfizer Laboratories.^[3, 4] The derivations of these fascinating structures followed solely from spectroscopic measurements, and were not supported either by degradative or crystallographic validations. Although the compounds manifest two interesting forms of biological activity (inhibition of farnesyl transferase and squalene synthase), the great interest that they have engendered from the synthesis community can best be comprehended in terms of their novel molecular lattice work and the implicit challenge of such structures to chemists. Indeed, there is already a burgeoning literature describing highly imaginative approaches to assembling **1** and **2** in the laboratory.^[5, 6] Clearly, any program which aspires to achieve a total synthesis of the CP compounds must transcend strictly regional issues and furnish provisions for all of the structural components of the natural products, including harmonization of competitive demands of various functional groups. Ideally, the synthesis program would be conducted with a view toward providing independent corroboration for the gross structure and configuration of **1** and **2**.

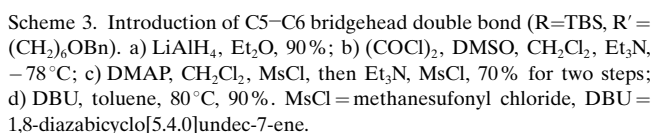
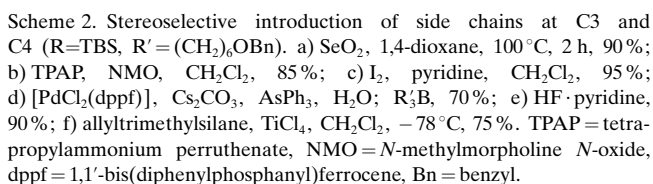
Recently, we disclosed an approach to the CP problem using an initial intermolecular α' -aldol condensation of a cyclohexenone with furylacetaldehyde derivative **3** (Scheme 1). This merger was followed by a bridging intramolecular Heck ring closure, leading to a consensus matrix structure (**4**) which either did or did not contain a functional group at C4 (**4a** and **4b**, respectively). In this context we could functionalize C5 by selenium dioxide mediated hydroxylation and exploit the resultant C5 β -hydroxyl group to introduce a potentially useful structural implement at C3 (**5**→**6**). Unfortunately, in the **4a** series, the selenium dioxide functionalization step lacks any regiochemical preference between C5 and the allylic methylene substituent on the C4 side chain. By contrast, if the aldol-Heck sequence was conducted on the C4-unsubstituted system (i.e., cyclohexenone itself), the resulting product **4b**, lacking the C4 side chain, suffers clean functionalization at C5, setting the stage for the Claisen transposition (→**7**). Unfortunately, with the C3 handle in place, we were unable to introduce the C4 substituent from any structure derived from **7** (e.g. **8**). Clearly, these problems required careful attention.

In our earlier report, we had reached systems of the type **9**. The oxo function in these structures could be exploited to create a quarternary center at C7, albeit with a suboptimal level of oxidation in its branches (see lactone **10**).^[6] Also, the furan ring was exploited, as planned, to provide a straightforward pathway to the 2,3-fused maleic anhydride appendage (→**11**).^[6] Neither of the most advanced compounds **10** or **11** had the bridgehead (C5–C6) olefin in place. While it seemed possible that some of our chemical implements could be channeled to gain access to the C5–C6 olefin, the point had not been demonstrated in practice. Also to be addressed was the potentially serious question as to the extent to which a bridgehead double bond would be restrictive of other required chemistry. Here we describe a program wherein these difficulties have been resolved, and all required elements for a global synthesis of the CP compounds or analogues appear to be in place.



employed a *B*-alkyl Suzuki strategy^[9, 10] to introduce the C4 side chain with the C5 oxo group already present ($\rightarrow$ **14**). In this way, the disastrous consequences of the regiorandom selenium-induced allylic dioxide hydroxylation of **4** were avoided. A variety of attempts to introduce a C3 substituent by conjugate addition to the enone linkage of **14** was to no avail. The logjam was broken as follows: A free alcohol functionality was exposed at C7 ($\rightarrow$ **15**). Presumably, this group plays a critical role in positioning the titanium tetrachloride derived Lewis acid moiety at the C5 oxo group. In the event, under these tightly defined circumstances, a Sakurai-type delivery of the β -oriented allyl group to C3 was accomplished.^[12] Moreover, stereospecific β -protonation at C4 (either with HCl or on workup) afforded **16**. In this way, the stereochemical relationship assigned to the CP compounds at C3 and C4 had been simulated.

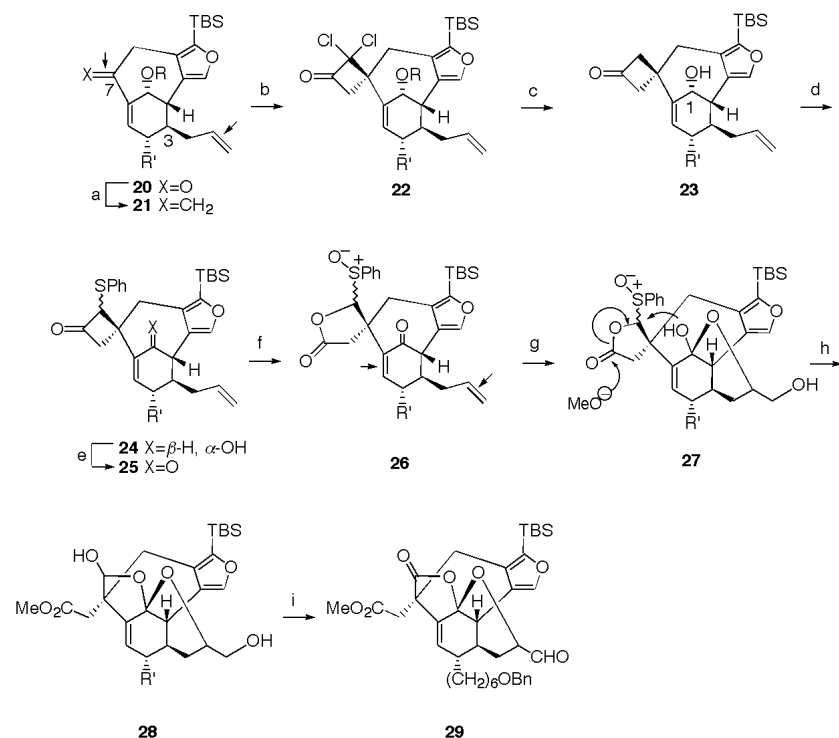
We turned next to installation of the C5–C6 double bond. Toward this end we could again exploit small but decisive differential reactivity margins. Thus, reduction of the C5 oxo group occurred from the β face ($\rightarrow$ **17**; Scheme 3). In a critical step, selective Swern



oxidation occurs at C7 to give monoketone **18**. At this stage, mesylation at C5 could be conducted ($\rightarrow$ **19**). Elimination of the mesyloxy functionality was achieved by the reaction of **19** with DBU, providing **20** with the C5–C6 (bridgehead) double bond in place.

It then transpired that with the bridgehead olefin moiety in nominal conjugation to the C7 oxo group,^[13] the geminal alkylation methodology which had previously been employed

to produce **10**^[7] broke down completely due to the base lability of the system. A new program was thus devised in response to the very restricted terrain available for feasible chemical elaboration (Scheme 4). Fortunately, it did prove possible to conduct a Tebbe olefination of **20** ($\rightarrow$ **21**).^[14]



Scheme 4. Construction of the quaternary center at C7 and the CP-263,114 building array (R = TBS, R' = (CH₂)₆OBn). a) Tebbe reagent, THF, $-78 \rightarrow -10^\circ\text{C}$, 90%; b) trichloroacetyl chloride, Zn, Et₂O, DME, ultrasound, 85%; c) 1. Zn, NH₄Cl, MeOH, ultrasound, 80%; 2. TBAF, THF, 0°C , 70%; d) PhSSPh, NaH/KH, THF, 80%; e) Dess–Martin periodinane, CH₂Cl₂, 90%; f) H₂O₂, MeOH, 70%; g) OsO₄, NMO, acetone/water, 70%; h) NaOMe, MeOH, 60%; i) (COCl)₂, DMSO, CH₂Cl₂, Et₃N, -78°C , 70%. DME = dimethoxyethane, TBAF = tetrabutylammonium fluoride.

Remarkably, it was possible to realize a [2+2] cycloaddition of dichloroketene at the exocyclic double bond in the presence of the allyl group.^[15] Moreover, and surprisingly, the reaction was highly stereoselective in the desired sense ($\rightarrow$ **22**). However, given the highly restricted menu of reactions feasible for the dichlorocyclobutanone functionality (it, too, is base labile), it was necessary to forgo the potential advantage of the geminal dichloro groups that are *syn* to the C1 bridge. Rather, both chlorine atoms were cleaved reductively and the C1 hydroxyl group was deprotected ($\rightarrow$ **23**). Possibly due to a directing effect of the free hydroxyl group, we could execute base-induced sulfonylation of **23** with diastereotopic specificity *syn* to the C1 bridge. At the kinetic level, it was possible to achieve stereoselectivity even within the required diastereotopic methylene group. However, in practice the configurational lability at this center was such that a diasteromeric mixture of **24** was employed in subsequent steps. Oxidation of **24** afforded **25**. While a regiospecific Baeyer–Villiger reaction, under the apparent control of the phenylsulfonyl group, could be conducted, the yield of the subsequent sequence of lactone cleavage and valence isomerization leading to **28** was rather low. This situation was much improved by further oxidation of the

phenylsulfonyl lactone to the corresponding sulfoxide **26**. With the bridgehead double bond deactivated by its adjacency to the C1 oxo group, it proved possible to selectively dihydroxylate the terminal allyl group of **26**. Treatment of the crude hydroxylation product (see valence tautomer **27**)^[16] with sodium methoxide in methanol afforded **28**, which on oxidation led to **29**.^[17]

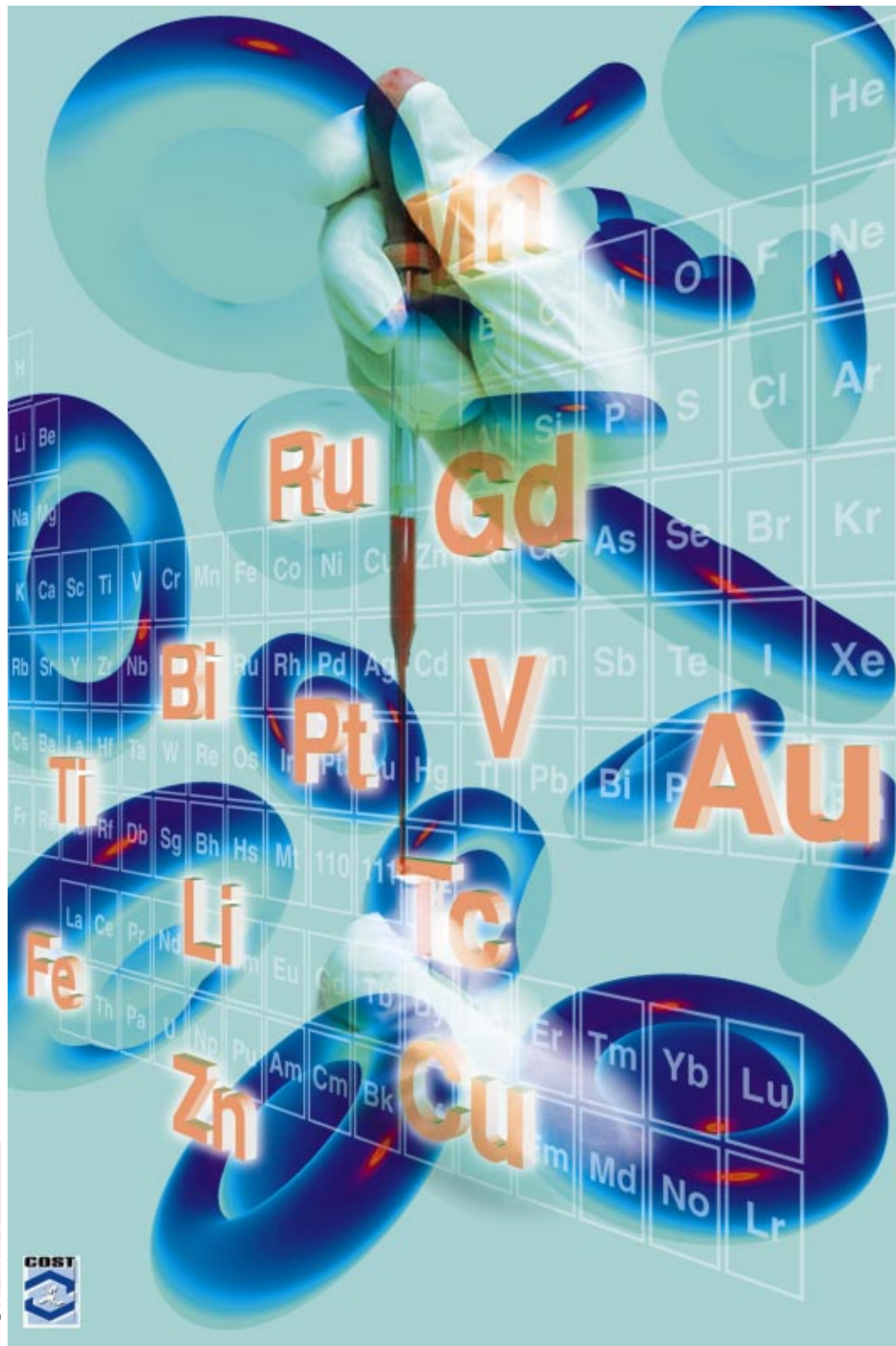
In various stages of these studies, we have demonstrated the viability of converting the furan moiety into the maleic anhydride unit. We have easily differentiated the aldehyde at C13 for relevant nucleophilic alkylation and, at an earlier stage, implemented the full CP side chain at C4. There remains now the still significant task of sequencing these capabilities in a fully harmonized program.

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- [17] Analysis of the NMR spectrum of aldehyde **29** suggests that the formyl group at C12 is in the α position. The stereochemical assignment for **2** presented the C13 oxo group as being in the β position. The stereochemical relationships between the two CP natural products at C12 have not been rigorously defined. These matters, as well as the order of thermodynamic stabilities at C12, will require further research.
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Metals in Medicine

Zijian Guo and Peter J. Sadler*

Bioinorganic chemistry is a rapidly developing field and there is enormous potential for applications in medicine, not only for the 24 or so essential elements, but also for nonessential and even radioactive elements. Medicinal inorganic chemistry offers real possibilities to pharmaceutical industries, which have traditionally been dominated by organic chemistry alone, for the discovery of truly novel drugs with new mechanisms of action. The field has been stimulated by the success of cisplatin, the world's best selling anticancer drug, and platinum complexes with reduced toxicity, oral activity, and activity against resistant tumors are currently on clinical trial. The organometallic complex titanocene dichloride is also being injected into patients, and Ru^{III} complexes have promising anti-

metastatic activity. The toxicity of Gd^{III} complexes can be controlled so that they can be safely injected in gram quantities as magnetic resonance imaging (MRI) contrast agents, and ligand design allows paramagnetic ions to be targeted to specific organs. Designed ligands also enable the targeting of radiodiagnostic (e.g. ^{99m}Tc) and radiotherapeutic isotopes (e.g. ¹⁸⁶Re). There has been recent progress in understanding the coordination chemistry and biochemistry of older metallo-drugs such as gold antiarthritic and bismuth antiulcer drugs, and further work might lead to their more effective use. Current areas with exciting clinical potential include manganese superoxide dismutase mimics, vanadium insulin mimics, ruthenium nitric oxide scavengers, lanthanide-based photo-

sensitizers, and metal-targeted organic agents. Our increasing knowledge of metal biochemistry will provide scope for the design of new drugs (both inorganic and organic) in many other areas too, for example neuropharmaceutical and anti-infective agents. Progress in medicinal coordination chemistry is heavily dependent on understanding not only the thermodynamics (equilibria and structures) but also the kinetics (and mechanisms) of reactions of metal complexes, especially under biologically relevant conditions.

Keywords: bioinorganic chemistry • coordination chemistry • drug research • medicinal chemistry • metallodrugs

1. Introduction

Biomedical inorganic chemistry ("elemental medicine") is an important new area of chemistry. It offers the potential for the design of novel therapeutic and diagnostic agents and hence for the treatment and understanding of diseases which are currently intractable (Figure 1).^[1–3] Inorganic elements play crucial roles in biological and biomedical processes, and it is evident that many organic compounds used in medicine do not have a purely organic mode of action; some are activated or biotransformed by metal ions including metalloenzymes,^[4] others have a direct or indirect effect on metal ion metabolism. In this review we focus on metal complexes, and especially on recent developments relating to platinum

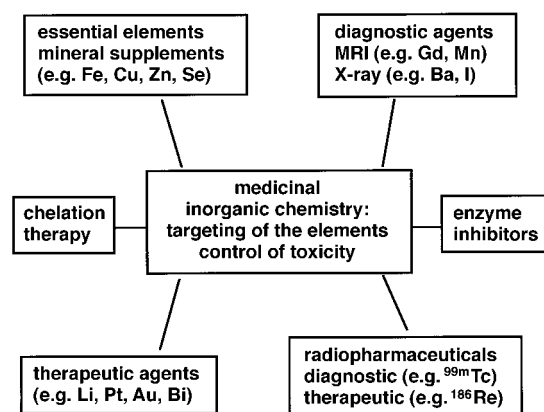


Figure 1. Some of the key areas of medicinal inorganic chemistry.

anticancer, gold antiarthritic, and bismuth antiulcer agents. First, in Section 2, wider applications involving metal complexes which are either used, or close to being used, clinically are described briefly to illustrate the scope of the subject.

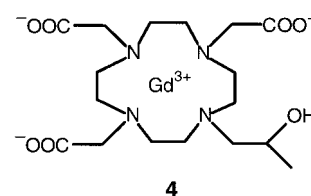
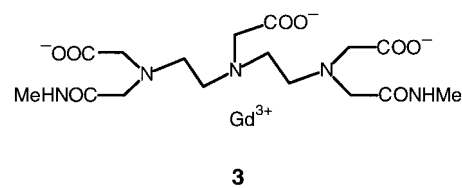
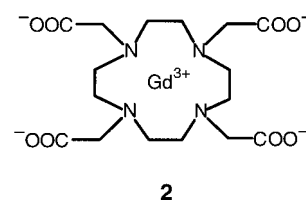
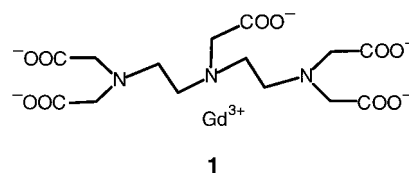
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2. Design of Diagnostic and Therapeutic Agents

2.1. MRI Contrast Agents

Magnetic resonance imaging is now a powerful tool in clinical diagnosis.^[5] Diseases can be detected from differences in ^1H NMR resonances (mainly of H_2O) between normal and abnormal tissues by administration of external paramagnetic agents. Most contrast agents contain Gd^{III} , Mn^{II} , or Fe^{III} , ions which have a large number of unpaired electrons (7, 5, and 5, respectively, high spin) and long electron spin relaxation times.^[6, 7]

Four Gd^{III} complexes have been approved for clinical use, and are widely used, for example, for the detection of abnormalities of the blood–brain barrier.^[15, 199] Complexes containing DTPA (see **1**, Magnevist) and DOTA ligands (see **2**, Dotarem) are ionic, whereas those of BMA-DTPA (see **3**, Omniscan) and HP-DOTA (see **4**, Prohance) are neutral; their low osmolality decreases the pain of the injections. All these agents are extracellular, and they diffuse rapidly into the interstitial space. The Gd^{III} center is nine-coordinate in each complex and contains one bound H_2O ligand. The crystal structure of $[\text{Gd}(\text{dota})]^-$ (**2**) is shown in Figure 2. Water exchange on Gd^{III} is dissociative,^[200] and steric crowding at the H_2O site enhances the exchange rate. Thus the H_2O exchange rate for $[\text{Gd}(\text{bma-dtpa})]$ (**3**), in which the distances between Gd and the amide oxygen atoms (2.44 Å) are longer than those between Gd and the carboxylate oxygen atoms in $[\text{Gd}(\text{dtpa})]^{2-}$ (**1**, 2.40 Å), is seven times less than for **1** (Table 1). The Gd^{III} complexes of DTPA and DOTA are thermodynamically more stable (Table 1) than those of BMA-DTPA and HP-DOTA, respectively. Complexes **2** and



4 have a higher kinetic stability than **1** and **3**. The stabilities are highly pH dependent. For example, $\lg K$ for complex **1** decreases by a factor of about 4 from pH 7.4 to 5. This could

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P. Sadler



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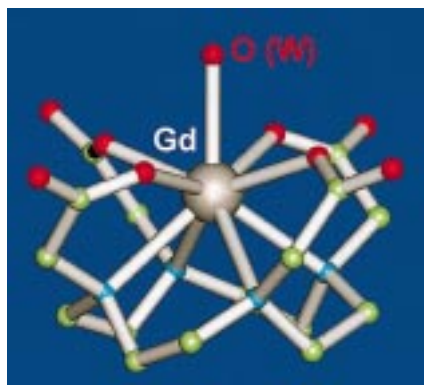


Figure 2. Crystal structure of $[\text{Gd}(\text{dota})]^-$ in $\text{Na}_2\text{-2}$, an MRI contrast agent clinically used for detection of abnormalities in the blood–brain barrier. Adapted from ref. [197]. W indicates water.

Table 1. The relaxivity R_1 (20 MHz) and other properties of clinical contrast agents.^[a]

Agent	$k_{\text{ex}}^{298[\text{b}]}$ [10^6 s^{-1}]	R_1 [$\text{mM}^{-1} \text{ s}^{-1}$]	$\lg K^{[\text{c}]}$	Ref.
$[\text{Gd}(\text{dtpa})]^{2-}$ (1)	3.3	4.5	22.5	[193]
$[\text{Gd}(\text{dota})]^-$ (2)	4.8	3.4	25.8	[194]
$[\text{Gd}(\text{bma-dtpa})]$ (3)	0.45	4.4	16.9	[194]
$[\text{Gd}(\text{hp-dota})]$ (4)		3.6	23.8	[194]
$[\text{Gd}(\text{bopta})]^{2-}$ (5)		4.4	22.5	[9]
$[\text{Mn}(\text{dpdp})]^{4-}$ (6)		2.8	15.1	[10, 195]

[a] Relaxivity is the enhancement of proton relaxation rate in aqueous solution per unit of concentration in mM. Each agent has one bound water except **6**, which has no bound water. [b] k_{ex}^{298} = the water-exchange rate at 298 K. [c] K = stability constant at 298 K.

be significant in some biological compartments, for example lysosomes where the pH value can be as low as 5. The derivatization of soluble and insoluble polysaccharides allows the delivery of multiple paramagnetic ions.^[8]

Complex **1** does not enter cells and is excreted almost exclusively by the kidney. Introduction of a benzyloxymethyl substituent on the α -C atom of a terminal acetate of DTPA as in BOPTA produces a Gd^{III} complex (**5**, Gadobenate, Figure 3) which enters hepatocytes and is excreted in bile.^[9] The

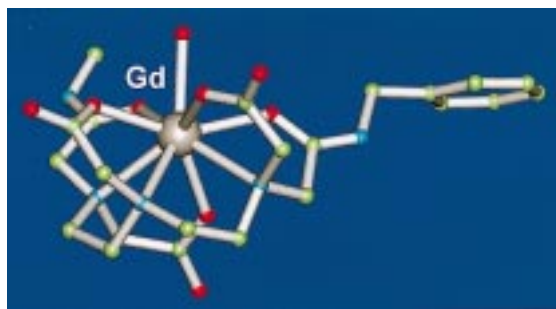


Figure 3. Crystal structure of $[\text{Gd}(\text{bopta})]^{2-}$ in $\text{Na}_2\text{-5}$, an MRI contrast agent on clinical trial for liver imaging. Adapted from ref. [9].

coordination sphere of Gd^{III} in **5** is almost identical to that in **1** (nine-coordinate, distorted tricapped trigonal prism), and both complexes have similar stabilities and relaxivities (Table 1).^[9]

The distorted octahedral complex **6** (Figure 4; Teslascan is the mangafodipir trisodium salt)^[10] is in clinical use for enhancing contrast in the liver (detection of hepatocellular carcinomas).^[11] The relaxivity of complex **6** (Table 1) is about

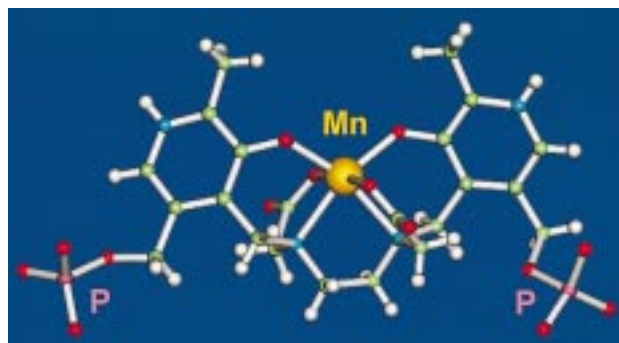
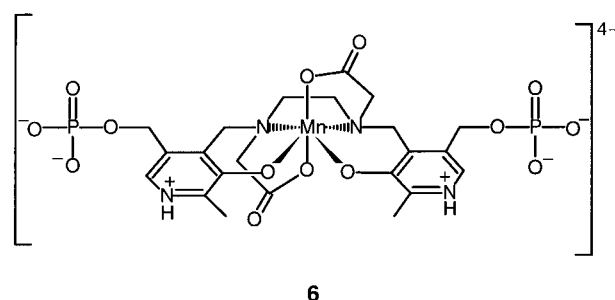


Figure 4. Crystal structure of $[\text{Mn}(\text{dpdp})]^{4-}$ in $\text{CaNa}_2\text{-6}$, an MRI contrast agent for liver imaging. Adapted from ref. [10].

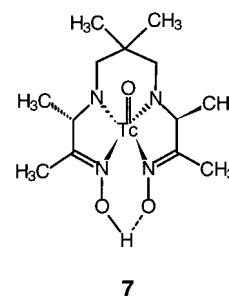


35 % greater than that of Mn complexes of DTPA and DOTA, which also do not contain directly coordinated water.^[12]

Superparamagnetic nanoparticles consisting of iron oxide coated with dextran are also being used as MRI contrast agents. The distribution of injected particles in the body is dependent on particle size: those of diameter 30 nm (Sinerem)^[13] are useful for blood pool imaging, of diameter 150 nm (Endorem)^[14] for specific liver imaging, and larger particles of diameter 300 nm (Lumirem)^[15] can be administered orally for gastrointestinal tract imaging, as can Gadolite,^[16] a zeolite containing trapped Gd^{III} .

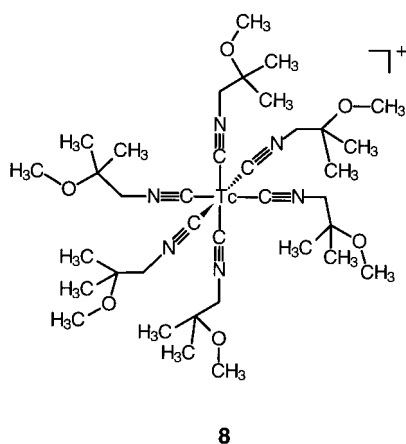
2.2. Radiopharmaceuticals

Clinical interest in radionuclides centers not only on high-intensity γ -ray emitters, especially $^{99\text{m}}\text{Tc}$ and ^{201}Tl , ^{111}In , ^{67}Ga , ^{51}Co , ^{51}Cr , and ^{169}Yb for diagnostic imaging, but also on β emitters, for example ^{89}Sr , ^{153}Sm , and ^{186}Re , for therapy.^[1,201] Many $^{99\text{m}}\text{Tc}$ -based radiopharmaceuticals and several other radionuclides are currently used in clinical diagnosis. Complex **7** ($^{99\text{m}}\text{Tc}^{\text{V}}(\text{dl-hm-pao})$), Ceretec is an approved cerebral perfusion imaging agent for evaluation of stroke. It is taken up by the brain and is transformed into a more hydrophilic species which is retained in the brain.



Intriguingly, the isomer containing *dl*-HM-PAO is retained in brain significantly longer than that containing the *meso* ligand.^[17]

Complex **8** ($[^{99m}\text{Tc}(\text{sestamibi})]^+$, Cardiolite) is used for myocardial perfusion imaging. It was designed on the basis

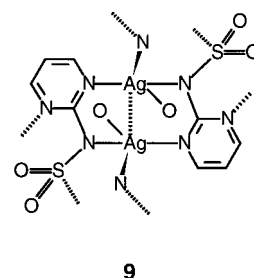
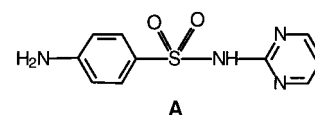


that lipophilic cationic complexes behave as potassium mimics and are taken up by the myocardium.^[18] The sequential metabolism of the six identical methoxy groups of **8** to hydroxyl groups in the liver leads to formation of ^{99m}Tc complexes with greater hydrophilicity which are not retained in myocardial tissues.^[19]

Monoclonal antibodies (mAbs) conjugated with radionuclides such as ^{111}In satumomab pentetide (which contains the murine mAb B72.3, which is directed to TAG-72, an antigen expressed by many adenocarcinomas) are used clinically for diagnosis of colorectal and ovarian cancer.^[20] Several other murine mAbs linked to ^{99m}Tc and ^{111}In are in clinical trials.^[21] Substantial progress has been made recently in the development of ^{99m}Tc -based receptor-specific radiopharmaceuticals.^[22] Encapsulation in fullerenes may also provide a novel method for the delivery of radionuclides to target sites.^[23, 24]

2.3. Antiinfective Agents

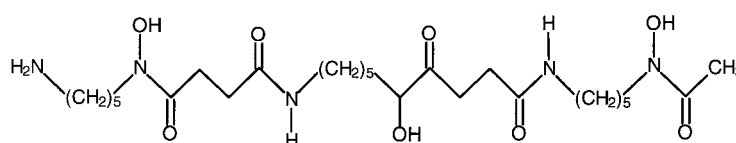
Silver and its compounds have long been used as antimicrobial agents in medicine. Silver is active at low concentrations and has a low toxicity. The practice of instilling the eyes of infants with 1% AgNO_3 solution immediately after birth is still common in some countries for prevention of ophthalmia neonatorum.^[25] Silver sulfadiazine **9**, made from Ag^+ and sulfadiazine **A**, is used clinically as an antimicrobial and antifungal agent. It is an insoluble polymeric compound which releases Ag^+ ions slowly, and is applied topically as a cream to prevent bacterial infections in cases of severe burns. Of industrial importance is the slow release of antimicrobial Ag^+ ions from inorganic or organic polymer matrices.^[26]



The mechanism of Ag^+ cytotoxicity is unknown. Cell-wall damage may be important, and it has been shown that Cys150 in phosphomannose isomerase, an essential enzyme for the biosynthesis of cell walls of *Candida albicans*, is a Ag^+ target in this organism.^[27] Silver-resistant bacteria are known,^[25] and progress in understanding the mechanism of resistance is now being made.^[202]

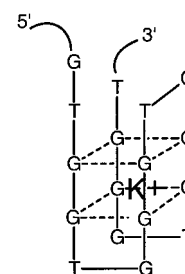
Antimony has been used for medicinal purposes for many centuries. Complexes of Sb^{III} are generally more toxic than those of Sb^{V} . Two Sb^{V} drugs, *N*-methylglucamine antimonate (Glucantime) and sodium stibogluconate (Pentostam), are used clinically for the treatment of leishmaniasis, a disease caused by intracellular parasites.^[28] The carbohydrates in the drugs may serve to deliver Sb^{V} to macrophages. The Sb^{V} complexes may be prodrugs for the more toxic Sb^{III} formed at or near the site of action. There is current interest in improving the stability, solubility, and efficacy of Sb drugs, and Sb^{III} and Sb^{V} complexes with yeast mannan are reported to be promising.^[29, 30]

The iron chelator desferrioxamine (**10**) is clinically approved for the treatment of malaria. Its activity may arise from disruption of Fe^{III} metabolism within the digestive vacuole of malaria parasites.^[31]



10

Several antisense oligonucleotides are potent inhibitors of HIV-1 integrase,^[32] and are currently on clinical trial. These have key sequences such as 5'-d(GTGGTGGGTGGGTGG-GT) (**11**, T30175), and are composed entirely of deoxyguanosine and thymidine. They fold up in the presence of K^+ ions to give four-stranded structures dominated by two stacked guanine-quartet motifs (Figure 5).^[33]

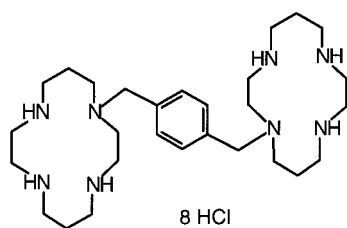


K-11

Figure 5. The folding of anti-HIV oligonucleotide **11** in the presence of potassium ions.

This structure is very stable under physiological conditions (e.g., **K-11** is resistant to serum nucleases with a half-life of 5 h) and is probably critical for biological activity.

Macrocyclic bicyclam ligands such as **12** (JM3100) are amongst the most potent inhibitors of HIV ever described, being active at nanomolar levels.^[34] Since they are nontoxic at

**12**

micromolar levels, they have a high selectivity index (ca. 10^5). The zinc complex of **12** is also active.^[35] The bicyclams appear to block HIV-1 entry and membrane fusion through interaction with the CXCR4 coreceptor during the early stages of the retrovirus replicative cycle.^[36]

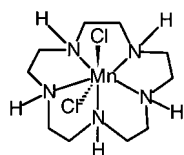
2.4. Superoxide Dismutase Mimics

The free radical superoxide, O_2^- , is a product of activated leukocytes and endothelial cells, and has been postulated to be a mediator of ischemia-reperfusion injury as well as inflammatory and vascular diseases. It can react with NO to form damaging peroxynitrite, ONO_2^- . The metalloenzyme superoxide dismutase (SOD) can destroy O_2^- : Cu,Zn-SOD in the cytoplasm of eukaryotic cells, Mn-SOD in mitochondria [Eqs. (1) and (2)]. However, the use of SOD in therapy is



limited by its short plasma half-life (clearance by the kidney) and inability to penetrate cell membranes (i.e., extracellular activity only). Low molecular mass mimics of SOD are

therefore of much potential pharmaceutical interest.^[37] For example, a

**13**

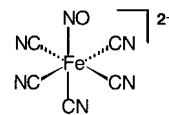
variety of Mn- and Fe-based porphyrins and macrocyclic complexes exhibit SOD mimic activity.^[38–41] Mn^{II} and Mn^{III} macrocycles appear to be particularly promising.^[42, 43] For example,

complex **13** (SC-52608) is able to scavenge superoxide and therefore effectively protect the regionally ischemic

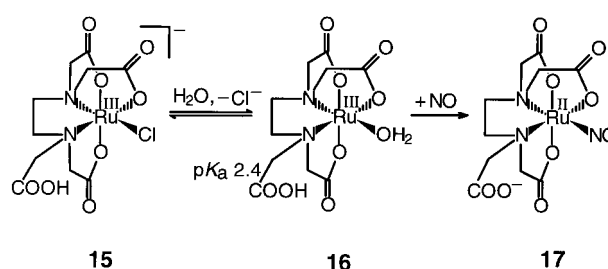
and reperfused myocardium from injury.^[44] Another example is manganese(III) 5,10,15,20-tetrakis(4-benzoic acid)-porphyrin (MnTBAP), which can protect against neurodegeneration and is therefore of potential interest for the treatment of brain diseases such as Parkinson's and Alzheimer's diseases.^[45]

2.5. Cardiovascular System

The low-spin Fe^{II} complex sodium nitroprusside (**14**) is the only clinically used metal–nitrosyl complex.^[46] It is often used to lower blood pressure in humans. Its hypotensive effect is evident within seconds after infusion, and the desired blood pressure is usually obtained within one to two minutes. It is also useful in cases of emergency hypertension, heart attacks, and surgery.^[47] The therapeutic effects of **14** depend on release of nitric oxide, which relaxes vascular smooth muscle. Activation in vivo may involve reduction to $[Fe(CN)_5(NO)]^{3-}$, which then releases cyanide to give $[Fe(CN)_4(NO)]^{2-}$ and then nitric oxide.^[48, 49]

**14**

Ruthenium complexes such as $K[Ru(Hedta)Cl]$ **K-15** (JM1226) have been proposed as nitric oxide scavengers for the control of NO levels under conditions of medical interest.

**15****16****17**

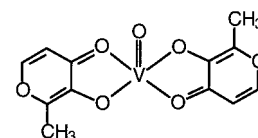
Complex **15** in water exists in equilibrium with the aqua species **16** (JM6245; the pK_a of the dangling arm carboxyl group is 2.4).^[50] Both complexes bind to NO very rapidly (rate constant $> 10^8 M^{-1} s^{-1}$ at body temperature 310 K) and tightly ($K > 10^8 M^{-1}$), forming the linear nitrosonium $Ru^{II}-NO^+$ adduct **17**.^[50]

Complex **16** has been shown to reverse the poor response of the artery to vasoconstrictor drugs,^[51] which is a major clinical problem in the treatment of patients with septic shock (caused by very high levels of circulating bacteria in the body). The excessive production of NO not only appears to be a major contributory factor in septic shock, but also in diabetes, arthritis, inflammation, and epilepsy.

2.6. Insulin Mimetics

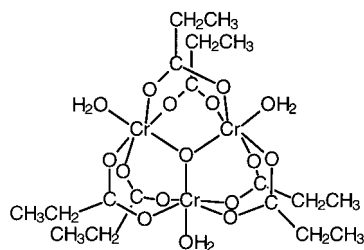
It was discovered nearly 20 years ago that V^V as vanadate and V^{IV} as vanadyl can mimic some of the effects of insulin (stimulate glucose uptake and oxidation as well as glycogen synthesis).^[52, 53] Vanadium complexes with organic ligands are often less toxic and can have improved aqueous solubility and lipophilicity.

The orally active complex bis-(maltolato) oxovanadium(IV) (**18**, BMOV)^[54] is three times more effective in vivo as an insulin-mimetic agent than $VOSO_4$.^[55] In the solid-state, complex **18** has a five-coordinate square-

**18**

pyramidal geometry with the oxo ligand in the axial position and *trans* maltolato ligands.^[56] In aerobic aqueous solutions, the complex is rapidly oxidized to the dioxovanadium(v) species.^[57]

Low molecular weight chromium-binding substance (LMWCr), a naturally occurring oligopeptide (ca. 1500 Da, consisting of Cr^{III}, Asp, Glu, Gly, and Cys in a 4:2:4:2:2 ratio), has been found to activate the insulin-dependent tyrosine kinase activity of the insulin receptor protein, with the activity being proportional to the Cr content of the oligopeptide (maximum activity with four Cr^{III} per oligopeptide).^[58] The trinuclear cation **19** can activate tyrosine kinase activity of the



19

insulin receptor protein in a fashion almost identical to that of LMWCr.^[59] Intriguingly when acetate replaces propionate in **19**, the complex does not activate but rather inhibits both the membrane phosphatase and kinase activity. Its potential for diabetes treatment has yet to be established.

2.7. Organic Drugs Targeted at Metals

Metal ions can play an important role in the mechanism of action of organic drugs, as is briefly illustrated by the following examples.

Organic drugs Galardin (glycomed), Ro319790, batimastat (BB-94), and BB-2516 are being evaluated in clinical trials for the treatment of diseases such as arthritis, cardiovascular disorders, and cancer. They are inhibitors of matrix metalloproteinases (MMPs), a family of zinc-dependent enzymes that degrade the major component of the extracellular matrix. Over-expression and activation of these enzymes have been linked with several diseases including cancer, arthritis, and multiple sclerosis. Inhibitors of these enzymes usually possess a group which can bind (chelate) to the active-site zinc(II) ion, and a peptide backbone which matches the requirements of the peptide cleavage site (Figure 6).^[60]

Batimastat, 4-(*N*-hydroxyamino)-(2*R*)-isobutyl-(3*S*)-[2-(thienylthiomethyl)succinyl]-*L*-phenylalanine-*N*-methylamide, is a broad spectrum inhibitor with nanomolar activity against MMPs, and is in phase II clinical trial for treatment of breast and ovarian cancer.^[61] The crystal structure of the catalytic domain of human neutrophil collagenase with bound batimastat (Figure 7) shows that batimastat coordinates to the catalytic Zn^{II} center in a bidentate manner through the hydroxyl and carbonyl oxygen atoms of the hydroxamate group.^[62]

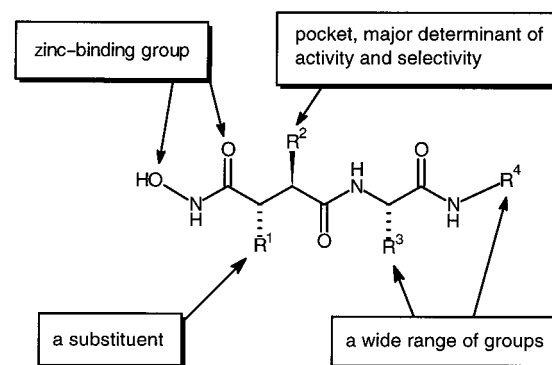


Figure 6. Backbone structure of matrix metalloproteinase inhibitors.

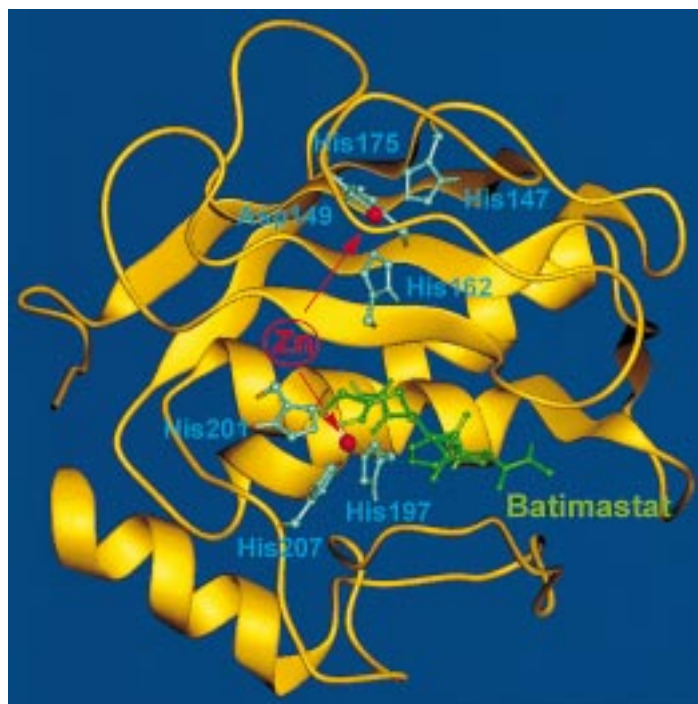
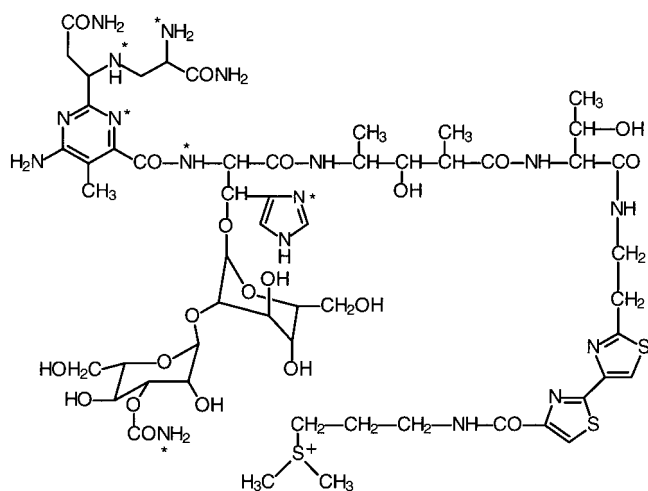


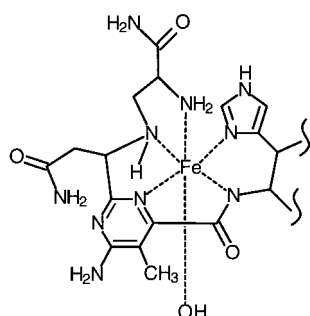
Figure 7. Crystal structure of the batimastat–human neutrophil collagenase complex, showing the inhibitor (green) coordinated to the catalytic Zn^{II} center. Adapted from ref. [62] (structure reference: PDBID, 1JAO).

The bleomycins (BLMs; e.g. A2 (**20**); the asterisks represent metal-binding sites) are a group of glycopeptide antibiotics which possess antitumor activity against several types of tumors. Bleomycin sulfate is used clinically in combination chemotherapy for the treatment of head and neck cancer, certain lymphomas, and testicular cancer.^[63]

The cytotoxicity of BLM is believed to result from its ability to bind iron (**20**-Fe),^[64] activate oxygen, and cleave DNA^[63] and possibly RNA.^[65] The ability of the Fe^{II}–BLM complex to bind to oxygen and produce oxygenated BLM species such as O₂[–]-Fe^{III}-BLM or O₂-Fe^{II}-BLM may be due to the presence of delocalized π electrons around iron and the strong iron–pyrimidine π backbonding.^[66, 67] Oxygenated BLM accepts an additional electron to form activated low-spin ferric-peroxide BLM (O₂^{2–}-Fe^{III}-BLM).^[66, 67] The structural features of Fe–BLM responsible for DNA (or RNA) degradation remain unclear.



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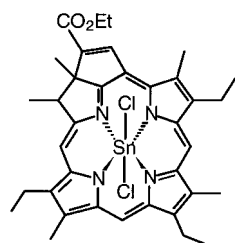


20-Fe

2.8. Photodynamic Therapy

Photodynamic therapy involves the treatment of diseased tissues and cells with a photosensitizer and visible light.^[203,204] Most of the clinical interest is focused on cancer, porphyrias, and hematological diseases, and various forms of jaundice. Photosensitisers are required which show some selectivity for photodamage to tumor tissue.

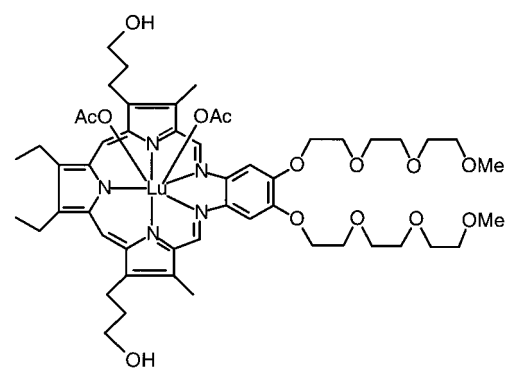
Tin(IV) ethyl etiopurpurin (**21**, SnET2)^[68] is a second-generation photosensitizer currently under clinical evaluation. It is preferentially bound to high-density lipoproteins in blood plasma.^[69] Complexes of texaphyrins (expanded porphyrins) with Cd^{II}, La^{III}, and Lu^{III} (**22**) are effective photosensitisers.^[70,71] They absorb strongly in the physiologically important far-red spectral region (700–800 nm), give rise to long-lived



21

triplet states, and are highly efficient in the production of singlet oxygen.

The lutetium complex **22** is currently on clinical trial as a photosensitizer for the treatment of cancer. This complex



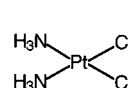
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possesses a strong broad absorption band centered at 732 nm.^[72] Upon absorption of light, it becomes activated to a long-lived triplet state complex that reacts with O₂ to generate cytotoxic singlet oxygen. Complex **22** is also in clinical trials as a photosensitizer for the treatment of atherosclerosis, the vascular disease caused by deposition of cholesterol and other fatty materials in the walls of blood vessels.

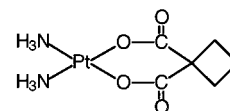
3. Anticancer Agents—Platinum

3.1. Clinical Platinum Complexes

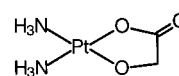
Platinum complexes are now amongst the most widely used drugs for the treatment of cancer.^[205,206] Four injectable Pt^{II} compounds have been approved for clinical use and several other *cis*-diam(m)ine complexes are on clinical trials, including an oral Pt^{IV} complex. Today cisplatin (**23**) is one of the



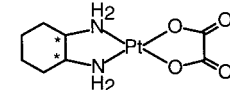
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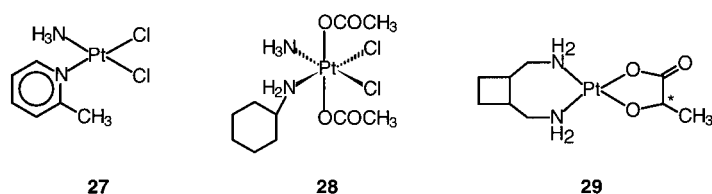
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most widely used anticancer drugs, together with the second-generation drug carboplatin [Pt(NH₃)₂(CBDCA-*O,O'*)] (**24**). The glycolato complex **25** (nedaplatin, 254-S) and oxalato complex **26** (oxaliplatin, *l*-OHP, which contains *R,R*-1,2-diaminocyclohexane, DACH) have been approved for clinical use in Japan and France, respectively. These drugs are particularly effective in combination chemotherapy for treatment of advanced lung, colorectal, and ovarian cancers.^[73,74]

The sterically-hindered complex **27** (ZD0473) is active (by injection and oral administration) against an acquired cisplatin-resistant subline of a human ovarian carcinoma xenograph,^[75] and entered clinical trials in 1997. It is less reactive



than cisplatin, for example inducing DNA interstrand cross-links in cells and binding to plasma proteins more slowly. The steric hindrance provided by the 2-methyl group, which lies over the top of the Pt-centered square plane, is evident in Figure 8.^[76] The hydrolysis rates of **27** are two to three times slower than those of cisplatin (Table 2).^[76]

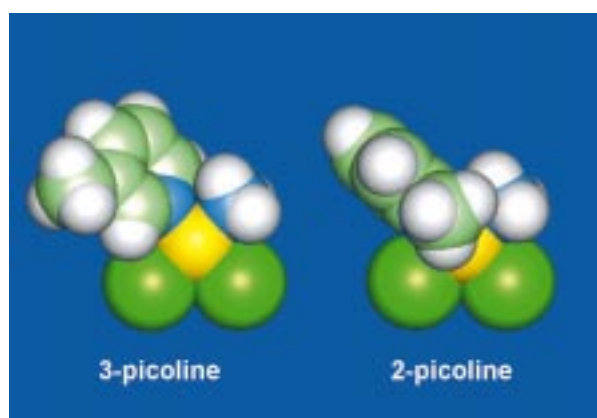


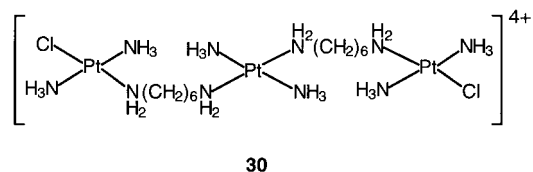
Figure 8. Crystal structure of the sterically hindered 2-picoline anticancer complex **27** in comparison with its 3-picoline analogue. The 2-methyl group in **27** lies directly over the Pt-centered square plane ($\text{H}_3\text{C}\cdots\text{Pt}$ 3.22 Å). Adapted from ref. [76].

Table 2. Rate constants k (Scheme 1) for hydrolysis of the platinum–picoline anticancer complex **27** in comparison with cisplatin (308 K, 0.1 M NaClO_4),^[76] in comparison with cisplatin (308 K, 0.32 M KNO_3).^[196]

Compound	k [$\times 10^{-6} \text{ s}^{-1}$]	Compound	k [$\times 10^{-6} \text{ s}^{-1}$]
2-picoline–Pt complex 27	k_{1a} : 31.9 ± 1.5 k_{1b} : 22.1 ± 1.4 k_{2a} : 73 ± 14 k_{2b} : 3.5 ± 2.5	3-picoline–Pt complex	k_{1a} : 44.7 ± 1.9 k_{1b} : 103 ± 4 k_{2a} : 35.0 ± 1.7 k_{2b} : 78 ± 60
cisplatin (23)	k_1 : 75.9		

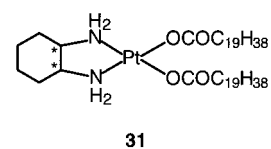
The orally active complex **28** (JM216) is currently in phase II clinical trials. It is reported to have superior in vitro and in vivo activity compared to cisplatin against human cervical, small cell lung, and ovarian carcinoma cell lines.^[77] When **28** is incubated with human plasma it is converted into at least six biotransformation products, which include mono- and dihydroxo Pt^{IV} complexes and the dichloroplatinum(II) complex $\text{cis-}[\text{PtCl}_2(\text{NH}_3)(\text{cyclohexylamine})]$ as the major metabolite.^[78, 79] Complex **29** (Lobaplatin, D-19466) was introduced into clinical trials in 1992.^[80] It is currently in phase II trials for treatment of cisplatin-resistant ovarian cancer,^[81] advanced head and neck cancers,^[82] and small-cell lung cancer.^[83]

The trinuclear complex **30** (BBR3464, counteranion = NO_3^-), in which the central Pt unit is capable only of hydrogen-bonding interactions with DNA is now on clinical



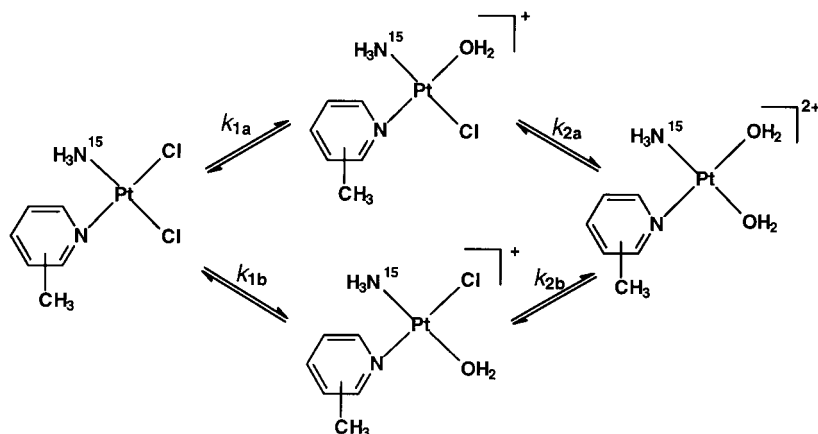
trial.^[84–86] The overall charge of +4 greatly enhances DNA affinity, characterized by long-range interstrand cross-linking up to six base pairs apart with significant unwinding and efficient, irreversible conversion of B- to Z-DNA. The adducts terminate DNA synthesis in vitro. The cytotoxicity of the complex is insensitive to the p53 status of cisplatin-resistant cells (effective against tumors carrying a mutant p53). It is reported to be up to 100-fold more potent than cisplatin against human tumor cell lines resistant to cisplatin.

cis-Bis(nonadecanoato)(*trans*-*R,R*-1,2-diaminocyclohexane)-platinum(II) **31** (N-DDP) is a liposome-incorporated lipophilic cisplatin analogue that has shown promising in vivo activity against tumors resistant to cisplatin and liver metastases,^[87] and is currently on clinical trial.



3.2. Platination of DNA

Guanine N7 is the most electron-rich site on DNA (most easily oxidized), and the major adducts of platinum drugs with DNA are 1,2-GpG and 1,2-ApG intrastrand cross-links. The properties of these adducts have been extensively characterized and reviewed.^[88] The NMR solution structure of $\text{cis-}[\{\text{Pt}(\text{NH}_3)_2\}^{2+}[\text{d}(\text{CTG}^*\text{G}^*\text{TCC})\cdot\text{d}(\text{GGACCAGG})]]$ (where * denotes Pt-bound G) indicates that the B-DNA backbone conformation is significantly altered to accommodate the platinated lesion (Figure 9).^[89] A recent X-ray crystal structure of the cisplatin adduct of the duplex



Scheme 1. Reaction steps in the hydrolysis of am(m)ineplatinum complexes.

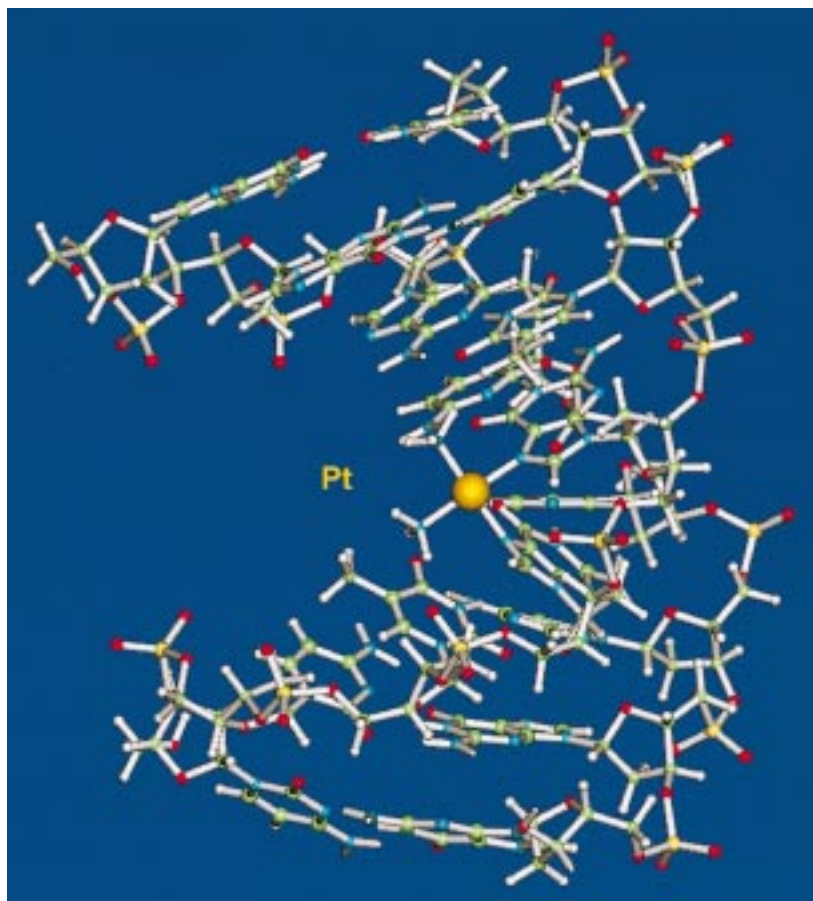


Figure 9. The refined structure based on NMR data of *cis*- $[\text{Pt}(\text{NH}_3)_2]^{2+}\{\text{d}(\text{CCTG}^*\text{G}^*\text{TCC})\cdot\text{d}(\text{GGACCAGG})\}$, showing that the DNA is significantly altered from the B form to accommodate the platination lesion^[89] (structure reference: PDBID, 1AU5).

$\text{d}(\text{CCTCTG}^*\text{G}^*\text{TCTCC})\cdot\text{d}(\text{GGAGACCAGAGG})$ (Figure 10) shows that cisplatin bends DNA by $35\text{--}40^\circ$ in the direction of the major groove with a dihedral angle of 26° between the two guanine rings.^[90, 91] The duplex adopts a juxtaposition of A-like and B-like helical DNA segments. It is notable that the conformation surrounding the GG platination site in the solid-state X-ray crystal structure^[91] is remarkably similar to that in solution.^[89, 92] The Pt ion is displaced from the planes of the coordinated G bases by about $0.8\text{ \AA}$, suggesting the presence of significant strain in this lesion. The nature and properties of minor cisplatin–DNA lesions which account for about 10% of the adducts, including 1,3-d(GpNpG) intrastrand and interstrand cross-links and monofunctional adducts, are less well understood.

Interstrand cross-linking of DNA occurs predominantly between two guanine residues on opposite strands, and requires a distance of approximately $3\text{ \AA}$ between two N7 atoms. The most common interstrand cross-linking sequences are 5'-CG and 3'-CG.^[93] In these sequences the two guanine residues are separated by at least $7\text{--}9\text{ \AA}$; therefore a large distortion of double-helical B-DNA is necessary to achieve the cross-linking. The solution NMR structure (Figure 11) of *cis*- $[\text{Pt}(\text{NH}_3)_2]^{2+}\{\text{d}(\text{CATAGCTATG})_2\}$ shows that the duplex undergoes a significant rearrangement at the lesion site so that the platinum atom is located in the minor groove.^[94] The deoxyribose of the platinated G residue is inverted so that O4' is pointing in the opposite direction compared to the remaining nucleotides. Moreover, the C residue which was originally base-paired to the platinated G is extruded and

becomes extra-helical (Figure 11). In the solution NMR structure of the interstrand cross-linked $[\text{Pt}(\text{NH}_3)_2]^{2+}\{\text{d}(\text{CCTCG}^*\text{CTCTC})\cdot\text{d}(\text{GAGAG}^*\text{CGAGG})\}$, the *cis*- $[\text{Pt}(\text{NH}_3)_2]^{2+}$ moiety is also located in the minor groove.^[95] The stacking of the two cross-linked guanine residues with the surrounding bases induces a bend of 40° towards the minor groove. A similar bend occurs in the X-ray crystal structure of this interstrand cross-link. The two cytosin residues are extruded from the double helix, and the platinum residue is embedded in a cage of nine water molecules.^[207]

The general mechanism of formation of GG cross-links on DNA by cisplatin is shown in Scheme 2. Cisplatin is hydrolyzed to give monoaqua (and diaqua) species with a half-life of 1.7 h at 310 K. The aqua complexes are believed to be the active species towards DNA.

Unexpected was the finding that the 5'-G monofunctional adduct formed during the reaction of cisplatin with the 14-mer DNA duplex $\text{d}(\text{ATACATGGTACATA})\cdot\text{d}(\text{TATGTACATGTAT})$ is very long lived with a half-life of 80 h at 298 K.^[96, 97] The lifetimes of the two monofunctional G adducts of the GG single strand are similar, suggesting that the three-dimensional structure of DNA plays a role in stabilizing the 5'-G adduct either by shielding the Cl ligand from hydrolysis, or by constraining the position of the incoming 3'-G N7 ligand. Molecular modeling studies demonstrate that hydrogen-bonding between the NH_3 ligands and carbonyl groups on DNA plays a major role in determining the orientation of the Pt–Cl bonds and their accessibility. Molecular mechanics calculations show that although the

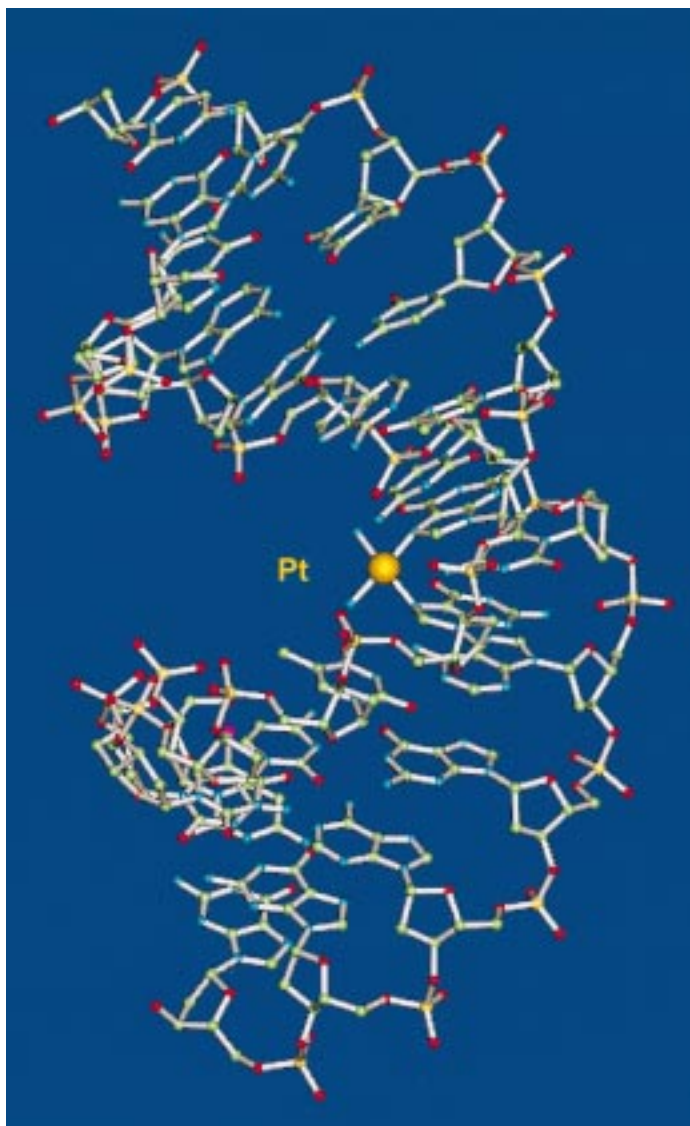


Figure 10. Crystal structure of *cis*-[[Pt(NH₃)₂]²⁺{d(CCTCTG*G*TCTCC)·d(GGAGACCAGAGG)}], showing that the DNA duplex is kinked by about 40° towards the major groove and has a juxtaposition of A-like and B-like helical DNA segments^[90, 91] (structure reference: PDBID, 1AIO).

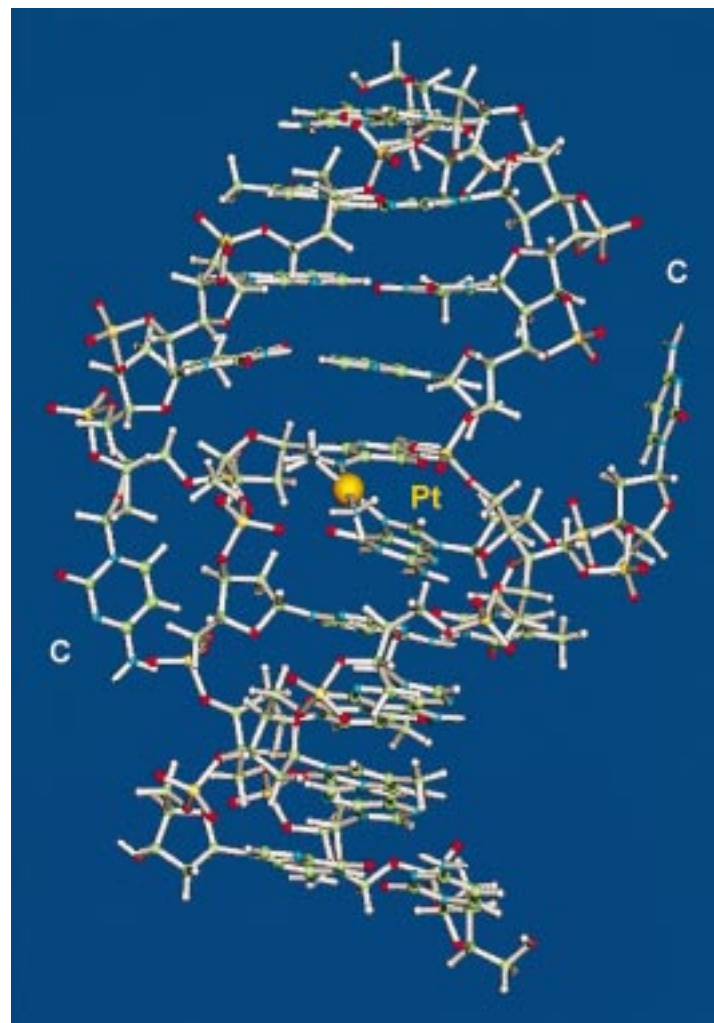
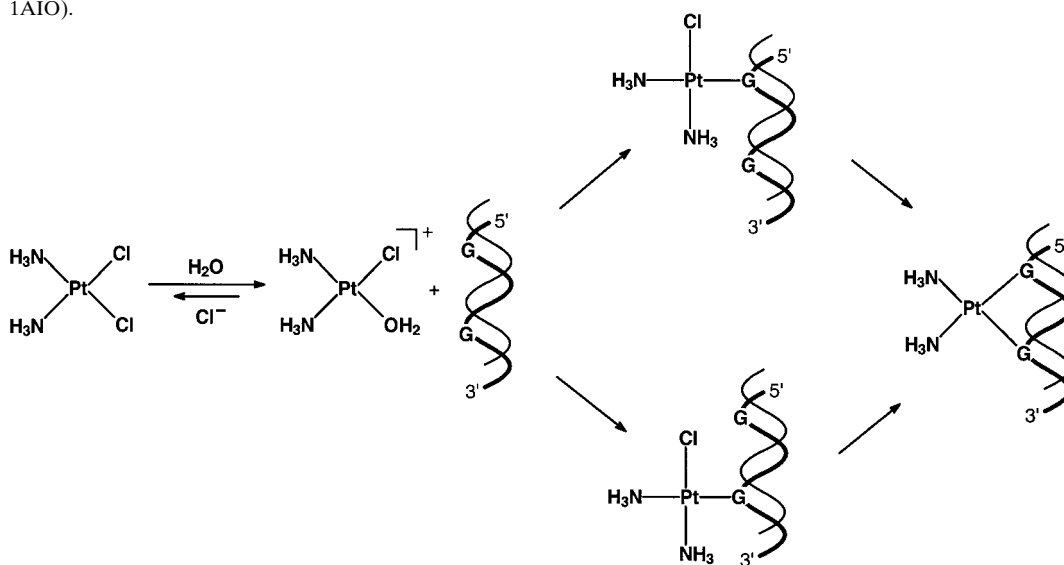


Figure 11. The refined structure based on NMR data of interstrand cross-linked *cis*-[[Pt(NH₃)₂]²⁺{d(CATAGCTATG)₂}. The deoxycytosine residues opposite to the platinated deoxyguanines are excluded and become extra-helical^[94] (structure reference: PDBID, 1DDP).



Scheme 2. Mechanism of reaction of cisplatin (**23**) with DNA.

chloride ligand in the monofunctional adduct faces outward, away from the helix, the aqua ligand which replaces it after hydrolysis faces inward on account of its strong hydrogen-bonding properties.^[97] Modeling of transition states is now required.

Kinetic analyses based on HPLC results allow the accurate determination of the rates of both platination and chelation steps for reactions of cisplatin diaqua with oligonucleotides.^[98, 99] For the double-stranded oligonucleotide d(TTGGCCAA)₂ the formation of the 5'-G monoadduct is faster than that of the 3'-G monoadduct, and macrochelate ring closure of the 5'-G monoadduct to give the bifunctional adduct (half-life of 3.2 h at 293 K) is much slower than that of the 3'-G monoadduct. The biological significance of long-lived monofunctional adducts on DNA remains to be determined, but these alone may be sufficient to kill cells if they are not repaired, which seems to be the case for the active *trans* iminoether complex **33** (see Section 3.4).^[100] Long-lived monofunctional adducts may also promote the formation of DNA–protein cross-links.

The stability of the Pt–N7G bond is known to be very high, and it can be broken only by very strong nucleophiles such as cyanide or thiourea. However, recent studies have shown that this bond can be labile in certain DNA adducts. The adduct *trans*-[Pt(NH₃)₂]²⁺[d(TCTACG*CG*TTCT)] (1,3-GG cross-link) is unstable at neutral pH and rearranges to form the linkage isomer *trans*-[Pt(NH₃)₂]²⁺[d(TCTAC*GCG*TTCT)] (1,4-CG cross-link).^[101] It was found subsequently that intra- and interstrand transplatin–DNA adducts undergo isomerization in both single- and double-stranded DNA.^[102–104] For example interstrand cross-links between a platinated 5'-G and the complementary C residue can be formed (Scheme 3).^[102]



Scheme 3. Rearrangement of a *trans*-[Pt(NH₃)₂]-DNA intrastrand cross-link (left) to an interstrand cross-link (right).

The isomerization of the intrastrand d(CCTG*G*TCC)·d(GGACCAGG) cross-link to a d(CCTG*GTCC)·d(GGACCAGG*) interstrand cross-link shows that even Pt–N7 bonds in Pt–1,2-GpG cross-links can be destabilized.^[89] The process appears to be assisted by Cl[–].

3.3. Protein Recognition

In tumor cells the excision repair of platinated DNA lesions may be protected by a class of proteins known as high mobility group (HMG) proteins.^[106, 107]

The protein HMG1 has three structural domains, two of them, domains A and B, are positively charged, and the third domain comprises a 30-amino-acid stretch of acidic residues at the C terminus. Several NMR structures of HMG domains have been determined.^[108, 109] Domain B of HMG1 consists of three α -helical regions joined by small loops, and folded into an L-shaped structure. The positively charged domain A and

the central domain B bind to DNA, while the acidic C-terminal domain interacts with histones.

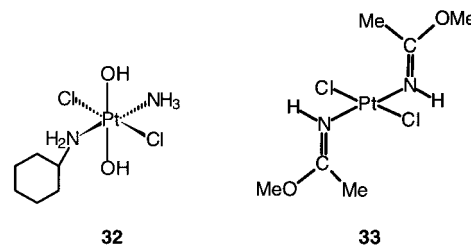
The binding affinity of HMG1 towards a series of cisplatin-modified 15-mer DNA duplexes d(CCTCTCN1G*G*N2TCTTC)·d(GAAGAN3CCN4GAGAGG) is highly dependent on the bases adjacent to the Pt lesion.^[110] The affinity for HMG1 domain A decreases by over two orders of magnitude in the order N2 = dA > T > dC. When N1 = N2 = dA, Pt–DNA binds 100-fold stronger to HMG1 domain A (K_d = 1.6 nM) than to HMG1 domain B (K_d = 134 nM). The HMG binding increases the bending of platinated DNA to as much as 90°. ^[111] NMR studies of a GG 14-mer platinated with cisplatin suggest that the kinked duplex binds in the elbow region of HMG1 domain A,^[112] and recent X-ray studies^[105] of HMG1 domain A bound to a 16-base-pair DNA fragment containing a cisplatin–DNA 1,2-d(GpG) intrastrand cross-link shows that the protein inserts a Phe side chain into the platinum site.

Nuclear protein, the linker histone H1, also binds much more strongly to cisplatin-modified DNA than to transplatin or unmodified DNA.^[113] Competitive binding of a cisplatin-modified 123-base-pair DNA fragment shows that histone H1 binding is stronger than that of HMG1. Also the promoter recognition factor TATA box-binding protein (TBP)/TFIID binds selectively to and is sequestered by cisplatin-damaged DNA. This may lure TBP/TFIID away from its normal promoter sequence explaining the inhibition of RNA synthesis.^[114] These proteins may therefore also play important roles in the mechanism of action of platinum drugs.

3.4. Active *trans* Complexes

Most of the work on the design of platinum anticancer complexes has concentrated on *cis*-diam(m)ine complexes, since it was discovered early on that *trans*-[PtCl₂(NH₃)₂] is inactive. However, the report that *trans*-[PtCl₂(py)₂] (py = pyridine) is potently cytotoxic has stimulated renewed interest in *trans* complexes.^[115]

The *trans* Pt^{IV} complex **32** is active against both murine and human subcutaneous tumor models,^[116] and efficiently promotes DNA interstrand cross-links and single-strand



breaks.^[117] These properties may account for its unusual cytotoxicity profile against cisplatin-resistant tumors.^[118] Intriguingly the corresponding *trans* Pt^{II} complexes (without the axial hydroxo groups) are inactive. Direct reactions of the Pt^{IV} complexes with DNA may be important, but in vivo reduction could produce reactive intermediates.

trans-Imino ether platinum complexes such as **33** have much higher antitumor activity than the *cis* analogues (OMe and Pt are *cis* with respect to C=N in the *Z* isomer, and *trans* in the *E* isomer).^[119, 120] The mechanism of action of these agents appears to be different from that of cisplatin and may be related to the properties of the imino ether ligands.^[121] Although these *trans* complexes react with DNA more slowly than cisplatin, they achieve the same level of DNA binding after 24 h. The *trans E,E* complex is the most effective in inhibiting DNA synthesis and cell proliferation, but does not induce large local DNA conformational changes.^[122] It preferentially forms monofunctional adducts at guanine residues in double-helical DNA even after long incubation times (48 h at 310 K).^[123a] The reactivity of the second chloride ligand in the monofunctional adduct is markedly reduced. The *trans E,E* monofunctional adducts are not recognized by HMG proteins.^[123b,c]

3.5. Biotransformation

L-Methionine (L-MetH) may play an important role in the metabolism of platinum anticancer drugs. The Pt^{II}–L-Met complexes form rapidly in plasma after injection of cisplatin into rats.^[124] The complex [Pt(Met-*S,N*)₂] has been detected in the urine of patients^[125] and exists predominantly as the *cis* isomer (*cis:trans* = 87:13) in solution (Figure 12).^[126]

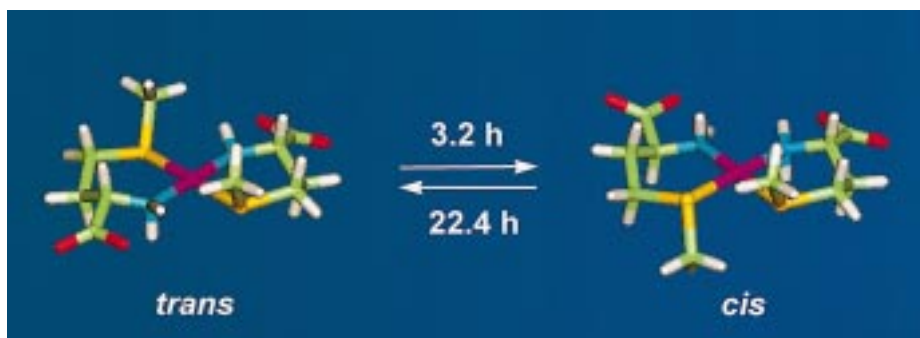


Figure 12. Isomerization of *R,R-trans*- and *S,S-cis*-[Pt(Met)₂], a metabolite of cisplatin. Adapted from ref. [126].

Human serum albumin (HSA) is a single-chain 66-kDa protein which contains 17 disulfide bridges and one free thiol group at Cys34, as well as six Met residues: M87, M123, M298, M329, M446, and M548. Reaction between cisplatin and HSA is thought to be the main route for platinum binding in human blood plasma.^[127] Recent data suggest that cisplatin reacts mainly with methionine residues of albumin, forming a Met-*S,N* macrochelate together with minor adducts with Cys34 and monodentate Met-*S* residues.^[128]

The reaction of carboplatin **24** with L-Met leads to a surprisingly stable ring-opened intermediate with a half-life of 28 h at 310 K.^[129] Similar ring-opened complexes appear to be present in urine after carboplatin administration.^[130] Therefore it is possible that the reaction of carboplatin with Met or its derivatives could provide an activation pathway for the drug. Binding of the methionine sulfur atom is reversible, and it can be displaced by guanine N7.^[208]

Intracellular thiols such as glutathione (the tripeptide γ -Glu-Cys-Gly, GSH), often present at concentrations of 3–10 mM, may inactivate platinum drugs. The major product from reaction between cisplatin and GSH is a high molecular mass polymer with a Pt:GSH ratio of 1:2.^[131] The formation of such polymers may be responsible for depletion of platinum from the circulation. The active export of cisplatin from cells is one of the major mechanisms of resistance, and a GS-X pump, an ATP-dependent export pump for GS-conjugates, is able to pump out GS–Pt complexes from tumor cells.^[132] Reaction between the cysteine-rich protein metallothionein (MT) and cisplatin leads to displacement of the ammine ligands and gives rise to Pt_{7–10}MT containing PtS₄ clusters.^[133] This provides another pathway for the inactivation of Pt drugs.

3.6. Photoactivation

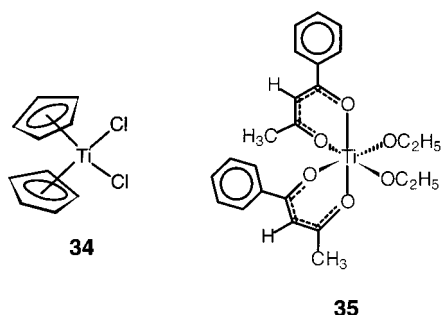
Both Pt^{II} and Pt^{IV} complexes have long been known to be photoreactive, and there is much scope for the application of photodynamic reactions in biochemistry and pharmacology.

A novel approach to DNA platination involves the use of iodoplatinum(IV) complexes which are activated by visible light.^[134] The toxicity of *trans,cis*-[Pt(en)(OAc)₂L₂] towards human bladder cancer cells is enhanced (by 35 %) when the treated cells are irradiated with light of $\lambda > 375$ nm. It has been shown that visible light can induce the aquation of this complex followed by photoreductive platination of guanosine monophosphate, in contrast to the dihydroxo analogue.^[135] Reactions of iodoplatinum(IV) ethylenediamine complexes with glutathione in the absence of light involve the initial attack of thiol on an iodide ligand of Pt^{IV} to generate reactive chelate ring-opened intermediates.^[136] Electron transfer driven labilization of *trans* ligands provides a new concept in drug design.

UV light induced cleavage of both intra- and interstrand cross-links involving Pt–G bonds has been observed. Irradiation of DNA modified by cisplatin with UV light ($\lambda > 300$ nm) can induce specific cross-links to the protein HMG1, thought to involve Lys6 in domain B with labilization of a Pt–purine bond.^[137, 138]

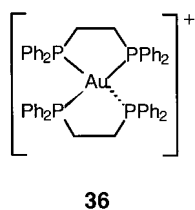
3.7. Other Metal Anticancer Agents

The antitumor activity of titanocene dichloride (**34**) was first recognised in 1979,^[139] and, since then, the activities of some other metallocenes (V, Nb, Mo, Fe, Ge, and Sn) have been reported.^[140] Complex **34** is active against a diverse range of human carcinomas, including gastrointestinal and breast carcinomas, but not against head and neck cancers, and is now in phase II clinical trials. There appears to be a lack of cross-resistance between **34** and cisplatin.^[141]



The bis(β -diketonato) Ti^{IV} complex **35** (budotitane, shown as the predominant *cis,cis,cis* isomer) entered phase I clinical trials in Germany in 1986 for the treatment of colon cancer.^[142] The complex is very susceptible to hydrolysis, and to minimize this, the coprecipitate of Cremophor EL, 1,2-propylene glycol in ethanol, and the drug in a ratio of 9:1:1 is dissolved in water prior to administration.^[142] The dose-limiting toxicity appears to be cardiac arrhythmia.^[143] Ti^{IV} binds strongly to human serum transferrin,^[144] and this protein could serve to deliver Ti^{IV} to cancer cells.

Tetrahedral bis(diphosphanyl)gold(I) complexes such as **36** are much less reactive, for example towards thiols, than linear Au^{I} antiarthritic complexes. Complex **36** (lactate salt) exhibits activity in a range of cancer models. It has a different mechanism of action to cisplatin, and is targeted to mitochondria, where it destroys membrane potentials. Complex **36** proved to be too cardiotoxic for clinical use, but the introduction of pyridylaryl substituents on P increases the hydrophilicity of bischelated Au^{I} diphosphane complexes, and gives rise to potent and selective activity towards cisplatin-sensitive and resistant ovarian tumor cells.^[145] It remains to be seen whether the cardiotoxicity is reduced.

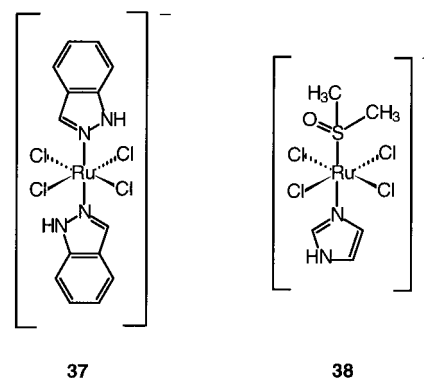


Gallium salts are known to exhibit anticancer activity, and Ga^{III} is probably delivered to tumor cells through the serum protein transferrin. Recent interest in gallium salts arises from their synergistic effect with cisplatin

in the treatment of lung cancer^[146] and carcinoma of the urothelium.^[147] Gallium(III) maltolate has recently entered clinical trials for the treatment of bone disease and related conditions.^[148]

Ruthenium(III) complexes are more active against metastases than against primary tumors, and Ru^{III} is also thought to be delivered to tumor cells through transferrin.^[149] The indazole complex **37**, which is especially active against colorectal tumors,^[150] binds reversibly to His253 of apolactoferrin, one of the Fe^{III} ligands in the iron-binding cleft of the N lobe, with displacement of a chloride ligand.^[151] Another promising candidate for clinical trial as an anti-metastatic agent is complex **38** (as the imidazolinium salt, NAMI-A).^[152] Ruthenium(III) complexes may be activated by reduction to Ru^{II} in vivo.

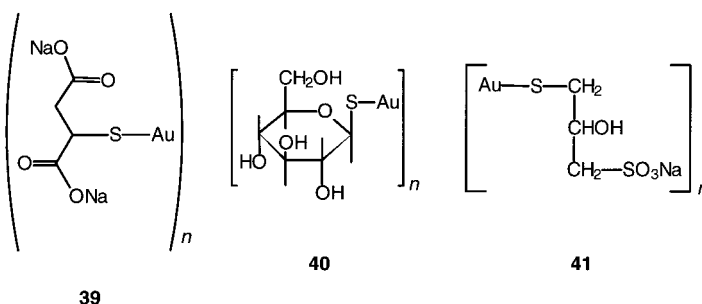
Both Ru^{II} and Ru^{III} complexes are known to bind preferentially to N7 of G on DNA, but can also bind to A and C residues.^[153, 154] Although most ruthenium antitumor agents have two reactive coordination sites, GG intrastrand cross-



links on DNA appear to be sterically unfavorable. The only example appears to be that of *trans*- $[\text{Ru}^{\text{II}}\text{Cl}_2(\text{dms})_4]$ with GpG, which has been structurally characterized by NMR spectroscopy.^[155] In this complex, the two N7-coordinated G residues adopt a head-to-head conformation and are strongly destacked.

4. Gold Antiarthritic Drugs

Several injectable 1:1 Au^{I} thiolato complexes are used clinically for the treatment of difficult cases of rheumatoid arthritis, including sodium aurothiomalate (**39**, Myocrisin), aurothioglucose (**40**, Solganol), and sodium aurothiopropionol sulfonate (**41**, Allochrysin).^[209]



Most of the gold thiolates have a gold-to-ligand ratio close to 1:1, and are polymeric complexes (e.g. chain or ring forms) with thiolate S-bridging and linear Au^{I} ions, as shown by EXAFS^[156] and WAXS data.^[157] Crystal structures of the gold thiolate drugs themselves have been elusive, the only example resulting from the recent elegant crystallization of **39** by Bau^[158] using techniques for crystallization of macromolecules. The linear S-Au-S units are arranged into polymeric double-helical chains (Figure 13). Hexameric Au_6S_6 rings of the type predicted previously^[159] have also been shown to exist in crystals with 2,4,6-tri(isopropyl)thiophenol as the thiolato ligand (Figure 14).^[160] The formulated gold thiolate drugs often contain a small molar excess (e.g. 10%) of thiol over Au^{I} , and readily undergo thiolate addition and thiolate exchange reactions.^[161]

The only oral gold antiarthritic compound in clinical use is the phosphane complex **42** (auranofin, Ridaura) shown in Figure 15. Complex **42** is also highly cytotoxic to tumor cells in culture, and is also reported to be active topically against psoriasis.^[162]

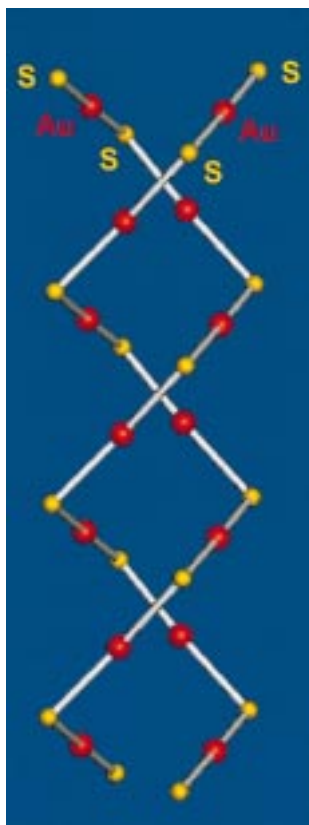


Figure 13. Crystal structure of **39** showing the double-helical chains. The right-handed helix shown contains thiomalate ligands with the *R* absolute configuration. Au–S bond lengths : 2.283, 2.286 Å; S–Au–S angles: 178.88°, 169.41°. Adapted from ref. [158].

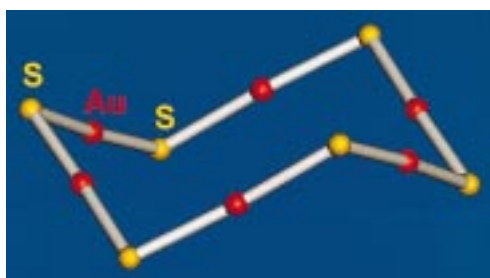


Figure 14. The hexameric ring structure in gold(I) 2,4,6-tri(isopropyl)thiophenolate. Substituents on S have been omitted for clarity. Au–S bond lengths: 2.28–2.29 Å. S–Au–S bond angles: 175–177°. Adapted from ref. [160].

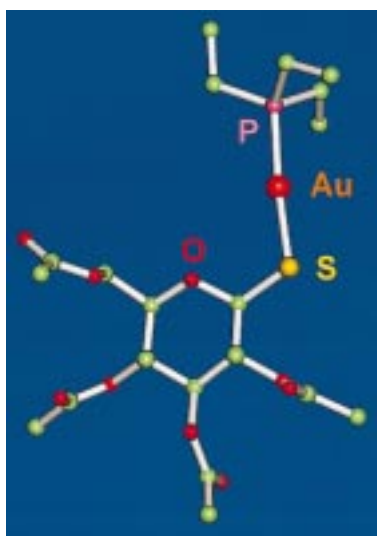


Figure 15. Crystal structure of the oral antiarthritic drug auranofin (**42**). Adapted from ref. [198].

A common metabolite found in the urine and plasma of patients treated with gold drugs is $[\text{Au}(\text{CN})_2]^-$,^[163] an ion which readily enters cells and can inhibit the oxidative burst of white blood cells. It may therefore be an active metabolite of gold drugs. It also exhibits anticancer and anti-HIV activity.^[209] The high Au content of the red blood cells of smokers receiving gold therapy has been attributed to the inhalation of HCN in smoke.^[164]

Under conditions mimicking red blood cell concentrations, Elder et al.^[165] have observed that $[\text{Au}(\text{CN})_2]^-$ reacts with GSH to form $[\text{Au}(\text{SG})_2]^-$, which is very stable. $[\text{Au}(\text{SG})_2]^-$ is likely to be formed inside cells both from gold thiolate drugs and from auranofin **42**, and may be responsible for the inhibition of enzymes such as the Se-enzyme glutathione peroxidase.^[166]

Compound **39** is a potent inhibitor of neutrophil collagenase, a zinc enzyme which contains Cys ligands in the metal-binding site.^[167, 168] Such interactions may be important in joint tissue.

Gold(I) has a very high affinity for thiolate S, but binds only weakly to O and N ligands. Hence proteins containing cysteine thiolate groups (especially those with low $\text{p}K_{\text{a}}$ values) are targets for Au^{I} , but binding of Au^{I} to DNA is weak. Moreover, thiolate exchange reactions are usually rapid. During therapy, Au levels in the blood typically reach $20 \mu\text{M}$, and Au is transported by albumin bound to Cys34. The rate of gold binding is determined by the rate of opening of the cleft containing Cys34,^[169, 170] and Au binding appears to induce a “flip-out” of this residue.^[171] Gold(I) drugs can bind strongly to thiol groups in DNA-binding proteins such as the transcription factors Jun–Jun and Jun–Fos, giving rise to the possibility that gold can regulate transcription factor activity.^[172]

The oxidation of Au^{I} to Au^{III} in vivo may be responsible for some of the toxic side effects of gold drugs.^[173, 174] In inflammatory situations, strong oxidants such as ClO^- and H_2O_2 are potentially available in vivo and can oxidize the metal centers in **39**, **40** and **42** to Au^{III} .^[175] Gold(III) has the unusual ability to deprotonate peptide amide groups even at low pH values (e.g. pH 2)^[176] and may modify peptide recognition by T cells. Gold(III) can oxidize disulfide bridges in albumin and insulin,^[177] and methionine residues of ribonuclease.^[178]

5. Bismuth Antiulcer Drugs

Bismuth compounds have been used for treating gastrointestinal disorders for more than two centuries.^[179] These include nitrate, salicylate salts, and colloidal bismuth subcitrate, all Bi^{III} compounds. Bismuth(V) is usually a strong oxidant. The structures of Bi drugs are largely unknown. The coordination number of Bi^{III} is highly variable (3–10), and the coordination geometry often irregular.^[180] Bi^{III} is highly acidic in water (first $\text{p}K_{\text{a}}$ ca. 1.5) and has a strong tendency to form stable hydroxo- and oxo-bridged clusters. Figure 16 shows the core structure of $[\text{Bi}_6\text{O}_4(\text{OH})_4](\text{NO}_3)_6 \cdot 4\text{H}_2\text{O}$, known since the 17th century as “magisterium bismuti” and used as a beauty care product, which crystallizes at pH values below 1.2.^[181]

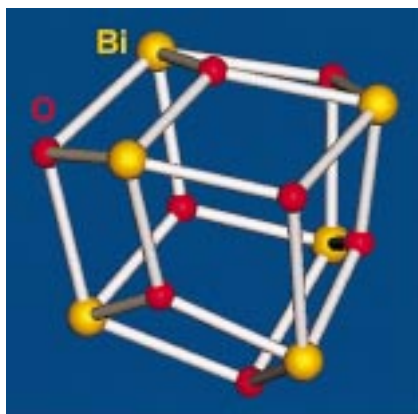


Figure 16. Crystal structure of $[\text{Bi}_6\text{O}_4(\text{OH})_4](\text{NO}_3)_6 \cdot 4\text{H}_2\text{O}$, showing hydroxo- and oxo-bridged Bi^{III} centers. Adapted from ref. [181].

The best understood in structural terms are the citrate complexes, for which several X-ray structures have been determined,^[182] although none has exactly the same composition as the drugs themselves. The dominant feature is the dimeric $[(\text{cit})\text{BiBi}(\text{cit})]^{2-}$ unit (H_4cit = citric acid), which contains bridging citrate anions. The Bi–O(alkoxide) bond is very short (2.2 Å) and strong, and a stereochemical role for the $6s^2$ lone pair is apparent in the structure. These dimers aggregate into chains and sheets in the crystal through a network of hydrogen bonds involving citrate, counter ions, and water to give polymers such as that shown in Figure 17. Such polymers may be deposited on the surface of ulcers. Bismuth(III) citrate complexes appear to be stable in solution over a pH range of about 3.5 to 7.5. $[\text{Bi}^{\text{III}}(\text{Hcit})]$ itself can be solubilized by a variety of amines,^[183] and the adduct with the organic histamine antagonist ranitidine, ranitidine bismuth citrate,^[184, 185] has recently been marketed as a new drug.

The antimicrobial activity of Bi^{III} against the bacterium *Helicobacter pylori* appears to be important for its antiulcer activity.^[186] This organism may also be involved in other conditions such as cancer. The biological effects of Bi^{III} are probably largely due to binding to proteins and enzymes. The binding of Bi^{III} to DNA is, as yet, unknown. There is a strong correlation between the strength of binding of Bi^{III} to a variety of ligands and that of Fe^{III} (Figure 18). Although Bi^{III} (ionic radius 1.03 Å) is larger than Fe^{III} (ionic radius 0.64 Å, high spin) it also binds strongly to the serum Fe^{III} transport protein transferrin.^[187] The strength of binding of transferrin to Bi^{III} can be correlated with the high acidity of Bi^{III} (Figure 19). Related proteins (periplasmic iron-binding proteins) are involved in Fe uptake by some virulent bacteria. Bismuth(III)

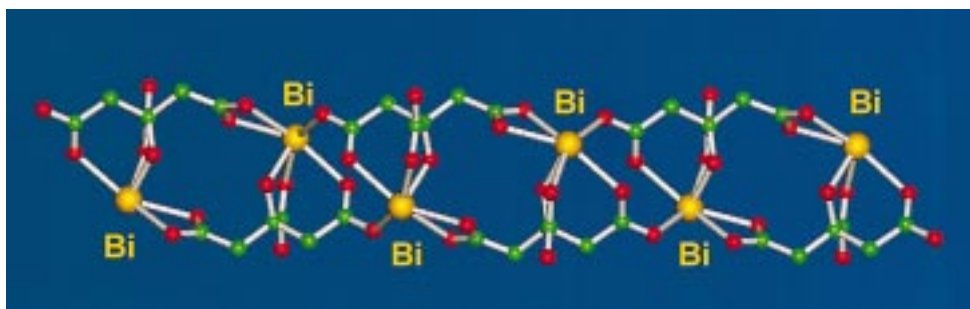


Figure 17. Crystal structure of the polymeric Bi–citrate complex $\text{Na}_2[\text{Bi}_2(\text{cit})_2]$. Adapted from ref. [182d].

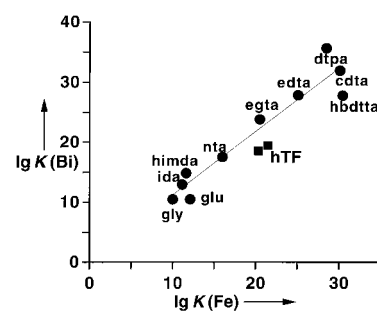


Figure 18. Linear free energy relationship for complexation of Fe^{III} and Bi^{III} with oxygen and nitrogen donor ligands. Adapted from ref. [187a]. hbdta = *N,N'*-bis(2-hydroxybenzyl)diethylenetriamine-*N,N',N''*-triacetate, cdta = *trans*-1,2-diaminocyclohexane, dtpa = diethylenetriamine pentaacetate, gly = glycine, glu = glutamate, ida = iminodithanoate, himda = *N*-(2-hydroxyethyl)iminodithanoate, nta = nitrilotriacetate, hTF = human transferrin, egta = ethylenebis(oxyethylenenitrilo)tetraacetate, edta = ethylenediaminetetraacetate.

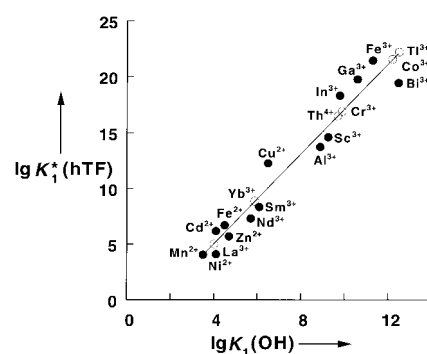


Figure 19. Correlation of the strength of metal binding to human serum transferrin hTF (bicarbonate independent binding constant) with metal ion acidity. Open circles are predicted values. Adapted from ref. [187b].

can readily displace Zn^{II} from the Cys-rich protein metallothionein, and bismuth metallothionein is stable even under strongly acidic conditions (pH 2).^[188]

Cysteine and glutathione may play a role in the transport of Bi^{III} in cells and biofluids. These thiols can prevent the precipitation of colloidal bismuth subcitrate (CBS) at pH 2.0, and animal studies have shown that simultaneous oral administration of bismuth salts and thiols produces a significant rise in the bismuth concentration in blood plasma.^[189, 190] The complex $[\text{Bi}(\text{SG})_3]$ is very stable ($\lg K = 29.6$) over a wide pH range (2–10) with binding through the S atom only.^[191] Exchange of GSH between free and bound forms is relatively rapid at biological pH (ca. 1500 s^{-1}).

6. Summary and Outlook

It is clear that inorganic chemistry will have an important role to play in medicine in the future. The range of elements traditionally available to the pharmacologist (largely H, C, N, O, F, S, P) is being widened to include all 24 elements thought to be essential to mammalian life together with many other elements in the periodic table, both stable and radioactive. Metal ions in particular play crucial roles in physiological processes and offer much scope for innovative drug design.

The success of cisplatin (the world's best selling anticancer drug) for the treatment of cancer is clear. The mechanism of action of platinum-based agents is much better understood than those of other widely used metallodrugs such as gold antiarthritic, bismuth antiulcer, lithium antidepressive (used by about 1 in 1000 of the population of the UK), or antimony antiparasitic agents. These elements all present significant challenges, as do elements new to the clinic such as Ti, V, Mn, Ru, and Gd.

The improved design of metallodrugs depends on a better knowledge of coordination chemistry under biologically relevant conditions, knowledge not only of the thermodynamics (equilibrium constants and structures of products) but also of the kinetics (mechanisms, pathways, ligand-exchange dynamics) of substitution and redox reactions. Biological systems often operate far from thermodynamic equilibrium. Understanding the nature of the interactions of metallodrugs with cell membranes, proteins, enzymes, and DNA is particularly important. Our increasing knowledge of the role of metals in genetic regulation and the nature of metal sensor, transport, and chaperone proteins will eventually have a major impact on drug design. For example, iron is a corepressor for the diphtheria toxin gene in virulent pathogenic bacteria,^[192] and inherited human diseases of copper transport can now be linked directly to genes encoding copper-transporting ATPases.^[210] There is much further scope for using metals to deliver biologically active ligands, for example NO, and the recent clinical introduction of titanocene dichloride illustrates that organometallic complexes too can have uses in medicine, provided they are formulated appropriately.

As a final illustration of how medicinal inorganic chemistry is likely to have a major impact on pharmacology, we highlight neuropharmacology. The roles of Na⁺, K⁺, and Ca²⁺ in neurochemistry are well-known, but it is also apparent that Fe and Cu enzymes can control neurotransmitter biosynthetic pathways, and there are millimolar levels of Zn²⁺ in the hippocampus during neurotransmission. Moreover, Mn is abundant in the brain in enzymes such as glutamine synthase and superoxide dismutase. We can speculate that the control of metal neurochemistry is vital for the prevention of neuronal degradation, and for an understanding, and perhaps effective treatment, of conditions such as Parkinson's disease, senile dementia, Alzheimer's disease, and even Creutzfeldt–Jakob disease (CJD). Prion protein, believed to be the cause of CJD, is thought to be a copper protein in vivo.^[211] We can expect medicinal inorganic chemistry to rise to such challenges.

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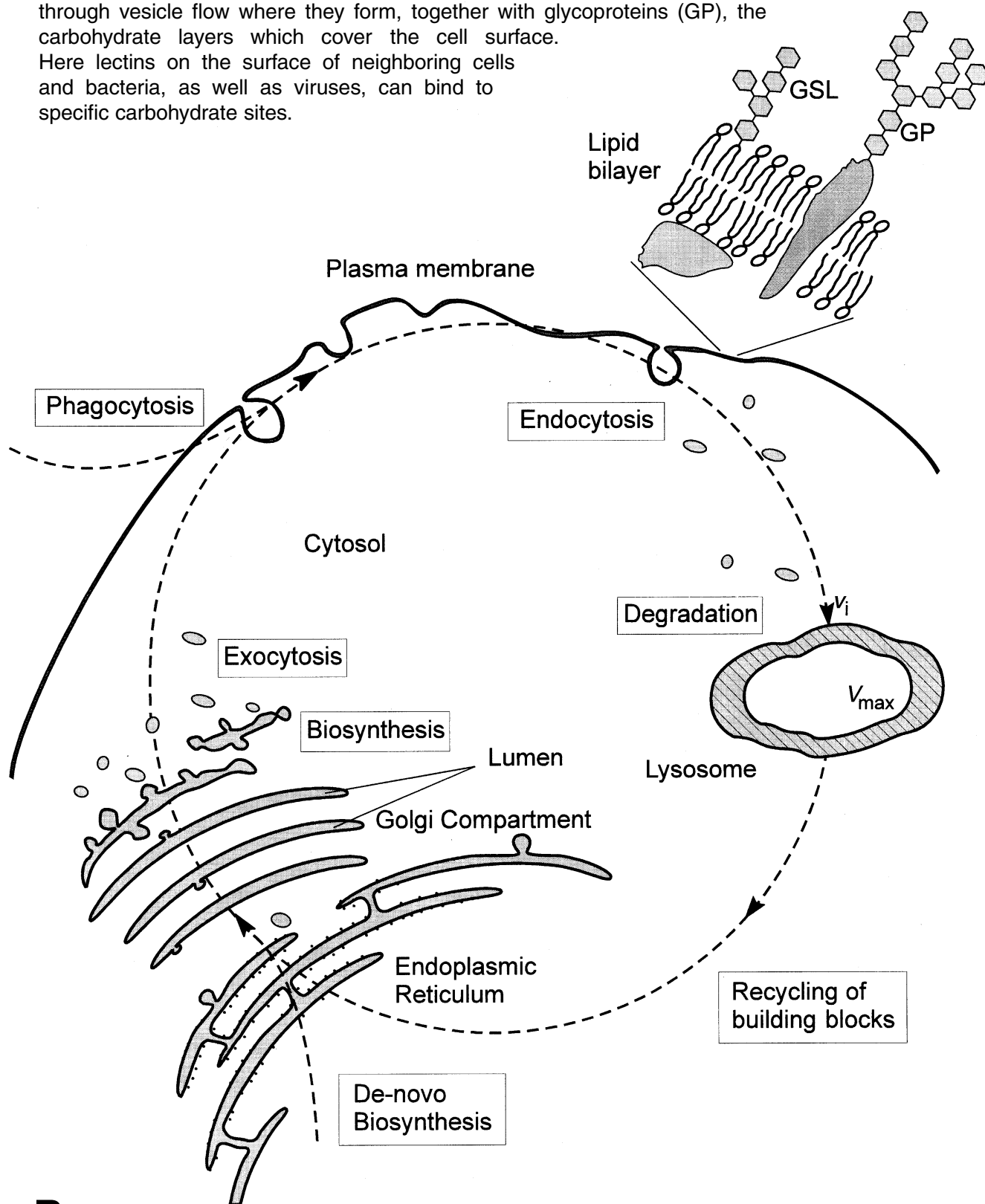
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The biosynthesis of glycosphingolipids (GSL) takes place at the intracellular membranes of endoplasmic reticulum and the Golgi apparatus in eukaryotic cells. Glycolipids reach the plasma membrane through vesicle flow where they form, together with glycoproteins (GP), the carbohydrate layers which cover the cell surface. Here lectins on the surface of neighboring cells and bacteria, as well as viruses, can bind to specific carbohydrate sites.



Building blocks of the plasma membrane can reach the lysosomes by endocytosis, where they break down. The glycolipid influx rate v_i and maximum activities V_{max} of the degrading system within the lysosome are fundamental parameters in human genetic diseases that result from defects in the decomposition of sphingolipids. As well as being used in the new synthesis of glycoproteins, the building blocks, which are produced by lysosomal degradation and released into the cytosol, can be reused in a recycling process.

Sphingolipids—Their Metabolic Pathways and the Pathobiochemistry of Neurodegenerative Diseases

Thomas Kolter* and Konrad Sandhoff*

Dedicated to Professor Hans-Jürgen Quadbeck-Seeger on the occasion of his 60th birthday

Sphingolipids are building blocks of the plasma membrane of eukaryotic cells. Their function is to anchor lipid-bound carbohydrates to cell surfaces and to construct the epidermal water permeability barrier. Intermediates in sphingolipid metabolism are involved

in the transduction of extracellular signals into the interior of cells. We give a review of the metabolism of these compounds in vertebrates, the intracellular topology of individual metabolic pathways, and the genetic diseases that occur as a result of defects

in these pathways and which frequently lead to degeneration of the nervous system.

Keywords: enzyme inhibitors • diseases • glycolipids • glycosidases • sphingolipids

1. Historical Introduction

Glycosphingolipids (GSLs), a class of natural products, were first described in the second half of the last century. The German physician J. L. W. Tudichum was able to isolate an organic base that he called sphingosine in addition to sugar and fatty acids by fractional crystallization of alcoholic brain extracts.^[1] The structure was elucidated by Carter in 1947.^[2] The isolation and naming of further brain lipids such as ceramide, sphingomyelin, and cerebroside are also attributed to Tudichum.^[3] At the end of the 1930s the Cologne biochemist Ernst Klenk discovered a new subgroup of acid sphingolipids in the brain tissue of patients who were suffering from amaurotic idiocy, a rare inherited disease. He called these new compounds gangliosides, and their acid component neuraminic acid.^[4] The structure of *N*-acetylneuraminic acid, also known as sialic acid^[5] or lactaminic acid, was elucidated by Richard Kuhn in 1962.^[6] The first structure elucidation of a ganglioside, GM1 (Figure 1) was made by Kuhn and Wiegandt in 1963.^[7]

The structural identification of GSLs and the investigation of its metabolism were both facilitated by autopsy material from patients who had suffered from rare metabolic defects. Sphingolipids accumulate in the course of these storage diseases as a consequence of mutations that lead to the

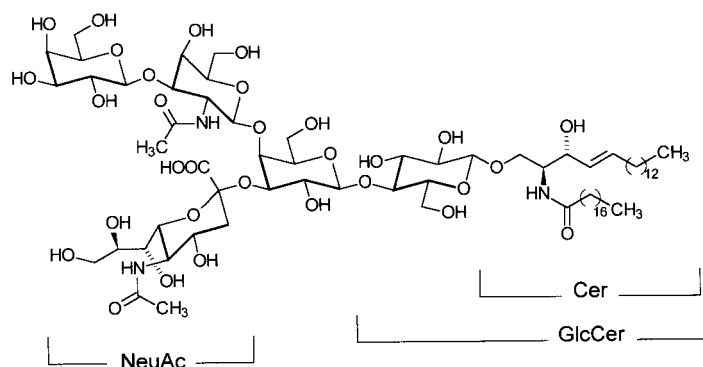


Figure 1. Structure of ganglioside GM1, an abundant glycosphingolipid bearing sialic acid in the adult human brain, which is also the intestinal cholera toxin receptor. The names of partial structures are indicated; heterogeneity within the lipid moiety is not indicated. NeuAc: *N*-acetylneuraminic acid (sialic acid), Cer: ceramide (*N*-acylsphingosine), GlcCer: glucosylceramide.

deficiency of proteins that are necessary for their lysosomal degradation. In our laboratory the analysis of these genetic diseases led to the identification of the molecular defects in Tay–Sachs disease, Sandhoff disease, and other sphingolipidoses (Section 6), as well as to a new hypothesis for the topology of endocytosis and lysosomal digestion (Section 5.4). It was also possible to demonstrate the function of activator proteins that are necessary for sphingolipid catabolism by the analysis of inherited diseases. Thus, the analysis of the AB-variant of GM2-gangliosidosis led to the identification of the GM2-activator protein (Section 6.2.4), and the detection of the specificity of saposins A–D (SAP-A–D) in vivo was only

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made possible with the help of the two only known patients with SAP-precursor deficiency (Section 6.11).

2. Structure and Function of Sphingolipids

The surfaces of eukaryotic cells are covered by a carbohydrate layer. This so-called glycocalyx consists of glycoproteins, glycolipids, and glycosaminoglycans. Ceramide, the component of most sphingolipids, functions essentially as the anchor of lipid-bound carbohydrates in eukaryotic cells.^[8–10] Ceramide itself consists of a long-chain amino alcohol, *D*-erythro-sphingosine, the amino group of which is acylated with a fatty acid. Carbohydrate-free sphingolipids also exist, such as ceramide itself, the membrane lipid sphingomyelin, or intermediates of sphingolipid metabolism such as sphingosine, sphinganine, or sphingosine-1-phosphate.

A number of GSLs have been found in nature that differ from each other in the type, number, and coupling of the individual carbohydrate building blocks. The GSL composition of an organism is species specific. The lipids can be classified into a few series (Figure 2 and 3) that are characteristic of species that are related in their evolutionary history.^[10] GSLs form cell-type specific patterns on the cell surface (Figure 4), which change with the differentiation stage and with viral or oncogenic transformations.^[11] The heterogeneity within this substance class is not limited to the carbohydrate moiety, however; the lipid anchor can also vary in alkyl chain length and the degree of unsaturation and hydroxylation.

GSLs on the cell surface are involved in cell-type specific adhesion processes.^[12] It is known that they can function as binding sites for toxins, viruses,^[13] and bacteria.^[14] These pathogens profit from the close spatial neighborhood of specific carbohydrate recognition sites on the cell surface and the plasma membrane. Cell adhesion phenomena of this type result from a binding of the carbohydrate moiety of the membrane-bound GSLs to lectins on the surface of neighboring cells. The formation of myelin layers around axons of

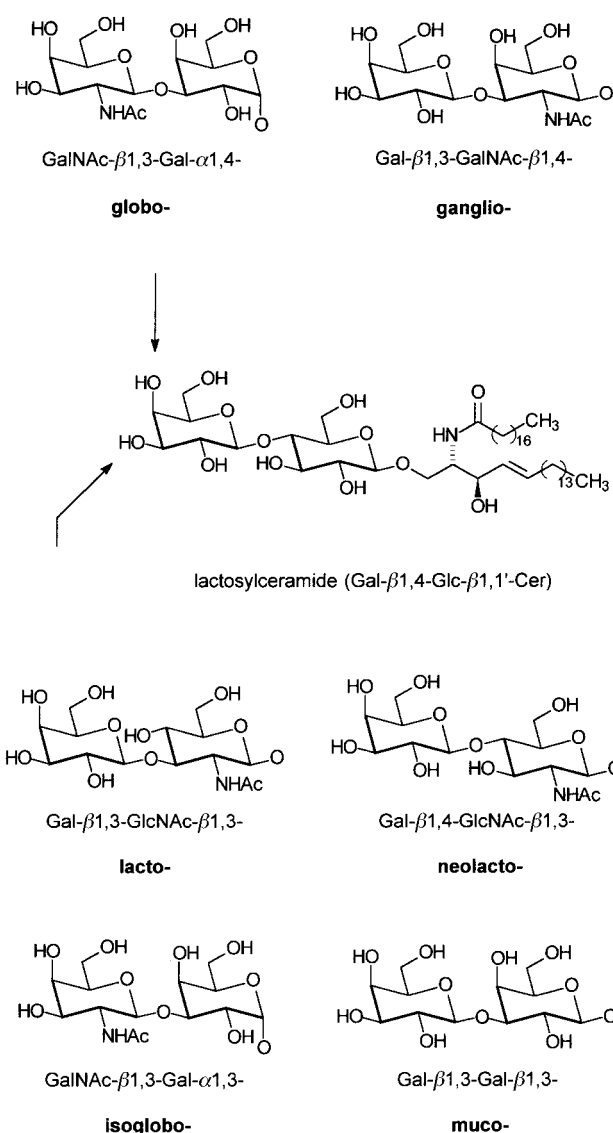


Figure 2. Structures and trivial names of the mammalian glycosphingolipid series derived from lactosylceramide.

Thomas Kolter was born in 1963 and studied Chemistry at the Universität of Bonn. He completed his PhD with A Giannis on the synthesis of peptide mimetics in 1993. His work with Konrad Sandhoff is in the area of lipid biochemistry. His particular interest lies in bioorganic aspects of glycolipid metabolism.



T. Kolter



K. Sandhoff

Konrad Sandhoff was born in 1939 and studied Chemistry at the Universität München. He completed his PhD under the guidance of H. Jatzkewitz and F. Lynen and then worked at the Max-Planck-Institut für Psychiatrie, München, the John-Hopkins University, Baltimore, and the Weizmann-Institut, Rehovot. In 1979 he became Professor of Biochemistry at the Kekulé-institut für Organische Chemie und Biochemie der Universität Bonn. The honors he has received include the naming of a human disease as "Sandhoff's disease", the award of the 1992 Richard-Kuhn Medaille der Gesellschaft Deutscher Chemiker (German Chemical Society) and the 1998 K.-J.-Zülch-Preises der Max-Planck-Gesellschaft. Since 1991 he has been the spokesman for the SFB 284 (glycoconjugate and contact structures of cell surfaces) and has been a member of the Editorial Board of Angewandte Chemie. His research interests are the analysis of lysosomal storage disorders and all aspects of the metabolism of glycolipids.

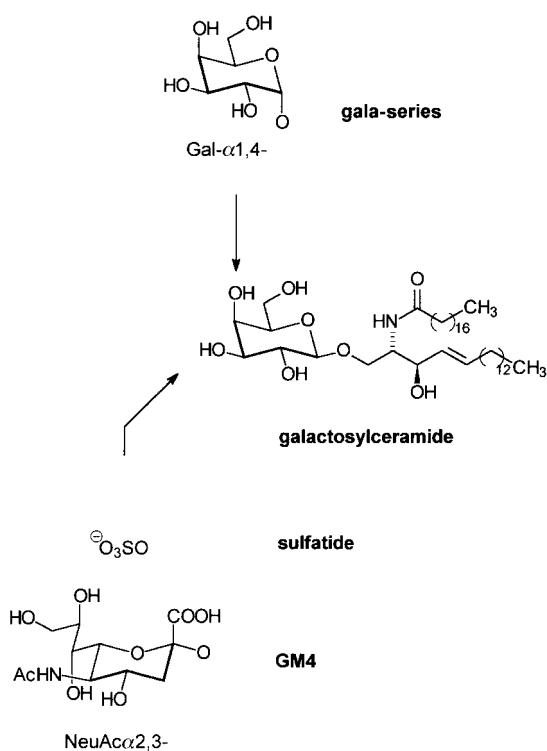


Figure 3. Structures and trivial names of the mammalian glycosphingolipid series derived from galactosylceramide.

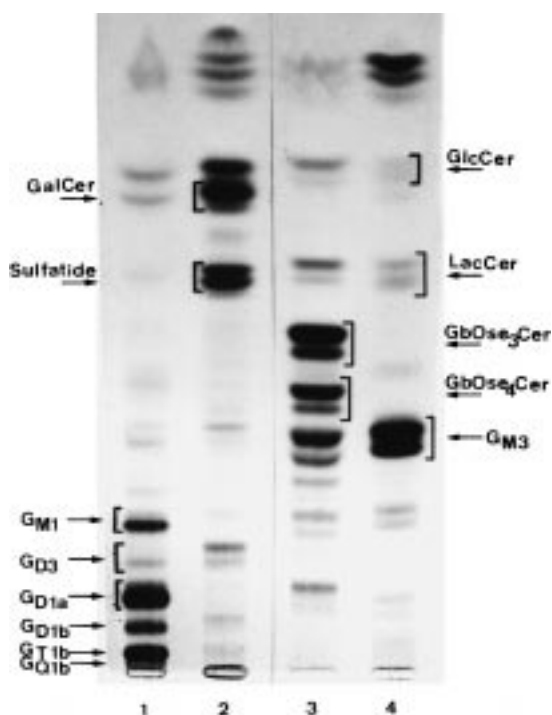


Figure 4. Biosynthetic labeling of cellular glycolipids with $[^{14}\text{C}]$ galactose.^[438] Cells were labeled by incubation with $[^{14}\text{C}]$ galactose ($2\ \mu\text{Ci mL}^{-1}$) for 48 h, harvested, and extracted. The glycosphingolipids were separated by thin layer chromatography and visualized by fluorography. Lane 1: -granule cells of murine cerebellum; Lane 2: oligodendrocytes; Lane 3: fibroblasts; Lane 4: neuroblastoma cells (B 104). The mobility of the standard lipids is indicated; see following figures for the abbreviations used.

neuronal cells is an example that is based upon this principle. In this case gangliosides on the surface of neuronal cells serve as ligands for the myelin-associated glycoprotein (MAG), which is located on the surface of myelinizing oligodendrocytes.^[15] Interactions of GSLs with receptors and enzymes, which, like themselves, are located in the same membrane have been described and are of possible physiological relevance.^[16] For example, ganglioside GM1 can activate the nerve growth factor,^[17] and ganglioside GM3 inhibits tyrosine phosphorylation of the epidermal growth factor receptor.^[18]

Many physiological processes can be affected by GSLs, for example embryogenesis, neuronal cell and leukocyte differentiation, cell adhesion, and signal transduction.^[19] Lipophilic intermediates of GSL metabolism such as sphingosine, ceramide, and their phosphorylated derivatives have been recently identified as novel signal substances.^[20, 21] Finally, complex GSLs form a layer on the extracytosolic faces of cellular membranes, which protects them from degradation and uncontrolled membrane fusion.^[22, 23] However, little is known about the precise function of individual sphingolipids in vivo. Numerous observations suggest that they are involved in biological processes, but in many cases clear evidence for their function is not available. Only minor disorders have been observed in the nervous system of genetically modified mice that cannot form complex gangliosides because of inactivation of GM2- and GD2-synthase;^[24] in contrast, severe functional disorders of the testes have been observed. Furthermore, inhibition of the biosynthesis of GSLs, which are derived from glucosylceramide, does not impair the development of fish^[25] and mice embryos.^[26] In contrast, however, the formation of glucosylceramide itself is essential for embryogenesis: genetically modified mice whose glucosylceramide synthase is missing die as early as the 19th day of embryogenesis.^[27] Galactosylceramide, sulfatides, and gangliosides are essential for myelin function (Section 3.1), and ceramides for skin function (Section 2.1). Conservation of GSL structure during evolution and the absence of inherited diseases arising from disorders in their biosynthesis also indicate that they fulfill essential functions for the living organism.

2.1. Skin Sphingolipids

Sphingolipids are essential for the function of human skin where they contribute to the formation of the water permeability barrier.^[28, 29] This barrier is localized in the horny layer of the epidermis and consists of a tightly stacked system of terminally differentiated keratinocytes (corneocytes) that are embedded in a highly organized multilamellar lipid matrix of free fatty acids, cholesterol, and ceramides. The structure of the ceramides involved here differ in a characteristic manner from the membrane anchors normally found in the GSLs of vertebrates (Figure 5 and 6). Thus, for example, unusually long fatty acid residues with up to 34 carbon atoms and sphingoid bases that are hydroxylated at various positions are found.^[30]

A characteristic sphingoid base of keratinocytes is phytosphingosine (*D-ribo*-4-hydroxysphinganine), which is otherwise

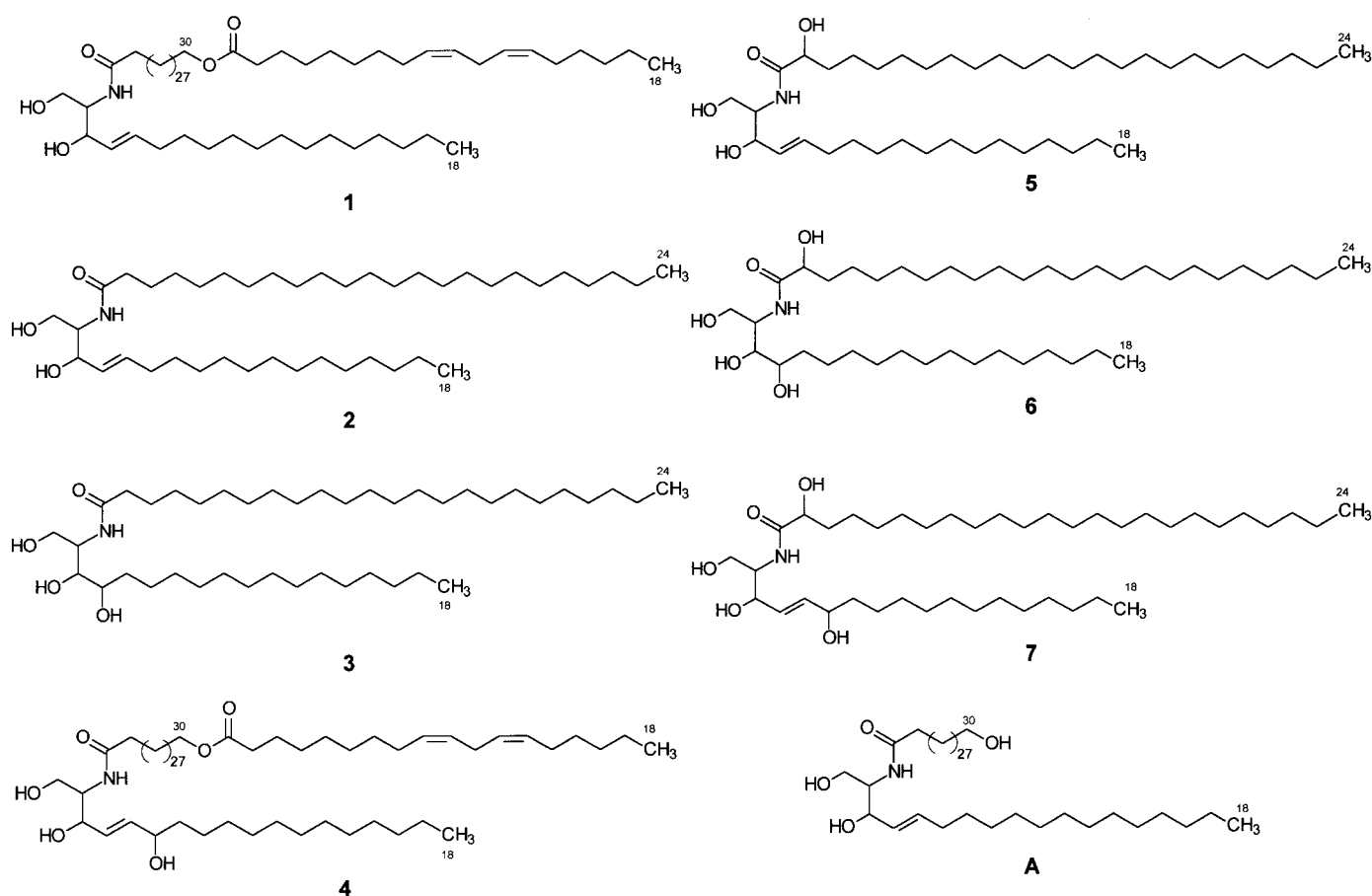


Figure 5. Sphingolipids of human skin (I): Structures of ceramides 1–4.^[30]

found as a component of the sphingolipids of yeasts and other lower eukaryotes. In humans it forms about 40% of the sphingoid bases of epidermal ceramide.^[31] The function of the additional free hydroxyl group is presumed to be to increase the rigidity of intercellular lipid aggregates through the formation of a large number of hydrogen bonds and hence reduce transepidermal water loss.^[32] Covalent bonds between the ceramides and proteins such as involucrin also contribute to the stability of skin.^[33] The biosynthesis of sphingolipids in human keratinocytes remains unclarified and is currently under investigation by us. In 1991 Elias and Menon hypothesized that the ceramides of intracellular lipid lamella are formed by extracellular degradation of complex GSLs. The lipid lamella originate in vivo from so-called Odland bodies that secrete their lipid content into the extracellular space.^[34] Odland bodies are ellipsoidal organelles, and in addition to lipid aggregates they also contain an array of lysosomal proteins that can be used for the degradation of precursor lipids.^[35]

We were able to elucidate details of this process during investigations with genetically modified mice that had no SAP precursor protein (Section 6.11) and which correspondingly exhibit a reduced capacity for degradation of glucosylceramides. A glucosyl derivative of ceramide (**1**; Figure 5) was identified in the epidermis, which is normally transferred onto the acidic amino acid side chains of proteins by transesterification followed by cleavage of the glucose residue.^[445] This is a key step for the formation of extracellular lipid aggregates

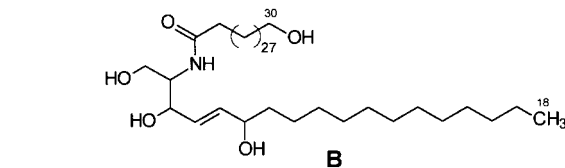


Figure 6. Sphingolipids of human skin (II): Structures of ceramides 5–7, A, and B.^[30]

that are essential for the epidermal water permeability barrier of land animals. The significance of sphingolipids for skin function is itself confirmed by an inherited disease: in rare cases of Gaucher's disease where there is a complete failure of glucocerebrosidase, the patients die of transepidermal water loss within a few hours of birth.^[36] Because of the significance of ceramides for skin function, there is interest in ceramides and ceramide replacements in the cosmetic industry.^[37] In this respect reference is made to the function of ceramide as a signal molecule (Section 5.1), which makes the use of ceramide itself appear problematical for skin care.

2.2 Glycosphingolipids and the Immune System

Glycolipids can be recognized by the immune system. Historically, the identification of the Forssman antigen as a GSL is particularly significant.^[38] Individual glycosphingolipids are blood group antigens; autoantibodies against gangliosides were known as the cause of peripheral neuropathies. For example, anti-GM1 and anti-GQ1b antibodies are regarded as

the cause of Guillain–Barre Syndrome. Anti-glycolipid antibodies can also be detected in other autoimmune diseases, for example, against sulfatide in insulin-dependent (type 1) diabetes.^[39] A number of glycolipids play a role as tumor-associated antigens and in the immunotherapy of individual cancer forms.^[40] α -Configured glycolipids have been described as potent immunostimulants.^[41] On the other hand, inhibition of sphingolipid biosynthesis by the natural product myriocin (Section 4.1) is associated with severe immunosuppression.^[42] Sphingolipids are also involved in signal transduction processes within the immune system.^[43]

3. Biosynthesis and Intracellular Topology

3.1. Sphingolipid Biosynthesis

GSL patterns that are characteristic for a cell type in a certain stage of development reside on the cell surface. Coordinated and interrelated processes are hence involved in the biosynthesis of these compounds, their degradation, and their intracellular transport.

Biological membranes consist of lipid bilayers in which the cytosolic and anticytosolic layers can be of different composition. GSLs and sphingomyelin are most frequently located on the outer half of the plasma membrane (Figure 7). Since their biosynthesis and degradation occurs in different cellular

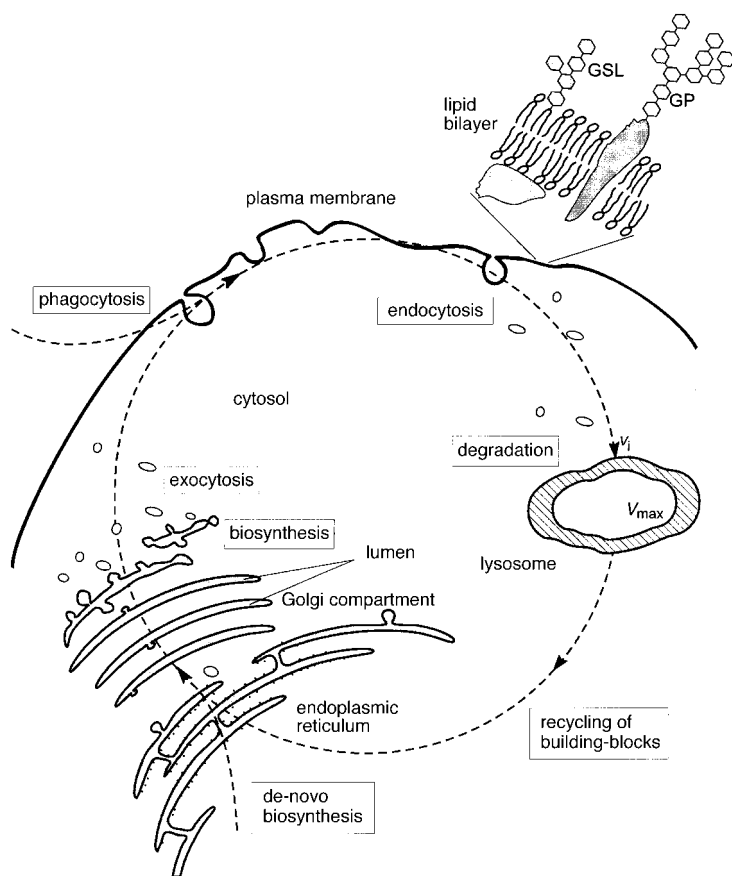


Figure 7. Intracellular metabolic flux of sphingolipids. GSL: glycosphingolipid; GP: glycoprotein; v_i = the influx rate of the substrate into the lysosome; for further information see the text.

organelles, they and their precursors are also found in intracellular membranes that are associated with the plasma membrane through membrane fusion processes. The enzymes that are involved in sphingolipid biosynthesis are membrane-bound proteins. Little is known of their structure, catalytic mechanism, biosynthesis, and regulation.

De novo biosynthesis of GSLs^[44] (Figure 8) takes place in the same compartments as glycoprotein biosynthesis. It is

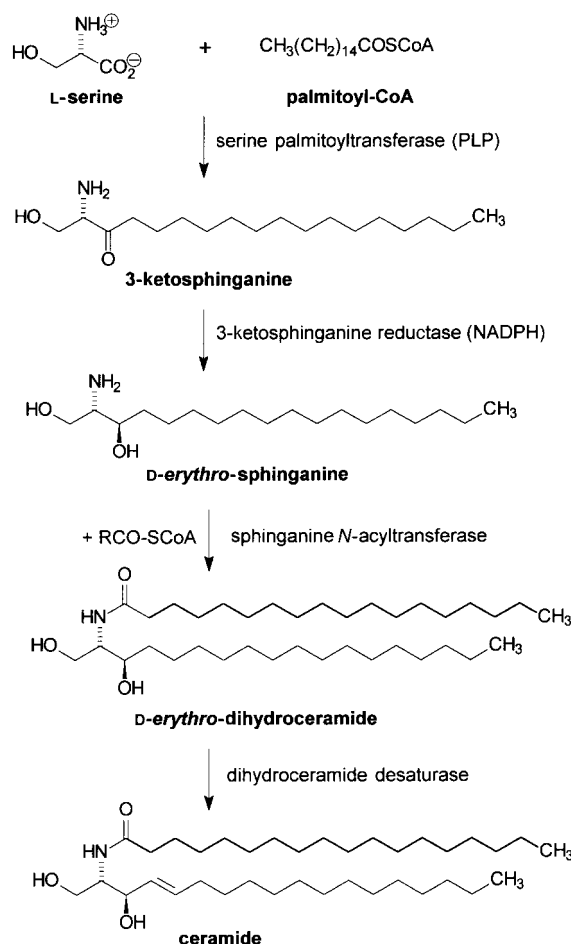


Figure 8. Ceramide biosynthesis. The formation of ceramide occurs on the cytosolic face of the membrane of the endoplasmic reticulum.^[44]

coupled to the intracellular vesicular transport of the growing lipid molecule that leads to the plasma membrane through the cisternae of the Golgi apparatus. It begins with the formation of ceramide on the membranes of the endoplasmic reticulum (ER). The condensation of the amino acid L-serine with a fatty acid activated as a coenzyme A derivative to give 3-ketosphinganine is catalyzed by serine palmitoyltransferase (SPT).^[45–47] SPT is a pyridoxal phosphate dependent enzyme and is mechanistically related to aminolevulinic synthase, which catalyzes the initial reaction of heme biosynthesis (see reference [100]). Serine palmitoyltransferase has a lower activity than the subsequent enzymes of ceramide biosynthesis and catalyzes the rate-determining step of this metabolic pathway. It preferentially uses fatty acid coenzyme A esters of a chain length of 16 C atoms so that a long-chain base with a C18 chain is formed. Serine palmitoyltransferase is the only enzyme of ceramide biosynthesis for which sequence

data are available from yeast^[48, 49] and humans.^[50] In the subsequent NADPH-dependent reaction, 3-ketosphinganine is reduced to *D-erythro*-sphinganine by 3-ketosphinganine reductase.^[46] Finally, sphinganine *N*-acyltransferase acylates sphinganine to dihydroceramide.^[51, 52] The enzyme^[53] exhibits a selectivity towards coenzyme A activated stearic acid and is also able to acylate sphingosine formed in the salvage pathway of sphingolipid degradation.^[54]

There are also reports of an enzyme activity that acetylates sphingosine after stimulation by platelet activation factor.^[55] Dihydroceramide is dehydrogenated to ceramide by dihydroceramide desaturase.^[56–58] The sequence of acylation and the introduction of the double bond was disputed for a considerable time, but it is now accepted that dihydroceramide is desaturated, not sphinganine. Sphingosine, the parent compound of the sphingolipids, is thus not an intermediate in sphingolipid biosynthesis, but is formed during sphingolipid degradation. The biosynthesis of ceramides containing phytosphingosine is unknown; however, the gene that is responsible for the biosynthesis of these compounds in yeast has been characterized.^[59]

Ceramide is the common precursor of GSLs and sphingomyelin. In the GSLs of vertebrates a glucose or galactose residue is linked β glycosidically in the 1-position of ceramide. Glycosyltransferases, which catalyze the formation of these compounds, transfer individual nucleotide-activated sugars to ceramide. The galactosylation of ceramide,^[60] which leads to the formation of glycolipids of the gala series, occurs mainly in the oligodendrocytes of the brain and in the kidney. Galactosylceramide (GalCer) and sulfatide (GalCer-3-sulfate) are present in high concentrations in the multilamellar layers of the myelin that surrounds the axons of neuronal cells.^[61] Knockout mice, in which the gene of ceramide galactosyltransferase was inactivated and which consequently cannot form any galactosylceramide or sulfatide, are still able, however, to construct myelin of essentially normal structure. In place of the absent GalCer they incorporate a GlcCer hydroxylated in the fatty acid moiety into their myelin. However, the stability and function of these myelin layers are considerably impaired.^[62, 63] The reaction of the 3'-OH group in the galactose residue of GalCer with sulfate activated as PAPS (3'-phosphoadenosine-5'-phosphosulfate) yields sulfatide.^[64]

The biosynthesis of most vertebrate GSLs does not require galactosylation, but the glucosylation of ceramide. GlcCer synthase has been isolated recently from rat liver,^[65] and the cDNA of the human protein has been cloned.^[66] It transfers a glucose residue from UDP-glucose to ceramide. The fatty acids that are found in GlcCer and GalCer differ in length and degree of hydroxylation, which can be attributed to the different cell types in which these structures are formed.^[9] Although GlcCer and GalCer synthase catalyze similar reactions, there is no sequence homology between the cDNAs that encode for these enzymes.^[67] Knockout mice, in which GlcCer synthase is absent and thus also the majority of complex GSLs, are unable to survive and die during embryogenesis.^[27] This finding demonstrates the vital functions of glycolipids in morphogenesis and embryogenesis. Lactosylceramide, the common precursor of five GSL series that are

found in vertebrates, is formed by the action of galactosyltransferase I, which transfers a galactose residue from UDP-galactose to glucosylceramide. The enzyme was recently purified from rat brain and cloned.^[68]

Ceramide is also the precursor of sphingomyelin, a structural component of the plasma membrane. It is a 1-ceramide phosphorylcholine that is located predominantly in the plasma membrane where it occurs mainly in the externally orientated layer. The sphingomyelin content of plasma membranes depends upon the cell type and can reach 25 mol %. Sphingomyelin differs functionally from glycerolipids such as phosphatidylcholine by a higher melting temperature. The reason for this lies in the higher proportion of saturated fatty acid chains and the existence of intermolecular hydrogen bonds between the 3-OH group and the amide N atom. Sphingomyelin is formed by transfer of the phosphorylcholine headgroup from phosphatidylcholine to ceramide during which diacylglycerol, an activator of protein kinase C, is released and secretory vesicles in the Golgi apparatus are formed.^[69] The mode of formation of sphingomyelin suggests a tight coupling between sphingolipid and glycerolipid metabolism. Indeed, an inverse correlation between the amounts of sphingomyelin and phosphatidylcholine is observed in many biological membranes.

3.2. Topology

Some experimental findings on the topology of sphingolipid biosynthesis are in part contradictory. The presumed most important transport pathways are described here. Thus, the first four steps of sphingolipid biosynthesis, which lead to the formation of ceramide, are catalyzed by membrane-bound enzymes at the cytosolic face of the endoplasmic reticulum (ER).^[70, 71] Since the formation of glucosylceramide occurs on the cytosolic face of the Golgi apparatus or a pre-Golgi compartment,^[72, 73] ceramide, or at least in part dihydroceramide too, must be transported from the ER to the Golgi apparatus. This occurs either by vesicle flow or by a protein-mediated transport process. In CHO cells (CHO = Chinese hamster ovary cells), a direct transport pathway appears to exist so that glucosylceramide can reach the plasma membrane from the cytosolic face of the Golgi apparatus.^[74] Introduction of the next sugar residue that leads to the formation of lactosylceramide occurs on the luminal site of the Golgi apparatus.^[75] This implicates a membrane translocation of glucosylceramide, which could be mediated by a protein, an as yet uncharacterized "flippase".^[76] The biosynthesis of higher gangliosides then occurs on the luminal face of the Golgi apparatus.^[77] Consequently, the glycan chains on the membrane-bound GSLs are orientated extracytosolically. This orientation is topologically equivalent to the situation in the plasma membrane, where the carbohydrate residues of complex glycolipids project into the extracellular space.

The bulk of sphingomyelin is formed on the luminal face of the Golgi compartment,^[78] although other sites of synthesis have been described.^[79] This requires an additional membrane translocation step at the ceramide stage. The formation of galactosylceramide with hydroxylated fatty acids in the side

chain appears to occur on the luminal face of the ER membrane, whereas non-hydroxylated GalCer species are formed on the cytosolic face of the cis-Golgi.^[80] The biosynthesis of sulfatide^[81] and digalactosylceramide^[80] takes place in the lumen of the Golgi apparatus.

3.3. Biosynthesis of Complex Gangliosides

Gangliosides are acid glycolipids of the ganglio or lacto series^[9] that contain one or more sialic acid residues. Gangliosides of the ganglio series are particularly prevalent on the surfaces of cells of the nervous system.^[82] Their biosynthesis is described briefly here. In both gangliosides and glycoproteins, sialic acids are only found in α -glycosidic linkage. On the other hand, most other sugars can be present as glycoconjugates in both the α and the β configuration. In a hypothesis we have correlated this observation with the specificity of sialyltransferases that use nucleoside monophosphate activated sugars, for example CMP-NeuAc as glycosyl donor, instead of the nucleoside diphosphate activated sugars used by other enzymes.^[83]

With the exception of GM4, gangliosides are derived structurally and biosynthetically from lactosylceramide. Further sugar residues, including sialic acid, are transferred stepwise to the growing glycan chains in the Golgi apparatus by membrane bound glycosyltransferases. Lactosylceramide and its sialylated derivatives GM3, GD3, and GT3 serve as precursors for complex gangliosides of the 0, a, b, and c series (Figure 9). Gangliosides from the c series are found only in trace amounts in human tissues. The stepwise glycosylation of these precursors is surprisingly performed by only a few nonspecific glycosyl transferases, which, as we have been able to demonstrate in enzyme kinetic experiments, transfer the respective sugar to glycosyl acceptors that differ only in the number of sialic acids bound to the inner galactose.^[84–86] Experiments *in vivo* have demonstrated that the early sialyltransferases I and II are far more specific for their glycolipid substrates than the late sialyltransferases IV and V as well as Gal-II and GalNAc transferase. This concept is supported by the observation that the cDNAs for GD3 and GT3 synthase are identical and that both GD3 and GT3 can be biosynthesized by the same enzyme in humans.^[87] The distribution of these glycosyltransferases within the cisternae of the Golgi apparatus has been investigated with the help of inhibitors of vesicular membrane flow. Monensin, a cationic ionophore, inhibits membrane flow between proximal and distal Golgi cisterna and leads to an increased biosynthetic galactose labeling of GlcCer, LacCer, GM3, GD3, and GM2, whilst the labeling of complex gangliosides is reduced. The addition of brefeldin A, which causes a fusion of the ER mainly with parts of the cis and medial Golgi, leads to a

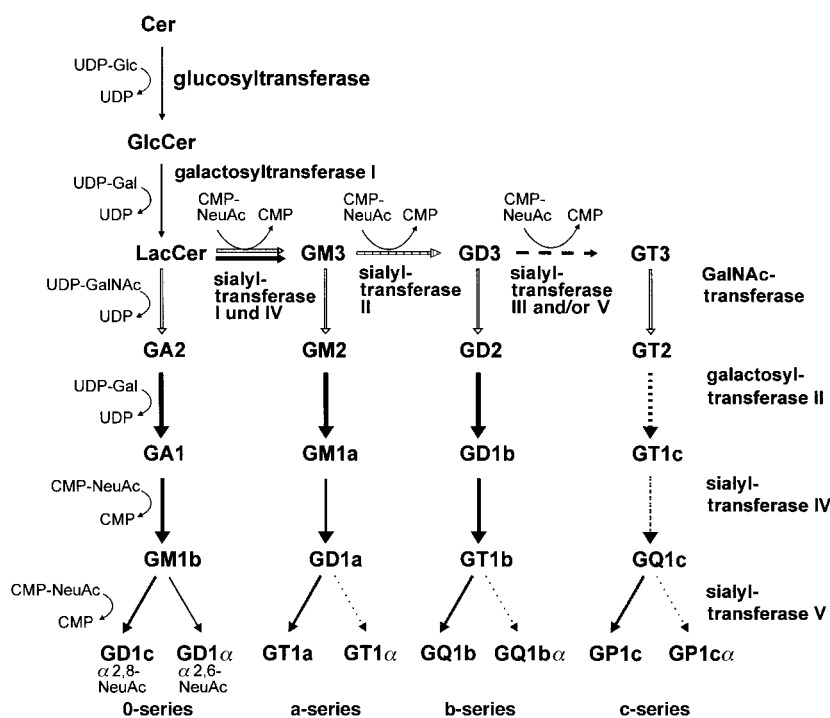


Figure 9. Biosynthetic scheme of complex gangliosides^[44] with consideration of reference [439]. The reaction steps are catalyzed by membrane-bound glycosyltransferases in the lumen of the Golgi apparatus.

reduced labeling of the gangliosides GM1a, GD1a, GD1b, GT1b, and GQ1b as well as to a lesser extent of sphingomyelin.^[88]

Although glycosyltransferase activity has been detected in many Golgi subcompartments,^[89] current data suggest that the precursor molecules GM3 and GD3 are formed in early Golgi compartments, and the complex gangliosides predominantly in the trans Golgi network.^[90] In addition to *de novo* biosynthesis, GSL can also be formed in salvage processes in which monosaccharides and especially sphingosine from GSL degradation are utilized.^[91, 92]

3.4. Regulation

Maintenance of stable GSL patterns on individual cell surfaces requires a precise control of GSL biosynthesis, degradation, and intracellular transport. Since not only regulated sphingomyelin degradation but also increased ceramide biosynthesis can be held responsible for cellular responses,^[93, 94] regulation of this metabolic pathway is of particular importance. Regulation of GSL metabolism and transport is not well understood, and there are only a few leads. Serine palmitoyltransferase appears to be the first control point in sphingolipid formation. The enzyme activity correlates with the relative amounts of sphingolipids that are found in different tissues.^[95] Since sphingosine reduces SPT activity in cultured neuronal cells, autoregulation of the enzyme appears to be possible.^[121] In addition, removal of skin lipids increases SPT activity.^[96]

During ontogenesis and cell transformation a correlation between GSL expression and the activity of glycosyl trans-

ferases that leads to their synthesis has been observed. Therefore, control of glycosyltransferases, possibly at the transcription level, appears to be a significant regulation point (review: reference [44]). Since most glycosyltransferases have been cloned in the recent past,^[97, 98] it is expected that information for an understanding of the control at the transcription level will be available in the near future. In addition to regulation at a genomic level, some observations also suggest epigenetic regulation mechanisms. Feedback control of several glycosyltransferases either by their respective reaction product or by an end product of the respective GSL series has been observed at least *in vitro*.^[44] The phosphorylation status of glycosyl transferases and the pH value of their environment^[99] can also affect their activity.

4. Inhibitors of Sphingolipid Biosynthesis

Low molecular weight inhibitors of sphingolipid biosynthesis^[100] (Figure 10) have been isolated from natural sources

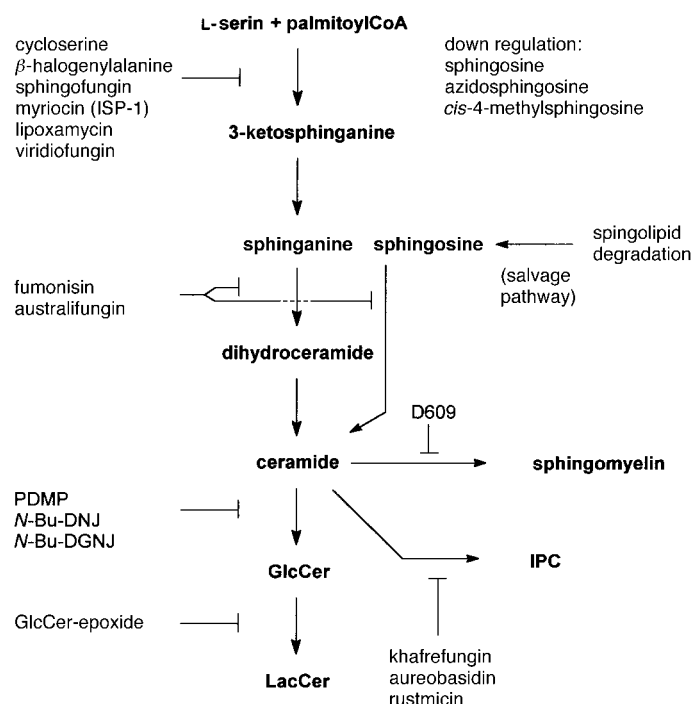


Figure 10. Flow scheme and inhibitors of sphingolipid biosynthesis (modified from reference [100]). IPC = inositolphosphorylceramide; *N*-Bu-DNJ = *N*-butyldeoxyonojirimycin; *N*-Bu-DGNJ = *N*-butyldeoxygalactonojirimycin; PDMP = 1-phenyl-2-decanoylamino-3-morpholinopropan-1-ol.

or synthesized chemically. Most act at an early stage of biosynthesis and have a lipid-like structure. These inhibitors have a greater membrane permeability than those with a carbohydrate basis. Together with inhibitors of sphingolipid degradation (Section 5.2), they are valuable tools in the investigation of this metabolic pathway. For a number of reasons there is interest and a requirement for low molecular weight inhibitors that specifically inhibit a certain step in sphingolipid biosynthesis.^[100] A possible application is the analysis of GSL function by switch-off experiments.^[101] An

interruption of sphingolipid biosynthesis at a certain stage can be achieved either with inhibitors, by the production of mutant cells, or with animals in which certain biosynthesis enzymes are absent.^[102] The effect of this method is twofold: the cell or organism is depleted of metabolites downstream of the inhibited or mutated enzyme. Additionally, metabolic intermediates upstream of the blocked stage accumulate, which allows investigations of their biological function. Compounds such as myriocin or 1-phenyl-2-decanoylamino-3-morpholinylpropan-1-ol (PDMP) were also of interest as tools in the investigation of the cell cycle.^[103]

Membrane permeating inhibitors of sphingolipid biosynthesis are interesting as therapeutic agents.^[100] Myriocin, an inhibitor of serine palmitoyltransferase, is currently one of the most potent immunosuppressants.^[42] Since carbohydrate structures of glycolipids form binding sites for bacteria, viruses, and toxins (Section 2), it is in principle possible to prevent diseases caused by these pathogens by inhibition of their synthesis. An alternative is inhibition of the biosynthesis of sialic acid, a frequent component of their recognition sites. Metastatic spread of cancer could also be favorably influenced in this way.^[104] In addition to the inhibition of sphingolipid biosynthesis of the host, there is also the possibility of inhibiting that of parasites in so far as they require sphingolipids for survival or reproduction. This indication was proposed as a new principle for the treatment of fungal infections^[100, 105] and malaria.^[106, 107] The inhibition of biosynthesis is also of particular significance for the treatment of sphingolipidoses (Section 6.14.3), as postulated^[108] by N. S. Radin and demonstrated experimentally by F. Platt^[431] (Section 6.14.3). With the help of suitable inhibitors, the function of lateral aggregates of sphingolipids in cellular membranes could also be analyzed. They lead to the formation microdomains,^[109] which are held responsible inter alia for the transport of GPI-anchored proteins (GPI = glycosylphosphatidylinositol).^[110]

4.1. Inhibitors of Serine Palmitoyltransferase

SPT is inhibited by suicide inhibitors of PLP-dependent enzymes (Figure 11) that are directed against the serine binding site. These include L-cycloserine,^[111, 112] as well as β -chloro- and β -fluoroalanine.^[113] Particularly high SPT activity has been found in human keratinocytes that can be inhibited by L-cycloserine and β -chloroalanine with IC_{50} values of 3.0 μ M and 25 μ M.^[114]

Compounds with a structural similarity to sphingolipids, the sphingofungins, have been isolated from a culture of *Aspergillus fumigatus* as compounds with fungicidal but not antibacterial activity. Sphingofungin B reduced the incorporation of [^{3} H]inositol into the sphingolipids of yeast (IC_{50} = 8 nM).^[115] This leads to growth inhibition and cell death. The action of sphingofungins can be abolished by the addition of phytosphingosine, but not by sphingosine. This is not unexpected, since the sphingolipids of yeast contain phytosphingosine in place of sphingosine as the most common long chain base. Sphingofungins inhibit SPT competitively with respect

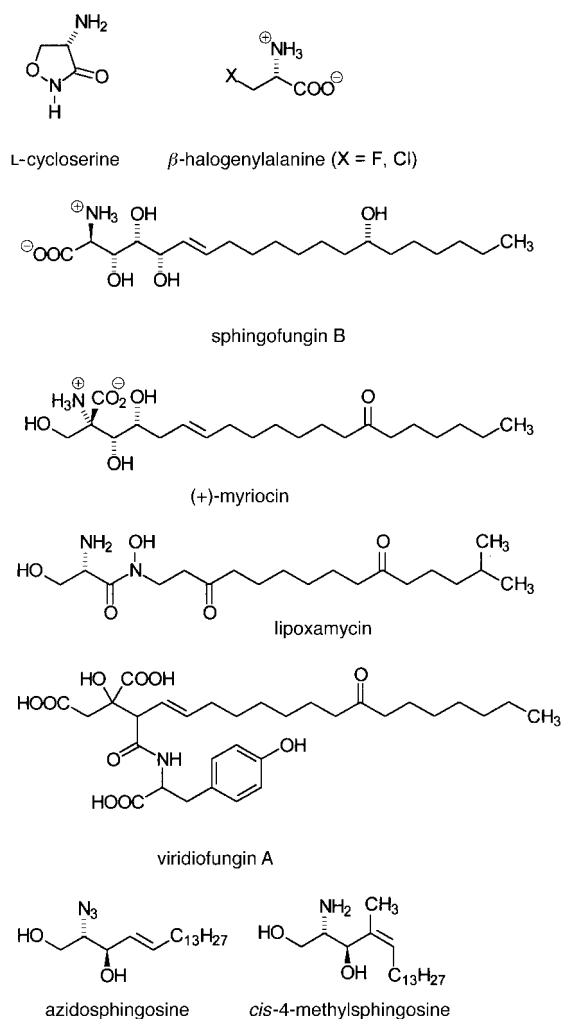


Figure 11. Inhibitors of serine palmitoyltransferase (SPT).

to serine.^[115] Sphingofungins E and F have been isolated from *Paecilomyces variotii*.^[116]

A further structural analogue of the sphingoid backbone is myriocin. It inhibits the biosynthesis of ceramide and the two most common GSLs in yeast, inositolphosphorylceramide (IPC) and mannosylIPC (MIPC).^[117] At the same time it reduces the transport rate of GPI-anchored proteins to the Golgi apparatus and the remodeling reaction of the GPI anchor to ceramide-containing structures. The SPT inhibitor myriocin is an extremely potent immunosuppressant; it has been shown to be identical to the antibiotic ISP-1 and thermozymocidin. Unlike the frequently used immunosuppressants cyclosporin and FK-506, ISP-1 does not interfere with the formation of interleukin-2 but suppresses the IL-2 dependent growth of a cytotoxic T-lymphocyte cell line of the mouse, CTLL-2. The SPT of these cells is inhibited non-competitively in vitro with an apparent inhibition constant of 0.28 nM.^[42] The inhibition of SPT is accompanied by a suppression of T cell growth, which can be abolished again by the addition of C2 ceramide, sphinganine, or sphingosine-1-phosphate, but not, however, by sphingomyelin, GlcCer, GalCer, or GM3. It is assumed that myriocin induces apoptosis in the CTLL-2 cells.^[118]

A secondary metabolite from actinomycetes, lipoxamycin, has also been described as a potent inhibitor of SPT ($IC_{50} = 21$ nM).^[119] Noteworthy are its high toxicity towards mice and its fungicidal activity, which can be abolished by the administration of sphinganine and phytosphingosine. In addition, the antifungal agent viridifungin, previously recognized as an inhibitor of squalene synthase in vitro, has also been proved to be an inhibitor of SPT.^[120]

Other agents reduce SPT activity in cultured cells without directly inhibiting the enzyme. These include D-erythro-sphingosine and the metabolically stable D-erythro-azido-sphingosine, which reduces SPT activity in primary cultured neuronal cells either with the natural *trans* or the unnatural *cis* double bond.^[121] The synthetic 4-methyl derivative of *cis*-sphingosine down regulates SPT activity ($IC_{50} = 10$ μ M), increases intracellular calcium ion concentration, and behaves as a potent mitogen in Swiss 3T3 fibroblasts. Moreover, the compound causes drastic morphological changes in cultured granule cells of the cerebellum and induces neuronal death. Both the *cis* configured double bond and the 4-methyl group are necessary for the action of the compound, since the corresponding *trans* and 5-methyl derivatives are inactive. All data suggest that a metabolite of *cis*-4-methylsphingosine is the actual active compound, namely its 1-phosphate. Sphingosine-1-phosphate is rapidly degraded by a lyase (Section 5.2); *cis*-4-methylsphingosine appears to be a prodrug for a metabolically stable analogue of sphingosine-1-phosphate.^[122]

4.2. Inhibitors of Sphinganine N-acyltransferase

Fusarium moniliforme is a fungus that frequently infects corn and cereals. Consumption of such contaminated agricultural products leads to various, often neurological, diseases in animals and has been implicated in the occurrence of esophageal cancer in humans.^[123–125] Fumonisin, mycotoxins from *F. moniliforme*, have been isolated as the active principle (Figure 12). Fumonisin B1 and B2 (which lack the 10-hydroxyl group of FB1) have been identified as inhibitors of sphinganine N-acyltransferase with IC_{50} values of about 0.1 μ M.^[126, 127] Fumonsinins are structural analogues of the sphingoid bases, which lack the 1-hydroxyl group. This contributes to the metabolic stability of these mycotoxins, since long chain bases can be cleaved after phosphorylation of the 1-position. The tricarballoyl residue and the 5-OH group are important but not decisive for inhibition. Fumonisin B3, which lacks the 5-OH group, is still active, but only in concentrations above 1 μ M. A similar value also applies to fumonisin B1H, which is obtained by cleavage of the tricarballoyl group by treatment with mild base. Different fumonisin analogues have been synthesized and shown to be substrates and inhibitors of the acyltransferase (IC_{50} about 10 μ M). The N-palmitoyl derivative of such an aminopentol inhibits acyltransferase ($IC_{50} = 9$ μ M) and is cytotoxic. Unlike other 1-deoxysphinganine derivatives, fumonisin B1 is not acylated by acyltransferase in vitro.^[128] Inhibition of ceramide formation by fumonisins is accompanied by an accumulation of the biosynthetic precursor sphinganine. Sphinganine may be responsible for some of the effects caused by the

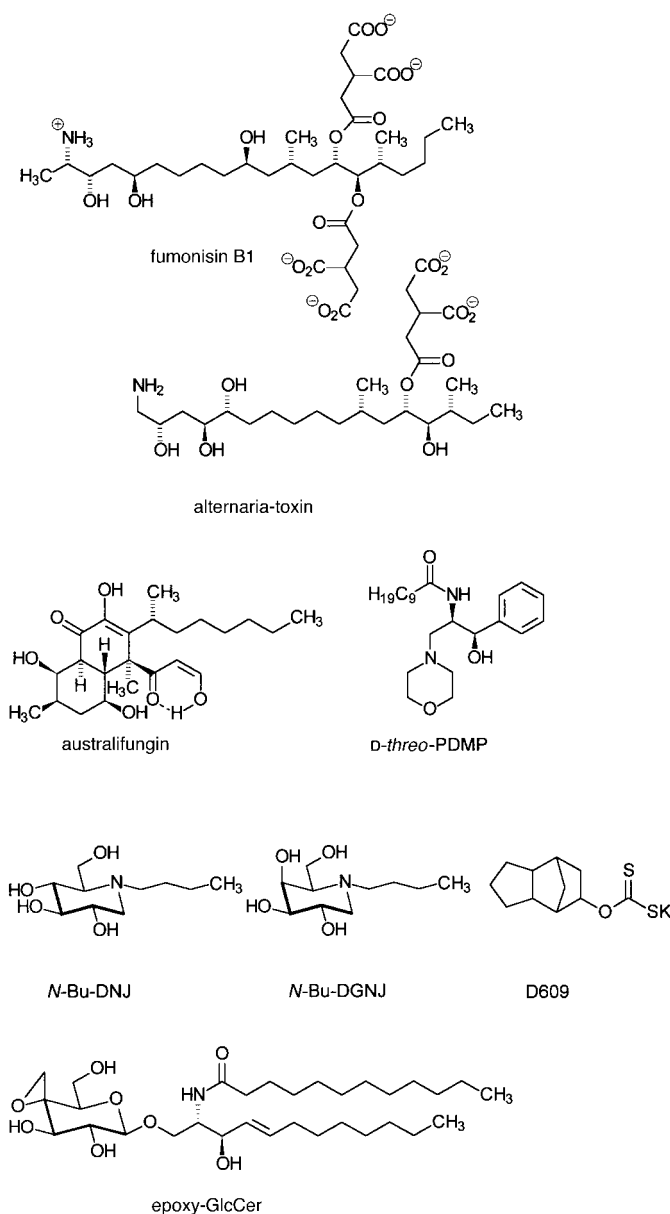


Figure 12. Further inhibitors of GSL biosynthesis. The absolute configuration of the tricarballoyl residue in fumonisin B1 and alternaria toxin is not shown.

fumonisin, since it is toxic and mitogenic even in low concentrations. The ratio of sphinganine to sphingosine in the serum of animals is effective as a diagnostic indicator for the consumption of fumonisin-contaminated feed.

Alternaria toxin is a phytotoxin with a structure similar to the sphingoid backbone. It inhibits sphingolipid biosynthesis at the level of dihydroceramide formation ($IC_{50} = 1 \mu M$), but as a result of its lower activity relative to fumonisin and its increased cytotoxicity it is of limited use for mammalian cell culture experiments.^[129]

The fungal metabolite australifungin has been described as an inhibitor of sphinganine *N*-acyltransferase in vitro. Its IC_{50} is still less or equal to that of fumonisin B1, according to the cell type used as enzyme source.^[130] Australifungin is a potent fungicide but shows no structural similarity to sphingoid bases.

4.3. Inhibitors of Glycosyltransferases

The introduction of carbohydrate structures into GSLs follows the formation of the membrane anchor. Unlike for most other glycosyltransferases,^[131] the early glycosylation steps on ceramide belong to the few cases for which inhibitors are available. Inhibition of ceramide biosynthesis itself is unsuitable for the analysis of GSL functions since it has several consequences: a depletion of GSLs in the plasma membrane, a reduction in sphingomyelin biosynthesis, and an increase in the concentration of the metabolites upstream of the inhibited step. Potent and specific inhibitors of GlcCer biosynthesis are suitable for functional analysis. Two classes of compounds have currently been described as inhibitors of GlcCer biosynthesis.^[132] *D*-threo-PDMP (*D*-threo-(1*R*,2*R*)-1-phenyl-2-decanoylamino-3-morpholino-1-propanol, Figure 12) is the most thoroughly studied representative of a series of GlcCer synthase inhibitors with a ceramide-like structure. *D*-threo-PDMP inhibits the formation of glycosylceramide in concentrations of 2.5 to $10 \mu M$ and it has already been used in functional studies.^[133]

D-threo-PDMP exhibits a mixed inhibitor profile relative to ceramide and is noncompetitive for the glycosyl donor. The apparent K_i value is $0.7 \mu M$. At concentrations above $25 \mu M$ the biosynthesis of sphingomyelin and the transport of secretory proteins are also inhibited.^[134] Stereoisomers and analogues of PDMP have been synthesized and investigated, including *D*-threo-1-morpholino-1-deoxyceramide (73 % inhibition of GlcCer synthase in Madin–Darby canine kidney (MDCK) cells at a concentration of $5 \mu M$ compared to 20 % inhibition by *D*-threo-PDMP).^[135] Concentrations of *D*-threo-PDMP above $100 \mu M$ or of its palmitoyl derivative PPMP above $10 \mu M$ are toxic to HL-60 cells. *D*-threo-PDMP exhibits a plethora of cellular effects, including inhibition of cell growth, which is possibly caused by ceramide accumulation and/or inhibition of sphingomyelin biosynthesis. The interruption of the cell cycle in the G1/S and G2/M phases by PDMP also appears to be attributable to cellular ceramide accumulation.^[136] Various other effects of PDMP, its stereoisomers, and analogues have been reviewed.^[133] It is metabolized by cytochrom P450.^[137] A further effect of PDMP, which is not attributable to modulation of sphingolipid metabolism, concerns membrane transport from the Golgi apparatus to the ER induced by brefeldin A, which is blocked by PDMP.^[138]

A synthetic derivative of the naturally occurring glycosidase inhibitor deoxynojirimycin, *N*-butyldeoxynojirimycin (*N*-Bu-DNJ), inhibits GlcCer formation with an IC_{50} value of $20 \mu M$. *N*-Bu-DNJ is known to be an inhibitor of HIV replication in vitro, and clearly acts as an inhibitor of viral glycoprotein processing. Butyldeoxygalactonojirimycin (*N*-Bu-DGNJ) is a related compound with improved selectivity ($IC_{50} = 40 \mu M$).^[139] Glycosidases such as β -gluco- and β -galactocerebrosidases, and α -glucosidases I and II are not, or only poorly, inhibited by the compound. Structure–activity relationships show that an alkyl chain length of three C atoms is necessary for inhibition of GlcCer synthase, and alkyl chain lengths of C4 and C6 are optimal. Longer chain compounds do indeed show a further improvement in inhibition in vitro, but have cytotoxic properties in vivo. The corresponding

derivatives of mannose, fucose, and *N*-acetylglucosamine are inactive. A great advantage of these compounds is their low toxicity and high metabolic stability. An application of *N*-butyldeoxynojirimycin in the treatment of sphingolipid storage disease will be discussed in relation to newer therapeutic uses in Section 6.14.3.

4.4. Inhibition of LacCer Synthesis

A synthetic GlcCer derivative with an additional epoxide function in the 4-position of the glucose residue causes an irreversible and concentration-dependent reduction in LacCer synthase activity in cultured cells.^[140] The biosynthetic labeling of GSLs downstream from GlcCer is reduced in primary cultured neuronal cells from chick embryos, whereas the labeling in GlcCer accumulates. The derivative with gluco configuration is active, whereas the compound with the galacto configuration shows no effect. The inhibition of LacCer formation by the epoxy compound in vitro is less pronounced: a concentration of 250 μM is necessary to achieve a 30% inhibition in enzyme activity. Therefore, it cannot be excluded that the effects observed in vivo may be attributed to the inhibition of a GlcCer translocator or transcription factor.

4.5. Inhibitors of Inositolphosphorylceramide (IPC) Synthase

Whereas in vertebrates a majority of sphingolipids are present in the form of GSL, in fungi compounds the binding of an inositol residue to ceramide by diphosphate diesters predominate. Inhibition of the biosynthesis of these compounds leads to a depletion in the organisms of structures that clearly fulfill essential functions for their survival.^[100, 145] It is possible that in fungi sphingolipids represent the only storage form of very long chain fatty acids that are necessary for the survival of these organisms.^[141] Ceramides accumulate through inhibition of IPC synthase, which at least in yeast leads to arrest of the cell cycle in the G1 phase.^[142] Thus, for example the fungal component khafrefungin (Figure 13) inhibits the biosynthesis of IPC in *Saccharomyces cerevisiae* and pathogenic fungi in picomolar and nanomolar concentrations and causes ceramide accumulation.^[143] In vitro IPC synthase is inhibited with an IC_{50} value of 0.6 nM, and the incorporation of inositol into the sphingolipids with an IC_{50} value of 150 nM. Unlike other inhibitors that inhibit the corresponding enzyme in fungi and mammals to the same extent, sphingolipid biosynthesis in mammals is not impaired by khafrefungin.

The antifungal agent aureobasidin A also inhibits the biosynthesis of IPC; its IC_{50} value is 0.2 nM.^[105] Aureobasidin in micromolar concentrations also inhibits the transferase, which transfers phosphorylinositol to mannosylinositol-phosphorylceramide.^[144] Another, more recently described inhibitor of IPC synthase is the macrolide rustimicin,^[145] also known as galbonolide A, which is also a potent fungicide. It inhibits the enzyme with an IC_{50} value of 70 pM. The suitability of the compound for the treatment of systemic

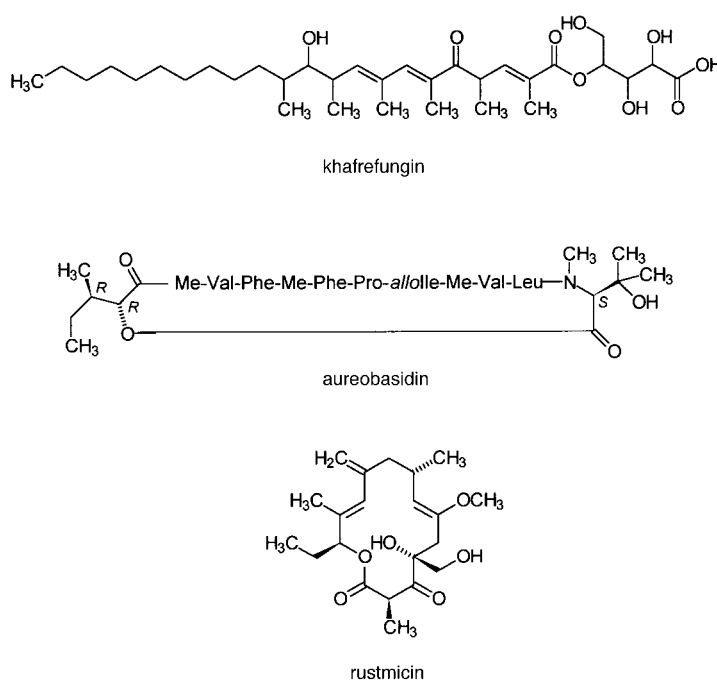


Figure 13. Inhibitors of inositolphosphorylceramide formation.

fungus infections is limited by its metabolic and chemical instability as well as by resistance phenomena.^[145]

4.6. Miscellaneous

D,L- α -fluoropalmitic acid also inhibits the biosynthesis of sphingolipids.^[146] A recent study has shown^[147] that azidothymidine (AZT), which is used as a chemotherapeutic agent in the treatment of HIV infections, inhibits the glycosylation of glycolipids and glycoproteins in clinically relevant concentrations of 1–5 μM . The primary cellular metabolite of AZT, the monophosphate, possibly inhibits the uptake of nucleotide sugars into the Golgi apparatus and in this way reduces the amount of complex acid GSLs. The toxic side effects of AZT, particularly in the maturation of blood cells, appear to be a consequence of changed glycosylation patterns and are not based on an inhibition of DNA replication.

Antisense oligodeoxynucleotides for GM2 synthase and GD3 synthase lead to a reduction of complex GSLs downstream from GM3 within the biosynthetic pathway. The treatment of human leukemia cell line HL-60 with antisense DNA results in their differentiation into monocytes and the accumulation of GM3.^[148] Since suitable low molecular weight inhibitors of glycosyltransferase are currently unavailable, this approach represents a highly promising tool for the clarification of GSL function.

Sphingomyelin synthase is inhibited in vitro by a xanthogenate, D609 (Figure 12).^[149] The compound has been known for some time as an inhibitor of a phosphatidylcholine-specific phospholipase C activity.^[150] Concentrations in the range 40–400 μM also inhibit sphingomyelin formation in transformed fibroblasts.^[149]

5. Sphingolipid Catabolism

5.1. Nonlysosomal Degradation: Sphingolipids and Signal Transduction

Constitutive sphingolipid degradation takes place in the lysosomes of the cell. An additional, highly regulated degradation pathway starting from sphingomyelin also exists, during the progression of which signal molecules are released (Figure 14).^[8] Sphingomyelin can be cleaved by sphingomyelinases^[151, 152] and an acid sphingomyelinase (Section 6.6) and

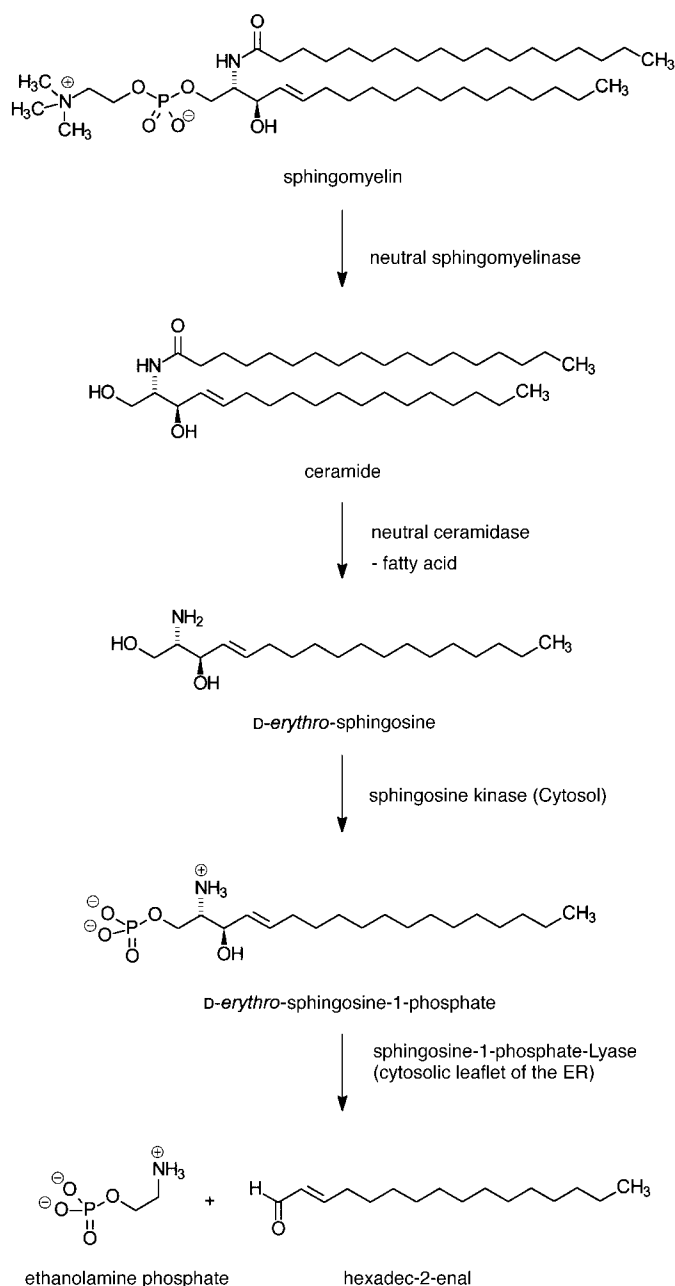


Figure 14. Nonlysosomal sphingolipid degradation.

a neutral sphingomyelinase have been cloned.^[153] Ceramide is formed during the sphingomyelinase-catalyzed reaction and can be cleaved into sphingosine and a long-chain fatty acid by

ceramidases of various subcellular localization (Section 6.10).^[154] Phosphorylation to ceramide-1-phosphate is also a possibility.^[155] Sphingosine itself can be phosphorylated at the 1-OH function by a cytosolic sphingosine kinase^[156, 157] to form sphingosine-1-phosphate, which, like the 1-phosphates of *D-erythro*-sphinganine and *D-ribo*-phytosphingosine, is cleaved in a pyridoxalphosphate-dependent reaction by the sphingosine-1-phosphate lyase situated on the cytosolic face of the ER membrane.^[158] The mechanism of the cleavage corresponds formally to a retroaldol addition. The gene of the lyase has been characterized from yeast and mouse amongst other sources.^[159, 160] In addition to the degradation of sphingoid-1-phosphates, the phosphatase reaction back to sphingosine has also been characterized.^[161, 162] A phosphatidic acid phosphorylase catalyzes the hydrolysis of sphingosine-1-phosphate, ceramide-1-phosphate, and lysophosphatidic acid.^[163]

A few findings suggest that ceramide plays a role comparable to that of the structurally similar and functionally complementary diacylglycerol (DAG). Together with inositol-1,4,5-trisphosphate, DAG is released from phosphatidylinositol-4,5-bisphosphate as a signal molecule by phospholipase C in response to an extracellular signal.^[164] The observation that sphingomyelin hydrolysis can also be induced by extracellular agents in various cell types such as lymphocytes, myelocytes, or fibroblasts has led to the discovery of the so-called sphingomyelin cycle (Figure 15).^[165, 166] The first indication of this came from the observation that vitamin D₃ induces the cleavage of sphingomyelin in HL-60 cells.^[167] Tumor necrosis factor α , γ -interferon, or interleukin-1, which act on receptors within the plasma membrane, also lead to the release of ceramide. The cellular and molecular effects of these extracellular agents, inhibition of cell growth, induction of differentiation, modulation of protein phosphorylation, and regulation of gene transcription, can be mimicked by the addition of an exogenic membrane-permeable ceramide derivative. The actions of this C₂-ceramide (*N*-acetylsphingosine) are not observed with the corresponding saturated compound, C₂-dihydroceramide.^[168] This result suggests a specific interaction between ceramide and a binding protein. The identity of the ceramide receptors and other molecules downstream within the signal flow is not unambiguously known. A ceramide-dependent kinase,^[169] a phosphatase,^[170] and a protein kinase C subtype^[171] are currently being investigated. Protein kinase c-Raf has been identified by photoaffinity labeling as a ceramide binding protein within this signaling pathway (see Section 5.1.1).

In general, ceramide mediates antimitogenic effects such as cell differentiation, cell-cycle arrest, and cell senescence. The most spectacular of the various effects that ceramide can initiate is programmed cell death.^[172] Unlike necrosis, programmed cell death (or apoptosis) is a well-defined process whose biochemical principles have only now been partially elucidated.^[173–175] It is controlled by receptor-mediated mechanisms, which themselves activate intracellular signal cascades and thus influence the phosphorylation status of target proteins and finally gene expression. In the strictest sense ceramide is not a second messenger whose action is terminated within a short time. Instead, it mediates a long lasting response to stress.^[20] In yeast too, sphingolipids appear

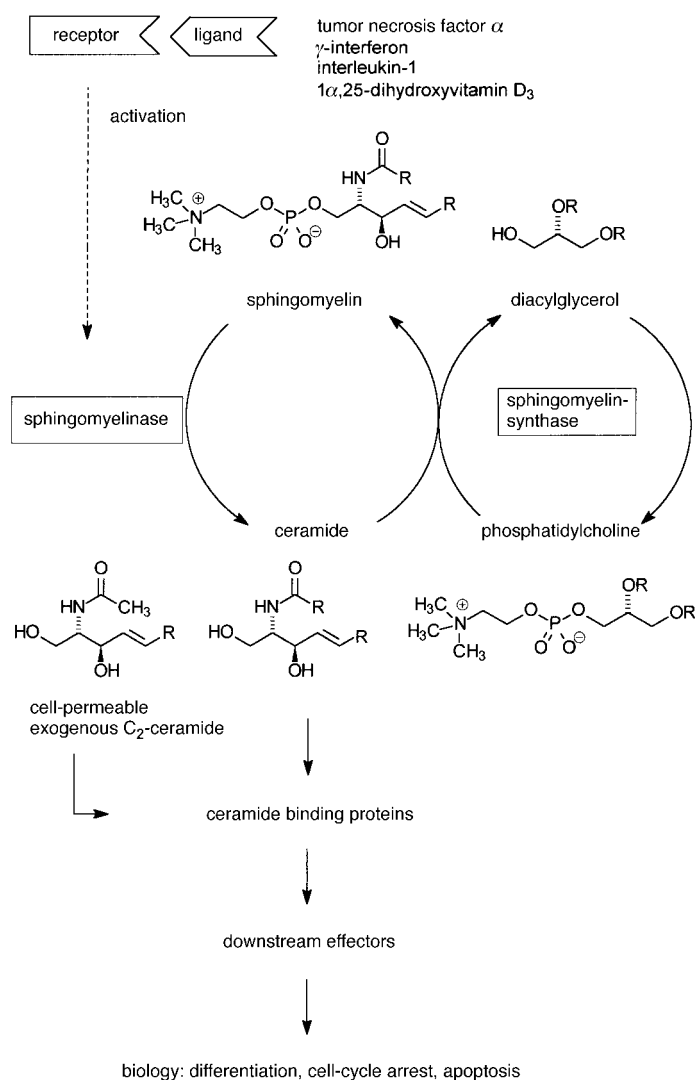


Figure 15. Sphingomyelin cycle (modified according to reference [165]). R = alkyl group.

to act as stress signals.^[176] The initiation of apoptosis by ceramide can be inhibited by the overexpression of the apoptosis inhibitor Bcl-2.^[177] The release of ceramide itself can also be suppressed by Bcl-2.^[178] Surprisingly, the presence of ganglioside GD3 appears equally necessary for the initiation of apoptosis by ceramide in hematogenic cells^[179] as the presence of acid sphingomyelinase.^[180] The evaluation of many published experiments remains difficult to interpret, however, since intracellular ceramide concentrations are usually determined by the diacylglycerolkinase reaction. However, this test is not specific enough. It showed, for example, an increase in ceramide concentration in experiments on FAS-induced apoptosis of T cells, although an independent mass spectrometric method detected no change.^[181]

The induction of apoptosis in U937 cells by ceramide is stereospecific: the D- and L-*threo* stereoisomers have proved to be more potent than the *erythro* compounds. In addition, experiments with N-octylsphingosine have shown that the amide carbonyl group is not necessary for the induction of apoptosis.^[182] The N-acetylsphingosine (C_2 -ceramide) fre-

quently used in experiments of this type is subject to metabolism;^[183] it can be acylated on the 1-OH group by an enzyme with transacylase or phospholipase A2 activity.^[184, 185] Ceramide can also be sulfated at the 1-position in marine organisms. This metabolic product inhibits human DNA topoisomerase I.^[186]

In addition to the degradation pathway discussed previously, ceramide can be converted into bioactive metabolic products, in particular sphingosine and sphingosine-1-phosphate.^[187] Unlike ceramide, sphingosine-1-phosphate mediates mitogenic effects.^[188] It induces proliferation of quiescent Swiss 3T3 cells and stimulates the release of calcium ions from intracellular sources. Since ceramide, sphingosine, and sphingosine-1-phosphate are metabolically coupled, it is not clear in every case which of these molecules is responsible for a certain effect and why this pathway is mitogenic in some cells and antiproliferative in others. Unlike the unknown identity of the ceramide receptors, a binding protein ($K_D = 8.1 \text{ nM}$) for extracellularly acting sphingosine-1-phosphate which mediates a part of its activity has been characterized on the cell surface.^[189, 190] It is the Edg-1 receptor, which is coupled with an inhibitory G protein. Its expression in COS cells leads to inhibition of adenylatecyclase and to activation of mitogen-activated protein kinase (MAPK), but not to the release of calcium ions. Sphingosine and lysosphingolipids, which lack the amide-bound fatty acid, are known to be inhibitors of protein kinase C.^[191] Sphingosine itself also appears to act apoptotically in some cells independent of ceramide.^[192] In the case of sphingosine and ceramide, the mechanisms that lead to apoptosis are clearly different, although dependent upon the cell type they can also act cooperatively.^[192]

5.1.1. Ligands of Sphingolipid Binding Protein

There is a requirement for metabolically stable ligands—agonists and antagonists—of sphingolipid binding proteins, for example of sphingosine-1-phosphate.^[193] With the help of a ceramide analogue photoaffinity ligand (Figure 16) it has been possible for the first time to identify protein kinase c-Raf as a ceramide binding protein that is involved in the signal cascade of interleukin-1 binding for the activation of the mitogen-activated protein kinase (MAPK).^[194] The photoaffinity ligand was radiolabeled with a specific radioactivity of $>2000 \text{ Ci mmol}^{-1}$.^[195] We have synthesized a 1-deoxy-1-ethyl-dihydroceramide as the first metabolically stable dihydroceramide with proapoptotic properties (Figure 16). The compound inhibits the proliferation of a human leukemic cell line with a IC_{50} value of $20 \mu\text{M}$. It is nontoxic over an extended period and it is able to induce apoptosis in this cell line.^[196]

5.2. Inhibition of Sphingolipid Degradation

The elucidation of GSL function on the cell surface is not the only motivation to influence sphingolipid metabolism. In addition to GSLs themselves,^[197–199] lipophilic intermediates of GSL degradation are involved in processes of signal transduction when extracellular signals are transmitted to intra-

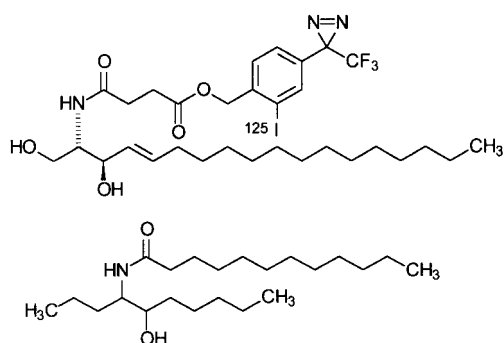


Figure 16. Ligands of ceramide binding protein ligands.

cellular regulatory systems (Section 5.1). A notable inhibitor of GSL degradation is conduritol B epoxide, which inhibits glucocerebrosidase (see Section 6.8).^[200] Although it has been possible for a long time to investigate the effects of a dysfunctional sphingolipid degradation in cells of sphingolipidosis patients or genetically modified animals, a covalent inhibitor represents a valuable, independent tool for the analysis of the active site of the enzyme.

5.2.1. Inhibition of Sphingomyelinases

Inhibitors of the sphingomyelinases involved in signal transduction are interesting, for example, as potential therapeutic agents in the treatment of inflammatory and autoimmune diseases. Since the identity of the enzyme is unknown, acid and neutral sphingomyelinases are targets for inhibitory studies. The activity of acid sphingomyelinase is necessary, for example for the infection of nonphagocytizing cells by *Neisseria gonorrhoea*.^[201] A few low molecular weight inhibitors of acid sphingomyelinase are known,^[202] including phosphatidylinositol-4',5'-bisphosphate, adenosine-3',5'-bisphosphate, and adenine-9- β -D-arabinofuranoside-5'-monophosphate ($IC_{50} = 1-5 \mu M$). The calcium channel antagonist SR33557 (Figure 17) has also been described as an inhibitor of acid sphingomyelinase activity (72 % inhibition at $30 \mu M$ in a leukemia cell line of the mouse, P388/ADR).^[203] Different sphingomyelin derivatives (3-deoxy-, 3-O-methyl- ($IC_{50} = 50 \mu M$), 3-O-ethyl-, 3-O-tetrahydropyranyl-, and *N*-demethylsphingomyelin) have been synthesized and investigated. They have been shown to be less active inhibitors of neutral and acidic sphingomyelinase.^[204] The synthesis of a phosphonate analogue of sphingomyelin as an affinity ligand for the purification of sphingomyelinases has been described.^[205] Ganglioside GM3 inhibits neutral sphingomyelinase with an IC_{50} value of $45 \mu M$.^[206] Glutathione is also an inhibitor of neutral sphingomyelinase (95 % inhibition at a concentration of $5 mM$).^[207] Scyphostatin, a constituent of *Dasyscyphus mollissimus*, has been described recently as an inhibitor of neutral ($IC_{50} = 1 \mu M$) and acid sphingomyelinase ($IC_{50} = 49 \mu M$).^[208] An interesting lead structure is thus available for optimization.

5.2.2. Ceramidase Inhibitors

Since the signal molecules ceramide and sphingosine/sphingosine-1-phosphate are metabolically coupled by differ-

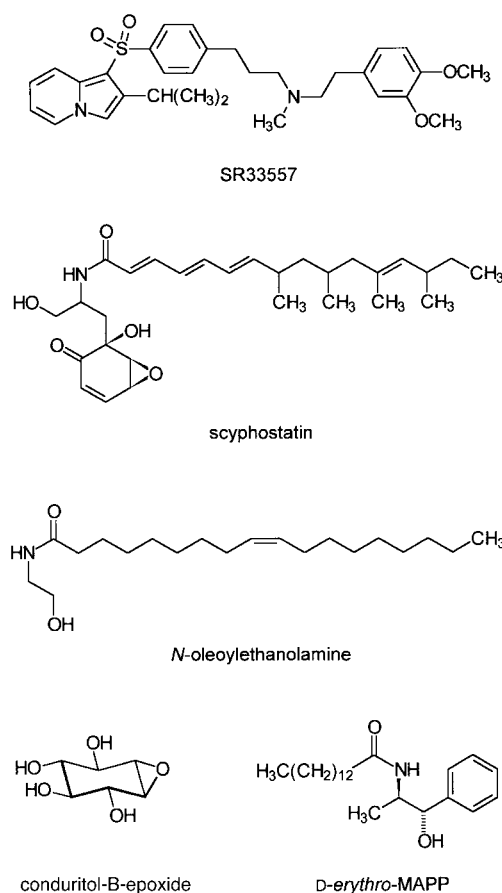


Figure 17. Inhibitors of sphingolipid degradation.

ent enzymes, among them different ceramidases, ceramidase inhibitors are of interest as tools in the investigation of signal transduction mechanisms. *N*-oleylethanolamine has been described as a ceramidase inhibitor,^[209] and (1*S*,2*R*)-*D*-erythro-2-(*N*-myristoylamino)-1-phenyl-1-propanol (MAPP) is a potent inhibitor of alkaline ceramidase with an IC_{50} value in the lower micromolar range.^[210]

5.2.3. Inhibitors of Sphingosine Kinase

Sphingoids with a *threo* configuration are able to inhibit sphingosine kinase.^[211] Since these compounds can be metabolized by acylation of the amino nitrogen atom and phosphorylation in the 1-position, they are only of limited use. *N,N*-dimethylsphingosine, an inhibitor of sphingosine kinase in blood platelets ($IC_{50} = 5 \mu M$),^[212] behaves differently. The compound can be phosphorylated in activated blood platelets,^[213] and the resulting 1-phosphate is a functional agonist of the sphingosine-1-phosphate receptor.^[214]

In addition to inhibitors of sphingolipid degradation, we have discovered a stimulator: 1-methylthiodihydroceramide at a concentration of $10 \mu M$ apparently reduces the *de novo* synthesis of ceramide in primary cultured neuronal cells by 90 %. Surprisingly, enzymes of ceramide biosynthesis are not affected by the compound; on the other hand, it stimulates sphingosinekinase by a factor of 2.5. The mechanism of the apparent biosynthesis inhibition is accordingly an accelerated degradation of biosynthetic intermediates such as sphinganine

and sphingosine. At the same time the compound interrupts the axonal growth of cultured hippocampus neurons in a way that is comparable to the direct inhibition of sphingolipid biosynthesis.^[215] A further activator of sphingokinase is 1 α ,25-dihydroxyvitamine D3.^[216]

5.2.4. Inhibitors of Sphingosine-1-phosphate Lyase

In order to be able to investigate the physiological functions of sphingosine-1-phosphate as a second messenger, metabolically stable analogues or inhibitors of sphingosine-1-phosphate lyase are required. Recently, *cis*-4-methylsphingosine has been identified as a prodrug of a metabolically stable sphingosine-1-phosphate analogue (Section 4.1). Stoffel and Grol^[217] have prepared a deoxysphinganine-1-phosphonate, which is a competitive inhibitor of the lyase ($K_i = 5 \mu\text{M}$, $K_M = 16 \mu\text{M}$). This extremely toxic compound is actually cleaved by the lyase, but at a rate that is slower by a factor of 10 than the natural phosphate. A homologue, a compound which is extended in the headgroup by a single carbon atom, has been synthesized^[218] and radiolabeled.^[219] The synthesis of the corresponding phosphoramidate^[220] and the unsaturated phosphonate have also been described.^[221] These compounds are potential ligands of the sphingosine-1-phosphate receptor. They possibly exhibit increased metabolic stability against cleavage by phosphatases. The synthesis of a radiolabeled 2-vinylidihydrosphingosine-1-phosphate, a potential irreversible inhibitor of the lyase, has also been described.^[222]

5.3. Lysosomal Sphingolipid Degradation

Constitutive degradation of GSLs takes place in the acid compartments of the cell, the late endosomes, and, in particular, in lysosomes.^[223, 224] The composition of the sphingolipids that enter the lysosomes from the cell surface is dependent upon the cell type. The neuronal plasma membrane, for example, is rich in gangliosides, whereas oligodendrocytes and Schwann cells, which form the myelin sheath around the axons of neuronal cells, are particularly rich in galactosylceramide and sulfatide. GSLs of the globo series such as globoside and globotriaosylceramide are found in different cell types of visceral organs. Finally, skin cells contain mainly ceramides and glucosylceramides with unusually long fatty acid residues. The individual sugar residues are sequentially cleaved from the nonreducing end of GSLs. Ceramide is finally formed from lower glycosylated sphingolipids, and is degraded to sphingosine and a long chain fatty acid. The building blocks thus released, namely monosaccharides, fatty acids, and sphingoid bases, are able to leave the lysosome. If because of a significant defect the function of one of the proteins involved in degradation fails, nondegradable lipid substrates accumulate in the lysosome. Unlike the water soluble oligosaccharides of glycoproteins, these are poorly soluble amphiphiles that cannot be removed and form poorly soluble aggregates in the lysosomes.

5.4. Topology of Endocytosis

In order that the GSLs of the cell surface can undergo intracellular degradation they must be transported to the lysosomes. Building blocks and fragments of the plasma membrane can reach the lysosomes by endocytosis.^[225] In this way, regions of the plasma membrane are budded to the intracellular vesicles as coated pits that can fuse with the early endosomes. Thus, components of the endosomal membrane are formed from previous regions of the plasma membrane. Consequently, the components of the plasma membrane reach the lysosomes as components of the lysosomal membrane by means of such a vesicular flow (Figure 18 A). Finally,

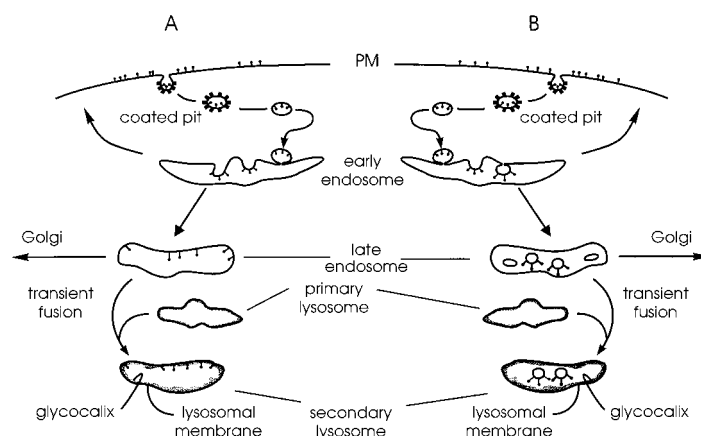


Figure 18. Models of endocytosis and lysosomal digestion of glycosphingolipids (GSLs) in the plasma membrane.^[228] a) Conventional model: the degradation of the GSLs of plasma membrane occurs selectively within the lysosomal membrane. b) Our alternative model: plasma membrane GSLs are incorporated into the membrane of intraendosomal vesicles (multi-vesicular bodies) during endocytosis. The vesicles reach the lysosomal compartment when late endosomes are fused transiently with primary lysosomes and are degraded there. PM = plasma membrane: ● GSL.

the degradation of the lipids must occur selectively within the lysosomal membrane without impairing their integrity. There is no plausible conception how this can happen, particularly since the inner face of the lysosomal membrane is protected by a lining of carbohydrates that are composed predominantly of lactosamine structures. This glycocalyx is formed from glycoproteins, the so-called “limps” (lysosomal integral membrane proteins) and “lamps” (lysosomal associated membrane proteins), which are components of the lysosomal membrane.^[226]

We have proposed an alternative model (Figure 18B), according to which components of the plasma membrane can also reach the intracellular digestion apparatus as components of intra-endosomal or intra-lysosomal vesicles and membranes.^[227] These could be formed by an invagination and budding off of certain regions of endosomal membrane that are particularly rich in previous components of the plasma membrane. By means of known fusion processes between the late endosomes and the lysosomes, the intra-endosomal vesicles thus formed could reach the lumen, that is the inner space of the lysosomes, where they are exposed to the digestive proteins. A number of observations support this mode of lysosomal membrane digestion.^[228, 229]

5.5. Mechanisms of Lysosomal Digestion

Lysosomal degradation of individual GSLs is carried out by exohydrolases that cleave the sugar moieties from the non-reducing end of the glycoconjugates. GSL degradation thus begins at a phase frontier, presumably at the surface of intralysosomal vesicles. These vesicles differ from the perimeter membrane by their curvature and their lipid composition, and consequently also by the lateral pressure operating at their surface. These factors can influence the enzymatic degradation of such structures quite considerably.^[230, 231] The hydrolases that degrade the glycolipids are enzymes which are dissolved in lysosol. However, their substrates are lipids that are present in a membrane bound form. Analyses of inherited diseases of sphingolipid degradation in the laboratory of Horst Jatzkewitz and in our laboratory have shown that in the case of GSLs with short oligosaccharide chains, the lipids to be degraded are no longer accessible to the hydrolases for steric reasons. A two component system of lipid and hydrolase is then no longer sufficient for GSL degradation *in vivo*, but an activator protein is also required. The enzymatic degradation of the ganglioside GM2 is thus initiated by a lysosomal binding protein, the GM2-activator.^[246] It acts as a weak surfactant that binds to the membrane-based ganglioside GM2 or structurally similar gangliosides to form stoichiometric, water-soluble complexes. On membrane or vesicle surfaces it clearly acts as a so-called “liftase”, which recognizes, binds and releases the membrane-based ganglioside from the membrane face so that they can be transported as substrates to the water-soluble, degrading enzymes, such as hexosaminidase A (Figure 19). The activator can only penetrate those lipid bilayers with a lateral pressure less than 25 mN m^{-1} .^[446] Further factors that can affect glycolipid degradation in addition to lateral pressure are curvature and composition of the lipid bilayer, where negatively charged lysosomal lipids such as bis(monoacylglycero)phosphate are of importance.^[230] The failure of the GM2-activator protein in a rare inherited disease, the AB-variant of GM2-gangliosidosis, leads to an accumulation of the ganglioside in neuronal cells, with fatal consequences for the patient.

There are four further sphingolipid activator proteins in addition to GM2-activator protein, called SAPA–D or saposines. As early as 1964 a protein was identified^[232] which was necessary for the hydrolytic cleavage of sulfatide (galactosylceramide-3-sulfate) by lysosomal arylsulfatase A. This sulfatide activator (SAP-B) is a small lysosomal glycoprotein that consists of 80 amino acids with an *N*-glycosidically-linked oligosaccharides and is stabilized by three disulfide bridges.^[233] Similar to the GM2-activator, it binds GSLs and acts as a GSL-transfer protein *in vitro*. Sulfatides and similar GSLs can be recognized on the surface of donor liposomes, bound in stoichiometric complexes, extracted from the membrane, and transported into the membranes of acceptor liposomes.^[234, 235] It evidently acts like the GM2-activator as a liftase in the lysosomes; the sulfatide activator can bind quite different GSLs of vesicular membranes, “lift” them from the membrane face, and present them as substrates to water soluble enzymes. The inherited defect of the sulfatide activator leads accordingly to a storage disease similar to

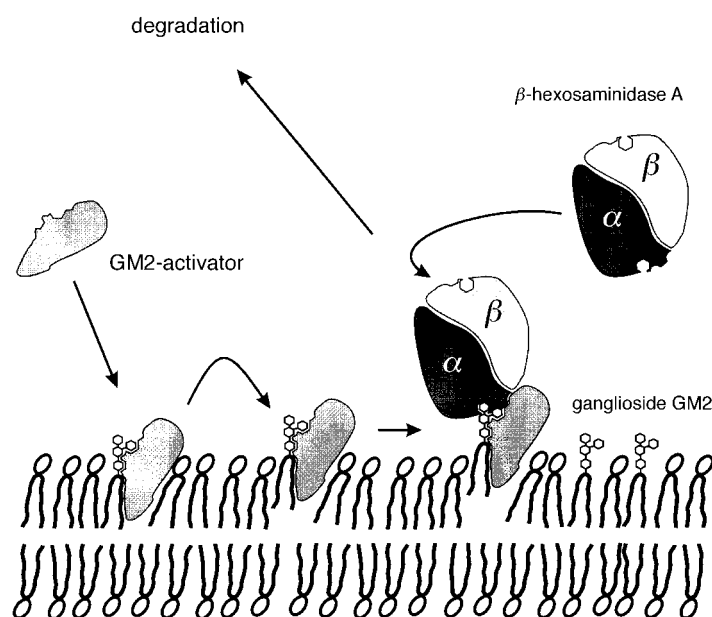


Figure 19. Model for GM2-activator stimulated hydrolysis of ganglioside GM2 by human hexosaminidase A (modified from reference [227]). The water-soluble hexosaminidase A does not attack membrane-bound ganglioside GM2 in the absence of the GM2-activator or suitable surfactants, but it cleaves ganglioside GM2 analogues that contain a short chain or no fatty acid residue (lysoganglioside GM2). These are bound less tightly to the lipid bilayer and are more water soluble than ganglioside GM2. Membrane-bound ganglioside GM2 (for example, that of intralysosomal vesicles) is only hydrolyzed in the presence of the GM2-activator. The GM2-activator binds one molecule of ganglioside GM2 and lifts it out of the membrane. The activator–ganglioside complex can then be recognized by the water-soluble hexosaminidase A and the lipid substrate is cleaved.

metachromatic leukodystrophy in which other GSLs accumulate in addition to sulfatide, for example globotriaosylceramide (Section 6.11). The protein-chemical and molecular biological analyses of the sulfatide activator (SAP-B) and a further activator protein, Gaucher factor (SAP-C), have shown that both proteins are formed together with two further activator proteins, SAP-A and SAP-D,^[236] by proteolytic processing from a common proprotein, the SAP precursor.^[237, 238] All four activator proteins, SAP A–D, are homologous to each other, have similar properties, but different, as yet, indeterminate functions.

Membrane-active sphingolipid activator proteins act amongst other things as GSL binding proteins and thus as liftases for membrane-bound glycolipids, which enables the interaction with membrane-bound GSL substrate and the respective exohydrolase. Their function thereby need not remain limited to that of a GSL binding protein; thus, for example, a direct activation of glucosylceramide β -glucosidase by SAP-C has been detected,^[239] and a specific interaction of the GM2-activator with hexosaminidase A has been demonstrated.^[260] The participation of activator proteins in GSL degradation can also contribute to the protection of cellular membranes. Lysosomal enzymes or their frequently already active proenzymes appear, even if diluted, in the extracellular space because of incomplete sorting mechanisms. There, lysosomal hydrolases that do not require an activator could slowly degrade GSLs on the cell surface. This process is

normally diminished by two factors, a neutral pH value on the cell surface, at which the lysosomal hydrolases exhibit only a low activity, and also by the necessary stimulation by means of lysosomal activator proteins which occur equally in low concentrations in the extracellular space.

6. Pathobiochemistry

The lysosomal degradation of sphingolipids is associated with the occurrence of a number of inherited diseases.^[240, 241] In general, the degradation of cellular components takes place mainly in the acid compartments of cells, in both lysosomes and endosomes. Here, macromolecules are degraded into their building blocks by hydrolytic enzymes. About 40 hydrolases are involved, including proteases, glycosidases, lipases, phospholipases, nucleases, phosphatases, and sulfatases. The material undergoing degradation can reach the lysosomes by way of endocytosis or autophagy. The degradation products leave the lysosomes to serve as energy sources or cellular components for further synthesis in other subcellular compartments. Their passage through the lysosomal membrane takes place by diffusion or with the help of transporter proteins.

The inherited dysfunction of one or more degradation steps leads to the accumulation of nondegradable material and to lysosomal storage diseases, which are classified according to the nature of the stored substances.^[242] A differentiation is thus made between sphingolipidoses, mucopolysaccharidoses, mucolipidoses, glycoprotein storage disease, and a lysosomal glycogen storage disease, Pompe's disease. The concept that congenital metabolic dysfunction can lead to inherited diseases is attributed to Garrod.^[243] In the example of Pompe's disease, Hers^[244] introduced the name lysosomal disease, which is characterized by an inherited defect of an acid hydrolase with lysosomal localization and an accumulation of nondegradable enzyme substrates. Today the concepts of Garrod and Hers must be expanded to acknowledge that not only enzymatic defects can lead to storage diseases, but also defects in transport^[245] and activator proteins.^[246] As in Krabbe's disease, the defect of a lysosomal hydrolase is not necessarily accompanied by the lysosomal concentration of its substrate. Here the concentration of an otherwise rarely occurring toxic substance, galactosylsphingosine, is increased in the oligodendrocytes (Section 6.9). In comparison to other metabolic pathways it is conspicuous that a variety of inherited diseases is known within the GSL degradation pathway (Figure 20). This must be attributed to the strictly sequential degradation pathway of these compounds where the use of alternative pathways is not possible during metabolic blockage. Thus, mice with the enzyme defect which gives rise to Tay–Sachs disease in humans are essentially healthy because, in contrast to humans, an alternative metabolic pathway exists (Section 6.2.5).

Lysosomal glycosidases are generally specific for the cleavage of a particular terminal residue, which may be present in different substance classes, but are less so for any particular aglycon. A consequence is that in addition to lipid substrates, glycoproteins or mucopolysaccharides may be

stored in a particular sphingolipidosis if the defective hydrolase is also necessary for their degradation pathway. A defect is known in humans for almost each of the individual degradation steps of sphingolipid catabolism. One exception is lactosylceramide degradation, which can be carried out by two enzymes in combination with two activator proteins.^[247] Accordingly, no single enzyme defect is known that leads to the isolated storage of lactosylceramide. In contrast, lactosylceramide is stored in addition to other lipids when several activator proteins fail (Section 6.11).

In the first instance, the clinical consequences of degradation dysfunction is dependent upon the cell type that is predominantly affected by the storage. Neuronal diseases are a consequence of ganglioside storage, whereas the accumulation of ceramide and glucosylceramide affects mainly the visceral organs and skin, and the storage of galactosylceramide and sulfatide affects the white substance of the brain. With the exception of Fabry's disease, the inheritance of sphingolipidoses is an autosomal recessive. In addition, there is also a further X-chromosome-inherited lysosomal storage disease where sphingolipid metabolism is not affected. In mucopolysaccharidosis II, known as Hunter's syndrome, α -iduronate sulfatase is defective. In addition to defects in enzyme and activator proteins, incorrect posttranslational modification can lead to the expression of lysosomal storage disease. This is the case in I-cell disease (mucopolipidosis II) and multiple sulfatase deficiency. A review of individual mutations that cause lysosomal storage disease is available.^[248]

6.1. GM1-gangliosidosis

The inherited defect of GM1 β -galactosidase expresses itself in humans as two diseases, GM1-gangliosidosis, which is characterized predominantly by neuronal ganglioside GM1 storage,^[249] and Morquio Type B syndrome, in which oligosaccharide accumulation in the viscera predominates.^[250] Their causes lie in the substrate specificity of the mutated β -galactosidase. The molecular defect that leads to GM1-gangliosidosis was identified as a β -galactosidase deficiency in 1967.^[251, 252] GM1- β -galactosidase is a monomeric protein with a molecular weight of about 70 kD, which together with sialidase and the so-called "protective protein" forms a ternary complex. In this form it is protected from premature proteolytic degradation.^[253] Mutations in the protective protein can also cause a secondary GM1-accumulation. The cDNA^[254] and the gene^[255] of GM1- β -galactosidase have been cloned.

It is possible to differentiate clinically between three forms of GM1-gangliosidosis. In type 1, the infantile form, neurological symptoms appear within the first six months of life, and lead to death within two years. Cherry-red spots on the patient's ocular fundus, facial dysmorphism, liver and spleen hypertrophy, and skeletal deformation are characteristic. The late infantile or juvenile form of the disease, the so-called type 2, progresses less severely, with a life expectancy for the patient of about 10 years. Bone growth disorders and cerebral symptoms occur after the first year of life. Patients with the adult form of the disease (type 3) develop mild, slowly

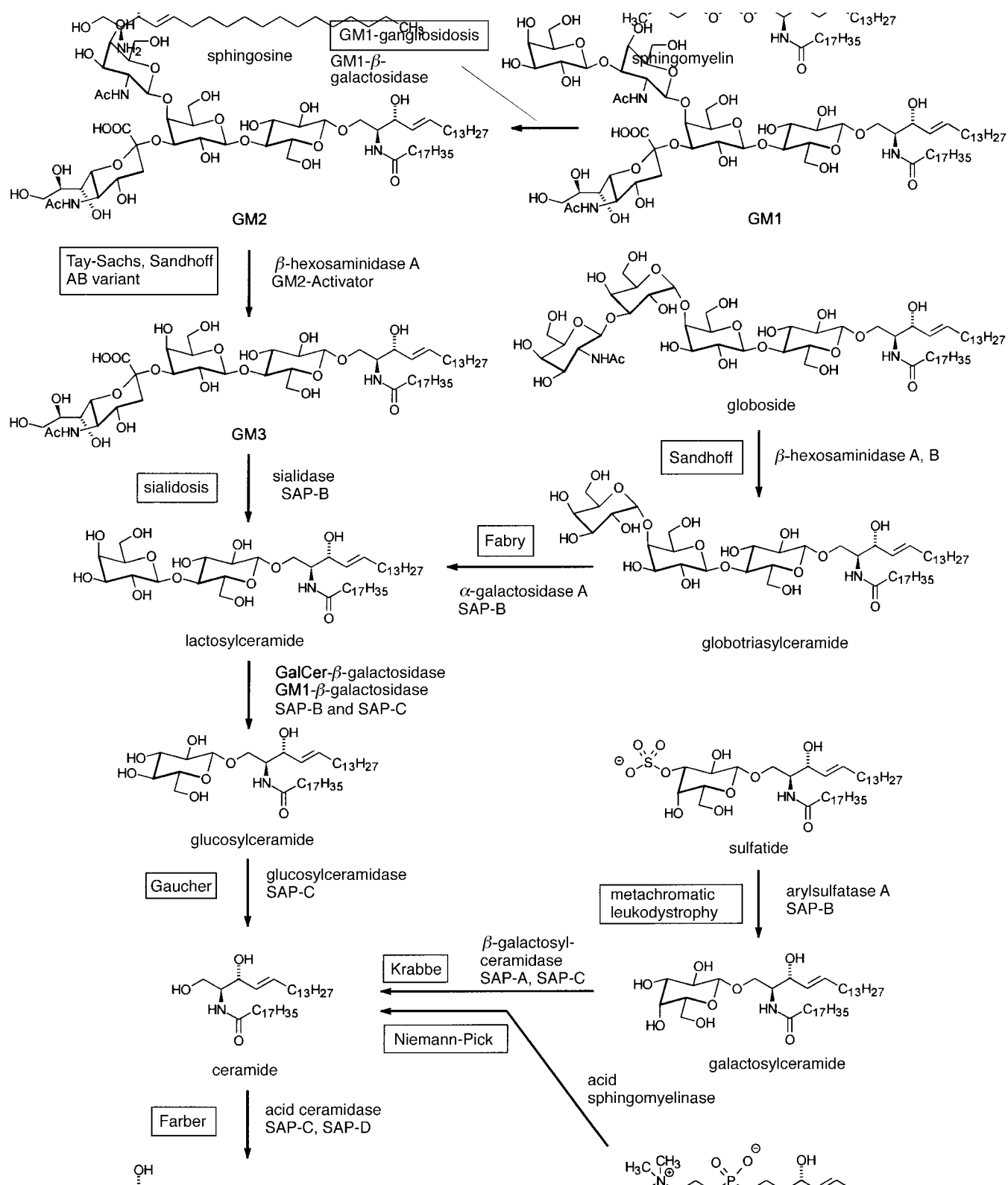


Figure 20. Lysosomal sphingolipid degradation.^[223] The eponyms of known metabolic diseases and those of sphingolipid activator proteins necessary for in vivo degradation are indicated. Heterogeneity in the lipid part of the sphingolipids is not indicated. Variant AB = variant AB of GM2-gangliosidosis (deficiency of GM2-activator protein); SAP = sphingolipid activator protein.

progressive neurological disorders with little involvement of the skeleton. Transitional forms are also known.

With a β -galactosidase defect, further enzyme substrates can accumulate in addition to ganglioside-GM1, including glycolipid GA1, oligosaccharides from glycoproteins, and intermediates of keratansulfate degradation. Depending upon the site of their biosynthesis, these substances are stored in different organs. GM1-storage in neurons leads to degeneration of the nervous system. The severity and progression of the disease correlates with the residual enzymatic activity in cells and body fluids as well as with the extent of substrate storage.^[256] The mutations that have been identified in infantile GM1-gangliosidosis patients (review: reference [248]) are heterogeneous, and none of the known defects occurs with special frequency. Recently, an authentic mouse model of GM1-gangliosidosis has been successfully developed.^[257, 258]

6.1.1. Morquio Type B Syndrome

Morquio Type B syndrome (review: reference [250]) is clinically a mild phenotype of the nonallelic type A syndrome in which *N*-acetylgalactosamine-6-sulfatase is defective, which results in the storage of keratansulfate. As in GM1-gangliosidosis, Morquio B syndrome is attributed to a defect in GM1- β -galactosidase. It is characterized by skeletal deformation without primary involvement of the central nervous system. Oligosaccharides with terminal galactose residues accumulate in the visceral organs and are secreted in the urine.

6.2. GM2-gangliosidoses

GM2-gangliosidoses are caused by a defect in the degradation of the ganglioside GM2.^[259, 263] Long term investigations have shown that three polypeptide chains are involved in the *in vivo* enzymatic cleavage of the terminal *N*-acetylgalactosamine residue, which are encoded by three different genes (Figure 19): the α and β chains of the β -hexosaminidases, and the GM2-activator protein. The β -hexosaminidases are dimeric proteins and cleave β -glycosidically-coupled terminal *N*-acetylglucosamine and *N*-acetylgalactosamine residues from glycoconjugates. The GM2-activator protein is required as a cofactor in the cleavage of ganglioside GM2. The α and β chains of β -hexosaminidases form three isoenzymes that differ in their subunit structures and substrate specificities. β -Hexosaminidase A has the $\alpha\beta$ subunit structure and degrades negatively charged and uncharged substrates. It has two, initially totally unexpected, active centers, one on the α chain, and one on the homologous β chain.^[260]

β -Hexosaminidase B with the subunit structure β_2 cleaves mainly *N*-acetylglucosamine and *N*-acetylgalactosamine residues from uncharged substrates such as glycolipid GA2, globotetraosylceramide, and oligosaccharides. β -Hexosaminidase S is a homodimer of α chains. It is of secondary significance for GM2 degradation and far less is known about its function *in vivo* than for the other isoenzymes. Investigations with genetically modified mice suggest that β -hexosaminidase S is involved in the degradation of glycoaminogly-

cans.^[261] We have classified GM2-gangliosidoses according to which enzyme is still detected in the tissues of patients.^[262] Mutations that affect the gene for the α -subunits of β -hexosaminidases can lead to the failure of β -hexosaminidases A and S. This defect is known as the B-variant of the GM2-gangliosidoses, and its infantile form is also known as Tay–Sachs disease. Mutations in the gene for the β subunit can lead to failure of β -hexosaminidases A and B, and storage of glycolipid GA2 and globotetraosylceramide in addition to ganglioside GM2. The respective disease is the 0-variant of the GM2-gangliosidoses, or Sandhoff's disease. Defects in the gene for the GM2-activator lead to AB-variants of the GM2-gangliosidoses.

6.2.1. B-Variant of GM2-gangliosidosis

The infantile form of the B-variant is better known as Tay–Sachs disease. The symptoms of Tay–Sachs disease were first described in 1881 by the British ophthalmologist Warren Tay, who reported on a case of amaurotic idiocy.^[263] Tay discovered a cherry-red spot in the retina of a one year old patient who was physically and mentally retarded. Before the turn of the century, the American neurologist Bernhard Sachs coined the term amaurotic familial idiocy and described the morphological characteristics of the disease. In 1962 Lars Svennerholm identified the main storage substance in Tay–Sachs disease within the nervous system to be the ganglioside GM2, the chemical structure of which was determined in the middle of the 1960s by Makita and Yamakawa,^[259] and Ledeen and Salsman.^[259] The underlying metabolic defect, the deficiency of the enzyme β -hexosaminidase A was identified in 1969.^[264, 265]

The classical Tay–Sachs disease is characterized by the onset of neurological symptoms in early childhood. The earliest symptoms occur at the age of three to five months. Motor weakness develops: sudden noise causes abnormal startle reflexes. At six to ten months attentiveness deteriorates, and motor dexterity and sight are lost. Macrocephaly and neurological disorders worsen until death occurs. Juvenile forms of the B-variant of GM2-gangliosidoses are characterized by motor disorders at the age of two to six years and advancing dementia in early childhood. A chronic form is made conspicuous by abnormalities in gait and posture and can commence at the age of two to five years. Neurological symptoms are manifest with advancing age, and the patient can live to an age of 40 years. Adult forms of the disease show a multiplicity of symptoms; neurological disorders such as spinal muscular atrophy and psychoses can occur. However, visual capabilities and intelligence are not impaired.

Both cDNA and gene^[266] of the α chain and cDNA^[267] and gene^[268, 269] of the homologous β chain have been characterized. The mature form of the α chain has a molecular weight of 56 kDa,^[270] that of the β chain 52 kDa.^[271] The β chain is cleaved proteolytically in the lysosomes of many cell types into two smaller fragments, β_a and β_b .

Tay–Sachs disease occurs particularly frequently in populations of Ashkenasi Jews (heterozygote frequency 1:27). Three mutated alleles within the α chain are responsible for 93 % of all mutated alleles. In total over 50 mutations on the α chain have been identified (review: reference [259]). The

form and severity of the disease correlate with the residual activity of the enzyme in the lysosome.^[272]

6.2.2. B1-Variant of GM2-gangliosidosis

The B1-variant of GM2-gangliosidosis^[273, 274] differs enzymologically from the B-variant by a changed substrate specificity of the mutated β -hexaminidase A. Synthetic uncharged substrates, which are used for diagnosis, are cleaved, whereas no activity is detected in respect to the natural substrate and negatively charged synthetic substrates. We have found that the function of the active site of the α chain is defective, whereas subunit association, enzyme processing, and the activity of the β chain activity are unimpaired. Mutations on three positions of the α chain lead to the B1-variant. Since at the protein level Arg178 is replaced by the amino acids Cys, His, and Leu in three mutations, it was assumed that Arg178 plays a role of the catalytic amino acid in the active site. This hypothesis has been refuted,^[275] but the precise role of Arg178 still remains unclear. Homozygous patients with B1-mutation present a juvenile disease progression; the late infantile form was found within compound heterozygotes with a null allele.

6.2.3. 0-Variant of GM2-gangliosidosis

The 0-variant of GM2-gangliosidosis is called Sandhoff's disease.^[246] We received the post-mortal tissue of the first case to be investigated and which was diagnosed as Tay–Sachs disease. However, the isolation and analysis of the storage substance exhibited an abnormal picture.^[276] Unlike the B-variant, it was noted that in addition to negatively charged glycolipids, mainly ganglioside GM2 and uncharged glycolipids were stored,^[262] in particular globoside in the visceral organs, glycolipid GA2 in neuronal tissue, and, as was determined later, oligosaccharides in the urine. All these storage lipids had a terminal β -glycosidically bound *N*-acetylgalactosamine residue in common. This feature suggested a possible metabolic defect. During studies on the degradation of the radiolabeled storage components we were able to detect an almost total loss of the β -*N*-acetylgalactosaminidase activities known at that time, that is, hexosaminidases A and B, in all the tissues investigated.^[276] This was the first described enzyme defect in a gangliosidosis. Later investigations showed that both hexosaminidase A and B are defective as a result of mutations on the gene of the common β chain.

The clinical and pathological picture of Sandhoff's disease corresponds essentially to Tay–Sachs disease, although hypertrophy of internal organs and bone deformation also occur. We have identified Glu355 as the catalytic amino acid in the active site of β -hexosaminidase B by photoaffinity labeling.^[277, 278]

6.2.4. AB-variant of GM2-gangliosidosis

The AB-variant of GM2-gangliosidosis is attributed to a deficiency of the GM2-activator. Early investigations showed us that in spite of the presence of β -hexosaminidase A and B

activities, a massive storage of the glycolipids GM2 and GA2 occurred in the brain.^[224] The enzymes isolated from patient tissue were able to cleave enzymatically the stored substances isolated from the same tissue when in the presence of a surfactant. This apparent paradox prompted us to seek an endogenous, that is natural, surfactant in post-mortal human tissue. In 1978 we were able to identify the molecular defect in the AB-variant as the deficiency of a cofactor, the GM2-activator protein.^[279] The AB-variant served as a prototype for the analysis of further activator deficiency diseases. Shortly thereafter we were successful in isolating the protein.^[280] The mature GM2-activator is a glycoprotein containing a polypeptide thread of 162 amino acids. This forms four disulfide bridges whose mode of coupling was recently elucidated by the combination of protein-chemical and mass spectrometric techniques.^[281] The cDNA and large regions of the gene are known.^[282] Mutations in AB-patients have been identified.^[283–285]

The clinical progression of the disease corresponds to that of Tay–Sachs disease. In comparison with Tay–Sachs disease, the appearance of symptoms is slightly delayed. Ganglioside GM2 and glycolipid GA2 are stored in AB-variant, but not, however, globoside^[262] or GD1a-GalNAc, a minor storage substance in the 0- and B-variants.^[286]

6.2.5. Animal Models of GM2-gangliosidosis

Different natural animal models of GM2-gangliosidosis are known (review: reference [259]). In addition, it has been possible more recently to develop mouse models for the individual forms of GM2-gangliosidosis.^[287] The targeted disruption of genes for the α chain and the β chain of hexosaminidases as well as the GM2-activator protein in embryo stem cells has led to animal models of the B-variant,^[288, 289] the 0-variant,^[290] and the AB-variant^[291] of GM2-gangliosidosis. Whereas the individual forms of GM2-gangliosidosis in humans differ phenotypically to only a slight extent, animal models show drastic differences in the progression and severity of the disease. The mouse with B-variant is phenotypically inconspicuous. In contrast, the mouse with 0-variant develops severe motor disorders, and life expectancy is greatly reduced. The cause lies in the specificity of the sialidase, which differs between mouse and humans.^[290] Murine sialidase accepts GM2 as a substrate and degrades it slowly to GA2 (Figure 21). This metabolic pathway plays no significant role in humans. GA2 can be degraded by the still intact β -hexosaminidase B, so that in the Tay–Sachs mouse the metabolic block can be partially circumvented in spite of a complete failure of β -hexosaminidase A. Only the failure of both isoenzymes, hexosaminidase A and B, leads to a symptomatology that corresponds to the human form of Sandhoff's disease. Murine sialidase can still convert GM2 into GA2, but GA2 can no longer be degraded since the responsible enzyme, β -hexosaminidase B, is defective.

The mouse deficient in GM2-activator expresses an intermediate phenotype, which is characterized by motor disorders and a normal life expectancy. Crossing mice of the B- and 0-variants leads to animals in which all three isoenzymes, hexosaminidases A, B, and S are defective. There is no known

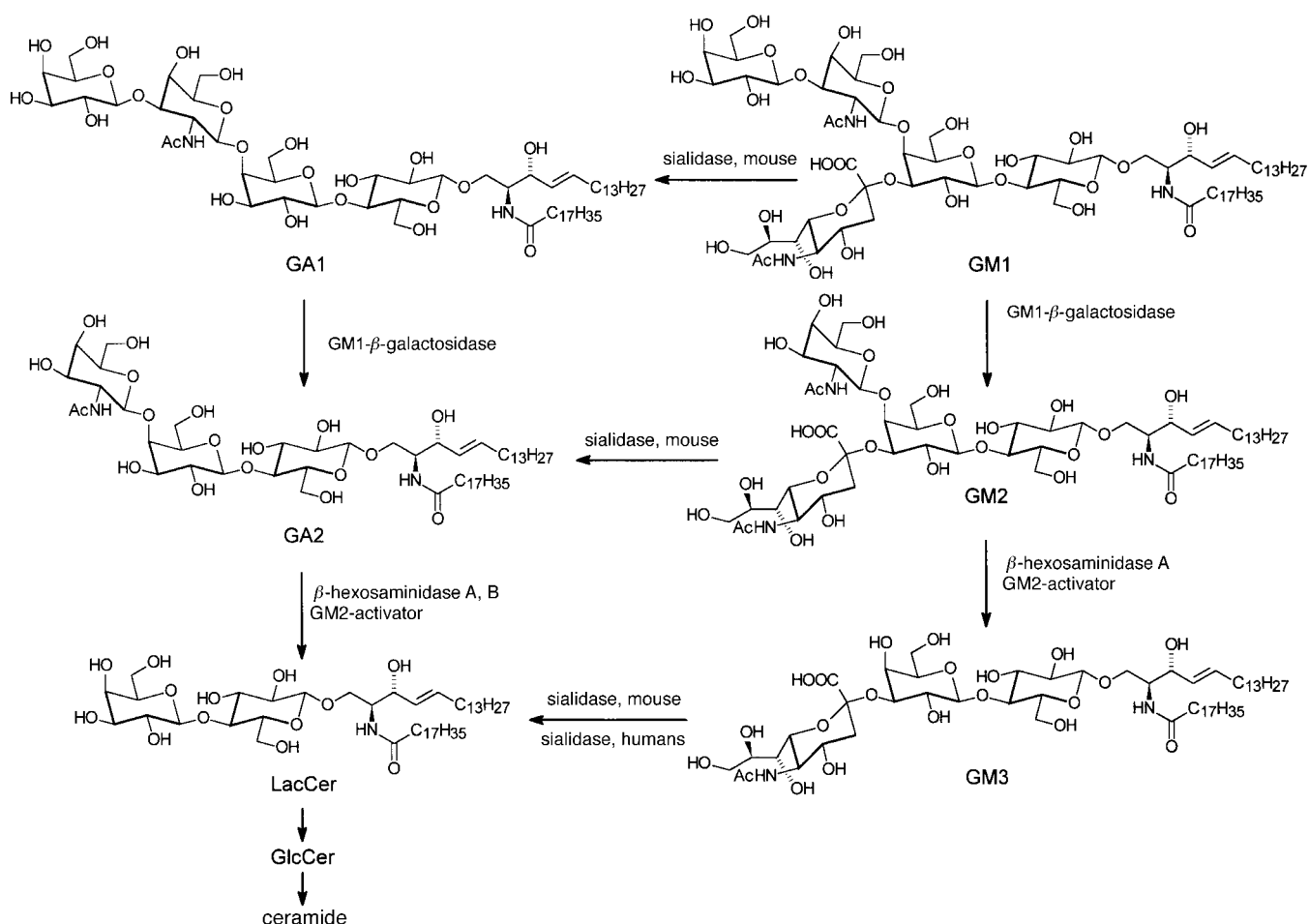


Figure 21. Degradation pathways of ganglioside GM1 in humans and mice.^[257, 290]

human disease that corresponds to this defect. In addition to neuronal glycolipid storage, these animals exhibit the phenotype of a mucopolysaccharidosis and excrete large amounts of glycosaminoglycans in their urine. Accordingly, hexosaminidases are responsible for the degradation of glycoaminoglycans, whereby in human patients with B- or 0-variant the presence of the respective, intact isoenzyme is sufficient to prevent storage of glycosaminoglycans. Only the failure of all three isoenzymes leads to an accumulation of nondegradable glycosaminoglycans.^[261] B-variant mice have gained in importance as a model for the therapy with low molecular weight enzyme inhibitors (Section 6.14.3).

6.3. Galactosialidosis

Galactosialidosis involves the secondary deficiency of two enzyme activities,^[292] β-galactosidase and sialidase (*N*-acetylneuraminidase). The primary defect is a result of a mutation within the gene of the so-called “protective protein”,^[293] which associates with the two other proteins to form a stable complex of more than 600 kD.^[294] The stoichiometry of the complex is still unknown; however, the presence of β-galactosidase protein is not essential for the stability of the complex. Accordingly, a deficiency of the “protective protein” leads to degradation of β-galactosidase and sialidase and

thereby to the storage of sialic acid and galactose-containing substrates, including oligosaccharides and gangliosides such as GM3 and GM1.^[292] Sialyloligosaccharides accumulate in the lysosomes and are finally excreted in the urine.

The “protective protein” combines different enzymatic activities. In addition to its property as a protective protein, it is a serine esterase, a carboxypeptidase (acid pH optimum), and a deamidase (neutral pH optimum). It has emerged that it is identical to a protein that is released after thrombin stimulation of blood platelets, and hydrolyzes, and thus inactivates, different peptide hormones with amidic C-termini such as oxytocin, endothelin, and substance P. On the other hand, it is identical to the lysosomal protease cathepsin A. The protease and protective functions are independent of each other, since a gene product with an inactivated catalytic site restores the degradative capacity of sialidosis fibroblasts. The “protective protein” is synthesized as a 54 kD precursor, which is processed proteolytically into two chains of 32 and 20 kD connected by disulfide bridges. In all patients so far examined, deamidase and carboxypeptidase activities of the “protective protein” are also defective. The crystal structure of the 108 kD dimer of the “protective protein” precursor has been established.^[295]

Since the molecular defect of GM1-gangliosidosis was identified as a β-galactosidase deficiency,^[251, 252] reduced β-galactosidase activity, which is not, however, attributable to

defective β -galactosidase, has been found in different atypical patients. In one of these patients Wenger et al. detected a combined deficiency in β -galactosidase and neuraminidase activities,^[296] which was later found in other patients with atypical GM1-gangliosidosis and sialidosis. A partial restoration of sialidase activity in cells with combined enzyme defect was achieved in cell fusion experiments with neuraminidase deficient cells.^[297] The nature of a postulated “corrective factor” was finally elucidated by d’Azzo et al.^[293] Galactosialidosis has been diagnosed in about 70 patients world wide (review: reference [292]). The symptoms in all patients are coarsened facial features, cherry-red spots on the ocular fundus, spinal column changes, foam cells in the spinal cord, and vacuolized lymphocytes. Three different forms of the disease are recognized phenotypically. The early infantile form leads to neurological disorders and death of the patient at an age of about eight months as a result of cardiac or renal failure. Hypertrophy of the liver and spleen, growth disorders, and cardiac damage are characteristic of the late infantile form, but there is no impairment of the nervous system. Most patients suffer from the juvenile/adult form, which is characterized amongst other things by mental retardation and neurological damage. A mouse model with defective “protective protein” corresponds biochemically essentially to the severest human form, but, in contrast, however, it exhibits unusually high β -galactosidase activity.^[298]

6.4. Sialidosis

Defects in lysosomal sialidase lead above all to an accumulation and excretion of sialylated oligosaccharides and glycoconjugates. Since gangliosides play only a subordinate role in storage substances, this is not a sphingolipidosis in its narrowest sense. Sialidases that accept gangliosides as substrates are localized on the cell surface.^[299–302] The infantile form of the disease, (type II sialidosis) is also called mucopolipidosis I and is characterized by skeletal deformation, hypertrophy of the internal organs, and mental retardation. A juvenile form (type II sialidosis) is also known as mucopolipidosis IV. The human cDNA of sialidase has been cloned.^[303]

6.5. Fabry’s Disease

A failure of α -galactosidase A underlies Fabry’s disease. The first patients were described independently in 1898 by the dermatologists Anderson and Fabry.^[304] The identity of the storage material was determined in the 1960s by Sweeley and Klionsky, and the nature of the enzymatic defect by Brady and Kint.^[304] Unlike the other sphingolipidoses, Fabry’s disease is inherited as an X-chromosomal disease. It leads to the accumulation of enzyme substrates with terminally α -glycosidically bound galactose. The enzyme is a homodimer of subunits of each 50 kD and was purified from liver and spleen.^[305, 306] The cDNA^[307] and gene^[308] of α -galactosidase A have been cloned.

Fabry’s disease is characterized by painful skin lesions and is manifested clinically in lipid deposition within the skin,

pains in the extremities, and renal failure. The symptoms can start in infancy or adolescence, but is most frequently an adult form. Heterozygote female carriers are symptomless or show a milder disease progression. Mainly globotriaosylceramide, but also digalactosylceramide and lipids with blood group B specificity are stored. Detectable residual activities of α -galactosidase A can be attributed to the presence of α -galactosidase B (α -N-acetylgalactosaminidase), which exhibits a slight overlap in substrate specificity with the Fabry enzyme. This enzyme is defective in Schindler disease, of which only three patients have been currently described. The nature of the storage substance and the pathogenesis of Schindler disease are unknown.^[309]

The lipids affected in Fabry patients are synthesized in nerve cells only to a small extent, so it is accordingly a systemic disease with neuronal damage only as a secondary effect. The storage sites are the epithelial cells of blood vessels, cells of the smooth musculature and the myocardium. The pathogenesis is caused by the blockage of small blood vessels by lipid deposition and manifests itself by angiokeratoma of the skin, renal failure, and cardiovascular disease. Lipid deposition in the brain of patients is attributed to the storage in blood vessels. However, the autonomic nervous system can be affected. The molecular causes that lead to the expression of Fabry’s disease are multifactorial. Gene rearrangements, point mutations, and splice site mutations have been found (review: reference [248]). The detection of the enzyme defect can be carried out by the determination of the enzyme activity in different sources, but the interpretation of this is hampered in female carriers, which results from the statistical inactivation of one of their X-chromosomes in the different cells and tissues.^[310] A mouse model of the disease has been recently described.^[311]

6.6. Niemann–Pick Disease

In 1914 the German physician Albert Niemann reported on a young patient who suffered from hepatosplenomegaly, lymphadenopathy, and impairment of the central nervous system and who died before reaching the age of two years.^[312] During subsequent histological studies, Ludwig Pick observed the occurrence of foam cells that were similar, but not identical, in appearance to those previously described in association with Gaucher’s disease. Ernst Klenk identified the storage substance as sphingomyelin, and in 1965 Brady detected a reduced sphingomyelinase activity in affected cells. In 1961 Crocker classified Niemann–Pick’s disease into three different types, A–C, to which further types were added later. Types A and B of Niemann–Pick disease are caused by a defect in acid sphingomyelinase (review: reference [312]) and are characterized by a lysosomal sphingomyelin storage. Type C of the disease does not involve a defect in acid sphingomyelinase.^[313] The neuronopathic type A occurs during childhood and is characterized by advancing psychomotor retardation and massive hypertrophy of the internal organs. Death occurs at the age of about three years. Patients who suffer from the non-neuronopathic type B of the disease also show organ enlargement, but almost no involvement of the

central nervous system is observed. The patients can reach adulthood.

Intermediate forms are characterized by an initially mild type B-like disease picture, which worsens with advancing years with involvement of the central nervous system.^[314] Patients of types A and B exhibit massive hypertrophy of the liver and spleen as well as characteristic storage cells in the bone marrow. We have purified to homogeneity and characterized the acid sphingomyelinase that is defect in types A and B of Niemann–Pick's disease in order to elucidate the molecular origin of the disease. It is a monomeric protein with a molecular weight of about 70 kD.^[315] The cDNA^[316] and the gene^[317] of the enzyme (Figure 22) have been cloned.

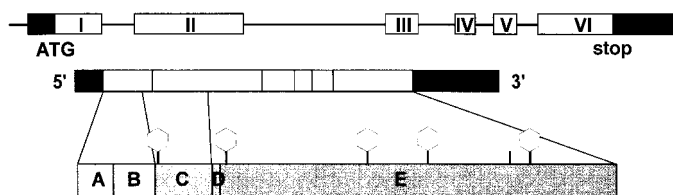


Figure 22. Genomic structure (top), cDNA (center), and protein domains (bottom) of acid sphingomyelinase.^[448] I–VI: exons, A: presequence, B: prosequence, C: activator domain, D: proline-rich region, E: catalytic domain. Hexagon: utilized *N*-glycosylation sites; ATG: translation start, stop: translation stop.

Niemann–Pick's disease (review: reference [312]) is a panethnic disorder, but it occurs more frequently in Ashkenasi Jews (heterozygote frequency 1:60). The differing disease pictures of types A and B can be fundamentally attributed to the different residual enzymatic activity of the acid sphingomyelinase.^[318] Unlike type A patients, patients with type B possess greater residual activity, which is still able to maintain an adequate turnover of sphingomyelin in the nervous system.^[319]

Acid sphingomyelinase cleaves sphingomyelin into ceramide and phosphorylcholine. This reaction was of special interest in the recent past since ceramide has been under discussion as a signal molecule in intracellular signal transduction. The role of acid sphingomyelinase in the signal-dependent induced generation of ceramide in the so-called sphingomyelin cycle is currently disputed.^[20] Unlike normal lymphoblasts, the lymphoblasts of patients with Niemann–Pick's disease and sphingomyelinase knockout mice exhibit no ceramide formation and no apoptosis in response to irradiation.^[320] On the other hand, acid sphingomyelinase appears to be necessary for FAS-induced apoptosis.^[180] Stimulation of cells that overexpress the magnesium-dependent neutral enzyme with tumor necrosis factor α leads to only a slight elevation of intracellular ceramide concentration.^[153]

Treatment of rats with tricyclic antidepressants leads to storage of sphingomyelin in visceral organs and a disease picture which resembles Niemann–Pick's type B.^[321] In cultured fibroblasts the antidepressant desipramine causes proteolytic degradation of mature lysosomal sphingomyelinase, but not of one of its precursor forms. A thiol protease is clearly responsible for this induced degradation.^[322] A mouse model has also been developed for the Niemann–Pick

disease, whose phenotype essentially corresponds to the human type A disease.^[323, 324]

6.7. Metachromatic Leukodystrophy and Multiple Sulfate Deficiency

Metachromatic leukodystrophy (MLD) is caused by a deficiency of arylsulfatase A (ASA) and leads to the accumulation of sulfatides in different organs (review: reference [325]). The name MLD comes from the metachromatic staining of the stored substances in histological slices. Sulfatide was isolated from human brain tissue by Blix in 1933, and the structure was determined by Yamakawa in 1962. The storage of sulfatide in MLD patients was detected by Jatzkewitz (review: reference [326]), and the sulfatase deficiency independently of each other by Austin and Jatzkewitz.^[3] Arylsulfatases are enzymes that can cleave artificial aryl sulfates. In MLD patients one of the different arylsulfatases is defective, namely arylsulfatase A,^[327] which cleaves sulfatides in the presence of a cofactor.^[322] The cofactor of arylsulfatase A, later called the SAP-B activator protein, was isolated from tissues of MLD patients.^[328] The disease has been divided into late infantile, juvenile, and adult forms.^[329] The adult form is rarer than the other two. The clinical symptoms arise at the end of the second or third decade. The leading symptoms are mental retardation, which leads to dementia, or behavioral abnormalities, which lead to the development of psychosis.^[330]

In both Krabbe's disease and MLD the myelin-forming cells of the central nervous system are affected, but unlike Krabbe's disease a significantly higher proportion of patients with the juvenile and adult forms are found in MLD. The reduction in the number of oligodendrocytes, which supply the nerve cells with the myelin sheath, is less severe than in Krabbe's disease. The function of peripheral organs is not impaired, but the nervous system is damaged by advancing demyelination. Increased concentrations of lysosulfatide (deacylated sulfatide) have been detected in the tissue of MLD patients. This cytotoxic compound is possibly an important factor in the pathogenesis of the disease.^[331] Macroscopically, a reduced volume of white substance is observed. Microscopically, a loss of myelin, a reduction in oligodendrocytes, and the occurrence of metachromatic granula have been found.

Arylsulfatase A aggregates at low pH values. The ASA gene has been cloned;^[332, 333] the incidence of the disease in the white population is estimated at 1:40000, the frequency of the leukodystrophy allele is about 0.5%.^[329] More than 30 different mutations that lead to MLD have been characterized in the ASA gene (review: reference [248]). The molecular cause for atypical forms of MLD lies in a deficiency of the sulfatide activator.^[334] MLD can be diagnosed by activity measurement of arylsulfatase A towards the artificial substrate 4-nitrocatechol sulfate in extracts of leukocytes or cultured fibroblasts.^[335] Previously, the detection of sulfatide in urine or the metachromatic staining of Schwann cells in nerve biopsies was used. Mice with artificially produced arylsulfatase A deficiency^[336] show only a mild extent of neurological and

neuropathological changes, although biochemically they resemble the severe late infantile phenotype in humans. The causes of this are still unclear.

6.7.1. Multiple Sulfatase Deficiency (Austin's Disease)

Numerous natural products of different substance classes contain alcohol groups that are modified as sulfate esters. These sulfate esters are cleaved by sulfatases, of which nine different enzymes have been characterized in humans and which, with the exception of steroid sulfatase, are localized in lysosomes (reviews: reference [337, 338]). The activities of all known sulfatases are greatly reduced in multiple sulfatase deficiency. The phenotype of the patients is characterized clinically and pathologically by a combination of MLD and mucopolysaccharidosis symptoms (review: reference [325]). Dermatan sulfate and heparan sulfate are excreted in the urine, and skeletal deformation, hepatosplenomegaly, and craniofacial abnormalities occur in addition to MLD symptoms. The primary defect of multiple sulfatase deficiency is not known, however, since expression of sulfatase cDNAs in fibroblasts of MSD patients leads to enzyme proteins with reduced catalytic activity, von Figura and co-workers have concluded that a defective co- or posttranslational modification is the cause of MSD.^[339] They were able to detect a defective protein modification in arylsulfatases A and B in MSD cells. This was the transformation of a cysteine into a formylglycine residue.^[340] This newly discovered modification is clearly responsible for the catalytic activity of the sulfatases. A presentation of this information has appeared in this journal.^[341]

6.8. Gaucher's Disease

Gaucher's disease is the most common of the sphingolipidoses and it is characterized by the deficiency of β -glucocerebrosidase and the accumulation of glucosylceramide (review: reference [342]). The disease was first described by Gaucher in 1882. The identification of the storage material was made by Aghion in 1934, and the identity of the defective enzyme was elucidated by Brady^[343] and Patrick^[344] in 1965. The glycoprotein glucocerebrosidase has a molecular weight of 59 to 67 kDa and consists of 497 amino acids. The complementary DNA of the enzyme was cloned in the middle of the 1980s.^[345, 346] Information on the active site of glucocerebrosidase was obtained with the help of competitive covalent inhibitors of the enzyme. The amino acid residue Asp443 was labeled^[348] with conduritol B epoxide (Figure 17).^[347] However, an involvement of this amino acid side chain in glycoside hydrolysis was excluded by site-specific mutagenesis.^[349] In contrast, Glu340 labeled with 2-deoxy-2-fluoroglucopyranose is clearly the nucleophilic residue in catalytic glycoside hydrolysis.^[350]

Gaucher's disease is classified by three types, I–III. The most frequent by far nonneuropathic form (type I) is clinically heterogeneous and has an incidence of 1 in 50 000 to 200 000 births. It has a higher frequency amongst Ashkenasi Jews; the carrier frequency is up to 1 in 10.^[351] The reticuloendothelial

system is mainly affected. The nervous system is also affected in the rarer, acute neuropathic type II of the disease. An intermediate, juvenile form III is also known as the subacute neuropathic form in which the neurological symptoms develop later and progress more slowly than in form II. Gaucher's disease is panethnic; type I is particularly frequent amongst the Ashkenasi Jews, and type III prevails in the Swedish province of Norrbotten.

Although the deficiency in glucocerebrosidase exists in all body cells, with the exception of the very rare neuropathic form, the phenotype is only significantly manifest in macrophages, which, because of the phagocytosis of, for example, erythrocytes, have especially large amounts of sphingolipids to degrade. The lipid storage leads to a characteristic morphology of the macrophages, which are called Gaucher cells. The appearance of storage cells in the liver, lymph nodes, and spleen causes hypertrophy of these organs. Infiltration of the bone marrow by Gaucher cells results in painful bone damage and the displacement of hematogenic cells. Glucosylceramide storage is accompanied by an elevated chitotriosidase activity in the plasma, which can be used for diagnostic purposes.^[352]

Four mutations are responsible for 80 % of all defects so far detected (review: reference [353, 248]). Two cases of Gaucher's disease are known that are not attributable to the deficiency of glucocerebrosidase, but to a deficiency of the sphingolipid activator protein SAP-C.^[354–356] The severity of the disease correlates inversely with the residual glucocerebrosidase activity that was found in cultured skin fibroblasts of Gaucher patients.^[357] Particularly high levels of glucocerebrosidase activity have been found in fibroblasts and placenta, and also in the chorion villi and cultured amnion cells that are important for prenatal diagnosis. The adult form of Gaucher's disease (type I) is currently the only sphingolipid storage disease for which a causal therapy can be undertaken.^[358–360] Glucocerebrosidase is used which has been obtained from human placenta or by genetic engineering and which has been equipped with targeting information for the mannose receptor on macrophages. The treatment leads to a normalization of blood parameters and a reduction in weight of the liver and spleen. Bone marrow transplantation can be carried out in patients with the severe infantile form. Animal models of infantile Gaucher's disease, type II^[361] as well as II and III^[362] have been described. Artificial model systems of Gaucher's disease have been generated with the help of conduritol B epoxide.^[363, 364]

6.9. Krabbe's Disease

Krabbe's disease is attributed to the deficiency of lysosomal galactocerebrosidase (review: reference [365]). Together with metachromatic leukodystrophy it belongs to the classical myelin diseases, since in both cases the degradation of glycolipids, galactosylceramide, or sulfatide, which are characteristic for the myelin sheath of nerve cells, is dysfunctional. The enzyme defect that underlies globoid cell leukodystrophy was elucidated in 1970.^[366]

The disease picture is manifested by neurological symptoms that usually start within the first six months of life. The patients die within two years. Adult disease forms are also known. The white substance of the central nervous system and peripheral nerves are the exclusive sites of the clinical and pathological manifestations of the disease. At the terminal stage the white substance contains almost no myelin. Oligodendroglia are replaced by astrocytes and abnormal globoid cells. Unlike all other storage diseases, there is no accumulation of the substrate of the defective enzyme in the cells particularly affected. On the contrary, the levels of galactosylceramide are unchanged or are less than normal, only the relative amount of galactosylceramide in myelin lipids is elevated. The cause lies in a rapid destruction of myelin-forming oligodendrocytes. Therefore, incomplete myelination, accompanied by rapid demyelination, means that galactosylceramide cannot accumulate. According to the so-called psychosine hypothesis, increased concentrations of the nondegradable galactosylsphingosine (psychosine), a lytic metabolite of sphingolipid metabolism, are responsible for the rapid depletion of the oligodendrocytes and the pathology of the disease.^[367, 368]

Galactocerebrosidase is a membrane-bound protein with a molecular weight of 50 kD.^[369] The cDNA^[370, 371] and gene^[372] of galactocerebrosidase have recently been cloned. An authentic animal model of the disease, the twitcher mouse, is known.^[373] The murine galactosylceramidase cDNA has been cloned and the twitcher mutation identified as a premature stop codon in the middle of the encoding sequence.^[374]

6.10. Farber's Disease

Farber's disease is caused by a deficiency in acid ceramidase and the ensuing accumulation of ceramide in various tissues (review: reference [375]). It is a rare inherited autosomal recessive disease; about 43 patients have been reported. Cell culture studies have shown that the sphingolipid activator proteins SAP-D^[376] and SAP-C^[447] are essential for lysosomal ceramide degradation. Farber's disease was first reported in 1947. During a symposium of the Mayo Foundation in the autumn S. Farber reported the case of a 14 month old girl with symptoms that were reminiscent of Niemann–Pick disease, but which differed from it through histological variations.^[375] Because of the most obvious symptom, the occurrence of lipid-containing nodes, the disease was called a lipogranulomatosis. During childhood the patients developed a painful swelling of the joints followed by subcutaneous nodes. An advancing hoarseness and feeding difficulties as a result of laryngeal changes are also characteristic. According to the localization of the lipogranuloma, functions of organs such as lung, heart, and kidney can be impaired. Impairment of the nervous system in most protracted forms is slight. The disease leads to death within the first year, but a longer time course is possible. The storage substance was identified as ceramide in 1969,^[377] and the underlying defect as a deficiency in acid ceramidase.^[378, 379] We have isolated the enzyme from human urine.^[380] It is a heterodimer from a α -subunit of 13 kDa and a glycosylated β -subunit of 40 kDa (Figure 23). The comple-

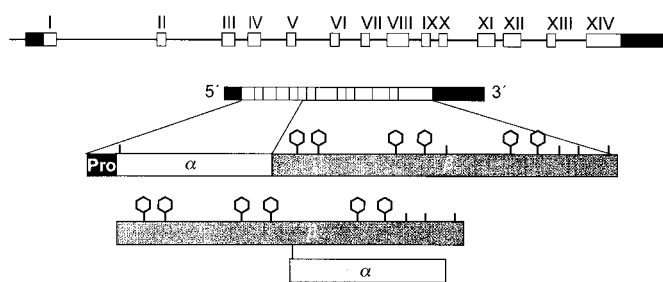


Figure 23. Gene, cDNA, and processing of acid ceramidase.^[449] I–XIV: exons; hexagon: utilized N-glycosylation sites.

mentary DNA has been cloned;^[381] it encodes for both subunits.

A number of subclasses of Farber's disease have been identified according to the clinical picture. All patients display lipid storage in the kidney, whereas the type and scope of deposition in the other tissues and organs can vary. The cause of the disease in six of the seven classes lies in a defect in lysosomal acid ceramidase. The seventh subtype is attributed to a mutation in the start codon of sphingolipid activator protein precursor.^[401] As a result, SAP-C and SAP-D which are required as cofactor for acid ceramidase fail, as do two further activators. Biochemically, the storage of ceramide in tissue^[377] and the increased secretion of ceramide in the urine of patients^[382] is characteristic of Farber's disease. The fraction of ceramide in subcutaneous nodes can reach 20 % of total lipid, and the ceramide content in the kidney is also elevated. The hydrolysis of ceramide is catalyzed by two other, non-lysosomal ceramidases, one of which achieves its optimum activity in a neutral medium,^[383] the other in an alkaline medium.^[379] The three ceramidases exhibit varying tissue distribution as well as different intracellular topology and substrate specificity.^[375, 384] Thus, neutral and alkaline ceramidase are unable to replace the defective lysosomal acid ceramidase in Farber's disease.^[378]

Results in cultured fibroblasts from Farber patients have shown that the course of Farber's disease correlates inversely with the extent of lysosomal ceramidase degradation.^[385] The residual activity of the enzyme in Farber patients is higher than that of the defective enzymes of the other respective sphingolipidoses; obviously, larger amounts of ceramide from sphingolipid degradation must be converted by the enzyme, so even a slight impairment of enzyme activity leads to substrate storage.

We have identified as the first molecular defect in the gene of a patient with Farber's disease a homoallelic point mutation, which leads to a Thr222-Lys exchange in the β -subunit of acid ceramidase.^[381] Diagnosis is carried out by determination of acid ceramidase activity with the help of the synthetic substrates *N*-[1-¹⁴C]oleoylsphingosine and *N*-[1-¹⁴C]lauroylsphingosine in the presence of surfactants,^[386] or by the analysis of ceramide storage after radiolabeling in cultured fibroblasts.^[387] In addition, morphological studies on biopsy and autopsy material have been carried out.

6.11. Sphingolipid Activator Protein Deficiency

The degradation of sphingolipids with short oligosaccharide chains requires the presence of small non-enzymatic proteins,

the so-called sphingolipid activator proteins (Section 5.5). Five of these small, heat-stable proteins are currently known, the GM2-activator and the four sphingolipid activator proteins SAP-A, SAP-B, SAP-C, and SAP-D, which are produced from a common precursor by proteolytic processing (Figure 24). The GM2-activator protein has already been

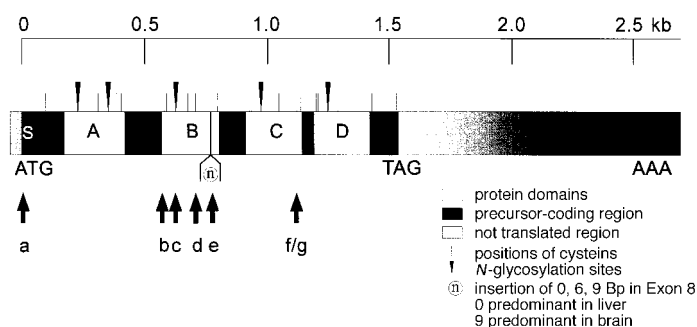


Figure 24. Structure of the SAP-precursor cDNA. The cDNA of the SAP-precursor encodes for a sequence of 524 amino acids (or 527 amino acids)^[388] including a signal peptide (S) of 16 amino acids for entry into the ER.^[227] The four domains of the precursor, SAP-A–D, correspond to the mature proteins found in human tissue: A = SAP-A or saposin A, B = SAP-B or SAP-1 or sulfatide activator protein or saposin B, C = SAP-C or SAP-2 or saposin C or glucosylceramidase activator protein, and D = SAP-D or saposin D or component C. ATG: translation start, TAG: translation stop, AAA: polyadenylation region. The positions of the known mutations (homoallelic) are indicated. a: A1T (Met1Leu);^[401] b: g-t-transversion within the 3'-acceptor splicing site at the transition from intron e to exon 6;^[440] c: C650T (Thr217Ile);^[441, 442] d: G722C (Cys241Ser);^[388] e: 33 BP insertion after G777 (11 additional amino acids after Met259);^[443, 444] f: G1154T (Cys385Phe);^[355] g: T1155G (Cys385Gly).^[356]

discussed in connection with the mechanisms of lysosomal digestion and the AB-variant of GM-gangliosidosis. The number of patients in whom a sphingolipid activator protein defect could be identified is small, and the clinical picture of each of such activator deficiencies is incomplete. Amongst the activators that are produced from the SAP-precursor, only one case of SAP-precursor deficiency is currently known, as well as isolated cases of SAP-B or SAP-C defects. The structure of the SAP-precursor gene is known.^[388, 389]

In addition to the already described SAP-B (Section 5.5), the activators SAP-C,^[239] SAP-D,^[236] and finally SAP-A have been isolated.^[390] SAP-C stimulates the activity of glucosylceramidase towards natural and synthetic substrates. Unlike SAP-B, glycoprotein SAP-C binds not only to lipids but also to the enzyme and stimulates it directly.^[391–393] It has a molecular weight of 20 kDa^[239] and in vitro it stimulates the degradation of galactosylceramide by galactosylceramide- β -galactosidase^[394] and sphingomyelin by acid sphingomyelinase.^[394–396]

Insights into the physiological function of the SAPs could only be obtained after investigation on patients in whom a certain SAP is absent. Thus, SAP-B deficient patients^[334, 397] show a drastic storage of sulfatide and the disease picture of a metachromatic leukodystrophy. In addition, these patients excrete increased amounts of globotriaosylceramide and digalactosylceramide in the urine.^[398] The deficiency of SAP-C is expressed in a massive storage of glucosylceramide; the clinical picture of the patients corresponds to that of a juvenile variant of Gaucher's disease.^[354, 399] Currently, no disease is

known that is caused by the sole deficiency or defect of SAP-A or SAP-D.

The case of a patient who died at 16 weeks and his fetal brother was particularly interesting. They displayed morphologically Gaucher-like storage cells in the bone marrow and a massive lysosomal storage of glucosylceramide, lactosylceramide, and ceramide in the liver.^[400] We were able to explain the molecular defect with the discovery in 1991 of a homoallelic mutation of the start codon of the SAP-precursor of ATG to TTG.^[401] This causes a complete failure of the precursor protein and all four SAPs A–D. The analysis of this case gave decisive support for our hypothesis on the function of the activator proteins and for the topology of endocytosis and lysosomal digestion. As expected, the tissues of the patient and fetus stored increased amounts of all glycolipids with short oligosaccharide chains (Section 5.5): neutral glycolipids such as mono-, di-, tri-, and tetrahexosylceramide in the liver, kidney, and cultured skin fibroblasts.^[402, 403] Sulfatide was stored in the kidney, and free ceramide in the liver and kidney. The amount of the gangliosides GM3 and GM2 was elevated in the liver, but not in the brain. The amount of phospholipid remained normal, as far as it has been investigated. Sphingomyelin metabolism was also not affected significantly in these patients.^[402] We postulated that the accumulation of the multivesicular bodies was morphologically conspicuous (see Figure 18).^[402] These could also be detected in cultured fibroblasts of the patient and as pathologically modified lysosomes under the electron microscope.^[229] Feeding the patient with the missing precursor protein rectified both the storage of glycolipid and the occurrence of intralysosomal storage vesicles.^[229] Genetically modified mice, which are homozygous for the inactivated gene of the SAP-precursor protein, correspond in their phenotype to the symptoms observed in humans.^[404]

6.12. Pathogenesis of Sphingolipidoses

Sphingolipidoses belong to those inherited diseases where a lot of information is available about their biochemical basis and primary causes. The nature of the storage substances and the underlying defects at a protein and nucleic acid level are extensively known. This is in contrast to their pathogenesis, where correlation of genotype and phenotype is not yet possible. On the one hand, as with many other diseases, defects in quite different structural genes can lead to clinically similar disease pictures. Thus, defects in the α chains of the hexosaminidase A and S, defects in the β chains of the hexosaminidases A and B, and defects in the GM-activator lead to disease pictures that earlier were collected together under the term amaurotic idiocy.^[263] On the other hand, different mutations in one and the same structural gene can lead to different disease courses that are sometimes even given different eponyms. Thus, severe forms of α -iduronidase defects are known as Hurler's disease, and the milder course as Scheie's disease.^[337] A β -galactosidase deficiency can cause a GM1-gangliosidosis with neurological damage or lead to Morquio type B disease in which no neurological involvement is observed, but mainly skeletal deformation is evident.

Even patients with identical mutations within the same structural gene, for example in that of arylsulfatase A,^[405] can show different clinical forms that are probably a result of a respective different genetic background. Knowledge of the primary defect at a DNA level is certainly a necessity, but in no way a sufficient condition for an understanding of the pathogenesis of these diseases. This applies especially to the adult forms of sphingolipidoses.^[318] Despite their enormous heterogeneity and the only indirect correlation between genotype and phenotype of a disease form, it is possible to correlate the genotype and phenotype of the disease and to specify a number of significant factors that influence the pathogenesis. One significant factor is clearly the cell type specific expression of individual sphingolipids. This entails that during storage dysfunction, storage occurs primarily in cells and tissue in which the lipid substrates of the mutated enzyme step are predominantly synthesized (for example, complex gangliosides in neurons) or by which they are taken up during phagocytosis (for example, glucosylceramide storage in macrophages in Gaucher's disease).

A further factor is the nature of the storage material. It is assumed that sphingolipids are generally nontoxic, and their accumulation is tolerated by the cells within wide limits. Only by mechanical damage is the cell function disrupted. A degradation disorder can also lead to the accumulation of morphogenetically active compounds^[406] or to a concentration of toxic lysoglycolipids.^[365] A decisive factor is the residual enzyme activity of the mutated degrading system in the lysosome. Numerous examples have confirmed that the different extent of a degradation disorder results in different clinical forms of the disease. The measured residual activity of the affected enzyme correlates with the form of the disease. The initial symptoms in neurolipidoses can be expected to arise when the function of the most severely affected nerve cells are impaired by storage. Accordingly, in the animal model of the AB-variant of GM2-gangliosidosis, only a defined population of nerve cells are observed in which GM2 accumulates.^[291] The theory of residual activity also explains that a minimal change in residual enzyme activity can cause major changes in substrate turnover.

6.12.1. Theory of Residual Enzyme Activity

The extent of residual enzyme activity is a significant pathogenetic factor. The activity of the degrading system can be reduced to different residual levels within the lysosomal compartment by different mutations and pathobiochemical mechanisms. The level of this residual activity should have a direct effect upon the pathogenesis of clinical forms. The respective mutated protein and the interrelated maximum activities ($V_{\max}$) of the degrading system form a decisive link between the genotype and phenotype of the respective disease form.

Starting from the Michaelis–Menten equation, we have calculated the substrate concentration $[S]_{\text{eq}}$ within the lysosome as a function of the residual activity under steady state conditions.^[407] The substrate concentration $[S]_{\text{eq}}$ at the steady state is approximately dependent upon only two factors [Eq. (1)], the influx rate of the substrate into the lysosome v_i

$$[S]_{\text{eq}} = K_M / (V_{\max} / v_i - 1)$$

and the enzyme kinetic parameters K_M and $V_{\max}$ of the degrading enzyme.

In normal cells the substrate concentration usually lies below the K_M value of the degrading enzyme, that is, the Michaelis–Menten constant. The K_M values of lysosomal enzymes usually are in the millimolar range. A reduction in enzyme activity to values of 20–50 % of the value of a normal cell does not affect the turnover rate v since the reduced enzyme activity is compensated by an increased substrate concentration and consequently a larger substrate saturation of the enzyme (Figure 25). The ratio v/v_i remains constant; this situation is met in heterozygous carriers of such inherited diseases. This compensation mechanism functions until the maximum degradation activity of the mutated enzyme falls below the value of substrate influx into the lysosome. At this threshold value ($v_{\max}^{\text{mut}}/v_i = 1$) all enzyme molecules exist in the form of the enzyme-substrate complex; a reduction in residual enzyme activity to this theoretical threshold value, however, does not lead to an irreversible substrate accumulation. This explains why probands with pseudodeficiency with, for example a loss of up to 90 % of activity of γ -hexosaminidase A or arylsulfatase A are asymptomatic. Only a reduction in enzyme activity below the critical threshold value causes storage of the corresponding lipid substrate, since only a part of the entering substrate can be degraded.

This model was checked by substrate-flux and enzyme-activity measurements in cultured skin fibroblasts from different patients with GM2-gangliosidosis and metachromatic leukodystrophy^[272] as well as with other sphingolipidoses.^[256, 318, 319, 357, 385] Below a threshold value the substrate turnover initially falls linearly, as expected, with falling enzyme activity. Patients of the clinically heterogeneous adult form then show a significantly higher turnover of storage substances than patients of the clinically otherwise manifested juvenile form, and in turn these shows a higher turnover than the again otherwise manifested infantile form of the disease. As a result, small differences in residual enzyme activity consequently correspond to the significant differences in the respective metabolic flux.

6.13. Molecular Diagnosis: Metabolic, Enzymatic, and Genetic Procedures

The diagnosis of sphingolipidoses was initially based on clinical and pathological findings. The disease was later diagnosed on the basis of the biochemically identified storage material. The emphasis of current diagnostic procedures rests upon the determination of the enzymatic activity of the individual hydrolytic enzymes.^[408, 409] Readily accessible clinical preparations of serum, leukocytes, cultured skin fibroblasts, or biopsy material serve as enzyme sources. Enzyme activities in amnion cells or chorion villi have been measured for prenatal diagnostic purposes. Metabolic procedures are irreplaceable for the diagnosis of diseases that may be attributed to the deficiency of enzymatically inactive sphingolipid activator protein. Diagnoses based on DNA analysis is

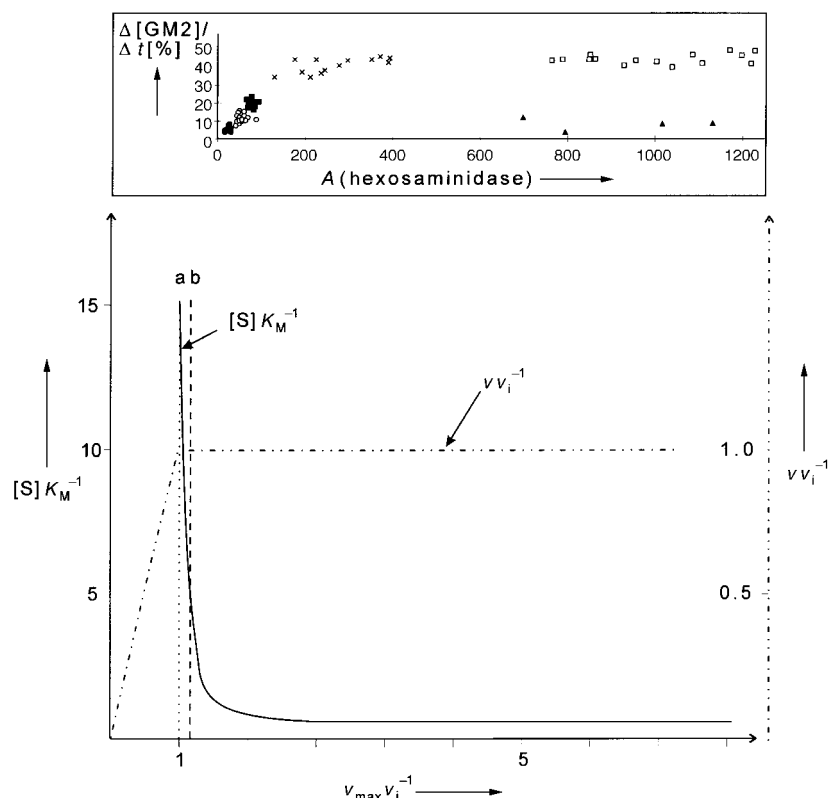


Figure 25. [223] Residual activity of a mutated enzyme and turnover of its substrate in the lysosomes.^[407] The substrate concentration $[S]$ is expressed as a multiple of the Michaelis constant K_M , the turnover rate v_i , and the maximum achievable enzyme activity ($v_{\max}$) as a multiple of the influx rate (v_i). — Substrate steady state concentration $[S]K_M^{-1}$; ••—•• Turnover rate of the substrate ($v v_i^{-1}$); •••• Critical threshold value of the enzyme activity (a). — Critical threshold value (b) of the enzyme activity with consideration of the limited solubility of the substrate. Above: residual activity A of hexosaminidases towards a ganglioside GM2 substrate (calculated as the amount of cleaved GM2 in vitro in $\text{pmol h}^{-1} \text{mg}^{-1} \text{AU}^{-1}$; AU = activator unit as defined in reference [280]) and turnover $\Delta[\text{GM2}]/\Delta t$ of ganglioside GM2 in fibroblast cultures of probands and patients with GM2 gangliosidosis.^[272] Skin fibroblasts of normal probands and patients with different forms of GM2-gangliosidosis and their carriers were fed with a culture of radiolabeled ganglioside GM2 for three days. The cells were then harvested, homogenized in water, and the following three parameters were determined: a) total incorporation of the substrate, ganglioside GM2. b) percentage of degraded substrate, ganglioside GM2. c) activity of hexosaminidase A towards the ganglioside GM2 in the presence of the GM2-activator. ● α -chain deficiency (variant B of GM2-gangliosidosis or Tay–Sachs disease, ● infantile form; ○ α -chain deficiency, juvenile form; ■ α -chain defect, adult form; ▲ activator deficiency (variant AB of GM2-gangliosidosis). Healthy probands: × carrier of GM2-gangliosidosis; □ normal controls.

possible if the mutations within the family of the patient are known.

A particularly readily accessible enzyme source for the determination of enzymatic activity is serum, from which the activity of some, but not all hydrolases can be determined. A disadvantage is that the level of activity of lysosomal enzymes in serum is generally lower than in cellular sources, and the stability can be reduced, particularly those of mutated proteins. Blood plasma is not generally useful as an enzyme source as lysosomal hydrolases can be inhibited by anti-coagulants. Leukocytes are a reliable and readily prepared enzyme source. For many questions it is advantageous to use lymphocytes, which by reason of their cellular homogeneity show a small scatter of measurement values. Cultured skin fibroblasts are another suitable enzyme source. Lysosomal hydrolases generally have low substrate specificity and their

activity need not only be determined with the help of natural substrates, which are frequently difficult to handle, but also can be measured with the help of synthetic substrates (Figure 26) that have suitable fluorogenic or chromogenic properties.^[408]

The demonstration of substrate storage in cultured patient cells after loading with radiolabeled substrate or catabolic substrate precursors is an additional diagnostic confirmation. For example, the degradation of ceramide^[410, 411] or ceramide precursors such as sulfatide^[410, 412, 413] and sphingomyelin^[414] can be investigated for the diagnosis of Farber's disease. Such lipid loading studies show high degradation rates of endogenous ceramide despite existing Farber's disease. This is ascribed in part to the high residual activity of the mutated acid ceramidase or nonlysosomal localized degradation of the respective lipids.^[414]

The comparison of biosynthetically labeled sphingolipids from patient cells with those of normal cells also permits the detection of sphingolipid storage. Serine radiolabeled in the 3-position allows the biosynthetic labeling of cellular sphingolipids. Sphingolipids whose degradation is disrupted contain stronger labeling after extended chase periods than those whose turnover is not impaired. A prerequisite is that a cell type is investigated in which the nondegradable lipid is also formed in significant amounts. An advantage of the method is that the enzymes and lipids occur in their natural, topologically correct sub-cellular environment. If lipid storage is detected with this cell system, its intensity correlates directly to the extent of the defect of the respective enzyme system. This also includes the detection of the failure of a cofactor whose deficiency is often overlooked in conventional diagnostic

procedures. However, in patients whose enzyme shows a high residual activity, biosynthetic labeling is inferior to direct determination of enzyme activity.

6.14. Therapy

With the exception of the adult form of Gaucher's disease, causal therapy of sphingolipidoses is currently not possible. The objective of a number of therapeutic activities in sphingolipidoses is the re-establishment of defective degradative capacity within the lysosome. This can be achieved either directly by enzyme replacement therapy, or indirectly by gene therapy or organ transplantation. The aim of enzyme replacement therapy in sphingolipidoses is the restoration of

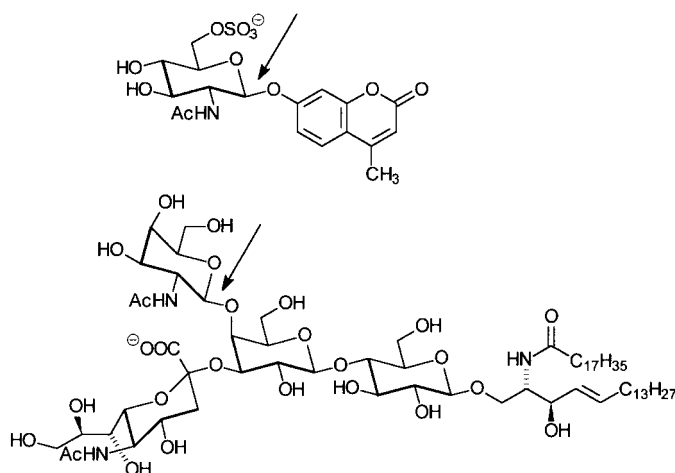


Figure 26. Structure of ganglioside GM2 and the synthetic substrate 4-methylumbelliferyl- β -D-N-acetylgalactosamine-6-sulfate. The arrow in each case indicates the bond that is cleaved by β -hexosaminidase A in the case of ganglioside GM2 in the presence of GM2-activator protein or surfactant.

nonexistent enzymatic activity by the exogenous addition of protein and thus alleviation of substrate storage or the prevention of further substrate accumulation. The concept of treating sphingolipidoses by the exogenous administration of the defective lysosomal enzyme is attributed to de Duve.^[415] The main hurdles in its way are, on the one hand, the localization of the target cells within central nervous system in which substrate storage is to be alleviated in most sphingolipidoses and, on the other hand, for a considerable time no techniques have been available for the isolation of the required proteins in sufficient amounts and purity. The latter problem can be overcome by the availability of cDNA sequences of lysosomal proteins for recombinant DNA technology. Further problems are posed by stability and immunogenicity of the administered preparations, their half-life in humans, uptake by the respective target organs, and last but not least, the costs of such therapy. Since an adequate penetration of the blood–brain barrier^[416] by systemically administered proteins cannot be presumed at the present time, enzyme replacement therapy remains for now restricted to diseases without primary impairment of the central nervous system.

The first sphingolipidosis that was treated successfully by enzyme replacement therapy is the adult form of Gaucher's disease (type I).^[358] The reason is that storage of glucosylceramide is mainly limited to Kupffer cells (hepatic macrophages) and other macrophages that express mannose receptors on their surfaces through which the exogenously administered protein targeted at the lysosome can be incorporated.^[417] There is no involvement of the nervous system because the patients have sufficiently high residual activity of the defective enzyme, glucosylceramidase, available. The initial difficulties encountered in the treatment of Gaucher's disease arose because the infused enzyme was taken up in the liver by hepatocytes, which incorporated an enzyme preparation isolated from human placenta through asialoglycoprotein receptors. The target cells of the enzyme, the Kupffer cells, were not reached by the enzyme. If,

however, an enzymatically modified enzyme preparation that exposes terminal mannose residues on its surface through partial glycosidase digestion is administered, it is incorporated by mannose receptors on the macrophages and is thus able to ameliorate a few of the symptoms of Gaucher patients. This includes regression of organ hypertrophy. The regression of skeletal deformations, which proceeds only very slowly, and the neuronopathic forms II and III remain problematical. A degeneration of the neurological state of the patient is indeed prevented in type III, but no improvement is achieved.^[358, 360] Antibody formation has been reported, but neutralization of the enzyme clearly does not occur.^[418]

6.14.1. Bone-Marrow Transplantation

The most significant obstacle in enzyme replacement therapy of neuropathic sphingolipidoses, namely passing the blood–brain barrier, would, in principle, be avoided by bone-marrow transplantation. Bone-marrow macrophages can cross the blood–brain barrier to a small extent and can serve as an enzyme source in the brain as microglia.^[419] Although there have been individual reports of successful attempts, bone-marrow transplantation does not yet represent a successful concept for the treatment of sphingolipidoses.^[420, 421] has led to an improvement in neurological symptoms and the regression of neuronal injury. The life expectancy of the twitcher mouse as an animal model of Krabbe's disease was increased by this form of treatment but recovery of the animal was not achieved.^[420] A clinical improvement of "late onset" lipidosis patients (Krabbe's disease, MLD) has been reported, whereas the therapy is contra-indicated in infantile patients.^[422]

6.14.2. Gene Therapy

Gene therapy applications in the treatment of sphingolipidoses are even less well advanced. Their objective is the insertion of a functional gene into the affected patient. The gene is inserted by means of suitable vectors into somatic cells that release a certain amount of the gene product into the environment. One major problem is the efficient transfer of therapeutic DNA into the central nervous system. Postmitotic cells including most neuronal cells are not transfected with retroviral vectors,^[423] so that other vectors must be employed for many purposes. However, this system is also the subject matter of investigations. For example, at the cell culture level, transduction experiments have been carried out with retrovirally mediated galactosylceramidase cDNA.^[424] Of the other different procedures for gene transfer,^[425] replication-deficient adenoviruses deserve a special mention, which stand out in that they are suitable vectors for the introduction of recombinant genes into neuronal cells. In respect of pathogenicity and predictability of the level of expression, they appear superior to systems that are based upon the *Herpes simplex* virus. In the mouse model of a lysosomal storage disease, the Sly syndrome (mucopolysaccharidosis VII), the visceral pathology could be corrected by adenovirus-mediated gene transfer. After injection of the recombinant virus into

the lateral ventricle of mice a transient increase in β -glucuronidase activity in the brain of the experimental animals could be detected.^[426, 450] Exemplary mention is also made to cell experiments in which GM2-storage in the fibroblasts of Tay–Sachs patients were eradicated after adenoviral-mediated gene transfer.^[427]

6.14.3. Glycosphingolipid Biosynthesis Inhibitors

Inherited diseases may be treated fundamentally at the metabolite level. One of these methods is called “substrate deprivation” or “substrate restriction”.^[27, 428] One of the important factors that affect the severity and form of sphingolipidoses is the relationship of residual enzyme activity ($V_{\max}$) to substrate influx (v_i) into the lysosome. As long as the biosynthesis of the substrate responsible for the defective degradation system continues, so does the pathological accumulation of the substrate in the lysosome. A change in the substrate influx into the lysosome (Figure 27) as

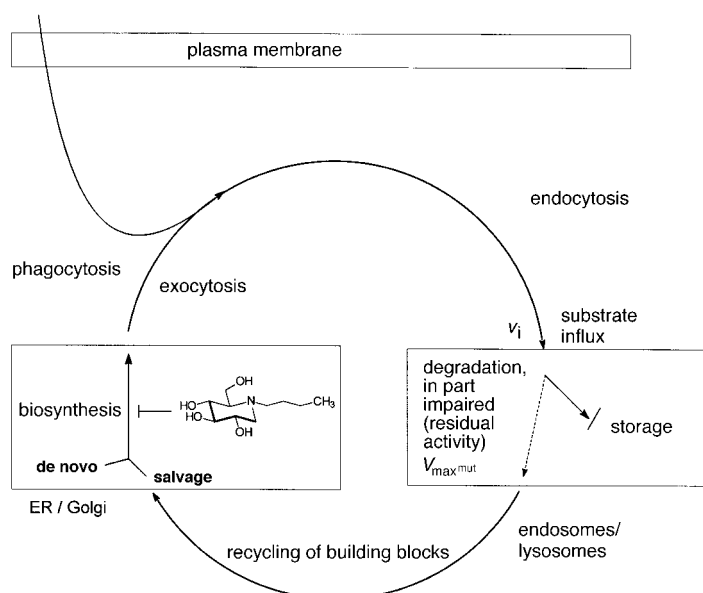


Figure 27. Substrate deprivation: the flow of substrate into the lysosome is reduced by inhibition of sphingolipid biosynthesis, whereas other therapy concepts aim to restore the degradative activity within the lysosome.

well as the lysosomal degradative capacity, can affect the course of the sphingolipidosis. It should be possible to restrict influx into the lysosome by inhibition of sphingolipid biosynthesis. It should thus be possible to positively affect both the severity and form of these diseases with the help of inhibitors of biosynthesis. This method requires that a minimal residual activity of the defective enzyme is present in the lysosome. This is the case in juvenile and adult forms, whilst in the infantile form no or only a little residual activity has been found.^[272] In the long term it may be assumed that nonexistent residual activity will be restored exogeneously, for example, by bone marrow transplantation, and at the same time the progression of the disease will be delayed by inhibition of substrate influx.

A number of low molecular weight inhibitors of GSL biosynthesis are known.^[100] One active compound is of

particular interest in connection with the therapy of sphingolipidoses.^[429, 430] This is the already known glycosidase inhibitor, *N*-butyldeoxynojirimycin, which has been investigated in the animal model of Tay–Sachs disease.^[431] Oral administration of the compound gives serum concentrations of 50 μM , which has already been demonstrated in human patients who have received this compound as part of an antiviral therapy. A 12 week treatment of Tay–Sachs mice has shown that this concentration is sufficient to achieve a 50% reduction in the storage of GM2 in the brain compared to untreated mice. Unlike untreated mice, after 16 weeks the neuronal cells of treated mice had only a few cytoplasmic membrane structures arising from lipid storage. Its low toxicity and its high bioavailability are important for the activity of the compound. It can be absorbed orally and it passes the blood–brain barrier so that sufficiently high concentrations are reached in the central nervous system to inhibit the formation of GSL to the desired extent (Figure 27).^[431] The success of the compound was not totally predictable, since it not only functions as an inhibitor of biosynthesis, but also as an inhibitor of glucocerebrosidase degradation.

Glucocerebrosidase was purified by affinity chromatography on *N*-alkyl-deoxynojirimycin sepharose,^[432] which would appear to make the suitability of the compound for the treatment of Gaucher's disease problematical. Tay–Sachs knockout mice have such a high residual enzyme activity that they do not fall ill and in this respect are suitable as a model system for checking the concept. The compound inhibits glucosyltransferase, which transfers glucose from uridine diphosphate glucose to ceramide (Section 4.3). Fundamentally, treatment with compounds of this type come into question in those patients who store sphingolipids that are derived biosynthetically from glucosylceramide. The successful use of this or similarly active compounds in human patients is at this time still hypothetical, but on the basis of the above considerations, there are realistic chances of treating sphingolipidoses successfully.

7. Future Prospects

Critical information on the pathogenesis and therapeutic methodology may be expected from animal models that were developed for the human form of sphingolipidoses.^[433, 434] For example, after hints on the involvement of the immune system in the pathogenesis of Krabbe's disease, double knockout mice that showed a significantly milder disease progression were obtained from the crossing of twitcher mice (an authentic animal model for Krabbe's disease) with MHC (major histocompatibility complex) class II deficient mice.^[435] Bone marrow transplantations with retrovirally modified cells has been tested in β -glucuronidase-deficient mice.^[436, 437] The phenotype of the galactosialidosis mouse model could be corrected by transgenic and normal bone marrow cells.^[298] These methods show that these recently available animal models are valuable aids for understanding the pathogenesis of sphingolipidoses. They are certainly of extraordinary value for the further development of novel therapeutic concepts. It may be assumed that these preclinical models will signifi-

cantly simplify the testing of concepts for the causal therapy of these diseases, and improved treatment possibility will follow as a result.

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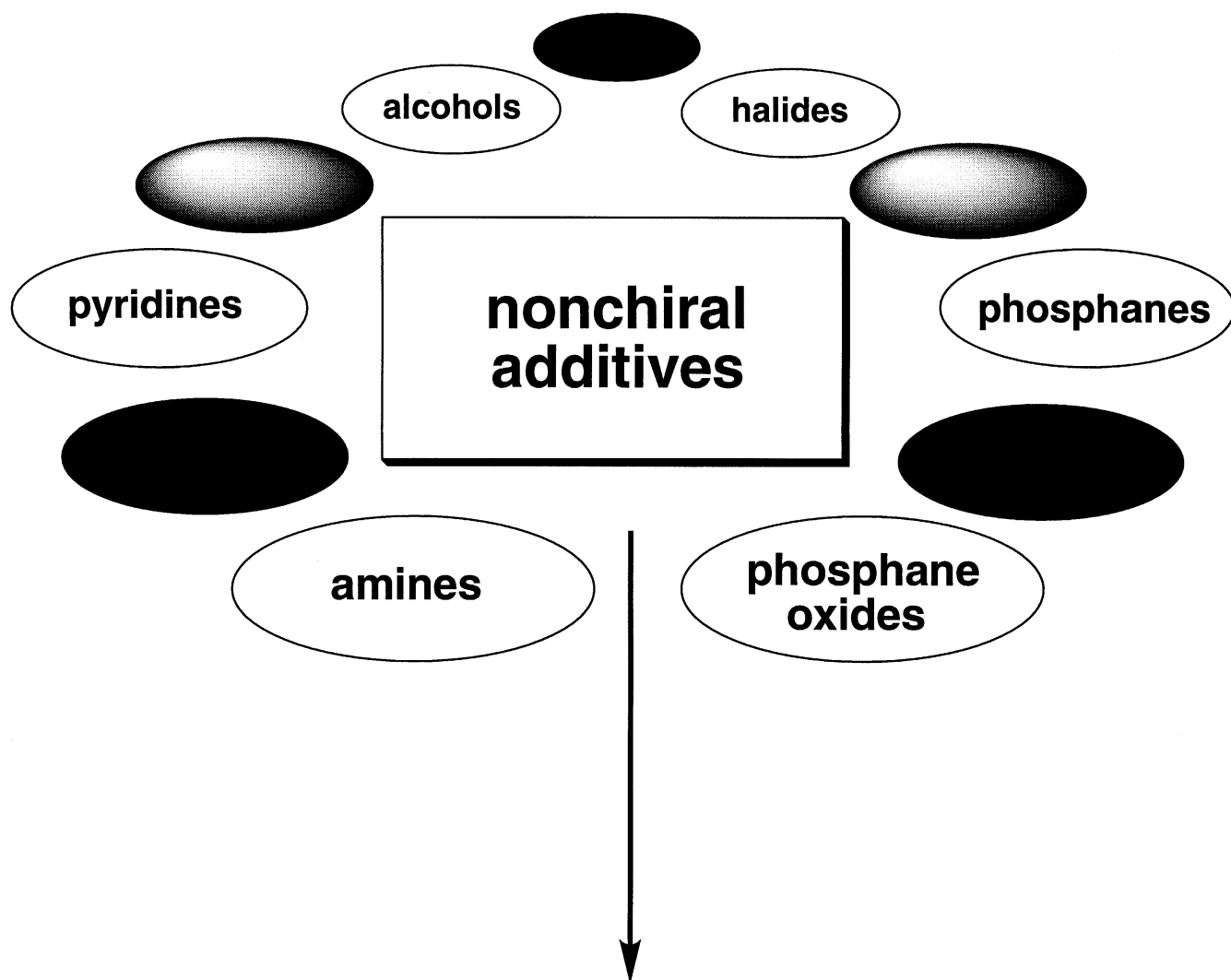
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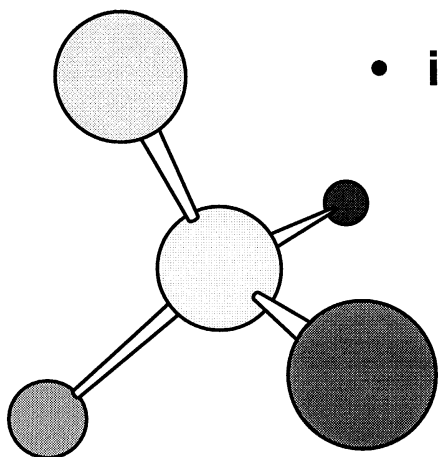
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**improved
asymmetric catalysis**



- **increased stereoselectivity**
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- **faster conversion**
- **modified reaction pathway**

Towards Perfect Asymmetric Catalysis: Additives and Cocatalysts

Erasmus M. Vogl, Harald Gröger, and Masakatsu Shibasaki*

The multiplication of chiral information is one of the most important tasks in organic chemistry. Perhaps the most elegant way to achieve this is by chiral catalysis. Although organic chemists developed a variety of asymmetric catalytic reaction systems, in many cases the efficiency of the catalysts (substrate/catalyst ratio, turnover number (TON), turnover frequency (TOF)) is not yet high enough for industrial applications. Asymmetric

catalytic systems are often very sensitive to small changes in reaction conditions. As a result several research groups have recognized that in many cases the addition of small (usually also catalytic) amounts of very simple achiral compounds can be beneficial for the yields and enantioselectivities obtained. This review intends to summarize this recent trend with experimental results from various laboratories covering a number of organic reactions

and additives. In some cases a variety of similar additives was carefully investigated with respect to achieving maximum selectivity. The success of the strategy can be recognized by a comparison of the results obtained with and without the additives.

Keywords: additives • asymmetric catalysis • asymmetric synthesis • synthetic methods

1. Introduction

Over the last few years the organic chemist has greatly benefited from asymmetric catalysis, which has not only allowed convenient access to chiral compounds, but has also produced a number of efficient asymmetric reactions.^[1] Nevertheless, transferring asymmetric catalytic methods to an industrial scale is unfortunately still a difficult and sluggish process. More or less successful applications of homogeneous catalytic asymmetric methodologies in industrial chemistry have only been reported for hydrogenations,^[2] isomerizations,^[3] cyclopropanations,^[4] and epoxidations.^[5] Although very often enantioselectivities of 90 to 95 % *ee* have been achieved for a variety of transformations in the research laboratory, this is usually not sufficient for industrial needs, particularly when it comes to pharmaceutical intermediates and products. For example, the certification of a pharmaceutical product requires compounds to have an enantiomeric purity of 99 % or more.^[6] And even if such a high enantioselectivity is not required in the case of, say, pesticides, the

catalyst productivity, catalyst activity, and catalyst stability can be even more important.

“How to make good asymmetric catalysis perfect” must therefore be the main concern for everybody, not only industrial chemists. Besides a subsequent (and expensive) purification step, by crystallization or separation of diastereomers, several interesting methodologies for optimization of chiral catalysis have recently been published. The underlying basic concept has already been successful in “classic industrial chemistry” for the optimization of yields and product purity: The addition of suitable achiral additives and cocatalysts,^[7] which support the chiral catalyst system, enhance the yield and, surprisingly, in many cases also enhance the enantioselectivity very efficiently.^[8] In reality it is much more than an optimization, and the fact that achiral additives can dramatically change the enantiomeric excess of the products makes the subject so exciting.

In the following, this concept will be illustrated with several very promising recent examples, hopefully highlighting its usefulness for obtaining enantiomerically pure compounds. Naturally, additives not only help to optimize a reaction by increasing good *ee* values to excellent *ee* values, but can also efficiently improve reactions with low enantioselectivity to modest (good, or even very good) enantioselectivity. This will be shown in this review as well. For methodological research this effect should be important as it can sometimes suddenly pave the way for new catalytic concepts. It should be emphasized here that in most cases (but not all) the improved methodologies may still be far from what would be desired by industrial chemists and may not fulfill the prerequisites for

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technically feasible catalytic processes. But this is hopefully just a matter of time.

2. Mechanistic Considerations

The improvement of a catalytic enantioselective reaction may sometimes appear to be modern alchemy. Naturally, a priori predictions of which from a variety of additives may be beneficial are hard to make. However, it is also hard to explain why an additive is beneficial (at least in many cases). Although a confusing variety of reasons and several mechanistic effects which influence the catalysis are likely to exist and due to the complexity of the resulting catalytic systems, which make reproducible results difficult to obtain, the role of additives can only be elucidated after mechanistic studies. Until now such mechanistic studies have been carried out in only a few cases. Therefore, only general reasons (usually vaguely stated in the original papers) for how additives can function may be outlined here.

a) Probably one of the most common effects of additives is to deoligomerize nonactive (or less active) catalyst structures, resulting in the formation of the desired active monomeric catalyst species (or analogous di- and trimeric catalyst species).^[9] The homogenization of a heterogeneous catalytic system through the addition of suitable donors therefore represents a way to increase the number of active sites.

b) Bases are also often considered to enable a faster dissociation of the formed catalyst–product complex by

ligand exchange reactions.^[10] In that way the active catalyst is regenerated more rapidly and the activity is increased, which can often result in higher *ee* values as the reaction can then be carried out at lower temperature.

c) In particular basic additives may be able to coordinate to the metal center permanently or temporarily, thereby changing the geometry of the catalyst in a favorable way. This may accelerate the desired reaction by turning the catalyst into a more active species,^[11] or increase the *ee* value owing to a different chiral geometry around the active center. In addition this may be one of the more predictable effects of additives, as molecular modeling might be employed to understand the resulting geometry.

d) Through tuning of the ligand sphere or the electronic properties of the environment of the catalyst, the mechanistic pathway of the reaction may also be changed completely. Accordingly, the presence of halides as counterions can lead to a more efficient (neutral) pathway in a palladium-catalyzed reaction (see Scheme 10).

e) Additives might also serve as a “buffer”, for example, to maintain the water concentration at a defined level. Therefore, molecular sieves are sometimes applied even in combination with water to keep the moisture of a reaction at an optimized level.^[12] Other effects of molecular sieves can also be postulated (surface-assisted desorption of the products, alkali metal exchange^[13]).

f) Another additive effect should be mentioned which perhaps represents a challenge as it might also be utilized deliberately: the design of a catalyst poison for an undesired

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catalyst species. That is, upon treatment of a mixture of transition metal complexes with an additive, the complex which shows an undesired (nonenantioselective) catalytic activity, reacts highly selectively with the additive, resulting in the formation of an “inert” transition metal complex without any catalytic activity. The catalytic activity of the desired catalytic species, however, should not be influenced by these additives.

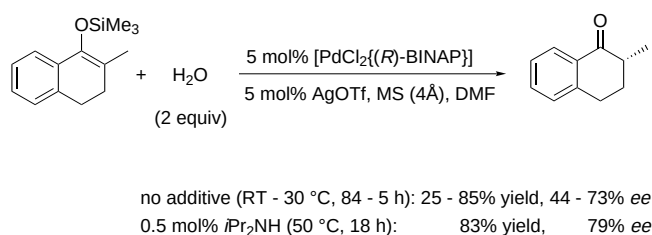
When trying to explain various additive effects, one should be well aware that our understanding is as yet rudimentary. The need for an automated screening process or even a combinatorial screening of additives should (if practically possible) also be considered. On the other hand, it would be desirable to obtain detailed mechanistic understanding of the role of additives.

3. Additives in Asymmetric Catalysis

3.1. Amines and Pyridines

Generally, nitrogen bases are the most common additives,^[14] and it has become usual practice to screen nitrogen bases (amongst other additives) to improve catalytic asymmetric processes. For the enantioselective hydrogenation of simple ketones catalyzed by a new Rh–diphosphane complex, such screening was carried out with careful variation of the Rh/additive ratio.^[15] For acetophenone as substrate the yield could be increased from 45 to 97 % and the enantiomeric excess from 57 to 95 % *ee* when 0.3 catalyst equivalents of 2,6-lutidine were added.

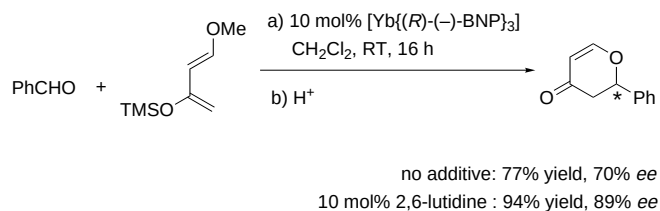
Also impressive is the finding by Nakai et al. that addition of a small amount of amine in a Pd-catalyzed protonation reaction to form chiral α -substituted carbonyl compounds gives improved results.^[16] In the absence of base only poorly reproducible yields and largely varying enantioselectivities have been obtained. In contrast, after addition of base the desired high yields and good enantioselectivities could always be reproduced (Scheme 1). Interestingly the positive effect of



Scheme 1. Protonation of silyl enol ethers.^[16] BINAP = 2,2'-bis(diphenylphosphanyl)-1,1'-binaphthyl; Tf = F₃CSO₂.

the additive was attributed to a quite unexpected scenario. Instead of activating the catalyst, the added diisopropylamine was causing a (desired) catalyst poisoning! The additive deactivates only the catalytic species that catalyzes the reaction with low or no enantioselection without altering the catalytic activity of the “surviving” desired Pd species much. This ensures that the reaction proceeds in high yields (83 %) and with good enantioselectivity (79 % *ee*).

Also for an asymmetric hetero-Diels–Alder reaction, catalyzed by a chiral Yb^{III}–phosphate complex, the addition of a base (here 2,6-lutidine) caused a jump from 70 to 89 % *ee* at room temperature (Scheme 2; BNP = 1,1'-binaphthyl-2,2'-



Scheme 2. Hetero-Diels–Alder reaction.^[17] TMS = Me₃Si.

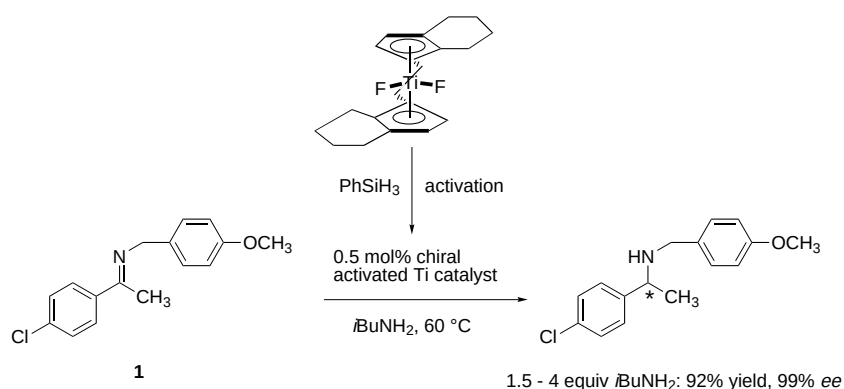
diyl phosphate).^[17] Since the reaction without an additive proceeds under heterogeneous conditions, and since the addition of bases increases the solubility of the catalyst in dichloromethane (catalyst solutions without precipitate are of advantage and generally seem to work better), the addition of basic ligands like hexamethyl phosphoramide (HMPA) has also been reported. However, in this case the reaction was seriously retarded, an effect we also observed for other systems. It is likely that when strongly coordinating additives are provided, the catalyst is easily deactivated or the catalyst structure collapses.

The asymmetric synthesis of chiral amines from imines is industrially important. An interesting additive effect has been observed recently by Buchwald et al., when investigating the asymmetric hydrosilylation of imines.^[18] Despite high TON and enantioselectivities, previous efficient hydrosilylation methods have been limited to imines that are not sterically hindered.^[19] This limitation has now been overcome by Buchwald et al. simply by adding an achiral base to the reaction mixture. Thus, in the presence of 0.05–1 mol % of a chiral titanium catalyst and isobutylamine (or *sec*-butylamine) as an additive, excellent enantioselectivities in the range of 91–99 % *ee* and enhanced yields of 86–97 % were obtained with a variety of sterically hindered substrates such as **1** (Scheme 3).

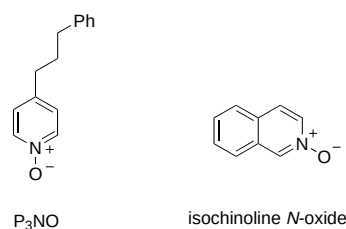
The importance of the additive can be recognized by looking at the model reaction with *N*-benzyl-1-indanimine. The authors found that the absence of an additive led to a conversion of only 5 %, whereas 100 % conversion and 92 % *ee* were achieved with isobutylamine as a base. Furthermore, in contrast to previous methods the high enantiomeric excesses do not depend on the *E/Z* ratios of the imines. Although the mechanistic course has not been clarified in detail, the added achiral amine appears to cleave the complex between the titanium catalyst and the chiral amino product. A representative example for the highly efficient asymmetric Buchwald hydrosilylation, in which an enantiomeric excess of 99 % was obtained, is shown in Scheme 3.

3.2. Pyridine *N*-Oxides

Solutions for industrial applications of chiral catalysis, here the manganese salen catalyzed asymmetric epoxidation of olefins, were recently reported by teams from Merck & Co.,

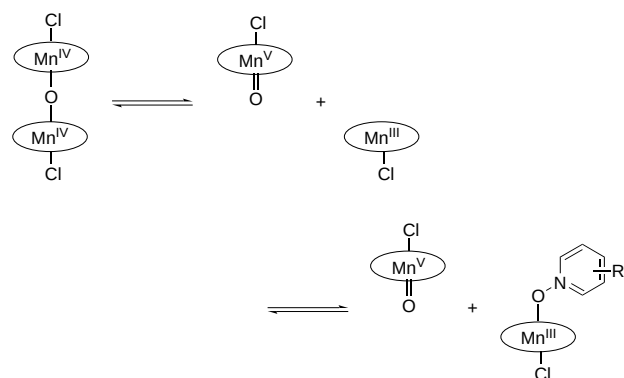
Scheme 3. Hydrosilylation of imines.^[18]

Inc.^[20] and SmithKline Beecham Pharmaceuticals.^[21] Both groups utilized pyridine *N*-oxide derivatives as additives; the oxidant was aqueous sodium hypochlorite. By screening several pyridine *N*-oxides, the Merck group found that for the chiral manganese salen catalyzed epoxidation of indene the addition of 4-(3-phenylpropyl)pyridine *N*-oxide (P_3NO , Scheme 4) gives the best results. The indene oxide product obtained is a chiral building block of the HIV protease inhibitor Indinavir.



Scheme 4.

In the case of a salen complex catalyzed enantioselective epoxidation to afford chiral 2,2-dimethyl-6-pentafluoroethylchromene oxide, the addition of isochinoline *N*-oxide allowed a decrease in catalyst loadings to a remarkable 0.1–0.4 mol%. In fact, the positive effect of the addition of pyridine *N*-oxide derivatives on enantioselectivity had been noted earlier.^[22] It was proposed that the effect of *N*-oxide additives could be due to the set of equilibria shown in Scheme 5.^[23] The active (salen) $Mn^V=O$ complex is thought to

Scheme 5. Proposed role of pyridine *N*-oxides in manganese-catalyzed epoxidations.^[23]

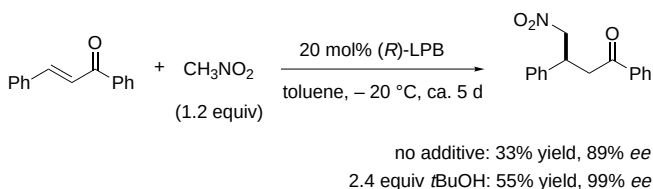
undergo a reversible coupling with a Mn^{III} complex to generate an inactive μ -oxo dimer. Through additive binding of the pyridine *N*-oxide derivative to the coordinatively unsaturated Mn^{III} complex, the equilibrium is shifted towards the Mn^V oxo intermediate. Owing to the increased concentration of the active Mn^V oxo species a rate acceleration of the epoxidation is then expected.

Very recently Komatsu et al. used pyridine *N*-oxide as an additive for the asymmetric aziridination of styrene derivatives by transfer of a nitrogen atom from a chiral nitridomanganese

complex.^[24] However, this reaction is not a catalytic process (yet).

3.3. Alcohols

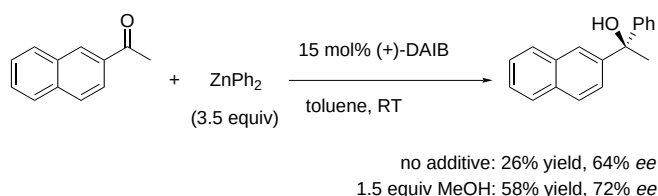
Even simple alcohols like *tert*-butyl alcohol have successfully been used as additives, and pronounced and surprising ligand effects could be observed.^[25] The asymmetric Michael addition of nitromethane to chalcone, catalyzed by a chiral lanthanum–potassium–BINOL complex (LPB complex; BINOL = 1,1'-binaphthol), proceeded in 33 % yield and with 89 % *ee*. After addition of 2.4 molar equivalents of *tert*-butyl alcohol (that is, 12 equivalents based on the catalyst), the yield increased to 55 % and the enantiomeric excess reached 99 % *ee* (Scheme 6). Here the exact amount of additive did

Scheme 6. Michael addition of nitromethane to enones.^[25]

not have a pronounced effect on the yield and enantiomeric excess (use of 20–480 mol % of additive gave comparable results). Similar improvements, using *tert*-butyl alcohol as an additive, were obtained with the substrate 4-chlorochalcone, suggesting that the effect may be general.

Also in the case of an asymmetric Heck reaction, the yields and *ee* values were surprisingly improved when *tert*-butyl alcohol was added.^[26] In terms of mechanism it was suggested that the additive might prevent a specific substrate–palladium interaction which produces oxidation side products.

It has also been reported that even when organometallic reagents are used, an additive like methanol, with an acidic proton, can have positive effects. Dosa and Fu investigated the catalytic asymmetric addition of $ZnPh_2$ to ketones, the chiral information being provided by an amino alcohol (3-*exo*-(dimethylamino)isoborneol, DAIB; Scheme 7).^[27] Although a promising level of enantioselectivity was observed in the

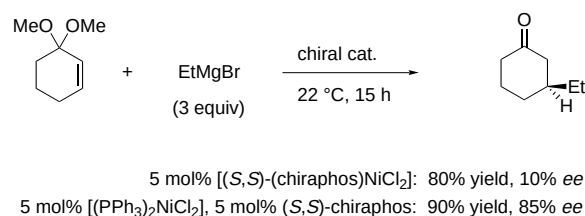
Scheme 7. Addition of ZnPh_2 to ketones.^[27]

absence of an additive (64% *ee*), the yield of the product was low (26%). In the presence of 1.5 equivalents of methanol the yield increased to 58% and the *ee* value reached 72% for the substrate shown in Scheme 7. For other substrates *ee* values up to 91% could be obtained in the presence of methanol, which was added to the DAIB/ ZnPh_2 /toluene mixture before the reaction, and may alter the nature of the zinc species in solution. As frequently seen when metal alkoxides are incorporated in the catalyst structure, there was a nonlinear relationship between the *ee* values of product and catalyst.

3.4. P-Based Additives

3.4.1. Phosphanes

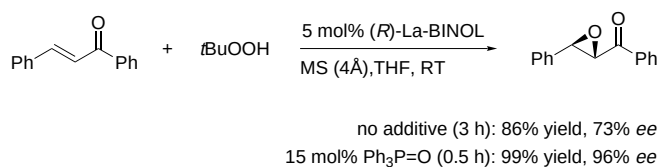
Surprisingly, there seems to be little limitation for beneficial effects of additives in terms of the type of reaction. A systematic investigation, this time of the influence of various achiral phosphane additives on the enantioselectivity of a Ni-catalyzed asymmetric addition of Grignard reagents to unsaturated cyclic acetals (Scheme 8), was reported by

Scheme 8. Addition of Grignard reagents to unsaturated cyclic acetals.^[28]

Hoveyda et al.^[28] Trying to avoid a “background reaction” arising from the action of an achiral catalyst, the authors first isolated (prior to use) the catalytically active (*S,S*)-(chiraphos) NiCl_2 complex prepared from [$(\text{PPh}_3)_2\text{NiCl}_2$] and (*S,S*)-chiraphos (chiraphos = 2,3-bis(diphenylphosphanyl)butane). However, later it was observed that in some cases the catalyst prepared in situ actually provided superior selectivity (see Scheme 8) due to the additional achiral phosphane ligands present in the mixture. Product *ee* values from lower than 5 up to 82% were then obtained for a test reaction when different phosphanes were added to the (*S,S*)-(chiraphos) NiCl_2 catalyst.

3.4.2. Phosphane Oxides

A pronounced ligand effect on the enantioselectivity in the chiral lanthanum complex catalyzed asymmetric epoxidation of enones was found by Inanaga and co-workers (Scheme 9).^[29] In the reaction of chalcone, simply addition of

Scheme 9. Epoxidation of enones.^[29]

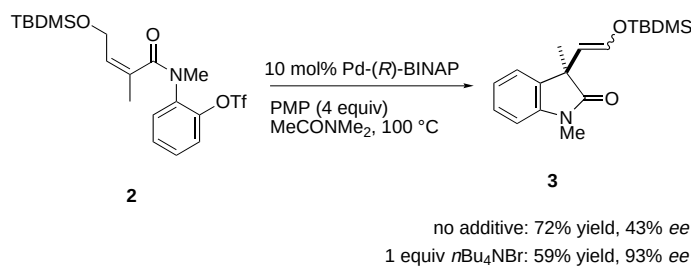
triphenylphosphane oxide led to an increase in the *ee* value of the epoxide products from 73% (no additive) to 96% with simultaneous improvement of the yield from 86 to 99%. It is perhaps interesting to point out the subtleness of the process by considering the precise nature of the additive. Whereas similarly good results have been obtained upon addition of tri-*p*-tolylphosphane oxide (95% yield and 94% *ee* after 1 h), with tri-*o*-tolylphosphane oxide the *ee* value dropped back to 73% (96% yield). With *n*-butylphosphane oxide as an additive the results have been virtually the same as without an additive (88% yield and 73% *ee*). The method was also found to be quite general for other enones. To rationalize the beneficial effect of Ph_3PO , it has been suggested that the additive might prevent an oligomerization of the catalyst (the metal precursor was added to a mixture of phosphane oxide and ligand), or it might depolymerize the catalyst after formation. The amount of molecular sieves (MS) was also found to have a strong influence on the *ee* value. Future experiments should prove important for clarifying how even molecular sieves can influence the *ee* value. To be able to carry out practical optimizations of catalytic processes, it would be necessary to know if, for example, owing to kinetic advantages a dissociation of the products is assisted or if molecular sieves perhaps just control the exact amount of water.^[30]

The addition of phosphane oxide was also very effective in the cyanosilylation of aldehydes utilizing a new Lewis base/Lewis acid catalyst (with general applicability to aromatic, aliphatic, and α,β -unsaturated aldehydes), and very high *ee* values have been obtained.^[31] In fact initially the *ee* values were below 10%, but after the addition of phosphane oxide and with very slow addition of the substrate (syringe pump) it was possible to obtain the products with up to 98% *ee* and even in improved yields.

3.5. Ionic Additives

3.5.1. Halides

A clear picture of the effect of salt additives, like tetrabutylammonium halides, was recently drawn by Overman and Poon for the asymmetric Heck reaction.^[32] The cyclization of the (*Z*)-butenamide triflate **2** with Pd-(*R*)-BINAP leads to (*R*)-oxindole **3** with 43% *ee* (Scheme 10). However, after addition of only one molar equivalent of tetrabutylammonium bromide, product **3** was obtained with a creditable 93% *ee*. In the presence of such a halide additive, the crucial insertion step of the reaction is diverted to a neutral pathway via a neutral intermediate. (Contrary to this, a cationic pathway is generally assumed to be favored in the presence of silver salts.) In the case of substrate **2**, the neutral



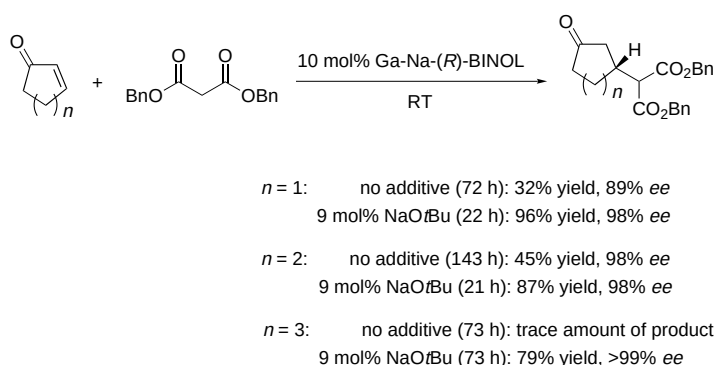
Scheme 10. Heck reaction.^[32] PMP = 1,2,2,6,6-pentamethylpiperidine; TBDMS = *t*BuMe₂Si.

pathway seems to proceed with a much higher degree of stereoselection than the cationic pathway, therefore yielding the product with high *ee* values. Such improved distinction between two competing pathways with a different degree of stereoselection seems to be one of the more common reasons why achiral additives can be so effective.

In a previous paper Ashimori and Overman showed that both enantiomers of a spirooxindole similar to **3** could be synthesized, although the same chiral information ((*R*)-BINAP) was used. Depending only on the additives, the *ee* value obtained changed from 71 % with Ag₃PO₄ to 66 % of the opposite enantiomer when 1,2,2,6,6-pentamethylpiperidine was present.^[33]

3.5.2. NaOR/BuLi

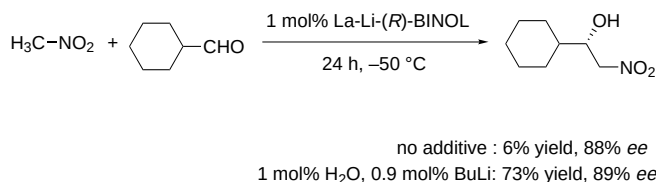
Heterobimetallic complexes have been reported to self-assemble with additional (catalytic) amounts of NaOR or BuLi to provide catalysts of improved efficiency.^[34] Results of the enhancement of the efficiency for an asymmetric Michael reaction utilizing a Ga-Na-(*R*)-BINOL complex are depicted in Scheme 11. When sodium dibenzyl malonate (9 mol %) was added instead of NaOR, the same effect could be obtained.



Scheme 11. Michael reaction.^[34]

By using a similar activation strategy, catalytic asymmetric nitroaldol reactions could also be improved considerably (Scheme 12). It was proposed that by combined addition of water and BuLi a LiOH species is generated that is incorporated into the structure of La-Li-BINOL.

Noyori et al. demonstrated previously that the addition of KO*t*Bu or KOH in combination with diamine ligands is very beneficial for the enantioselective hydrogenation of aromatic



Scheme 12. Nitroaldol reaction.^[34]

ketones. Very excellent yields and *ee* values have been reported utilizing the BINAP–Ru^{II} catalyst system.^[35]

Since, for example, BuLi as an additive is very reactive, it may not be applicable for too many other systems. It may, however, be suggested that the addition of quite reactive species to a catalyst mixture might in general still be interesting initially, as they may be transformed into truly beneficial additives in situ (BuLi to LiOH).

3.5.3. Acetic Acid and Halides

A new catalyst system currently applied technically for homogeneous enantioselective hydrogenation was recently developed by a research team of Ciba-Geigy.^[36] Acetic acid and iodide (added as Bu₄NI) were found to be crucial additives. For the enantioselective synthesis of a number of important acyl anilide pesticides—the most important example being the herbicide metolachlor^[37]—an asymmetric hydrogenation of *N*-arylimines can be utilized. However, the activity of existing chiral catalytic systems had been far too low. The use of a new class of iridium ferrocenyl diphosphane complexes, which turned out to be very stable under reaction conditions, gave very active and productive catalysts. However, the breakthrough on the way to a production process of metolachlor came only with the addition of both acetic acid and iodide. Ligand optimization showed that [(Ir(cod)Cl)₂/xylyphos^[38] (cod = 1,5-cyclooctadiene, xylyphos = (*R*)-1-[(*S*)-2-(diphenylphosphanyl)ferrocenyl]ethylidene(3,5-dimethylphenyl)-phosphane) was the optimal catalyst, and under a hydrogen pressure of 80 bar and with a remarkable substrate/catalyst ratio of 750 000–1 000 000 complete conversion could be reached within three to four hours at 50 °C. The optical yields have only been moderate (78% *ee*), but the TOF was excellent (350 000 h^{−1}). It is difficult to explain why iodide and acid have such a remarkable (synergetic) effect. Until now, the mechanism responsible could not be clarified in detail.

4. Summary and Outlook

A number of very recent examples of how addition of, for example, nitrogen bases, alcohols, phosphanes, phosphane oxides, pyridine *N*-oxides, halides, or acid can positively effect asymmetric catalytic reaction systems was discussed. In our approach we deliberately covered all sorts of organic reactions, knowing that each case may be different and mechanistic similarities may not exist even for the same additive.

Only weakly coordinating ligands (for example, phosphane oxides or pyridine *N*-oxides) could perhaps be seen among the most promising additives. In some cases such ligands might

temporarily occupy the empty reactive sites of catalyst complexes while the catalyst is “at rest”. This could perhaps be developed to effect a general catalyst protection or stabilization.^[39]

To date, in many instances additives have been found by chance rather than by logical prediction. We imagine that the recent developments and reaction systems selected could provide a pool of ideas for optimizing industrial processes, as well as being of practical value for similar research on asymmetric reactions in general.^[40] Clearly the most urgent goal of academic research in asymmetric catalysis is to rationally reduce the amount of catalyst—and additives may play a crucial role in this process.

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RNA and the Small Molecule World

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Our understanding of the flow of biochemical information from deoxyribonucleic acid (DNA) to ribonucleic acid (RNA) and then to proteins has evolved enormously in the last few decades.^[1, 2] DNA sequences coding for specific genes are transcribed in the cell nucleus to yield heterogeneous nuclear RNAs. These primary transcripts are processed to yield mature messenger RNAs that are then transported to the cytoplasm where translation, namely, protein synthesis, takes place in the ribosomes. The transport, stability, cellular localization, and translation efficiency of individual messenger RNA molecules are all well controlled. This multi-level regulation, mediated by numerous RNA-binding proteins and ribonucleoprotein complexes, ultimately governs gene expression and its functional manifestation.^[3] RNA is therefore no longer viewed as a passive carrier of genetic information. Rather, it is appreciated as a structurally and functionally sophisticated biomolecule intimately involved in key cellular processes. As such, it is quickly becoming a central target for drug design.^[4, 5] It is illustrative to follow how RNA has assumed this pivotal role in modern bioorganic chemistry.^[6]

Experimental evidence correlating RNA with protein synthesis started emerging in the late 1930s from the laboratories of Caspersson and Brachet. In their 1939 *Nature* paper, Caspersson and Schultz stated: "The presence of pentose nucleotides in high concentrations in rapidly dividing tissues is probably thus a general phenomenon".^[7] At about the same time, small, RNA-rich particles, initially named microsomes and later ribosomes, had been noticed by Claude.^[8] In 1946 (at the first major biological symposium focusing on nucleic acids), Brachet put forward the hypothesis that these granules are responsible for protein biosynthesis.^[9] Classic electron microscopy work by Palade,^[10] and pulse-chase experiments by Zamecnik,^[11] provided the necessary experimental support for this hypothesis almost a decade later.^[12]

In summarizing a 1947 *Experientia* paper by Boivin and Vendrely, an anonymous editor concluded: "Through catalytic actions the macromolecular desoxyribonucleic acids govern the building of macromolecular ribonucleic acids, and, in turn, these control the production of cytoplasmic enzymes".^[13] This statement, although formulating the es-

sence of biological information flow, did not specify the exact components and mechanisms involved. After the 1953 elucidation of the double helical structure of DNA by Watson and Crick,^[14] attention turned to the roles RNA plays in gene expression. The view held in the late 1950s was that each gene controls the synthesis of a single specialized ribosome, which in turn governs the synthesis of a specific protein. In this scheme, epitomized as the one gene–one ribosome–one protein hypothesis, ribosomal RNA was postulated to be the template for protein synthesis. The difficulties encountered with this model led Jacob and Monod to hypothesize that the information is carried by a short-lived RNA molecule and that the ribosomes were nonspecialized structures.^[15] Their 1961 prediction was soon confirmed by the discovery of messenger RNA (mRNA), a labile template that turns over rapidly.^[16] The fundamentals of translation began to unfold when a soluble RNA, named transfer RNA (tRNA), was shown to be involved in protein synthesis by carrying an activated amino acid,^[17, 18] an observation that supported Crick's adaptor hypothesis.^[19] In 1966, the genetic code—an algorithm relating triplets of nucleotides in genes to specific amino acids in proteins—was established.^[20]

The central machinery for protein biosynthesis, where both decoding and peptidyl transferase take place, is the ribosome: a complex of ribosomal RNA (rRNA) and numerous proteins.^[21] This large and as yet unsolved structure comprises 85 % of all cellular RNA. Intriguing experiments reported by Noller and co-workers in 1992 suggested that protein-depleted rRNA has, by itself, a peptidyl transferase activity.^[22] These fascinating observations, indicating catalytic activity of rRNA, have been very recently substantiated by Watanabe et al. They showed that the six domains of the 23S rRNA of *E. coli* stimulated peptide-bond formation when assembled together in the absence of any ribosomal proteins.^[23] Taken together with the well-characterized RNA enzymes (ribozymes),^[24] RNA is once more taking center stage as a key functional biomolecule.^[25]

The functional diversity of RNA can be attributed to the intricate three-dimensional folds it can assume through a multitude of secondary structures and tertiary interactions.^[26] This structural sophistication, together with the spatial projection of functional groups and the electrostatic field generated by the RNA fold, create potential binding pockets for ions, small molecules, and proteins.^[27] Ideally, one should be able to take advantage of this promising recognition capacity to identify common binding motifs and utilize them

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for the design of new RNA binders as modulators of cellular functions.^[5]

The search for small molecules as RNA binders has been inspired by early observations that indicated the ability of antibiotics to interfere with protein biosynthesis.^[28] In a seminal paper published in 1987, Noller and Moazed showed that several classes of antibiotics, among them aminoglycosides, interact with functional sites on 16S rRNA.^[29] This discovery, suggesting the direct binding of low molecular weight drugs to rRNA, triggered a renewed interest in RNA–ligand recognition. Since then, aminoglycoside antibiotics have been shown to interact with a number of functional RNA molecules including group I introns,^[30] hammerhead ribozymes,^[31] and the human hepatitis delta virus ribozyme.^[32] Their binding to the A-site analogue of 16S rRNA has been established,^[33–35] and their interactions with tRNA have been studied in detail.^[36] Aminoglycoside-specific RNA aptamers have been selected in vitro,^[37] and NMR studies have revealed the structures of the complexes with their cognate aminoglycosides.^[38] Certain aminoglycoside antibiotics and synthetic ligands have been shown to interfere with RNA–protein binding,^[39–43] Issues of specificity in RNA–ligand interactions,^[44] as well as binding mechanism and dynamics have been recently addressed.^[45–47] Although the challenges are still enormous, RNA has become a promising and fertile target for ligand design.^[5]

Werstuck and Green have recently added an exciting new dimension to this rapidly growing area of RNA recognition. They have demonstrated that gene expression can be controlled in living cells through specific interactions between RNA and small molecules.^[48] Specifically, Werstuck and Green engineered unique RNA sequences into bacterial and mammalian mRNA and mediated essential cellular functions by adding small and cell-permeable molecules. Although artificially constructed, the systems reported, provide a proof of principle and the first demonstration that small molecules can act as cellular regulators at the mRNA level.

Werstuck and Green have chosen RNA aptamers, short RNA sequences that are selected in vitro to bind small molecules with high affinity and selectivity, as the targeted sites. Initially, aptamers recognizing tobramycin and kanamycin, two aminoglycoside antibiotics, have been employed (Figure 1). Plasmids expressing these RNA aptamers have been constructed by cloning the corresponding DNA sequences into a T7 RNA polymerase-driven expression vector. An *Escherichia coli* strain containing an inducible T7 RNA polymerase was then transformed with these plasmids. To test for the in vivo function of these RNA sequences, Werstuck and Green have looked for a drug-resistant phenotype. Thus, bacteria expressing the kanamycin-binding aptamer were able to grow in the presence of kanamycin A, the cognate bactericidal antibiotic. In contrast, the growth of untransformed bacteria and a strain producing a tobramycin-binding aptamer was negligible. Similarly, bacteria containing the tobramycin-recognizing aptamer were able to grow in the presence of tobramycin, the

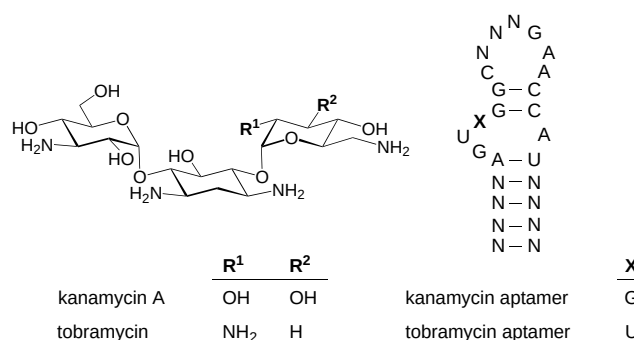


Figure 1. Aminoglycoside antibiotics and their corresponding RNA aptamers.^[48]

cognate antibiotic, while their growth was significantly repressed by kanamycin A, the noncognate small molecule.^[48]

This series of experiments established that a drug-resistant bacterial strain can be realized by the expression of a drug-binding RNA molecule. These RNA aptamers generated in vivo act as a scavenger of a substance that otherwise would be toxic to the cell. In certain respects, the approach taken by Werstuck and Green is reminiscent of actual resistance mechanisms that operate in bacteria. The most prominent mechanism for the acquired microbial resistance to aminoglycosides is the inactivation of these antibiotics by plasmid-encoded enzymes.^[49] Numerous enzymes that phosphorylate, adenylylate, or acetylate the aminoglycosides have been identified. It is worth noting that these aminoglycoside-modifying enzymes have severely hampered the clinical usefulness of aminoglycoside antibiotics.

To demonstrate the utilization of small molecules as modulators of eukaryotic gene expression, Werstuck and Green have focused on regulating the translation initiation.^[48] The template for this process, the eukaryotic mRNA, is commonly posttranscriptionally modified at the 5'-end by a 7-methylated guanosine nucleotide, which acts as a cap. Translation is typically preceded by a scanning process through the 5'-untranslated region (UTR)—the mRNA sequence spanning between the 5'-cap and a start codon (Figure 2). In this key process a pre-initiation complex composed of a ribosomal subunit, initiation factors, and a charged initiator tRNA^{Met}, migrates along the 5'-UTR until it encounters a start codon, normally the first AUG triplet. Translational regulation via the 5'-UTR can therefore be mediated by several mechanisms, including modification at

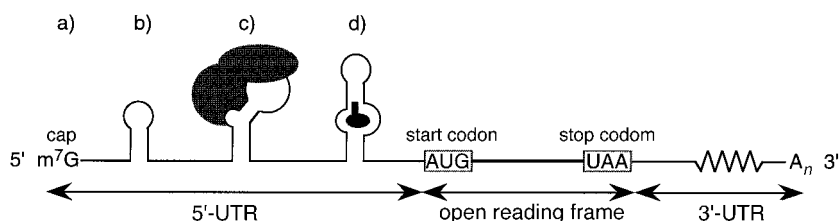


Figure 2. Schematic illustration of functional elements and their organization within eukaryotic mRNA. Translational regulation at the 5'-UTR can be mediated by a) modification of the cap structure, b) a stable secondary structure, c) RNA–protein interactions, and, as hypothesized by Werstuck and Green, d) interactions between RNA and small molecules. Note that additional regulation elements that exist in the 3'-UTR are not shown.^[3b]

the cap structure (Figure 2a), the introduction of stable secondary structures into the UTR (Figure 2b), and through protein–RNA binding within the UTR (Figure 2c).^[3b, 50] Werstuck and Green hypothesized that the incorporation of a stable small molecule–aptamer complex into the 5'-UTR may impede the initiation apparatus by blocking the scanning processes or the ribosome–mRNA binding (Figure 2d).

Aminoglycoside antibiotics are highly charged at physiological pH values, relatively impermeable, and are known to interfere with translation. Werstuck and Green have therefore selected RNA aptamers that specifically bind the benzimidazole-containing Hoechst dye H33258, a known DNA minor groove binder (Figure 3). Two aptamers were then inserted

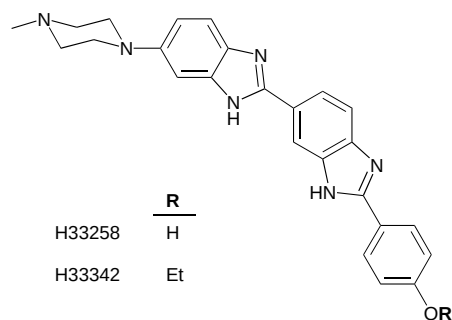


Figure 3. Structure of Hoechst dyes H33258 and H33342. H33258 was used to generate RNA aptamers, and the more soluble derivative H33342 was used to regulate gene expression in living cells that express the corresponding aptamer.^[48]

into the 5'-UTR of a mammalian β -galactosidase expression plasmid, and Chinese hamster ovary cells were cotransfected with the engineered vector and a luciferase reporter gene. To test for the in vivo function of these RNA aptamers, Werstuck and Green have analyzed for β -galactosidase and luciferase activities after translation took place, as an indication for the successful expression of these genes. In the absence of a Hoechst dye, the incorporated aptamers did not influence gene expression, which indicated that the translation machinery had not been affected by the presence of these “foreign” constructs within the 5'-UTR. In contrast, in the presence of increasing concentrations of H33342 (a closely related more soluble Hoechst dye; Figure 3), the β -galactosidase activity had dropped substantially with no effect on the luciferase control activity.^[48]

This set of experiments illustrates how small organic molecules can, in principle, be used to control gene expression in living cells. The ability to regulate the translation of exogenous genes with small molecules that act as a switch opens up exciting opportunities. It may be beneficial for gene therapy applications, where genes containing a built-in switch are introduced and their expression is kept under the control of the cognate small molecule. It may also be useful for manipulating specific genes as a tool to investigate the biological outcome of their expression.

The elegant use of RNA aptamers to create controllable genes circumvents a major problem of RNA binding specificity. RNA aptamers, selected under highly stringent conditions, typically show extremely high affinity and specificity for their

cognate ligands. This is not the case with “naturally occurring” RNA sequences and their known ligands. Aminoglycoside antibiotics, for example, are rather nonspecific RNA binders. They bind numerous RNA sequences with similar affinity. Their promiscuous binding is likely to result from their recognition of similar RNA folds rather than sequences.^[5] In certain cases aminoglycoside binding is accommodated by a conformational change in the RNA host.^[35c] These issues, together with the minute cellular amount of mRNA relative to rRNA and tRNA, makes the de novo design of highly selective mRNA binders a formidable task. The in vivo utilization of small molecules as regulators of endogenous gene expression remains a major challenge. It is likely to get resolved as our knowledge of RNA structure, folding, and recognition advances.

The area of small molecule recognition by RNA is growing rapidly as fast screening methods for evaluating RNA affinity and specificity of potential binders are developed.^[51] Studies of structure–activity relationships of combinatorial libraries complemented by novel naturally occurring products will further advance our understanding of the interactions between RNA and small molecules. As our knowledge of RNA–protein interactions evolves, new RNA targets will be identified and targeted by small molecules. We are likely to encounter small molecules that thrive as specific RNA binders.

The last two decades have brought bacterial and viral infections to center stage. Numerous new viruses have appeared. Their effect may be inapparent (for example, hepatitis G), yet in other cases it might have devastating consequences (for example, HIV).^[52] Concurrently, there has been a resurgence of previously known viral and bacterial diseases, as well as an emergence of resistant mutants of well-studied pathogens.^[53] These changes have produced new targets for antiviral and antibacterial therapy and present a major challenge to the scientific community. The thrilling days of antibiotics discovery, less than 60 years ago, have now been replaced by a deep concern for the future, and the necessity to uncover new approaches for antiviral and antibacterial therapy. Since RNA molecules play key roles in protein synthesis, transcriptional regulation, and viral replication novel antibiotic and antiviral agents may well be found within small molecules that target RNA sites.

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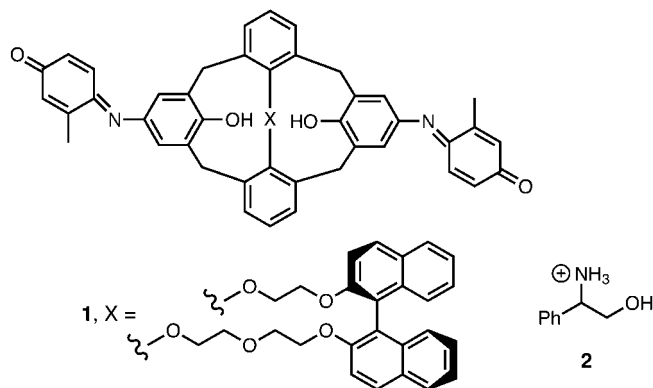
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Asymmetric Phase-Transfer Catalysis

Adam Nelson*

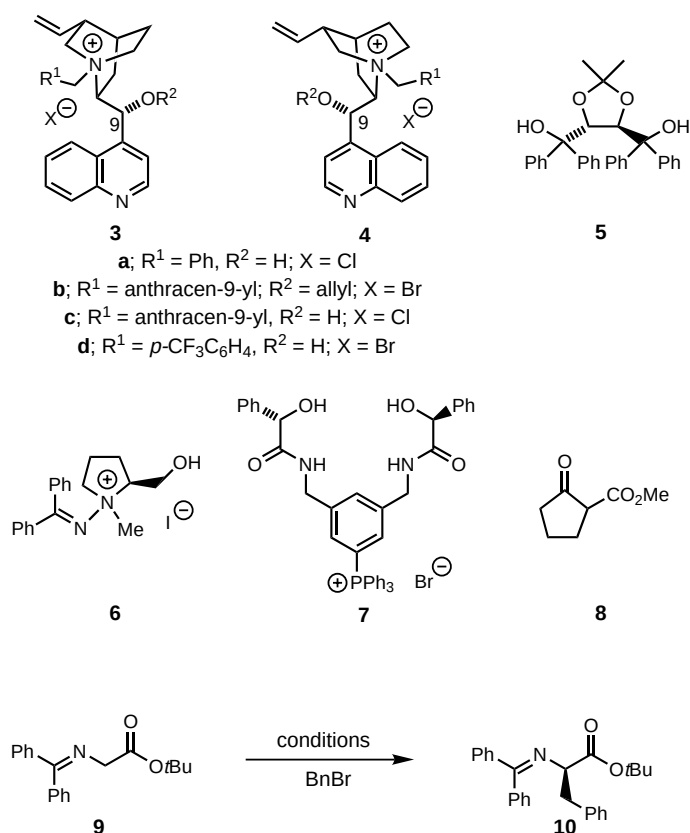
The selective recognition of enantiomeric ions and the efficient transmission of stereochemical information between contacting ions remain important goals for many chemists.^[1–3] For example, the molecular sensor^[1] **1** can distinguish between the enantiomers of primary ammonium ions **2** and reports this



information as a discernible color change; (*S*)-camphor-10-sulfonate can direct the enantioselective folding of a protonated polyguanine into helices of predominantly one handedness.^[2]

Phase-transfer catalysts can be effective tools for accelerating^[4] and controlling the regiochemistry^[5] of synthetic reactions. Although isolated examples of highly enantioselective phase transfer catalyzed reactions have been known for many years,^[6] some “high” enantioselectivities claimed by early workers have been attributed to trace impurities with high optical rotations.^[7] Recently, however, the structural features of cinchonidinium (**3**) and cinchoninium salts (**4**) which are necessary for effective asymmetric phase-transfer catalysis (PTC) have been unravelled.^[3]

A key discovery was that the size of the R¹ substituent of salts **3** and **4** has a profound effect on the enantioselectivity of alkylation of the glycine derivative **9** (Scheme 1, Table 1). The benzyl cinchoninium salt **3a** induces a modest level of enantioselectivity in the reaction of **9** with benzyl bromide



Scheme 1. Alkylation of **9** under PTC conditions; see Table 1 for details.

(entry 1, Table 1).^[8] Corey et al.^[3] as well as Lygo and Wainwright^[9] have found that by changing the quaternary ammonium substituent R¹ to the bulkier anthracen-9-ylmethyl group, good to excellent levels of enantioselectivity can be obtained through the use of either solid–organic or aqueous–organic biphasic systems (entries 2–4, Table 1). An important feature of this methodology is that the cinchonidinium salts **3** and the cinchoninium salts **4** induce almost equal levels and opposite senses of enantioselectivity (compare

Table 1. Asymmetric PTC alkylation of **9** with benzyl bromide.

Entry	Cat. ^[a]	<i>T</i> [°C]	<i>t</i> [h]	Reagents	Solvent	Product	<i>ee</i> [%]	Yield [%]	Ref.
1	3a	25	22	NaOH	H ₂ O	(<i>R</i>)- 10	66	75	[8]
2	3b	–78	22	CsOH · H ₂ O	CH ₂ Cl ₂	(<i>R</i>)- 10	99.5	73	[3]
3	3c	25	18	KOH	H ₂ O/PhMe	(<i>R</i>)- 10	89	63	[9]
4	4c	25	18	KOH	H ₂ O/PhMe	(<i>S</i>)- 10	91	68	[9]

[a] In each case 10 mol % of the catalyst was used.

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entries 3 and 4, Table 1). Optically active unnatural amino acids^[10] can be prepared by alkylation of **9**, and an extended enolate has also been alkylated with high levels of enantioselectivity using this technology.^[11]

A new approach to the design of phase-transfer catalysts has recently been reported; Corey et al. studied the X-ray

crystal structures of cinchonidinium salts and formulated a model which explains the highly enantioselective alkylation of the enolate of **9**.^[3] The positive charge of ammonium salts is delocalized onto the carbon and hydrogen atoms surrounding the quaternary nitrogen atom; the enolate **11** is in close contact with the chiral ammonium salt and is alkylated highly enantioselectively. A model^[3] which has been proposed to explain the efficient transfer of stereochemical information between the ammonium salt **3b** and the enolate **11** is summarized in Figure 1.

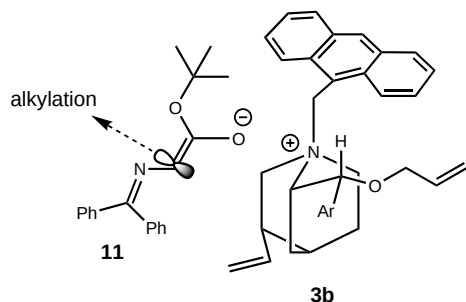
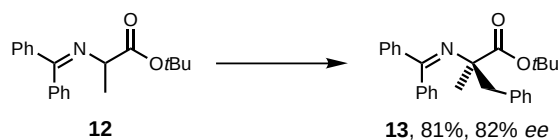


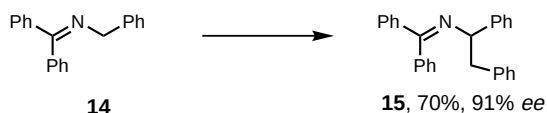
Figure 1. Transition state proposed to explain the enantioselective alkylation of the enolate **11**.

Although alkylations of the glycine derivative **9** have become standards by which chiral phase-transfer catalysts are judged, other asymmetric alkylations under PTC conditions have been studied. For example, 10 mol % of the TADDOL **5** can mediate asymmetric alkylation of **12**, giving the product **13** with up to 82% *ee* (Scheme 2; TADDOL = $\alpha,\alpha,\alpha',\alpha'$ -tetraaryl-2,2-dimethyl-1,3-dioxolan-4,5-



Scheme 2. Conditions: 10 mol % of **5**, BnBr, NaOH, PhMe, 15–24 h, room temperature.

dimethanol).^[12] The hydrazone salt **6** (2 mol %) greatly accelerates the alkylation of the Schiff base **14**, and alkylated products **15** are obtained with high enantiomeric excess (Scheme 3).^[13] Manabe has prepared the chiral quaternary

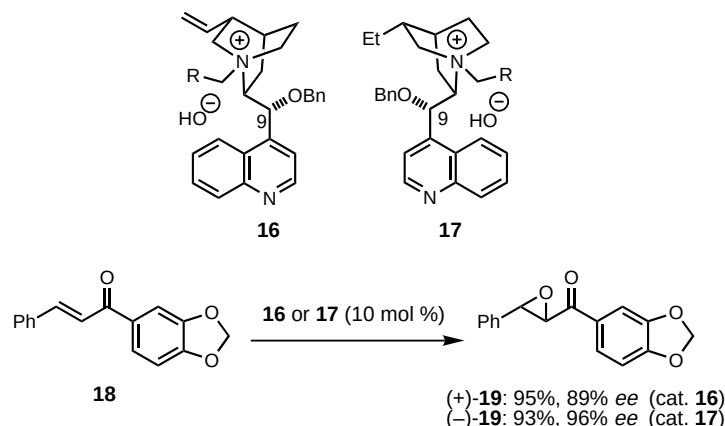


Scheme 3. Conditions: 2 mol % of **6**, BnBr, K₂CO₃, KOH, CH₂Cl₂, 24 h, room temperature.

phosphonium salt **7** with a multiple hydrogen bonding site; this salt accelerates the alkylation of the ketoester **8**, giving products with about 40% *ee* at room temperature.^[14]

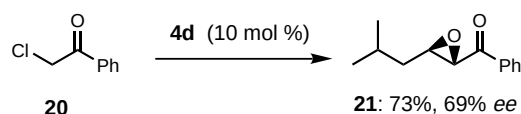
Chiral phase-transfer catalysts have now been exploited in a wide range of reactions with anionic intermediates. For example, the salts **16** and **17** have been used to catalyze the

nucleophilic epoxidation of enones (e.g. **18**) to give either enantiomer of epoxides such as **19** (Scheme 4).^[15] Once again, the large anthracen-9-ylmethyl substituent is thought to have a profound effect on the enantioselectivity of the process.



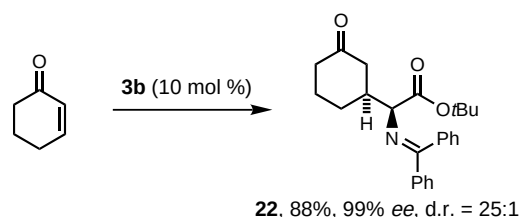
Scheme 4. Conditions: 11 % aq. NaOCl, PhMe, 48 h, room temperature. R = anthracen-9-yl.

Epoxides similar to **19** can also be made enantioselectively by an asymmetric Darzens reaction under PTC conditions; here, the cinchoninium salt **4d** allowed the epoxide **21** to be prepared with reasonably high enantiomeric excess (Scheme 5).^[16] Michael additions, too, can be rendered



Scheme 5. Conditions: *i*BuCHO, LiOH · H₂O, Bu₂O, 134 h, 4 °C.

asymmetric by adding a chiral phase-transfer catalyst. For example, in the presence of 10 mol % of **3b**, the enolate of **9** added to cyclohexenone with excellent diastereoselectivity to give the ketoester **22** with greater than 99% *ee* (Scheme 6).^[10]



Scheme 6. Conditions: CsOH · H₂O, **9**, CH₂Cl₂, –78 °C.

Several families of efficient chiral phase-transfer catalysts are now available for use in asymmetric synthesis. To date, the highest enantiomeric excesses (> 95% *ee*) are the exclusive preserve of salts derived from cinchona alkaloids with an anthracen-9-ylmethyl substituent on the bridgehead nitrogen atom (e.g. **3b**, **4b**). These catalysts will be used to improve the enantioselectivity of existing asymmetric PTC reactions and will be exploited in other anion-mediated processes both in the laboratory and industrially.

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Keywords: alkylations • asymmetric catalysis • epoxidations
• molecular recognition • phase-transfer catalysis

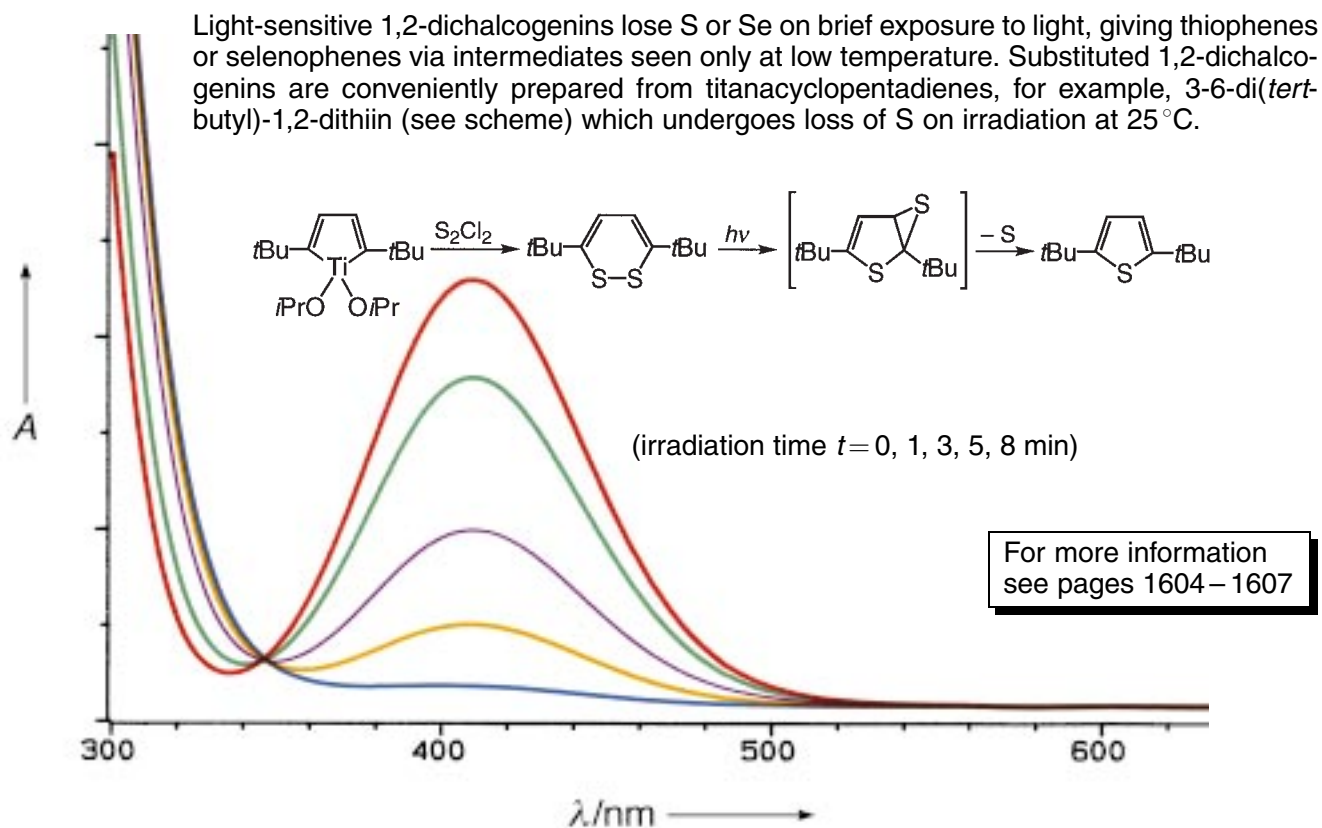
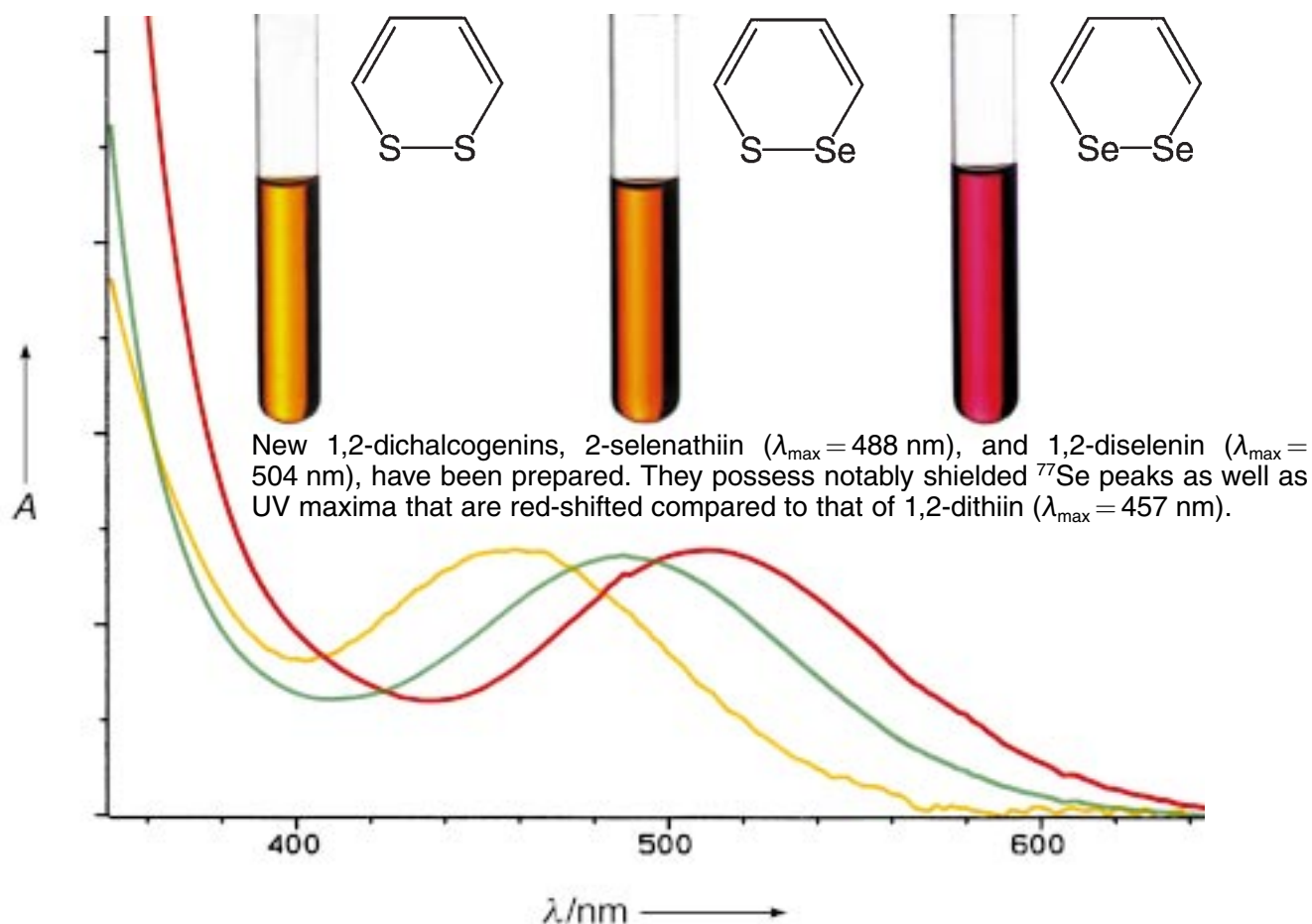
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Deposition of Data from X-Ray Structure Analyses

In order to make life easier for authors and referees the Cambridge Crystallographic Data Centre (CCDC) and the Fachinformationszentrum Karlsruhe (FIZ) have unified their procedures for the deposition of data from single-crystal X-ray structure analyses.

Prior to submitting a manuscript please deposit the data for your compound(s) **electronically** at the appropriate data base, that is, at the CCDC for organic and organometallic compounds and at the FIZ for inorganic compounds. Both data bases will be pleased to provide help (see our *Notice to Authors* in the first issue of this year). In general, you will receive a depository number from the data base within two working days after electronic deposition; please include this number with the appropriate standard text (see our *Notice to Authors*) in your manuscript. This will enable the referees to retrieve the structure data quickly and efficiently if they need this information to reach their decision.

This is now the uniform procedure for manuscripts submitted to the journals *Advanced Materials*, *Angewandte Chemie*, *Chemistry—A European Journal*, *the European Journal of Inorganic Chemistry*, and *the European Journal of Organic Chemistry*.



Triple Helicate—Tetrahedral Cluster Interconversion Controlled by Host–Guest Interactions**

Markus Scherer, Dana L. Caulder, Darren W. Johnson, and Kenneth N. Raymond*

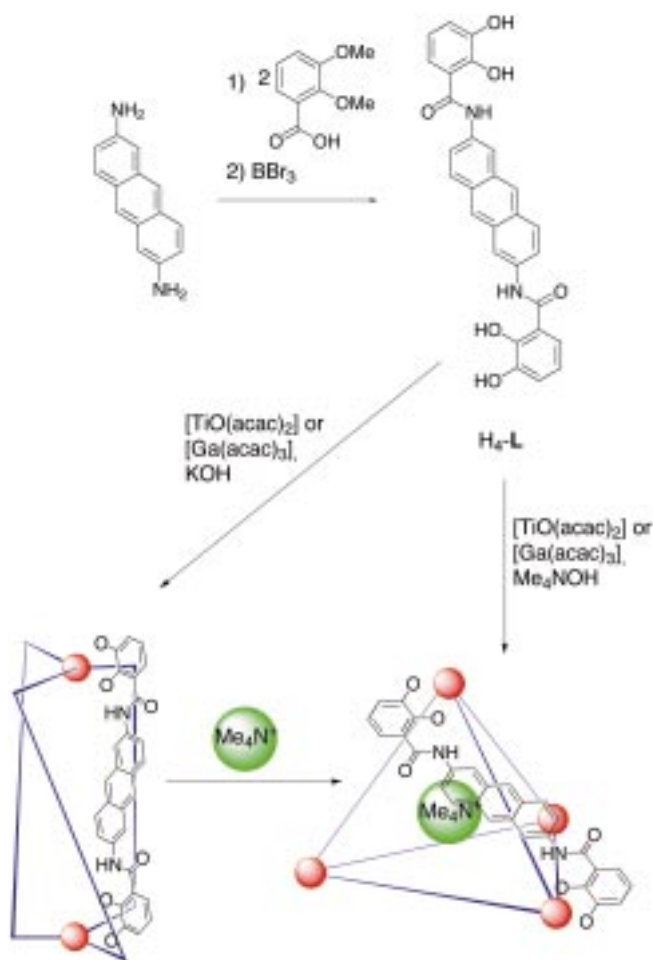
Dedicated to Professor Thomas Kruck on the occasion of his 65th birthday

Whereas natural clusters and synthetic analogues^[1] utilize hydrogen bonds and other numerous weak interactions, the use of stronger, highly directional, and well-studied metal–ligand interactions has recently provided many examples of clusters with a range of sizes, stoichiometries, and symmetries.^[2] We have recently demonstrated that a variety of high-symmetry coordination clusters can be rationally designed and synthesized in high-yield self-assembly reactions.^[3–12] Careful consideration of the geometric requirements of a particular symmetry and stoichiometry for a cluster can lead to the controlled formation of M_2L_3 triple helicates (D_3),^[8–10] M_4L_4 tetrahedra (T),^[7] and M_4L_6 tetrahedra (S_4 and T).^[6, 11]

Here we present an example of a bis(bidentate) catecholamide ligand whose geometry will allow for the formation of both an M_2L_3 triple helicate and an M_4L_6 tetrahedron. In the absence of a guest, an M_2L_3 triple helicate forms; however, in the presence of Me_4N^+ , the entropically disfavored M_4L_6 tetrahedron self-assembles to form a host–guest complex with Me_4N^+ encapsulated in the tetrahedral cluster cavity. Even more remarkably, the triple helicate can be quantitatively converted into the tetrahedron by addition of Me_4N^+ (Scheme 1).

The ligand H_4-L is based on a 2,6-diaminoanthracene backbone and is similar to a previously reported ligand system derived from 1,5-diaminonaphthalene.^[5, 6] For these types of backbones the catecholate binding units are offset when the ligand is in the conformation required to form a helicate, thus disfavoring helicate formation.^[13] Although the horizontal offset between the catecholate groups is exactly the same in both ligands, the extended length of H_4-L allows for greater flexibility in constructing the M_2L_3 structure.

Extensive molecular modeling of both the M_2L_3 and M_4L_6 structures was performed using the CAChe^[14] system (MM3) before beginning ligand synthesis. Results of these calculations indicated that both structures appeared equally plausible. These initial modeling investigations and the previous work with the 1,5-bis(2,3-dihydroxybenzamido)naphthalene



Scheme 1. Reaction of H_4-L with $[TiO(acac)_2]$ or $[Ga(acac)_3]$ leads to an M_2L_3 helicate in the absence of Me_4N^+ guest and an M_4L_6 tetrahedron in the presence of Me_4N^+ guest. The helicate can be converted into the tetrahedron simply by addition of Me_4N^+ .

ligand led us to believe that the M_2L_3 triple helicate structure could be formed by H_4-L , but that a strong host–guest interaction might push the equilibrium towards formation of the M_4L_6 tetrahedron.

Ligand H_4-L was synthesized according to established methods by coupling of 2,3-dimethoxybenzoyl chloride to 2,6-diaminoanthracene followed by deprotection with BBr_3 . Reaction of H_4-L (3 equiv), $[TiO(acac)_2]$ (2 equiv; $acac$ = acetylacetonate), and KOH (4 equiv) in methanol afforded after recrystallization orange microcrystals that analyze as $K_4[Ti_2-L_3] \cdot DMF \cdot 2H_2O$ (see Experimental Section). The highly symmetric ^1H NMR spectrum exhibits downfield-shifted ligand resonances indicative of metal complex formation. Without high-resolution mass spectrometry results or a solid-state structure, however, there is no way to distinguish between a $K_4[Ti_2-L_3]$ or a $K_8[Ti_4-L_6]$ structure.^[15] Fortunately, the high-resolution electrospray mass spectrum shows that $K_4[Ti_2-L_3]$ does indeed form in solution.

A single-crystal X-ray diffraction study of this compound confirmed that the helicate structure is maintained in the solid state.^[16] The $[Ti_2-L_3]^{4-}$ tetraanion is a homochiral triple helicate ($\Delta\Delta$ or $\Lambda\Lambda$ at the metal centers) with a Ti–Ti distance of 16.7 Å (Figure 1). Disregarding the anthracene

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Figure 1. Stereoview of the crystal structure of $[\text{Ti}_2\text{-L}_3]^{4-}$.

backbones of the ligand, the tetraanion has approximately D_3 molecular symmetry. However, the threefold axis is lost because two of the anthracene rings are oriented with their edges directed into the cluster interior, while the third is oriented approximately perpendicular to the plane bisecting the angle between the two former anthracene ring planes. This third ligand is substantially nonplanar. Despite the anthracene ring (and all other aromatic rings) being restrained in the least-squares refinement to be flat, the deviations of the carbon atoms from the best-fit plane (RMS deviation 0.12) are significantly above the random deviations (RMS deviation 0.02–0.03) observed for the carbon atoms of the catechol rings and the remaining two anthracene rings. In addition to this bend of the anthracene ring, the nitrogen atoms of the amide moieties show even greater deviation (≈ 0.25 Å) from the anthracene least-squares plane. In effect, this ligand is bowed out from the cluster interior.

In each of the three ligands, the catechol rings are not coplanar with the anthracene backbone. The angles between the least squares planes calculated for the catechol rings and the anthracene backbone are $61.0(0.6)^\circ$ and $18.0(1.0)^\circ$ for the first ligand, $44.9(0.6)^\circ$ and $6.6(1.1)^\circ$ for the second ligand, and $72.4(0.7)^\circ$ and $44.9(0.8)^\circ$ for the third ligand. The third ligand, which shows the most substantial twisting about the amide N–C bond, is the one previously described as being curved.

It is apparent from the crystal structure that this particular ligand allows for the formation of the M_2L_3 structure, but “just barely.” The third ligand shows substantial strain, as evidenced by the curvature of the aromatic backbone and the twists of the catechol rings with respect to the anthracene backbone. The ideal symmetry of a triple helicate (D_3), therefore, is not possible. As mentioned above, the ^1H NMR spectrum of $\text{K}_4[\text{Ti}_2\text{-L}_3]$ shows a single high-symmetry product on the NMR timescale. Variable-temperature NMR experiments are underway to determine if the asymmetry seen in the solid-state structure can also be observed at low temperature in solution.

Previous results suggested that alkylammonium cations might be suitable guests for the proposed tetrahedral cluster host.^[5, 6] Reaction of $\text{H}_4\text{-L}$ (3 equiv), $[\text{TiO}(\text{acac})_2]$ (2 equiv), and Me_4NOH (4 equiv) in methanol leads to an orange precipitate that analyzes as $[\text{Me}_4\text{N}]_8[\text{Ti}_4\text{-L}_6] \cdot 3\text{DMF} \cdot \text{H}_2\text{O}$. The ^1H NMR spectrum of this product shows a single, highly symmetric product with two Me_4N^+ resonances in a ratio of 7:1 ($\delta = 3.97, -2.60$). The presence of the extremely upfield shifted Me_4N^+ resonance ($\delta = -2.6$) whose relative intensity corresponds to one Me_4N^+ cation to six ligands can be interpreted as a direct indication of the encapsulation of one

Me_4N^+ cation by the tetrahedral cluster $[\text{Ti}_4\text{-L}_6]^{8-}$.^[6, 7, 17] In turn, this encapsulation indicates successful formation of the tetrahedral cluster.

^{13}C NMR spectroscopy and high-resolution electrospray mass spectrometry results support the assignment of this species in solution as the $[\text{Ti}_4\text{-L}_6]^{8-}$ cluster. Two resonances ($\delta = 54.54, 49.32$) for the Me_4N^+ cation are observed in the ^{13}C NMR spectrum. In the ES mass spectrum all peaks can be assigned to the tetrahedral $[\text{Ti}_4\text{-L}_6]^{8-}$ cluster; these peaks are for the compositions $[\text{Me}_4\text{N}^+]_n[\text{Ti}_4\text{-L}_6]^{(8-n)-}$ ($n = 2-6$) and $\text{H}_m[\text{Me}_4\text{N}^+]_n[\text{Ti}_4\text{-L}_6]^{(8-m-n)-}$ ($m = 1, n = 3-4; m = 2, n = 0, 2, 3$). Due to the high resolution of the results from the experiment, the charge states of all the signals (except those with 6 – charge) were confirmed by inspection of the peak separations. For example, the signal for $[\text{Me}_4\text{N}^+]_5[\text{Ti}_4\text{-L}_6]^{3-}$ at m/z 1140.4 has a peak separation of 0.3 amu, indicative of a 3 – charge state.

A single-crystal X-ray diffraction study of this compound confirmed that the tetrahedral structure is maintained in the solid state.^[18] Crystallographic analysis of $(\text{Me}_4\text{N})_8[\text{Ti}_4\text{-L}_6] \cdot 4\text{H}_2\text{O} \cdot 2\text{DMF} \cdot 2\text{C}_4\text{H}_8\text{O}_2 \cdot x\text{solvent}$ showed it to be a high-symmetry cluster in which the four Ti^{IV} ions are in a tetrahedral array bridged by six ligands (Figure 2). The octaanion is a racemic mixture of homochiral tetrahedra ($\Delta\Delta\Delta\Delta$ or $\Lambda\Lambda\Lambda\Lambda$ at the metal centers) with an average Ti–Ti distance of 16.1 Å. Additionally, a Me_4N^+ cation was found encapsulated within the cavity of this cluster.

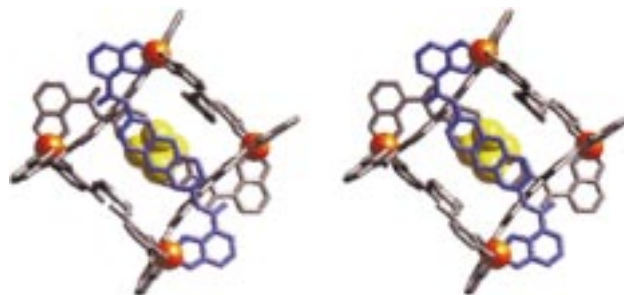


Figure 2. Stereoview of the crystal structure of $\text{Me}_4\text{N}^+ \subset [\text{Ti}_4\text{-L}_6]^{8-}$.

The tetranuclear complex has crystallographic D_2 symmetry and idealized T symmetry. There is little twisting about the amide moiety in the tetrahedral cluster as compared to the helicate; the angles between the least-squares planes calculated for the anthracene and catecholate rings range from only 9.4° to 12.7° . The ligands are, however, bowed in towards the cavity, presumably in order to make van der Waals contact with the small Me_4N^+ cation. The deviations of the carbon atoms of the anthracene rings from the best-fit planes (RMS deviation 0.11 Å) are significantly above the random deviations (RMS deviation 0.03–0.04 Å) observed for the carbon atoms of the catechol rings. In addition to this bend of the anthracene rings, the nitrogen atoms of the amide moieties show even greater deviation (≈ 0.29 Å) from the anthracene least-squares planes. Furthermore, in the tetrahedron the catechol binding units of each ligand are pointed in towards one another (antiparallel coordinate vectors); in the helicate the ligands are contorted to allow the two catecholate binding units of each ligand to align in roughly the same direction (parallel coordinate vectors). This represents an elegant proof

of principle for our rational design of these two types of systems.^[13]

Since the only difference in the two systems described is the absence or presence of Me_4N^+ , it should be possible to transform a triple helicate into a tetrahedral cluster simply by addition of Me_4N^+ . To test this hypothesis, the gallium(III) analogues were prepared because of the greater lability of Ga^{III} compared to Ti^{IV} . Upon addition of 20 equivalents of Me_4NCl to a solution of $\text{K}_6[\text{Ga}_2\text{-L}_3]$ in D_2O , complete transformation of the helicate into the tetrahedral cluster occurred. This was monitored by ^1H NMR spectroscopy over the course of five days (pD 6.5, $T = 40^\circ\text{C}$; Figure 3). Similar transformation studies at higher pD values (pD 7.5) and lower temperatures (room temperature) resulted in lower conversion rates due to the slower kinetics of metal–ligand rearrangement under these conditions.

In summary, we have shown that two different clusters, a triple helicate and a tetrahedron, can be prepared using identical ligand and metal components.^[19–22] Both structures conform to the rational design we have proposed for such clusters, and the relative energies are consistent with model predictions. Simply the addition of an appropriate guest is enough to shift the equilibrium from the entropically preferred helicate to the tetrahedron. More remarkably, the triple helicate can be quantitatively transformed into the tetrahedron by simple addition of this guest, Me_4N^+ . While we have demonstrated control of cluster synthesis by control of ligand geometry, these results show that we can also systematically use host–guest interactions in the rational design and synthesis of supramolecular clusters.

Experimental Section

General: All NMR spectra were measured with a Bruker 500-MHz spectrometer. Chemical shifts are reported as in ppm downfield from tetramethylsilane (TMS).

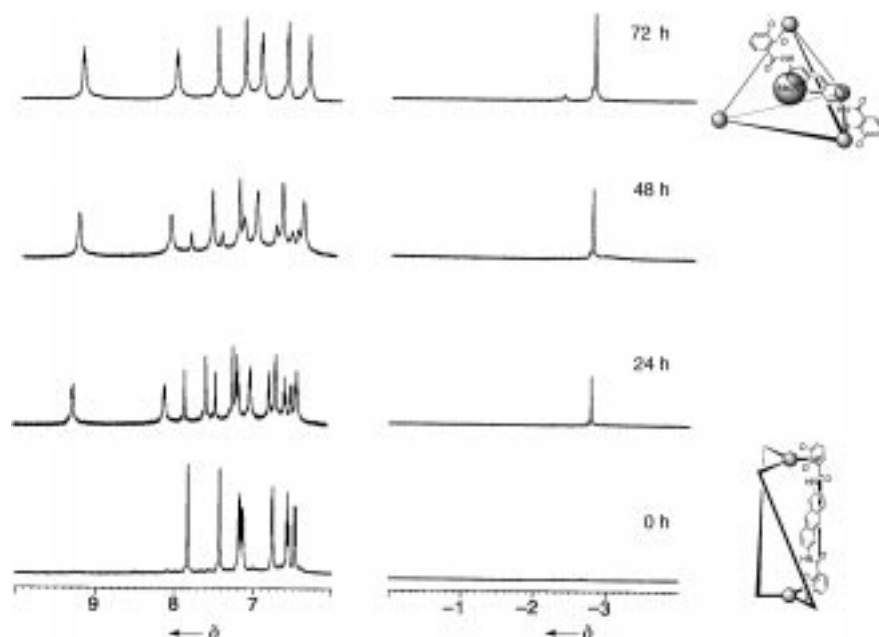


Figure 3. ^1H NMR spectra of helicate–tetrahedral cluster interconversion (D_2O , pD 6.5). Addition of Me_4N^+ to a solution of $\text{K}_6[\text{Ga}_2\text{-L}_3]$ leads to quantitative transformation of $\text{Me}_4\text{N}^+ \subset [\text{Ga}_4\text{-L}_6]^{12-}$.

$\text{Me}_4\text{-L}$: Under a N_2 atmosphere, 2,6-diaminoanthracene^[23] (706 mg, 3.4 mmol) was added all at once to 2,3-dimethoxybenzoyl chloride^[24] (1.36 g, 6.78 mmol) in dry CH_2Cl_2 (60 mL) followed by the addition of excess Et_3N (1.5 mL). The mixture was stirred for 20 h and then successively washed with 1N HCl, 10% aq NaOH, and brine. After drying of the solution over MgSO_4 and removal of the solvent under vacuum, a yellow powder was obtained. The product was further purified by layering a solution of the powder in CHCl_3 with Et_2O to yield yellow microcrystals (0.78 g, 43%). ^1H NMR (500 MHz, $[\text{D}_6]\text{DMSO}$): $\delta = 10.47$ (s, 2H), 8.62 (s, 2H), 8.42 (s, 2H), 8.02 (d, 2H), 7.64 (d, 2H), 7.19 (m, 6H), 3.85 (d, 12H); ^{13}C NMR (125 MHz, $[\text{D}_6]\text{DMSO}$): $\delta = 166.55, 153.87, 136.45, 132.15, 131.89, 129.93, 129.18, 125.75, 124.96, 122.13, 121.26, 121.15, 115.46, 112.76, 62.06, 56.78$; FAB-MS (positive ion): m/z : 537 (MH^+ , 100%); elemental analysis calcd (found) for $\text{C}_{32}\text{H}_{28}\text{N}_2\text{O}_6 \cdot 1.5\text{H}_2\text{O}$: C 68.19 (68.25), H 5.54 (5.52), N 4.97 (5.01).

$\text{H}_4\text{-L}$: Boron tribromide (1.25 mL, 12.2 mmol) was added by syringe to a N_2 -purged solution of $\text{Me}_4\text{-L}$ (0.37 g, 0.77 mmol) in dry CH_2Cl_2 at -78°C . The mixture was allowed to warm to room temperature and was stirred overnight under a N_2 atmosphere. The volatile components were removed under vacuum, and the resulting solid was hydrolyzed with water and stirred for 2 h at 100°C . The yellow-green solid was collected by filtration, washed with water and Et_2O , and dried under vacuum (0.32 g, 93%). ^1H NMR (500 MHz, $[\text{D}_6]\text{DMSO}$): $\delta = 11.61$ (brs, 2H), 10.57 (s, 2H), 9.47 (brs, 2H), 8.53 (s, 2H), 8.48 (s, 2H), 8.08 (d, 2H), 7.72 (d, 2H), 7.49 (d, 2H), 7.00 (d, 2H), 6.80 (t, 2H); ^{13}C NMR (125 MHz, $[\text{D}_6]\text{DMSO}$): $\delta = 168.25, 148.57, 146.62, 135.25, 131.29, 129.74, 128.88, 125.74, 122.70, 119.45, 118.99, 118.94, 117.77, 117.17$; FAB-MS (positive ion): m/z : 481 (MH^+ , 100%); elemental analysis calcd (found) for $\text{C}_{28}\text{H}_{20}\text{N}_2\text{O}_6 \cdot 4\text{H}_2\text{O}$: C 60.87 (60.93), H 5.11 (5.07), N 5.07 (5.04).

$\text{K}_4[\text{Ti}_2\text{-L}_3]$: The ligand $\text{H}_4\text{-L}$ (150 mg, 0.312 mmol) was suspended under oxygen-free conditions in CH_3OH (30 mL). A 0.508N KOH solution in CH_3OH (817 μL) was added by a pipetteman. $[\text{TiO}(\text{acac})_2]$ (55 mg, 0.21 mmol) was added as a powder, after which the solution gradually turned orange. The mixture was stirred overnight, and the solvent subsequently removed under vacuum to afford a red solid, which was purified by recrystallization from DMF/ Et_2O (156 mg; 89%). Crystals suitable for analysis by X-ray diffraction were obtained by the slow vapor diffusion of Et_2O into a solution of the complex in DMF/THF. ^1H NMR (500 MHz, $[\text{D}_6]\text{DMSO}$): $\delta = 11.66$ (s, 6H), 8.01 (s, 6H), 7.68 (s, 6H), 7.30 (d, 6H), 7.15 (d, 6H), 7.13 (d, 6H), 6.50 (t, 6H), 6.39 (d, 6H); ^{13}C NMR (125 MHz, $[\text{D}_6]\text{DMSO}$): $\delta = 165.25, 161.11, 160.09, 135.45, 132.09, 129.16, 129.13, 125.04, 122.78, 117.78, 117.05, 116.96, 115.33, 113.89$; ESI-MS (positive ion): m/z : 1567.3 ($\text{H}_4\text{K}[\text{Ti}_2\text{-L}_3]^+$, 97%), 1529.4 ($\text{H}_3[\text{Ti}_2\text{-L}_3]^+$, 83%), 765.2 ($\text{H}_6[\text{Ti}_2\text{-L}_3]^{2+}$, 100%); elemental analysis calcd (found) for $\text{K}_4[\text{Ti}_2\text{-L}_3] \cdot \text{DMF} \cdot 2\text{H}_2\text{O}$: C 58.84 (58.76), H 3.32 (3.25), N 5.48 (5.41).

$\text{K}_6[\text{Ga}_2\text{-L}_3]$: Prepared as above using $[\text{Ga}(\text{acac})_3]$. The product was recrystallized by layering a solution in CH_3OH with Et_2O (98%). ^1H NMR (500 MHz, $[\text{D}_4]\text{MeOH}$): $\delta = 8.01$ (d, 6H), 7.39 (d, 6H), 7.17 (d, 6H), 7.11 (d, 6H), 6.87 (d, 6H), 6.73 (d, 6H), 6.42 (t, 6H); ^{13}C NMR (125 MHz, $[\text{D}_6]\text{DMSO}$): $\delta = 164.65, 161.06, 160.01, 134.45, 131.38, 129.01, 128.89, 125.01, 122.28, 117.18, 117.06, 116.98, 115.31, 113.22$; ESI-MS (positive ion): m/z : 1576 ($\text{H}_7[\text{Ga}_2\text{-L}_3]^+$, 100%); elemental analysis calcd (found) for $\text{K}_4[\text{Ga}_2\text{-L}_3] \cdot \text{DMF}$: C 55.69 (55.76), H 2.95 (2.89), N 5.26 (5.28).

$(\text{Me}_4\text{N})_8[\text{Ti}_4\text{-L}_6]$: Prepared as above using $[\text{TiO}(\text{acac})_2]$ and Me_4NOH instead of KOH. The orange precipitate was collected by filtration and recrystallized by layering a solution in DMF with Et_2O (73 mg, 61%). Single crystals suitable for X-ray diffraction were grown by the slow vapor diffusion of ethyl acetate into a wet solution of the complex in DMF. ^1H NMR (500 MHz, $[\text{D}_6]\text{DMSO}$): $\delta = 14.79$ (s, 12H), 9.47 (d, 12H), 7.86 (d, 12H), 7.49 (s, 12H), 7.29 (s, 12H), 6.79 (d,

12H), 6.32 (d, 12H), 6.23 (t, 12H), 3.97 (s, 84H), -2.55 (12H); ^{13}C NMR (125 MHz, $[\text{D}_6]\text{DMSO}$): $\delta = 168.34, 159.94, 157.98, 138.94, 130.35, 128.39, 127.81, 123.01, 122.03, 113.95, 112.87, 112.73, 112.31, 112.12; 54.54, 49.32$; ESI-MS (negative ion): m/z : 1747.2 ($(\text{Me}_4\text{N})_6[\text{Ti}_4\text{-L}_6]^{2-}$), 1140.4 ($(\text{Me}_4\text{N})_5[\text{Ti}_4\text{-L}_6]^{3-}$), 1116.0 ($(\text{H}(\text{Me}_4\text{N})_4[\text{Ti}_4\text{-L}_6]^{3-})$), 1091.7 ($(\text{H}_2(\text{Me}_4\text{N})_3[\text{Ti}_4\text{-L}_6]^{3-})$), 818.3 ($(\text{H}(\text{Me}_4\text{N})_3[\text{Ti}_4\text{-L}_6]^{4-})$), 836.8 ($(\text{Me}_4\text{N})_4[\text{Ti}_4\text{-L}_6]^{4-}$, 100%), 800.6 ($(\text{H}_2(\text{Me}_4\text{N})_2[\text{Ti}_4\text{-L}_6]^{4-})$), 654.4 ($(\text{Me}_4\text{N})_3[\text{Ti}_4\text{-L}_6]^{5-}$), 533.4 ($(\text{Me}_4\text{N})_2[\text{Ti}_4\text{-L}_6]^{6-}$), 509.1 ($(\text{H}_2[\text{Ti}_4\text{-L}_6]^{6-})$); elemental analysis calcd (found) for $[\text{Me}_4\text{N}]_8[\text{Ti}_4\text{-L}_6] \cdot 3\text{DMF} \cdot 2\text{H}_2\text{O}$: C 64.39 (64.46), H 5.61 (5.56), N 8.26 (8.32).

$\text{K}_4(\text{Me}_4\text{N})_8[\text{Ga}_4\text{-L}_6]$: Prepared as above using $[\text{Ga}(\text{acac})_3]$, KOH, and Me_4NCl . The product was precipitated by reducing the volume of the solution slowly to 5 ml. The yellow precipitate was collected by filtration and dried under high vacuum (61 mg, 91%). ^1H NMR (500 MHz, $[\text{D}_6]\text{DMSO}$): $\delta = 14.79$ (s, 12H), 9.39 (d, 12H), 7.81 (d, 12H), 7.46 (s, 12H), 7.21 (s, 12H), 6.78 (d, 12H), 6.26 (d, 12H), 6.08 (t, 12H), 3.95 (s, 84H), -2.60 (s, 12H); ^{13}C NMR (125 MHz, $[\text{D}_6]\text{DMSO}$): $\delta = 167.88, 159.67, 157.60, 138.56, 130.04, 127.89, 127.81, 123.01, 122.03, 113.95, 112.87, 112.73, 112.31, 112.02, 54.70, 49.97$.

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$\beta = 113.571(1)^\circ$, $V = 11984.5(5)$ Å³, $Z = 4$, $\mu = 0.345$ mm^{−1}, $F(000) = 4712$, $\rho_{\text{calcd}} = 1.256$ Mg m^{−3}, $2\theta_{\text{max}} = 37.7^\circ$. The data were integrated using SAINT.^[26] Of the 23943 reflections collected, 13861 were Friedel unique ($R_{\text{int}} = 0.0753$). The structure was solved by direct methods and refined on F^2 using SHELXTL.^[27] Data were corrected for Lorentz and polarization effects. Outliers with $\Delta I/\sigma > 5$ ($\Delta I/\sigma > 10$ if only two equivalents) were rejected, resulting in 224 reflections (0.90% of the data) being removed. An empirical absorption correction was applied using SADABS^[28] (ellipsoidal model, $T_{\text{max}} = 0.928$, $T_{\text{min}} = 0.750$). The structure was modeled as a racemic twin (50:50) in the non-centrosymmetric space group *Cc* (no. 9).^[29] Inspection shows that the cluster does actually crystallize in the non-centrosymmetric space group *Cc*, not its centrosymmetric counterpart *C2/c*. The twofold axis that would run through the cluster is only approximate: The ligand that would be perpendicular to this twofold axis is significantly distorted from twofold symmetry. The catecholate rings and the anthracene ring are offset from the would-be symmetry axis. The lack of high-angle data resulted in a low-resolution (1.1 Å) structure that necessitated the use of numerous restraints. Weighting scheme: $1/[\sigma^2(F_o^2) + (0.2911p)^2 + 74.6955p]$, where $p = (F_o^2 + 2F_c^2)/3$. Final $R1 = 0.1468$ for 7925 Friedel unique data (767 parameters, 487 restraints, $2.72^\circ < 2\theta < 37.7^\circ$); for all 13861 data, $wR_2 = 0.4780$, GOF = 1.103; max./min. residual density $+1.553/-0.606$ e Å^{−3} (the largest difference peak was located 0.82 Å from Ti1 between the two coordinating catechol oxygen atoms).^[18b]

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A Simple Lithographic Approach for Preparing Patterned, Micron-Scale Corrals for Controlling Cell Growth**

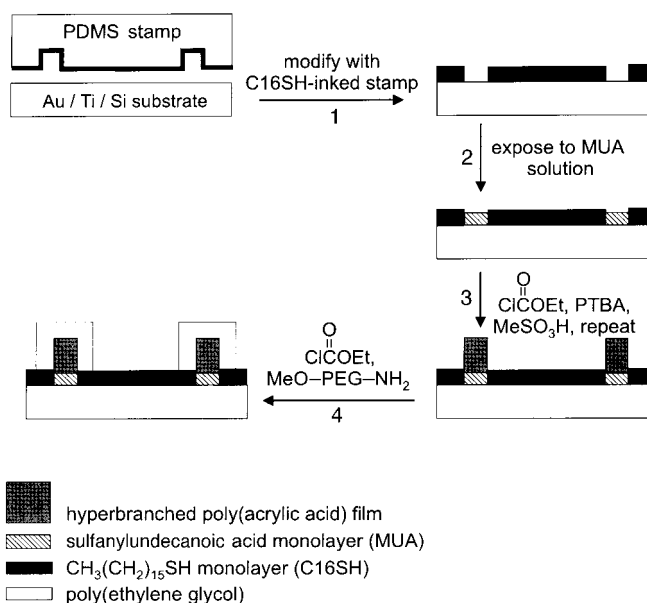
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In this report we demonstrate that a simple, three-step lithographic process—involving microcontact printing ($\mu\text{-CP}$), polymer grafting, and subsequent polymer functionalization—results in patterned surfaces exhibiting excellent cell

adhesion and spatial definition. The patterned substrates consist of cell corrals having lateral dimensions of $63\ \mu\text{m}$ and wall heights of $54 \pm 4\ \text{nm}$. The corrals have hydrophobic, methyl-terminated *n*-alkanethiolate bottoms, which promote cell adhesion, and the walls consist of hydrophilic poly(acrylic acid)/poly(ethylene glycol) (3-PAA/PEG) nanocomposite polymers that resist cell encroachment. Peritoneal IC-21 murine macrophage cell growth occurs only within the boundaries of the corrals. This approach provides a simple and chemically flexible route to local definition of cells on surfaces.

Formation of biological tissue structures, such as nerves and blood vessels, require micron-scale morphologic control and spatial positioning of cells. To achieve this on artificial substrates, the adsorption and adhesion of cells and proteins have been studied on self-assembled monolayers (SAMs) made up of alkanethiolates or alkylsilanes and on thin polymer films.^[1–5] There have been a smaller number of studies focused on the adhesion and motility of cells on patterned substrates.^[6–11]

Our approach for exerting micron-scale control over cell adhesion involves four steps (Scheme 1).^[12] After preparation of a suitable poly(dimethylsiloxane) (PDMS) stamp from a TEM-grid master (TEM = transmission electron microscopy),



Scheme 1. Formation of micron-scale corrals with hydrophobic alkanethiolate bottoms and walls consisting of a hydrophilic three-layer poly(acrylic acid)/poly(ethylene glycol) nanocomposite.

an evaporated gold substrate is patterned with a hexadecanethiol (C16SH) SAM by soft lithography.^[13] Second, the unmodified regions of the gold substrate are filled with a SAM of 11-sulfanylundecanoic acid (also called mercaptoundecanoic acid, MUA). Next, the terminal acid groups of the MUA-patterned portions of the mixed monolayer are activated and treated with α,ω -diamino-substituted poly(*tert*-butyl acrylate) ($\text{H}_2\text{NR-PTBA-RNH}_2$) to yield the amide-grafted polymer layer. Hydrolysis of PTBA with MeSO_3H yields the first layer of PAA, and two additional cycles of activation, grafting, and hydrolysis yields a hyperbranched

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three-layer PAA film (3-PAA). The details of this synthesis and the properties of the resulting films have been reported previously.^[14] These films contain a high density of acid groups, which can subsequently be functionalized with methoxypoly(ethylene glycol)amine (MeO-PEG-NH₂) by simple amidation chemistry.

Fourier transform infrared–external reflection spectroscopy (FT-IR–ERS), ellipsometry, contact-angle measurements, and X-ray photoelectron spectroscopy (XPS) were performed on macroscopic regions of a partially patterned substrate. This battery of analytical methods confirms that PAA grafting and subsequent functionalization with PEG occurs only on MUA-modified regions of the gold surface. These studies also indicate that covalent attachment of PEG occurs primarily at the surface of the 3-PAA film. For example, the peak at 1730 cm⁻¹ in Figure 1a arises from the acid carbonyl group of 3-PAA. After grafting of PEG to the surface, new amide bands appear at 1645 and 1545 cm⁻¹ (Figure 1b), but the somewhat diminished acid carbonyl peak remains, indicating only partial derivatization of the 3-PAA film. Exposure of

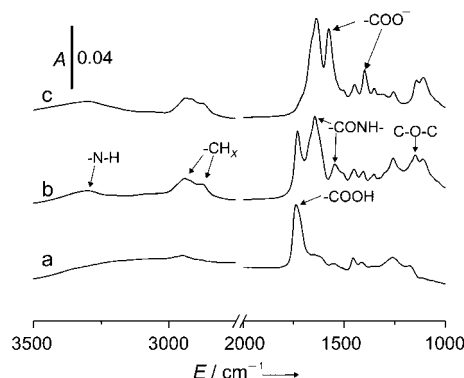


Figure 1. FT-IR–ERS spectra of a) a 3-PAA film; b) the film in a) after derivatization with MeO-PEG-NH₂, immersion into a 0.1M ethanolic solution of HCl to convert unchanged carboxylate groups into the acid form, washing with CH₂Cl₂, and sonication in ethanol to remove physisorbed material from the surface; and c) the film in b) after derivatization with PEG, immersion in aqueous 0.1M CsOH, and rinsing with water and ethanol.

these films to an aqueous CsOH solution resulted in the disappearance of the peak at 1730 cm⁻¹ and appearance of new carboxylate peaks at 1580 and 1400 cm⁻¹ (Figure 1c), confirming the presence of unfunctionalized acid groups within the interior of the composite. In Figure 1b the presence of a C-O-C stretching band at 1145 cm⁻¹, peaks at 2878 and 2940 cm⁻¹ due to the CH₃ and CH₂ stretching modes of PEG, the N-H stretch at 3303 cm⁻¹, and the decrease in the broad OH stretch around 3100 cm⁻¹ are additional support for PEG grafting on 3-PAA films. Ellipsometric and tapping-mode AFM measurements indicate that prior to PEG grafting the 3-PAA film is 23 ± 3 nm thick,^[12] but after addition of PEG the composite film thickness increases to 54 ± 4 nm. Additionally, the water contact angle increases from 21° to 27°. Interestingly, there is no change in the water contact angle after soaking of this film in aqueous CsOH solution, even though the film still contains acid groups that have not yet reacted; in the absence of the PEG layer the water contact

angle decreases from 21° to less than 5° upon deprotonation of acid groups with CsOH. These results strongly suggest the absence of unchanged acid groups on the surface of the 3-PAA/PEG nanocomposite film. Additional evidence comes from surface-sensitive X-ray photoelectron spectroscopy (XPS) studies of CsOH-treated 3-PAA/PEG films; there is no signal corresponding to Cs, whereas a prominent Cs 3d doublet for PEG-free 3-PAA films appears. Control experiments carried out on a C16SH SAM, rather than on the reactive, acid-terminated MUA monolayer, indicate no growth of PAA and no grafting of PEG.

Prior to cell immobilization all films are sterilized with UV light. Ellipsometry and IR spectroscopy indicate that sterilization does not damage the films chemically or topographically. Figure 2a shows a bright-field micrograph of macrophage cells adhered to an unpatterned hyperbranched 3-PAA film. Adhesion and growth of cells occur because the 3-PAA film contains a high density of acid functional groups. Surface

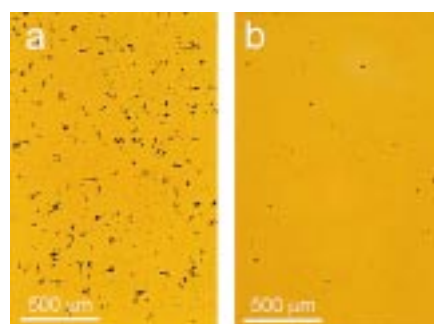


Figure 2. Optical micrographs of macrophage cells seeded onto polymer-modified gold surfaces. a) Adhesion and growth on hyperbranched 3-PAA polymer films grafted onto a self-assembled MUA monolayer. b) Inhibition of adhesion and growth on a 3-PAA film after grafting a layer of PEG to the 3-PAA surface.

sulfonate, hydroxyl, and carboxyl groups have all been demonstrated to promote cell attachment and growth.^[15] Figure 2b is an optical micrograph of a 3-PAA/PEG film that was seeded with cells at the same density used for Figure 2a. These data clearly demonstrate that cells neither adhere to, nor grow on, the 3-PAA/PEG films. The absence of cell adhesion on the PEG surface is consistent with the exclusion of protein adsorption, which has been attributed in part to the large excluded volume of PEG chains.^[16] These results imply that the relatively thick PEG layer must fully cover the underlying 3-PAA film and that the 3-PAA film, in turn, fully coats the MUA-functionalized gold surface.

Figure 3 shows a bright-field optical micrograph of a square-grid pattern (derived from a TEM-grid master) consisting of C16SH and 3-PAA/PEG on a gold substrate. The grid is a 3-PAA/PEG polymer film and the bottoms of the corrals consist of a C16SH SAM (last frame of Scheme 1).

A micrograph of macrophage cells seeded onto a patterned 3-PAA film is presented in Figure 4a. That macrophage cells grow on both the methyl-terminated SAM and across the 3-PAA corral walls is anticipated by Figure 2a. Figure 4b shows an optical micrograph of a patterned surface in which the corral walls have been modified with PEG. It indicates that PEG inhibits cell adhesion and growth, and that the cells



Figure 3. Optical micrographs of a gold surface patterned using μ -CP followed by polymer grafting. The grid is composed of a 54-nm-thick, PEG-grafted 3-PAA polymer composite, and the squares contain a 1.8-nm-thick *n*-hexadecanethiol (C16SH) monolayer.



Figure 4. Optical micrographs of a) cells grown onto a patterned film having 3-PAA walls (no PEG) and C16SH bottoms: cells adhere to and grow over the 3-PAA walls; b–d) macrophage cells patterned onto the PEG-modified substrate shown in Figure 3: cells are confined within the corrals, and there is no evidence for cellular interactions between corrals.

are confined to the methyl-terminated SAM surface. These results clearly demonstrate that simple polymer chemistry can be used to control growth; note especially that no part of the cell body escapes the corral. Figures 4c and 4d are higher resolution micrographs of macrophage-patterned regions of the surface. The macrophage cells in these images possess the correct morphology. For example, the peripheral skirt of the cytoplasm and the surface ridges of the cell are evident. Additionally, the cells are well spread, as evidenced by the clearly defined extended processes. Close examination of the macrophage in Figure 4d reveals that it grows only to the boundary of the 3-PAA/PEG corral wall even though the wall is much thinner than the cell. Macrophage cell growth is dependent on the anchorage, so as long as there is surface

available the cells will continue to grow. In the case of a fixed area, cell survival is a function of cell-seeding density. At low densities, more surface is available for cell proliferation. We have been able to grow cells for over three days at a density of 1.0×10^4 cells mL^{-1} .

In summary, we have made use of a simple soft-lithography technique and covalent grafting of suitable polymers to prepare micron-scale corrals for cell adhesion and growth. The strict control of cell position and function is a fundamental focus in the development of applications ranging from cellular to tissue engineering,^[17] and for biosensing applications. Sequential grafting with polymeric building blocks facilitates a rapid increase in the height of the corral walls, and PEG grafting is so complete that there is no evidence of cell growth outside the corrals. Because the interiors of the corral walls contain unchanged carboxylic acid groups, they can selectively bind metal ions; entrap organic molecules, drugs, and enzymes;^[18] or be chemically modified to prevent permeation of aqueous solutions between corrals.^[19] Thus, these cell corrals provide new platforms for chemical sensing applications and for tailoring polymer surface properties^[20] for a wide variety of biomedical applications. Moreover, this simple patterning strategy may lead to more complex structures such as ensembles of organized tissues, including nerves and blood vessels.

Experimental Section

Methoxypoly(ethylene glycol)amine (MeO-PEG₅₀₀₀-NH₂) was grafted on 3-PAA by immersing the 3-PAA film in 10 mL of dried DMF, to which 200 μL of *N*-methylmorpholine and 200 μL of ethyl chloroformate were added while stirring. After 10 min the substrate was removed, rinsed with ethyl acetate, dried under N₂, and then immersed in a solution of MeO-PEG₅₀₀₀-NH₂ in DMF/CH₂Cl₂ (9/1; 0.1 g of MeO-PEG₅₀₀₀-NH₂ in 9 mL DMF and 1 mL CH₂Cl₂) for 1 h. The slide was then removed, dipped into a 0.1 M ethanolic solution of HCl for 10 min to protonate unfunctionalized carboxylate groups and remove salts, dipped in CH₂Cl₂ for 1 h, sonicated in CH₂Cl₂ for 10 min, washed with EtOH to remove physisorbed material from the surface, and finally dried under flowing N₂.

The polymer surfaces were sterilized by placing the substrates under 245-nm UV light for 2 h. SV-40-transformed peritoneal IC-21 mouse macrophages were cultured in RPMI-1640 media supplemented with fetal bovine serum and an antibiotic/antimycotic solution at 37 °C. Cells were plated on supported thin films at a density of 1.0×10^5 cells per mL in all experiments except for cells plated on 3-PAA patterned films (1.0×10^4 cells per mL). After 24 h of incubation at 37 °C in a 5% CO₂ incubator, the substrate-confined cells were put in 10% formalin for 24 h. They were then dehydrated through a series of ethanol washes, treated with hexamethyldisilazane, and imaged by optical microscopy.

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Pleated Sheets and Turns of β -Peptides with Proteinogenic Side Chains

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The properties of peptides and proteins depend on their three-dimensional structure, which itself is determined by the sequence of the amino acids, that is, the primary structure. The mechanisms of formation and the parameters determining the stability of secondary structures of proteins—including not only the helix, the pleated sheet, and the turn, but also the random-coiled region—are not yet fully understood.^[1] In contrast, β -peptides (oligomers of β -amino acids^[2]) adopt predictable^[3] secondary structures that can also be identified by calculations.^[4] This also holds for β -peptides whose backbones are not conformationally restricted by cyclic residues. Thus, β -peptides composed of more than five homochiral β^2 -, β^3 -, or *like*- $\beta^{2,3}$ -amino acids^[5] with proteinogenic side chains form a 3_{14} helix in methanol with all substituents in lateral positions;^[3] on the other hand, chains consisting of (*R*)- β^2 /(*S*)-

β^3 or *unlike*- $\beta^{2,3}$ residues were expected to adopt an extended conformation, with formation of pleated sheets (Figure 1 a); in β -peptide sections with (*S*)- β^2 / β^3 or with *geminally* disubstituted β^2 -amino acid moieties we have observed the

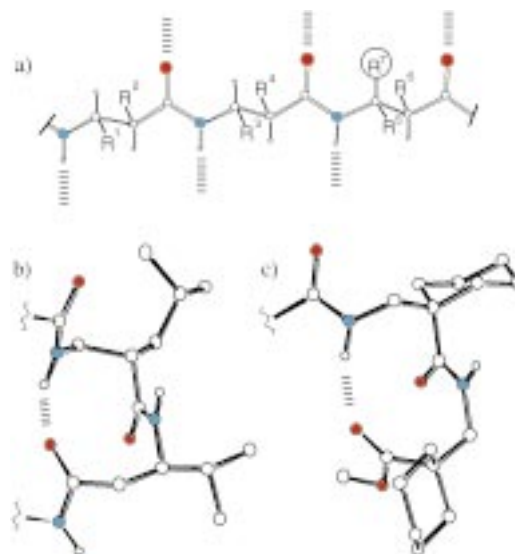
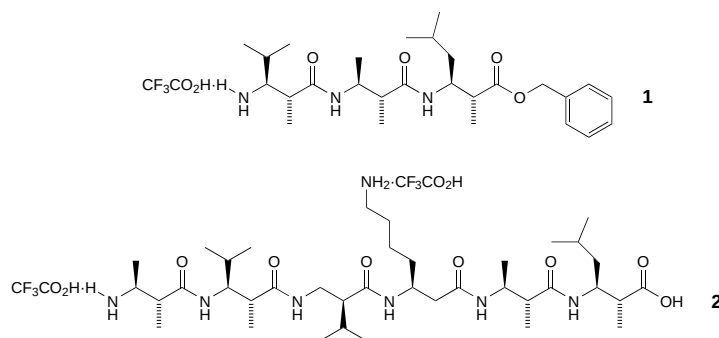


Figure 1. a) Model of a fully extended β -peptide chain. β -Amino acid sequences with R^1 to $R^6 \neq H$, $R^7 = H$ (*unlike*- $\beta^{2,3}$, type I) or, for example, $R^1, R^4, R^5 \neq H$, $R^2, R^3, R^6, R^7 = H$ ((*S*)- β^2 /(*R*)- β^3 , type II) preclude a β -peptide from adopting the 3_{14} -helical secondary structure; the linear arrangement is enforced.^[2, 3, 6] Geminal disubstitution, for example R^5 and $R^7 \neq H$, prevents both the formation of the helix and the aggregation to a pleated sheet (see 14-membered hydrogen-bonded turns in Figure 2 and the discussion in reference [3]). b), c) Ten-membered hydrogen-bonded turns found in the β^2 - β^3 -segments of the 12/10/12 helix^[3, 7] or formed by geminally disubstituted β -peptides.^[8]

formation of ten-membered hydrogen-bonded rings (Figure 1 b, c). These results and conclusions have provided guidance in our search for β -peptidic parallel and antiparallel pleated sheets and turns, and we have prepared (by known methods^[3, 9]) the β -peptides **1** (in solution) and **2** (on solid phase), containing *unlike*- $\beta^{2,3}$ -amino acids. Herein, we report their structures.



Unsurmountable solubility problems arise upon chain elongation with formation of β -peptides such as **1** (type I in Figure 1 a), so that attempted dimerizing coupling of **1** to give a hexapeptide was unsuccessful.^[10] The crystal structure and crystal packing of **1**, which indeed forms sheets, are shown in Figure 2.^[11] The parallel amide planes in the individual strands

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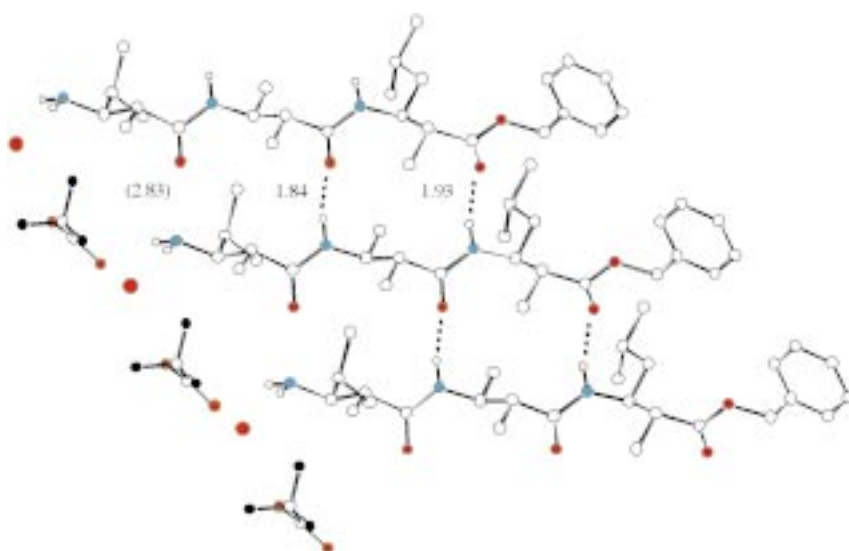


Figure 2. Parallel pleated sheet structure of β -tripeptide ester **1**·H₂O. The angles N–H···O are 159.7 and 165.6°; the distance N···O is shown for the N-terminal hydrogen bond. The X-ray structure analysis was performed by P. Seiler.

are connected through CHR–CHMe ethane moieties, in which R and Me are antiperiplanar as are HN and CO; 14-membered hydrogen-bonded rings connect the strands in the *parallel* pleated sheet structure. In contrast to α -peptidic pleated sheets, where neighboring O=C bonds point in opposite directions, these bonds are unidirectional in the β -peptide structure, leading to a polar packing that might be an additional reason for the low solubility of compounds of this type (cf. cyclo- β -tetrapeptides^[12]).

We chose the turn (Figure 1b) composed of a β^2/β^3 -dipeptide sequence (with the side chains of valine and lysine) for the construction of an *antiparallel* pleated sheet arrangement. This turn has the same substitution pattern as the central ten-membered hydrogen-bonded ring of the 12/10/12 helix.^[3, 7] Two dipeptide segments of *unlike*- $\beta^{2,3}$ -amino acids, which enforce the extended conformation, were attached on each end of this unit and were supposed to form intra- rather than intermolecular hydrogen bonds. In fact, the resulting β -peptide **2** is well soluble, even in water (due to the ω -aminobutyl group^[13]).

The structure of hexapeptide **2** in CD₃OH was determined by 2D NMR spectroscopy. All resonances in the ¹H NMR spectrum were assigned unequivocally by the evaluation of DQF-COSY and TOCSY measurements. The coupling con-

stants between the protons of the peptide backbone are collected in Table 1. The *J* values of the backbone protons of amino acids **2** and **5** are large, implying an antiperiplanar arrangement of the corresponding NH and C(β)-H as well as C(β)-H and C(α)-H protons; that there is more than one preferred conformation of the terminal amino acids **1** and **6** is suggested by their smaller *J* values. However, the values observed for the central amino acids **3** and **4** indicate a completely different, nonextended conformation, and they resemble those found for the ten-membered hydrogen-bonded ring of the 12/10/12 helix.^[3, 7] ROESY measurements were used to obtain information about the distances between the protons, and the volumina of 20 NOE cross-peaks were classified in three distance categories (Table 2). Long-range NOEs

Table 1. Coupling constants for β -hexapeptide **2** (CD₃OH, 500 MHz).^[a]

Amino acid	<i>J</i> (NH, C(β)-H)	<i>J</i> (C(α)-H, C(β)-H)
1	–	7
2	10.1	9.7
3	<i>J</i> (NH, C(β)-H ^{Si}) 7.4 <i>J</i> (NH, C(β)-H ^{Re}) 3.8	<i>J</i> (C(β)-H ^{Si} , C(α)-H) 3.8 <i>J</i> (C(β)-H ^{Re} , C(α)-H) 10.7
4	9.0	<i>J</i> (C(β)-H, C(α)-H ^{Si}) 4.1 <i>J</i> (C(β)-H, C(α)-H ^{Re}) 9.6
5	10.6	9.2
6	7	7

[a] The backbone CH₂ protons of amino acids **3** and **4** were assigned stereospecifically. The large values for the amino acids **2** and **5** suggest an extended conformation, whereas the *J* values for **1** and **6** indicate several preferred conformations. The coupling constants in residues **3** and **4** are in agreement with a well-defined, nonextended conformation.

between amino acids **1** and **6** as well as between **2** and **5** are especially significant. An extended section of the structure is suggested by NOEs between NH(**3**), C(α)-H(**2**) and NH(**2**), as well as between NH(**6**), C(α)-H(**5**), and NH(**5**). The 20 NOEs and 12 *J* values were used as distance and torsion-angle restraints in molecular dynamics simulations according to the simulated annealing method (programme X-PLOR). The resulting conformations converged to a unique structure of peptide **1** (Figure 3): β -peptide **2** adopts a hairpin arrange-

Table 2. NOEs of β -hexapeptide **2** extracted from the 150ms-ROESY spectrum (CD₃OH, 500 MHz).^[a]

H atom		H atom		NOE	H atom		H atom		NOE
NH	2	CHMe ₂	2	m	NH	5	α -Me	5	w
NH	2	C(α)-H	2	m	NH	5	β -Me	5	m
NH	2	C(β)-H	1	w	NH	5	C(α)-H ^{Si}	4	m
NH	2	C(α)-H	1	s	NH	5	C(α)-H	5	m
β -H ^{Si}	3	CHMe ₂	3	m	NH	5	C(β)-H	4	m
β -H ^{Si}	3	C(α)-H	3	m	NH	6	C(α)-H	6	m
NH	3	C(β)-H	2	m	NH	6	CHMe ₂	6	m
NH	3	C(α)-H	2	s	C(α)-H	1	C(β)-H	6	w
NH	4	α -H ^{Si}	4	w	C(α)-H	2	C(β)-H	5	m
NH	4	α -H ^{Re}	4	s	δ -Me	2	α -Me	5	w

[a] The NOEs were classified in three distance categories: s (strong, <3 Å), m (medium, <3.5 Å), and w (weak, <4.5 Å).

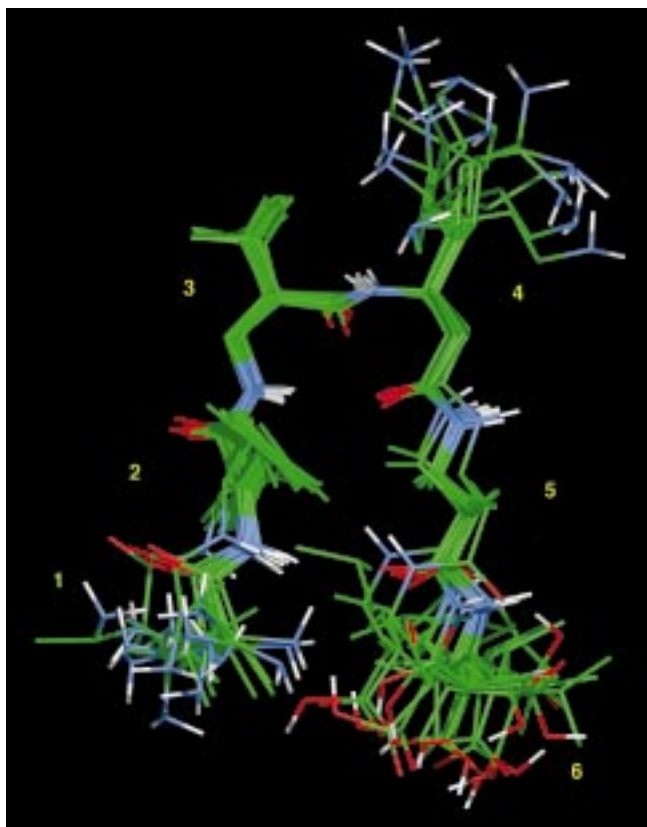


Figure 3. Structure of β -peptide **2** as determined by NMR spectroscopy (CD_3OH). Bundles of the 15 lowest energy conformers showing no significant violation of the experimental restraints (NOE and J values). According to this analysis, the C- and N-terminal amino acids **1** and **6** are rather flexible, and two conformations are observed for **6** that are in perfect agreement with the experimental data. The figure was generated by MOLMOL.^[14]

ment,^[15] a secondary structural element of α -peptides which is frequently responsible for interactions with receptors.^[16]

The results described herein now complete the demonstration that oligomers (β -peptides) consisting of the homologues of simple proteinogenic amino acids form helices, pleated sheets, and turns, just like the α -peptidic proteins. However, the β -peptidic secondary structures are not only of larger variety (three different helices have already been discovered^[2b, 3]) and of much more pronounced stability (even with short chain lengths^[3, 17]), but they are also predictable and amenable to rational planning and theoretical calculations. Furthermore, β -peptides were found to be stable to the most aggressive peptidases.^[18] Thus, the door is wide open to large structures (β -proteins, β -enzymes) and to β -peptidic drugs.

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- [10] Peptide **1** is only soluble in hot MeOH; the β -heptapeptide H-(S)- β^2 -HVal-(R)- β^3 -HAla-(S)- β^2 -HPhe-(R)- β^3 -HPhe-(S)- β^2 -HVal-(R)- β^3 -HAla-(S)- β^2 -HLeu-OH of type II (Figure 1a) also shows very poor solubility.
- [11] Crystal structure data of **1**: $\text{C}_{29}\text{H}_{64}\text{N}_3\text{O}_6\text{F}_3 \cdot \text{H}_2\text{O}$, $M_r = 607.72$. Crystals were grown by slow evaporation of a solution of **1** in methanol. Crystal size: $0.20 \times 0.10 \times 0.10 \text{ mm}^3$, orthorhombic, space group $P2_12_12_1$ (no. 19), $a = 6.0636(7)$, $b = 20.191(4)$, $c = 27.139(4) \text{ \AA}$, $V = 3322.7(9) \text{ \AA}^3$, $Z = 4$, $\rho_{\text{calc}} = 1.22 \text{ g cm}^{-3}$, $\mu = 0.816 \text{ mm}^{-1}$, $2.73 < \theta < 59.94^\circ$; of 3074 reflections observed, 2658 were unique; Nonius CAD4 diffractometer (193 K, $\lambda(\text{CuK}\alpha) = 1.5418 \text{ \AA}$). The structure was solved by direct methods (SHELXS86; G. M. Sheldrick, *Acta Crystallogr. Sect. A* **1990**, *46*, 467–473) and refined by full-matrix least-squares analysis (G. M. Sheldrick, *SHELXL93, Program for the Refinement of Crystal Structures*, Universität Göttingen, **1993**); $w = 1/[\sigma^2(F_o^2) + (0.0710 P)^2 + 0.83 P]$, where $P = (F_o^2 + 2 F_c^2)/3$ (heavy atoms anisotropic, hydrogen atoms isotropic, where hydrogen positions are based on stereochemical considerations). $R(F) = 0.039$, $wR(F^2) = 0.105$ for 392 variables and 2409 reflections with $I > 2\sigma(I)$. 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-113798. 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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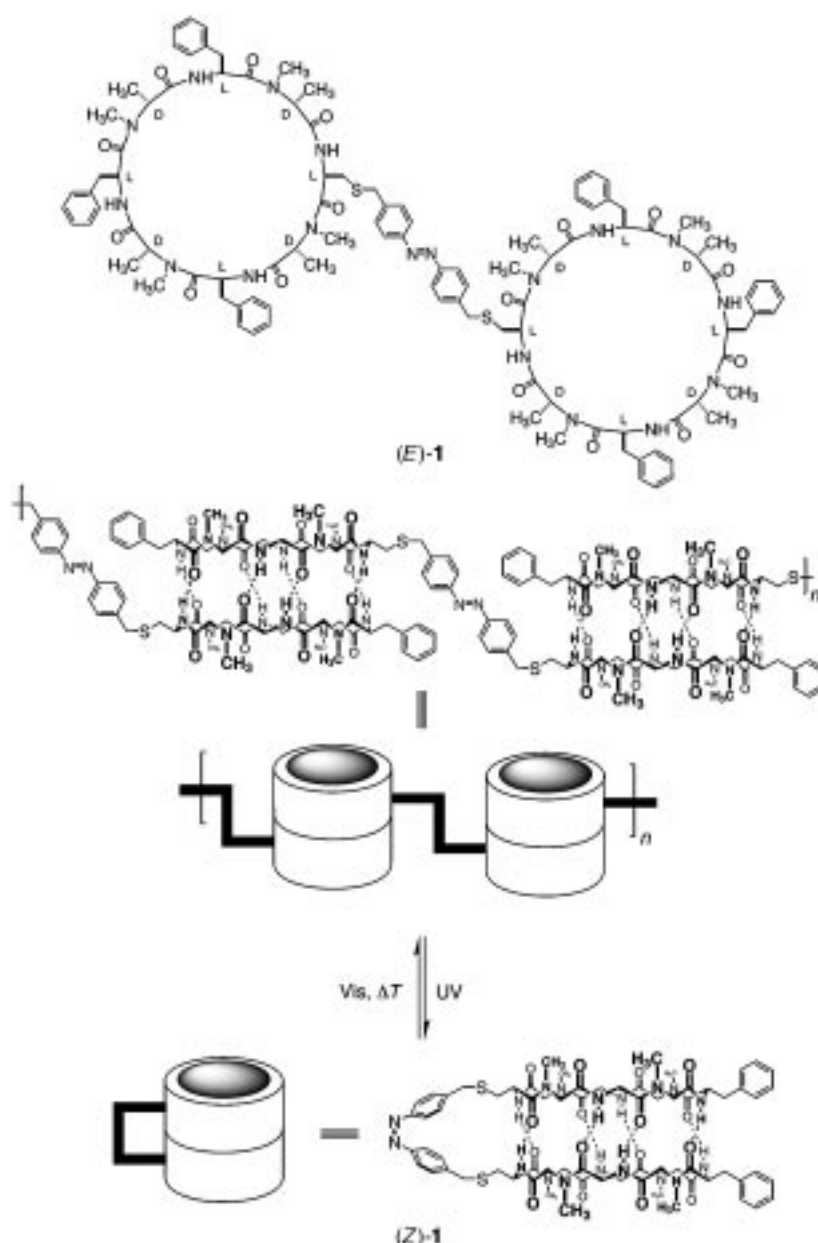
Photoswitchable Hydrogen-Bonding in Self-Organized Cylindrical Peptide Systems**

Martin S. Vollmer, Thomas D. Clark, Claudia Steinem, and M. Reza Ghadiri*

Molecular self-organization is of paramount importance for the development of functional materials.^[1, 2] The desire to control self-assembly processes by external signals, such as light, has led to the design of numerous photochromic supramolecular systems.^[3] Among the various photoswitchable molecules, azobenzenes are utilized extensively in supramolecular chemistry, catalysis, and material science because of their efficient, reversible $E \rightarrow Z$ photoisomerization that leads to large changes in molecular geometry.^[4–12]

Here we report the synthesis and characterization of the novel peptide system **1** (Scheme 1), in which E/Z isomerization of the azobenzene subunit results in photo-switchable hydrogen bonding, which allows the controlled conversion between inter- and intramolecularly assembled cylindrical structures both in solution and in thin films at the air–water interface.

The peptide system **1** is composed of two cyclic octapeptides with alternating D- and L- α -amino acids bridged by an azobenzene moiety. The peptides were designed to give good solubility in nonpolar organic solvents, to offer a reactive nucleophile (cysteine side chain) for the linkage with the azobenzene, and to limit the self-assembly to dimeric β -sheetlike structures by selective backbone N-methylation.^[13] This system was also engineered to exhibit the following structural and kinetic properties. In nonpolar organic solvents, the E isomer of azobenzene ((E)-**1**) was foreseen to exist as an “assembly pool” comprised of dimers and higher oligomers, formed as a result of intermolecular hydrogen bonding and hence concentration dependent (Scheme 1). Irradiation with



Scheme 1. Peptide sequence employed in this study and schematic representation of the photochemical switching between inter- ((E)-**1**) and intramolecularly ((Z)-**1**) hydrogen-bonded cylindrical aggregates (for clarity most side chains are omitted).

UV light was expected to cause $E \rightarrow Z$ isomerization and conversion from intermolecular assemblies into the single intramolecularly hydrogen-bonded species (Z)-**1**. Molecular modeling studies indicated that the thioether spacer connecting the peptide and azobenzene moiety would allow optimal formation of eight intramolecular hydrogen bonds. Furthermore, the Z isomer was predicted to confer resistance against thermal $Z \rightarrow E$ isomerization as a consequence of the increased stability offered by intramolecular hydrogen bonding.

The azobenzene peptide system **1** was synthesized and purified using standard techniques.^[14] The characterization was performed by UV/Vis, NMR, and FT-IR spectroscopy, as well as electrospray ionization mass spectrometry (ESI-MS).^[15, 21, 23] The photoisomers (E)-**1** and (Z)-**1** show UV/Vis absorption bands characteristic of the azobenzene chromo-

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phore (Figure 1). The $\pi-\pi^*$ absorption band between 330 and 390 nm of (*E*)-**1**^[16] is red shifted by about 6 nm relative to the reference compound (*E*)-4,4'-dimethylazobenzene, which leads to a more selective excitation (96%) of the *E* isomer

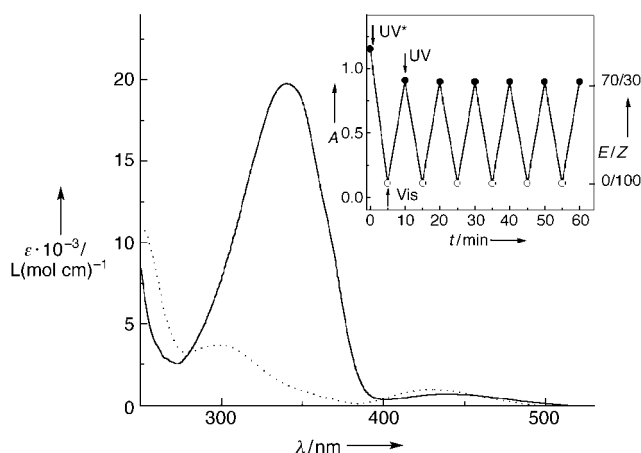


Figure 1. UV/Vis spectra of (*E*)-**1** (—; *E*:*Z*=90:10, photostationary equilibrium in ambient daylight) and (*Z*)-**1** (····; *E*:*Z*=0:100, photostationary equilibrium at 366 nm) in chloroform at 293 K. The inset shows switching cycles from (*Z*)-**1** (*E*:*Z*=0:100) to (*E*)-**1** (*E*:*Z*=70:30) by UV and Vis irradiation (5 min), respectively (the first UV irradiation is marked with an asterisk). The absorbance *A* was monitored after each irradiation step.

at 366 nm. Remarkably, UV irradiation of (*E*)-**1** at 366 nm^[17] causes a quantitative conversion to the *Z* isomer as shown by ¹H NMR spectroscopy. Examples of quantitative *E*→*Z* photo-isomerization are rare.^[18] In general, azobenzenes display *E*:*Z* ratios in the range of 50:50^[5] to 10:90^[3, 19] at the photostationary equilibrium (UV irradiation). On the other hand, irradiation of (*Z*)-**1** with visible light leads to the *Z*→*E* isomerization with an *E*:*Z* ratio of approximately 85:15 at the photostationary equilibrium.^[20]

The 1D and 2D ¹H NMR studies establish that (*Z*)-**1** has the expected monomeric hydrogen-bonded cylindrical structure.^[13] The 1D NMR spectrum of (*Z*)-**1** is concentration-independent and shows a single set of sharp resonances (Figure 2a).^[21] The ROESY spectrum displays only ROE cross peaks (Figure 3a), which indicates a unique structure with no exchange processes detectable on the *R*₁ time scale.^[22] Additionally, the position of the signals corresponding to the *ortho* (δ =6.56) and *meta* (δ =7.07) protons confirms the *Z* stereochemistry of the azobenzene.^[18] The 1D NMR spectrum of (*E*)-**1** is more complicated than that of (*Z*)-**1**, and denotes, as expected, the presence of several species (Figure 2b).^[23] Azobenzene signals now appear in regions diagnostic of a *E* configuration,^[18] however, both *ortho* (δ =7.32–7.50) and *meta* (δ =7.70–7.85) protons display multiple resonances. In addition, the ROESY spectrum of (*E*)-**1** shows numerous exchange peaks (Figure 3b). These data indicate that (*E*)-**1** predominantly forms intermolecular aggregates such as dimers and/or higher oligomers.

FT-IR studies of (*E*)-**1** and (*Z*)-**1** further support the predominance of hydrogen-bonded β -sheet assemblies in chloroform. The IR data display only one amide A band ($\tilde{\nu}_{\text{N-H}}$) at 3308 cm⁻¹ (indicative of hydrogen bonding), the

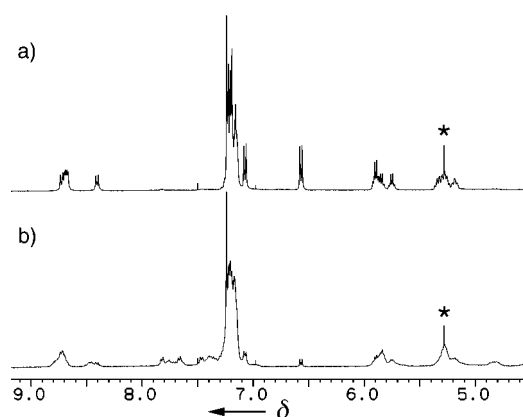


Figure 2. The NH and C^αH regions of the one-dimensional ¹H NMR spectra (400 MHz, 293 K, CDCl₃) of a) (*Z*)-**1** (the spectrum was recorded 24 h after switching to the *Z* isomer) and b) a 90:10 mixture of (*E*)-**1** and (*Z*)-**1**. The residual CH₂Cl₂ is marked with an asterisk. a) A single set of sharp resonances are present for (*Z*)-**1**, with downfield shifts for the NH (δ =8.41–8.72) and C^αH signals (δ =5.19–5.89) relative to the related non-assembled cyclic peptides, which indicates the formation of a tight hydrogen-bonded β -sheet structure. The ³*J*_{N,α} values are all greater than 8.6 Hz, characteristic of β -sheets. b) In the case of (*E*)-**1** several sets of resonances are observed, which is consistent with the presence of multiple oligomeric intermolecular assemblies. The NH and C^αH signals exhibit downfield shifts similar to (a); however, C^αH resonances at δ >5 can be clearly seen, which likely arise from monomeric (*E*)-**1**.

amide I_⊥ band at 1630 cm⁻¹, and the amide II_⊥ band at 1525 cm⁻¹, which are characteristic of β -sheet structures.^[13] The expected signals of non-hydrogen-bonded NH stretches in (*E*)-**1** could not be observed by FT-IR spectroscopy. The association behavior of (*E*)-**1** was also studied by ESI-MS. In addition to signals characteristic for the monomer,^[14] the appearance of the signals for [2M+H+Na]²⁺ as well as [2M+H+K]²⁺ established the presence of a dimer in the assembly pool.

The photoisomerization of **1** is completely reversible in both directions. This can be demonstrated by irradiation of (*E*)-**1** in chloroform solution first with UV light (366 nm, 5 min) followed by visible light (5 min). The absorption was monitored at 340 nm after each irradiation step (inset of Figure 1). The chosen time intervals for irradiation allow reversible switching between an *E*:*Z* ratio of 0:100 to 70:30 and with longer Vis irradiation^[20] between 0:100 to 85:15.

It was expected that (*Z*)-**1** would be more resistant to thermal *Z*→*E* isomerization than (*Z*)-4,4'-dimethylazobenzene as a result of intramolecular hydrogen bonding. Therefore we investigated the rate constants for the thermal *Z*→*E* isomerization at different temperatures by UV/Vis spectroscopy and calculated the activation energy *E*_a from the Arrhenius plot (Table 1). The thermal *Z*→*E* isomerization of (*Z*)-**1** at 293 K in the dark is 7.5 times slower than that of (*Z*)-4,4'-dimethylazobenzene.

With regard to thin films as components for optical devices, we also investigated the self-organization of the peptide system **1** at the air–water interface. Remarkably, both the *E* and *Z* isomer form very stable thin films. The respective isotherms are readily distinguished by plateau regions at 12.5 mN m⁻¹ and 14.0 mN m⁻¹, respectively (Figure 4). Reversible isomerization of **1** within the film is facile as well.

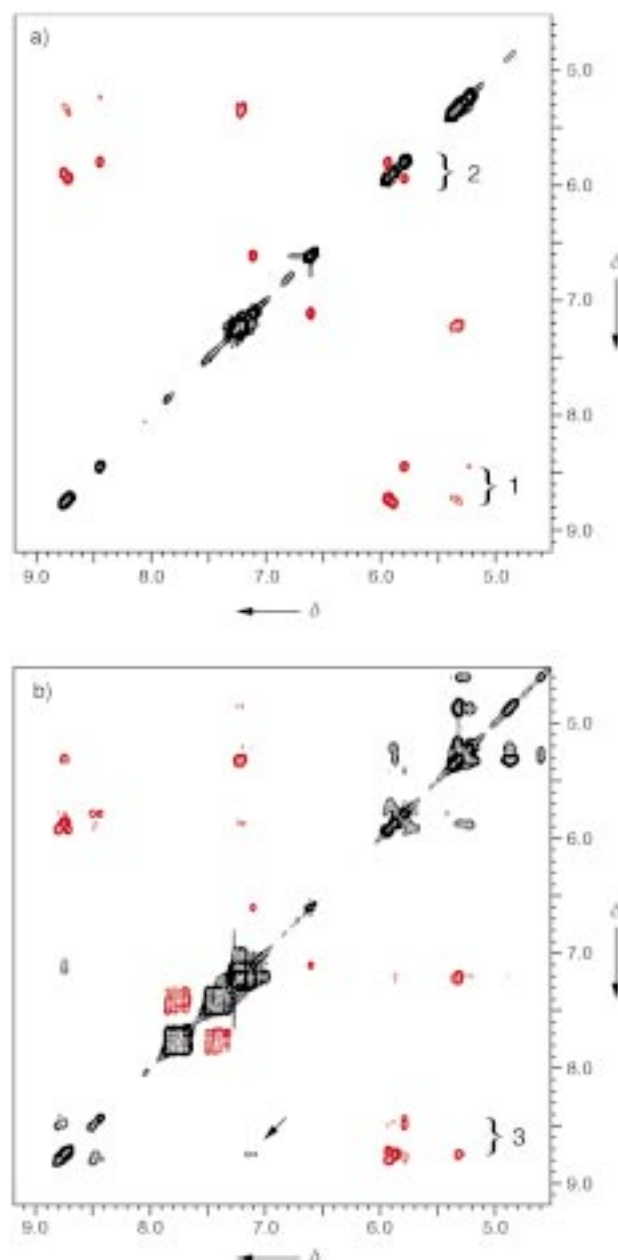


Figure 3. ROESY spectra of (Z)-**1** and a 90:10 mixture of (E)-**1** and (Z)-**1** showing NH, aromatic H, and C^αH regions. ROE cross peaks are shown in red, while exchange cross peaks are in black. a) (Z)-**1** (400 MHz, 293 K, CDCl₃). Only ROE cross peaks are observed, which supports the idea of a unique, monomeric species. Area 1 shows strong $d_{\alpha-N}$ ($i, i+1$) ROE cross peaks (downfield) and weaker intramolecular residue signals (upfield), consistent with the expected β -sheet structure. Furthermore, region 2 displays strong $d_{\alpha-\alpha}$ cross-strand ROE signals diagnostic of antiparallel β -sheets. b) (E)-**1**:(Z)-**1** = 90:10 (500 MHz, 293 K, CDCl₃). In addition to ROE cross peaks, numerous exchange peaks are observed. For example, area 3 shows a complicated pattern of $d_{\alpha-N}$ ROE signals and d_{N-N} exchange peaks, which reflects the presence of multiple interconverting oligomeric species. The exchange cross peaks marked with an arrow occur between hydrogen-bonded and free NH resonances, the latter possibly arising from monomeric (E)-**1**.

Switching cycles were carried out while maintaining either a constant area or surface pressure. Under constant area conditions the surface pressure increases by 1.2 mN m⁻¹ upon conversion from the E into the Z isomer (inset of Figure 4).

Table 1. Rate constants k [s⁻¹] of the thermal Z $\rightarrow$ E isomerization of (Z)-**1** and (Z)-4,4'-dimethylazobenzene (AZO) at different temperatures, as well as the activation energy E_a [kcal mol⁻¹] and the Arrhenius constant $\ln A$ [s⁻¹].^[a]

	$k_{293\text{ K}}$	$k_{303\text{ K}}$	$k_{313\text{ K}}$	$k_{323\text{ K}}$	E_a	$\ln A$
(Z)- 1	2.9×10^{-7}	1.1×10^{-6}	3.6×10^{-6}	1.1×10^{-5}	23.4	25.0
AZO	2.2×10^{-6}	7.0×10^{-6}	2.2×10^{-5}	7.1×10^{-5}	22.4	25.3

[a] All experiments were performed in duplicate. The statistical errors are <5%.

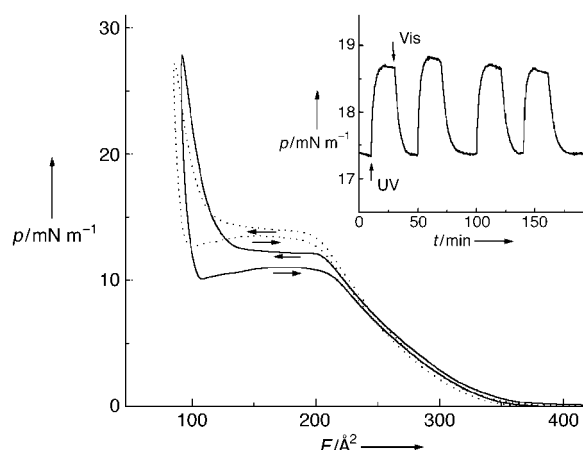


Figure 4. Surface pressure area isotherms (compression and expansion) of (E)-**1** (—) and (Z)-**1** (····) on water at 293 K; F is the area per molecule. The arrows indicate the direction of compression and expansion. The inset shows four photoinduced isomerization cycles of the peptide film at the air–water interface under constant area conditions. The area per molecule was adjusted to 175 Å² after equilibration of the peptide film for 10 h on the air–water interface at 0 mN m⁻¹.

Peptide films were transferred onto a solid support, such as mica and quartz glass, for analysis of the two-dimensional structure by scanning force microscopy and UV/Vis spectroscopy. We observed self-organized photoswitchable peptide films on these surfaces as well.^[24]

Altogether, the described azobenzene–peptide **1** provides a new photochromic supramolecular system that permits reversible switching between inter- and intramolecular hydrogen bonds both in solution and in thin films. We anticipate that this structure-based switching principle together with the quantitative conversion into the Z isomer will have a number of potential applications including photoswitchable transmembrane channels and ionophores. Furthermore, self-organization of photoswitchable peptides on solid substrates can provide a potential entry to novel photoactive materials as components for optical, electronic, and sensor devices.

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- [15] For analytical investigations the peptide solutions in CH₂Cl₂ were dried over molecular sieves. After removal of the CH₂Cl₂ **1** was dissolved in CHCl₃ and CDCl₃ (previously dried over P₂O₅).
- [16] Solutions of (*E*)-**1** in chloroform in daylight contain small amounts of (*Z*)-**1** (*E*:*Z* = 90:10, as determined by ¹H NMR spectroscopy).
- [17] Irradiation at 366 nm was performed with a 4 W UV lamp. At a concentration of 0.05 mm the photostationary equilibrium was obtained after 5 min.
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- [20] Irradiation with visible light was performed with a 15 W fluorescence lamp (Philips F15T8/CW). At a concentration of 0.05 mm the photostationary equilibrium was obtained after 20 min.
- [21] ¹H NMR data for (*Z*)-**1** (400 MHz, 293 K, ca. 3 mm in CDCl₃): L-Phe¹: 8.68 (d, *J* = 9.1 Hz, NH); 5.32 (m, CH); 2.88–3.04 (m, C^βH₂); 7.14–7.25 (m, C^{δ,ε}H); D-MeN-Ala²: 2.79 (s, NCH₃); 5.85 (q, *J* = 6.5 Hz, C-H); 1.03 (d, *J* = 6.8 Hz, C^βH₃); L-Phe³: 8.72 (d, *J* = 8.7 Hz, NH); 5.27 (m, C^αH); 2.88–3.04 (m, C^βH₂); 7.14–7.25 (m, C^{δ,ε}H); D-MeN-Ala⁴: 2.76 (s, NCH₃); 5.89 (overlap., C^αH); 0.98 (d, *J* = 7.0 Hz, C^βH₃); L-Phe⁵: 8.69 (d, *J* = 8.7 Hz, NH); 5.29 (m, C^αH); 2.88–3.04 (m, C^βH₂); 7.14–7.25 (m, C^{δ,ε}H); D-MeN-Ala⁶: 2.76 (s, NCH₃); 5.75 (q, *J* = 7.0 Hz, C^αH); 0.92 (d, *J* = 7.0 Hz, C^βH₃); L-Cys⁷: 8.41 (d, *J* = 8.6 Hz, NH); 5.19 (m, C^αH); 2.67 (2.18, m, C^βH₂); 3.56 (brs, SCH₂C₆H₄N=); 7.07 (d, *J* = 8.1 Hz, SCH₂C₆H₄N=); 6.56 (d, *J* = 8.1 Hz, SCH₂C₆H₄N=); D-MeN-Ala⁸: 3.43 (s, NCH₃); 5.89 (overlap., C^αH); 1.23 (d, *J* = 7.0 Hz, C^βH₃).
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A Chiral Molecular Based Metamagnet Prepared from Manganese Ions and a Chiral Triplet Organic Radical as a Bridging Ligand**

Hitoshi Kumagai and Katsuya Inoue*

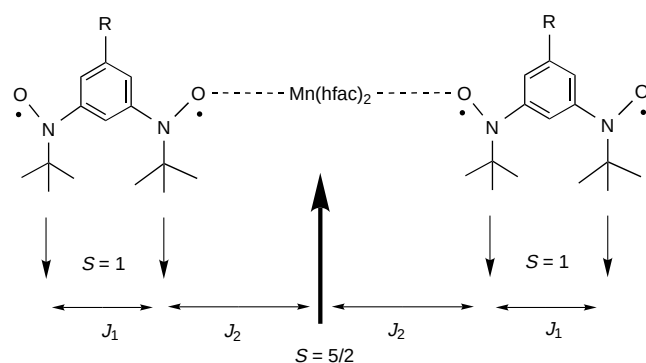
The design of molecular materials with interesting optical and/or magnetic properties has been one of the major challenges of the last few years.^[1, 2] In 1984 Barron and Vrbancich gave the name “magneto-chiral dichroism” (MChD) to the relationship between natural optical activity and magnetic field induced circular dichroism.^[3] In 1997 Rikken and Raupach observed the MChD effect for tris-(3-trifluoroacetyl-(±)-camphorato)europium(III) in the paramagnetic state.^[4] The MChD effect depends on the magnitude of the magnetic moment. It is important to make fully chiral molecule-based magnets, which are expected to exhibit a strong MChD effect. Although novel properties are expected for such compounds, there are only a few examples of molecule-based chiral magnetic material.^[1, 5–7]

Recently, a strategy of using π -conjugated polynitroxide radicals with high-spin ground states as bridging ligands for magnetic metal ions was applied to assemble and align electron spins on a macroscopic scale.^[8–11] The crystal structures and the magnetic structures of these complexes

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are known. The dimensionality of the complex as well as the sign and magnitude of the exchange coupling between neighboring spins can be readily tuned by this strategy.^[12] We are able to synthesize one-dimensional complexes by using bis-monodentate bisaminoxyl radicals as bridging ligands and manganese(II) hexafluoroacetylacetonate ($[\text{Mn}^{\text{II}}(\text{hfac})_2]$; Scheme 1).^[8, 9] The biradical **A** ($\text{R}=\text{H}$, Scheme 1) has been established to have a triplet ground state with a large intramolecular ferromagnetic coupling of $J_1/k_B > 300 \text{ K}$,^[13] where J_1 is defined as an intramolecular exchange parameter in the Heisenberg Hamiltonian $\hat{H} = -2J_1\hat{S}_a \cdot \hat{S}_b$ for the spins $\hat{S}_a$ and $\hat{S}_b$ in the same molecule of **A** ($\text{R}=\text{H}$). In the complex, two *tert*-butylaminoxyl groups are rotated out of the plane of the phenylene ring in a conrotatory manner; each molecule **A** ($\text{R}=\text{H}$) in the crystal has no symmetry element and is therefore chiral (that is, *R*- or *S*-configured). The one-dimensional polymeric chains are isotactic, as all units are of the same chirality. The crystal lattice as a whole is achiral because enantiomeric chains are present.^[8–11] Here we report the synthesis, structure, and magnetic properties of a novel chiral heterospin system which behaves as a metamagnet below 5.4 K.

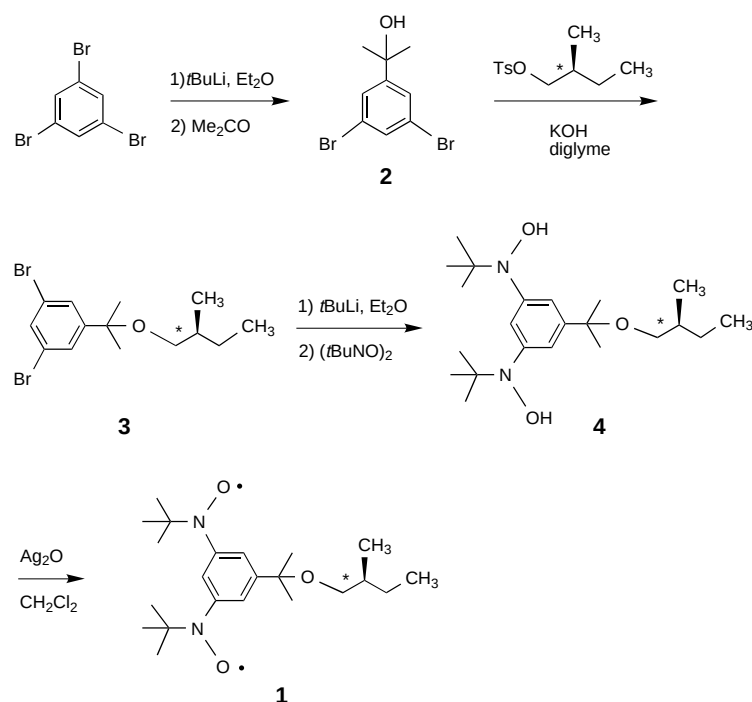


Scheme 1. Schematic representation of the one-dimensional chain formed by Mn^{II} ions and bridging biradicals **A**, which are ferromagnetically coupled.

The chiral biradical **1** was prepared according to Scheme 2. It was mixed with an equimolar amount of dehydrated $[\text{Mn}(\text{hfac})_2]$ in diethyl ether/*n*-heptane, and the mixture was concentrated. Deep brown block crystals were obtained upon storage for one day at -30°C .

An X-ray crystal structure analysis of a triclinic crystal of the complex revealed the formation of a one-dimensional, helical structure (Figure 1).^[14] The oxygen atoms of the aminoxyl moieties of **1** are ligated to two different manganese ions to form a one-dimensional helical chain along the *c* crystal axis. Each manganese ion has an octahedral coordination sphere in which the aminoxyl oxygen atoms from two different biradical molecules are in *trans* sites. The bisaminoxylbenzene unit is in a chiral conformation and forms a protein α -helix-type *R*-helical structure.

The UV/Vis spectra of the complex $[\mathbf{1} \cdot \text{Mn}^{\text{II}}(\text{hfac})_2]_n$ were measured in solution (hexane) and in the crystalline state



Scheme 2. Synthesis of **1**.

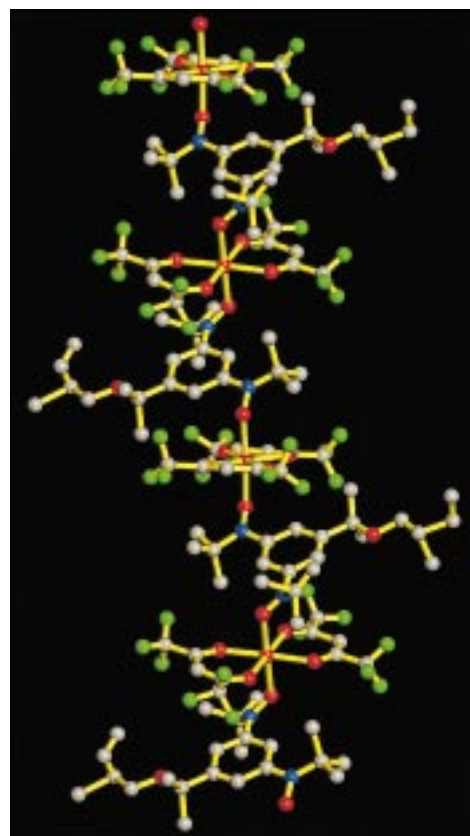


Figure 1. X-ray crystal structure of $[\mathbf{1} \cdot \text{Mn}^{\text{II}}(\text{hfac})_2]_n$. The hydrogen atoms are omitted for clarity. Gray: carbon, blue: nitrogen, red: oxygen and manganese, green: fluorine.

(KBr disk). Both spectra exhibit absorptions around 300 nm and 455 nm, and are very similar to each other and different from that of **1**. Therefore it is reasonable to consider that the

complex retains the interaction between units of **1** and $\text{Mn}^{\text{II}}(\text{hfac})_2$ in hexane. The solution of $[\mathbf{1} \cdot \text{Mn}^{\text{II}}(\text{hfac})_2]_n$ in hexane exhibits an optical rotation of $[\alpha]_{589}^{25} = -314$ ($c = 0.00635$ in hexane)), which is an indication that $[\mathbf{1} \cdot \text{Mn}^{\text{II}}(\text{hfac})_2]_n$ is chiral in solution.

The temperature dependence of the magnetic susceptibility of $[\mathbf{1} \cdot \text{Mn}^{\text{II}}(\text{hfac})_2]_n$ was investigated in a magnetic field of 5000 Oe. The μ_{eff} value of $4.91 \mu_{\text{B}}$ at 300 K is smaller than the theoretical value of $6.43 \mu_{\text{B}}$ for paramagnetic isolated spins of two 1/2 spins of organic radical and one 5/2 spin of $d^5 \text{Mn}^{\text{II}}$ and larger than the value of $3.87 \mu_{\text{B}}$ for two 1/2 spins of organic radicals and 5/2 spins of $d^5 \text{Mn}^{\text{II}}$ in antiferromagnetic coupling. Together with the lack of a minimum at lower temperature, the room-temperature μ_{eff} value suggests the occurrence of strong ($J_{\text{NO-Mn}}/k_{\text{B}} < -300 \text{ K}$) antiferromagnetic coupling between the Mn^{II} ions and the aminoxyl radical ligands. When the measurement was carried out in a magnetic field of 5 Oe, the magnetic susceptibility showed a cusp at 5.4 K. The magnetization at 1.8 K revealed metamagnetic behavior (Figure 2); the response of $[\mathbf{1} \cdot \text{Mn}^{\text{II}}(\text{hfac})_2]_n$ to the magnetization was not sensitive to the weak applied magnetic field below

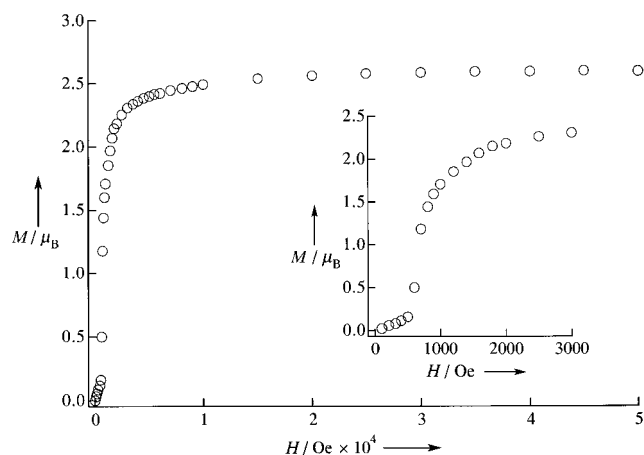


Figure 2. Dependency of the magnetic moment of a polycrystalline sample of $[\mathbf{1} \cdot \text{Mn}^{\text{II}}(\text{hfac})_2]_n$ on the applied field at 1.8 K. Inset: enlargement for the applied magnetic field range 0–3000 Oe.

about 500 Oe; this behavior is characteristic of an antiferromagnet. A sharp rise in magnetization and an approach to saturation, which is characteristic of a ferromagnet, was observed at higher applied magnetic field (inset of Figure 2). A saturation magnetization value of about $2.7 \mu_{\text{B}}$ was reached at 1.8 K and 3 T. When the interaction between the manganese(II) ion and **1** is antiferromagnetic ($J_2 < 0$ in Scheme 1), the magnetic susceptibility of $[\mathbf{1} \cdot \text{Mn}^{\text{II}}(\text{hfac})_2]_n$ is expected to be $3 \mu_{\text{B}}$ ($5/2 - 2/2 = 3/2$), which is in good agreement with the observed value. This magnetic behavior is similar to that observed for a $\text{Mn}^{\text{II}}(\text{hfac})_2$ complex with a nonchiral biradical.^[9]

It is concluded that with $[\mathbf{1} \cdot \text{Mn}^{\text{II}}(\text{hfac})_2]_n$ a R-helical, one-dimensional, molecule-based metamagnet has been prepared. It not only contains an S-configured chiral carbon center, but also an R-configured C_2 chiral skeleton of the organic ligand. The interaction between the manganese ions and the bisaminoxyl radical ligands is expected to be strongly antiferromag-

netic. The temperature dependence of the magnetization and the magnetization curve revealed that the heterospin system behaves as a metamagnet below 5.4 K. Attempts to measure the MChD effect for this complex are in progress.

Experimental Section

4: Compound **3**^[15] was treated by *tert*-butyllithium, and the mixture was stirred for 1 h. Nitroso-*tert*-butane was added to give **4** as a white powder; m.p. 169.8–170.2 °C (decomp.). MS: m/z : 380; ^1H NMR (270 MHz, CDCl_3): $\delta = 7.40$ (brs, 2H), 7.05 (s, 1H), 6.88 (s, 2H), 2.8–3.0 (m, 2H), 1.5–1.7 (m, 1H), 1.4–1.5 (m, 2H), 1.35 (s, 6H), 1.07 (s, 18H), 0.86 (s, 3H), 0.83 (s, 3H).

1: Compound **4** was oxidized with silver oxide in dichloromethane at 0 °C. The resulting product was purified by column chromatography on silica gel eluted with dichloromethane and then diethyl ether. MS: m/z : 378; HR-MS: m/z calcd. for $\text{C}_{22}\text{N}_2\text{O}_3\text{H}_{38}$: 378.2882, found: 378.2869; EPR (9.4507 GHz, CH_2Cl_2 , room temperature): $g = 2.0055$, $a_{\text{N}} = 13.1 \text{ G}$; UV/Vis (CH_2Cl_2): λ_{max} (ϵ) = 285 nm (20200); $[\alpha]_{436}^{25} = -1026$ ($c = 0.02775$ in hexane).

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1,2-Dichalcogenins: Simple Syntheses of 1,2-Diselenins, 1,2-Dithiins, and 2-Selenathiin**

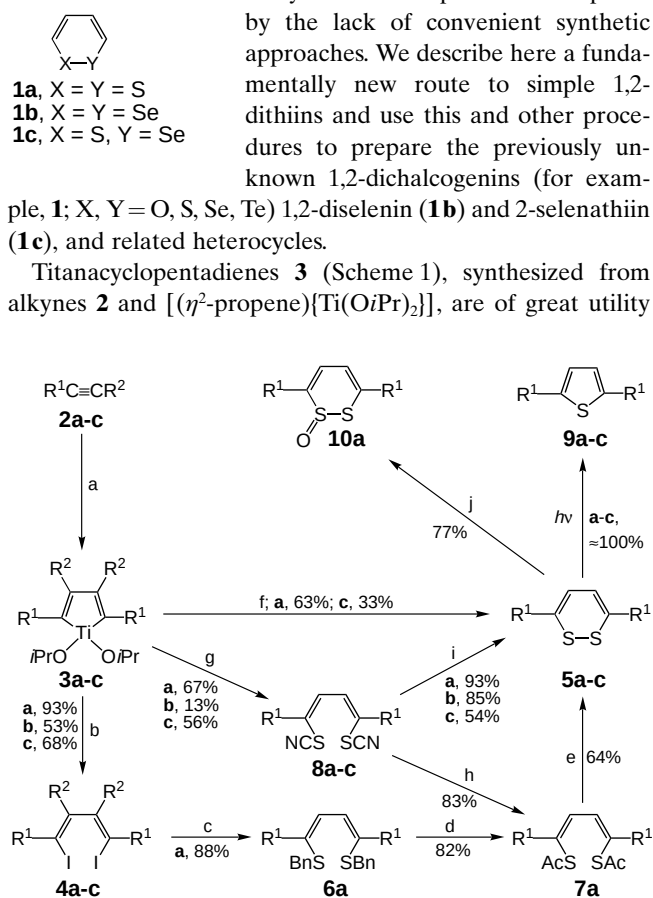
Eric Block,* Marc Birringer, and Chunhong He

Dedicated to Professor Werner Schroth
on the occasion of his 70th birthday

Since the first reports in 1964–1967 of the synthesis of 1,2-dithiin (**1a**)^[1] and isolation of 1,2-dithiin-containing thiurubins from *Asteraceae* species,^[2] synthetic and natural 1,2-dithiins have attracted considerable attention.^[3] These wine-red 8 π -electron heterocycles, which decompose with light or heat to thiophenes, show light-induced antibiotic or insecticidal activity with appropriate substituents.^[4] The detailed

study of these compounds is hampered by the lack of convenient synthetic approaches. We describe here a fundamentally new route to simple 1,2-dithiins and use this and other procedures to prepare the previously unknown 1,2-dichalcogenins (for example, **1**; X, Y = O, S, Se, Te) 1,2-diselenin (**1b**) and 2-selenathiin (**1c**), and related heterocycles.

Titanacyclopentadienes **3** (Scheme 1), synthesized from alkynes **2** and $[(\eta^2\text{-propene})\{\text{Ti}(\text{O}i\text{Pr})_2\}]$, are of great utility



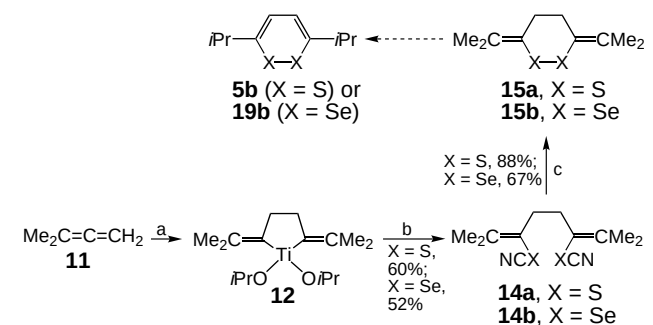
Scheme 1. Synthesis of the 1,2-dithiins **5a–c** from alkynes **2a–c** via titanacyclopentadienes **3a–c**; oxidation and photolysis of **5a–c**. a: $\text{R}^1 = t\text{Bu}$, $\text{R}^2 = \text{H}$; b: $\text{R}^1 = i\text{Pr}$, $\text{R}^2 = \text{H}$; c: $\text{R}^1 = \text{TMS}$, $\text{R}^2 = \text{H}$. Reagents: a) $\text{Ti}(\text{O}i\text{Pr})_4$, $i\text{PrMgCl}$; b) I_2 ; c) $n\text{BuLi}$, $(\text{BnS})_2$; d) LDMAN, AcCl ; e) KOH , MeOH , O_2 or I_2 ; f) S_2Cl_2 ; g) $(\text{SCN})_2$, CH_2Cl_2 ; h) LiAlH_4 , AcCl ; i) TBAF or SmI_2 , THF; j) $m\text{CPBA}$.

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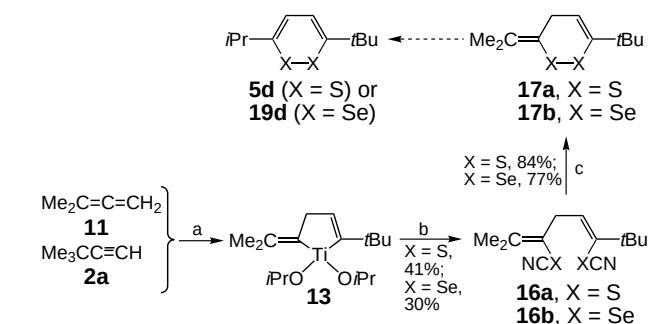
[**] This work was supported by grants from the National Science Foundation, the National Institutes of Health, and the United States Department of Agriculture. We thank Professors Scott Denmark and Werner Schroth for helpful suggestions and DeYou Sha and Zhixing Shan for experimental assistance.

in organic synthesis.^[5] The availability of (*Z,Z*)-1,4-diiodo-1,3-butadienes **4** from **3**^[5e] suggested a useful approach to the 1,2-dithiins **5** through lithiation followed by treatment with dibenzyl disulfide (BnSSBn) or other sulfur electrophiles. The terminal alkynes 3,3-dimethylbutyne, 3-methylbutyne, and trimethylsilylethyne (**2a–c**; $\text{R}^2 = \text{H}$; $\text{R}^1 = t\text{Bu}$, $i\text{Pr}$, and TMS , respectively) afford **3a–c**, which give the diiodo compounds **4a–c** on reaction with I_2 . Sequential treatment of **4a** with $n\text{BuLi}$, BnSSBn , lithium dimethylaminonaphthalene (LDMAN), acetyl chloride, and KOH/MeOH affords 3,6-bis(*t*butyl)-1,2-dithiin (**5a**; 46%) via **6a** and **7a**. By using thiocyanogen ($(\text{SCN})_2$) instead of I_2 and cyclizing with Bu_4NF or SmI_2 ,^[6] one obtains **5a–c** from **3a–c** (62, 13, and 30%, respectively) via **8a–c**. On reaction with sulfur monochloride (S_2Cl_2) **3a** affords **5a** (63%) and thiophene **9a** (16%); similarly, **3c** gives **5c** (33%) and **9c** (33%). The dithiins **5a–c** give thiophenes **9a–c** on brief exposure to light. Oxidation of **5a** gives the *S*-oxide **10a** (77%).^[7]

Ti-mediated cyclizations of 3-methyl-1,2-butadiene (**11**) or a mixture of **11** and **2a**, via **12** and **13**^[5d] followed by trapping with $(\text{SCN})_2$ ($\rightarrow$ **14a** and **16a**, respectively) and ring closure give **15a**^[8] and **17a**, respectively, with exocyclic double bonds (Schemes 2 and 3, respectively). The reactions work equally

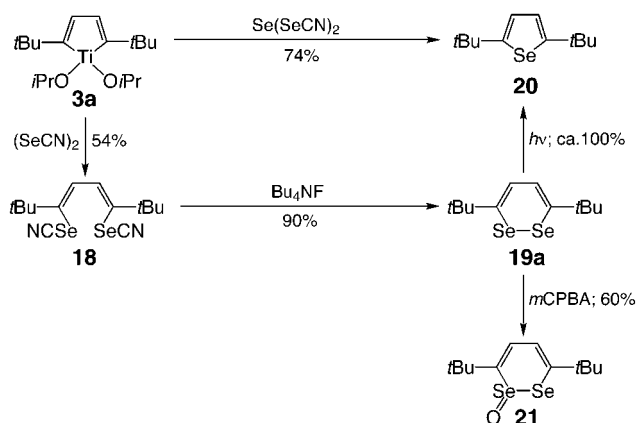


Scheme 2. Synthesis of **15a** and **15b** from **11**. Reagents: a) $\text{Ti}(\text{O}i\text{Pr})_4$, $i\text{PrMgCl}$; b) $(\text{SCN})_2$ or $(\text{SeCN})_2$ ($\text{X} = \text{S}$ and Se , respectively), CH_2Cl_2 ; c) Bu_4NF or SmI_2 .



Scheme 3. Synthesis of **17a** and **17b** from a mixture of **11** and **2a**. Reagents: a) $\text{Ti}(\text{O}i\text{Pr})_4$, $i\text{PrMgCl}$; b) $(\text{SCN})_2$ or $(\text{SeCN})_2$ ($\text{X} = \text{S}$ and Se , respectively), CH_2Cl_2 ; c) Bu_4NF or SmI_2 .

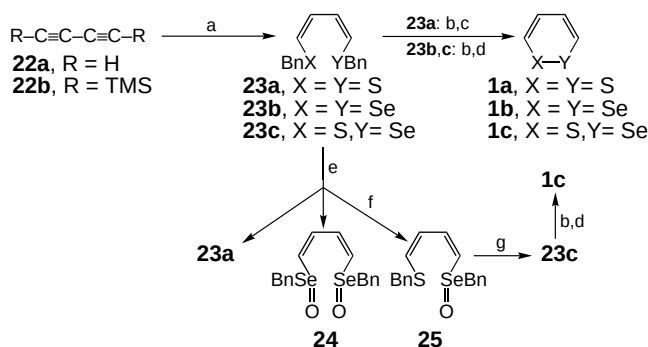
well with **3a**, **12**, and **13** using selenocyanogen ($(\text{SeCN})_2$)^[9a] to afford 3,6-bis(*t*butyl)-1,2-diselenin (**19a**; 49%) from **2a** (**2a** $\rightarrow$ **3a** $\rightarrow$ **18** $\rightarrow$ **19a**, Scheme 4), or **15b** from **11**, and **17b** from **2a/11**. Visible light converts **19a** into selenophene **20**, which can also be prepared from **3a** and $\text{Se}(\text{SeCN})_2$.^[9b]



Scheme 4. Synthesis of **19a** from **3a**, as well as oxidation and photolysis of **19a**.

Oxidation of **19a** gives seleno seleninate **21** (60%), which has NMR spectra similar to those of *S*-oxide **10**.^[10]

1,2-Dithiin (**1a**), first prepared from 1,3-butadiyne (**22a**) via (Z,Z)-1,4-bis(benzylthio)-1,3-butadiene (**23a**, Scheme 5),^[1b] is more easily synthesized from 1,4-bis(trimethylsilyl)-1,3-butadiyne (**22b**).^[11] 1,2-Diselenin (**1b**) is readily prepared by reaction of **22b** with BnSeNa , debenzylation (Li/NH_3) of



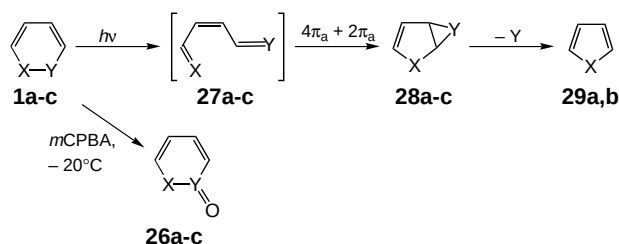
Scheme 5. Synthesis of **1a–c** from **22a, b**. Reagents: a) $\text{BnXNa}/\text{BnYNa}$ ($\text{X}, \text{Y} = \text{S}$ or Se); b) Li or Na/NH_3 ; c) I_2 ; d) O_2 , hexane/hexadecane; e) H_2O_2 , THF; f) chromatography; g) $\text{Na}_2\text{S}_2\text{O}_3$, MeOH .

(Z,Z)-1,4-bis(benzylseleno)-1,3-butadiene (**23b**), removal of hydrocarbons from an aqueous solution of the dilithium salt by hexane extraction, and air oxidation in the presence of hexane/hexadecane. The volatile, but easily polymerized, **1b** is purified, after removal of hexane, by vacuum distillation from hexadecane (b.p. 250 °C) into a trap chilled with liquid N_2 (Figure 1). Solutions for NMR analysis are prepared by addition of CDCl_3 prior to vacuum transfer of **1b**. The preparation of **1c** is accomplished by treating **22b** with a mixture of BnSNa and BnSeNa and oxidizing the mixed product with excess H_2O_2 . The *Se*-oxide **25** of (Z,Z)-1-benzylseleno-4-benzylthio-1,3-butadiene (**23c**) can then be separated from the bis(*Se*-oxide) **24** and unoxidized **23a**. Reduction of *Se*-oxide **25** with thiosulfate to **23c**, and treatment of **23c** with Na/NH_3 followed by work-up as above gives **1c**. Solutions of **1b** and **1c** in hexadecane can be used as sources of these compounds for gas-phase or matrix studies.^[12]



Figure 1. Small-scale apparatus for the distillation of **1a–c** from solution in hexadecane. Solutions of **1a–c** can be prepared by addition of the desired solvent to the hexadecane solution prior to distillation. The ruler is 15 cm long.

The structures of **1b** and **1c** follow from their spectroscopic data, which are surprisingly similar to those of **1a**, and by their facile oxidation to 1,2-diselenin 1-oxide (**26b**) and 2-selenathiin 2-oxide (**26c**, Scheme 6), respectively, the NMR shifts of which are quite similar to those of 1,2-dithiin 1-oxide (**26a**).^[7a, 13] Light converts **1b** into selenophene **29b**, and **1c** to thiophene **29a**, presumably via intermediates **27** and **28**,^[12] as seen in the photochemistry of **1a**.^[3b]



Scheme 6. Photolysis and oxidation of **1a–c**. a: $\text{X} = \text{Y} = \text{S}$; b: $\text{X} = \text{Y} = \text{Se}$; c: $\text{X} = \text{S}, \text{Y} = \text{Se}$.

Comparison of the UV maxima of the parent 1,2-dithiin **1a** (457) and **5a–c** (406, 420, 478 nm, respectively) reveals an interesting hypsochromic shift for **5a, b** and a bathochromic shift for **5c** relative to **1a**. The UV maxima of **1b** (504 nm) and **1c** (488 nm) are bathochromically shifted relative to that of **1a**, as is that of **19a** (447 nm) relative to that of **5a**.^[14] Bathochromic UV shifts are observed as the exocyclic positions of the two double bonds in **15a, b** are independently changed to endocyclic, for example, in the series **15a, 17a** (both 320 nm) $\rightarrow$ **5a** and **15b** (390 nm) $\rightarrow$ **17b** (414 nm) $\rightarrow$ **19a**. There is little change in the positions of the chemical shifts of the olefinic CH ^1H and ^{13}C atoms in the NMR spectra of **17a, b** relative to those in **5a** and **19a**, which is consistent with the nonaromatic character of **19a**. The ^{77}Se NMR peaks in the spectra of **1b** ($\delta = 119$), **19a** ($\delta = 165$), and **1c** ($\delta = 228$) show they are strongly shielded relative to model compounds (PhSeSePh ($\delta = 464$), PhSeSPh ($\delta = 526$),^[15] **15b** ($\delta = 326$), **17b** ($\delta = 287/415$), selenophene **29b** ($\delta = 565$)). Similarities in the spectroscopic data for 1,2-diselenins **1b** and

19a and 1,2-dithiins **1a** and **5a**, respectively, suggest that the structures are also quite similar, for example, *twist* conformations with $\phi(\text{CXXC}) \approx 50^\circ$ and $\phi(\text{CCCC}) \approx 30^\circ$.^[3c]

Experimental Section

Reactions were run under argon in anhydrous solvents, with CDCl_3 as the NMR solvent, and Na_2SO_4 or MgSO_4 as dessicants; **5a**, **8a**, **18**, and **23b**, **c** gave satisfactory CH analyses. Light-sensitive compounds **1b**, **c**, **5a**, and **19a** were handled under red light.

8a: Solutions of 3,3-dimethyl-1-butyne (15 mmol, 1.9 mL) and $i\text{PrMgCl}$ (18 mmol, 9.0 mL, 2.0 M in Et_2O) were added to a stirred mixture of $\text{Ti}(\text{O}i\text{Pr})_4$ (9.0 mmol, 2.4 mL) in Et_2O (100 mL) at -78°C . After 1 h at -78°C and 2 h at -30°C , $(\text{SCN})_2$ (15 mmol; from Br_2 and $\text{Pb}(\text{SCN})_2$ in CH_2Cl_2 at 0°C) in CH_2Cl_2 (45 mL) was added dropwise at -78°C . The mixture was warmed to 25°C over 1.5 h, and stirred for 1 h before cooling to 0°C . The mixture was quenched (saturated NH_4Cl), filtered, and extracted with Et_2O . The organic layer was washed (saturated NaHSO_3 , NaHCO_3 , NaCl), dried, and concentrated in vacuo. Purification by chromatography (hexane: Et_2O 80:20) gave **8a** as a light yellow solid (1.45 g, 67%). M.p. $78-79^\circ\text{C}$; ^1H NMR (300 MHz): $\delta = 1.30$ (s, 18H), 7.13 (s, 2H); ^{13}C NMR (75 MHz): $\delta = 28.9, 40.4, 110.3, 131.2, 142.9$; GC-MS (70 eV): m/z : 280 [M^+], 265, 222, 166, 149; FT-IR (KBr): $\tilde{\nu} = 2155\text{ cm}^{-1}$ ($\text{SC}\equiv\text{N}$).

5a: Bu_4NF (0.55 mmol, 0.55 mL, 1.0 M in THF) was added slowly to **8a** (0.25 mmol, 70 mg) in THF (12 mL) at 0°C . The mixture was stirred at 25°C for 2 h, quenched (saturated NH_4Cl), diluted (Et_2O), washed (NH_4Cl and NaCl solutions), dried, and concentrated. Purification by chromatography (hexane) gave **5a** as a light-sensitive red solid (53 mg, 93%). M.p. $76-77^\circ\text{C}$; ^1H NMR (300 MHz): $\delta = 1.20$ (s, 18H), 6.16 (s, 2H); ^{13}C NMR (75 MHz): $\delta = 28.7, 37.7, 122.0, 146.5$; GC-MS (70 eV): m/z : 228 [M^+], 213, 196, 181, 172, 57; UV (CH_2Cl_2): $\lambda_{\text{max}} = 406\text{ nm}$ ($\epsilon = 195$).

18: ($\text{SeCN})_2$ (12 mmol; from AgSeCN and I_2 in 25 mL THF at 0°C)^[9a] was added to the titanacyclopentadiene prepared as for **8a** from 3,3-dimethyl-1-butyne (12 mmol). Work-up afforded **18** (1.2 g, 54%). M.p. $134-135^\circ\text{C}$; ^1H NMR (300 MHz): $\delta = 1.30$ (s, 18H), 7.05 (s, 2H); ^{13}C NMR (75 MHz): $\delta = 29.3, 40.8, 100.8, 133.2, 145.4$; GC-MS (70 eV): m/z : 374 [M^+]; FT-IR (KBr): $\tilde{\nu} = 2148\text{ cm}^{-1}$ ($\text{SeC}\equiv\text{N}$).

19a: As with **5a**, Bu_4NF (1.1 mmol) was added to **18** (0.5 mmol). Work-up gave **2** as a red solid (146 mg, 90%). M.p. $84-85^\circ\text{C}$; ^1H NMR (300 MHz): $\delta = 1.23$ (s, 18H), 6.20 (s, 2H); ^{13}C NMR (75 MHz): $\delta = 29.2, 38.6, 124.7, 144.18$; ^{77}Se NMR (57 MHz): $\delta = 165$; LC-MS (70 eV): m/z : 324 [M^+], 245, 172, 115, 73; UV (CH_2Cl_2): $\lambda_{\text{max}} = 447\text{ nm}$ ($\epsilon = 235$).

23b: A suspension of BnSeSeBn (2.0 g, 5.9 mmol) in MeOH (25 mL) at 0°C was treated with NaBH_4 (1.1 g, 29.5 mmol) in small portions. Compound **22b** (0.57 g, 2.95 mmol) was then added. The mixture was refluxed for 4 h, cooled to 0°C , and filtered. Recrystallization of the precipitate ($\text{MeOH}:\text{CH}_2\text{Cl}_2$ 5:2) afforded **23b** (0.9 g, 86%) as off-white plates. M.p. $124-125^\circ\text{C}$; ^1H NMR (300 MHz): $\delta = 3.92$ (s, 4H), 6.44 (dd, $^3J_{\text{cis}} = 7, ^5J = 1.9\text{ Hz}$, 2H), 6.52 (dd, $^3J_{\text{cis}} = 7, ^5J = 1.9\text{ Hz}$, 2H), 7.2 (m, 10H); ^{13}C NMR (75 MHz): $\delta = 29.2, 123.1, 126.0, 127.6, 127.7, 127.9, 137.5$; ^{77}Se NMR (57 MHz): $\delta = 315$; MS (70 eV): m/z : 394 [M^+].

1b: Compound **23b** (0.98 g, 2.8 mmol) was added to liquid NH_3 (70 mL) in a 100-mL 3-necked flask at -78°C followed by the addition of lithium (0.2 g, 28 mmol) in small pieces. The blue solution was stirred for 1.5 h at -60°C , quenched (MeOH), and evaporated. The residue was dissolved under argon in degassed aqueous 10% KOH solution (150 mL), which was extracted with degassed hexane ($3 \times 20\text{ mL}$) to remove toluene. The aqueous solution was then covered with hexane (70 mL) and oxygen carefully bubbled through the solution until the hexane phase turned red. The extraction process was repeated twice with fresh hexane ($2 \times 70\text{ mL}$). After drying the hexane extract, hexadecane (15 mL) was added, and the hexane was evaporated (water pump vacuum, 15°C). CDCl_3 (2 mL) was added and the volatiles were transferred at 0.02 Torr to a liquid nitrogen cooled "flash distillation" trap (Figure 1). Yield 68% (deep red solution; by NMR with 1,4-dioxane standard); ^1H NMR (300 MHz): $\delta = 6.10$ (m, 2H, H3,6), 6.27 (m, AA'BB' multiplets with $^3J_{3,4/5,6} = 9.1, ^4J_{3,5/6,4} = 0, ^3J_{4,5} = 5.1, ^5J_{3,6} = 1.9\text{ Hz}$, 2H, H4,5); ^{13}C NMR (75 MHz): $\delta = 112.8$ (C3,6), 132.2

(C4,5); ^{77}Se NMR (57 MHz, Me_2Se): $\delta = 119$; UV (hexane): $\lambda_{\text{max}} = 504$ ($\epsilon = 107$) nm; GC-MS (70 eV): m/z : 212 [M^+].

23c: As for **23b**, BnSeNa (from 8.55 g, 25 mmol BnSeSeBn) and BnSNa (from 6.2 g, 50 mmol α -toluenethiol and 150 mmol NaOMe in 120 mL MeOH) were refluxed with **22b** (7.0 g, 36 mmol) for 24 h. A precipitate containing **23a-c** in a 1:1:2 ratio (10.8 g) formed at 0°C . Oxidation of a portion of this solid (1.22 g) in THF (11 mL) with 30% H_2O_2 (0.6 mL) at 25°C for 2 h precipitated **24** (0.33 g). The structure was determined from its ^{77}Se NMR ($\delta = 878$) and FT-IR spectra (film; $\tilde{\nu} = 785\text{ cm}^{-1}$; $\text{Se}=\text{O}$). Chromatography of the filtrate (silica gel; $\text{CH}_2\text{Cl}_2:\text{MeOH}$ 19:1) gave solid **25** (400 mg, yield 61%) which was characterized from its ^{77}Se NMR ($\delta = 867$) and FT-IR spectra, $\tilde{\nu} = 802\text{ cm}^{-1}$. Reduction of **25** (1.1 mmol) with sodium thiosulfate (0.7 mL, 1 M) in MeOH (10 mL) for 16 h gave **23c** (292 mg, yield 77%) as off-white needles. M.p. $120-121^\circ\text{C}$; ^1H NMR (300 MHz): $\delta = 3.89$ (s, 2H, SCH_2Ph), 3.92 (s, 2H, SeCH_2Ph), 6.05 (d, 1H, $^3J = 9.6\text{ Hz}$, $\text{SCH}=\text{CH}$), 6.17 (dd, $^3J = 10.3, 9.6\text{ Hz}$, 1H, $\text{SCH}=\text{CH}$), 6.40 (d, $^3J = 9.4\text{ Hz}$, 1H, $\text{SeCH}=\text{CH}$), 6.64 (dd, $^3J = 10.3, 9.4\text{ Hz}$, 1H, $\text{SeCH}=\text{CH}$), 7.2-7.3 (m, 10H); ^{13}C NMR (75 MHz): $\delta = 30.1$ (SCH_2Ph), 38.1 (SeCH_2Ph), 124.6 ($\text{SCH}=\text{CH}$), 126.8 ($\text{SeCH}=\text{CH}$), 127.7 ($\text{SCH}=\text{CH}$), 128.3 ($\text{SeCH}=\text{CH}$), 128.6, 128.8, 128.8, 130.2 (Ph); ^{77}Se NMR (57 MHz): $\delta = 312$; MS (70 eV): m/z : 346 [M^+].

1c: As for **1b**, but using sodium instead of lithium. Yield 56% (orange solution; by NMR with 1,4-dioxane standard). ^1H NMR (300 MHz): $\delta = 6.00$ (dd, $^3J_{\text{cis}} = 7.9\text{ Hz}$, $^5J = 1.7\text{ Hz}$, 1H), 6.26-6.29 (m, 3H); ^{13}C NMR (75 MHz): $\delta = 114.0$ ($\text{SeCH}=\text{CH}$), 119.3 ($\text{SCH}=\text{CH}$), 129.7 ($\text{SCH}=\text{CH}$), 132.0 ($\text{SeCH}=\text{CH}$); ^{77}Se NMR (57 MHz, Me_2Se): $\delta = 228$; UV (hexane): $\lambda_{\text{max}} = 488\text{ nm}$; GC-MS (70 eV): m/z : 164 [M^+].

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Lanthanide Nitrido Borates with Six-Membered B₃N₆ Rings: Ln₃B₃N₆**

Olaf Reckeweg and H.-Jürgen Meyer*

Nitrido borates of the lanthanides contain BN_x units in which boron tends to adopt coordination number three as in the structure of hexagonal boron nitride (α-BN). The coordination number four is only found for boron in the cubic form of boron nitride (β-BN). The coordination number two is reported for a large number of compounds, for example, of the type A₃(BN₂)₂ with A = Ca, Sr, or Ba.^[1] Under pressure these compounds, together with hexagonal boron nitride, form an eutectic mixture, thus catalyzing the

conversion of α-BN into its cubic modification (β-BN).^[2] The BN₂^{3−} ions (16 electrons) of the A₃(BN₂)₂ structure type are isoelectronic and isostructural to CO₂. The same can be said of BN₃^{6−} (24 electrons)^[3] and CO₃^{2−} or B₂N₄^{8−} (34 electrons) and C₂O₄^{2−}.

Some nitrido borates of the lanthanides exhibit short metal–metal distances and interesting electric or magnetic properties. For Ln³⁺, the compounds Ln₃B₂N₄ (Ln = La, Ce, Pr, Nd)^[4] have one electron per formula unit in the conduction band.^[5] These compounds as well as Ce₃B₃N₆ have been obtained as black, crystalline substances. Crystals of La₃B₃N₆ are colorless and transparent.

The crystal structures of the isostructural title compounds Ln₃B₃N₆ (Ln = La or Ce) contain the six-membered B₃N₆ ring with three exocyclic nitrogen atoms. This ring can be regarded as a fragment from a layer of hexagonal boron nitride. The triclinic crystal structures of Ln₃B₃N₆ are built up of lanthanide atoms and B₃N₆ rings in a rather complicated way (Figure 1).^[6] The B₃N₆ units are centered in layers near *x* = 0 and 1. Two of the three crystallographically different Ln atoms

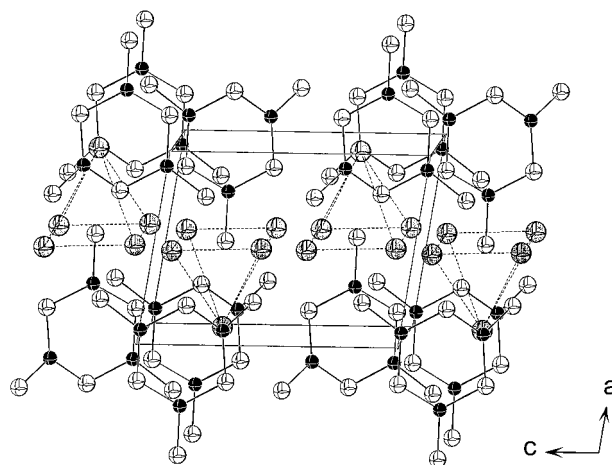


Figure 1. Section of the crystal structure of Ln₃B₃N₆ (Ln = La, Ce) with six-membered B₃N₆ rings.

in the structure build puckered layers (near *x* = 0.5) which are alternately capped from above and below by the third Ln atom. This results in a distorted square-pyramidal arrangement of metal atoms. Each of these motifs is occupied by one exocyclic N atom (5 × *d*(La–N) = 255–265 pm, 5 × *d*(Ce–N) = 252–263 pm) of the B₃N₆ ring. This kind of square-pyramidal arrangement of metal atoms is typical for the coordination environment of terminal N atoms in various nitrido borate ions.^[5, 7] The shortest Ln–Ln distances of 355 pm in La₃B₃N₆ and of 350 pm in Ce₃B₃N₆ are shorter than those in the corresponding metal (the shortest distances here are 375 pm for La and 365 pm for Ce). Short metal–metal distances have also been observed in related compounds LnBN₂ of the smaller lanthanides (Ln = Pr, Nd, Sm, Gd), whose structures are based on a different packing order.^[7] In these structures the nitrido borate units are piled to form ∞¹[B₃N₆] columns with the stacking sequence like that of α-BN.

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As the calculated electronic structure of $\text{La}_3\text{B}_3\text{N}_6$ shows, $\text{Ln}_3\text{B}_3\text{N}_6$ compounds with Ln^{3+} ions should be (transparent) insulators with a band gap on the order of 4 eV (Figure 2).^[8] The crystals we have obtained for the lanthanum compound

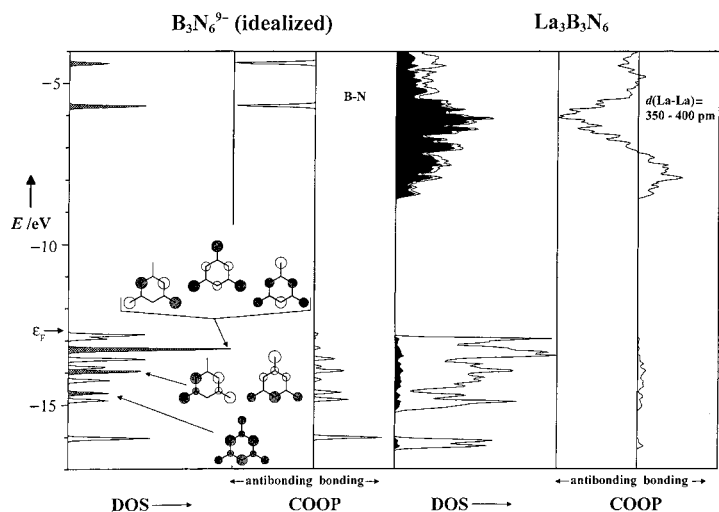


Figure 2. Left: Density of states (DOS) of an idealized $\text{B}_3\text{N}_6^{9-}$ structure (D_{3h} , $d(\text{B-N}) = 148$ and 145 pm). States having π symmetry are drawn in crosshatch, and some corresponding molecular orbitals exhibiting π symmetry are displayed. Crystal orbital overlap populations (COOP) are projected to show the B–N bonding, nonbonding, and antibonding interactions in the $\text{B}_3\text{N}_6^{9-}$ unit. Right: Calculated density of states (DOS) of $\text{La}_3\text{B}_3\text{N}_6$ (La contributions to the DOS of $\text{La}_3\text{B}_3\text{N}_6$ are shown in black) and La–La overlap populations (COOP). The Fermi level for $\text{La}_3\text{B}_3\text{N}_6$ is above the bonding B_3N_6 block (at about -12.7 eV).

are colorless and transparent, while crystals of the cerium compound appeared black. A possible explanation for this observation might be that the cerium ions do not exist in the configuration Ce^{3+} ($[\text{Xe}]4f^15d^0$), but in the different state $\text{Ce}^{4+}(\text{e}^-)$ ($[\text{Xe}]4f^05d^1$). Following this assumption, the surplus electron would occupy the conduction band. The expected ionic radii (for the coordination number eight) are 118 pm for La^{3+} and 114 pm for Ce^{3+} .^[9] The clearly smaller value of 97 pm reported for Ce^{4+} cannot be found in the structure of $\text{Ce}_3\text{B}_3\text{N}_6$. The cerium–nonmetal distances in $\text{Ce}_3\text{B}_3\text{N}_6$ are only 1–5 pm shorter than the lanthanum–nonmetal distances in $\text{La}_3\text{B}_3\text{N}_6$. Instead of the effective ionic radii, the molar volume may be considered as an indicator for the valence state of the metal. The decrease of the molar volume from LaBN_2 ($V_m = 30.5 \text{ cm}^3 \text{ mol}^{-1}$) to CeBN_2 ($V_m = 29.7 \text{ cm}^3 \text{ mol}^{-1}$) follows the trend of the lanthanide contraction. However, this change is not big enough to justify the assumption of a participation of the $[\text{Xe}]4f^05d^1$ configuration for the cerium compound.^[10] The correct configuration and the charge of cerium in $\text{Ce}_3\text{B}_3\text{N}_6$ will have to be determined with different techniques.^[11]

The deviation of the B_3N_6 rings from their ideal symmetry (D_{3h}) is remarkable (C_s). The B–N distances in the six-membered ring (146–150 pm, av 148 pm) are somewhat longer than those between boron and exocyclic N atoms (144 pm). For comparison the B–N distances in hexagonal BN are about 145 pm, and in borazine ($\text{B}_3\text{N}_3\text{H}_6$)^[12] and tris(dimethylamino)borazine ($(\text{NHBNMe}_2)_3$)^[13] they are about 143 pm. Borazine, like benzene, has six π electrons. Because of the

differences in electronegativity and size between boron and nitrogen, there is only a limited delocalization of the six π electrons in borazine. This explains the spontaneous addition reactions with HX or X_2 ($\text{X} = \text{halide}$).^[14] The $\text{B}_3\text{N}_6^{9-}$ unit has nine p orbitals that could form π bonds. For these orbitals we found three bonding combinations similar to those in borazine, three nonbonding combinations (these six orbital combinations are shown in Figure 2) and three antibonding combinations.^[15] The nonbonding orbitals can be considered lone pairs of the nitrogen atoms. Following group theory their combinations can be viewed as one a and one e state that are close to each other in energy. An expected result of our MO calculations^[15] was that the exocyclic nitrogen atoms of an idealized $\text{B}_3\text{N}_6^{9-}$ ion have a larger negative charge (-2.3) than nitrogen atoms in the six-membered ring (-1.8). The ring conformation or deformation in the solid state not only governed by the criterion of minimizing the energy of the B_3N_6 unit, but also by the goal to form a close-packed arrangement of B_3N_6 units and Ln atoms. This can be seen, for example, in the structure of $\text{La}_5\text{B}_4\text{N}_9$, where B_3N_6 units adopt a chair conformation, or in the structures of LnBN_2 ($\text{Ln} = \text{Pr}, \text{Nd}, \text{Sm}, \text{Gd}$), where the B_3N_6 units are nearly planar.^[5, 7]

Only one boron atom in the B_3N_6 unit of $\text{Ln}_3\text{B}_3\text{N}_6$ ($\text{Ln} = \text{La}, \text{Ce}$) is coordinated in a trigonal-planar fashion by nitrogen atoms. The ring distortion in the title compounds is more pronounced than in solid borazine (C_2 symmetry),^[12, 16] which has only very little effect on the electronic structure of $\text{La}_3\text{B}_3\text{N}_6$.^[8] The occupied energy levels of an idealized $\text{B}_3\text{N}_6^{9-}$ structure (D_{3h}) can be compared with the calculated density of states for $\text{La}_3\text{B}_3\text{N}_6$. Some of these energy levels split for the real B_3N_6 unit in $\text{La}_3\text{B}_3\text{N}_6$, for example the twofold degenerate σ combination near -16 eV. If the interactions with the Ln atoms are taken into consideration, some of the energy levels are lowered because of Ln– B_3N_6 bonding interactions (Figure 2). The conduction band is dominated by metal states and contains only small contributions from the orbitals of the nonmetal atoms. A formal description of $\text{B}_3\text{N}_6^{9-}$ ions is supported by the large band gap between the valence band and the conduction band. An analysis of the La–La overlap populations in the structure exhibits bonding states in the conduction band (see COOP in Figure 2, right). Therefore, it seems possible that cerium exists as Ce^{4+} in $\text{Ce}_3\text{B}_3\text{N}_6$ and that the compounds $\text{Ln}_3\text{B}_3\text{N}_6$ could be chemically reduced.

Like borazine, the $\text{Ln}_3\text{B}_3\text{N}_6$ compounds with their B_3N_6 unit may be possible candidates for addition reactions in solution. Another interesting aspect would be the use of $\text{Ln}_3\text{B}_3\text{N}_6$ for the conversion of hexagonal BN into its cubic modification.^[2]

Experimental Section

A general approach for synthesizing metal nitrido borates is by direct reaction of metal nitride with α -BN. For this purpose CeN was prepared by the reaction of cerium metal (Strem, 99.9%) under flowing ammonia (at 1027 K, 24 h). LaN was purchased (Alfa, 99%). α -BN (ABCR, 99.8%) was degassed under vacuum conditions before use (at 675 K). CaCl_2 was employed as a flux (dried for 4 h at 675 K under flowing HCl). All employed reagents and reaction containers were handled in a glove box under dried argon.

$\text{Ln}_3\text{B}_3\text{N}_6$ was synthesized by the reaction of LnN ($\text{Ln} = \text{La}$ or Ce) with α -BN at 1500 K in a CaCl_2 flux (molar ratio 1:5:1). Arc-welded tantalum ampoules (Plansee) were used as reaction containers. The ampoules were

sealed in evacuated silica tubes to shield them from moisture and air. $\text{La}_3\text{B}_3\text{N}_6$ was obtained as colorless, transparent single crystals, and $\text{Ce}_3\text{B}_3\text{N}_6$ as black single crystals with defined faces but irregular shape.

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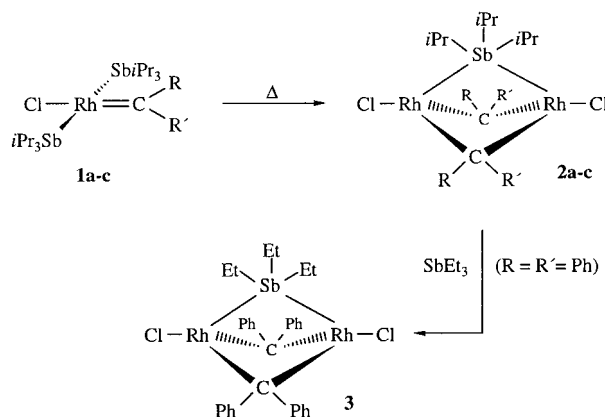
Keywords: electronic structure • lanthanides • materials science • nitrido borates • solid-state structures

Formation of Novel Dinuclear Mixed-Valence Rhodium Complexes by Intramolecular Migration of a Chelating Ligand**

Ulrich Herber, Birgit Weberndörfer, and Helmut Werner*

Dedicated to Professor Nils Wiberg
on the occasion of his 65th birthday

One of the most surprising results of recent work from our laboratory was that trialkylstibanes can function as bridging ligands in dinuclear transition metal complexes. In the course of investigations concerned with the reactivity of square-planar carbenerhodium(I) compounds **1**, which were obtained from *trans*- $[\text{RhCl}(\text{C}_2\text{H}_4)(\text{SbiPr}_3)_2]$ and diazoalkanes $\text{RR}'\text{CN}_2$ in excellent yields,^[1] we discovered that these compounds are thermally quite labile and react upon heating in benzene at 80 °C by partial elimination of SbiPr_3 to afford the Rh_2 complexes **2** (Scheme 1).^[2] The notable feature is that the



Scheme 1. **a:** $\text{R} = \text{R}' = \text{Ph}$; **b:** $\text{R} = \text{R}' = p\text{-Tol}$; **c:** $\text{R} = \text{Ph}$, $\text{R}' = p\text{-Tol}$.

coordination geometry around the two metal centers of **2**, which are formally in the oxidation state +I, is distorted tetrahedral and that this geometry is preserved in the reactions with SbMe_3 or SbEt_3 (formation of **3**).^[2] In textbooks and standard references it was generally considered as highly improbable^[3] that trialkylstibanes as well as their phosphorus and arsenic counterparts can undergo a bridging coordination mode, and this expectation seemed to be confirmed by numerous studies on the chemistry of ligands of the general type ER_3 ($\text{E} = \text{P}, \text{As}, \text{Sb}$).^[4]

Following our initial studies on the reactivity of **2** toward CO , CNtBu , SbR_3 , PiPr_3 etc.^[2, 5] we were interested to know whether the dinuclear structure with the core molecular fragment $\text{Rh}(\mu\text{-SbiPr}_3)(\mu\text{-CPh}_2)_2\text{Rh}$ would be maintained if the axial chloro ligands are replaced by chelating anions. The outcome of this substitution would be of course an increase in

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the coordination number of rhodium. We knew already that the reaction of **2a** with NaC_5H_5 leads to the formation of the dinuclear complex $[(\text{C}_5\text{H}_5)_2\text{Rh}_2(\mu\text{-CPh}_2)_2]$, which does not contain a bridging stibane unit.^[2] A different process takes place, however, if acetylacetonate (acac) is used as substrate. The dirhodium compound **4** (Scheme 2) is formed from **2a** and an equimolar amount of $[\text{Ti}(\text{acac})]$. The structure of **4** (which is thermally stable up to 144°C) is shown in Figure 1.^[6] Owing to the increase of the coordination number at Rh1, the stibane as well as one of the diphenylcarbene ligands are linked to the two metal centers in an unsymmetrical fashion. The bond lengths Sb–Rh1 and Sb–Rh2 differ by about $0.35\text{ \AA}$ and those between the carbene carbon atom C2 and Rh1 and Rh2, respectively, by approximately the same value. Compared to the distances in the starting material **2a**, the distance Sb–Rh1 is about $0.19\text{ \AA}$ shorter and the distance Sb–Rh2 about $0.18\text{ \AA}$ longer, which is probably due to the different types of anionic ligands. Quite unexpectedly, also the acetylacetonate is coordinated in an unsymmetrical mode [Rh1–O1 $2.064(3)\text{ \AA}$, Rh1–O2 $2.274(4)\text{ \AA}$] thereby the shorter bond Rh1–O1 being opposite to the shorter bond Rh1–C1. The Rh–Rh distance in **4** is about $0.15\text{ \AA}$ longer than in compound **2a** but still lies in the range of other rhodium(II) complexes with a metal–metal bond.^[7]

The reaction of **2a** with $[\text{Ti}(\text{acac})]$ in the molar ratio of 1:2.4 affords the symmetrical dinuclear compound **5** (Scheme 2), which after recrystallization from pentane is isolated as a brown, slightly air-sensitive solid in 86% yield. The related complex **6** with two trifluorinated acetylacetonate ligands is

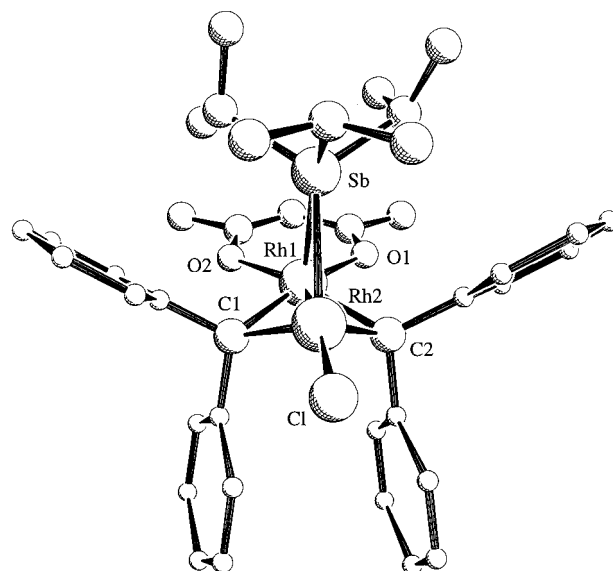
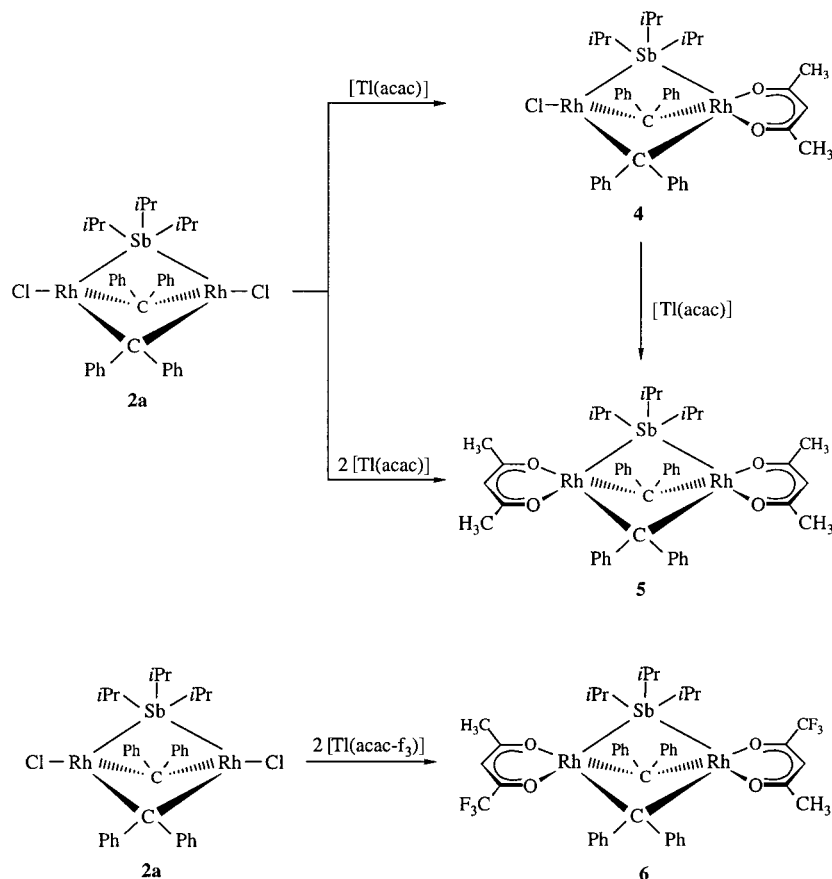


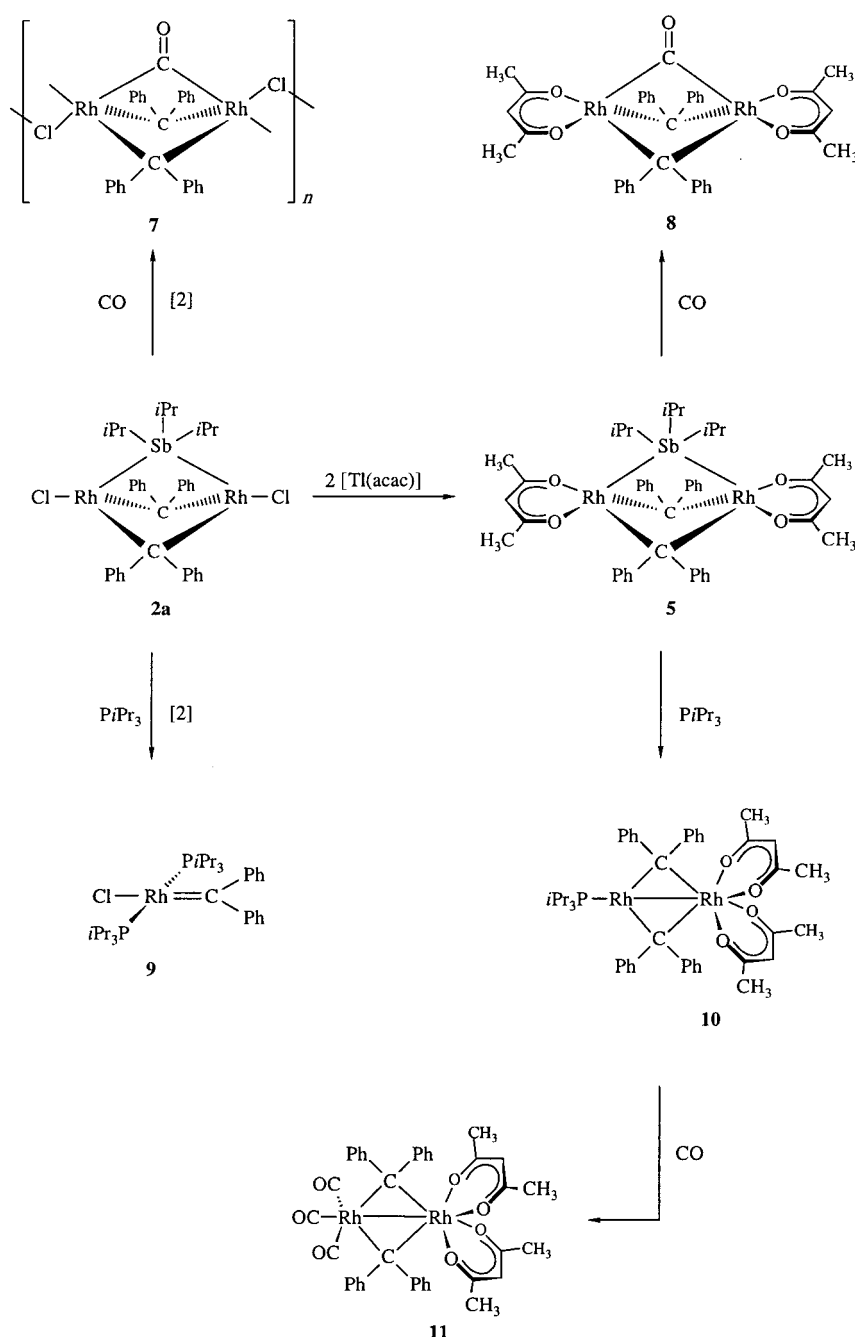
Figure 1. Molecular structure of **4** in the crystal (the carbon atoms of the phenyl groups were reduced in size for clarity). Selected bond lengths [$\text{\AA}$] and angles [$^\circ$]: Rh1–Rh2 $2.6796(6)$, Rh1–C1 $2.030(4)$, Rh1–C2 $2.187(4)$, Rh1–O1 $2.064(3)$, Rh1–O2 $2.274(4)$, Rh1–Sb $2.498(1)$, Rh2–Sb $2.846(1)$, Rh2–C1 $2.053(4)$, Rh2–C2 $1.825(4)$, Rh–C1 $2.483(1)$; Rh1–Sb–Rh2 $59.77(2)$, Sb–Rh1–Rh2 $66.57(2)$, Sb–Rh2–Rh1 $53.66(2)$, Rh1–Rh2–C1 $171.55(3)$, Rh1–C1–Rh2 $82.0(2)$, Rh1–C2–Rh2 $83.3(2)$, C1–Rh1–O1 $167.0(1)$, C2–Rh1–O2 $165.9(1)$, O1–Rh1–O2 $78.5(1)$, C1–Rh1–C2 $83.2(2)$, C1–Rh2–C2 $92.3(2)$.

obtained in a similar way. A remarkable feature is the different reactivity of **2a** and **5** toward CO (Scheme 3). While the dichloro compound **2a** reacts with carbon monoxide to give the presumably polymeric complex **7**,^[8] upon treatment of **5** with CO in benzene the triply bridged dinuclear compound **8** is cleanly formed. The exchange of one bridging ligand takes place without any further rearrangement of the molecular skeleton. Characteristic data of **8**^[9] are the CO stretching frequency at 1842 cm^{-1} in the IR spectrum and the signal for the $\mu\text{-CO}$ carbon atom at $\delta = 182.8$ in the ^{13}C NMR spectrum.

The difference in the behavior of **2a** and **5** toward triisopropylphosphane is even more striking than toward CO. Recently, we reported that treatment of **2a** with $\text{P}(\text{iPr})_3$ leads by substitution of the stibane and cleavage of the bridging bonds to the stable mononuclear complex **9**.^[2] The bis(acetylacetonate) compound **5** reacts much more slowly than **2a** with an excess of $\text{P}(\text{iPr})_3$ and affords after 4 h at 60°C in benzene the dinuclear compound **10** in about 70% yield. Compound **10** is a brown, thermally stable solid which is modestly air-sensitive and soluble in most common organic solvents. Both the ^1H and the ^{13}C NMR spectra of **10** display two sets of signals for the protons and carbon atoms of the $\text{OC}(\text{CH}_3)_2$ moieties of the acetylacetonate ligands, which points



Scheme 2.



Scheme 3.

to an unsymmetrical structure of the dinuclear complex. This proposal has been confirmed by the X-ray crystal structure analysis (Figure 2).^[6] One of the chelating ligands has migrated from one metal center to the other and its position is occupied by the triisopropylphosphane. The stibane ligand has been replaced while the bridging diphenylcarbene units are maintained. The distances between the less coordinated metal center Rh1 and the carbene atoms C1 and C2 are 0.17 Å shorter than those between Rh2 and C1 and C2, and they differ only slightly to the Rh–C bond lengths of **2a** (2.003(1) and 2.007(3) Å). The distance Rh1–Rh2 of **10** is nearly the same as in compound **4**. Similarly to **4**, also in compound **10** the acetylacetonate ligands are not coordinated in a symmetrical fashion and, therefore, in both six-membered chelate

rings the Rh–O bond lengths differ by 0.08–0.10 Å. The bond axis Rh2–Rh1–P is not exactly linear which is probably a consequence of the steric repulsion between the phenyl and the isopropyl groups.

The unsymmetrical dinuclear complex **10**, in which one of the rhodium centers (Rh1) formally has the oxidation state zero and the other (Rh2) the oxidation state +II, reacts with Lewis acids as well as with Lewis bases. While the composition of the products from **10** and HCl or $\text{CF}_3\text{SO}_3\text{Me}$, respectively, is not yet established, upon treatment of **10** with CO in pentane the tricarbonyl complex **11** is formed in excellent yield. We note that despite the use of a considerable excess of CO no cleavage of the carbene bridges occurs. Typical features for **11** are the three CO stretching vibrations at 2056, 2016, and 1965 cm^{-1} in the IR spectrum which support the proposed structure shown in Scheme 3.

To the best of our knowledge, compounds **10** and **11** are the first representatives of a novel type of mixed-valence dinuclear $\text{Rh}^0\text{--Rh}^{\text{II}}$ complexes with bridging carbene ligands.^[10] Dinuclear complexes with $\text{Rh}(\mu\text{-CR}_2)_2\text{Rh}$ as a core building block were already known,^[7, 8, 11] but in none of these compounds the metals have a different oxidation state. Current work is not only aimed to broaden the scope of unsymmetrical $\text{Rh}^0\text{--Rh}^{\text{II}}$ complexes, but also to find out whether these compounds possess a related reactivity to the rhodium(II) complexes, discovered and intensively investigated by Maitlis et al.,^[12] with CH_2 and CHR as bridging ligands.

Experimental Section

4: A solution of **2a** (78 mg, 0.09 mmol) in acetone (15 mL) was treated at 0 °C with $[\text{Ti}(\text{acac})]^{[13]}$ (27 mg, 0.09 mmol) and then allowed to warm to room temperature and stirred for 30 min. The solvent was removed in vacuo and the residue was extracted with pentane (3×10 mL). The combined extracts were concentrated to about 2 mL, and the concentrated solution was chromatographed on Al_2O_3 (neutral, activity grade V). A colorless fraction (which was thrown away) was eluted with pentane and then a red-brown fraction with benzene. The latter was concentrated to dryness in vacuo, and the residue was recrystallized from acetone/diethyl ether (1:1) to give brown crystals; yield 61 mg (73 %); m.p. 144 °C (decomp); ^1H NMR (400 MHz, C_6D_6):^[14] $\delta = 5.57$ (s, 1H, CH of acac), 1.99 (s, 6H, CH_3 of acac), 1.63 (sept, 3H, $^3J(\text{H,H}) = 7.4$ Hz, SbCHCH_3), 0.99 (d, 18H, $^3J(\text{H,H}) = 7.4$ Hz, SbCHCH_3); $^{13}\text{C}\{^1\text{H}\}$ NMR (100.6 MHz, C_6D_6): $\delta = 188.7$ (s, CO of acac), 1773 (m, CPh_2), 101.3 (s, CH of acac), 28.3 (s, CH_3 of acac), 24.8 (s, SbCHCH_3), 21.4 (s, SbCHCH_3).

5: A solution of **2a** (84 mg, 0.10 mmol) in acetone (15 mL) was treated with $[\text{Ti}(\text{acac})]$ (73 mg, 0.24 mmol) and stirred for 2 h at room temperature. The

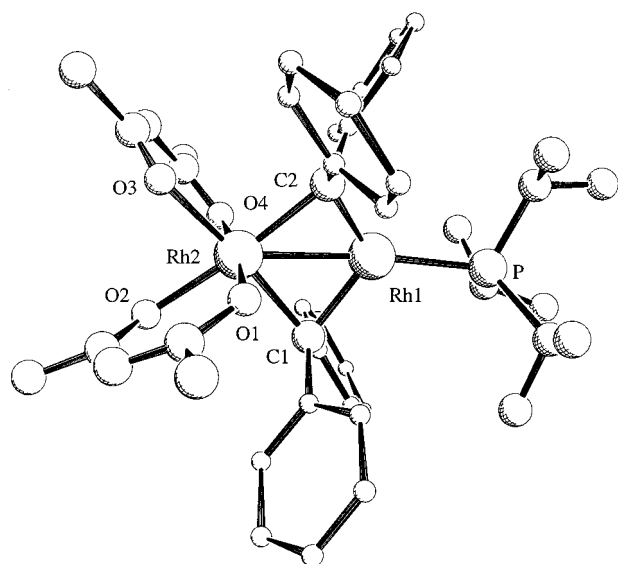


Figure 2. Molecular structure of **10** in the crystal (the carbon atoms of the phenyl groups were reduced in size for clarity). Selected bond lengths [Å] and angles [°]: Rh1–Rh2 2.6317(7), Rh1–P 2.332(2), Rh1–C1 1.982(6), Rh1–C2 1.982(5), Rh2–C1 2.158(5), Rh2–C2 2.153(6), Rh2–O1 2.008(4), Rh2–O2 2.114(4), Rh2–O3 2.115(4), Rh–O4 2.037(4); Rh2–Rh1–P 169.91(4), Rh1–C1–Rh2 78.8(2), Rh1–C2–Rh2 78.9(2), P–Rh1–C1 122.4(2), P–Rh1–C2 125.3(2), C1–Rh1–C2 103.7(2), C1–Rh2–C2 92.6(2), O1–Rh2–O2 92.2(2), O3–Rh2–O4 90.0(2), O1–Rh2–O4 170.0(2), O1–Rh2–O3 83.3(2), O2–Rh2–O4 82.6(2), O2–Rh2–O3 83.5(2), O2–Rh2–C2 173.1(2), O3–Rh2–C1 169.8(2).

work-up procedure was the same as described for **4**. Recrystallization from pentane (–78 °C) gave brown crystals; yield 85 mg (86%), m.p. 55 °C (decomp); ¹H NMR (400 MHz, C₆D₆): δ = 5.58 (s, 2H, CH of acac), 2.00 (s, 12H, CH₃ of acac), 1.91 (sept, 3H, ³J(H,H) = 7.4 Hz, SbCHCH₃), 0.96 (d, 18H, ³J(H,H) = 7.4 Hz, SbCHCH₃); ¹³C{¹H} NMR (100.6 MHz, C₆D₆): δ = 188.4 (s, CO of acac), 176.8 (t, ¹J(Rh,C) = 20.8 Hz, CPh₂), 100.6 (s, CH of acac), 28.6 (s, CH₃ of acac), 25.6 (s, SbCHCH₃), 21.3 (s, SbCHCH₃). Compound **6** was prepared in a similar way; yield 77%; ¹⁹F NMR (188.3 MHz, C₆D₆): δ = –75.0 (s).

8: A stream of CO was passed through a solution of **5** (85 mg, 0.09 mmol) in benzene (10 mL) for 15 s at room temperature. After the solution was stirred for 30 min, the solvent was removed in vacuo, and the residue was recrystallized from CH₂Cl₂/pentane (5 °C) to give red crystals; yield 59 mg (89%); m.p. 48 °C (decomp); IR (C₆H₆): ν̄(CO) = 1842 cm^{–1}; ¹H NMR (400 MHz, C₆D₆): δ = 5.36 (s, 2H, CH of acac), 1.76 (s, 12H, CH₃ of acac); ¹³C{¹H} NMR (100.6 MHz, C₆D₆): δ = 189.5 (s, CO of acac), 182.8 (m, μ-CO), 101.5 (s, CH of acac), 27.9 (s, CH₃ of acac).

10: A solution of **5** (105 mg, 0.11 mmol) in benzene (20 mL) was treated with P*i*Pr₃ (105 μL, 0.55 mmol) and stirred for 4 h at 60 °C. After the mixture was cooled to room temperature, the solvent was removed in vacuo, the residue was dissolved in pentane (3 mL) and the solution was chromatographed on Al₂O₃ (neutral, activity grade V). A colorless fraction (which was thrown away) was eluted with pentane and then a brown fraction with benzene. The latter was concentrated to dryness in vacuo, and the residue was recrystallized from pentane (5 °C) to give brown crystals; yield 67 mg (68%); m.p. 75 °C (decomp); ¹H NMR (400 MHz, C₆D₆): δ = 4.86 (s, 2H, CH of acac), 1.90 (m, 3H, PCHCH₃), 1.84, 1.42 (both s, 12H, CH₃ of acac), 0.83 (m, 18H, PCHCH₃); ¹³C{¹H} NMR (100.6 MHz, C₆D₆): δ = 186.4, 183.8 (both s, CO of acac), 160.5 (m, CPh₂), 98.7 (s, CH of acac), 27.7, 26.7 (both s, CH₃ of acac), 25.4 (m, PCHCH₃), 22.5 (m, PCHCH₃); ³¹P NMR (162.0 MHz, C₆D₆): δ = 40.5 (dd, ¹J(Rh,P) = 257.7, ²J(Rh,P) = 5.9 Hz).

11: A stream of CO was passed through a solution of **10** (150 mg, 0.17 mmol) in pentane (15 mL) for 10 s at room temperature. After the solution was stirred for 15 min, the solvent was removed in vacuo, and the residue was recrystallized from diethyl ether/pentane (1:3) at 5 °C to give red crystals; yield 116 mg (83%); m.p. 111 °C (decomp); IR (C₆H₆):

ν̄(CO) = 2056, 2016, 1965 cm^{–1}; ¹H NMR (200 MHz, C₆D₆): δ = 4.55 (s, 2H, CH of acac), 1.71, 1.21 (both s, 12H, CH₃ of acac).

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- [10] It was mentioned by a referee that the CPh₂ groups could be considered as dianionic ligands. The metal centers would then be Rh^{II} and Rh^{IV}. The total electron count would be unaltered due to this formalism.
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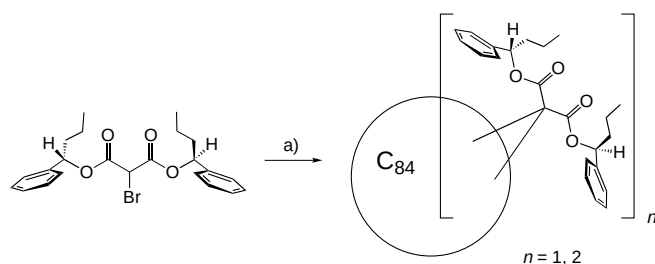
Chemistry of C₈₄: Separation of Three Constitutional Isomers and Optical Resolution of D₂-C₈₄ by Using the “Bingel–Retro-Bingel” Strategy**

Jeanne Crassous, José Rivera, Nicolette S. Fender, Lianhe Shu, Luis Echegoyen*, Carlo Thilgen, Andreas Herrmann, and François Diederich*

Although C₈₄ is the third most abundant fullerene,^[1] its chemistry remains remarkably unexplored. Computational studies revealed that this higher fullerene has 24 theoretically possible constitutional isomers that obey the isolated pentagon rule (IPR), with a D₂- and a D_{2d}-symmetrical isomer being the most stable ones.^[2] These two isomers are indeed the most abundant ones in the C₈₄ fraction of fullerene soot, with a ratio of D₂-C₈₄:D_{2d}-C₈₄ of approximately 2:1, but their chromatographic separation is extremely difficult and, for several years, they had only been isolated and characterized as

a mixture by ¹³C NMR spectroscopy^[3] and other methods. The successful separation by recycling high performance liquid chromatography (HPLC) and full characterization of these two isomers was only reported recently by Shinohara and co-workers.^[4] The structure of the D_{2d} isomer was unambiguously proven by Balch et al. who prepared the [(η²-D_{2d}-C₈₄)Ir-(CO)Cl(PPh₃)₂]·4C₆H₆ complex and solved its X-ray crystal structure.^[5] This is the only characterized derivative of a C₈₄ isomer reported thus far. Although four possible structures exist that fit the ¹³C NMR data of the major D₂ isomer,^[3,4] the experience with the isomers of another higher fullerene, C₇₈,^[6] and preliminary 2D NMR (INADEQUATE) data of the C₈₄ isomers^[7a] suggest that the compound with the calculated lowest energy structure is the one isolated in all published work. Both ¹³C NMR studies^[3c,7] and the analysis of endohedral ³He complexes of the C₈₄ fraction of the fullerene soot by ³He NMR spectroscopy^[8] have provided evidence for the presence of minor amounts of additional isomers, but none of them have been isolated to date. Here, we describe a powerful new protocol to isolate pure constitutional isomers of higher fullerenes by the intermediacy of defined covalent adducts,^[9] by using the recently introduced Bingel–retro-Bingel reaction sequence.^[10,11] This protocol takes advantage of the fact that covalent adducts of isomeric higher fullerenes are much easier to separate than the parent unfunctionalized carbon spheroids. By this method we not only isolated the two known major constitutional isomers of C₈₄ but also succeeded in the first isolation and spectroscopic characterization of a third minor isomer in pure form. Furthermore, we describe the application of this protocol to the optical resolution of D₂-C₈₄.^[12] Also, contradictory reports in the literature concerning the electrochemistry of C₈₄ isomers^[13,14] are clarified.

The C₈₄ fraction (52 mg, 0.052 mmol)^[15] of soot enriched in higher fullerenes, provided by Hoechst AG, was treated in *o*-dichlorobenzene (*o*-DCB) at 20 °C with bis[(*S*)-1-phenylbutyl]-2-bromomalonate^[16] in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; Scheme 1). Subsequent purification by column chromatography (SiO₂, CH₂Cl₂/hexane (1/1) → CH₂Cl₂) yielded four fractions containing, according



Scheme 1. Formation of mono- and bis-adducts of various C₈₄ isomers by Bingel cyclopropanation. a) C₈₄, DBU, *o*-DCB, Ar, 20 °C, 14 h.

to matrix-assisted laser-desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS), pure C₈₄, mono-adducts, bis-adducts, and tris-adducts, respectively. The mono-adduct fraction was further separated by HPLC ((*S,S*)-Whelk-O, 250 × 10 mm, hexane/CH₂Cl₂ (7/3), flow rate 2 mL min⁻¹) to give four main products **1a–1d** of which two (**1a** (0.8 mg, 1 % and **1d** (2.5 mg, 3.5 %)) were isolated and characterized

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(Table 1). The bis-adduct fraction was separated (HPLC, Macherey-Nagel Nucleosil 100-7 SiO₂, 250 × 21 mm, hexane/CH₂Cl₂ (7/3), flow rate 6 mL min⁻¹) to give six main products **IIa–IIf**, of which four (**IIa** (13.8 mg, 15 %), **IId** (6 mg, 7 %), **IIe** (3 mg, 3.5 %), and **IIf** (7 mg, 8 %)) were isolated and characterized. The symmetries of these compounds were determined by ¹H and ¹³C NMR spectroscopy (Table 2).

Table 1. Products isolated from the Bingel reaction of the C₈₄ fraction in the fullerene soot (Scheme 1).

Adducts	HPLC fraction	Yield [%]	Symmetry ^[a]	Derivative of	Assigned structure
mono-add. ^[b]	Ia	1	C ₂	D _{2d} -C ₈₄	1
	Id	3.5	C ₁	new isomer	none
bis-add. ^[c]	IIa	15	D ₂	D _{2d} -C ₈₄	2
	IIb	7	C ₂	D ₂ -C ₈₄	3a–8a
	IIe	3.5	C ₂	D ₂ -C ₈₄	3b–8b
	IIf	8	C ₁	new isomer	none

[a] The symmetry assignment was based on the number of signals seen for the PhCHO groups (¹H and ¹³C NMR) and for the bridgehead and methano bridge C(sp³) atoms (¹³C NMR). [b] Fractions **Ib** and **Ic** are the main products but were extremely difficult to separate. [c] Fractions **IIb** and **IIc** are severely overlapping and could not be separated; the amount of material in the mixture of these fractions is comparable to that in fraction **IIa**.

Based on the experiences with the functionalization of other higher fullerenes, nucleophilic cyclopropanation of C₈₄ was expected to occur preferentially at the 6-6 bonds (bonds between two 6-membered rings) that are the most curved ones and have the highest π bond order.^[9] In both D₂-C₈₄ and D_{2d}-C₈₄ there are ten 6-6 bonds of more pronounced local curvature^[9b, 15, 17] and three of these bonds also possess the highest π bond order (Figure 1).^[17] All possible structures of mono- and bis-adducts generated by the addition of either an achiral or a chiral malonate to the ten most curved bonds in both isomers were constructed with molecular models and their symmetries determined. This analysis revealed that the C₂-symmetrical mono-adduct from fraction **Ia** could correspond to one of four structures: three derivatives of D_{2d}-C₈₄ and one of D₂-C₈₄. An additional, extremely valuable structural selection criterion was provided by the circular dichroism (CD) spectra of the nonracemic compounds. Optically active fullerene derivatives with achiral fullerene cores or functionalization patterns display weak Cotton effects ($\Delta\epsilon$ values around 1–10 M⁻¹ cm⁻¹), while those having inherently chiral cores or functionalization patterns show large Cotton effects with $\Delta\epsilon$ values of up to 200 M⁻¹ cm⁻¹ and higher.^[9, 16] The CD spectrum of the mono-adduct from fraction **Ia** exhibited only weak Cotton effects with $\Delta\epsilon$ values up to 3 M⁻¹ cm⁻¹. Of the three possible C₂-symmetrical mono-adducts of achiral D_{2d}-C₈₄, only structure **1**, which results from nucleophilic addition to the C(32)–C(53) bond with the highest π bond order, displays an achiral functionalization pattern. Therefore, this structure is assigned to the mono-adduct in fraction **Ia** (Scheme 2). It is noteworthy that formation of the [(η^2 -D_{2d}-C₈₄)Ir(CO)Cl(PPh₃)₂]·4C₆H₆ complex had also occurred at this bond.^[5]

Table 2. Selected physical data of C₈₄ mono- and bis-adducts.^[a]

1 (product from fraction **Ia**): UV/Vis (CH₂Cl₂): λ_{max} [nm] (ϵ , [M⁻¹ cm⁻¹]) = 704 (380), 637 (670), 555 (sh, 1200), 525 (sh, 1500), 478 (2300), 435 (sh, 3500), 410 (sh, 6700), 384 (11500), 340 (sh, 10000), 296 (21800), 276 (24900), 255 (sh, 38100); ¹H NMR (CDCl₃, 500 MHz): δ = 7.46–7.29 (m, 10H), 6.15 (t, J = 7.1 Hz, 2H), 2.16–2.08 (m, 2H), 2.00–1.82 (m, 2H), 1.49–1.21 (m, 4H), 0.98 (t, J = 7.4 Hz, 6H); ¹³C NMR (CDCl₃, 125.8 MHz, 40 mm [Cr(acac)₃], acac = acetylacetonate): δ = 163.70, 148.00, 147.79, 146.39, 145.98, 145.81, 144.70, 143.65, 143.57, 143.39, 143.27, 143.23, 142.61, 142.58, 142.37, 141.80, 141.40, 141.27, 139.16, 138.85, 138.48, 138.46, 136.13, 135.86, 135.38, 135.00, 134.83, 134.02, 133.76, 133.73, 128.36, 128.26, 126.84, 79.57, 58.95, 37.69, 31.71, 18.65, 13.91.

2 (product from fraction **IIa**): UV/Vis (CH₂Cl₂): λ_{max} [nm] (ϵ , [M⁻¹ cm⁻¹]) = 720 (2300), 646 (1800), 570 (2600), 485 (sh, 5300), 458 (10400), 430 (13700), 395 (31500), 363 (sh, 17400), 310 (sh, 17400), 310 (sh, 53300), 286 (87900), 268 (sh, 105500), 257 (sh, 111000); ¹H NMR (CDCl₃, 500 MHz): δ = 7.47–7.28 (m, 20H), 6.22 (t, J = 7 Hz, 4H), 2.22–2.14 (m, 4H), 2.01–1.94 (m, 4H), 1.48–1.37 (m, 4H), 1.33–1.22 (m, 4H), 1.01 (t, J = 7.4 Hz, 12H); ¹³C NMR (CDCl₃, 125.8 MHz, 40 mm [Cr(acac)₃]): δ = 163.94, 146.72, 146.51, 143.65, 143.58, 143.26, 143.21, 142.03, 142.01, 141.68, 141.56, 139.86, 139.85, 138.92, 137.86, 136.39, 136.24, 136.03, 135.74, 134.60, 134.56, 128.39, 128.28, 128.01, 126.90, 126.31, 126.28, 79.65, 58.29, 49.31, 37.76, 18.71, 13.70.

Product from fraction **IIb**: UV/Vis (CH₂Cl₂): λ_{max} [nm] (ϵ , [M⁻¹ cm⁻¹]) = 620 (2100), 550 (4400), 470 (8300), 447 (10700), 418 (14500), 399 (15600), 377 (17300), 327 (sh, 31600), 305 (sh, 43600), 264 (93300); ¹H NMR (CDCl₃, 500 MHz): δ = 7.32–7.14 (m, 20H), 6.06–6.02 (m, 4H), 2.09–2.00 (m, 4H), 1.89–1.81 (m, 4H), 1.47–1.42 (m, 4H), 1.37–1.26 (m, 4H), 0.95 (t, J = 7.4 Hz, 6H), 0.94 (t, J = 7.4 Hz, 6H); ¹³C NMR (CDCl₃, 125.8 MHz, 40 mm [Cr(acac)₃]): δ = 163.66, 163.53, 148.53, 146.54, 146.07, 145.48, 144.83, 144.68, 144.37, 144.20, 144.09, 143.97 (2 ×), 143.76, 142.93, 142.38, 142.17, 141.91, 141.40, 141.28, 141.10 (2 ×), 140.24, 140.15, 139.77, 139.53, 139.18, 138.98, 138.84, 138.81, 138.75, 137.98, 137.94, 136.87, 136.21, 136.14, 134.58, 134.33, 133.99, 133.17, 133.15, 132.13, 131.87, 131.59, 128.30, 128.22, 128.10, 126.94, 126.69, 126.64, 79.51, 79.21, 58.78, 56.23, 47.12, 37.65, 37.58, 18.56, 13.56.

Product from fraction **Id**: UV/Vis (CH₂Cl₂): λ_{max} [nm] (ϵ , [M⁻¹ cm⁻¹]) = 569 (2200), 482 (sh, 5400), 454 (6900), 405 (10500), 358 (13000), 320 (sh, 18800), 267 (sh, 46800); ¹H NMR (CDCl₃, 500 MHz): δ = 7.40–7.27 (m, 10H), 6.09–6.05 (m, 2H), 2.11–2.05 (m, 2H), 1.91–1.85 (m, 2H), 1.50–1.30 (m, 4H), 0.98 (t, J = 7.5 Hz, 6H), 0.96 (t, J = 7.5 Hz, 6H); ¹³C NMR (CDCl₃, 125.8 MHz, 40 mm [Cr(acac)₃]): δ = 163.81, 161.41, 152.91, 148.00, 147.55, 147.19, 147.07, 146.91, 146.36, 146.23, 146.21, 145.96, 144.44, 143.57, 143.44, 143.34, 142.94, 142.84, 142.70, 142.56, 142.18, 141.89, 141.88, 141.83, 141.46, 141.41, 141.36, 141.30, 140.11, 140.06, 140.03, 139.48, 139.16, 139.14, 139.08, 138.90, 138.88, 138.75, 138.49, 135.82, 135.48, 135.07, 133.72, 133.44, 132.99, 132.96, 132.70, 131.46, 128.38, 128.36, 128.26, 128.14, 126.88, 126.72, 79.39, 79.34, 59.31, 58.37, 37.71, 37.61, 18.66, 18.30, 13.91, 13.64.

Product from fraction **IIe**: UV/Vis (CH₂Cl₂): λ_{max} [nm] (ϵ , [M⁻¹ cm⁻¹]) = 704 (600), 617 (sh, 1300), 502 (sh, 4700), 457 (7800), 399 (20200), 390 (sh, 20000), 364 (sh, 20000), 320 (sh, 35400), 295 (55400), 265 (sh, 75900); ¹H NMR (CDCl₃, 500 MHz): δ = 7.40–7.19 (m, 20H), 6.13 (t, J = 7.0 Hz, 1H), 6.09 (t, J = 7.0 Hz, 1H), 5.95 (t, J = 7.0 Hz, 1H), 5.91 (t, J = 7.0 Hz, 1H), 2.17–2.00 (m, 2H), 2.00–1.82 (m, 4H), 1.82–1.70 (m, 2H), 1.50–1.20 (m, 8H), 0.97 (t, J = 7.4 Hz, 3H), 0.95 (t, J = 7.4 Hz, 6H), 0.91 (“t”, J = 7.4 Hz, 3H), 0.88 (“t”, J = 7.4 Hz, 3H); ¹³C NMR (CDCl₃, 125.8 MHz, 40 mm [Cr(acac)₃]): δ = 163.95, 163.90, 163.40, 163.29, 147.29, 147.20, 147.05, 146.77, 146.67, 146.48, 146.31, 146.13, 145.85, 145.75, 145.49, 145.37, 145.35, 145.23, 144.94, 144.90, 144.86, 144.73, 144.63, 144.49, 144.45, 144.41, 144.09, 143.43, 143.21, 143.19, 141.90, 141.75, 141.71, 141.60, 141.52, 141.49, 139.50, 139.42, 139.22, 139.19, 138.79, 138.74, 138.36, 138.33, 138.14, 138.11, 137.87, 137.59, 137.01, 136.89, 136.62, 136.36, 135.25, 135.19, 134.97, 134.94, 134.79, 134.69, 134.48, 134.43, 133.49, 133.44, 132.96, 132.83, 131.56, 131.43, 128.70, 128.65, 128.23, 128.14, 128.09, 128.07, 127.88, 126.84, 126.76, 126.56, 126.39, 79.26, 79.22, 79.19, 79.02, 58.94, 56.60, 56.52, 49.11, 37.74, 37.54, 37.50, 18.55, 18.52, 18.47, 18.42, 13.81, 13.55, 13.49, 13.47.

[a] CD spectra and correct MALDI-TOF mass spectra were also obtained for each compound. [b] The data for the products from fraction **IIb** and **IIe** are very similar; the latter will be reported in a full paper.

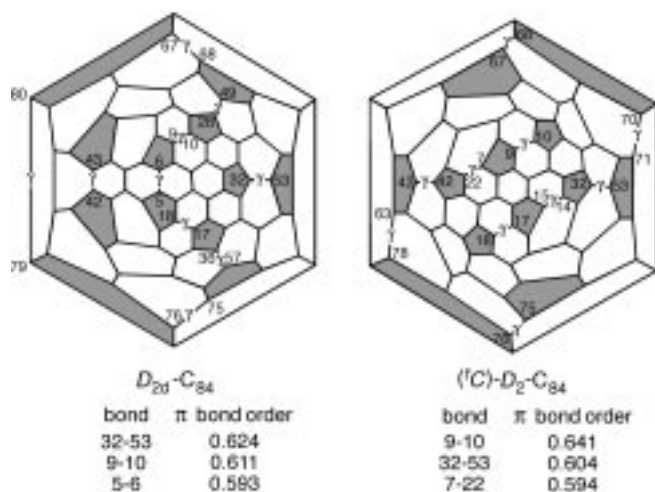
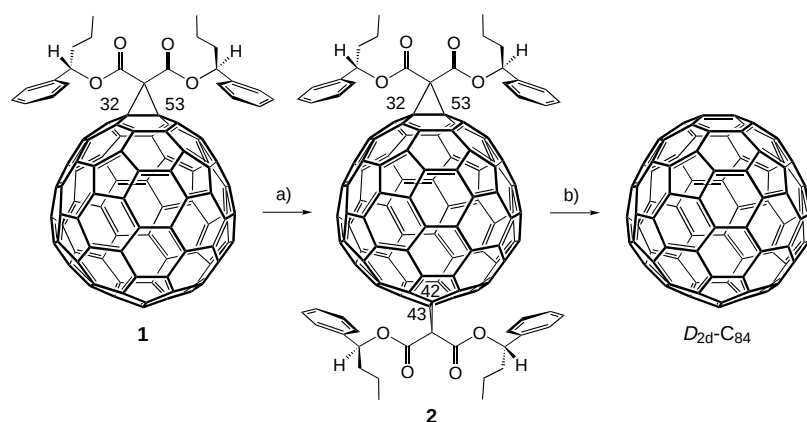


Figure 1. Schlegel diagrams of D_{2d} - C_{84} and $(1C)$ - D_2 - C_{84} showing the ten most curved bonds, which are of the γ type according to a previously introduced qualitative model to evaluate local curvature.^[15] The highest π bond orders in D_{2d} - C_{84} are at the γ -type bonds C(32)–C(53) (π bond order = 0.624), C(9)–C(10) (0.611), and C(5)–C(6) (0.593), and in D_2 - C_{84} at the γ -type bonds C(9)–C(10) (0.614), C(32)–C(53) (0.604), and C(7)–C(22) (0.594).^[17] The IUPAC numbering scheme for the C_{84} isomers^[18] as well as the previously introduced configurational descriptor system ($1C$; f = fullerene, C = clockwise) to describe the absolute configuration of inherently chiral fullerenes or fullerenes with a chiral functionalization pattern are used.^[19]

The D_2 -symmetrical bis-adduct from fraction **IIa** also displayed only weak Cotton effects ($\Delta\epsilon$ values lower than $3\text{ M}^{-1}\text{ cm}^{-1}$) in the CD spectrum and, in view of its abundance (Table 1) and the results of the retro-Bingel reaction described below, must be a derivative of D_{2d} - C_{84} with an achiral functionalization pattern. The CD and NMR spectral properties unambiguously support the formation of bis-adduct **2**, which results from cyclopropanation at C(32)–C(53) and C(42)–C(43) (Scheme 2). It is reasonable to assume that **2**, which features the remarkable structure of a D_2 -symmetrical bis-adduct having an achiral fullerene core, is formed by cyclopropanation of **1**. Compounds **1** and **2** are the first fully characterized organic derivatives of C_{84} .

The products of fractions **II d** and **II e** are C_2 -symmetrical bis-adducts, which feature mirror-image CD spectra with



Scheme 2. Formation of the D_{2d} - C_{84} bis-adduct **2** from mono-adduct **1** and generation of the parent fullerene by retro-Bingel reaction. a) Bis[(S) -1-phenylbutyl]-2-bromomalonate, DBU, o -DCB. b) Controlled potential electrolysis, CH_2Cl_2 .

large Cotton effects. Such spectra are characteristic of a pair of diastereoisomers, with the chiroptical contributions of the enantiomeric, inherently chiral fullerene cores or functionalization patterns largely dominating those of the chiral addends.^[16] The UV/Vis spectra of the two diastereoisomers (Table 2) differ considerably from those of the D_{2d} - C_{84} bis-adduct **2**, which suggests that they are derivatives of D_2 - C_{84} . This was clearly confirmed after the retro-Bingel reaction and the electrochemical investigations on the parent fullerene enantiomers (see below). A total of six C_2 -symmetrical constitutional isomers, each consisting of a pair of diastereoisomers (**3a/3b**–**8a/8b**) are possible structures for the products from fractions **II d** and **II e**, which cannot be further distinguished at this stage on the basis of the number of NMR resonances.^[20]

The UV/Vis spectra of the C_1 -symmetrical mono- and bis-adducts of fractions **II d** and **II f** again differed significantly from those of the derivatives of both D_2 - and D_{2d} - C_{84} . This first indication that these products originated from cyclopropanation of a hitherto unknown constitutional isomer of C_{84} was further substantiated by the retro-Bingel reaction and electrochemical investigations described below.

Electrochemical investigations by cyclic voltammetry (CV) or Osteryoung square wave voltammetry (OSWV) in CH_2Cl_2 (+0.12 M Bu_4NPF_6) revealed that the mono- (fractions **Ia** and **Id**) and bis-adducts (fractions **IIa** and **II d–f**) of C_{84} each underwent at least four reductions, with only the first one being fully reversible at 298 K in most cases. Chemical reversibility improves considerably at lower temperature.

The retro-Bingel reaction was performed in CH_2Cl_2 in a degassed H-cell on the compounds from fractions **Id**, **IIa**, and **II d–f**. The mono- and bis-adducts were reduced by controlled potential electrolysis (CPE) on a Pt mesh (100 mesh, 6.5 cm^2) working electrode at potentials 0.1 V more negative than the third reduction potential, and then re-oxidized at 0 V versus Ag. For the mono-adduct, the net charge transferred after reduction followed by re-oxidation corresponded to two electrons per molecule, while for the bis-adducts, six electrons were transferred per molecule. Almost all of the formed C_{84} precipitated when the re-oxidized solution was left overnight. The C_{84} isomers were purified by chromatography on neutral alumina (toluene) to afford the isolated products in yields between 22 and 94%.

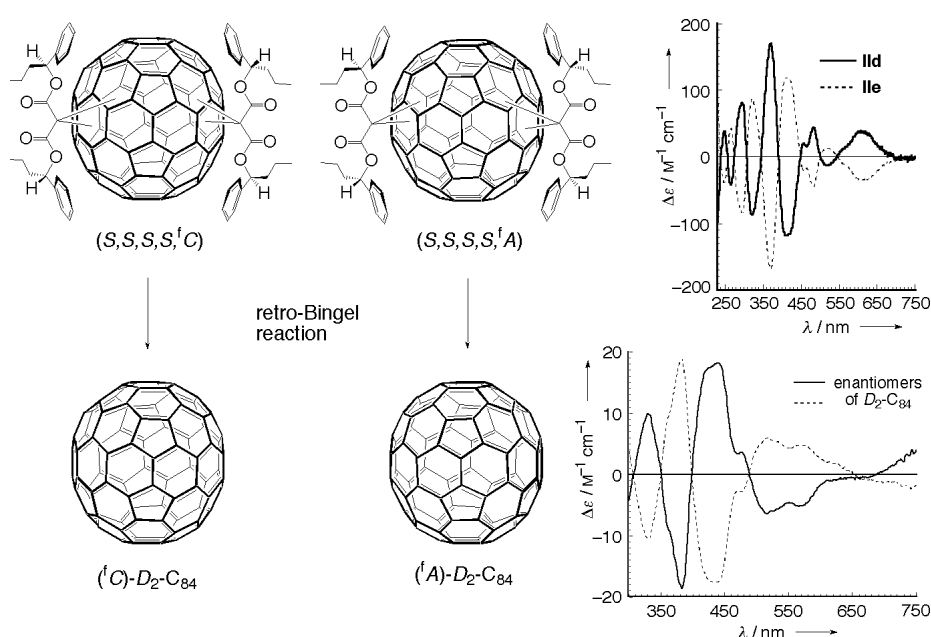
Analysis of this material by MALDI-TOF MS, however, revealed that the retro-Bingel reaction had not only provided the pure C_{84} isomers, but that the corresponding methanofullerenes $C_{84}\text{CH}_2$, which resulted from addition of the CH_2Cl_2 solvent to the fullerene di- or trianions, had also been formed in 10–20% relative yield during CPE. The electro-synthesis of methanofullerenes from C_{60} and dihalogenated reagents such as CH_2I_2 had been reported previously by some of us.^[21] The methanofullerenes could be separated from the new isomer and from the D_{2d} - and D_2 - C_{84} isomers by HPLC, either on a Buckyclutcher column ($250 \times 4.6\text{ mm}$; toluene, flow rate 2 mL min^{-1}) or on a Lichrosorb Si60

SiO₂ column (250 × 10 mm; hexane/toluene (9/1), flow rate 1 mL min⁻¹).

The retro-Bingel reaction of the product in fraction **IIa** produced the known *D*_{2d} isomer (Scheme 2), which displayed its characteristic bright green color with the corresponding UV/Vis spectrum^[4] and was CD-inactive. Controlled potential electrolysis of the diastereoisomeric pair from fractions **II d** and **II e** gave rise to the two enantiomers of *D*₂-C₈₄, and their mirror image CD spectra (Scheme 3) correspond nicely to those reported by Hawkins et al. for samples obtained by kinetic resolution through asymmetric osmylation.^[12] With $\Delta\epsilon$ values up to 20 M⁻¹ cm⁻¹, the inherently chiral *D*₂-C₈₄ enantiomers feature much weaker Cotton effects than the enantiomers of the smaller, inherently chiral *D*₂-symmetrical C₇₆ for which $\Delta\epsilon$ values up to 200 M⁻¹ cm⁻¹ have been measured.^[11] This may be a consequence of the fact that the chirality arises in *D*₂-C₇₆ from its oblong, helically twisted structure,^[6] while *D*₂-C₈₄ is rather round-shaped and only slightly distorted from achiral *D*_{2d}-C₈₄. A theoretical CD spectrum has been calculated by Zerbetto and co-workers for *D*₂-C₈₄^[22] but, unlike the case of C₇₆,^[23] it does not allow an accurate determination of the absolute configuration by comparison with the experimental spectrum.

The retro-Bingel reaction of the products from fractions **Id** and **IIf** yielded a novel C₈₄ isomer that is CD-inactive and whose UV/Vis spectra differ from those of the *D*₂- and *D*_{2d}-C₈₄ isomers (Figure 2). Unfortunately, the sample amounts were not sufficient for a structural characterization by ¹³C NMR spectroscopy, so the symmetry of the isomer remains unknown.

The redox properties of the three constitutional C₈₄ isomers were investigated in dry pyridine (+0.1 M Bu₄NPF₆) by OSWV in a micro-electrochemical cell.^[24] Table 3 shows the peak potentials (in V versus ferrocene/ferrocenium (Fc/Fc⁺)). The potentials for the first four reductions of the *D*_{2d} and *D*₂ isomers are quite similar as is the relative constant spacing (about 400 mV) between the different reduction waves. A nicely resolved, much more negatively shifted fifth reduction wave separated by some 800 mV from the fourth wave, is additionally observed for the *D*_{2d} isomer, which clearly indicates that the lowest unoccupied molecular orbital (LUMO) is very close to being doubly degenerate



Scheme 3. Preparation of the pure enantiomers of *D*₂-C₈₄ by retro-Bingel reaction of the bis-adducts from fractions **II d** and **II e**, and CD spectra of the diastereoisomeric bis-adducts (top right) as well as of the fullerene enantiomers (bottom right) in CH₂Cl₂. The absolute configurations of the fullerene spheroids (^fC and ^fA; f = fullerene, C = clockwise, A = anticlockwise) reflect the structural drawings, but have not been assigned experimentally.

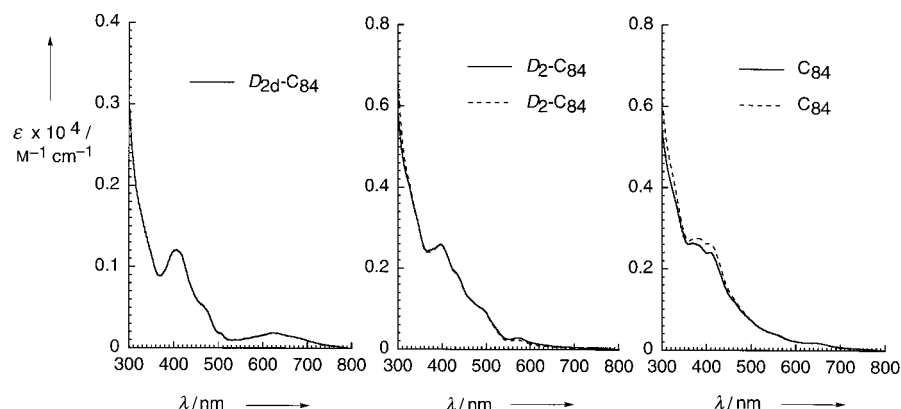


Figure 2. UV/Vis spectra of *D*_{2d}-C₈₄ (left), the two enantiomers of *D*₂-C₈₄ (middle), and the new achiral isomer of as yet unidentified structure (right). For the new isomer, the UV/Vis spectra of both samples prepared by retro-Bingel reaction of the products from fractions **Id** and **IIf**, are shown.

Table 3. Peak potentials *E*_p for constitutional isomers of C₈₄ measured by OSWV in pyridine (+ 0.1 M Bu₄NPF₆).

<i>E</i> _p	Assigned C ₈₄ isomers			ref. [14]	
	<i>D</i> ₂	this work ^[a] <i>D</i> _{2d}	new isomer	<i>D</i> ₂	<i>D</i> _{2d}
<i>E</i> ^[b]					
<i>E</i> ¹	-0.56	-0.55	-0.42	-0.65	-0.46
<i>E</i> ²	-0.88	-0.86	-0.73	-0.98	-0.77
<i>E</i> ³	-1.24	-1.20	-1.46	-1.34	-1.58
<i>E</i> ⁴	-1.67	-1.59	-1.90	-1.75	-1.98
<i>E</i> ⁵		-2.40			

[a] At 295 K. [b] Potential [V] versus Fc/Fc⁺.

with a LUMO+1 much higher in energy. The redox properties of the third, new isomer differ significantly from those of the *D*₂ and *D*_{2d} isomers, with a large gap being observed between the second and third reduction waves.

This indicates that the LUMO is nondegenerate for this isomer.

The electrochemical data for the D_2 and D_{2d} isomers are in full agreement with those previously reported^[13] except for a recent publication by Anderson et al.^[14] The latter group investigated the electrochemistry of two isomers (Table 3) that were separated by HPLC and, on the basis of the relative abundances and previous work, were assigned the D_2 - and D_{2d} -symmetrical structures. While the redox properties of the D_2 isomer match those reported here, the ones assigned to the D_{2d} isomer differ from those determined by us and others^[13] for this fullerene and rather match those of what we call here the “new isomer”. On the basis of our findings we conclude that Anderson et al. had not isolated D_{2d} -C₈₄ but rather the “new isomer” in addition to the D_2 -symmetrical one.

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A Polymer-Supported Phosphazene as a Stable and Practical Reagent in the Three-Component Synthesis of Substituted (Cyclopentadienyl)-tricarbonylrhenium Complexes**

Filippo Minutolo and John A. Katzenellenbogen*

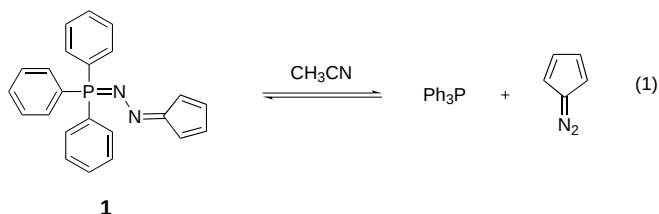
In recent years the use of reagents and catalysts bound to inorganic and organic solid supports has rapidly become an area of intense research activity,^[1] since they present several obvious advantages over their soluble counterparts: they can be removed from the reaction mixture by simple filtration and, often, can be recycled and used again. Herein we report the preparation and the use of a polymer-bound stabilized diazocyclopentadiene (C₅H₄N₂) analogue, which can be used as a safe and storable source of C₅H₄N₂ in the synthesis of substituted cyclopentadienyl–Re(CO)₃ complexes.

The use of “free” C₅H₄N₂ in the synthesis of halogen-substituted cyclopentadienyl complexes of rhenium was

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reported more than twenty years ago.^[2] Recently, however, we found that $C_5H_4N_2$ reacts simultaneously with a $[fac-Re(CO)_3]^+$ precursor^[4] and external nucleophiles, such as carboxylates^[3a] efficiently, to produce acyloxy-substituted cyclopentadienyl- $Re(CO)_3$ complexes in a one-pot reaction (three-component reaction) and in short times. We then extended the scope of this reaction to the synthesis of carbon-substituted cyclopentadienyl- $Re(CO)_3$ complexes by utilizing boronic acids as carbon nucleophiles;^[3b] this transformation represents a new method for C–C bond formation that is based on boronic acids, but does not require a catalyst such as palladium. These results were very encouraging, especially in view of a possible use of this reaction for radiolabeling biologically interesting molecules with cyclopentadienyltricarbonyl complexes containing ^{186}Re , ^{188}Re , and ^{99m}Tc radionuclides. However, a major limitation for an application of the three-component synthesis to the routine preparation of radiopharmaceuticals was the high instability of $C_5H_4N_2$.^[5] In fact, this key reagent must always be stored in solution,^[6] usually in pentane, at low temperatures (for example, $-40^\circ C$), in the dark; yet, in spite of all these precautions, it cannot be stored for longer than a few weeks, because of its rapid decomposition. All these aspects constituted a serious limitation to the practical utilization of this reaction in medical facilities. Therefore, we started to look for alternative reagents that possessed the same good reactivity of $C_5H_4N_2$, together with better stability and handling properties. We focused our attention on an adduct of $C_5H_4N_2$ with triphenylphosphane, phosphazine **1**,^[7] which was known to be a stable compound under ambient conditions (moist air, room temperature, light, shocks).^[8] As a matter of fact, **1** is used in the gravimetric titration of solutions of diazocyclopentadiene.^[8b] We were pleased to find that **1** readily dissociates into Ph_3P and free diazo compound when dissolved in acetonitrile, which is the solvent used in our reaction [Eq. (1)].^[3] At $23^\circ C$

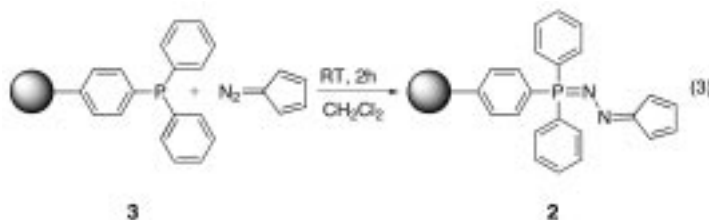


the dissociation of **1** is considerable [Eq. (2), the K_{diss} value is obtained by NMR analysis of a solution of **1** in $[D_3]$ acetonitrile at equilibrium], and under typical reaction conditions

$$K_{diss} (23^\circ C) = \frac{[C_5H_4N_2][Ph_3P]}{[1]} = 4.0 \times 10^{-3} \text{ mol L}^{-1} \quad (2)$$

($80^\circ C$), further dissociation generates levels of free $C_5H_4N_2$ that are sufficient for an efficient reactivity, as we could verify in preliminary experiments with carboxylates. However, the presence of free triphenylphosphane in the reaction mixture, produced by the dissociation of **1**, made the purification of the products very awkward, because it was difficult to separate the phosphane from most of the product complexes. This resulted in a decrease in the product yields when **1** was used in place of free $C_5H_4N_2$.

To overcome this problem, we envisaged the use of a polymer-bound phosphazine **2**, so that the phosphane formed at the end of the reaction could be removed by filtration, thereby considerably simplifying the work-up and purification procedure. The heterogeneous reagent **2** was prepared by treating a dichloromethane suspension of triphenylphosphane bound to a polystyrene support **3**^[9] with a pentane solution of $C_5H_4N_2$ [Eq. (3)]. The functionalization of the polymer was very efficient (85 %) after only 2 hours at room temperature.



The amount of $C_5H_4N_2$ in the heterogeneous reagent **2** was determined by several methods. Determination of the amount of nitrogen (%N) by elemental analysis can be used as a measurement of the extent of functionalization, but elemental analysis by itself does not distinguish between the $C_5H_4N_2$ that is covalently bound and thereby stabilized as a phosphazine and material that may simply be trapped in the polymeric network, but still remaining as a free unstable diazo compound. In order to be sure that all unreacted $C_5H_4N_2$ had been washed out of the polymer, we analyzed **2** by IR and solid-state NMR spectroscopy. Free $C_5H_4N_2$ has a very strong, characteristic IR absorption around 2100 cm^{-1} which arises from the asymmetric stretching of the cumulated C–N–N double bonds. The absence of such a band in the product polymer **2** (as was also the case in the monomeric phosphazine **1**), indicated that the amount of nitrogen found by elemental analysis could be entirely attributed to polymer-bound phosphazine. In fact, other typical bands for phosphazines were found in the IR spectrum of **2** at $\tilde{\nu} = 1517 (\nu_{C=N})$, $1102 (\nu_{P=N})$, and $974 \text{ cm}^{-1} (\nu_{N-N})$.

To further validate these results, we conducted a solid-state “magic angle spinning” (MAS) ^{31}P NMR analysis of **2** (Figure 1). The ^{31}P spectrum shows two peaks, an intense one at $\delta = 21.10$, which corresponds to functionalized P atoms, and a weaker one at $\delta = -7.03$, which arises from unreacted phosphane groups present in the polymer. The observed chemical shifts compare favorably to those found in the ^{31}P NMR spectra of their soluble counterparts in $CDCl_3$: free phosphazine **1** gives a resonance at $\delta = 23.23$, and Ph_3P at $\delta = -2.29$. The ^{31}P MAS NMR spectrum of the starting polymeric phosphane **3** also showed a single peak at $\delta = -6.77$, which confirmed the identity of the weaker peak shown in Figure 1.

As we expected, the striking feature of **2** was its great stability. This material shows no sign of decomposition (elemental analysis, NMR and IR spectroscopy) after many months (up to 11 as of yet) of storage on the bench, exposed to ambient conditions without any particular precautions. To test its reactivity, **2** was then used in the three-component reaction with several nucleophiles. The yields obtained using heteroatom nucleophiles (Br^- and carboxylates) according to

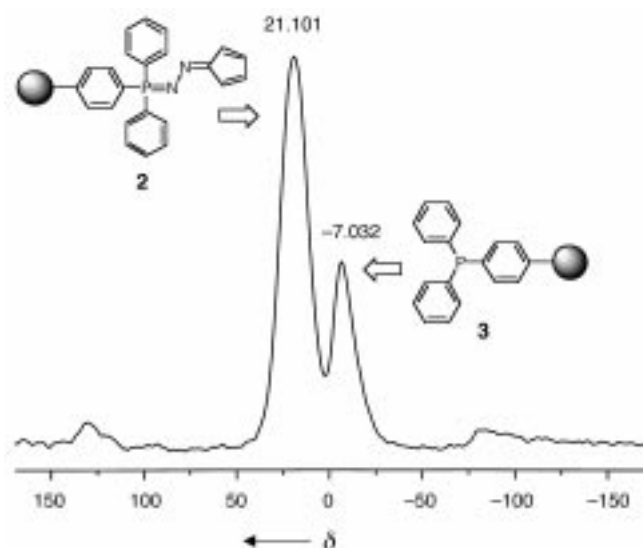


Figure 1. ^{31}P MAS NMR (121 MHz) spectrum of **2**.

Equation (4) ($\text{L} = \text{CH}_3\text{CN}$) are summarized in Table 1. Interestingly, **2** showed the same good reactivity as free $\text{C}_5\text{H}_4\text{N}_2$.^[3a]

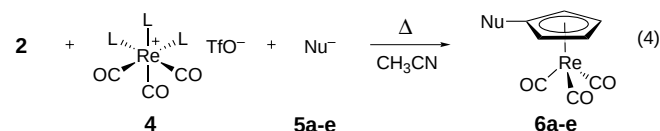


Table 1. Comparison of the yields of the isolated substituted cyclopentadienyl– $\text{Re}(\text{CO})_3$ complexes **6** obtained with heteroatom nucleophiles **5** from free diazocyclopentadiene or the polymer-supported reagent **2** [Eq. (4)].

Entry	Nu [−]	Product	Yield [%] ^[a] with $\text{C}_5\text{H}_4\text{N}_2$ ^[b]	Yield [%] ^[c] with 2
1	Br^- 5a	6a	67	71
2	$\text{CH}_3\text{C}(=\text{O})\text{O}^-$ 5b	6b	69	67
3	$\text{CH}_3\text{CH}(\text{OH})\text{C}(=\text{O})\text{O}^-$ 5c	6c	59	60
4	$\text{HO}-\text{C}_6\text{H}_4-\text{C}(=\text{O})\text{O}^-$ 5d	6d	72	71
5	$\text{Ph}-\text{CH}_2-\text{C}(=\text{O})\text{O}^-$ 5e	6e	60	64

[a] Yields of complexes purified by column chromatography are based on the initial rhenium precursor $(\text{Et}_4\text{N})_2[\text{ReBr}_3(\text{CO})_3]$ ^[10] used to prepare compound **4** in situ.^[3a] [b] See ref. [3a]. [c] 0.050 mmol of **4**, 0.10 mmol of nucleophile, 39 mg of **2** (containing 0.075 mmol of $\text{C}_5\text{H}_4\text{N}_2$), 2.5 mL of CH_3CN , 80 °C, 45 min; carboxylates **5b**, **5d**, and **5e** were generated in situ by treating the correspondent carboxylic acid (0.1 mmol) with 0.2 mmol of Et_3N ; commercially available sodium salt of **5c** was used.

The results obtained in the more recently described reaction involving boronic acids as carbon nucleophiles [Eq. (5)] are reported in Table 2. Here, the yields obtained with **2** are generally slightly lower (apart from entry 2) than

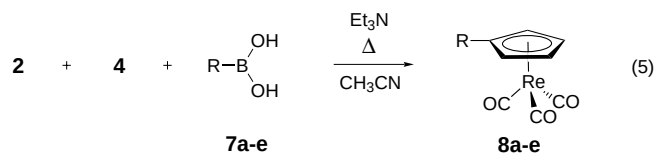


Table 2. Comparison of the yields of the isolated substituted cyclopentadienyl– $\text{Re}(\text{CO})_3$ complexes **8** obtained with boronic acids **7** using free diazocyclopentadiene or the polymer-supported reagent **2** [Eq. (5)].

Entry	Boronic acid	R	Complex	Yield [%] ^[a] with $\text{C}_5\text{H}_4\text{N}_2$ ^[b]	Yield [%] ^[c] with 2
1	7a		8a	64	53
2	7b		8b	34	41
3	7c		8c	74	56
4	7d		8d	51	45
5	7e		8e	72	50

[a] See Table 1. [b] See ref. [3b]. [c] 0.050 mmol of **4**, 0.50 mmol of boronic acid, 1.0 mmol of Et_3N , 39 mg of **2** (containing 0.075 mmol of $\text{C}_5\text{H}_4\text{N}_2$), 2.5 mL of CH_3CN , 80 °C, 45 min.

the ones obtained with $\text{C}_5\text{H}_4\text{N}_2$,^[3b] but are still in a respectable range (around 50 %), considering that this complex transformation results in the simultaneous formation of a carbon–carbon σ bond and an η^5 -cyclopentadienyl–rhenium bond. It is important to emphasize the great tolerance of this reaction towards a wide variety of functional groups, including alcohols, phenols, amides, and ketones. Moreover, this reaction is tolerant to sulfur-containing heterocycles such as thiophenes as shown by the synthesis of a novel thienyl-substituted complex **8e** (Table 2, entry 5).

In conclusion, the solid-phase reagent **2** described herein combines great safety, handling, and excellent storage properties, with good reactivity in the synthesis of substituted cyclopentadienyl– $\text{Re}(\text{CO})_3$ complexes. Its consequent suitability for kit-formulations should facilitate the use of the three-component reaction for the preparation and use of these radiopharmaceuticals that contain organometallic complexes of Re^I and, by chemical analogy, $\text{Tc}^{I[11]}$ in medical facilities, applications that would not be possible if it were necessary to use highly unstable reagents such as free diazocyclopentadiene. Furthermore, the availability of this supported/stabilized cyclopentadienyl-ring precursor should make the synthesis of half-sandwich complexes containing other metals practical.

Experimental Section

Diazocyclopentadiene (1.78 M in pentane) was prepared by a literature method.^[6b] Polymer-supported triphenylphosphane **3** and all other chemicals were obtained from commercial sources unless otherwise specified. Boronic acid (**7d**) was prepared from 1-nonyne by a literature method.^[12] Characterization data and purification details of complexes **6a**,^[2] **6b–e**,^[3a] and **8a–d**^[3b] have already been reported.

2: A 1.78 M pentane solution of diazocyclopentadiene (4 mL, 1.2 equiv) was added to a well stirred suspension of the heterogeneous phosphane **3** (2.0 g, ≈ 5.6 mmol of P) in CH_2Cl_2 (15 mL) at room temperature. The mixture was stirred for a further 2 h, after which time Et_2O (30 mL) was added and the orange insoluble polymer was collected by filtration, washed with Et_2O (3×10 mL), and dried under vacuum to yield **2** (2.4 g) as an orange powder. Elemental analysis revealed 5.39 %N, which corresponded to a 85 % functionalization of the phosphane groups. IR (CH_2Cl_2 film): $\tilde{\nu} = 1517$ (C=N), 1102 (P=N), 974 cm^{-1} (N=N); ^{31}P MAS NMR (121 MHz, neat solid, external reference: H_3PO_4 , see Figure 1): $\delta = 21.10$ (phosphazine), -7.03 (phosphane).

8e (Table 2, entry 5): m.p. 86°C ; R_f (silica gel, hexane) 0.14; IR (KBr): $\tilde{\nu} = 2018$ (CO_{asym}), 1910 (CO_{sym}); ^1H NMR (500 MHz, CDCl_3): $\delta = 7.32$ (dd, $J = 5.0, 2.9$ Hz, 1H, H5 thienyl), 7.28 (dd, $J = 2.9, 1.5$ Hz, 1H, H2 thienyl), 7.07 (dd, $J = 5.0, 1.5$ Hz, 1H, H4 thienyl), 5.67 (pseudo t, $J = 2.2$ Hz, 2H, Ha $\text{C}_5\text{H}_4\text{Ar}$), 5.37 (pseudo t, $J = 2.2$ Hz, 2H, H β $\text{C}_5\text{H}_4\text{Ar}$); ^{13}C NMR (126 MHz, CDCl_3): $\delta = 194.04$ (CO), 132.72 (C4 thienyl), 126.64, 126.20, 121.59 (C3 thienyl), 103.91 (C4 $\text{C}_5\text{H}_4\text{Ar}$), 84.01, 81.63 (C3 $\text{C}_5\text{H}_4\text{Ar}$); MS (70 eV): m/z (%): 418 (100) [M^+ , ^{187}Re], 416 (62) [M^+ , ^{185}Re], 390 (36) [$M^+ - \text{CO}$, ^{187}Re], 388 (23) [$M^+ - \text{CO}$, ^{185}Re], 362 (60) [$M^+ - 2\text{CO}$, ^{187}Re], 360 (37) [$M^+ - 2\text{CO}$, ^{185}Re].

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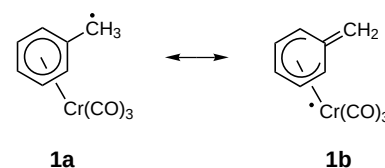
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Memory of Chirality in Electron Transfer Mediated Benzylic Umpolung Reactions of Arene–Cr(CO)₃ Complexes**

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Dirk Bernicke, Stephan Siegel, and Anja Pfletschinger

Since the discovery of arene–tricarbonylchromium complexes in 1958^[1] this class of compounds has been studied extensively. It has been shown that the $\text{Cr}(\text{CO})_3$ group activates the arene ligand in different ways to facilitate transformations that cannot be achieved with the free arenes.^[2] Furthermore, it has been demonstrated that novel and competitive strategies for the enantioselective synthesis of complex organic molecules are possible if both the chemical and stereochemical properties of such complexes are exploited.^[3, 4] While the vast majority of applications of arene– $\text{Cr}(\text{CO})_3$ complexes are based on polar reactions involving coordinatively saturated (18 valence electron (VE)) anionic or cationic intermediates,^[2] it has only recently been realized that transformations involving $\text{Cr}(\text{CO})_3$ -complexed benzylic radicals are also useful for organic synthesis.^[5]

A theoretical investigation carried out in this laboratory suggests that the parent benzyl radical complex **1a** is better described as the 17 VE resonance structure **1b**. This structure clearly indicates the presence of an exocyclic C–C double bond and shows delocalization of a significant portion of the spin density onto the chromium atom (Scheme 1).^[6]

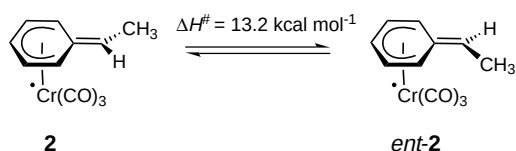


Scheme 1. Resonance structures for the $\text{Cr}(\text{CO})_3$ -complexed benzyl radical.

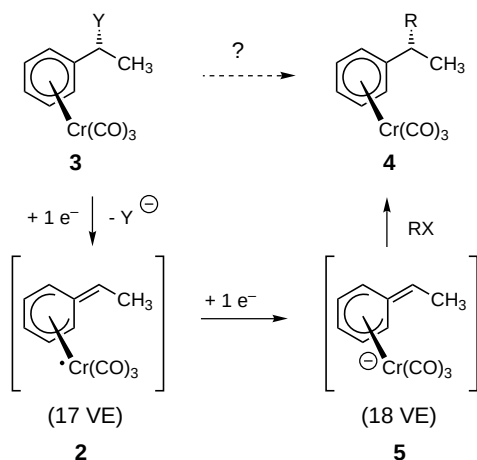
As a consequence, derivatives of **1** that carry an additional substituent in the benzylic position (for example, **2**) are planar chiral radical species and should exhibit a significant degree of configurational stability. Indeed, the barrier for the racemization of **2** was calculated to be $13.2 \text{ kcal mol}^{-1}$ (Scheme 2),^[7] which corresponds to a half-life of about one minute at -78°C .^[8]

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 Scheme 2. Racemization of the planar chiral radical intermediate **2**.

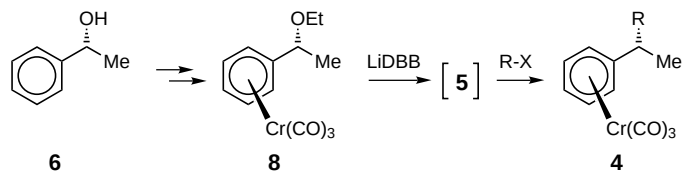
Based on these predictions we envisaged that, provided one could generate the radical intermediate **2** in a stereocontrolled fashion, for example, from a chiral precursor of type **3**, it would be possible to convert **2** into chiral products, such as **4**, without much loss of stereochemical information (Scheme 3). This transformation (**3**→**4**) would represent a novel reaction where “memory of chirality” would apply, even though a (normally notoriously labile) radical intermediate is involved.^[9]


 Scheme 3. Concept for the formation of **4** from **3**.

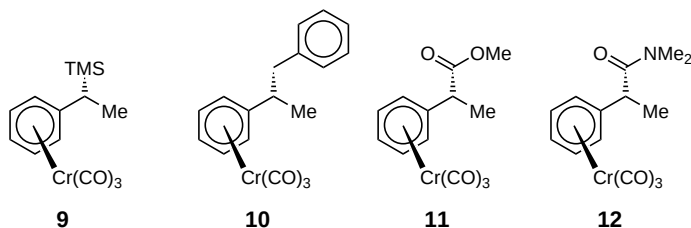
In accordance with our program on electron transfer driven transformations of transition metal π complexes,^[10] the plan was to generate **2** from **3** by a single electron reduction and to immediately convert this 17 VE intermediate into the anionic 18 VE species **5** by a second electron transfer. The latter species represents a configurationally stable benzylic anion^[11] that should react with electrophiles to give products of type **4** with overall retention of configuration (Scheme 3). Herein we disclose the first experimental evidence for the validity of this concept by describing an efficient method for the stereospecific umpolung^[12] of 1-arylalkanol–Cr(CO)₃ derivatives.

Starting from readily available (*R*)-1-phenylethanol (**6**; 91 % *ee*)^[13] the complex **8** (91 % *ee* (HPLC); $[\alpha]_D^{20} = +30$; $c = 0.10$ in CHCl₃; Table 2) was obtained by formation of the ethyl ether (EtBr, NaH, THF, sealed tube, 7 h, 88 %) followed by complexation (Cr(CO)₆, Bu₂O, THF, 65 h, 72 %). Treatment of **8** with 2.1 equivalents of lithium 4,4'-di-*tert*-butylbiphenyl (LiDBB)^[14, 15] in THF at –78 °C afforded a solution of the anion **5**, which was treated with a variety of electrophiles to give rise to the desired products **4** (Scheme 4).

In all instances, the reactions were first carried out with racemic mixtures in order to obtain reference material for analysis of the *ee* by means of HPLC on a chiral column.^[16] The results of the various experiments conducted in the


 Scheme 4. Synthesis of **4**.

optically active series, summarized in Table 1, show that the products **9**–**12** (Table 2) were obtained in fair yields and, as anticipated, with a high degree of retention of the absolute stereochemical information.


 Table 1. Results of the experiments with **8** (91 % *ee*)^[16] according to Scheme 4.

Entry	RX	Product	Yield ^[a] [%]	<i>ee</i> ^[b] [%]	<i>ee</i> ^[c] [%]	$[\alpha]_D^{20}$ (concn)
1	TMSCl	9	72 %	87 %	98 %	+ 25 ($c = 0.08$) ^[d]
2	PhCH ₂ Br	10	37 %	87 %	87 %	+ 34 ($c = 0.09$)
3	MeOC(O)Cl	11	67 %	86 %	98 %	– 52 ($c = 0.05$) ^[d]
4	Me ₂ NC(O)Cl	12	57 %	84 %	> 99 %	– 15 ($c = 0.04$) ^[d]

[a] Yields after chromatographic purification. [b] *ee* value of the chromatographically pure products. [c] *ee* value after one recrystallization from heptane or hexane. [d] Values refer to the recrystallized samples, concentration is given in g per 100 ml CHCl₃.

From the fact that all products were obtained almost without racemization, it is concluded that all three parts of the process, namely, 1) the reductive generation of the radical intermediate **2**, 2) its reduction to the anion **5**, and 3) the final alkylation, occur with a high degree of stereochemical control (Scheme 3). As no external chiral environment is involved in these reactions, the origin of the stereoselectivity can only be ascribed to the preservation of the chiral information in both the radical and the anionic intermediates. Hence during the process, the chirality of the molecule is temporarily transferred from a stereogenic center to a chiral plane and finally back to a center.^[17] This represents an example of self-regeneration of a stereo center.^[18]

To demonstrate the value of this new methodology we have completed a five step enantioselective synthesis of the sesquiterpene (+)- α -curcumene (Scheme 5).^[19] The alcohol **13** ($[\alpha]_D^{20} = +53$; $c = 0.9$ in CHCl₃) was obtained in 93 % *ee* by enantioselective reduction of cheap, commercially available *p*-methylacetophenone.^[13] Formation of the ethyl ether and attachment of the chromium unit as described previously afforded **14**. Treatment of **14** with LiDBB and then 5-iodo-2-methyl-2-pentene gave the desired product, which on oxidative removal of the Cr(CO)₃ unit afforded (+)- α -curcumene (**15**) with an enantiomeric purity of approximately 90 %

Table 2. Characteristic data for compounds **8**, **9**, **10**, **11**, **12**, and **14**. IR (ATR); ¹H NMR (400 MHz, CDCl₃); ¹³C NMR (67.7 MHz, CDCl₃ with DEPT).

8: M.p. 44 °C (heptane); IR: $\tilde{\nu}$ = 1961, 1874 cm⁻¹; ¹H NMR: δ = 1.26 (t, *J* = 7.0 Hz, 3H), 1.46 (d, *J* = 7.0 Hz, 3H), 3.64 (dq, *J* = 9.5, 7.0 Hz, 2H), 4.16 (q, *J* = 7.0 Hz, CH), 5.26–5.40 (m, 4H), 5.54–5.63 (m, 1H); ¹³C NMR: δ = 15.4 (CH₃), 22.9 (CH₃), 65.1 (CH₂), 75.1 (CH), 91.2 (CH), 91.5 (CH), 91.6 (CH), 91.9 (CH), 92.8 (CH), 114.0 (C), 232.9 (CO); HR-MS calcd for C₁₃H₁₄O₄Cr: 286.0297, found: 286.0297.

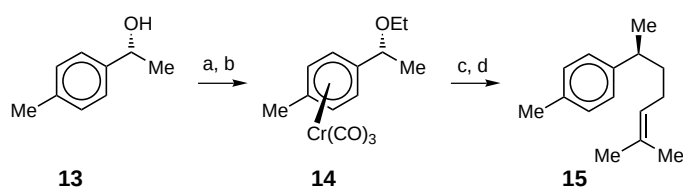
9: M.p. 105 °C (hexane); IR: $\tilde{\nu}$ = 1959, 1865 cm⁻¹; ¹H NMR: δ = 0.80 (s, 9H), 2.85 (d, *J* = 7.0 Hz, 3H), 2.46 (q, *J* = 7.0 Hz, 1H), 5.58–5.70 (m, 2H), 5.74–5.84 (m, 1H), 5.86–5.99 (m, 2H); ¹³C NMR: δ = -3.4 (CH₃), 13.9 (CH), 28.3 (CH₃), 88.9 (CH), 90.5 (CH), 92.9 (CH), 93.3 (CH), 93.6 (CH), 120.7 (C), 233.7 (CO); HR-MS calcd for C₁₄H₁₈O₃SiCr: 314.0430, found: 314.0429.

10: M.p. 50–51 °C (heptane); IR: $\tilde{\nu}$ = 1960, 1870 cm⁻¹; ¹H NMR: δ = 1.22 (d, *J* = 6.0 Hz, 3H), 2.66–2.75 (m, 2H), 2.82–3.90 (m, 1H), 5.04–5.08 (m, 1H), 5.18–5.40 (m, 4H), 7.06–7.10 (m, 2H), 7.19–7.33 (m, 3H); ¹³C NMR: δ = 19.7 (CH₃), 40.2 (CH), 45.1 (CH₂), 91.3 (CH), 91.9 (CH), 92.2 (CH), 92.5 (CH), 93.5 (CH), 118.0 (C), 126.4 (CH), 128.3 (2 × CH), 129.4 (2 × CH), 139.0 (C), 233.2 (CO); HR-MS calcd for C₁₈H₁₆O₃Cr: 332.0505, found: 332.0505.

11: M.p. 44 °C (hexane); IR: $\tilde{\nu}$ = 1957, 1863, 1730 cm⁻¹; ¹H NMR: δ = 1.50 (d, *J* = 7.0 Hz, 3H), 3.40 (q, *J* = 7.0 Hz, 1H), 3.72 (s, 3H), 5.24–5.42 (m, 4H), 5.49–5.54 (m, 1H); ¹³C NMR: δ = 17.7 (CH₃), 43.8 (CH), 52.4 (CH₃), 91.3 (2 × CH), 92.4 (CH), 94.3 (CH), 109.7 (C), 173.3 (CO), 232.5 (CO); HR-MS calcd for C₁₃H₁₂O₃Cr: 300.0090, found: 300.0091.

12: M.p. 117–118 °C (heptane); IR: $\tilde{\nu}$ = 1957, 1868, 1640 cm⁻¹; ¹H NMR: δ = 1.45 (d, *J* = 8.0 Hz, 3H), 2.99 (s, 3H), 3.18 (s, 3H), 3.66 (q, *J* = 8.0 Hz, 1H), 5.20–5.24 (m, 1H), 5.25–5.30 (m, 1H), 5.38–5.42 (m, 1H), 5.56–5.62 (m, 1H); ¹³C NMR: δ = 19.8 (CH₃), 35.9 (CH₃), 37.8 (CH₃), 40.0 (CH), 91.0 (2 × CH), 93.8 (CH), 94.2 (CH), 94.8 (CH), 111.3 (C), 172.4 (CO), 232.9 (CO); HR-MS calcd for C₁₄H₁₅O₄Nr: 313.0406, found: 313.0411.

14: M.p. 36 °C (hexane); IR: $\tilde{\nu}$ = 1957, 1870 cm⁻¹; ¹H NMR: δ = 1.23 (t, *J* = 7.0 Hz, 3H), 1.42 (d, *J* = 6.5 Hz, 3H), 2.18 (s, 3H), 3.57 (dq, *J* = 9.5, 7.0 Hz, 1H), 3.64 (dq, *J* = 9.5, 7.0 Hz, 1H), 4.08 (q, *J* = 6.5 Hz, 1H), 5.12 (d, *J* = 7.0 Hz, 1H), 5.14 (d, *J* = 7.0 Hz, 1H), 5.44 (d, *J* = 7.0 Hz, 1H), 5.61 (d, *J* = 7.0 Hz, 1H); ¹³C NMR: δ = 15.4 (CH₃), 20.4 (CH₃), 23.2 (CH₃), 65.0 (CH₂), 75.0 (CH), 91.5 (CH), 91.8 (CH), 92.7 (CH), 93.5 (CH), 109.7 (C), 111.0 (C), 233.3 (CO); HR-MS calcd for C₁₄H₁₆O₄Cr: 300.0454, found: 300.0451.



Scheme 5. a) EtBr, NaH, THF, sealed tube, 50 °C, 7 h, 70%; b) Cr(CO)₆, Bu₂O, THF, 145 °C, 30 h, 76%; c) LiDBB, THF, -78 °C, then 5-iodo-2-methyl-2-pentene, 27%; d) air, sunlight, 99%.

(HPLC). The analytical and spectroscopic data of **15** were in agreement with those reported in the literature.^[19] In this example the overall stereochemical retention was unequivocally established by determining the absolute configuration of the product **15** from its molecular rotation ($[\alpha]_D^{20}$ = +31; *c* = 0.95 in CHCl₃).^[20]

In conclusion, we have developed a new, convenient method for the stereospecific umpolung of readily available chiral 1-arylalkanol–Cr(CO)₃ derivatives. This was accomplished by exploiting the configurative stability of the Cr(CO)₃-complexed benzylic radical and anion intermediates. Having demonstrated the usefulness of this novel concept, we

are now investigating the scope of the methodology by using different substrates and electrophiles. This investigation will include the use of other transition metal π complexes.^[21]

Experimental Section

Lithium (ca. 40 mg, excess) was added under an argon atmosphere to a solution of 4,4'-di-*tert*-butylbiphenyl (188 mg, 0.71 mmol) in dry THF (7 mL) in a Schlenk tube equipped with a glass stirring bar. The mixture was stirred rapidly at 0 °C for 3 h. The blue/green solution was then transferred by cannula to a second Schlenk tube cooled to -78 °C leaving the excess lithium metal behind. A solution of **8** (100 mg, 0.35 mmol) in dry THF (2 mL) was injected into the solution of LiDBB and the resulting dirty red solution was stirred at -78 °C for 30 min before the appropriate electrophile (2 equiv) was added under argon with a syringe. After stirring the reaction mixture for 1 h at -78 °C it was quenched by careful addition of wet *tert*-butyl methyl ether (20 mL) and water (5 mL). The mixture was allowed to warm to room temperature before more water (30 mL) was added. The organic phase was separated and the aqueous layer washed with more *tert*-butyl methyl ether (2 × 20 mL). The combined organic extracts were dried (MgSO₄) and the solvent removed in vacuo to give a brown-yellow residue. Chromatography of the residue (SiO₂, EtOAc/hexane (5/95), then to more polar mixtures of EtOAc/hexane) afforded the desired products.

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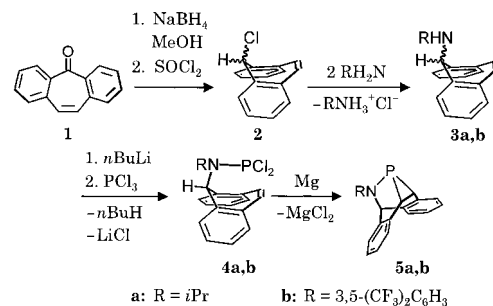
Very Stable Phosphiranes**

Jürgen Liedtke, Sandra Loss, Gilles Alcaraz,
Volker Gramlich, and Hansjörg Grützmacher*

*Dedicated to Professor Edgar Niecke
on occasion of his 60th birthday*

Phosphiranes are strained three-membered PC_2 -heterocycles^[1] which are potentially interesting ligands for homogeneous transition metal catalyst complexes. Their small sum of bond angles at the phosphorus center ($\Sigma \angle (\text{P})$: 240–275) should make them good π acceptors; however, these heterocycles are usually labile towards [2+1] cycloreversions into phosphinidines, RP, and olefins.^[2] Although in recent years some rather stable phosphiranes bearing bulky substituents at the phosphorus center (i.e. *t*Bu, adamantyl, mesityl, supermesityl, $\text{N}(\text{SiMe}_3)_2$) have been prepared,^[3] their use in catalysis was found to be limited because of ligand decomposition.^[4] In order to suppress this undesired cycloreversion reaction, we searched for a simple high-yield synthesis of phosphiranes, in which the PC_2 ring is embedded in a polycyclic framework.^[5]

Starting from the dibenzoannelated tropolone **1**, amines **3a** and **3b** are obtained following known literature procedures.^[6] After lithiation and subsequent reaction with PCl_3 the dichloroaminophosphanes **4a** and **4b** are isolated as colorless crystals in more than 90% yield. Using commercially available magnesium turnings and thf as solvent, dehalogenation of **4a** and **4b** led to gram quantities of the amino-substituted^[7] phosphiranes **5a** and **5b** in excellent yields (> 90%; Scheme 1).



Scheme 1. Synthesis of **5a** and **5b**.

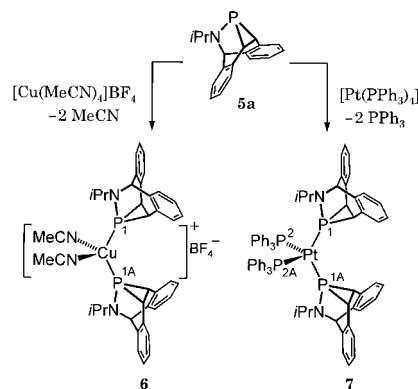
Formally these compounds are formed by an intramolecular [2+1] cycloaddition of an R_2NP phosphinidene unit to the C–C double bond of the central seven-membered ring of

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the dibenzotropyliene unit, which has a rigid boat conformation as shown for **4a** and **4b**.^[8] In **5a** and **5b** the PC₂ ring is part of a tetracyclic cage and, with reference to the analogous hydrocarbon C₉H₁₀ (barbarlane), we name these phosphiranes phosphazabarbaralanes (*BABAR*-Phos). They form colorless crystals which melt without decomposition and can be handled in air. In contrast to 1-phenylphosphirane, PhP(CH₂)₂, **5a** and **5b** do not react even with an excess of strong alkylating agents such as MeOSO₂CF₃ to form phosphiranium salts,^[9] nor do they react with sulfur in refluxing toluene to give thiophosphoranes.

To answer the question, how *BABAR*-Phos binds to d¹⁰ transition metal centers, we treated **5a** with [Cu(MeCN)₄]BF₄ and [Pt(PPh₃)₄]. The bis(*BABAR*-Phos) complexes **6** and **7** were obtained quantitatively (Scheme 2). While d⁸ platinum(II) metal complexes of the unsaturated phosphirene PhP(CPh)₂ are known, attempts to prepare electron-rich d¹⁰ complexes containing intact phosphiranes gave only ring-opened products.^[10]

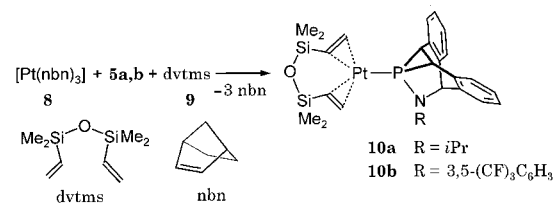


Scheme 2. Complexes with the ligand **5a**.

The binding properties of *BABAR*-Phos **5a** were qualitatively compared: triphenylphosphane, Ph₃P [$\Sigma \chi(P) \approx 303$], as the better σ donor, readily displaces the phosphirane **5a** from the copper complex **6**. Consequently, attempts to prepare *BABAR*-Phos complexes from triphenylphosphane coinage metal complexes, [(Ph₃P)_nM]⁺ (M = Cu, Ag, Au) failed. However, in solutions of platinum complex **7**, free PPh₃ is observed by ³¹P NMR spectroscopy in addition to [Pt(*BABAR*-Phos)₂(PPh₃)] ($\delta(^{31}\text{P}) = -109$, $^2J(\text{P,P}) = 74$ Hz,

$^1J(\text{Pt,P}) = 4454$ Hz; $^{15}J(\text{P,P}) = 74$ Hz, $^1J(\text{Pt,P}) = 4374$ Hz)); thus the *BABAR*-Phos ligand remains bonded to platinum. It is assumed that phosphorus to metal σ donation is the more important component in the complexation of phosphanes in coinage metal complexes. On the other hand, metal-to-ligand π donation is believed to make the predominant electronic contribution in the binding of phosphanes to zero-valent platinum.^[1b] Thus our experiments suggest a poor σ -donating but rather high π -accepting strength for **5a**.

As shown in Scheme 3, the d¹⁰ platinum *BABAR*-Phos olefin complexes **10a** and **10b** are readily accessible by substitution of norbornene (nbn) in [Pt(nbn)₃] (**8**). The



Scheme 3. Synthesis of the complexes **10a** and **10b** from **5a** and **5b**, respectively.

compound **10a** was isolated and characterized by single-crystal X-ray structure analysis (Figure 1).^[11a] The structures of **5b**, **6**, and **7**^[11b] have also been determined and selected bond lengths and angles are listed in Table 1.

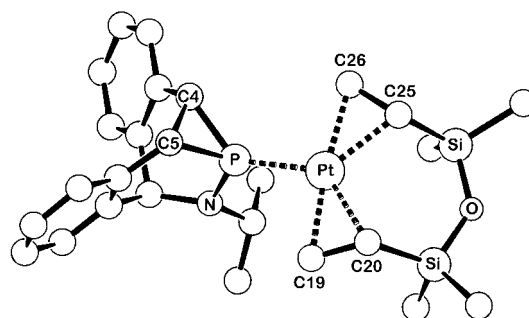


Figure 1. Molecular structure of **10a**. Selected bond lengths [Å] and angles [°] (see also Table 1; X1 = midpoint of the C19–C20 bond, X2 = midpoint of the C25–C26 bond): Pt–C19 2.141(7), Pt–C20 2.133(6), Pt–C25 2.152(7), Pt–C26 2.127(7), C19–C20 1.405(9), C25–C26 1.416(11); P–Pt–X1 111.95(6), P–Pt–X2 114.15(6), X1–Pt–X2 133.90(4).

Table 1. Selected structural data of **5b**, **6**, **7**, and **10a**.

	<i>a</i> ₁ [Å]	<i>a</i> ₂ [Å]	<i>b</i> [Å]	<i>c</i> [Å]	<i>d</i> [Å]	α [°]	β_1 [°]	β_2 [°]	$\Sigma \chi$ [°]	γ_1 [°]	γ_2 [°]	γ_3 [°]
5b	1.850(3)	1.863(3)	1.532(4)	1.738(2)	–	48.8(1)	98.8(1)	99.0(1)	246.5	–	–	–
6 Cu ^[a]	1.821(5)	1.849(5)	1.544(6)	1.676(4)	2.269(1)	49.8(2)	101.5(2)	103.1(2)	254.4	119.8(1)	127.2(2)	134.2(2)
7 Pt ^[a]	1.831(5)	1.856(5)	1.558(7)	1.699(4)	2.281(1)	49.9(2)	99.5(2)	101.0(2)	250.4	124.8(1)	125.2(2)	131.5(2)
10a Pt	1.812(6)	1.821(7)	1.576(9)	1.684(6)	2.241(2)	51.4(3)	101.7(3)	103.1(3)	256.2	119.8(2)	130.0(2)	131.4(2)

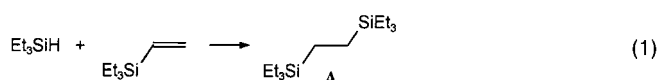
[a] Averaged data of both *BABAR*-Phos ligands are given.

Table 2. Selected physical data for **4a,b**, **5a,b**, **6**, **7**, and **10a,b** (δ in ppm, J in Hz).

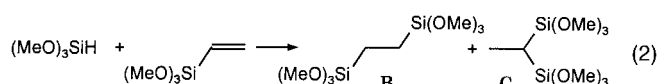
	$\delta(^{31}\text{P})$	$\delta(^1\text{H})$ PCHCH	$\delta(^{13}\text{C})$ PCHCH	M.p. [$^{\circ}\text{C}$]
4a	168.7	7.12	130.7	114
4b	154.7	7.21	130.6	122
5a	–153.5	2.65, $^2J(\text{P,H}) = 22$	21.3, $^1J(\text{P,C}) = 38$	85
5b	–146.0	3.12, $^2J(\text{P,H}) = 20$	22.4, $^1J(\text{P,C}) = 38$	147
6	–133.5	3.08, $^2J(\text{P,H}) = 15$	20.7, $^1J(\text{P,C}) = 30.2$	(decomp) > 176
7	–111, $^2J(\text{P,P}) = 66$, $^1J(\text{Pt,P}) = 4038$ (P1, 1A); 13.5, $^2J(\text{P,P}) = 66$, $^1J(\text{Pt,P}) = 4254$ (P2, 2A).	2.3 (m)	27.7, $^1J(\text{P,C}) = 23$	(decomp) > 180
10a	–74, $^1J(\text{Pt,P}) = 4203$	2.85, $^2J(\text{P,H}) = 5.7$, $^3J(\text{Pt,H}) = 13.8$	23.1, $^1J(\text{P,C}) = 15$, $^2J(\text{Pt,C}) = 18$	(decomp) > 95
10b	–76, $^1J(\text{Pt,P}) = 4218$	3.02, $^2J(\text{P,H}) = 6.0$, $^3J(\text{Pt,H}) = 12.8$	25.2, $^1J(\text{P,C}) = 18$, $^2J(\text{Pt,C}) = 15$	–

The bond parameters show the expected trends: The Pt–P1/1A lengths (2.281(1) Å in **7**, 2.241(2) Å in **10a**) are shorter than the Pt–P2/2A (PPh₃) bond lengths (2.324(1) Å) in **7**. In all compounds the ring internal P–C bonds (a_1, a_2) and P–N (c) bonds become shorter when the phosphorus lone pair is involved in complexation. At the same time, the basal C–C bond, b , becomes longer and the sum of bond angles, $\Sigma\angle$, at phosphorus becomes larger. These effects are most pronounced in the three-coordinate platinum(0) complex **10a**.

Given that Pt⁰ olefin complexes are known hydrosilylation catalysts, we mixed [Pt(nbn)₃] (**8**) with a slight excess of dtms **9** in benzene as solvent and then added one equivalent of phosphane to afford the catalyst precursor. By ³¹P NMR spectroscopy, the complexes **10a** and **10b** were the only species detected in these solutions. For the hydrosilylation reaction (1) 14 mol %, and for reaction (2) 1.4 mol % catalyst were used, respectively



	Conversion to A [%]	t [h]	T [$^{\circ}$]
Pt/PPh ₃	< 3	72	25
Pt/ 5a	100	72	25
Pt/ 5b	100	< 24	25



	Conversion [%]	B [%]	C [%]	t [h]	T [$^{\circ}$]
Pt/PPh ₃	100	> 90	< 10	24	25
Pt/ 5a	100	83	17	< 24	25
Pt/ 5b	100	78	22	< 8	25

The phosphirane ligands **5a** and **5b** significantly enhance the catalytic activity when compared to PPh₃ under identical conditions. Specifically, 1) no conversion was observed in reaction (1) with PPh₃ and 2) use of BABAR-Phos **5b** bearing an electron-withdrawing group bound to the nitrogen center, reduces the conversion time by a factor of about 3. In the mixtures, the intact complexes **10a** and **10b** were observed by ³¹P NMR spectroscopy even after longer time periods (> 5 d). The formation of elemental platinum was not observed.

Summarizing the results described above, we have shown that very stable, nonsensitive phosphiranes may be easily prepared in high yields when the three-membered PC₂ ring is incorporated into a polycyclic cage. BABAR-Phos ligands allow the synthesis of low-valent platinum metal complexes to which they tightly bind. Some of these complexes are indeed effective hydrosilylation catalysts and their activity may be tuned by the substituents bound to the nitrogen center.

Experimental Section

Selected physical data of all compounds described in the following are given in Table 2.

General procedure for 4a,b: *n*BuLi (25 mL of a 1.6 M hexane solution; 40 mmol) was added to a solution of the amine **3a** or **3b** (40 mmol) in Et₂O at –30 °C. The reaction mixture was allowed to warm to room temperature, and then added dropwise to a solution of PCl₃ (5 mL, 57 mmol) in Et₂O (20 mL) at –20 °C. All solvents were removed in vacuum, the residue was suspended in toluene (50 mL), and then filtered over celite. Subsequently the solvent was removed in vacuum to give nearly pure compounds which were recrystallized from Et₂O (**4a**) or toluene (**4b**) (yields > 90 %).

5a,b: A solution of **4a** or **4b** (29 mmol) in THF (100 mL) was stirred in the presence of Mg turnings (1 g, 41 mmol) at room temperature. After 5 h, dry dioxane (5 mL) was added to precipitate MgCl₂. The solvents were evaporated, the residue was dissolved in toluene (100 mL), and filtered over celite. The toluene phase was concentrated to 10 % of its volume and hexane was added to precipitate spectroscopically pure **5a** or **5b** as slightly yellow powders (95 % yield; **5a**: 7.7 g, **5b**: 12.4 g), which were recrystallized from Et₂O/hexane.

6: Complex **6** was obtained quantitatively by mixing [Cu(CH₃CN)₄]BF₄ (100 mg, 0.3 mmol) and **5a** (168 mg, 1.2 mmol) in CH₃CN (15 mL). Crystals suitable for an X-ray structure analysis were obtained at –20 °C.

7: Complex **7** was prepared from [Pt(PPh₃)₄] (0.2 g, 0.4 mmol) and **5a** (0.225 g 0.8 mmol) in toluene (10 mL). After the solvent had been dried in vacuum, the remaining yellow powder was washed with hexane (3 × 5 mL) to remove free PPh₃. Drying in vacuum gave pure **6** as a yellow powder in 92 % yield.

10a: Complex **10a** was obtained in 70 % yield (97 mg) from [Pt₂(dvtms)₃]^[12] (100 mg, 0.11 mmol) and **5a** (60 mg, 0.21 mmol) in toluene (2 mL). Crystals were grown from concentrated hexane solutions.

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Study of the Interactions between Poly(vinyl pyrrolidone) and Sodium Dodecyl Sulfate by Fluorescence Correlation Spectroscopy

Ralf Nörenberg,* Jürgen Klingler, and Dieter Horn

Dedicated to Professor Hans-Jürgen Quadbeck-Seeger on the occasion of his 60th birthday

Interactions between polymers and surfactants in aqueous solutions are attracting much attention at present as many formulations and industrial processes make simultaneous use of polymers and surfactants for their complementary or even synergistic roles.^[1] In many of these applications the optimization of the properties of the polymer in the presence of interference from other chemicals demands a method that is capable of monitoring the association behavior in complex media, in a wide concentration regime, and with less effort than the presently available methods permit. There have been a lot of experimental techniques explored in the past to study the complex formation.^[2–6] All of them, however, have their specific limitations. The first method used to study the complex formation was NMR-self-diffusion by monitoring the hydrodynamic size of surfactant molecules.^[7] However, this method is limited in the concentration range accessible and also takes a lot of effort.

With the ongoing development of optical correlation techniques, measurements of the self-diffusion coefficient of fluorescing molecules by fluorescence correlation spectroscopy (FCS)^[8] became a practical laboratory technique in the last few years.^[9] Since fluorescence is a very sensitive technique measurements of the self-diffusion coefficient can be extended to very low concentrations.^[10] We present herein the first study of the complex formation of sodium dodecyl sulfate (SDS) with poly(vinyl pyrrolidone) (PVP) by FCS to demonstrate its applicability for associating colloidal systems. The complexes of SDS with PVP belong to the best studied model systems in the literature and often serve as a reference system.

Fluorescence correlation spectroscopy uses fluctuations in the numbers of fluorescent molecules (or particles) in a very small observation volume to obtain information on the diffusive properties of these molecules.^[11] The resulting autocorrelation functions $G(t)$ were fitted to the theoretical Equation (1).^[10]

$$G(t) = 1 + \frac{1}{N} \left(\frac{1}{1 + \frac{4Dt}{r^2}} \right) \quad (1)$$

In this equation N is the average number of fluorescent molecules, D the thermal diffusion coefficient of the molecules and r the radial width of the laser focus. The value of r was obtained by calibrating the instrument with the dye Rhodamin 6G, by assuming a diffusion coefficient of this dye of $2.8 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$. The diffusion coefficients in this study

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were converted into apparent hydrodynamic diameters according to the Einstein equation [Eq. (2)] to give more intuitively accessible results.

$$d = \frac{kT}{3\pi\eta D} \quad (2)$$

Here d is the hydrodynamic diameter of the diffusing molecule, k the Boltzmann constant, T the absolute temperature, and η the viscosity of the medium. The diameters given below are the average of ten measurements for each sample (typical standard deviation 5 %).

It is possible to study a wide range of polymer and surfactant concentrations with FCS. Figure 1 shows the results obtained by monitoring the self-diffusion coefficient of the

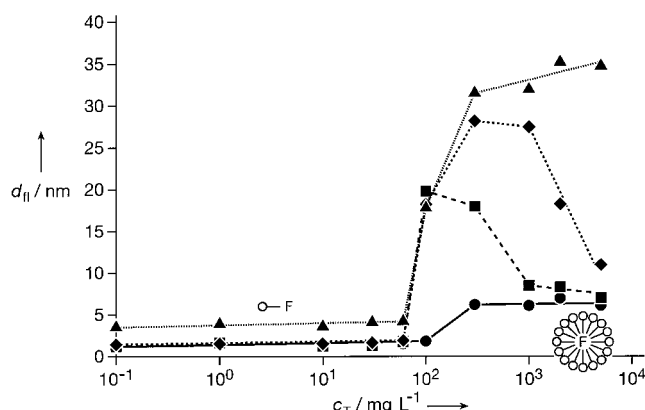


Figure 1. Apparent hydrodynamic size of the fluorescent surfactant SDecS (d_H) as a function of SDS concentration (c_T) for several concentrations of Kollidon K90 ($M_w = 10^6$ Da). Polymer concentration: $\bullet = 0$, $\blacksquare = 0.03$, $\blacklozenge = 0.3$, $\blacktriangle = 3$ g L $^{-1}$.

fluorescence-labeled surfactant molecules in solutions with different concentrations of Kollidon K90 (from 30 mg L $^{-1}$ to 3 g L $^{-1}$). The corresponding curve for pure SDS is shown as a reference.

At very low surfactant concentrations the molecules are solubilized by individual solvation shells. In this system the diffusion coefficient of the labeled SDecS molecules is constant and resembles the hydrodynamic size of a single molecule with a diameter of about 1.1 nm. At a concentration of about 300 mg L $^{-1}$ (about 1.0 mM) the apparent size of the labeled surfactant begins to increase steeply up to a new level of about 5 nm. This concentration is about the critical micelle concentration (CMC) of SDS under the applied conditions and coincides with the CMC measured with other techniques, when the salt concentration dependence is accounted for by Equation (3) with $c_{\text{salt}} = 0.5$ M.^[12]

$$\ln c_{\text{CMC}} = -7.3 - 0.5 \ln c_{\text{salt}} \quad (3)$$

In addition, the hydrodynamic size found for the surfactant aggregates at high concentrations is in reasonable agreement with light scattering data. The steepness of the function originates from the highly cooperative nature of the micelle-forming process.

The behavior of the FCS signal changes significantly in the presence of 30 mg L $^{-1}$ PVP in the solution. Again, at low concentrations the size of the fluorescing moiety corresponds

to the hydrodynamic size of a single surfactant molecule. At intermediate concentrations, however, a sudden rise in hydrodynamic diameter occurs up to values of about 10 nm. This process occurs at concentrations of 80 mg L $^{-1}$ (0.3 mM surfactant), which is significantly lower than the CMC of the bare molecule and is in accordance with the critical association concentration (CAC) obtained by surface tension measurements.^[13] At higher surfactant concentrations the apparent size of the aggregates decreases. We explain this behavior by a saturation of the binding capacity of PVP by the surfactant. Additional surfactant molecules form micelles as in the PVP-free case.

The CAC, identified from the steep increase of the hydrodynamic size, does not change upon increasing the polymer concentration even further to 300 mg L $^{-1}$ and 3 g L $^{-1}$. This result provides clear evidence that the CAC of the system is independent of the polymer concentration, as was shown with fluorescence probe studies.^[4a]

We find a different behavior of the dependencies of the hydrodynamic size on the surfactant interaction at the three presented polymer concentrations. At low surfactant concentration the hydrodynamic sizes coincide with the hydrodynamic size of the single surfactant molecule. The increase takes place in a similar fashion. The hydrodynamic sizes differ, however, in the next concentration steps.

A decrease of the hydrodynamic size down to the bare surfactant micelle size appears at low polymer concentration. At intermediate polymer concentration the fall-off is less pronounced and the maximum of the curve reaches a higher size. At the highest polymer concentration studied we find, instead of a fall-off regime, a plateau at a hydrodynamic diameter of 35 nm in the case of Kollidon K90. This value corresponds roughly to the coil diameter of the PVP under these conditions.

If there are a lot of molecules molecularly solvated or bound in bare surfactant micelles, the average value for the hydrodynamic size is lowered. We attribute the above mentioned fall-off process to an increased contribution of small micelles to the average particle size. This picture is sketched in Figure 2.

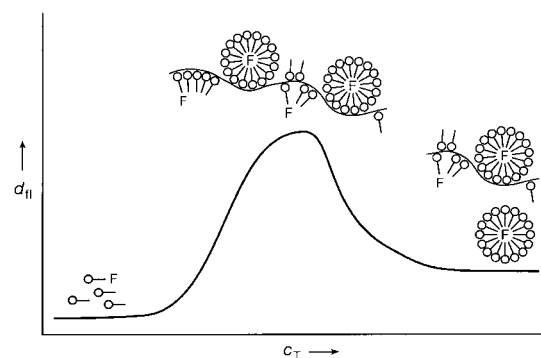


Figure 2. Schematic representation of the interpretation of the hydrodynamic size with the surfactant concentration. At low surfactant concentrations the hydrodynamic size corresponds to a free surfactant molecule. The curve bends at the CAC of the complex. The hydrodynamic size increases as a consequence of an increased contribution of polymer-bound surfactants until the maximum surfactant load of the polymer is reached. The decrease is attributed to the increased contribution of pure surfactant micelles.

As in the case of NMR self-diffusion, one can calculate the measured diffusion coefficient by a weighted sum over the individual diffusion coefficients D_{free} , D_{micelle} , and D_{mm} for the surfactant molecules that are free, bound in surfactant micelles, and in mixed polymer–surfactant micelles, respectively [Eq. (4)].^[14]

$$D_{\text{obs}} = w_{\text{free}} D_{\text{free}} + w_{\text{micelle}} D_{\text{micelle}} + w_{\text{mm}} D_{\text{mm}} \quad (4)$$

Equation (4) can be fitted to the titration curves to yield the relative amounts of free, micelle-bound, and polymer-bound surfactant molecules. Figure 3 shows analogous data for the

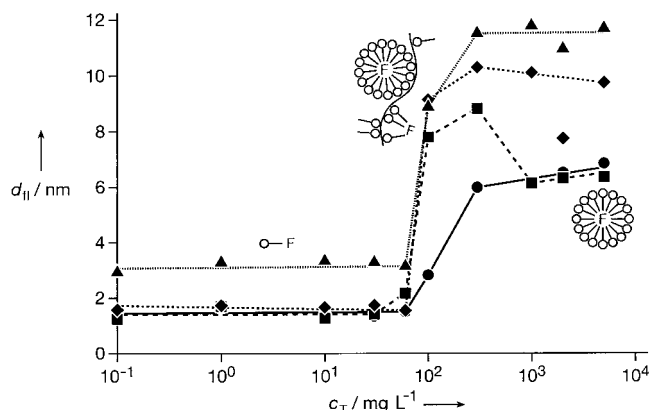


Figure 3. Apparent hydrodynamic size of the fluorescent surfactant SDecS (d_H) as a function of SDS concentration (c_T) for several concentrations of Kollidon K30 ($M_w = 5 \times 10^4$ Da). Polymer concentration: ● = 0, ■ = 0.03, ◆ = 0.3, ▲ = 3 g L⁻¹.

shorter chain PVP ($M_w = 5 \times 10^4$ Da). This Figure also includes the curve for the pure surfactant as a reference. A sudden increase in particle size at concentrations well below the CMC of the pure surfactant is again observed on adding polymer to the solution. This critical surfactant concentration is the same as with the higher M_w PVP discussed above. The other features of the results are similar, too: For all polymer concentrations studied we find the same CAC of about 80 mg L⁻¹ surfactant. At a polymer concentration of 30 mg L⁻¹ the formation of a weak maximum in the size is observed. This result is a consequence of the formation of the PVP complex with the SDS micelles. At higher surfactant concentrations the detected size of the fluorescent probe decreases to the value of the bare micelles. Here again the argument holds that at a certain concentration of SDS the polymer is saturated in its micelle-binding capacity and additional surfactant forms small micelles that contribute significantly to the overall signal. At higher polymer concentrations a plateau in the apparent micelle size of 12 nm is observed, which correlates well with the hydrodynamic size of the polymer.

In all cases, either with the high molecular weight or with the low molecular weight polymer, we find maxima, which allow us to calculate the composition of the PVP–SDS complex at saturation. From the corresponding concentrations we find a saturation value of about 3.3 g SDS per gram of PVP for all polymer concentrations and types. Murata et al.

report a value of 3.4 g SDS per gram of PVP from a binding isotherm determination.^[12]

The measurements presented here show the first application of FCS for the study of polymer–surfactant interactions. The experiments described herein show the formation of micelles and mixed polymer–surfactant micelles on a molecular level. In contrast to other methods, these measurements can be performed in a very dilute solution. Under these conditions viscosity effects arising from interacting polymer tails and chains and charge interactions between two anionically loaded PVP molecules do not have to be considered. We observe the CAC of the system to be 80 mg L⁻¹ surfactant, and independent of the size and concentration of the PVP. The presence of a maximum in the size versus surfactant concentration profile indicates that there is an upper limit for the amount of surfactant micelles that can be accommodated by PVP. The further use and development of this technique will include the application to more complex systems.

Experimental Section

We used two samples of technical poly(vinyl pyrrolidone) (BASF) with average molecular weight $M_w = 10^6$ Da (Kollidon K90, K value (viscosity measurement) 90) and $M_w = 5 \times 10^4$ Da (Kollidon K30, K value 30). The fluorescent probe was a terminally fluorescence labeled SDecS that is analogous to SDS in its formation of micelles and surface active properties (Molecular Probes Ltd., catalogue No. D-3829) used at 10 nM (0.005 mg L⁻¹) in a mixture with normal SDS (Aldrich). All chemicals were used as obtained without further purification.

The experiments were performed on a commercial FCS apparatus (ConfoCor, Carl Zeiss Jena; illumination: 488 nm; water immersion objective: 63×, 1.2 NA; detection pinhole: 45 nm). The fluorescence correlation function of each sample was measured ten times with a measurement duration of 60 s each. The experiments were carried out in 0.5 M NaCl solution (filtered through a 0.2 μm filter) at 24 °C. All solutions were equilibrated after preparation for at least 1 h before measurement. No change of FCS results between 1 h and 24 h after preparation of the samples was observed.

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 [14] Strictly speaking, this calculation is only valid for fast exchange (ca. 1 ms) between the bound and the free surfactant fraction on the timescale of the correlation function. For slow exchange, the autocorrelation function [Eq. (1)] will consist of two components with different diffusion coefficients. No significant deviation from Equation (1) was observed in our experiments, however.

[RuCl₂{PPh₂(2,6-Me₂C₆H₃)}₂]: A Neutral 14-Electron Ruthenium(II) Complex with Two Agostic Interactions**

Walter Baratta,* Eberhardt Herdtweck, and Pierluigi Rigo

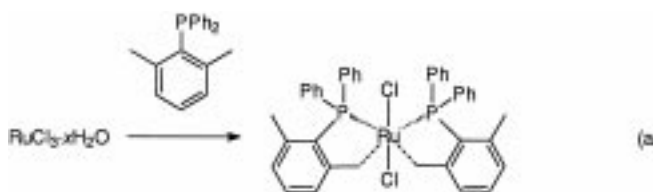
Coordinatively unsaturated transition metal complexes, which play a key role in many catalytic processes, have been often obtained with bulky tertiary phosphanes (PR₃) as ligands that provide steric protection to the empty coordination sites at the metal. The steric crowding forces the R group of the ligand in close proximity to the metal center to induce an agostic interaction^[1] between a C–H bond in the R group and the metal atom, thus preventing dimerization or solvent coordination. Ruthenium(II) complexes with small phosphanes are generally six-coordinate 18-electron species of the type [RuX₂(PR₃)₄] (X = halide), whereas with the bulky triarylphosphanes five-coordinate 16-electron complexes such as [RuCl₂(PPh₃)₃] are formed.^[2] La Placa and Ibers noted that the close approach of one *ortho*-phenyl hydrogen atom to the metal effectively blocks a coordination site at the ruthenium center.^[3] It can be anticipated that when the *ortho*-hydrogen atom of the phenyl rings of PPh₃ are substituted with methyl groups, stronger contacts would occur; the CH₃ group is forced close to metal center, and an agostic interaction is favored. With this in mind, we have been investigating ruthenium(II) complexes with *ortho*-methyl-substituted arylphosphane ligands.

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We report herein the isolation and characterization in solution and in the solid state of the formally four-coordinate complex [RuCl₂{PPh₂(2,6-Me₂C₆H₃)}₂] (**1**), in which the two empty coordination sites on the metal are occupied by one of the methyl groups of each xylyl substituent. No neutral monomeric bis-phosphane 14-electron species have been reported until now, the complexes of stoichiometry [RuCl₂(PR₃)₂] (R = Ph, 3-C₆H₄SO₃H)^[4,5] being binuclear derivatives with Ru(μ-Cl)Ru bridges. Recently, the dihydrogen complexes [RuXX'(H₂)L₂] (X, X' = halogen, H; L = PPr₃, PtBu₂Me) have been used as suitable sources of 14-electron complexes [RuXX'L₂] for the preparation of ruthenium(II) carbene and vinylidene derivatives.^[6] Complex **1** is the first example of a neutral ruthenium complex with two agostic interactions, and it can be compared with the recently reported cationic complex [Ru(Ph)(CO)-(PtBu₂Me)₂]⁺[BAR'₄][−] (Ar' = 3,5-(CF₃)₂C₆H₃).^[7]

Reaction of ruthenium trichloride hydrate with a threefold molar amount of (2,6-dimethylphenyl)diphenylphosphane in refluxing methanol afforded the purple, diamagnetic ruthenium complex **1** in good yield [Eq. (a)]. Compound **1** is



thermally stable in solution for many hours at room temperature if no air is admitted. The ³¹P{¹H} NMR spectra of **1** in different solvents and at different temperatures show only one sharp signal. Therefore, solvent coordination to the ruthenium center or formation of dimeric complexes of the type [Ru₂Cl₂(μ-Cl)₂(PR₃)₄] (R = Ph, 3-C₆H₄SO₃H),^[4,5] having two nonequivalent phosphane ligands, can be excluded. Moreover, the temperature dependence of the ¹H NMR spectra shows the fluxional behavior of **1** in solution. In fact, above 50 °C the resonances of the methyl protons appear as a broad signal (δ = 1.92 in C₂Cl₄D₂ at 80 °C). Upon lowering of the temperature the signal splits into two broad resonances of equal intensity (δ = 2.37 and 1.37 in C₂Cl₄D₂ at 20 °C), indicating that the xylyl rotation is slow on the ¹H NMR time scale. Furthermore, below −40 °C the resonance at δ = 1.37 becomes sharp, while that at δ = 2.37 splits into a doublet (J(H,P) = 5.1 Hz). Consequently the low-field signal can be attributed to the protons of the methyl group close to the ruthenium atom that couple with one phosphane phosphorus atom. No further splitting of this signal was observed even at −90 °C, so rotation of the interacting CH₃ group cannot be frozen out. The ¹³C{¹H} NMR spectrum of **1** in CDCl₃ at 20 °C shows two nonbinomial quintets for the *ipso*-carbon atoms of the xylyl and the phenyl groups at δ = 134.5 and δ = 132.0, respectively, which are in agreement with a *cis* arrangement of the two phosphane ligands.^[8] At room temperature the spectrum of **1** exhibits two broad signals for the methyl carbon atoms at δ = 23.7 and 11.7. Whereas the first resonance becomes sharp below −20 °C, and is therefore assigned to the noninteracting methyl groups (CH₃ of the free phosphane:

$\delta = 23.8$), the second absorption, which is attributed to the interacting methyl group, splits into a triplet with a $|J_{\text{trans}}(\text{C,P}) + J_{\text{cis}}(\text{C,P})|$ value of 23.6 Hz due to $^2J(\text{P,P})$ virtual coupling (CD_2Cl_2 at -40°C).^[9] These data can be compared with those of a methyl group bound through a δ -agostic interaction in an azine phosphane ruthenium(II) complex ($J(\text{C,P}) = 13.6$ Hz).^[10] Furthermore, a ^{13}C NMR INEPT experiment carried out on **1** exhibits a broad quartet at $\delta = 10.5$ with a $^1J(\text{C,H})$ value of about 117 Hz, a significantly low value^[1a] compared to the $^1J(\text{C,H})$ value of 127 Hz shown by the other sharp quartet at $\delta = 23.8$ (CDCl_3 at -40°C). Consequently, all these findings are in agreement with the presence of two strong $\text{Ru} \cdots \text{H}-\text{C}$ interactions in the stereochemically rigid complex **1**.

To establish the previous formulation with two methyl groups close to the metal center,^[11] an X-ray diffraction study was carried out on a single crystal of the solvate **1**·toluene (Figure 1a).^[12] In the discrete monomeric units of **1** the ruthenium center is in a nearly octahedral environment with two *trans* chloride atoms and two *cis* phosphane ligands. The coordination sphere of ruthenium is completed by two methyl

groups of the two xylyl substituents. Complex **1** is nearly C_2 -symmetric, with the twofold axis bisecting the $\text{P1}-\text{Ru}-\text{P2}$ angle. The $\text{Ru}-\text{Cl}$ and the $\text{Ru}-\text{P}$ bond lengths are in agreement with those reported for phosphaneruthenium(II) chloride complexes.^[13] The chloro atoms are significantly bent away from the phosphorus atoms, presumably to minimize steric interactions with the phenyl groups, resulting in a $\text{Cl1}-\text{Ru}-\text{Cl2}$ bond angle of $168.00(2)^\circ$. The $\text{Ru}-\text{P}-\text{C}_{\text{ipso}}$ angles for the xylyl groups of **1** are on average $109.37(5)^\circ$, which is about 7.44° smaller than the $\text{Ru}-\text{P}-\text{C}_{\text{ipso}}$ angles of the phenyl groups (mean value $116.81(4)^\circ$). The five atoms Ru , P1 , P2 , C10 , and C40 are almost coplanar (distances to the mean plane less than ± 8 pm) and the atom pairs $\text{C11}/\text{C12}$ and $\text{C41}/\text{C46}$ are in C_2 -symmetrical positions with respect to this plane ($+44/+53$ and $-50/-50$ pm, respectively). Owing to the steric crowding the xylyl groups are all bent back, and hinged about the $\text{P1}-\text{C10}$ and $\text{P2}-\text{C40}$ axes at an angle of 20.3° and 20.6° , respectively; one phenyl ring lies above and the other below the principal coordination plane (Figure 1b).

The X-ray analysis clearly confirms that complex **1** shows two agostic $\text{Ru} \cdots \text{H}-\text{C}$ interactions, through the coordination of one methyl group of each phosphane ligand. The shortest $\text{Ru} \cdots \text{C}(10/40)$ distances are on average $265.1(2)$ pm, a value which is within the range of very strong agostic interactions. In fact, in the cationic complex $[\text{Ru}(\text{Ph})(\text{CO})(\text{PrBu}_2\text{Me}_2)]^+[\text{BAR}_4']^-$ the distances of the two methyl groups involved in an agostic interaction are 287 and 288 ppm,^[7] while the reported $\text{Ru}-\text{C}$ bond lengths for alkyl- and arylruthenium(II) derivatives are in the range of 204–222 pm.^[14]

Apparently, in **1** the phosphane $\text{PPh}_2(2,6\text{-Me}_2\text{C}_6\text{H}_3)$ behaves as a “bidentate” ligand to give a five-membered ring in which a methyl group acts as two-electron donor, yielding a formally pentacoordinate methyl carbon atom.^[15] However, only a neutron diffraction experiment of **1** will provide clear-cut evidence of the coordination mode of the bridging methyl groups. With the related mono-*ortho*-methylated phosphanes $\text{PPh}_n(2\text{-MeC}_6\text{H}_4)_{3-n}$ ($n = 0-2$), we were unable to obtain complexes of the type $[\text{RuCl}_2(\text{PR}_3)_2]$. Moreover, **1** presents strong similarities to the dihalogenruthenium(II) complexes with unsymmetrical bidentate ligands containing phosphorus and a second weaker donor functionality such as oxygen. In particular, the ligands PPh_2Ar ($\text{Ar} = 2\text{-MeOC}_6\text{H}_4$, $2,6\text{-(MeO)}_2\text{C}_6\text{H}_3$) form the six-coordinate complexes $[\text{RuCl}_2(\text{PPh}_2\text{Ar})_2]$,^[16] which have geometrical features similar to **1**.

Complex **1**, which is accessible on a gram scale, appears to be attractive for manifold catalytic applications owing to the availability of two free coordination sites for substrate binding. Accordingly, **1** promptly reacts with carbon monoxide at ambient conditions to yield carbonylruthenium derivatives. We are currently investigating the extension of the coordination chemistry of the “chelating” phosphane $\text{PPh}_2(2,6\text{-Me}_2\text{C}_6\text{H}_3)$ to the neighboring elements.

Experimental Section

All operations were carried out with dry solvents and under an argon atmosphere. The NMR spectra were recorded on a Bruker AC200 spectrometer.

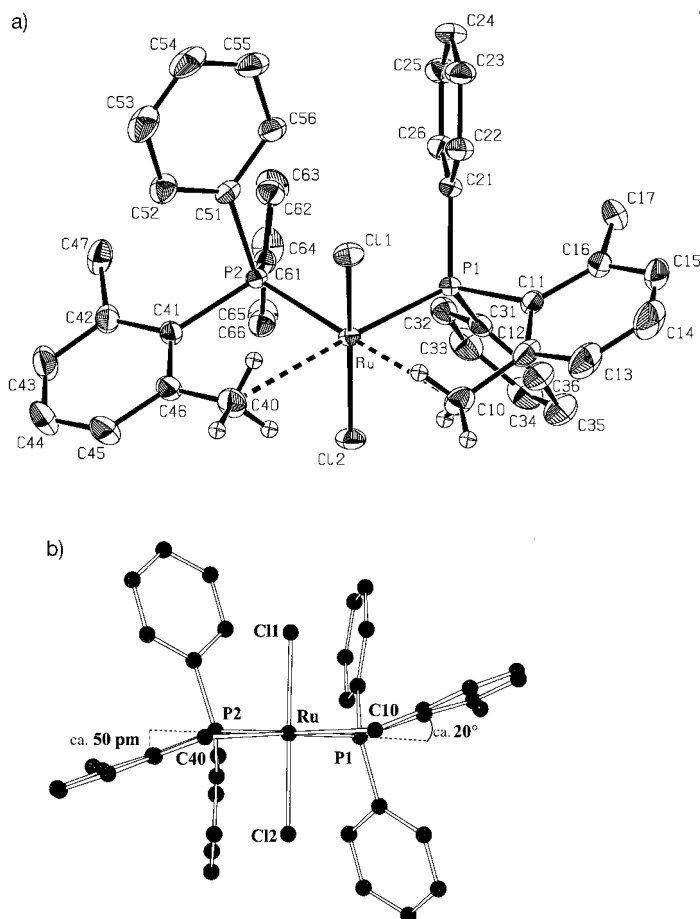


Figure 1. a) ORTEP drawing of the structure of **1**. Thermal ellipsoids are at the 50% probability level. Selected bond lengths [pm] and angles $^\circ$: $\text{Ru}-\text{Cl1}$ 239.94(4), $\text{Ru}-\text{Cl2}$ 239.76(4), $\text{Ru}-\text{P1}$ 225.82(4), $\text{Ru}-\text{P2}$ 225.25(3), $\text{Ru} \cdots \text{C10}$ 265.3(2), $\text{Ru} \cdots \text{C40}$ 264.9(2); $\text{Cl1}-\text{Ru}-\text{Cl2}$ $168.00(2)^\circ$, $\text{Cl1}-\text{Ru}-\text{P1}$ $94.69(2)^\circ$, $\text{Cl1}-\text{Ru}-\text{P2}$ $92.93(2)^\circ$, $\text{Cl2}-\text{Ru}-\text{P1}$ $92.42(2)^\circ$, $\text{Cl2}-\text{Ru}-\text{P2}$ $95.05(2)^\circ$, $\text{P1}-\text{Ru}-\text{P2}$ $101.91(1)^\circ$, $\text{C10} \cdots \text{Ru} \cdots \text{C40}$ $102.18(5)^\circ$, $\text{P1}-\text{Ru} \cdots \text{C10}$ $78.17(4)^\circ$, $\text{P2}-\text{Ru} \cdots \text{C40}$ $77.94(4)^\circ$; b) Perspective view of **1** approximately parallel to the mean plane made up of Ru , P1 , P2 , C10 , and C40 .

1: RuCl₃·xH₂O (Aldrich) (0.300 g, 1.2 mmol) was dissolved in methanol (10 mL) by bringing the mixture to a boil. PPh₂(2,6-Me₂C₆H₃) (1.00 g, 3.44 mmol) was added, and the solution was refluxed for 1 h to afford a purple precipitate. After filtration the product was washed with diethyl ether and dried under reduced pressure. Yield: 0.550 g (60%). ¹H NMR (200.1 MHz, CD₂Cl₂, –50 °C, TMS): δ = 7.45–6.75 (m, 26H; aromatic protons), 2.37 (d, J(H,P) = 5.1 Hz, 6H; CH₃), 1.30 (s, 6H; CH₃); ¹³C{¹H} NMR (50.3 MHz, CD₂Cl₂, –50 °C, TMS): δ = 142.6 (s; CMe), 142.1 (pseudo t, J(C,P) = 8.8 Hz; CMe), 133.7 (nonbinomial quint., *ipso*-C₆H₃), 132.8 (pseudo t, J(C,P) = 4.3 Hz; *o*-C₆H₃), 131.4 (nonbinomial quint., *ipso*-C₆H₃), 131.0 (s; *p*-C₆H₃), 130.2 (brs; *m*-C₆H₃), 129.7 (s; *p*-C₆H₃), 128.6 (pseudo t, J(C,P) = 5.3 Hz; *m*-C₆H₃), 127.9 (pseudo t, J(C,P) = 5.1 Hz; *m*-C₆H₃), 23.7 (s; CH₃), 10.1 (pseudo t, J(C,P) = 11.8 Hz; CH₃); ³¹P{¹H} NMR (81.0 MHz, CD₂Cl₂, –50 °C, H₃PO₄): δ = 56.2; elemental analysis calcd for C₄₀H₃₈Cl₂P₂Ru: C 63.8, H 5.1; found: C 63.1, H 5.0.

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- [11] Although the IR spectrum (KBr) of **1** shows weak absorption bands at 2736, 2676, 2615, and 2574 cm^{–1}, which are in the range of agostic C–H stretching frequencies,^[1a] these are more likely combination bands.
- [12] Crystal structure analysis of **1**·toluene: C₄₀H₃₈Cl₂P₂Ru·C₇H₈; *M*_r = 844.80, triclinic, space group *P*1̄, *a* = 1060.63(7), *b* = 1279.63(10), *c* = 1498.12(10) pm, α = 90.202(8), β = 89.943(8), γ = 96.509(9)°, *V* = 2020.2(2) × 10⁶ pm³; *Z* = 2; ρ_{calcd} = 1.389 g cm^{–3}, *F*₀₀₀ = 872, μ = 6.3 cm^{–1}. Suitable crystals were obtained by cooling a concentrated solution of **1** in toluene. Preliminary examination and data collection were carried out on a STOE Imaging Plate Diffraction System equipped with a rotating anode (NONIUS FR591) and graphite-monochromated MoK_α radiation. Data were collected at 193 K within the θ range 2.65° < θ < 27.75°. A total of 33 597 reflections were collected. After merging (*R*_{int} = 0.0263), 8706 independent reflections remained and were used to refine 653 parameters. Data were corrected for Lorentz and polarization effects. The structure was solved by a combination of direct methods and difference-Fourier syntheses. All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were located by difference Fourier synthesis and refined with individual isotropic displacement parameters. Full-matrix least-squares refinements were carried out by minimizing Σw(*F*_o² – *F*_c²) (SHELXL-93) and converged (all data) with *R*1 = 0.0235, *wR*2 = 0.0569, GOF = 1.063, and shift/error < 0.002. Neutral atom scattering factors for all atoms and anomalous dispersion corrections for the non-hydrogen atoms were taken from *International Tables for X-Ray Crystallography*.^[17] All calculations were performed on a DEC 3000 AXP workstation with the STRUX-V^[18] system, including the programs PLATON-92, PLUTON-92,^[19] SIR-92,^[20] and SHELXL-93.^[21] 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-111368. 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 Fe^{III}/Oxo Cubane Contained in an Octanuclear Complex of *T* Symmetry That Is Stable Over Five Oxidation States**

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Thermodynamic stability is a desired property of materials prepared for commercial applications, but, at the same time, chemical versatility is required for the manifestation of interesting properties such as catalytic activity. A reconciliation of these opposite characteristics is typically achieved by the coating of large surfaces or particles with inert substances on a macroscopic and microscopic scale. Nature applies similar techniques at the macromolecular level when metal active centers are protected inside the organic part of metalloproteins. We propose that a molecular-level analogue of this principle, placing redox-active metal clusters in a chemically inert shell, will result in ideal building blocks for the construction of durable supercluster assemblies with electron transfer properties.

Pyrazolates are convenient bridging ligands for the synthesis of polynuclear complexes in which, owing to the aromatic character of the ligand, the chemical activity is restricted to the metal centers.^[1] Here we present the characterization and initial studies of [Fe₈(μ⁴-O)₄(μ-pz)₁₂Cl₄] (**1**: pz = pyrazolate anion, C₃H₃N₂⁻), a Fe^{III} complex of *T* symmetry that is assembled in a one-pot reaction from simple starting materials and is stable over five oxidation states. Complex **1** contains a redox-active Fe₄O₄ core protected inside a Fe–pyrazolate coat.

The X-ray crystallographic characterization of **1** showed the whole complex and one-half of an interstitial hexane molecule per asymmetric unit. The core of this octanuclear complex consists of a Fe₄O₄ cubane with distorted octahedral *fac*-O₃N₃-Fe units. Four trigonal-bipyramidal ON₃Cl-Fe units are coordinated around the core. Each of the four outer Fe atoms is connected to three of the inner Fe atoms through μ-pz bridges, and each inner Fe atom is likewise connected to three outer Fe atoms (Figure 1). To connect the equatorial positions of each outer Fe atom to the *fac* sites of three inner Fe atoms, the twelve pyrazolate bridges adopt a propellerlike rotation in the same direction around each of the four C₃ axes of **1**.

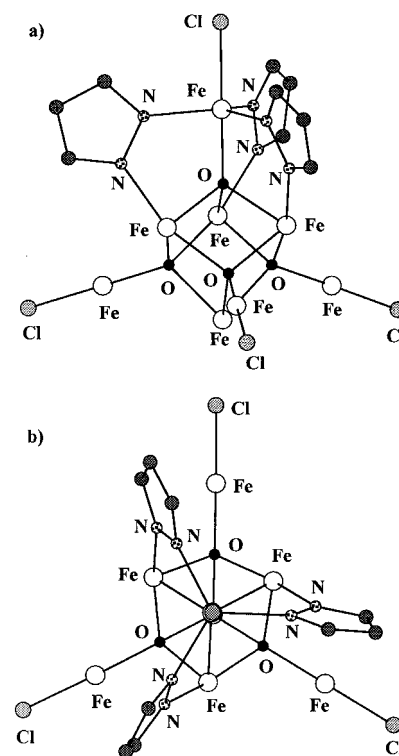


Figure 1. Crystal structure of **1**. Shown is the Fe₈O₄Cl₄ moiety plus three of the twelve pyrazolate groups. Selected bond lengths [Å] and angles [°]: for the Fe₄O₄ cube: Fe–O 2.022(4)–2.056(4), Fe⋯Fe 3.059(1)–3.088(1), Fe–N 2.048(5)–2.070(5); Fe–O–Fe 97.1(2)–98.7(2), O–Fe–O 81.1(2)–82.4(1); for the outer Fe atoms: Fe–O 1.944(4)–1.963(4), Fe–Cl 2.267(2)–2.276(2), Fe–N 2.007(5)–2.025(5); N–Fe–N 114.2(2)–124.6(2), Fe–N–N 119.1(4)–121.6(4). a) Side view, b) view down a C₃ axis.

The eight Fe^{III} atoms are located on C₃ axes at positions defining two concentric tetrahedra with average Fe⋯Fe edges of 3.074(2) and 5.853(4) Å. The μ⁴-O atoms, which connect the network of eight Fe atoms, are expected to be efficient mediators of antiferromagnetic coupling and should account for the relatively low effective magnetic moment of 6.52 μ_B of **1**.^[2] Within each (μ⁴-O)Fe₄ group the O atoms are displaced from the centers towards the bases of the Fe₄ trigonal pyramids. Similar, but asymmetric, (μ⁴-O)Fe₄ units have been found in the structures of a hexanuclear Fe^{III}–oxo/alkoxo aggregate and a tetranuclear Fe^{III}–formamidinato complex.^[3, 4] The Fe₈O₄ part of **1** resembles the core of the [Fe₁₆MO₁₀(OH)₁₀(OCOPh)₂₀] complexes (M = Mn, Co), which contain (μ⁴-O)₃(OH)Fe₃M cubanes.^[5] A mixed-valent [Co₈O₄(O₂CPh)₁₂(solvent)₄] complex as well as a heterometallic [V₄Zn₄O₄(O₂CPh)₁₂(thf)₄] complex bear striking structural resemblance to **1**; their metal and oxygen atoms are arranged as in **1**, but they have carboxylate in place of pyrazolate groups and solvent molecules in place of chlorides.^[6, 7] The symmetry of the former is lowered by the coordination of two different solvent molecules to the outer Co centers, while the coincidence of the μ-O₂CPh groups of the latter with the planes of symmetry of the M₈O₄ tetrahedron classifies this complex in the T_d group.

While the Fe₈O₄Cl₄ skeleton of **1** is in a tetrahedral arrangement, the propellerlike rotation of the μ-pz groups eliminates the mirror planes of T_d symmetry, reducing the

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overall symmetry of **1** to that of the T point group. A consequence of this symmetry is that **1** occurs in two enantiomeric forms, that cocrystallize here as a racemic mixture, the interconversion of which requires the rearrangement of all twelve pyrazolato bridges by simultaneous rotation about the four C_3 axes of **1**. The arrangement of twelve pyrazole rings in the outer part of **1** gives this molecule an approximate appearance of a sphere with a diameter of about 12 Å and provides a hydrophobic surface that is responsible for the high solubility of **1** in nonpolar solvents (Figure 2). A related $(\mu^4\text{-O})\text{M}_4$ core supported by pyrazolato bridges has been described in the structure of $[\text{Co}_4\text{O}(3,5\text{-Me}_2\text{pz})_6]$.^[8]

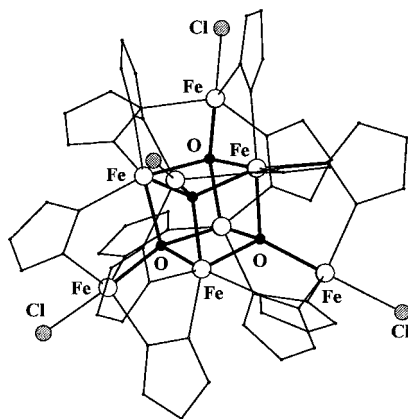


Figure 2. Representation of the structure of **1** in which the Fe–O bonds are emphasized. The Fe, O, and Cl atoms are shown as spheres of arbitrary radii, and the pyrazolato rings as a wireframe. Hydrogen atoms are not shown.

The Fe_4O_4 core of **1** is the first example of an all- Fe^{III} cubane. Consistent with the +3 valence, the Fe–O bonds of **1** (av 2.040(4) Å) are shorter than those of lower valent analogues. Some Fe/O cubanes containing Fe^{II} or mixed $\text{Fe}^{\text{II/III}}$ cores are known; they contain four- and six-coordinate Fe centers in FeO_4 , FeO_4N_2 , or FeO_6 environments and $(\text{Fe}_4)^{8+}$, $(\text{Fe}_4)^{9+}$, or $(\text{Fe}_4)^{10+}$ oxidation states.^[9–11] The existence of three examples of Fe_4^{II} , one each of $\text{Fe}_3^{\text{II}}\text{Fe}^{\text{III}}$ and $\text{Fe}_2^{\text{II}}\text{Fe}_2^{\text{III}}$ and now the first example of a Fe_4^{III} cubane, suggests that the remaining permutation, $\text{Fe}^{\text{II}}\text{Fe}_3^{\text{III}}$, should be stable with the appropriate set of peripheral ligands. Indeed, all of the above species can be readily generated by stepwise electrochemical reduction of **1**.

The electrochemical study of **1** in the range of –2.60 to +1.60 V shows three reversible reduction processes at –0.43, –0.78, and –1.07 V (versus Fc/Fc^+ ; Fc = ferrocene). A fourth reduction at –1.38 V is irreversible at 285 K, but becomes quasi-reversible in chilled solution (Figure 3). As no oxidation is observed, it is evident that **1** retains its structural integrity, in its neutral or some anionic form, over the 4.20-V window studied here. The complete electrochemical reversibility of the first three reductions, at ambient or low temperature, indicates that no significant structural rearrangement accompanies those electron transfer processes. The unusual stability of **1** over five oxidation states can be accounted for by the encapsulation of the Fe_4O_4 core inside the outer shell of four

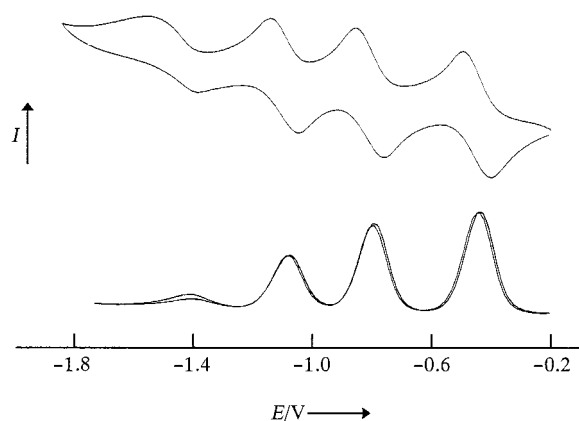


Figure 3. Voltammetry of **1** (in 0.1 M $\text{Bu}_4\text{NPF}_6/\text{PrCN}$, scan rate 100 mV s^{-1} , $T = 223$ K, Pt-disk working electrode, measured versus Fc/Fc^+). Top: Cyclic voltammogram, bottom: cyclic alternating current scan.

interlocked $\text{Fe}(\mu\text{-pz})_3\text{Cl}$ groups, forcing its structural integrity in a fashion similar to the wrapping of apoferritin around the Fe/O cluster of ferritin.^[12] In contrast to the abundant Fe_4S_4 systems,^[13] the corresponding Fe_4O_4 cubane units have not been encountered in nature to date. However, the spontaneous assembly from mononuclear precursors, as well as the stability and rich electrochemistry of **1**, anticipate the discovery of an electron transfer protein based on a Fe_4O_4 active center.

The four tetrahedrally arranged chlorine atoms at the outer shell of **1** are readily substituted by anionic or neutral ligands in simple metathesis reactions. This provides a convenient means by which to manipulate the size and solubility of the octanuclear cluster, as well as to connect octanuclear units through bridging ligands into covalent supramolecular assemblies.^[14] Preliminary studies show that such derivatives of **1** retain the redox characteristics of their parent compound with minimal variations of the $E_{1/2}$ values, pointing to the Fe_4O_4 core as the site of the redox activity.

Experimental Section

1: To anhydrous FeCl_3 (0.180 g, 1.11 mmol) in CH_2Cl_2 (15 mL) is added 3,5- Me_2pzH (0.375 g, 3.90 mmol) under N_2 followed by Kpz (0.355 g, 3.35 mmol). The reaction mixture is exposed to air for several days, and dark red air-stable **1** is precipitated by addition of hexane after reduction of the solvent volume to about 5 mL (yield > 30 %). M.p. 565 K (determined by differential thermal analysis); elemental analysis found (calcd for $1 \cdot 0.5\text{C}_6\text{H}_{14}$): C 31.14 (31.19), H 2.81 (2.87), N 22.59 (22.39), Cl 9.62 (9.45), Fe 29.43 (29.76); UV/Vis/NIR (CH_2Cl_2): $\lambda_{\text{max}} = 359$ nm; IR (KBr disk): $\tilde{\nu} = 1490$ (m), 1417 (m), 1362 (s), 1268 (s), 1169 (s), 1145 (m), 1078 (w), 1045 (vs), 963 (w), 915 (w), 894 (w), 763 (s), 615 (m), 555 (m), 476 cm^{-1} (s); IR (polyethylene disk): $\tilde{\nu} = 349$ (s), 331 (s), 308 cm^{-1} (s). The strong peak at 476 cm^{-1} is tentatively assigned to a Fe–O stretch. Magnetic moment: $\mu_{\text{eff}} = 6.52 \mu_{\text{B}}$, calculated from a Faraday balance susceptibility measurement at 290 K; FAB-MS (m/z): 1457.6 $[\text{M}]^+$, 1420.6 $[\text{M} - \text{Cl}]^+$, 1388.5 $[\text{M} - \text{pz}]^+$, 1353.6 $[\text{M} - (\text{Cl,pz})]^+$, 1321.5 $[\text{M} - 2\text{pz}]^+$, 1286.5 $[\text{M} - (2\text{pz,Cl})]^+$. Red, parallelepiped crystals appropriate for X-ray diffraction study grow from a solution of **1** in $\text{CH}_2\text{Cl}_2/\text{hexane}$.

X-ray crystal structure analysis of **1**: Rigaku-AFC6S diffractometer, $\lambda(\text{MoK}\alpha) = 0.71069$ Å; space group $P\bar{1}$ (no. 2), $a = 12.367(5)$, $b = 12.508(5)$, $c = 20.794(4)$ Å, $\alpha = 77.45(3)$, $\beta = 80.80(3)$, $\gamma = 70.27(3)^\circ$, $V = 2942(2)$ Å³, $Z = 2$, $\rho_{\text{calcd}} = 1.694$ g cm^{-3} , $\mu = 21.53$ cm^{-1} ; of 8873 unique reflections, 5239 were observed ($I > 3\sigma(I)$), $\omega - 2\theta$ scans, $6 < 2\theta < 50^\circ$, $T = 295$ K. Structure solution: data corrected for Lorentz and polarization effects; an empirical

absorption correction based on azimuthal scans was applied. The structure was solved by direct methods (SHELXS-86) followed by Fourier cycles, $R = 0.036$, $R_w = 0.027$ for 712 variable parameters, $GOF = 1.73$. 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-101766. 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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Organic Nitrates of Isoprene as Atmospheric Trace Compounds**

Gerhard Werner, Jürgen Kastler, Ralf Looser, and Karlheinz Ballschmiter*

Isoprene (2-methyl-1,3-butadiene) is a terpene building block which is emitted preferentially by oaks, but also by other plants, as well as algae.^[1] As such, it was examined early on with respect to its broad reaction potential as a butadiene derivative in connection with natural smog formation in the atmosphere.^[2] It is by far the single compound biologically emitted in the largest amounts. On a global average, the annual emission of isoprene is an estimated 450 Tg (1 Tg = 1×10^6 tons).^[3] After the addition of an OH radical to an isoprene double bond, follow-up reactions with oxygen in the atmosphere lead to the formation of methacrolein ($\text{H}_2\text{C}=\text{C}(\text{CH}_3)\text{CHO}$) and methyl vinyl ketone ($\text{H}_2\text{C}=\text{CHCOCH}_3$).^[4] Both are also formed in the reaction of isoprene with ozone^[5] and were detected in the ambient air; their concentration in the atmosphere shows strong seasonal fluctuations with a maximum in the summer.^[4, 6]

According to smog chamber experiments, further products of the reaction of isoprene in the OH/O₂/NO system are 3-methylfuran, hydroxymethyl vinyl ketone, C5-carbonyl compounds, and unspecified organic nitrates.^[7] 3-Methylfuran could be detected in forest air.^[8] The identification of a peroxyacetyl nitrate analogue of isoprene was the entry of the chemistry of isoprene into the group of “odd nitrogen compounds” (NO_x).^[9] Smog chamber experiments on the reaction of isoprene with NO₃ radicals in the dark, simulating night chemistry, led to the known products methacrolein, methyl vinyl ketone, and C5-hydroxycarbonyl compounds as well as C5-aldonitrates such as O₂NOCH₂C(CH₃)=CHCHO (**13**, **14**), C5-hydroxynitrates such as O₂NOCH₂C(CH₃)=CHCH₂OH (**5**, **6**), and C5-hydroperoxynitrates such as O₂NOCH₂C(CH₃)=CHCH₂OOH, as shown by atmospheric pressure ionization tandem mass spectrometry (API-MS/MS).^[10, 11] This range of products indicates the possibilities of the night chemistry of isoprene in NO₂- and ozone-rich air, in which NO₃ radicals are formed. Such an atmospheric situation usually builds up from traffic emissions when there is a high amount of sunshine during the day (areas of high pressure in temperate zones such as Europe; normal weather conditions in California).

If one considers the reactions that a compound can in principle undergo in the atmosphere, and if the corresponding

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possible reaction products are available as reference substances, the analytical parameters of the compound can be examined and optimized substance specifically for effective enrichment, separation and detection. This strategy allowed us to detect long-chain alkyl nitrates and a broad spectrum of dinitrates and hydroxynitrates of short-chain hydrocarbons in continental and marine air down to the lower ng m^{-3} range for the first time.^[12] Table 1 summarizes the possible isomers of the dinitrates, hydroxynitrates, and aldo/ketonitrates of isoprene retaining one of the double bonds. We obtained the following compounds in microsyntheses: **2–7**, **11–14**, **16–18**. The corresponding synthetic routes, starting out from epoxides and bromo and hydroxy derivatives of isoprene, are based on the respective literature procedures.^[13] Because of the variety of possible mono- and multifunctional organonitrates, Schneider and Ballschmiter^[14] developed a systematic nomenclature in short style based on the parent hydrocarbon which deviates from the IUPAC nomenclature. This is to be found in the penultimate column of Table 1. It is also used in the captions of the chromatograms in Figure 1.

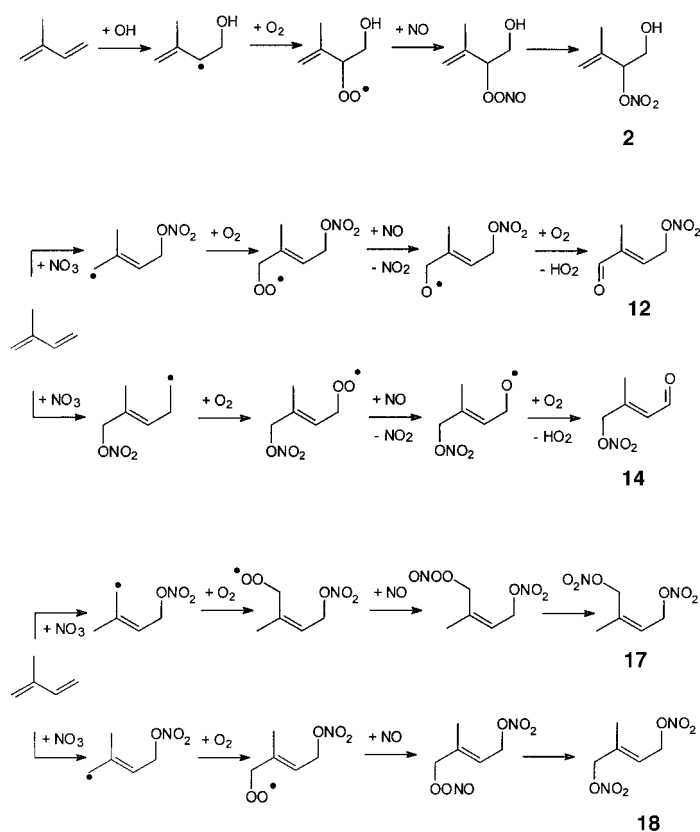
We report on the structure-specific synthesis of organic nitrates of isoprene, on the stability of these compounds under the conditions of a high-volume adsorptive sample collection on silica gel, and on a determination in the ng m^{-3} range by means of high-resolution gas chromatography/negative chemical ionization-mass spectrometry (HRGC/NCI-MS), which is preceded by a group-selective pre separation by means of normal-phase HPLC.^[15] With this method, the following five organic nitrates of isoprene retaining one of the double bonds were detected in ambient air for the first time: *cis*- and *trans*-2-methyl-2-butene-1,4-dinitrate (**17** and **18**, respectively), 3-methyl-2-nitrooxy-3-butene-1-ol (**2**), *trans*-2-methyl-4-nitrooxy-2-butenal (**12**), and *trans*-3-methyl-4-nitrooxy-2-butenal (**14**).^[16] According to a first quantitative estimate, the concentrations of the identified compounds lie in the lower ppt(v) range at a few ng m^{-3} . Thus, besides the two dinitrates **17** and **18**, the C5-hydroxynitrates ($\text{H}_2\text{C}=\text{C}(\text{CH}_3)\text{CH}(\text{ONO}_2)\text{CH}_2\text{OH}$) and C5-aldonitrates ($\text{O}_2\text{NOCH}_2\text{CH}=\text{C}(\text{CH}_3)\text{CHO}$ and $\text{O}_2\text{NOCH}_2\text{C}(\text{CH}_3)=\text{CHCHO}$) of isoprene,^[10, 11] so far described only in chamber experiments, have now also been detected in the ambient air for the first time with compounds **2**, **12**, and **14**.

The method of formation of the described organic nitrates of isoprene can be formulated as shown in Scheme 1. The proposed mechanisms for isoprene^[11, 17] as well as those already

Table 1. Hydroxynitrates, aldo/ketonitrates, and dinitrates of isoprene which can be formed under retention of one of the double bonds.^[a]

Entry	IUPAC nomenclature	Our nomenclature	Structure
1	3-methyl-1-nitrooxy-3-buten-2-ol 1	2M1en3OH4C4	
2	3-methyl-2-nitrooxy-3-buten-1-ol 2	2M1en4OH3C4	
3	<i>cis</i> -2-methyl-4-nitrooxy-2-buten-1-ol 3	c-2M2en1OH4C4	
4	<i>trans</i> -2-methyl-4-nitrooxy-2-buten-1-ol 4	t-2M2en1OH4C4	
5	<i>cis</i> -3-methyl-4-nitrooxy-2-buten-1-ol 5	c-2M2en4OH1C4	
6	<i>trans</i> -3-methyl-4-nitrooxy-2-buten-1-ol 6	t-2M2en4OH1C4	
7	2-methyl-2-nitrooxy-3-buten-1-ol 7	2M3en1OH2C4	
8	2-methyl-1-nitrooxy-3-buten-2-ol 8	2M3en2OH1C4	
9	3-methyl-1-nitrooxy-3-buten-2-on 9	2M1en3O4C4	
10	3-methyl-2-nitrooxy-3-butenal 10	2M1en4O3C4	
11	<i>cis</i> -2-methyl-4-nitrooxy-2-butenal 11	c-2M2en1O4C4	
12	<i>trans</i> -2-methyl-4-nitrooxy-2-butenal 12	t-2M2en1O4C4	
13	<i>cis</i> -3-methyl-4-nitrooxy-2-butenal 13	c-2M2en4O1C4	
14	<i>trans</i> -3-methyl-4-nitrooxy-2-butenal 14	t-2M2en4O1C4	
15	2-methyl-2-nitrooxy-3-butenal 15	2M3en1O2C4	
16	3-methyl-3-butene-1,2-dinitrate 16	2M1en3,4C4	
17	<i>cis</i> -2-methyl-2-butene-1,4-dinitrate 17	c-2M2en1,4C4	
18	<i>trans</i> -2-methyl-2-butene-1,4-dinitrate 18	t-2M2en1,4C4	
19	2-methyl-3-butene-1,2-dinitrate 19	2M3en1,2C4	

[a] Compounds **2–7**, **11–14**, and **16–18** were synthesized in the course of this work.



Scheme 1. Atmospheric decomposition of isoprene in NO_x -containing air, initiated by OH or NO_3 radicals, leading to the formation of hydroxynitrates, dinitrates, and aldo/ketonitrates.

proved for aliphatic hydrocarbons are shown.^[18] The subsequent behavior of these compounds is characterized by an addition of OH or NO_3 radicals to the double bond with the corresponding follow-up reactions. In the daytime, photolytic decay is also possible. As NO_2 and the corresponding alkoxy radical are generated in the course of this decay, the formation of the direct precursors of **12** and **14** by degradation of **18** is feasible. However, it has been shown that for unsaturated nitrates photolysis is of lesser importance than degradation by OH radicals.^[19]

The investigations into the optimization of the analytical procedure showed that **12** slowly decomposes on silica gel, even when the sample is cooled. Likewise, the reference compounds *cis*- and *trans*-2-methyl-4-nitrooxy-2-buten-1-ol (**3** and **4**, respectively) are not sufficiently stable for the described workup. Their occurrence in ambient air can therefore neither be proved nor excluded at present. We are working on an optimization of the method for these compounds and at the same time attempting to increase the frequency of sample collection. The air samples we checked so far for the presence of organonitrate derivatives of isoprene were all collected on the campus of the University of Ulm, which is located at an elevation of 610 m above sea level at the southern decline of the Swabian Mountains above the city of Ulm in a wooded area mainly populated by oak trees. Around the university buildings a large area is designated as parking space. The air samples were collected in this area.

The conditions of the high-volume sample collection (50–100 m^3) on ultrapure silica gel have already been described in detail.^[20] The desorption of the polar organic nitrates was done with 300 mL of pentane/acetone (4:1 w/w). The workup of the extracts by means of an NP-HPLC group separation with a nitrate phase^[15] already results in an extensive separation according to the functional groups present. The subsequent separation of the LC fractions by high-resolution capillary gas chromatography with mass-selective detection of the respective isoprene nitrates by indicator fragments (Table 2) after negative chemical ionization mass spectrometry (NCI-MS) takes up previously described analytical

Table 2. The respective six highest (methane) NCI-MS signals (m/z) of five isoprene nitrates identified in air samples.^[a]

Entry	Isoprene nitrate	m/z [amu] (rel. signal height [%])
1	2	101 (100), 46 (30), 99 (9), 77 (7), 102 (6), 71 (4)
2	12	98 (100), 62 (88), 99 (42), 46 (12), 69 (7), 97 (6)
3	14	62 (100), 98 (77), 99 (43), 46 (13), 69 (7), 97 (6)
4	17	62 (100), 46 (18), 98 (5), 99 (4), 101 (2), 97(1)
5	18	62 (100), 46 (22), 98 (7), 99 (7), 81(2), 71(2)

[a] The values in parentheses state the relative heights of the signal as a percentage relative to the height of the most intense signal.

procedures.^[15] The retention behavior of the target compounds was determined with the corresponding reference substances by means of high-resolution capillary gas chromatography. Identical retention times on two phases of different polarity and the respective substance-specific NCI-MS signal were rated as positive proof of the occurrence in the sample of ambient air. The result of the analysis of a sample of 50 m^3 of air that was collected on May 28, 1998 between 3:00am and 7:00am on the university campus is shown as an example in Figure 1. Six HRGC/(methane)-NCI-MS chromatograms of the NP-HPLC fraction containing the aldo/ketonitrates as well as the hydroxynitrates are shown.^[16] The identification of the dinitrates was carried out in analogy. The individual capillary gas chromatograms were generated by detection at different mass traces which are characteristic for the molecules (selected ion monitoring, SIM). Only by means of this selective mass spectrometric detection which eliminates the influence of interfering components did the identification of organonitrate derivatives of isoprene in the atmosphere become possible, to our knowledge for the first time. It is our aim to quantify the identified compounds and to become able to analyze the so far elusive isoprene nitrates in air samples by modifying the described procedure.

Experimental Section

Figure 1 shows gas chromatograms of an air sample which were obtained with an MN-Optima-1701 capillary column (Macherey-Nagel, Düren, Germany; length 50 m, inner diameter 0.32 mm, film thickness 0.21 μm , carrier gas helium 4.6). After the on-column injection on an HP-5890 gas chromatograph (Hewlett Packard, Palo Alto, USA), the separation took place in the temperature-programmed mode (40 °C (5 min), 3 °C min^{-1} , 160 °C (30 min)). NCI-MS in the selected-ion mode (reactant gas methane, $10^{-3.8}$ mbar) on a VG Micromass TS 250 mass spectrometer (VG Tritech,

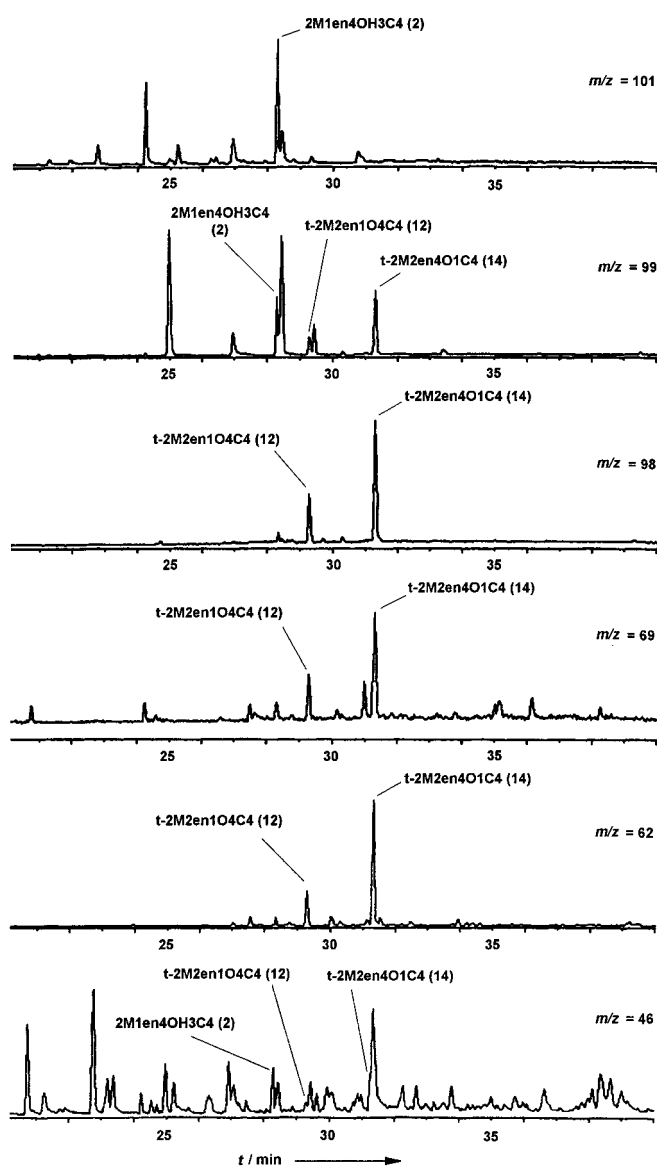


Figure 1. HRGC/(methane)-NCI-MS chromatograms of an air sample (hydroxy- and aldo/ketonitrates fractions, respectively, of the HPLC group separation), recorded in the SIM mode for six different m/z ratios. Two aldonitrates (12, 14) and one hydroxynitrate (2) were identified.

Manchester, Great Britain) was used for detection (ionization energy 50 eV, source temperature 100 °C).

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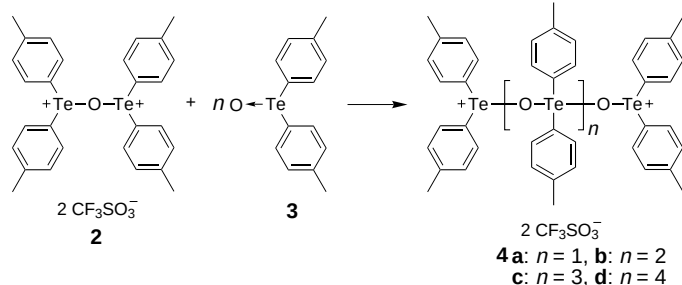
Keywords: atmospheric chemistry • gas chromatography • isoprene • mass spectrometry • organic nitrates

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Nucleophilic Addition of Telluroxides to a Cationic Ditelluroxane: Oligotelluroxanes**

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Linear polymers with oxo bridges between main group elements or transition metals, such as siloxanes and titanoxanes, have attracted considerable attention with regard to materials science.^[1] Polytelluroxanes $\text{XPh}_2\text{Te}[\text{OTePh}_2]_n\text{O}-\text{TePh}_2\text{X}$ (**1**), in which tellurium units are linked by oxo bridges and possess a hypervalent bonding character,^[2–4] have not been extensively explored, because a general method for their synthesis was not available. Ditelluroxanes **1** ($n=0$) were synthesized by the thermal dehydration of diaryltellurium hydroxide halides and related compounds $\text{Ar}_2\text{Te}(\text{OH})\text{X}$ ($\text{X}=\text{Cl}, \text{Br}, \text{NSC}, \text{etc.}$).^[5] Domasevitch et al. reported the synthesis of the tritelluroxane **1** ($n=1$, $\text{X}=\text{nitrosocarbamylcyanomethanide}$) by the dehydration procedure.^[6, 7] In the recently reported μ -oxo-bis[bis(4-methylphenyl)tellurium(IV)] bis(trifluoromethanesulfonate) (**2**), the tellurium atoms exhibit cationic character in solution.^[8] These tellurium cations can react with nucleophiles, for instance, with bis(4-methylphenyl) telluroxide (**3**), to produce oligotelluroxanes **4**. Because the terminal Te atoms in the polytelluroxanes carry a positive charge, further addition of **3** can lengthen the chain of **4**. Here we report the synthesis and characterization of the oligotelluroxanes **4** ($n=1-4$) from the ditelluroxane **2** and the telluroxide **3** (Scheme 1).



Scheme 1. Synthesis of **4** from **2** and n equivalents of **3**.

The reaction of the ditelluroxane **2** with n equivalents of the telluroxide **3** in dry CH_2Cl_2 at room temperature afforded the oligotelluroxanes **4** (>95% yield) as a white powder after

precipitation with n -hexane. Representative results for the formation of the oligotelluroxanes are listed in Table 1. The tritelluroxane **4a**, the tetratelluroxane **4b**, and the pentatelluroxane **4c** were the only products when stoichiometric

Table 1. Oligotelluroxanes **4a–d** from the ditelluroxane **2** and the telluroxide **3**.

3 [equiv]	Time [h]	Product ^[a] (ratio) ^[b]	Yield [%]
1	12	4a	> 95
2	12	4b	> 95
3	12	4c	> 95
4	72	4d + 4c (1:2.5)	> 95

[a] **4a** = tritelluroxane, **4b** = tetratelluroxane, **4c** = pentatelluroxane, **4d** = hexatelluroxane. [b] Determined by integration of ^{125}Te NMR spectrum of the reaction mixture.

amounts of **2** and **3** reacted at room temperature in CH_2Cl_2 . Four equivalents of **3** and one equivalent of **2** in CH_2Cl_2 for 72 h at room temperature gave a mixture of the penta- and the hexatelluroxane **4d** in the ratio of 2.5:1. The oligotelluroxanes **4** can be converted back to the starting material **3** quantitatively by treatment with aqueous NaOH (2M) in CH_3CN at room temperature.

The ^1H NMR spectra of **4a–d** in CD_3CN or $[\text{D}_8]\text{THF}$ at room temperature exhibit one set of AA'BB' signals for the aromatic protons, whereas two well-resolved sets of AA'BB' signals are present for **4a** at -40°C and for **4b** at -90°C . For **4c** and **4d**, two sets of multiplets are observed at -90°C . Well-resolved sharp peaks in the ^{125}Te NMR spectra appear at -40°C for **4a** and at -90°C for **4b–d**.^[9]

The ^{125}Te NMR chemical shifts and integration ratios of **4a–d** are summarized in Table 2. The signals of the terminal telluronium groups and the inner tellurane units in the

Table 2. ^{125}Te NMR chemical shifts and integration ratios of **4a–d**.^[a]

Telluroxane	δ	Ratio
2 ^[8]	1288.4	
4a	1311.9, 1124.7	2:1
4b	1263.4, 1063.4	2:2
4c	1242.2, 1087.0, 992.9	2:2:1
4d	1224.2, 1096.2, 990.1	2:2:2

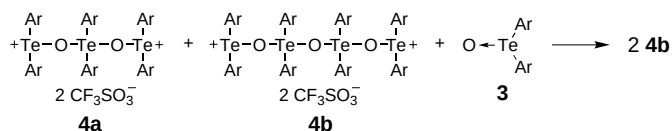
[a] Measured in CD_3CN at -40°C for **2** and **4a** and in $[\text{D}_8]\text{THF}$ at -90°C for **4b–d**.

backbone of **4** appear in the downfield region of $\delta = 1312-1224$ ^[8] and in the upfield region of $\delta 1125-990$,^[10] respectively. Chemical shifts of both the telluronium and tellurane moieties are shifted upfield with increasing degree of oligomerization. This distinctive change in chemical shift strongly suggests that the cationic character of the terminal telluronium moieties in **4** becomes less pronounced with increasing degree of oligomerization. Therefore, the reactivity of **4** with respect to **3** decreases in the order **4a** > **4b** > **4c** > **4d**. The competitive reaction of a 1:1 mixture of the tritelluroxane **4a** and the tetratelluroxane **4b** with one equivalent of **3** afforded **4b** as the sole product (Scheme 2). Thus, the degree of oligomerization can be controlled by means of the ratio of **3** to **2**.

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Scheme 2. Competitive reaction of a 1:1 mixture of **4a** and **4b** with one equivalent of **3**.

The molecular and electronic structure of oligotelluroxanes was evaluated for model compounds **5a–d**^[11] by B3LYP DFT calculations.^[12, 13] The geometries of **5a–d** optimized in C_{2v} symmetry with a zigzag skeletal structure (Figure 1).^[14] The

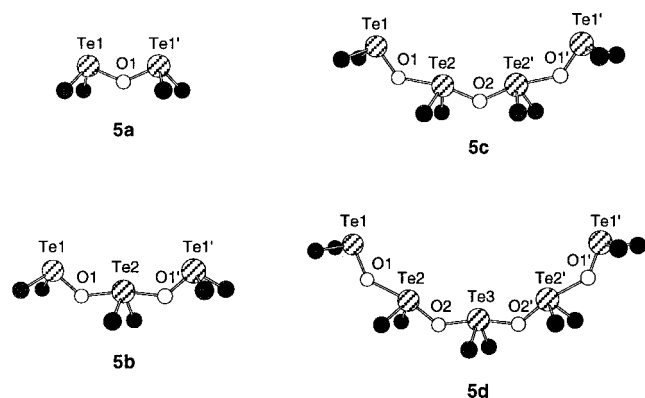


Figure 1. Optimized C_{2v} structures of oligotelluroxanes **5a–d**; hydrogen atoms omitted for clarity. Selected bond lengths [Å] and angles [°]: **5a**: Te1–O1 1.964; Te1–O1–Te1' 133.0. **5b**: Te1–O1 1.895, O1–Te2 2.133; Te1–O1–Te2 134.3, O1–Te2–O1' 168.1. **5c**: Te1–O1 1.868, O1–Te2 2.263, Te2–O2 1.999; Te1–O1–Te2 136.4, O1–Te2–O2 168.0, Te2–O2–Te2' 134.8. **5d**: Te1–O1 1.854, O1–Te2 2.363, Te2–O2 1.943, O2–Te3 2.090; Te1–O1–Te2 138.6, O1–Te2–O2 168.6, Te2–O2–Te3 135.7, O2–Te3–O2' 167.0.

atomic charges of the Te atoms and the LUMO energies are listed in Table 3.^[15] The positive charge on the terminal Te atoms decreases slightly with increasing degree of oligomer-

Table 3. Atomic charges on the Te atoms and the energy of the LUMO in the model compounds **5a–d**.

Telluroxane	Atomic charge			LUMO [eV]
	Te1	Te2	Te3	
5a	+1.860			–11.36
5b	+1.847	+1.861		–8.64
5c	+1.841	+1.873		–7.11
5d	+1.838	+1.878	+1.885	–6.15

ization. In all compounds, the LUMO is delocalized along the Te–O backbone and has antibonding character between adjacent Te and O atoms. This could be a reflection of the fact that the backbone of strongly polarized Te–O bonds is a delocalized σ -electron system. The energy of the LUMO increases in the order **5a** < **5b** < **5c** < **5d**. The calculated energy changes for formation of **5b** from **5a** and dimethyl telluroxide, **5c** from **5b**, and **5d** from **5c** are –74.2, –48.9, and –37.5 kcal mol^{–1}, respectively. These results explain the reactivity trends of the telluroxanes **2** and **4a–d**.

Slow diffusion of *n*-hexane into a solution of a mixture of **4a** and **2** in ethyl acetate gave single crystals of the 1:1 complex of

4a and **2**, the crystal structure of which was determined by X-ray diffraction analysis (Figure 2).^[16] In the **4a** unit, the terminal atoms Te1 and Te3 and the central atom Te2 form a telluronium and a tellurane moiety, respectively. Assembly of **4a** and **2** by interaction of their terminal Te atoms with two

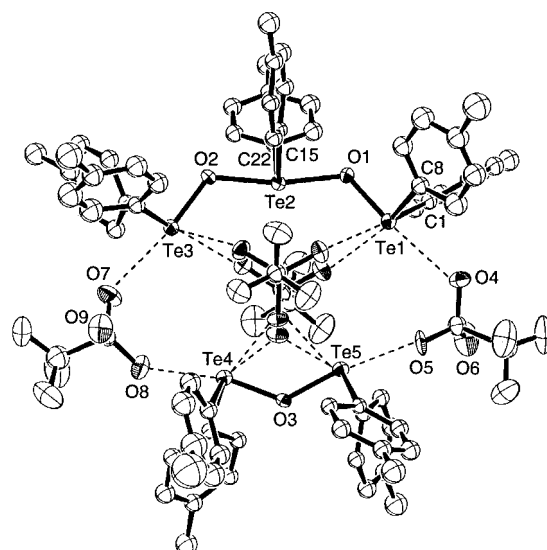


Figure 2. ORTEP plot of the 1:1 complex of **4a** and **2** (15% probability thermal ellipsoids); hydrogen atoms omitted for clarity. Selected bond lengths [Å] and angles [°]: Te1–O1 1.890(8), Te2–O1 2.089(8), Te2–O2 2.116(8), Te3–O2 1.896(8), Te4–O3 1.916(8), Te5–O3 1.964(7), Te1...O4 2.79(1), Te3...O7 2.76(1), Te4...O8 2.55(1), Te5...O5 2.555(9); Te1–O1–Te2 125.3(4), Te2–O2–Te3 122.0(4), Te4–O3–Te5 131.0(4), O1–Te1–C1 93.5(4), O1–Te1–C8 93.3(5), C1–Te1–C8 98.0(5), O1–Te2–O2 167.8(3), O1–Te2–C15 84.8(4), O1–Te2–C22 89.1(4), C15–Te2–C22 97.5(5), O1–Te1...O4 170.7(4), O2–Te3...O7 173.2(4), O3–Te4...O8 166.5(4), O3–Te5...O5 169.2(4).

counterions affords a 14-membered pseudoring with interatomic Te...O distances in the range of 2.55–2.79 Å. All the atoms of this ring are nearly coplanar. The other two counterions are accommodated in the cavity of the macrocycle.

We have demonstrated the selective formation of the cationic oligotelluroxanes **4** by reaction of the ditelluroxane **2** as initiator with the diaryl telluroxide **3** as monomer under mild conditions. Studies on the extension of this reaction to polytelluroxanes and the application of **4** as a building block for molecular assembly^[17] are underway.

Experimental Section

4: To a mixture of **2** (100.0 mg, 0.107 mmol) and *n* equivalents of **3** ($n \times 34.9$ mg, $n \times 0.107$ mmol; $n = 1–4$) under an argon atmosphere was added dry CH_2Cl_2 (2 mL) at room temperature. The resulting solution was stirred for 12 h at room temperature and then poured into *n*-hexane (50 mL) to precipitate the oligotelluroxane **4** (>95% yield) as a white powder.

4a: M.p. 216–218 °C (decomp); ^1H NMR (270 MHz, CD_3CN , –40 °C): δ = 2.33 (s, 6H), 2.34 (s, 12H), 7.14, 7.47 (AA'BB', J = 8.1 Hz, 8H), 7.27, 7.44 (AA'BB', J = 8.6 Hz, 16H); ^{125}Te NMR (85.2 MHz, CD_3CN , –40 °C): δ = 1124.7, 1311.9 (integration ratio 1:2); FAB-MS: m/z (%): 1111 (13) [$M - \text{CF}_3\text{SO}_3$]⁺, 785 (39) [$M - 3 - \text{CF}_3\text{SO}_3$]⁺, 329 (58) [$3 + \text{H}$]⁺, 312 (100) [$3 - \text{O}$]⁺; elemental analysis calcd for $\text{C}_{44}\text{H}_{42}\text{F}_6\text{O}_8\text{S}_2\text{Te}_3 \cdot \text{H}_2\text{O}$ (%): C 41.36, H 3.47; found: C 41.09, H 3.37.

4b: M.p. 98–100 °C (decomp); ^1H NMR (270 MHz, $[\text{D}_8]\text{THF}$, -90°C): δ = 2.30 (s, 12H), 2.32 (s, 12H), 7.18, 7.74 (AA'BB', J = 8.4 Hz, 16H), 7.25, 7.67 (AA'BB', J = 8.4 Hz, 16H); ^{125}Te NMR (85.2 MHz, $[\text{D}_8]\text{THF}$, -90°C): δ = 1063.4, 1263.4 (integration ratio 1:1); FAB-MS: m/z (%): 1437 (2) $[\text{M} - \text{CF}_3\text{SO}_3]^+$, 1111 (36) $[\text{M} - 3 - \text{CF}_3\text{SO}_3]^+$, 785 (64) $[\text{M} - 23 - \text{CF}_3\text{SO}_3]^+$, 329 (71) $[\text{M} - 33 - \text{CF}_3\text{SO}_3]^+$, 312 (100) $[\text{M} - \text{O}]^+$; elemental analysis calcd for $\text{C}_{38}\text{H}_{56}\text{F}_6\text{O}_9\text{S}_2\text{Te}_4 \cdot \text{H}_2\text{O}$ (%): C 43.44, H 3.65; found: C 43.25, H 3.50.

4c: M.p. 94–97 °C (decomp); ^1H NMR (270 MHz, $[\text{D}_8]\text{THF}$, -90°C): δ = 2.24 (s, 12H), 2.28 (s, 12H), 2.32 (s, 6H), 7.05–7.35 (m, 20H), 7.58–7.88 (m, 20H); ^{125}Te NMR (85.2 MHz, $[\text{D}_8]\text{THF}$, -90°C): δ = 992.9, 1087.0, 1242.2 (integration ratio 1:2:2); FAB-MS: m/z (%): 1437 (1.4) $[\text{M} - 3 - \text{CF}_3\text{SO}_3]^+$, 1111 (15) $[\text{M} - 23 - \text{CF}_3\text{SO}_3]^+$, 785 (36) $[\text{M} - 33 - \text{CF}_3\text{SO}_3]^+$, 329 (76) $[\text{M} - 33 - \text{CF}_3\text{SO}_3]^+$, 312 (100) $[\text{M} - \text{O}]^+$; elemental analysis calcd for $\text{C}_{72}\text{H}_{70}\text{F}_6\text{O}_{10}\text{S}_2\text{Te}_5 \cdot \text{H}_2\text{O}$ (%): C 44.82, H 3.76; found: C 44.87, H 3.69.

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Synthesis and Characterization of a Unimolecular Capsule**

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Host molecules that completely surround other molecules make use of either strong covalent bonds as in carcerands^[1] and cryptophanes,^[2] or weak hydrogen bonds as in self-assembling capsules.^[3] The former type offers the kinetic stability needed to isolate reactive intermediates^[4] and restrict molecular motions,^[5] while the latter type shows the dynamic lability useful in recognition^[6] and catalysis.^[7] We describe

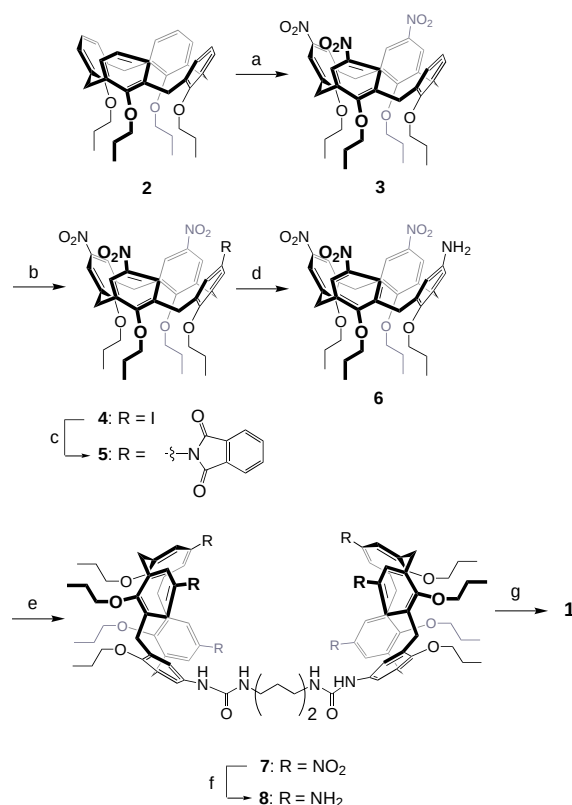
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here a system of intermediate stability^[8] that blends the characteristics of the two.

Calixarenes with urea substituents on their upper rims^[9] dimerize through a cyclic array of complementary hydrogen-bond donors and acceptors ("upper rim" indicates the opening of a calixarene that is not functionalized with hydroxyl groups). The resulting systems feature well-defined cavities that reversibly encapsulate smaller molecules.^[10] Connecting two of these calixarenes through their upper rims (to give, for example, **1**; Figure 1) requires special spacers: They must be long enough to reverse the direction of the divergent bonds of the adjacent urea substituents (arrows in Figure 1, **C1**), but short enough to minimize the loss of entropy due to restriction of freely rotating single bonds. Modeling suggested a hexamethylene spacer. This system allows several types of assembly: 1) intramolecular folding to the capsule **C1**, 2) assembly of dimers **1·1**, and 3) oligomerization to higher order systems **1_n** (Figure 1), which are either cyclic or linear.

The synthesis^[11] of **1** followed well-practiced procedures developed by Reinhoudt et al.^[12] for the selective functionalization of the upper rim of calix[4]arenes (Scheme 1). Nitration of the known tetrapropoxycalix[4]arene **2**^[12a, 13] with 60% HNO₃ and acetic acid in CH₂Cl₂ afforded the trinitrocalixarene **3**^[14] in 92% yield. Treatment of **3** in boiling chloroform with silver trifluoroacetate and iodine provided the monoiodinated species **4**. Copper-mediated aromatic substitution with phthalimide gave **5**,^[15] which was deprotected by hydrazinolysis to the amine **6**. Two molecules of **6** were coupled with 1,6-diisocyanatohexane to form the covalently bound hexanitro dimer **7**^[16] in 33% yield. Reduction of the six nitro functionalities with Raney Ni/H₂ ($\rightarrow$ **8**) followed by treatment with excess (*n*-heptyl)phenylisocyanate in CH₂Cl₂ afforded **1**.^[17]



Scheme 1. Synthesis of **1**: a) 60% aq HNO₃, CH₃CO₂H, CH₂Cl₂, RT, 4 h, 92%; b) AgO₂CCF₃ (2 equiv), I₂ (2 equiv); CHCl₃, 60 °C $\rightarrow$ reflux, 82%; c) phthalimide (2 equiv), Cu₂O (2 equiv), 2,4,6-trimethylpyridine, reflux, 24 h, 45%; d) excess H₂NNH₂·H₂O, toluene/C₂H₅OH, reflux, 65%; e) O=C=N(CH₂)₆N=C=O (0.5 equiv), CH₂Cl₂, 12 h, 33%; f) Raney Ni, H₂, toluene/C₂H₅OH, reflux, 24 h, quant.; g) C₇H₁₅PhN=C=O (12 equiv), CH₂Cl₂, 4 h, 48%.

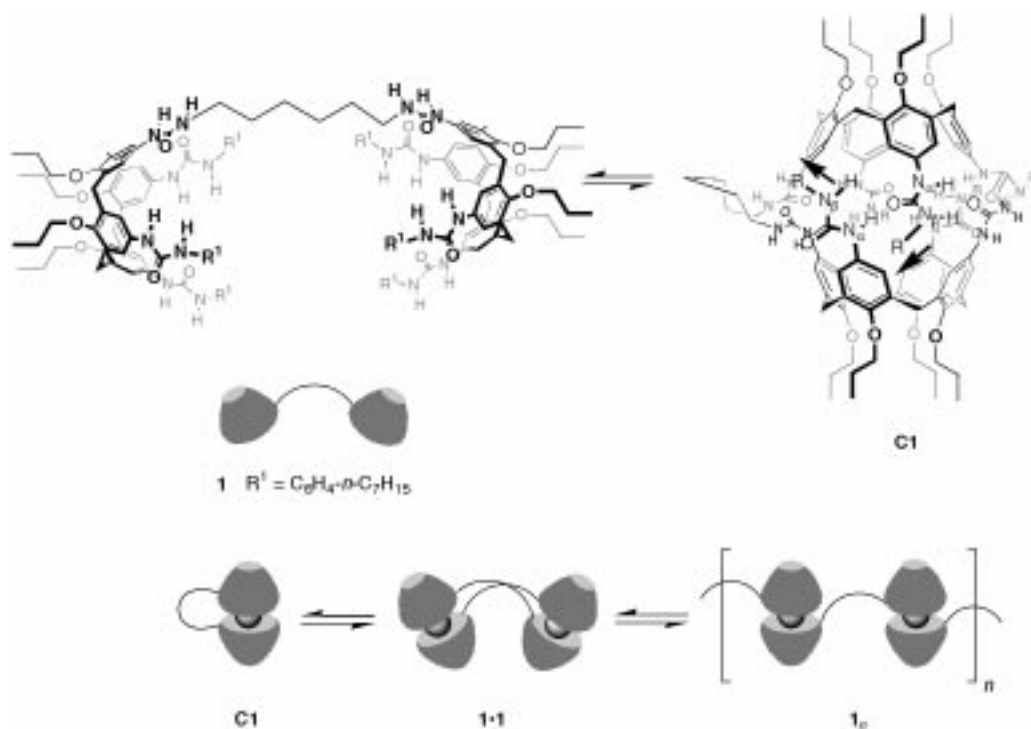


Figure 1. Bridged calixarene dimer **1** and the different options for its self-assembly.

Compound **1** and its complexes were characterized by a combination of ¹H NMR spectroscopy (Figure 2) and electrospray mass spectrometry (ESI-MS, Figure 3). The former method takes advantage of the downfield shifts of host NH resonances that signal hydrogen-bonded assemblies and the upfield shifts of guest resonances characteristic of encapsulation. The latter method takes advantage of ammonium salt guests: They are readily encapsulated and simultaneously provide ion labels for the complexes.^[18] This method has been used to identify related encapsulation complexes,^[19] and to characterize other weakly bound synthetic assemblies^[20] in the gas phase.

The ¹H NMR spectrum of **1** in [D₆]DMSO (Figure 2 a)

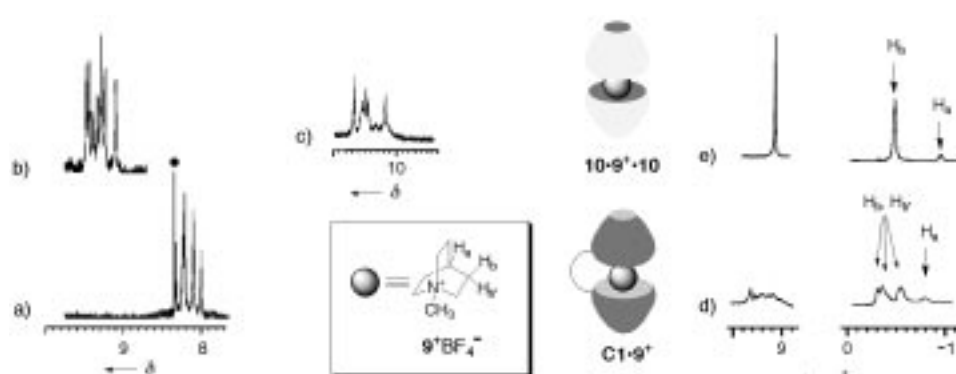


Figure 2. The downfield regions of the ^1H NMR spectra (600 MHz) of **1** a) in $[\text{D}_6]\text{DMSO}$ (an extra peak for remaining CHCl_3 is marked with a dot) and b) in CDCl_3 with a downfield shift of the urea NH signals for the assembly. c) Portion of the spectrum of $\mathbf{11}_2 \cdot \mathbf{1}$ in CDCl_3 . The upfield and downfield regions of the ^1H NMR spectra (600 MHz, CDCl_3) of d) $\mathbf{C1} \cdot \mathbf{9}^+$ with separate signals for H_b and e) $\mathbf{10} \cdot \mathbf{9}^+ \cdot \mathbf{10}$; these spectra show only a signal set of signals for $\mathbf{9}^+$.

shows the signals expected for an “open” system, that is, one in which no intramolecular hydrogen bonds compete with the solvent for the urea hydrogen atoms. The resonances for the urea NH protons adjacent to an aryl group appear as expected between $\delta = 8\text{--}9$ as five peaks in a 1:2:2:1:1 ratio of intensities. The urea hydrogen atom adjacent to the aliphatic tether appears upfield at $\delta = 5.79$. In contrast, in CDCl_3 (Figure 2b), a less competitive solvent, the urea NH signals shift downfield ($\Delta\delta \approx 1$), indicating a molecular assembly held together by a seam of hydrogen bonds. The relative simplicity and sharpness of these downfield signals suggested the formation of a discrete assembly rather than a polymer.^[21] However, the rest of the NMR spectrum is broadened and cannot be clearly assigned to any one of the possible assemblies.

Addition of cationic guests, for example *N*-methyl quinuclidinium ($\mathbf{9}^+$; counterion BF_4^-), to these NMR samples gave further evidence of capsule formation. Unlike neutral guests, the cation competes successfully with the solvent for entrance into the interior of the capsule due to cation– π interactions,^[18] and new signals for the encapsulated ammonium ion of the $\mathbf{1} \cdot \mathbf{9}^+$ complex appear upfield at $\delta = -0.2$ to -0.4 (Figure 2d).

The ESI mass spectrum from a solution of **1** and $\mathbf{9}^+$ in CHCl_3 showed a base peak corresponding to $\mathbf{C1} \cdot \mathbf{9}^+$ (m/z 2902, Figure 3a). Comparison of the measured isotope pattern with those calculated for $\mathbf{C1} \cdot \mathbf{9}^+$ and $\mathbf{1} \cdot (\mathbf{9}^+)_2 \cdot \mathbf{1}$ (inset of Figure 3a) confirms that the ion bears only one charge, and rules out larger assemblies such as $[\mathbf{1} \cdot \mathbf{9}^+]_n$, where $n = 2$. This result was also found for a highly concentrated sample of **1** ($2 \times 10^{-4}\text{ M}$), for which both NMR and ESI mass spectra were obtained. Accordingly, in the concentration range $5 \times 10^{-3}\text{ M}$ to $5 \times 10^{-5}\text{ M}$, intramolecular formation of **C1** is preferred over the formation of intermolecular assemblies.^[22]

It is unlikely that the ions observed for **1** originate from the fragmentation of oligomeric assemblies that may exist in solution. Rather, multiple MS experiments on heterodimer formation, guest selectivity, and collision-induced decomposition, which are described elsewhere,^[19] reinforce the evidence that the capsule's structure in solution is retained in the gas phase.

The ^1H NMR spectrum, conclusively assigned to **C1**, illustrates the complexity generated by adding a simple alkyl bridge. The tether breaks the symmetry of the capsule, and all urea protons adjacent to the heptylphenyl groups are heterotopic.^[23] A total of six downfield signals is expected.^[24] However, the eight distinct signals visible (Figure 2b) declare that the symmetry of the capsule may not be so simple. Molecular modeling suggests the cavity of the capsule may be deformed by the tether, thereby reducing the symmetry. At present there is no strong

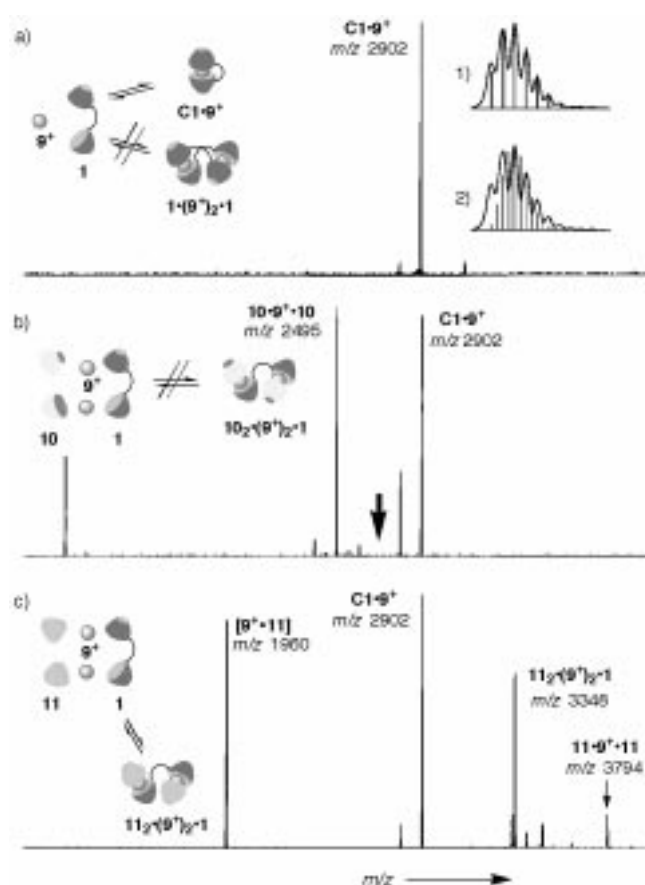
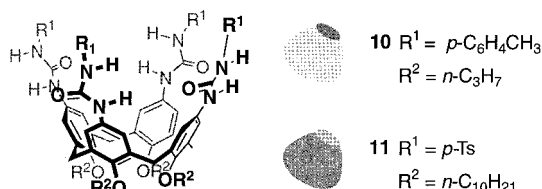


Figure 3. ESI mass spectra of solutions in CHCl_3 of a) **1** ($2.5 \times 10^{-5}\text{ M}$) with $\mathbf{9}^+\text{BF}_4^-$ ($7.5 \times 10^{-5}\text{ M}$) (the insets show the measured isotope pattern and those calculated for $\mathbf{C1} \cdot \mathbf{9}^+$ (1) and $\mathbf{1} \cdot (\mathbf{9}^+)_2 \cdot \mathbf{1}$ (2)), b) **1** ($2.5 \times 10^{-5}\text{ M}$) and **10** ($5 \times 10^{-5}\text{ M}$) with $\mathbf{9}^+\text{BF}_4^-$ ($2.5 \times 10^{-4}\text{ M}$), and c) **1** ($2.5 \times 10^{-5}\text{ M}$) and **11** ($5 \times 10^{-5}\text{ M}$) with $\mathbf{9}^+\text{BF}_4^-$ ($2.5 \times 10^{-4}\text{ M}$).

evidence for this in the NMR spectrum, but the broadened guest signals of encapsulated $\mathbf{9}^+$ may reflect such a loss of symmetry (Figure 2d).^[23] The symmetric calixarene complex $\mathbf{10} \cdot \mathbf{9}^+ \cdot \mathbf{10}$ shows a much simpler spectrum for both the host and the guest (Figure 2e). Deuterium labeling of the methyl

group of 9^+ led to the assignments for the visible guest signals as shown (Figure 2).

The effects of the tether on the stability of the capsule were addressed through competition experiments with other urea-substituted calixarenes. Studies of Böhmer et al.^[25] had shown that simple calixarene dimers such as **10**·**10** disproportionate to form heterodimeric species in the presence of similar calixarene dimers, and do so in an entropically driven (statistical) manner. What is expected for **C1** in the presence of, say, **10**? Intuition hints that heterodimerization is disfavored because it leads to a reduction in the number of



particles. In the experiment, a 1:1 mixture of **10** and **1** gave no new signals for other assemblies such as **10**₂·**1** in the ¹H NMR spectrum. Nor did the ESI mass spectrum of this mixture with 9^+ as a label show a peak corresponding to **10**₂·(9^+)₂·**1** (m/z 2699, arrow in Figure 3b); only signals for the two homodimers were observed.

In contrast, the sulfonylurea derivative **11** ($p\text{-Ts} = p\text{-toluenesulfonyl}$) in the presence of **1** gave a dumbbell system; four new peaks ($\delta = 10.5\text{--}10.8$) from the NH protons adjacent to the sulfonyl group of the heterotrimer **11**₂·**1** replace the signals of **C1** in the NMR spectrum (Figure 2c). The ESI mass spectrum of this mixture, again with 9^+ as the guest, showed an intense signal for the dumbbell-like structure **11**₂·(9^+)₂·**1** (m/z 3348, Figure 3c). Arylurea and sulfonylurea calixarenes such as **10** and **11** are known to prefer the heterodimers because of the well-matched acid/base properties of their hydrogen bonding sites.^[23]

In summary, a combination of ¹H NMR spectroscopy and ESI mass spectrometry shows that **1** exists as primarily the intramolecularly assembled capsule **C1**. Its encapsulation behavior and stability toward denaturants indicates that the addition of even a simple aliphatic tether can have profound effects on the shape of the calixarene cavity. It may be possible to apply these findings to create an optically active cavity—the goal of our current efforts.

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- [15] **5**: ¹H NMR (300 MHz, CDCl₃): $\delta = 8.81\text{--}8.78$ (m, 6H), 8.68–8.65 (m, 2H), 8.40 (s, 2H), 7.61 (s, 2H), 5.51 (dd, $J = 3.5$ Hz, 13.6 Hz, 4H), 5.11–5.08 (m, 2H), 4.83–4.78 (m, 6H), 4.34 (d, $J = 12.3$ Hz, 4H), 2.95–2.87 (m, 8H), 2.08–1.94 (m, 12H); ¹³C NMR (75 MHz, CDCl₃): $\delta = 166.6, 162.4, 143.5, 142.7, 136.7, 135.5, 134.1, 133.3, 131.4, 126.4, 126.3, 124.5, 124.0, 123.9, 123.5, 77.8, 77.5, 77.4, 31.0, 23.2, 23.1, 109.4, 10.3, 9.9$; HR-MS calcd for C₄₈H₅₄N₄O₆Cs⁺ [$M + Cs^+$]: 915.3098, found: 915.3127.
- [16] **7**: ¹H NMR (300 MHz, CDCl₃): $\delta = 7.64\text{--}7.60$ (brs, 16H), 4.46 (dd, $J = 1.2, 24.0$ Hz, 8H), 4.16–4.14 (m, 8H), 3.81–3.82 (m, 8H), 3.35–3.23 (m, 12H), 1.91–1.79 (m, 16H), 1.28–1.24 (m, 8H), 1.07–0.81 (m, 24H); HR-MS calcd for C₈₈H₁₀₄N₁₀O₂₂Cs⁺ [$M + Cs^+$]: 1785.6381, found: 1785.6510.
- [17] **1**: ¹H NMR (600 MHz, [D₆]DMSO, DMSO): $\delta = 8.29$ (s, 2H), 8.20 (s, 2H), 8.19 (s, 4H), 8.07 (s, 4H), 7.97 (s, 2H), 7.24 (d, $J = 6.0$ Hz, 4H), 7.19 (d, $J = 4.2$ Hz, 8H), 7.02 (d, $J = 6.0$ Hz, 4H), 6.28 (d, $J = 4.2$ Hz, 8H), 6.89 (s, 4H), 6.81 (s, 4H), 6.72 (s, 2H), 6.69 (s, 2H), 5.79 (s, 2H), 4.32 (t, $J = 6.9$ Hz, 8H), 3.79–3.76 (m, 12H), 3.72 (brs, 4H), 3.36–3.30 (m, 28H), 3.30–3.16 (m, 6H), 3.08 (dd, $J = 1.8, 27$ Hz, 4H), 2.98–2.96 (m, 4H), 2.54–2.46 (m, 14H), 2.00–1.85 (m, 8H), 1.49–1.47 (m, 8H), 1.24–1.22 (m, 20H), 1.00–0.94 (m, 6H), 0.85–0.81 (m, 6H); MS calcd for C₁₇₂H₂₃₀N₁₆O₁₆Cs⁺ [$M + Cs^+$]: 2908, found: 2908.

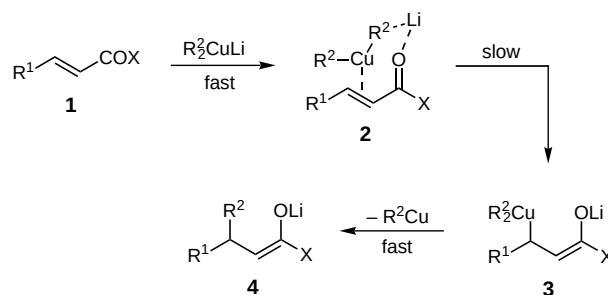
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The Mechanism of 1,4- and 1,6-Cuprate Additions: The First Determination of Activation Parameters**

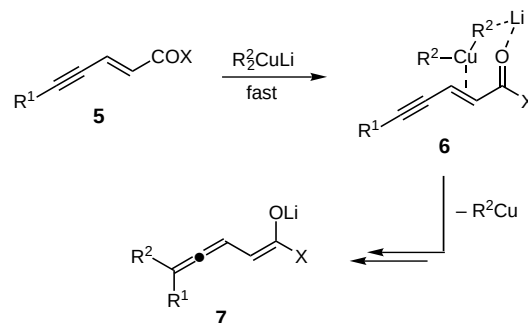
Johannes Canisius, Andreas Gerold, and Norbert Krause*

Michael additions of organocuprate reagents are among the most reliable methods for regio- and stereoselective coupling of C–C bonds. In addition to the classical 1,4-cuprate additions to enones, enoates, and acetylenic esters, recently 1,6-, 1,8-, 1,10-, and 1,12-additions to acetylenic Michael acceptors have been intensively studied.^[1] The discovery of

these new reaction classes, as well as advances in stereoselective cuprate additions,^[2] has led to an increasing interest in the structure of organocuprate reagents^[3] and the mechanisms of their reactions. Low-temperature NMR spectroscopy has been particularly well suited for mechanistic studies of these reactions,^[1, 4–6] and has provided evidence for the intermediacy of π complexes **2** in the 1,4-cuprate addition to enones and enoates **1**.^[4, 5] Further along the reaction pathway to product **4**, the rate-limiting step is likely an oxidative addition resulting in the formation of the σ copper(III) species **3**; this reaction pathway is in agreement with quantum-chemical calculations,^[7] and recently evidence for copper(III) intermediates in biological systems has been obtained experimentally.^[8]



Interestingly, 1,6-cuprate additions to electron acceptor substituted enynes **5** also result in π complexes **6** with coordination of the cuprate to the C–C double bond, even though the transfer of the alkyl moiety R^2 occurs at the acetylenic carbon atom.^[6] The similarity between the π complexes **2** and **6** presumably leads to further analogies in the reaction pathways of the 1,4- and 1,6-additions, in which several short-lived intermediates take part in the formation of the 1,6-addition product **7** from the π complex **6**.^[1, 6] To obtain

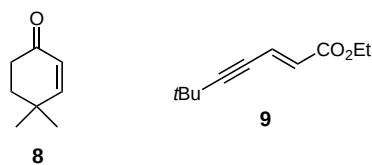


information about the rate-determining step of these reactions, we have performed a direct kinetic study for 1,4-additions of organocuprates to enones and 1,6-additions to enynes and determined the first set of activation parameters for these reactions.^[9] These measurements indicate not only the analogies between the two reaction mechanisms, but also allow a comparison of the reactivity of various Michael acceptors.

Enone **8** and enynoate **9** were chosen as model substrates for the kinetic studies; qualitative studies had shown that these two Michael acceptors have comparable reactivities towards cuprates, allowing the kinetic measurements to be

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carried out at similar temperatures. The reaction with lithium dimethylcuprate ($\text{Me}_2\text{CuLi} \cdot \text{LiI}$) was carried out in a double-walled flask between -43 and -65°C in diethyl ether, that is, under normal conditions for preparative application of cuprate additions (see Experimental Section). After addition of the substrate to the cuprate solution, the π complex (**2** or **6**) is immediately formed; NMR spectroscopic studies have indicated that this step is practically quantitative.^[4–6] In the rate-determining step the π complex transforms into the enolate (**3** or **7**). The progress of the reaction was determined by removing aliquots at specific time intervals; the aliquots were hydrolyzed and analyzed by gas chromatography (in this way, the substrate **8** or **9** was recovered quantitatively from the remaining π complex). In an effort to minimize the systematic error in aliquot sampling and analysis, an internal standard of tetradecane was used in the reaction mixture. A typical plot of concentration versus time for these experiments is depicted in Figure 1.

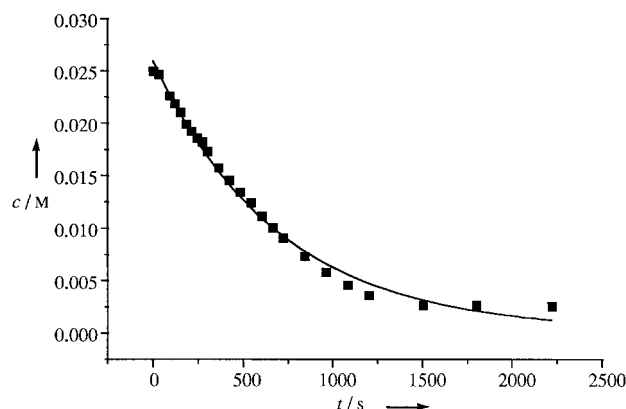


Figure 1. Time dependence of the substrate concentration for the reaction of enyne **9** with $\text{Me}_2\text{CuLi} \cdot \text{LiI}$ ($T = 219\text{ K}$; starting concentrations: $c_0(\text{cuprate}) = 0.05\text{ M}$, $c_0(\text{9}) = 0.025\text{ M}$ in diethyl ether).

The measured values were fitted using the exponential function $c = c_0 \exp(-bt)$. In the case of a first-order reaction ($v = k[\pi \text{ complex}]$) the calculated slope b should be independent of the cuprate concentration (therefore $b = k$), whereas for a higher order reaction (for example $v = k[\pi \text{ complex}][\text{cuprate}]$) a strong dependence on the cuprate concentration is expected. Actually, for the two Michael acceptors **8** and **9**, the values measured for b with several different initial cuprate concentrations were the same within experimental error;^[10] therefore, the 1,4-addition^[9a] and the 1,6-addition occur in an *intramolecular manner* from the respective π complexes.

To determine the activation parameters for the Michael addition to the model substrates **8** and **9**, the kinetic measurements were carried out at several temperatures between 204 and 230 K. The resulting rate constants^[11, 12] show errors of 3–

30%, with most lying within 10% of the value of k . Problems mainly occurred in runs at low temperature as the repeated withdrawal of aliquots sometimes led to decomposition of the cuprate. The rate constants result in practically parallel lines in the Arrhenius plot (Figure 2) with the following activation parameters:

- 1,4-addition of $\text{Me}_2\text{CuLi} \cdot \text{LiI}$ to enone **8**:
 $E_A = 76 \pm 7\text{ kJ mol}^{-1}$, $\lg A = 16 \pm 2$ (correlation coefficient $r = -0.985$)
- 1,6-addition of $\text{Me}_2\text{CuLi} \cdot \text{LiI}$ to enynone **9**:
 $E_A = 70 \pm 7\text{ kJ mol}^{-1}$, $\lg A = 15 \pm 2$ (correlation coefficient $r = -0.975$)

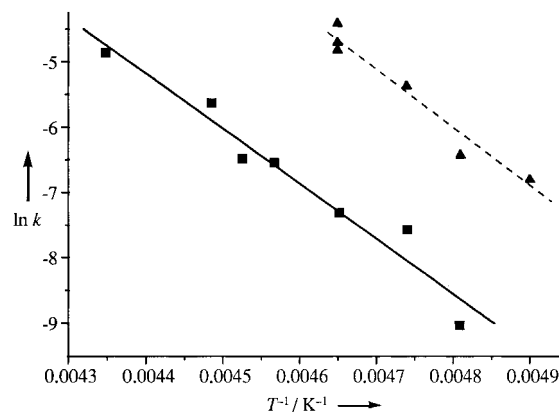
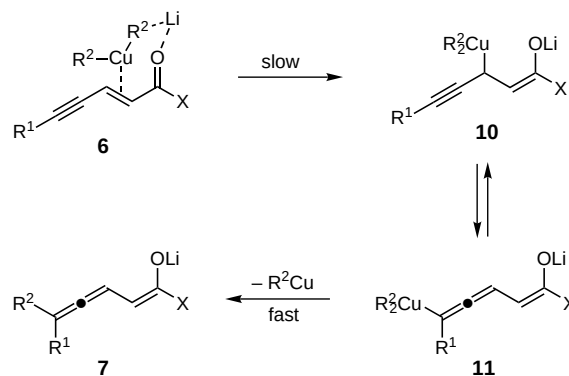


Figure 2. Arrhenius plot for the 1,4-addition of $\text{Me}_2\text{CuLi} \cdot \text{LiI}$ to enone **8** ($\blacktriangle$) and for the 1,6-addition to enynone **9** ($\blacksquare$).

The activation parameters for the Michael acceptors **8** and **9** are identical within the limits of error. Comparable data for other copper-mediated reactions are not available; it is noteworthy, however, that the values measured here for the preexponential term ($\lg A = 15–16$) lie in the region typical for the opening of small rings.^[13] Direct conclusions concerning analogous rate-determining steps based upon the similarity of activation parameters are not possible; nevertheless, it seems conceivable that both the 1,4-cuprate addition to enone **8** and the 1,6-addition to enyne **9** proceed through the oxidative addition of the substrate to produce a σ copper(III) species. On this premise the following mechanistic model arises for the 1,6-addition to acceptor-substituted enynes: In the rate-determining step of the reaction the copper π complex **6** decomposes into the copper(III) species **10**, which



could be in equilibrium with the allenecopper compound **11**. The two intermediates can undergo reductive elimination to produce the 1,4-addition product from **10** and the 1,6-adduct from **11**. The experimentally observed exclusive formation of the 1,6-addition product may indicate that the hypothetical equilibrium lies on the side of intermediate **11**, or that the reductive elimination of **11** occurs much faster than from **10**.^[14]

In conclusion, it is noted that kinetic investigations provide useful insight into the mechanistic pathways of the cuprate additions. The activation parameters determined here for the first time indicate that strong analogies exist between the reactions of various Michael acceptors.

Experimental Section

The kinetic measurements were conducted under an argon atmosphere in a double-walled flask with a double-walled dropping funnel; both were cooled with a Kryomat RUK90 made by Lauda. The temperature in the reaction flask was measured using a PT-100 thermometer and held constant to within ± 0.5 K. $\text{Me}_2\text{CuLi} \cdot \text{LiI}$ was prepared in the double-walled flask by addition of MeLi (2 equiv, salt-free; ca. 1.5 M solution in diethyl ether) to a suspension of CuI (1 equiv) in diethyl ether. The cuprate solution was cooled to the reaction temperature, and a solution of the Michael acceptor and the internal standard tetradecane was likewise cooled in the dropping funnel. At the timepoint $t=0$ the substrate was added in one shot to the cuprate (end volume: 40 mL; starting concentration of substrate and cuprate: 0.02–0.10 M); warming due to the formation of the π complex could be held to within 0.5 K. Aliquots were withdrawn at specific time intervals with a pipet that was precooled in liquid nitrogen; the aliquots were immediately hydrolyzed and analyzed by gas chromatography.

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- [11] Rate constants k for the 1,4-addition of $\text{Me}_2\text{CuLi} \cdot \text{LiI}$ to the enone **8**: 0.0011 ± 0.0002 s^{−1} (204 K), 0.0016 ± 0.0005 s^{−1} (208 K), 0.0046 ± 0.0003 s^{−1} (211 K), 0.009 ± 0.001 s^{−1} (215 K), 0.0080 ± 0.0005 s^{−1} (215 K), 0.009 ± 0.001 s^{−1} (215 K), 0.012 ± 0.001 s^{−1} (215 K).
- [12] Rate constants k for the 1,6-addition of $\text{Me}_2\text{CuLi} \cdot \text{LiI}$ to the enyne **9**: 0.00012 ± 0.00004 s^{−1} (208 K), 0.00052 ± 0.00008 s^{−1} (211 K), 0.00067 ± 0.00008 s^{−1} (215 K), 0.00145 ± 0.00008 s^{−1} (219 K), 0.00154 ± 0.00005 s^{−1} (221 K), 0.0036 ± 0.0009 s^{−1} (223 K), 0.0078 ± 0.0003 s^{−1} (230 K).
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- [14] Analogous mechanistic models can be designed for cuprate additions to any other Michael acceptor in which further rearrangement steps of the type **10** → **11** are necessary to produce the experimentally observed regioisomer.^[1]

η^5 -Phospholylgallium: The First Monomeric Polyhapto Compound between a Phospholyl Ligand and a Main Group Metal**

Andreas Schnepf, Gregor Stöber, Duncan Carmichael, François Mathey,* and Hansgeorg Schnöckel*

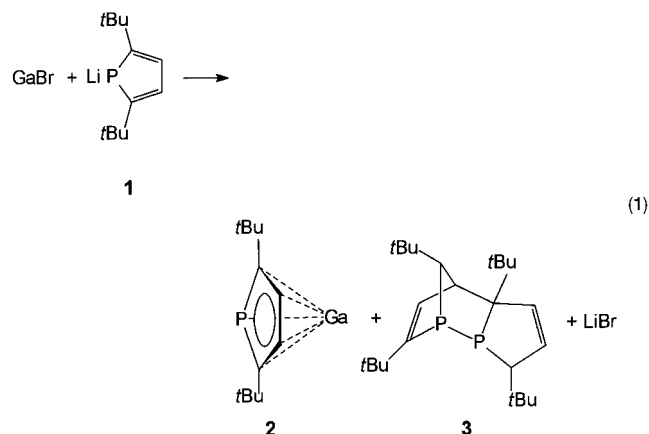
Metastable, donor-stabilized Ga^{I} halide solutions are important precursors for subvalent gallium species.^[1] For instance, Ga^{I} chloride solutions have provided access to the first organometallic compounds of gallium(II), $\text{GaCp}^{[2]}$ and $\text{GaCp}^*^{[3]}$ ($\text{Cp} = \text{C}_5\text{H}_5$, $\text{Cp}^* = \text{C}_5\text{Me}_5$), which incorporate highly symmetrically bound η^5 -Cp ligands. Simple neutral complexes involving main group elements bound to an aromatic

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ligand containing one phosphorus atom are unknown, except for alkali metal compounds.^[4] However, transition metals form many compounds of this type.^[5] The absence of simple complexes containing the elements of Group 13 is surprising because such compounds could be interesting precursors for III–V semiconductors. An earlier attempt to employ indium as the group III component did not allow this problem to be resolved.^[6] Consequently we were motivated to investigate the synthesis and characterization of the first η^5 -phospholyl-gallium complex. We decided to use lithium 2,5-bis(*tert*-butyl)phospholide **1**, because the steric hindrance at its phosphorus lone pair tends to promote η^5 coordination.^[7]

Cocondensation^[1] of the high-temperature molecule GaBr with a toluene/THF mixture provides a metastable solution of Ga^I bromide, which was allowed to react with lithium 2,5-bis(*tert*-butyl)phospholide **1** at -78°C [Eq. (1)]. Slowly



warming the reaction mixture to room temperature resulted in a black residue, which was formed from the excess gallium reagent, and a pale orange solution. After the solvent was removed in vacuo, the products were extracted with pentane.

The ^{31}P NMR spectrum of the pentane extract indicated that two products are formed in the reaction; a singlet at $\delta = 67$ for the principal product and two doublets at $\delta = -16.7$ and -30.2 with a coupling constant of 214 Hz for the by-product. Further workup of the pentane extract by fractional crystallization allowed colorless crystals of by-product **3** to be removed. The X-ray structure determination of these crystals shows this compound to be the Diels–Alder dimerization product of the protonated phosphole.^[8] The major reaction product was obtained in the form of a pale yellow oil, which has precluded its characterization by X-ray diffraction to date. Its ^{69}Ga NMR spectrum gave a relatively sharp signal at $\delta = -653$ showing a half-height linewidth of 1926 Hz, indicative for η^5 coordination at gallium (GaCp*: ^{69}Ga NMR ($[\text{D}_8]$ toluene): $\delta = -653$).^[3]

Further support for this formulation was obtained from ab initio calculations,^[9] the results of which are presented in Figure 1. An η^5 -Ga^I-phospholyl structure **2** is obtained, in which the gallium atom is displaced from the axis perpendicular to the phospholyl centroid because of the greater van der Waals radius of phosphorus than carbon. NMR chemical shifts^[9] were also calculated by the ab initio method, and these are in good agreement with the experimental data (Table 1).

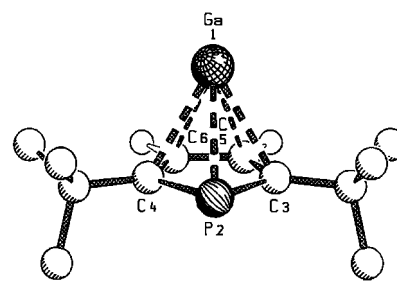


Figure 1. Structure of **2** calculated by density functional theory: Ga–P 266.2, Ga–C3 256.7, Ga–C5 253.4, P–C 179, C3–C5 142.2, C5–C6 138.7 pm.

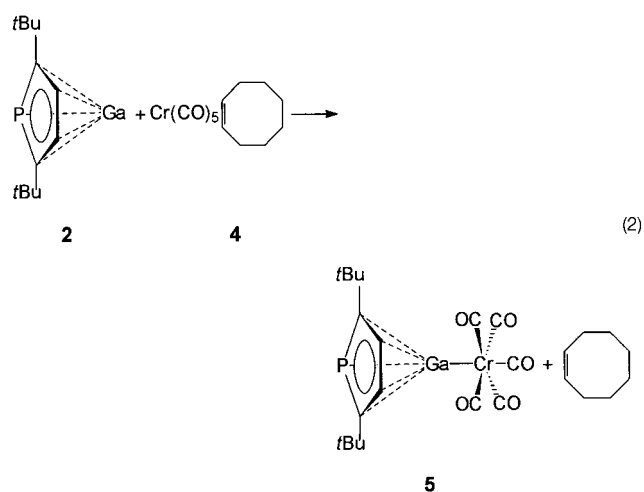
Table 1. Comparison of the calculated and observed NMR chemical shifts for **2**.

	δ Calculated	δ Experiment [ppm]
^{69}Ga	–655	–653
^{31}P	73	67
^{13}C	116.3, 176.4	118.9, 164.6

Further data supporting formulation **2** were obtained from the mass spectrum of the oil, whose molecular ion (m/z 266.1, 264.1 [M^+]) and base peak (m/z 251.1, 249.1 [$M^+ - \text{CH}_3$]) show the anticipated $^{69}\text{Ga}/^{71}\text{Ga}$ isotope patterns.

To further substantiate these results by X-ray structure analysis, we attempted to synthesize a crystalline derivative. One possible target compound would be a transition metal carbonyl complex in which one CO ligand is replaced by the Ga^I compound. A series of analogous GaCp* derivatives have been described recently.^[10]

Treatment of a solution of **2** in hexane with $\text{Cr}(\text{CO})_5$ –cyclooctene [Eq. (2)] furnished yellow crystals of the desired



product in 80% yield. The results of the X-ray structure analysis (Figure 2) show the adduct to have the anticipated η^5 -phospholylgallium(i) chromium pentacarbonyl structure **5**. A tilting of the phospholyl plane with respect to the Ga–Cr axis is clearly visible, and is reflected in the different gallium–ring distances. Thus, the Ga–P bond length of 248.9(1) pm is longer than the Ga–C lengths of 234.5(3) (C–*t*Bu) and 237.2(5) pm for (C–H). A comparison of the structures calculated for **5** and the uncomplexed phospholylgallium(i)

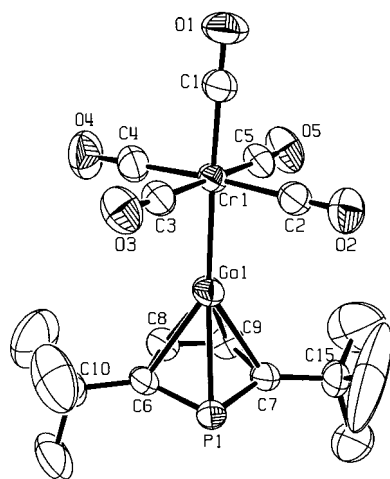


Figure 2. ORTEP diagram of the structure of **5**. For clarity, all hydrogen atoms except those of the phospholyl ligand have been omitted. Selected bond lengths [pm] and angles [°]: Ga1–P1 248.9(1), Ga1–C6 234.6(3), Ga1–C8 237.9(5), Ga1–Cr1 239.0(1), Cr1–C1 186.3(3), Cr1–C2 190.4(4), Cr1–C3 188.3(3), Cr1–C4 188.7(4), Cr1–C5 189.7(3), O1–C1 113.8(4), O2–C2 114.2(4), O3–C3 114.6(4), O4–C4 114.5(5), O5–C5 114.7(4), P1–C6 170.6(4), P1–C7 173.7(3), C6–C8 148.6(6), C7–C9 149.0(7), C8–C9 139.8(7), C1–Cr1–Ga1 177.5(1), C3–Cr1–C5 176.9(2), C6–P1–C7 95.6(2), C8–C6–P1 110.2(3).

compound **2** shows that the Ga–ring distances decrease by approximately 20 pm upon coordination to the Cr(CO)₅ fragment.^[11] This is consistent with the trends observed for the corresponding Ga–Cp* compound which have been extensively discussed elsewhere.^[12] Equally, the similarities between the Ga–Cr lengths in **5** (239 pm) and the corresponding Cp* complex (240 pm) illustrate that the bonding scheme which we have proposed previously^[12, 13] can be extrapolated to **5**.

Consideration of the pentacarbonylchromium fragment reveals a close similarity between **5** and its Cp* analogue, which is apparent from the pattern of the IR spectrum in the carbonyl region. That the pattern is shifted to lower wave-number in the phospholyl case may be attributed to the electron-donating properties of the phosphorus.^[10, 13]

The results presented show that phospholylgallium(i) compounds may be synthesized by the reaction of Ga^IBr with an appropriate lithium phospholide and that they are stable enough to be handled at room temperature. Additionally, mass spectrometric studies have confirmed that **2** is quite volatile. This property augurs well for the use of **2** as a CVD precursor for GaP.^[14]

Experimental Section

2: Lithium 2,5-bis(*tert*-butyl)phospholide **1** (250 mg, 0.723 mmol) was dissolved in toluene (10 mL) and cooled to –78 °C. To this suspension, GaBr solution (2.5 mL, 0.3 M, 0.75 mmol GaBr) was slowly added at –78 °C. Allowing the mixture to warm slowly to room temperature produced a black precipitate and a pale orange solution which, when evaporated to dryness under high vacuum, yielded a black residue. This was extracted with pentane to give a pale orange solution from which the by-product **3** was separated by crystallization. This left the product **2** in the form of a light orange oil. Yield: 134 mg, 70%. ⁶⁹Ga NMR (92 MHz, [D₆]benzene, 25 °C): δ = –653 (*h*_{1/2} = 1926 Hz); ³¹P NMR (100 MHz, [D₆]benzene, 25 °C): δ = 67.13 (s, 1P); ¹³C NMR (63 MHz, [D₆]benzene, 25 °C): δ = 32.6 (2C), 34.96 (6CH₃), 118.86 (2CH), 164.6 (d, *J* = 51 Hz, 2C);

¹H NMR (250 MHz, [D₆]benzene, 25 °C): δ = 1.32 (s, 12H), 6.72 (d, ³*J* = 4.1 Hz, 2H); MS (70 eV): *m/z* (%): 266.1 (43, [M⁺]), 264.1 (65, [M⁺]), 251.1 (79, [M⁺ – CH₃]), 249.1 (100, [M⁺ – CH₃]), 196.1 (53), 181.1 (17), 140.1 (68), 125 (46), 103 (13), 70.9 (45), 68.9 (62), 57.1 (82). **3:** ³¹P NMR (100 MHz, [D₆]benzene, 25 °C): δ = –30.20 (d, ²*J* = 214 Hz, 1P), –16.70 (d, ²*J* = 214 Hz, 1P); ¹³C NMR (63 MHz, [D₆]benzene, 25 °C): δ = 29.95 (3CH₃), 30.35 (3CH₃), 32.59 (6CH₃), 33.57 (C), 35.50 (C), 37.56 (2C), 58.54 (CH), 60.92 (CH), 65.69 (CH), 75.75 (C), 133.385 (CH), 139.95 (CH), 151.07 (C); ¹H NMR (250 MHz, [D₆]benzene, 25 °C): δ = 1.03 (s, 9H), 1.07 (s, 18H), 1.21 (s, 9H), 2.23 (m, 1H), 2.54 (m, 1H), 3.21 (m, 1H), 5.67 (m, 2H), 6.08 (m, 1H); MS (70 eV): *m/z* (%): 392.4 (100, [M⁺]), 335.3 (8, [M⁺ – *t*Bu]), 196.2 (54, [M⁺/2]), 140.1 (40, [M⁺/2 – *t*Bu]), 57.1 (18, [*t*Bu]).

5: Phospholylgallium(i) **2** (132.5 mg, 0.5 mmol) dissolved in *n*-hexane (30 mL) was added to cyclooctene chromium pentacarbonyl **4** (150 mg, 0.5 mmol). The reaction mixture was refluxed for 1 h, whereupon a yellow solution was formed. After removal of the free cyclooctene in vacuo, a crystalline residue was obtained. Further crystallization from hexane gave yellow crystals of **5** (183 mg, 0.4 mmol 80%). Phospholylgallium(i)chromium pentacarbonyl **5**: ³¹P NMR (100 MHz, [D₆]benzene, 25 °C): δ = 41.97 (s, 1P); ¹³C NMR (63 MHz, [D₆]benzene, 25 °C): δ = 30.94 (2C), 33.90 (6CH₃), 126.6 (2CH), 163.4 (d, *J* = 54 Hz, 2C); ¹H NMR (250 MHz, [D₆]benzene, 25 °C): δ = 1.22 (s, 12H), 6.90 (d, ³*J* = 3.7 Hz, 2H); IR (toluene): $\tilde{\nu}$ = 2022 (m) [ν_s (CO)₄], 1936 (sh) [ν (CO)_{ax}], 1870 (vs) [ν_{as} (CO)₄] cm^{–1}; MS (70 eV): *m/z* (%): 457.9 (9, [M⁺]), 455.9 (12, [M⁺]), 444.1 (0.3, [M⁺ – CH₃]), 442.2 (0.5, [M⁺ – CH₃]), 402.0 (6.5, [M⁺ – 2CO]), 399.9 (8.8, [M⁺ – 2CO]), 317.9 (68, [M⁺ – 5CO]), 315.9 (100, [M⁺ – 5CO]), 196.1 (23), 140 (30), 70.9 (5.6), 68.9 (9).

Crystal structure data for **3**: P₂C₂₄H₄₂, *M_r* = 392.52; crystal dimensions 0.8 × 0.7 × 0.5 mm, orthorhombic, space group *P*2(1)2(1)2(1), *a* = 11.9890(7), *b* = 12.800(1), *c* = 15.5492(8) Å, *V* = 2386.2(3) Å³, *Z* = 4, ρ_{calcd} = 1.093 g cm^{–3}, μ_{Mo} = 1.88 cm^{–1}, 2 θ_{max} = 559.184 observed reflections, 5485 unique reflections, absorption correction: semiempirical (min./max. transmission 0.2598/0.2185), *R*₁ = 0.036, *wR*₂ = 0.084. Stoe Stadi4 diffractometer (MoK α radiation, (λ = 0.71073 Å), 200 K). The structure was solved by direct methods and refined against *F*² for all observed reflections. Programs: Shelxs and Shelxtl (G. M. Sheldrick, Universität Göttingen).

Crystal structure data for **5**: GaCrPO₅C₁₇H₂₀, *M_r* = 457.02; crystal dimensions 0.8 × 0.6 × 0.46 mm, triclinic, space group *P*1̄, *a* = 6.6462(13), *b* = 9.929(2), *c* = 15.670(3) Å, α = 88.88(3), β = 79.51(3), γ = 85.42(3)°, *V* = 1013.5(3) Å³, *Z* = 2, ρ_{calcd} = 1.498 g cm^{–3}, μ_{Mo} = 1.966 cm^{–1}, 2 θ_{max} = 60°, 9164 observed reflections, 5934 unique reflections, no absorption correction, *R*₁ = 0.0484, *wR*₂ = 0.122. Stoe Stadi4 diffractometer (MoK α radiation, (λ = 0.71073 Å), 200 K). The structure was solved by direct methods and refined against *F*² for all observed reflections. Programs: Shelxs and Shelxtl (G. M. Sheldrick, Universität Göttingen).

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-112168 (for **5**) and CCDC-112169 (for **3**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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- [9] The ab initio calculations were performed with the TURBOMOLE^[16] package using TZVP^[17] (**2**) or SVP (**5**) basis sets.^[18] Geometries were optimized by using the RI-DFT module^[19] (BP-86-Functional^[20]) in the C_s point group. NMR chemical shifts were calculated at the SCF level,^[21] using geometries optimized at the RI-DFT level. **2**: $E = -2730.201229$ au, Ga–P 266.2, Ga–C 256.7 and 253.4, P–C 179, C–C 138.1, C–C 142.2 pm, C–P–C 90.9, P–C–C 110.4, C–C–C 114.1°. **5**: $E = -4346.064324$ au, Ga–P 249.9, Ga–C 246.3 and 242.1, Ga–Cr 241.4, P–C 184.9, C–C 138.7, C–C 143.4 pm, C–P–C 88.9°, P–C–C 110.9°, C–C–C 114.6°.
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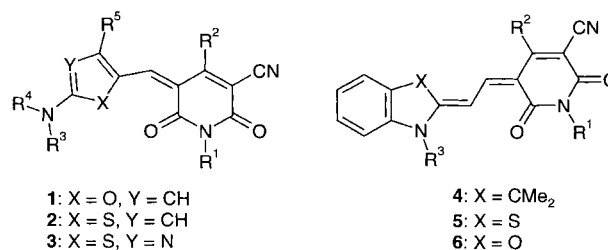
Design, Synthesis, and Evaluation of a Dye Library: Glass-Forming and Solid-State Luminescent Merocyanines for Functional Materials**

Frank Würthner,* Rüdiger Sens, Karl-Heinz Etzbach, and Günther Seybold

Dedicated to Professor Hans-Jürgen Quadbeck-Seeger on the occasion of his 60th birthday

In the development of functional dyes, after the identification of a suitable chromophore extensive variations of the substituents usually have to be carried out before a satisfactory product is obtained for the desired area of application. This optimization of the lead structure traditionally takes place according to combinatorial rather than rational principles, as stability, solubility, affinity, and compatibility properties are hard to predict.

Recently we reported on the dyes **1–4**,^[1] which possess a very interesting chromogenic system for several high-technology applications.^[2] These chromophores with an electronic



structure at the mesomeric center between neutral and zwitterionic electron distribution have high polarizabilities, high dipole moments, and exhibit absorption spectra with sharp bands ($\epsilon_{\max} > 100\,000 \text{ L mol}^{-1} \text{ cm}^{-1}$, half widths $\Delta\tilde{\nu}_{1/2} < 1500 \text{ cm}^{-1}$) that give rise to exceptionally brilliant magenta hues.^[3] This resulted in hitherto unattainable refractive index modulations in photorefractive materials^[1] as well as brilliant hues in thermal dye transfer printing and in electrophotography (color copiers).^[2]

High concentrations of colorant are necessary in photorefractive materials, ribbons for thermal dye transfer printing, and toners for color copiers, which leads to problems due to

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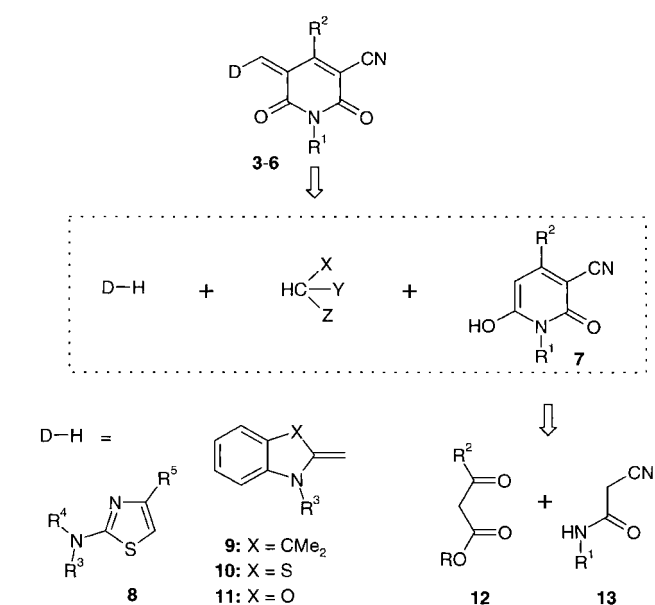


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the marked tendency of these dipolar chromophores to aggregate and crystallize. Our aim was now to optimize these properties, which are hard to predict, by variation of the substituents in a combinatorial fashion. It was advantageous that in terms of their molecular properties the lead structures **1–6** developed by rational concepts were all similarly well suited to the desired applications;^[1] thus, further treatment of the furan and thiophene dyes **1** and **2**, respectively, which are less easily accessible in view of their precursors, became unnecessary.

Readily accessible starting materials, which can be produced in one-pot syntheses from industrially available precursors by simple alkylation or multicomponent condensation reactions,^[4] include the dialkylamino thiazoles **8**,^[5] the methylene bases **9–11**,^[6] and the hydroxypyridones **7**.^[7] Their further reaction, usually consisting of a Vilsmeier formylation of the donor bases **8–11** and subsequent condensation with **7**, is, however, troublesome and requires prior purification of **8–11**, which is only possible by means of time-consuming chromatography in the case of longer chain alkyl substituents $R^3–R^5$. Therefore our aim was to find a method with which a wide variety of structurally variable, unpurified starting materials **7–11** would react in a simple sequence leading directly to the dyes **3–6** and with which the pure products could be isolated directly from the reaction mixtures.

Prompted by the recent description of a formylation–condensation sequence for the synthesis of merocyanines in which orthoformates^[8] were used, we examined a series of formic acid derivatives in diverse solvents for their suitability for the synthesis of the dyes **3** and **4** from the reactants **7** and **8** or **9**, respectively (Scheme 1).^[9] Indeed, we were able to obtain almost quantitative yields with orthoformates and some other activated formic acid derivatives as long as the starting material was pure. Impure, and in particular not completely anhydrous, donor components **8** or **9**, on the other



Scheme 1. Retrosynthesis of the merocyanine dyes **3–6**.

hand, led to rapid hydrolysis of the formylating agent and thus to impure products and losses in yield. Finally, the little-known formylating agent dimethylformamide in acetic anhydride^[10] proved to be the ideal reagent, since it is extremely tolerant towards the use of impure starting materials.^[11]

Simple heating of equimolar amounts of the starting materials in acetic anhydride in the presence of 1.5 equivalents of dimethylformamide [Eq. (1)] led to the formation of the merocyanine dyes **3** and **4** in almost quantitative yield (HPLC) (Tables 1 and 2). Even the synthesis of derivatives

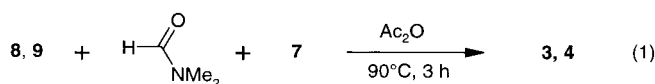


Table 1. Thiazole dyes **3** and their optical and thermal properties.^[a]

	R^1	R^2	$R^3 = R^4$	R^5	$\lambda_{\max}$ [nm] ^[b]	Color of the solid	Luminescence of the solid ^[c]	DSC (type, m.p., T_g [°C]) ^[d]
3a	Me	Me	Bu	Ph	536	red	+	D, 229
3b	Bu	Me	Bu	Ph	536	green ^[e]	0	A, 182, –
3c	hex	Me	Bu	Ph	536	green ^[e]	0	A, 140, –
3d	Me	Me	Bu	chex	529	red	0	B, 182, 24
3e	Bu	Me	Bu	chex	529	red	+	A, 162, –
3f	Bu	Ph	Bu	chex	530	red	+	A, 205, –
3g	Bu	Me	Bu	<i>i</i> Pr	528	red	0	B, 154, 10
3h	Me	Me	Bu	<i>t</i> Bu	533	light red	+	A, 170, –
3i	Bu	Me	Bu	<i>t</i> Bu	534	orange	++	A, 173, –
3j	Me	Me	Et	neop	526	red	0	D, 244
3k	Bu	Me	Bu	neop	530	orange	++	A, 146, –
3l	Bu	Me	Bu	<i>o</i> -MeC ₆ H ₄	529	red	(+)	B, 174, 28
3m	Bu	Me	Bu	<i>p</i> -MeOC ₆ H ₄	542	green ^[e]	0	B, 183, 33
3n	Bu	Me	Bu	2-furyl	565	brass-colored ^[e]	0	A, 204, –
3o	Bu	Me	Bu	2-thienyl	555	green ^[e]	0	A, 203, –
3p	Bu	Me	Bu	3-thienyl	542	bronze-colored ^[e]	0	A, 178, –

[a] hex = *n*-hexyl, chex = cyclohexyl, neop = neopentyl. [b] In dichloromethane. All alkyl-substituted derivatives are characterized by a single, extremely narrow absorption band, for example **3g** ($\epsilon_{\max} = 110\,000 \text{ L mol}^{-1} \text{ cm}^{-1}$, $\Delta\tilde{\nu}_{1/2} = 1250 \text{ cm}^{-1}$). Substituents capable of conjugation in the 4-position at the thiazole ring, on the other hand, lead to bathochromic shifts and broader bands, for example **3b** ($\epsilon_{\max} = 89\,000 \text{ L mol}^{-1} \text{ cm}^{-1}$, $\Delta\tilde{\nu}_{1/2} = 1800 \text{ cm}^{-1}$), **3o** ($\epsilon_{\max} = 64\,000 \text{ L mol}^{-1} \text{ cm}^{-1}$, $\Delta\tilde{\nu}_{1/2} = 2200 \text{ cm}^{-1}$). [c] After excitation with UV light of 366 nm: 0 no, (+) weak, + distinct, ++ intense luminescence. [d] The DSC measurements were carried out with heating and cooling rates of 10 K min^{-1} . A: Recrystallization upon cooling; B: Glass formation upon cooling, but recrystallization during the second heating cycle; C: Glass formation upon cooling and no recrystallization during the second heating cycle; D: Decomposition upon melting. [e] Bronze shiny colors.

Table 2. Indoline **4**, benzothiazole **5**, and benzoxazole dyes **6**, as well as their optical and thermal properties.^[a]

	R ¹	R ²	R ³	X	λ_{max} [nm] ^[b]	Color of the solid	Luminescence of the solid ^[c]	DSC (type, m.p., T_g [°C]) ^[d]
4a	Me	Me	Me	CMe ₂	522	light red	(+)	D, 310
4b	Bu	Me	Me	CMe ₂	522	red	(+)	A, 253, –
4c	hex	Me	Me	CMe ₂	523	red	+	B, 204, 64
4d	<i>p</i> -MeC ₆ H ₄	Me	Me	CMe ₂	524	red-brown	(+)	D, > 300
4e	BnOPr	Me	Me	CMe ₂	525	red	(+)	B, 171, 55
4f	Bu	Me	Bu	CMe ₂	527	red	+	B, 196, 46
4g	Bu	Me	<i>i</i> Pent	CMe ₂	527	red	++	A, 218, –
4h	Ethex	Me	Bu	CMe ₂	526	red	+	B, 162, 37
4i	Bu	Pr	Bu	CMe ₂	528	red	++	B, 160, 30
4j	Bu	Me	<i>i</i> Pr	CMe ₂	526	red	(+)	B, 221, 73
4k	Ethex	Me	<i>i</i> Pr	CMe ₂	526	red	+	C, 161, 58
5a	Bu	Me	Et	S	533	red	+	D, 310
5b	Ethex	Me	Et	S	533	red	+	D, 278
6	Bu	Me	Et	O	495	orange	+	D, 286

[a] hex = *n*-hexyl, BnOPr = (CH₂)₅OCH₂Ph, Ethex = 2-ethylhexyl, *i*Pent = isopentyl. [b] In dichloromethane. All dyes are characterized by one single, extremely narrow absorption band, for example **4f** ($\epsilon_{\text{max}} = 130\,000 \text{ L mol}^{-1} \text{ cm}^{-1}$, $\Delta\tilde{\nu}_{1/2} = 1300 \text{ cm}^{-1}$), **5a** ($\epsilon_{\text{max}} = 137\,000 \text{ L mol}^{-1} \text{ cm}^{-1}$, $\Delta\tilde{\nu}_{1/2} = 1150 \text{ cm}^{-1}$), **6a** ($\epsilon_{\text{max}} = 145\,000 \text{ L mol}^{-1} \text{ cm}^{-1}$, $\Delta\tilde{\nu}_{1/2} = 1150 \text{ cm}^{-1}$). [c, d] See footnotes of Table 1.

with sterically demanding substituents, such as neopentyl- and *tert*-butylthiazoles, was possible without complications. When the reactions were carried out in concentrated solutions, almost all dyes crystallized upon cooling in > 80 % yield, and analytically pure products were obtained through simple washing with alcohol.^[12] With the strongly basic and oxidation-sensitive methylene bases **10** and **11** it proved effective to start from the more stable hydroiodides and to convert these in an analogous way to dyes **5** and **6**, after addition of 1 equivalent of potassium acetate.

Tables 1 and 2 provide an overview of the structural variations we carried out on the dyes **3–6**, the extension of which to a large library displaying a diverse range of substituents should be readily accessible by an automated parallel synthesis. In terms of our objective, that is the identification of positions in the chromophore and substituents that lead to as low a tendency as possible to crystallize, the compounds presented herein that contain the “standard” substituents methyl and *n*-butyl and in general one varied position should be sufficient.

Differential scanning calorimetry (DSC)^[13] was used to characterize the crystallization behavior, wherein the dyes were first melted and then examined for recrystallization or formation of an amorphous glass during the subsequent cooling and a second heating cycle. Figure 1 shows the results of two typical DSC experiments. Whereas most low molecular weight compounds recrystallize from the melt on cooling, compounds **4c** and **4k** form amorphous glasses. Besides the absence of exothermic recrystallization during the cooling cycle (not shown), the course of the DSC plot is characterized by a glass transition at 64 °C (**4c**) and 58 °C (**4k**), respectively, on reheating. In the case of **4c**, recrystallization occurs at a higher temperature, which can be interpreted as the expression of a noticeable tendency to crystallize. This tendency is completely suppressed in **4k**; thus, once melted, the substance only alternates between the melted and the glassy state without recrystallizing even in repeated heating–cooling cycles. This behavior is exceptional for dyes, which are among the substances most prone to crystallization because of

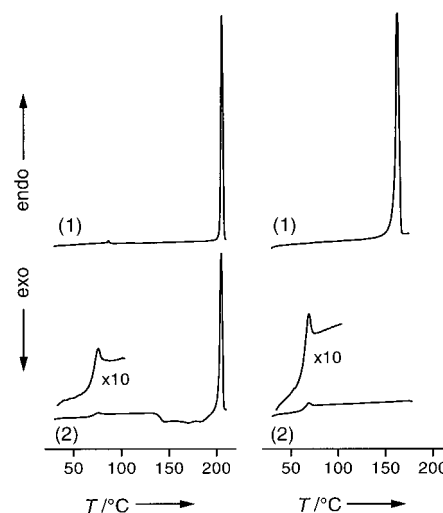


Figure 1. DSC measurements on **4c** (left) and **4k** (right). The heat flows for the first (1) and the second heating cycle (2) are shown. The heating and the cooling rates were 10 K min^{−1} each. The glass transitions are clearly visible in the magnified section. endo: heat absorption, exo: heat emission.

their high dipole moments and a pronounced tendency towards π – π stacking.

Tables 1 and 2 summarize the DSC results for all the dyes. The colorants were divided into A) crystallizing, B) glass-forming, recrystallizing, and C) glass-forming, non-recrystallizing substances. In accordance with earlier findings for other classes of compounds,^[14] the only dye of the C type, **4k**, is characterized by several sterically demanding substituents that prevent the ordered stacking of the π faces. This interpretation is also supported by the high solubility of the compound in all organic solvents and its almost identical color in solution and in the solid state. The tendency to glass formation is already apparent for the parent skeletal structure, as demonstrated by a number of other indoline dyes **4**, in which one or two *n*-alkyl chains already suffice for amorphous solidification (type B). In contrast, only a few thiazole dyes **3** form glasses upon cooling (**3d**, **g**, **l**, **m**). No systematic trend is apparent, especially as the compounds

with sterically demanding *tert*-butyl and neopentyl substituents crystallize readily (type A).

Another property listed in Tables 1 and 2 is the very intense photoluminescence exhibited by some of the dyes in the solid state. This finding was surprising to us as solutions of **3–6** do not fluoresce. For many fluorophores the opposite behavior is typical, that is despite intensive fluorescence in solution, radiationless deactivation takes place in the crystalline state. Evidently, these properties are also influenced considerably by packing effects, which have a decisive influence on the relaxation paths for the excitation energy. Many of the nonluminescent dyes listed in Table 1 are planar molecules whose bronze or green colors in the solid state indicate strong π – π interactions in the crystal. The dyes with sterically demanding substituents, on the other hand, form red crystals that shine intensively in numerous cases.^[15, 16]

The dye library obtained through a highly efficient multi-component synthesis led us to colorants with glass-forming and solid-state photoluminescent properties. Sterically demanding substituents proved crucial to both properties by determining the packing of the functional π systems, thus paving the way for the exploitation of these interesting dyes in new materials.

Experimental Section

Typical procedure for the synthesis of the merocyanine dyes **3** and **4**: A thiazole **8** (0.05 mol) or a methylene base **9** (0.05 mol), respectively, a hydroxypyridone **7** (0.05 mol), and dimethylformamide (0.075 mol, 5.5 g) were heated to 90 °C in acetic anhydride (20–30 mL) for about 3 h. The solid which precipitated upon cooling to room temperature was filtered off, washed thoroughly with 2-propanol and/or aqueous ethanol until the color of the filtrate changed from violet to red, and subsequently dried in a vacuum-drying cabinet at 50 °C.

For the physical characterization, the dyes were recrystallized from acetic anhydride, toluene, or toluene/hexane mixtures.

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- [11] In numerous reactions, starting materials with purities (NMR, GC) <80% were used and nevertheless very good yields were obtained and pure products isolated. Difficulties were encountered with the most readily soluble dyes when these did not crystallize from the reaction mixtures in the presence of solubility-promoting impurities or when greater amounts of 2-propanol, which became necessary in the washing procedure, led to losses in yield.
- [12] Because of the high influence of the purity on the investigated properties (see Tables 1 and 2), the dyes were recrystallized once more.
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- [15] Compounds of the structural types **4–6** also show intensive luminescence in solid solutions, that is in textile dyeing on polyester. A detailed discussion of the competition between radiationless deactivation and photoluminescence, which is evidently strongly dependent on the rigidity of the matrix, should become possible based on the emission spectra of solid samples and dyed textile fibers as well as on crystal structure analyses.
- [16] As extremely small concentrations of impurities already function as traps in the solid state, the data given in Tables 1 and 2 should not be overinterpreted. Especially the substances marked (+) could show better emission properties given a higher purity.

Total Synthesis of (–)-Bafilomycin A₁: Application of Diastereoselective Crotylboration and Methyl Ketone Aldol Reactions**

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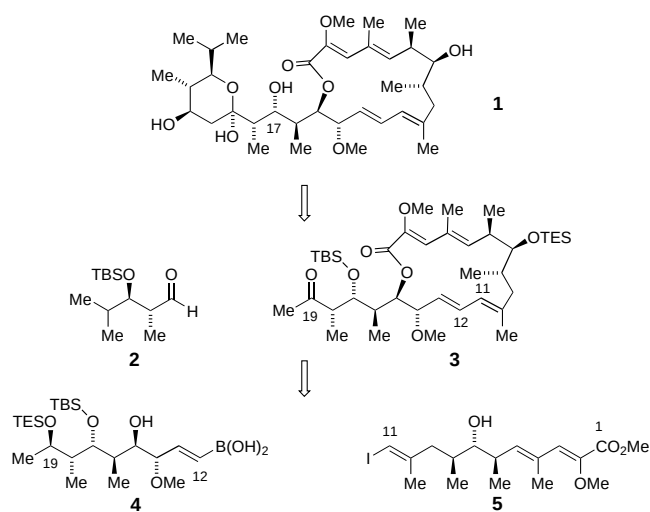
Bafilomycin A₁ (**1**), a member of the hygrolide family of macrolide antibiotics that includes the concanamycins^[1] and the hygrolidins,^[2] is a potent vacuolar ATPase inhibitor that displays broad antibiotic activity.^[3] First isolated in 1983 by Werner et al. from a culture of *Streptomyces griseus* sp. *sulphureus*,^[4] the stereochemistry of the bafilomycins was proposed based on NMR data^[5] and was subsequently verified

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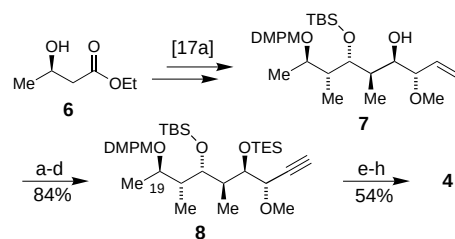
in the case of **1** by X-ray crystallography.^[6] Bafilomycin A₁ contains two distinct structural features characteristic of the hygrolide macrolides: a sensitive 6-membered hemiketal that participates in a hydrogen bond network (between the C17 hydroxyl group and the carbonyl group of the 16-membered lactone) and the C2–C5 diene system that incorporates a C2 methyl enol ether. The important biological properties and interesting structural features of these molecules have stimulated considerable interest. Total syntheses of bafilomycin A₁ have been recorded by Evans and Calter^[7] and Toshima et al.,^[8] and syntheses of concanamycin F and hygrolidin have been accomplished by Toshima et al.^[8] and Yonemitsu et al.,^[9] respectively. In this communication we report a highly convergent synthesis of bafilomycin A₁ (**1**) following the strategy summarized in Scheme 1.



Scheme 1. Retrosynthetic analysis of bafilomycin A₁ (**1**). (see reference [10] for abbreviations).

We envisioned that bafilomycin could be assembled from three key fragments: the known aldehyde **2**,^[11] vinylboronic acid **4**, and the vinyl iodide **5**, which in turn would be constructed utilizing highly diastereoselective aldehyde crotylboration reactions developed in our laboratory.^[12] This highly convergent approach would allow for the use of a Suzuki cross-coupling reaction^[13] to generate an appropriately protected macrocyclization precursor. While the Stille reaction^[14] has found great utility in the assembly of natural products, including in the earlier bafilomycin syntheses,^[7, 8] there are limited applications of the Suzuki reaction for late-stage union of complex intermediates, the most notable of the limited examples being recorded in the palytoxin synthesis by Kishi et al.^[15] and the rutamycin synthesis by Evans et al.^[16] The aldol reaction between **2** and **3**, which serves as the final C–C bond forming event in the synthesis, provides an additional opportunity to explore the factors that control the stereochemistry of fragment assembly in methyl ketone aldol reactions.^[17]

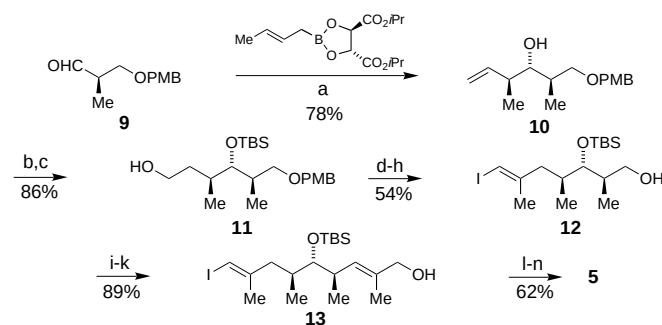
The vinylboronic acid **4** was prepared from olefin **7**, which was synthesized from commercially available ester **6** by the previously described diastereoselective allylmatalation sequence (Scheme 2).^[17a] The presence of the free hydroxyl



Scheme 2. Synthesis of the C12–C20 fragment **4**. a) TES-OTf, 2,6-lutidine, CH₂Cl₂, –50 °C, 1 h, 99%; b) OsO₄, NMO, THF, pH 7 buffer, 16 h; c) Pb(OAc)₄, EtOAc, 0 °C, 10 min; d) (MeO)₂P(O)CHN₂, *t*BuOK, THF, –78 °C → RT, 15 min, 85% (three steps); e) DDQ, CH₂Cl₂, pH 7 buffer, 0 °C, 25 min, 94%; f) TFA, THF, H₂O, 0 °C, 2.5 h, 88%; g) TES-Cl, CH₂Cl₂, pyridine, –40 °C → RT, 10 h, 92%; h) catecholborane, 8 mol % 9-BBN, THF, 60 °C, 4 h, then pH 7 buffer, RT, 1 h, 71%. (See reference [10] for abbreviations.)

group in **7** prevented direct elaboration of the olefin to the vinylboronic acid unit of **4**. In addition, removal of the DMPM ether in the presence of the C10–C13 diene at later stages of the synthesis proved to be problematic. Therefore, the hydroxyl group of **7** was protected as a TES ether, the terminal olefin was then cleaved oxidatively, and the resulting aldehyde was elaborated to acetylene **8** in 84% overall yield with the Seyferth–Gilbert reagent.^[18] The DMPM and TES ethers were removed by sequential exposure of **8** to DDQ (94%) and TFA (88%), then the less hindered C19 hydroxyl group was protected as a TES ether (92%). Finally, treatment of the resulting alkyne with catecholborane and a catalytic amount of 9-BBN^[19] produced the vinylboronic acid **4** after aqueous workup (71% yield).

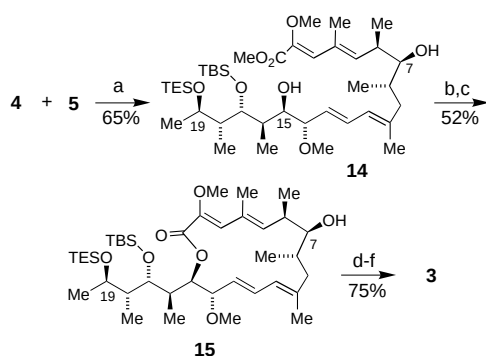
The vinyl iodide **5** was synthesized starting from the known aldehyde **9** (Scheme 3).^[20] Installation of the difficult *anti*–*anti* stereotriad^[21] was accomplished by our diastereoselective aldehyde crotylboration methodology^[12] in the mismatched manifold.^[22] Thus, the reaction of **9** and (*R,R*)-diisopropyl tartrate (*E*)-crotylboronate provided an 85:15 mixture of **10** and the undesired 3,4-*anti*-4,5-*syn* diastereomer (78% yield of



Scheme 3. Synthesis of the C1–C11 fragment **5**. a) Toluene, –78 °C, 8 h, 78%; b) TBS-OTf, 2,6-lutidine, CH₂Cl₂, –50 °C, 30 min, 99%; c) catecholborane, (Ph₃P)₃RhCl, THF, –5 °C, 30 min, then MeOH, 1 N NaOH, H₂O₂, RT, 2 h, 87%; d) (COCl)₂, DMSO, Et₃N, CH₂Cl₂, –78 → 0 °C, 30 min, 99%; e) CBr₄, PPh₃, CH₂Cl₂, 30 min, 89%; f) *n*BuLi, THF, –78 → 0 °C, 15 min, 99%; g) DDQ, CH₂Cl₂, pH 7 buffer, 0 °C, 20 min, 96%; h) Cp₂ZrCl₂, AlMe₃, Cl(CH₂)₂Cl, 60 °C, 14 h, then I₂ at –30 °C, 1 h, 65%; i) (COCl)₂, DMSO, Et₃N, CH₂Cl₂, –78 → 0 °C, 30 min; j) Ph₃PCH(Me)CO₂Et, toluene, 60 °C, 15 h, 90% (two steps); k) DIBAL-H, THF, –78 °C, 3.5 h, 99%; l) MnO₂, CH₂Cl₂, RT, 18 h, 99%; m) KHMDS, THF, (*i*PrO)₂P(O)CH(OMe)CO₂Me, [18]crown-6, 0 °C → RT, 8 h, 85%; n) TBAF, THF, RT, 2 h, 82%. (See reference [10] for abbreviations.)

10). Alcohol **10** was protected as the TBS ether (99 %) and then was converted into the primary alcohol **11** (87 %) through a Rh^I-catalyzed hydroboration with catecholborane.^[23] Oxidation of **11** by the standard Moffatt–Swern^[24] protocol provided the corresponding aldehyde that was transformed to the alkyne through the Corey–Fuchs protocol (89 %).^[25] The PMB group was removed with DDQ (96 %), and then the alkynol intermediate was converted into vinyl iodide **12** using Negishi's carbocyclization methodology (65 %).^[26] The yield of **12** for this five step sequence was 54 % (attempts to perform the Negishi carbometallation sequence before the PMB group was removed were largely unsuccessful). The vinyl iodide **12** was elaborated to the allylic alcohol **13** in 89 % yield by a sequence of Moffatt–Swern oxidation,^[24] stabilized Wittig olefination, and DIBAL-H reduction. This intermediate was oxidized to the enal by MnO₂ (99 %), then the dienolate unit of **5** was introduced with greater than 95:5 (*Z,E:E,E*) selectivity through a Horner–Wadsworth–Emmons reaction with (*i*PrO)₂P(O)CH(OMe)–CO₂Me (85 %).^[27] Finally, removal of the TBS group with TBAF provided **5** (82 %).

With **4** and **5** in hand, the C11–C12 bond of **14** was generated in 65 % yield using Kishi's modification of the Suzuki cross-coupling reaction (Scheme 4).^[28] Hydrolysis of the methyl ester and treatment of the unpurified seco acid



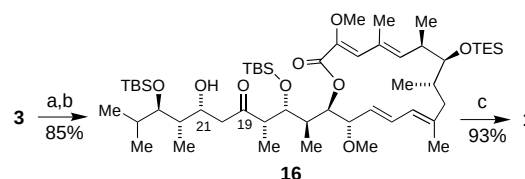
Scheme 4. Fragment assembly and synthesis of methyl ketone **3**. a) 1.0 equiv of **4** and **5**, 20 mol % Pd(PPh₃)₄, aq TIOH, THF, RT, 30 min, 65 %; b) 1 N KOH, dioxane, 80 °C, 1.5 h; c) 2,4,6-trichlorobenzoyl chloride, *i*Pr₂EtN, THF, then DMAP, toluene, reflux, 24 h, 52 % (two steps); d) TES–OTf, 2,6-lutidine, CH₂Cl₂, –50 °C, 20 min, 85 %; e) TFA, THF, H₂O, 5 °C, 6 h, 90 %; f) Dess–Martin reagent, pyridine, CH₂Cl₂, 0 °C, 4 h, 98 %. (See reference [10] for abbreviations.)

with 2,4,6-trichlorobenzoyl chloride and *i*Pr₂NEt in THF formed the mixed anhydride; the THF was then replaced with toluene, DMAP added, and the reaction mixture heated at reflux for 24 h. This provided macrolactone **15** in 52 % yield.^[29] The hindered C7 hydroxyl group of **15** was protected as a TES ether (85 %), then the C19 hydroxyl group was selectively deprotected (90 %) and oxidized to methyl ketone **3** in 98 % yield by using the Dess–Martin reagent.^[30]

Very poor yields of the macrolactone were obtained when the macrolactonization was attempted on seco acids with the C7–OH group protected as TBS or TES ethers. We speculated that a silyl group on the axial C7–OH group might experience serious nonbonded interactions with the equatorial C6 and C8

methyl groups in the pre-cyclization conformation,^[31] however comparable problems were not noted in the earlier bafilomycin^[8] and hygrolidin^[9] syntheses. Unfortunately, the attempted cyclization of a triol with the C7, C15, and C19 alcohols unprotected provided a 20-membered lactone preferentially. Moreover, attempted oxidation of the C19-hydroxyl group of macrocyclic intermediates containing an unprotected C7–OH group led to the preferential oxidation of the latter group.^[32] These factors dictated the careful orchestration of protecting groups for the C7, C15, and C19 hydroxyl groups of intermediates **14**, **15**, and **3**.^[33]

The completion of the synthesis required that we perform a stereoselective aldol reaction between aldehyde **2** and the methyl ketone unit of **3**. Previous studies of methyl ketone aldol reactions have revealed that the stereoselectivity is highly dependent on the metal enolate and the β -hydroxy protecting group of the aldehyde.^[17a–c] In particular, a β -silyl ether in the aldehyde component leads to diminished Felkin selectivity with metal enolates. Studies by Evans et al.^[11] and Paterson et al.,^[34] as well as unpublished work from our laboratory,^[35] demonstrated that aldehydes such as **2** undergo highly Felkin-selective aldol reactions under Mukaiyama conditions.^[36] Accordingly, methyl ketone **3** was treated with a premixed solution of TMSCl and Et₃N (1:1) at –78 °C followed by LiHMDS to generate the TMS enol ether (Scheme 5).^[37] Exposure of a mixture of the crude enol silane



Scheme 5. Synthesis of bafilomycin A₁. a) TMS–Cl, Et₃N, LiHMDS, CH₂Cl₂, –78 °C, 30 min; b) **2**, BF₃·OEt₂, –78 °C, 30 min, 85 %; c) TAS–F, DMF, H₂O, RT, 4 h, 93 %. (See reference [10] for abbreviations.)

and aldehyde **2** in CH₂Cl₂ to BF₃·OEt₂ for 30 min at –78 °C provided a >95:5 mixture of aldol products favoring **16** (69 %; 85 % based on recovered **3**). Finally, deprotection of aldol **16** with TAS–F^[38] in wet DMF provided synthetic bafilomycin A₁ (**1**) that was identified by comparison with a sample of natural bafilomycin A₁ (¹H, ¹³C NMR, and IR spectroscopy, optical rotation, and TLC mobility in several solvent systems). Use of TAS–F for this final deprotection^[38b] thus solves a problem that has complicated earlier efforts in this area.^[7–9]

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- [10] Abbreviations: 9-BBN = 9-borabicyclo[3.3.1]nonane; DDQ = 2,3-dichloro-5,6-dicyanoquinone; DIBAL-H = diisobutylaluminum hydride; DMAP = 4-dimethylaminopyridine; DMPM = 3,4-dimethoxybenzyl; DMS = dimethyl sulfide; DMSO = dimethyl sulfoxide; HMDS = hexamethyldisilazane; NMO = 4-methylmorpholine *N*-oxide; PMB = *para*-methoxybenzyl; TAS-F = tris(dimethylamino)sulfonium difluorotrimethylsilicate; TBAF = tetrabutylammonium fluoride; TBS = *tert*-butyldimethylsilyl; TES = triethylsilyl; TFA = trifluoroacetic acid; TMS = trimethylsilyl.
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Selective Hydrovinylation of Styrene in a Membrane Reactor: Use of Carbosilane Dendrimers with Hemilabile P,O Ligands**

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Despite the numerous advantages of homogeneous catalysis, a major drawback remains in the need for efficient catalyst recovery. For many catalytic applications seen today, the finding of an elegant and convenient solution to this problem had been crucial for their commercialization. Catalyst recovery also becomes increasingly important in fine chemicals production when sophisticated ligands are used, whose cost often exceeds that of the noble metal used.

The use of dendritic materials is currently generating enormous attention in a number of areas in science and

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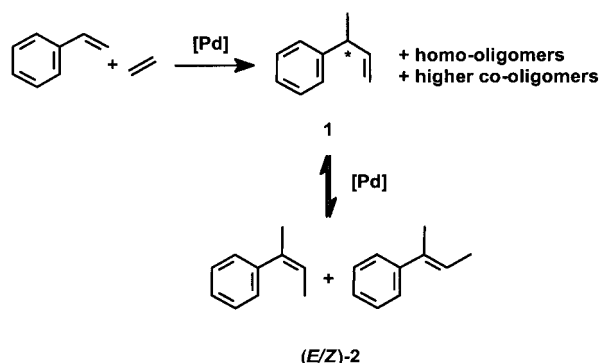
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technology.^[1–4] This includes transition metal containing dendrimers^[3] and their use in catalysis.^[5] Earlier we presented a carbosilane dendrimer decorated with 12 organonickel groupings as an active catalyst for the Kharasch addition of CCl_4 to alkenes.^[4] Both the well defined structure and the possibility of attaching a large number of active sites with respect to the total molecular weight make these dendritic materials especially interesting for catalyst immobilization.

Hydrovinylation of olefins^[6] provides an easy access to building blocks for fine chemicals.^[7] Recently we reported the first asymmetric hydrovinylation with a ligand bearing a stereogenic phosphorus atom. Enantioselectivities of up to 86 % *ee* at room temperature were achieved.^[8] In the hydrovinylation of styrene (Scheme 1) high activity and selectivity

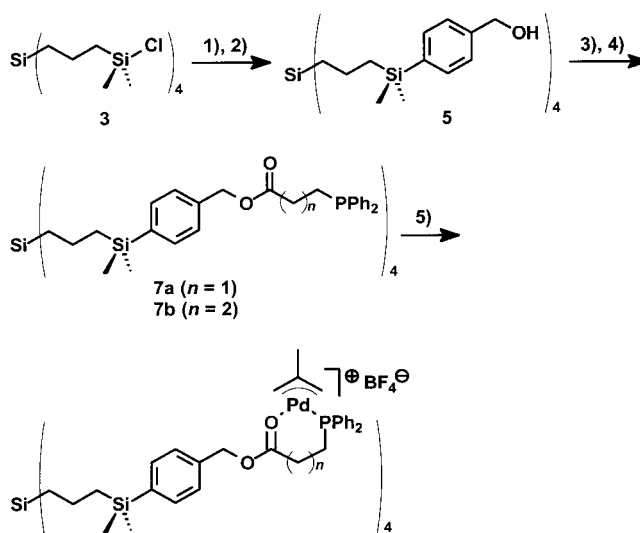


Scheme 1. Hydrovinylation of styrene and side reactions.

was observed with phosphanylcarboxylic acid derivatives.^[9] However, isomerization of the chiral product 3-phenylbut-1-ene (**1**) to internal achiral olefins (*E/Z*)-**2** takes place at higher conversions. Therefore, it would be useful to run the reaction continuously at lower conversion.

Herein we report on the synthesis of dendritic carbosilanes functionalized with various ω -diphenylphosphanylcarboxylic acid ester end groups. These dendritic hemilabile ligands were applied successfully in the palladium-catalyzed hydrovinylation of styrene. The new dendritic Pd-catalysts derived from hemilabile P,O ligands are stable under the reaction conditions, whereas similar complexes of monodentate ligands are susceptible to decomposition, namely to the formation of palladium black. For the first time the hydrovinylation was carried out continuously in a pressure membrane reactor using a nanofiltration membrane, which gave rise to the selective formation of the desired, chiral 3-phenylbut-1-ene (**1**).

In order to obtain a functionalized dendrimer suitable for catalysis in the hydrovinylation of styrene we coupled the chlorosilane $\text{G}_0\text{-SiMe}_2\text{Cl}$ **3** with a protected linker group, 4-bromobenzyl-*tert*-butyldimethylsilyl ether (Scheme 2). Lithiation with *t*BuLi followed by reaction with **3** afforded **4** in 89 % yield. Desilylation of **4** afforded the dendritic polyol **5** in 74 % yield. Coupling of **5** with the phosphanoxycarboxylic acid chlorides $\text{ClC(O)(CH}_2)_n\text{CH}_2\text{P(O)Ph}_2$ ($n=1,2$) afforded compounds **6a** and **6b**. The model compounds $\text{PhCH}_2\text{O-C(O)(CH}_2)_n\text{CH}_2\text{P(O)Ph}_2$ **8a** ($n=1$) and **8b** ($n=2$) were prepared in high yields in an analogous manner by coupling the corresponding acid chlorides with benzylic alcohol.^[10] The



Scheme 2. Synthesis of the functionalized carbosilane dendrimers. 1) $\text{LiC}_6\text{H}_4\text{CH}_2\text{OSiMe}_2(t\text{Bu})$, Et_2O , -20°C ; 2) $\text{Et}_3\text{N}\cdot 3\text{HF}$, THF, 17 h; 3) $\text{Ph}_2\text{P(O)CH}_2(\text{CH}_2)_n\text{C(O)Cl}$, 4-dimethylaminopyridine (DMAP), DMF, RT; 4) HSiCl_3 , NEt_3 , benzene, reflux, 17 h; 5) $[(\eta^3\text{-C}_4\text{H}_7)\text{Pd(cod)}]\text{BF}_4$, CH_2Cl_2 , 0°C , 1 h.

dendritic phosphane oxides **6a** and **6b** and the model compounds **8a** and **8b** were converted into the phosphane compounds **7a** and **7b** and **9a** and **9b**, respectively by reduction with trichlorosilane.^[11] The catalysts were prepared in situ by treatment of the ligands with equimolar amounts of $[(\eta^3\text{-C}_4\text{H}_7)\text{Pd(cod)}]\text{BF}_4$ (cod = 1,5-cyclooctadiene).

High selectivity to the codimers was observed when the resulting catalysts were applied in batch reactions (Table 1).

Table 1. Hydrovinylation of styrene: comparison of dendritic ligands and model compounds.^[a]

Entry	Ligand	Conv. [%] ^[b]	Yield [%] ^[c]	$S_{(1+2)}$ [%] ^[d]	$S_{(1)}$ [%] ^[e]
1	$\text{G}_0\text{-(CH}_2)_2\text{PPh}_2$ (7a)	68.1	56.8	97.1	85.8
2	$\text{C}_6\text{H}_5\text{CH}_2\text{OCO(CH}_2)_2\text{PPh}_2$ (9a)	96.9	49.5	90.7	56.4
3	$\text{G}_0\text{-(CH}_2)_3\text{PPh}_2$ (7b)	99.9	0.2	91.5	4.7
4	$\text{G}_0\text{-(CH}_2)_3\text{PPh}_2$ (7b)	3.4 ^[f]	3.2	95.0	99.9
5	$\text{C}_6\text{H}_5\text{CH}_2\text{OCO(CH}_2)_3\text{PPh}_2$ (9b)	99.9 ^[f]	4.4	93.4	0.2
6	$\text{G}_0\text{-(CH}_2)_3\text{PPh}_2$ (7b) ^[g]	8.1	7.6	96.3	98.3

[a] Conditions: T = room temperature; reaction time = 17 h; initial pressure 30 bar; $\text{P/Pd} = 1$; styrene/ $\text{Pd} = 500\text{--}1000$, CH_2Cl_2 (20 mL), styrene (4 mL; 34.8 mmol). [b] Conversion of styrene. [c] Yield of **1**. [d] $S_{(1+2)} = [(\text{yield } (1) + \text{yield } (2))/\text{conv.}] \times 100$. [e] $S_{(1)} = [(\text{yield } (1))/(\text{yield } (1) + \text{yield } (2))] \times 100$. [f] $t = 3$ h. [g] Continuous run for $t = 9$ h, for the conditions see Figure 1.

The model compounds $\text{PhCH}_2\text{OC(O)(CH}_2)_n\text{CH}_2\text{PPh}_2$ **9a** ($n=1$) and **9b** ($n=2$) showed higher activity than the corresponding G_0 dendritic phosphanyl esters **7a** ($n=1$) and **7b** ($n=2$) (entries 2 and 5 versus entries 1 and 4). The catalysts become more active as the Pd-P,O chelate ring increases in size from the six-membered ($n=1$) to the seven-membered ring ($n=2$), as a consequence of the decreased stability of the hemilabile chelate.

Complete conversion and almost complete isomerization of 3-phenylbut-1-ene (**1**) into the *E/Z* mixture of achiral

2-phenylbut-2-ene ((*E/Z*)-**2**) was observed within 17 h for the ligands derived from diphenylphosphanylbutyric acid **7b** and **9b** ($n=2$) (entries 3 and 5 in Table 1). However, as the isomerization is a consecutive reaction that occurs only at considerably high conversion with the phosphanylcarboxylic acid ester type of ligands, it can be suppressed efficiently by limiting the conversion (entry 4 in Table 1).

Therefore the next step was to run the catalysis in a continuous high-pressure membrane reactor with the new dendritic phosphane–Pd catalysts derived from **7a** and **7b**. In preceding experiments the retention of the dendritic model compound G_0 -Si(Me)₂(*t*Bu) **4** in the membrane reactor was determined to be 85%. Although this retention is still far from being sufficient for practical purposes, the results under catalytic conditions with the dendritic Pd-catalyst derived from ligand G_0 -(CH₂)₃PPh₂ **7b** look very promising (entry 6 in Table 1, Figure 1). It should be noted also that this catalyst is already much larger ($M_w=2867.97$ g mol⁻¹) than **4** ($M_w=1314.62$ g mol⁻¹).

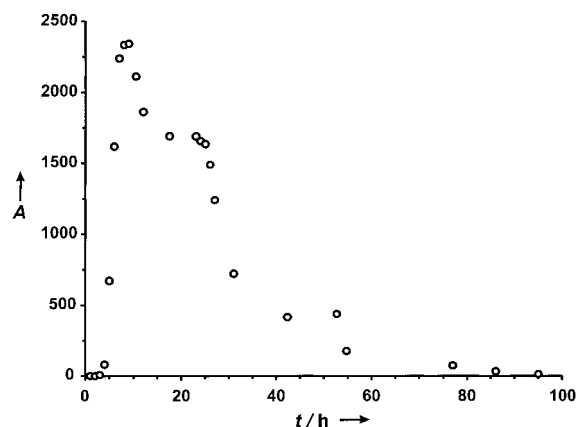


Figure 1. Continuous hydrovinylation of styrene in a membrane reactor using dendrimer **7b**. Conditions: $T=23^{\circ}\text{C}$, $p=30$ bar, 0.05 mmol Pd, P/Pd=1, flow rates: ethene solution 2.5 mL h⁻¹ (10M), styrene solution 2.5 mL h⁻¹ (1.8M), $\tau=4$ h, MPF-60 NF membrane (Koch Int., Düsseldorf, Germany). A =space time yield [mg L⁻¹ h⁻¹].

It takes about 9 h before the system reaches its maximum productivity of 2.3 g L⁻¹ h⁻¹.^[12] It can clearly be seen that the catalyst is washed out of the reactor, assuming that the decreased activity correlates with a lower concentration of the dendritic G_0 -Pd₄ catalyst. This decrease is accelerated most probably by the formation of small amounts of Pd (black) on the membrane surface, which can be seen after reaction.

Though the retention of the G_0 dendrimer is modest, the desired 3-phenylbut-1-ene (**1**) was produced over a period of 80 h. Most important is the observation that hardly any isomerization or other side products could be detected in the product solution (entry 6 in Table 1). Under these conditions a highly selective conversion is achieved by using a dendritic G_0 -Pd₄ catalyst and a continuous reaction mode, though the yields are quite low so far. It can be expected that the dendritic G_1 -Pd₁₂ catalyst derived from the next generation of ligands (G_1 -(CH₂)₃PPh₂)₁₂ will already allow sufficient hold back by a nanofiltration membrane to give efficient catalyst immobilization.

Experimental Section

Preparation of the cationic allyl-Pd-phosphane complexes: A solution of 1.00 equivalent of the phosphorus P(O) ligand (0.05 mmol) in CH₂Cl₂ was added to a solution of 0.05 mmol of $[(\eta^3\text{-C}_4\text{H}_7)\text{Pd}(\text{cod})]\text{BF}_4^{[13]}$ in CH₂Cl₂. After stirring the mixture for 60 min at 0 °C the resulting solution was used for catalysis.

Hydrovinylation

Batch reaction: The cold catalyst solution (0.05 mmol of $[(\eta^3\text{-C}_4\text{H}_7)\text{Pd}(\text{P(O))}]\text{BF}_4$ in 20 mL of CH₂Cl₂) was transferred into a 75-mL stainless steel autoclave by a syringe with a stainless steel cannula. The autoclave was cooled in an ice bath. Chilled styrene (4 mL, 34.8 mmol) was added and the autoclave was pressurized with ethylene (30 bar). After the reaction the autoclave was slowly vented, the reaction mixture was separated from the catalyst, and higher oligomers were purified by flash chromatography on basic alumina. The products were analyzed by gas chromatography.

Continuous catalysis: The membrane (MPF-60 NF membrane, Koch Int., Düsseldorf, Germany), stored in ethanol, was rinsed with acetone and carefully transferred into the membrane reactor. After the membrane had been thoroughly flushed by several 100 mL portions of CH₂Cl₂, the reactants (ethylene solution 1.8M in CH₂Cl₂, flow rate 2.5 mL h⁻¹; styrene solution 10M in CH₂Cl₂, flow rate 2.5 mL h⁻¹) were pumped through the reactor. The catalyst solution (0.05 mmol of $[(\eta^3\text{-C}_4\text{H}_7)\text{Pd}(\text{P(O))}]\text{BF}_4$ in 2 mL of CH₂Cl₂) was injected through an HPLC injection valve. Samples of the outgoing product solution were taken continuously and analyzed by gas chromatography.

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- [10] The structure of the novel functionalized dendrimers were confirmed by elemental microanalysis, ^1H , ^{13}C , and ^{31}P NMR spectroscopy, IR spectroscopy, and FAB-MS.
- [11] The compounds were fully characterized by ^1H , ^{13}C , and ^{31}P NMR spectroscopy, IR spectroscopy, FAB-MS, and elemental microanalysis. Elemental microanalysis of **7a** did not give satisfying results because of its air sensitivity.
- [12] There is also a significant induction period in the batch reactions, but this is much shorter than that obtained here. As the catalyst is fed into the reactor together with the styrene, complexes other than that initially present in the batch reactions could be formed. This is presently under investigation.
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Self-Assembly of Quinodimethanes through Covalent Bonds: A Novel Principle for the Synthesis of Functional Macrocycles**

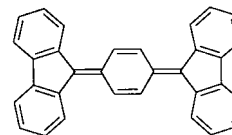
Junes Ipaktschi,* Rahman Hosseinzadeh, and Peter Schlaf

Dedicated to Professor Fritz Vögtle on the occasion of his 60th birthday

The development of methods for the synthesis of very large molecules such as dendrimers, supramolecules, and nanostructures with specific functions has increasingly become the center of attention in recent years.^[1, 2] We have developed a

synthetic strategy which is based on a directed tetramerization of basic building blocks in the final synthetic step.^[3] With this method it is possible to obtain large and uniform molecular structures in one step from small molecules.

We could recently demonstrate that the quinodimethane derivative **1** assembles itself to a spherical cyclic tetramer in high yield.^[3, 4] This spontaneous self-assembly process is driven by the four weak $\text{C}_{\text{sp}^3}\text{--}\text{C}_{\text{sp}^3}$ bonds between the fluorene units that are formed during the tetramerization, and also by the *gauche* arrangement of these units to one another.^[3, 5] Furthermore, these products have a remarkable property: Upon addition of energy they undergo a reversible color change to blue-violet as the reverse reaction to form the quinodimethane units occurs to a certain extent.^[3]



1

We have tested this synthetic strategy towards the construction of highly branched systems as well as multifunctional metallocenes, which combine the reversible color change with the potential applications of side chains. We report herein the preparation of metallocene **3** containing 16 ferrocene units on the periphery of the molecule, as well as the synthesis of dendrimer **5**.

The key compounds for the synthesis of **3** and **5** are diol **2** and dichloride **4**, respectively.^[6] These compounds can be obtained in only a few steps from the corresponding substituted fluorenone derivatives and 1,4-dilithiumbenzene.

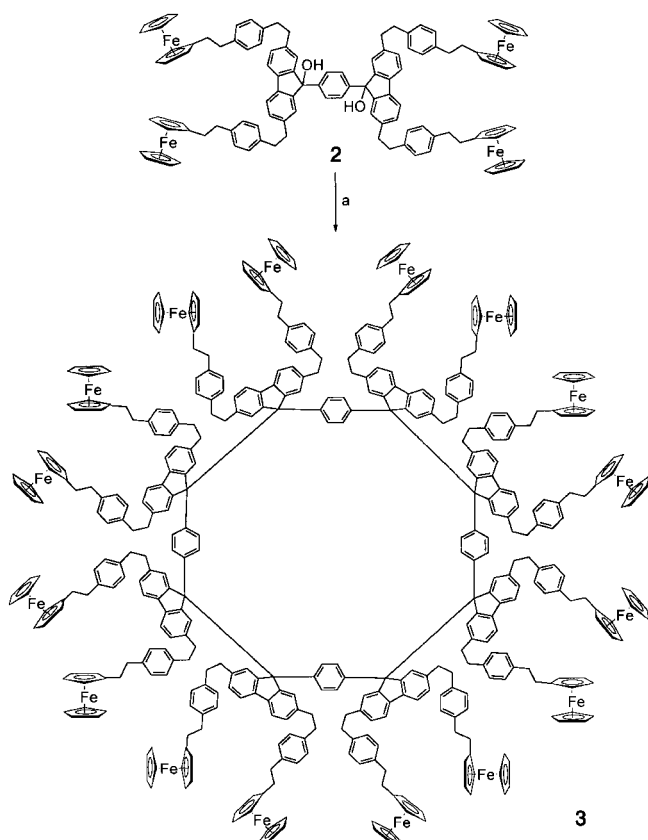
Reduction of diol **2** with a SnCl_2/HCl solution at room temperature in THF as the solvent led to the macrocycle **3** in 82% yield (Scheme 1). The results of the molecular mass determination using the analytical ultracentrifuge are in agreement with the molecular formula of $\text{C}_{448}\text{H}_{400}\text{Fe}_{16}$. NMR spectroscopic data confirm the structure of **3**.^[7] Owing to the S_4 symmetry of the molecular conformation, the ^{13}C NMR spectrum of **3** shows two characteristic signals at $\delta = 65.45$ and 65.24 for the C9 atoms of the fluorene units. Important information about the constitution of the macrocycle can be obtained from the ^1H NMR spectrum, which shows two signals for the protons of the inner phenyl rings at $\delta = 8.70$ and 8.26 (broad doublets) and two singlets each at $\delta = 5.93/5.90$ and $7.85/7.72$.^[8] Because of the S_4 symmetry of the molecule, the protons on the unsubstituted Cp ring of the ferrocenyl groups show up as four singlets at $\delta = 4.09$, 4.07 , 3.98 , and 3.97 .

It was determined by cyclovoltammetry that all ferrocene units of **3** are reversibly oxidized at $E_{1/2} = 0.49\text{ V}$.^[9] A redox splitting of consecutive electron transfer processes was not observed. The number of electrons transferred per molecule of **3** was determined by addition of $[(\eta^6\text{-C}_6\text{H}_6)_2\text{Cr}]$ in a ratio of 1:16 and comparison of the respective peak currents, which showed an approximate ratio of 1:1. The deviation in the peak currents is caused by the different diffusion coefficients of the two molecules, which result from the differences in size.

The principle of the synthesis of **3** was applied towards the preparation of **5**. In this case, the synthesis started from dichloride **4**, which afforded **5** in 63% yield upon reaction

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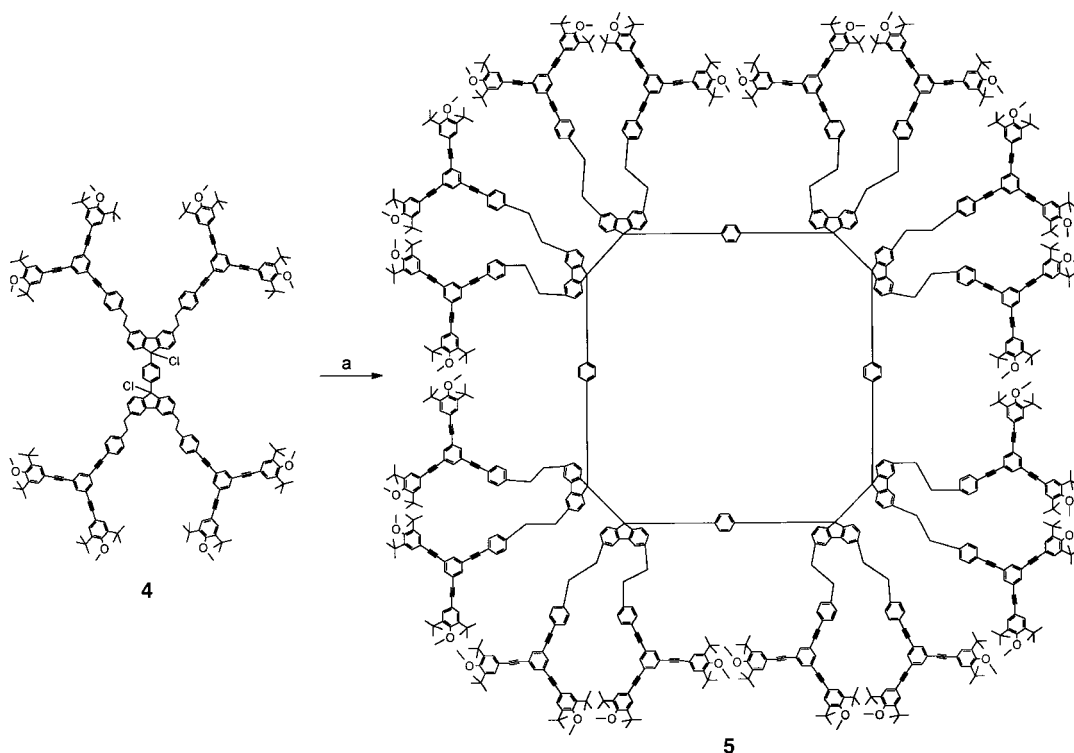
Scheme 1. a) 3.7 M SnCl_2 /conc. HCl solution, THF, 4 h, reflux.

with $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ (Scheme 2). The molecular mass of 12642 Dalton was confirmed with matrix-supported laser desorption/ionization time of flight (MALDI-TOF) mass spectrom-

etry. As in the previous example, the large substituents did not prevent or alter the desired tetramerization process. Owing to the substitution pattern at the fluorene ring, the intramolecular dynamics of the molecule leads to the observation of a time-averaged NMR spectrum for **5**, which, as a result, appears to have a molecular symmetry twice as high as the symmetry of **3**. Thus, two of the four protons of the inner phenyl ring appear as a broad singlet^[8] at $\delta = 8.06$ in the ^1H NMR spectrum, and the signals for H2/H7 and H1/H8 of the fluorene units appear at $\delta = 6.52$ and 6.06 .^[10] Additional NMR spectroscopic indications for the seemingly higher symmetry of **5** in comparison to that of **3** are the number of signals for the protons of the *tert*-butyl groups ($\delta = 1.39$ and 1.37), the methoxy groups ($\delta = 3.65$ and 3.63), and the C9 atoms of the fluorene units ($\delta = 64.97$).

We assume that during the reduction of **2** or **4** with SnCl_2 the corresponding substituted quinodimethane derivative of type **1** is formed first. This derivative subsequently tetramerizes selectively to give **3** and **5**, respectively, in a stepwise process. We believe that these macrocycles are formed in very high yields because the newly formed, relatively weak C–C σ bonds are reversibly cleaved during the cyclization process.^[11] This would mean that errors in the construction process can easily be reversed and corrected. In addition, steric factors that lead to the *gauche* arrangement of the fluorene units linked at the 9-position facilitate a ring closure to **3** and **5**.

Analogous to the parent system, **3** and **5** turn blue-violet upon heating above about 50°C in a solvent such as cyclohexane (**3**: $\lambda_{\text{max}} = 582\text{ nm}$; **5**: $\lambda_{\text{max}} = 587\text{ nm}$). Upon cooling the solution becomes colorless. Under an inert atmosphere, this process can be repeated infinitely. The blue-violet color also appears upon grinding of these compounds.



Scheme 2. a) $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$, benzene, 30 min, RT.

The herein discussed principle of self-assembly of four basic units to form one large macrocyclic molecule can be used as a basis for the synthesis of novel macromolecules which also exhibit the characteristic to change their color reversibly upon mechanical impact.

Experimental Section

3: Diol **2** (150 mg, 88 μmol) was dissolved in degassed THF (20 mL) in a Schlenk tube. A SnCl_2/HCl solution (2 mL, 5.3 mmol) was added, and the resulting mixture was stirred under reflux for 4 h. After removal of the solvent, the residue was suspended in CH_2Cl_2 (5 mL) and purified by chromatography on silica gel with CH_2Cl_2 /petroleum ether (1/2–1/1). A yellow powder (120 mg, 18 μmol , 82%) was obtained; m.p. 150 °C (decomp.). ^1H NMR (400 MHz, CDCl_3): δ = 8.70 (br d, J = 7.70 Hz, 4H), 8.26 (br d, J = 7.70 Hz, 4H), 7.85 (br d and s, 8H), 7.72 (s, 4H), 7.48–6.55 (m, 84H), 6.14 (s, 16H), 5.93 (s, 4H), 5.90 (s, 4H), 4.12–3.80 (m, 64H), 4.09 (s, 20H), 4.07 (s, 20H), 3.98 (s, 20H), 3.97 (s, 20H), 2.95–2.15 (m, 128H); ^{13}C NMR (100 MHz, CDCl_3): δ = 147.93–137.11 (22 signals for C), 133.08–118.19 (22 signals for CH), 89.22–67.20 (13 signals for Cp), 65.45 (C), 65.24 (C), 38.56–31.21 (14 signals for CH_2); molecular mass calcd for $\text{C}_{448}\text{H}_{400}\text{Fe}_{16}$: 6677.7; found: 7200 ± 800 (analytical equilibrium–ultracentrifugation); elemental analysis calcd for $\text{C}_{448}\text{H}_{400}\text{Fe}_{16}$: C 80.58, H 6.04; found: C 80.28, H 6.00.

5: Dichloride **4** (161 mg, 50 μmol) was stirred for 30 min with $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ (1 g) in degassed benzene (5 mL) under argon in a Schlenk tube at room temperature. After that, the mixture was allowed to stand for about 14 h. The solution was filtered through ALOX (basic) and the filtrate was evaporated. The residue was filtered through silica gel with CH_2Cl_2 /*n*-pentane (1/1). A light orange solid (100 mg, 7.9 μmol , 63%) was obtained; m.p. 180 °C (decomp.). ^1H NMR (400 MHz, CD_2Cl_2): δ = 8.06 (brs, 8H), 7.63–7.58 (m, 24H), 7.56 (d, J = 1.4 Hz, 16H), 7.51 (d, J = 1.3 Hz, 16H), 7.46–7.30 (m, 112H), 7.21 (brs, 8H), 7.18 (d, J = 8.1 Hz, 16H), 7.02 (d, J = 8.2 Hz, 16H), 6.95 (d, J = 7.6 Hz, 8H), 6.52 (d, J = 7.5 Hz, 8H), 6.06 (d, J = 7.5 Hz, 8H), 3.65 (s, 48H), 3.63 (s, 48H), 3.07–2.84 (m, 32H), 2.83–2.64 (m, 32H), 1.39 (s, 288H), 1.37 (s, 288H); ^{13}C NMR (100 MHz, CD_2Cl_2): δ = 160.86–140.80 (11 signals for C), 133.85–129.03 (6 signals for CH), 124.78–120.60 (5 signals for C), 119.32 (CH), 117.41 (C), 91.85–86.74 (8 signals for $\text{C}\equiv\text{C}$), 64.97 (C), 64.76 (OMe), 64.75 (OMe), 38.37 (CH_2), 37.95 (CH_2), 36.05 (C), 36.03 (C), 32.12 (Me); MS (MALDI-TOF): m/z : 12643.9; elemental analysis calcd for $\text{C}_{928}\text{H}_{976}\text{O}_{32} \cdot 3\text{CH}_2\text{Cl}_2$: C 86.71, H 7.67; found: C 86.69, H 8.10.

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- [8] For derivatives which are substituted at positions 2 and 7 on the fluorene ring the inner phenyl protons show four broad doublets (*ortho* und *meta* coupling), whereas the fluorene protons show four groups each with one singlet and two doublets. In the ^{13}C NMR spectrum the eight C9 atoms of the fluorene unit show two signals at δ = 65–66. For the 3,6-substituted derivatives the protons of the inner phenyl rings show two broad singlets (only *meta* coupling), whereas the protons of the fluorene unit form two groups of one singlet and two doublets each. The eight C9 atoms in the ^{13}C NMR spectrum give rise to one signal at $\delta \approx 65$. The characteristic signals of these compounds spread over the range of $\delta \approx 8.70$ –5.60 in the ^1H NMR spectrum. The individual assignments of all signals is difficult as they are partially hidden by substituent absorptions or overlap which each other. Therefore, the low-field and high-field signals were used for the structure elucidation.
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Tuning Dihydrogen Bonds: Enhanced Solid-State Reactivity in a Dihydrogen-Bonded System with Exceptionally Short H...H Distances**

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The unconventional hydrogen bonds (dihydrogen bonds) between the hydridic hydrogen centers, or more accurately the σ -bonding electrons of M–H bonds (M = B, Ga, Ir, Re, W, Os), and traditional hydrogen-bonding partners H–X (X = N, O) have received considerable attention in the last five years.^[1] Very recently, we demonstrated that dihydrogen bonds can be used for topochemical assembly of covalent materials, because of their ability to react by H₂ loss, trading the weak H...H interactions for strong covalent bonds.^[2] Solid-state decomposition of the triethanolamine (TEA) complex of NaBH₄, whose crystal structure shows multiple dihydrogen bonds between the BH₄[−] ions and the OH groups from TEA, proved to be topochemical, leading to a polymeric trialkoxyborohydride which is not achievable from solution or melt. However, the relatively low melting point and solid-state reactivity of this dihydrogen-bonded system made its solid-state decomposition extremely slow (weeks!), which hampered the systematic study of this topochemical process. Moreover, a phase transition preceding the H₂ loss complicated the topochemical relationship between the structures of the initial complex and the final decomposed material.

A special consideration of the acidity and basicity of the protonic and hydridic partners is essential, in order to allow the crystallization of these systems prior to their decomposition by H₂ loss, but still to induce relatively high solid-state reactivity. Substitution of Li for Na in the NaBH₄·TEA complex appeared to be the most straightforward way to tune the solid-state reactivity in this particular system. The Li⁺ would complex the TEA more strongly, making the OH sites more acidic and consequently more reactive. Here we describe the structure and reactivity of LiBH₄·TEA (**1**), a dihydrogen-bonded system with very short H...H distances and enhanced solid-state reactivity toward H₂ loss with topochemical covalent bond formation.

The complex **1** was crystallized by slow evaporation of a 1:1 TEA/LiBH₄ mixture in 2-propanol, as a white crystalline compound which does not melt up to 300 °C. It differs spectroscopically^[3] from LiBH₄, suggesting different environments for the BH₄[−] and Li⁺ ions. The X-ray crystal structure^[4] of **1** is presented in Figure 1. Each Li⁺ is pentacoordinated by the N and three O atoms from a TEA molecule and by an O atom from a different TEA molecule, forming {Li⁺(tea)}₂

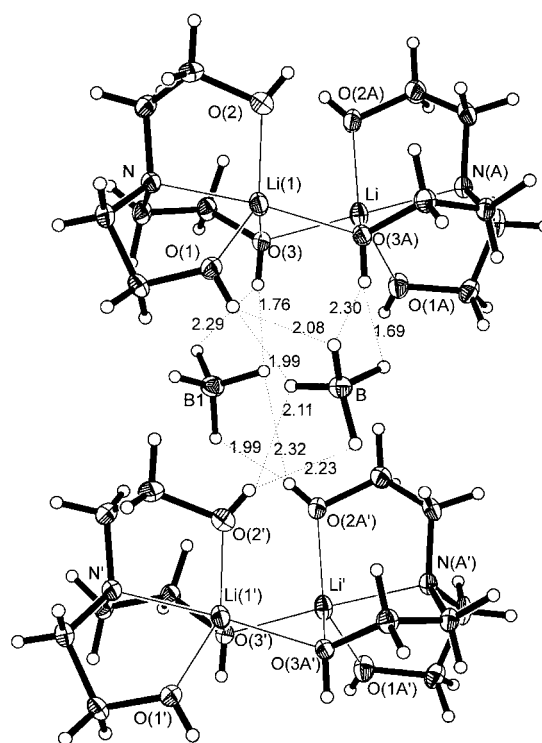


Figure 1. Dihydrogen bonding network in crystals of **1**. H–H contact distances [Å] are not normalized. Selected bond lengths [Å]: O(1)–Li(1) 2.05, O(2)–Li(1) 1.94, O(3)–Li(1) 2.11, N–Li(1) 2.16, O(3A)–Li(1) 2.05, O(1A)–Li 1.95, O(2A)–Li 1.97, O(3A)–Li 2.17, N(A)–Li 2.15, O(3)–Li 2.02, O(1)–H(1) 0.81, O(2)–H(2) 0.74, O(3)–H(3) 0.87, O(1A)–H(1A) 0.88, O(2A)–H(2A) 0.75, O(3A)–H(3A) 0.89, B–H(B) 1.11, 1.16, 1.13, 1.11, B1–H(B1) 1.14, 1.06, 1.15, 1.18.

dimers, in contrast to the Na analogue, where {Na⁺(tea)}_n chains were present.^[2]

Multiple dihydrogen bonds connect the dimers through BH₄[−] pairs, giving rise to extended ribbons. The dihydrogen bonding network is asymmetrical: one BH₄[−] ion forms hydrogen bonds with three OH groups (O(2'), O(1), and O(3A)) in a total of six dihydrogen bonds, while the second BH₄[−] ion only forms hydrogen bonds with two of the remaining OH groups (O(2A') and O(3)) in a total of four dihydrogen bonds. The O(1A)H groups are not involved in any dihydrogen bonding; instead they form conventional hydrogen bonds with O(1)s from the neighboring ribbon, creating thus overall two-dimensional layers. The H–H contact distances range between 1.69 and 2.32 Å.^[5] All hydrogen atoms were unambiguously located from the difference Fourier map, making these distances sufficiently reliable. The shortest dihydrogen bonds are 1.69 and 1.76 Å, which after the normalization of the O–H and B–H bonds become 1.62 and 1.67 Å, respectively.^[6] These are the shortest H–H distances reported so far for dihydrogen bonds.^[7] Notably, both OH groups involved in these two dihydrogen bonds contain bridging O atoms coordinating two Li⁺ ions, which undoubtedly results in increased acidity of their corresponding protons, and thus enhanced hydrogen-bonding ability. All the other OH groups complex only one Li⁺ ion, leading thus to H–H separations that are significantly longer. The OH...H–B angles range between 75.8 and 106.1° (av 95.0°), while

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the O–H...HB angles are larger, ranging between 130.0 and 170.7° (av 150.8°).

The very short H–H distances and the expected acidity increase of the OH groups due to their complexation of Li⁺ ions predicted enhanced solid-state reactivity for this system. Indeed, when heated for 1 h at 120 °C under argon, the complex completely decomposed, as indicated by X-ray powder diffraction (Figure 2), ¹¹B solid-state MAS NMR and IR spectra of the resulting material. Only 24 h were necessary for decomposition at 82 °C, the temperature

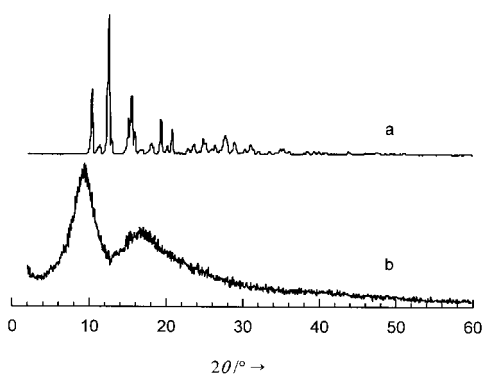


Figure 2. X-ray powder diffraction pattern of **1** (a) and final decomposed material (b).

used for the solid-state decomposition of NaBH₄·TEA, which took about six weeks! Three moles of H₂ per mole of **1** were lost, as indicated by the hydride content of the initial complex and final decomposed material. The resulting solid is insoluble in common organic solvents and does not melt up to 300 °C, indicating a polymeric nature. Its ¹¹B solid-state MAS NMR spectrum exhibits a single peak at δ = –4.6, which, together with the hydride content of one per molecule, suggests a trialkoxyborohydride structure for the decomposed material. The ν_{B–H} of 2247 cm^{–1} for the same material is also significantly shifted from the 2290 cm^{–1} in the initial complex. No phase transition was observed in this case when the decomposition was monitored by X-ray powder diffraction.

The loss of three moles of H₂ from **1** points to a topochemical relationship between the final decomposed material and the initial complex, where two BH₄[–] ions and six OH groups are in close proximity due to the crystal packing imposed by the dihydrogen bonding network. Unlike in the NaBH₄·TEA case where two-dimensional covalent layers were likely to have formed, a topochemical reaction in the present example is expected to yield a one-dimensional covalent structure, according to the crystal structure of **1**. However, due to the modest crystallinity of the final material^[8] (Figure 2b), a definitive knowledge of its structure remains elusive.

In direct contrast, decomposition of **1** in DMSO at 120 °C under argon for four days, yielded a material with no hydridic hydrogen left as shown by its hydride content and IR spectrum. Its ¹¹B solid-state MAS NMR chemical shift (δ = –5.9) is slightly different from the corresponding value in the solid-state decomposition product. In a parallel reaction run in [D₆]DMSO, unconverted BH₄[–] and completely alcoholized

B(OR)₄[–] were the only boron species found by ¹¹B NMR spectroscopy in solution throughout the reaction. Such behavior is expected for a reaction in which the BH_x(OR)_{4–x}[–] intermediates are more reactive than the starting BH₄[–], and susceptible to disproportionation.^[9] Thus, the decomposition process in solution differs qualitatively from that in the solid state, demonstrating again the ability of dihydrogen bonding to exert topochemical control in the BH...HO to B–O conversion.

In summary, we have described a new dihydrogen-bonded system with extremely short H...H distances, presumably induced by the increased acidity of the proton donor OH sites due to strong complexation by Li⁺ ions. This system also displays the high solid-state reactivity expected from these structural characteristics. The present study offers the best defined example to date of topochemical assembly of covalent materials using dihydrogen bonds, a developing application of this new and intriguing intermolecular interaction.

Experimental Section

1: LiBH₄ (0.22 g, 10 mmol) and TEA (1.4 mL, 10 mmol) were dissolved in 2-propanol (20 mL). Slow evaporation of most of the solvent under argon followed by addition of CH₂Cl₂ (20 mL) afforded 0.95 g (56 %) of complex **1**. X-Ray quality crystals were grown by diffusion of a diethyl ether layer into a solution of **1** in 2-propanol. ¹H NMR (300.1 MHz, [D₃]CH₃CN, 25 °C): δ = 4.21 (s, 3H; OH), 3.60 (t, J = 5.1 Hz, 6H; CH₂), 2.58 (t, J = 5.1 Hz, 6H; CH₂), –0.23 (q, J (B,H) = 81.0 Hz, 4H; BH₄); ¹³C NMR (125.89 MHz, [D₃]CH₃CN, 25 °C): δ = 53.5 (CH₂), 58.4 (CH₂); ¹¹B NMR (96.23 MHz, [D₃]CH₃CN, 25 °C, B(OCH₃)₃): δ = –55.98 (quintet, J(B,H) = 80.9 Hz; BH₄); ¹¹B MAS NMR (128.33 MHz, 5997 Hz, 25 °C, B(OH)₃): δ = –48.7 (Δν_{1/2} = 1586 Hz); ⁷Li MAS NMR (155.44 MHz, 6000 Hz, 25 °C, LiCl): δ = 2.8 (Δν_{1/2} = 1735 Hz); IR (KBr): ν̄ = 2290 cm^{–1} (B–H); elemental analysis calcd for C₆H₁₉O₃NBLi (%): C 42.15, H 11.11, N 8.19; found: C 42.39, H 11.56, N 8.17.

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- [4] Crystal data for **1**: Data were collected on a Siemens SMART CCD diffractometer with MoK_α radiation (λ = 0.71073). The structure was solved by direct methods and refined by full-matrix least-squares on F² using SHELXTL package (structure analysis program 5.1, Bruker AXS, Inc., Madison, WI, **1997**). Absorption corrections were applied using SADABS, part of the SHELXTL package. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were located from

difference Fourier map and refined isotropically. Crystal dimensions: $0.44 \times 0.23 \times 0.21$ mm; $T = 173$ K; monoclinic, space group $P2_1$, $a = 7.7156(5)$, $b = 17.0039(11)$, $c = 7.8656(5)$ Å, $\beta = 98.133(1)^\circ$, $V = 1021.55(11)$ Å³, $Z = 4$, $\rho_{\text{calc}} = 1.112$ g cm⁻³; 5330 reflections collected, 3229 independent; $2\theta_{\text{max}} = 50^\circ$; 370 parameters; $R_1 = 0.0417$, $wR_2 = 0.0768$ for $I > 2\sigma(I)$; residual electron density: 0.145 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-105289. 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).

- [5] The typical normalization of O–H and B–H bonds to 0.96 and 1.21 Å, respectively, leads to the more realistic H–H distances of 1.62–2.28 Å.
- [6] These corrections are still likely to overestimate the H–H contact distances as they represent typical values for noninteracting partners. A similar analysis of the three close BH...HO contacts in the X-ray structure of NaBH₄·2H₂O (NaBD₄·2D₂O) yielded distances 0.04 Å longer on average than the final neutron diffraction values.
- [7] We found the 1.73 Å as the shortest reliable H–H contact distance for a dihydrogen bond reported so far (ref. [1a]). Another value of 1.68 Å was reported, but the disorder of the proton donor solvent as well as the uncertainty in its N–H proton location make this distance somewhat less reliable. See: B. P. Patel, W. Yao, G. P. A. Yap, A. L. Rheingold, R. H. Crabtree, *Chem. Commun.* **1996**, 991–992.
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Contactless Electrodeposition of Palladium Catalysts**

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In principle, electrodeposition offers a convenient method of modulating the properties of metal catalysts. It has been used extensively to prepare metal catalysts on conductive substrates such as carbon, metals, or conductive oxides.^[1–4] Underpotential deposition to form surfaces with modified catalytic behavior could be considered a related technology.^[5, 6] A critical limitation of electrodeposited catalysts is that the deposited area is essentially limited to the electrode surface. To increase the available surface area of electrodeposited catalysts, electrodeposition of metal structures within polymers such as polyvinylpyridine,^[7, 8] poly(vinylacetic acid),^[9] and nafion^[10–13] has been actively explored. A recently developed, related approach is electrodeposition in thin gel coatings.^[14] However, such approaches are limited to

coatings only a few micrometers thick because of the difficulty of assuring an ohmic or electron-hopping contact with the growing electrodeposit while maintaining a highly permeable structure for rapid diffusion of the reagents and high surface area. Furthermore, small isolated deposits cannot usually be obtained by this method because of the necessity of having an electrically contiguous structure that extends from the electrode into the matrix. Here we describe an electric-field method for preparing electrodeposited catalysts which does not require physical contact between the power source and the deposits. This approach, referred to as bipolar electrodeposition, not only enables electrodeposition across several centimeters of the support but also allows the position of the deposit to be controlled.

Other attempts to increase the thickness of electrodeposited catalysts include the use of electrically conductive polymers such as polypyrrole,^[15–17] polyaniline,^[18–21] or viologen-based polymers.^[22] However, due to anisotropic field distributions and the finite resistivity of the conductive polymer, it is unlikely that homogeneous electrodeposition will be possible in deposits more than a few micrometers thick with this approach. Furthermore, the choice of support is limited to conductive polymers in electrical contact with an electrode.

One method that avoids electrical contact with the conductive support is photoelectrodeposition onto semiconductor particles. This has been used extensively as a method of electrodepositing catalytically active metals (e.g. Au, Pt, Pd, Ag, Rh, Ir) onto a dispersed semiconducting support (e.g. TiO₂, ZnO, SnO₂, ZrO₂, ThO₂, CdS, WO₃).^[23–39] In this case photons promote electrons from the valence band into the conduction band of the semiconductor particles to give a situation in which anodic and cathodic processes occur on different regions of the same particle. Although this method was successful in producing catalysts, it requires a semiconductor whose bandgap is tuned to the wavelengths that can penetrate into the sample. Furthermore, due to absorption of light, homogeneous exposure across large volumes is not possible without prolonged mixing.

Here we describe a novel method for creating electrodeposited catalysts over large volumes of nonconductive supports with a much more homogenous distribution than available by other electrodeposition techniques. Our method relies on the polarization of conductive micrometer-scale particles in a nonconductive matrix. This represents another application of our approach to toposselectively (site-selectively) functionalize or otherwise modify particles by using electric fields. We earlier demonstrated toposselective electrodeposition onto isolated millimeter-^[40, 41] and micrometer-sized^[42] copper particles to form wires with diameters as small as 0.3 μm. We refer to the objects generated from such toposselective manipulation as toposomes.^[43]

The preparation of the catalyst is shown schematically in Figure 1. Graphite particles (nominally 1–2 μm in diameter) were dispersed onto one side of 60-μm-thick cellulose paper by nebulizing an acetone suspension of the particles. The papers were then stacked to a thickness of 0.5 cm and sandwiched between two flat graphite electrodes with dimensions of 2 × 2 cm. After immersion in a 1 mM solution of PdCl₂

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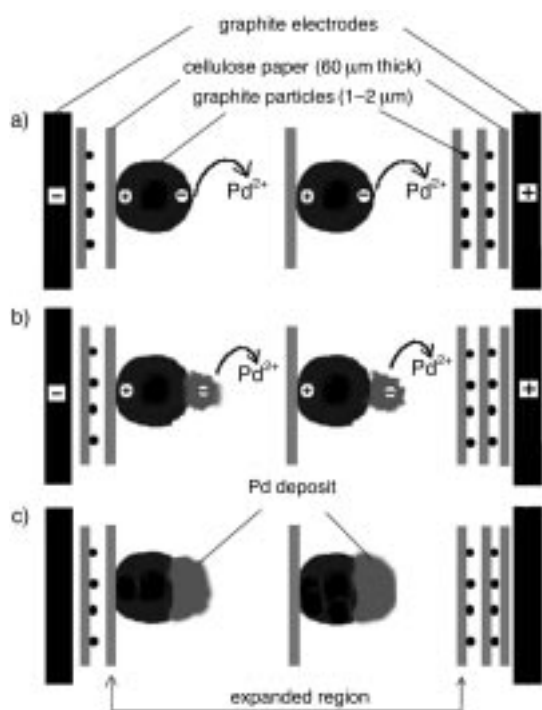


Figure 1. Schematic representation of the preparation of the bipolar electrodeposited catalyst. a) An electric field is generated across graphite particles sandwiched between layers of thin cellulose paper by applying a voltage drop with two flat graphite electrodes. b) Since the system is bathed in a 1 mM solution of PdCl_2 in toluene/acetonitrile (1/1), Pd is electrodeposited on the cathodically polarized region of each graphite particle. c) After the field is removed, the catalyst is washed and dried. It can now be used as a catalytically active material.

in toluene/acetonitrile (1/1), an electric field of controlled intensity and duration was applied by imposing a voltage drop between the graphite electrodes. The paper support was then washed thoroughly with acetone to remove unchanged salt and dried.

Application of an electric field polarizes the electrically isolated graphite particles and results in separate anodic and cathodic regions on the same particle (Figures 1 and 2). As expected, the reduction process involves electrodeposition of the metal. Although we have not yet identified the oxidation processes, the involvement of the solvents, the halide counterions, or adventitious water is likely.

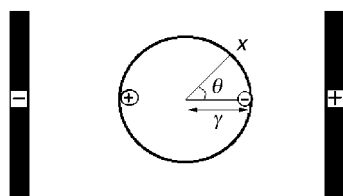


Figure 2. Polarization of a spherical particle in an electric field. See text and Equations (a) and (b).

To a first approximation, for a spherical particle of radius r exposed to an electric field E , an overpotential η is induced at a position x , expressed as an angle θ relative to the direction of

the applied electric field.^[44, 45] [Eq. (a)]. It follows that the maximum potential difference ΔV_{max} across the particle is given by Equation (b). Significant deviations from Equation (b) are expected for nonspherical particles such as the graphite platelets in the present work. However, Equation (b) does suggest that the particle radius and the electric field required to induce electrodeposition in a given system are inversely proportional to one another.

$$\eta_x = Er \cos \theta \quad (\text{a})$$

$$\Delta V_{\text{max}} = 2Er \quad (\text{b})$$

Since each particle serves as both anode and cathode, the process is referred to as bipolar electrochemistry.^[44–54] This phenomenon has been investigated for applications in metal recovery,^[46–49] electrosynthesis,^[50–53] and electrochemical etching of diamond.^[54] However, in these cases, millimeter-scale particles or disks were used because the field required to induce a sufficient overpotential at the particle surface increases with diminishing particle radius [see Eq. (b)]. This is particularly difficult in aqueous salt solutions, for which high conductivity prevents the application of high fields at the feeder electrodes. Micrometer and submicrometer metal particles have been used to carry out bipolar electrochemistry, but aprotic solvents or conductivity water must be used.^[55–57] Bipolar electrodeposition onto electrically isolated micrometer-scale particles has received limited attention.^[42] Although aqueous media are generally too conductive for particles of this size, organic solvents such as toluene/acetonitrile offer a sufficiently resistive environment for high fields (5000 V cm^{-1}) to be applied while still allowing electrodeposition to occur.

A key concept in the present work is to control the properties of catalytic systems by modulating electric field parameters during their preparation. As a first step, the hydrogenation of crotonaldehyde with palladium as catalyst was employed as a model reaction. Figure 3A shows the effect of the electric field intensity on the amount of Pd deposited, as monitored by atomic absorption spectroscopy (AAS). A strong correlation between the amount of Pd deposited and catalytic activity for hydrogenation can be seen in Figure 3B. Exposure to electric fields in the absence of graphite particles led to no increase in Pd content (see Figure 3A, control curve). Apparently, some palladium is not removed by thorough washing, and there are contributions from the paper and from the graphite itself. This residual palladium is presumably still in ionic form as no catalytic activity is detected for such samples (Figure 3B).

Two types of assay were used to investigate the effect of electric field strength on the activity of the catalyst. In Figure 3B the same total weight of support was used, whereas in Figure 3C the total amount of Pd was kept constant. Both methods clearly show a strong correlation between the electric field intensity during catalyst preparation and the catalytic activity. Note that in Figure 3C, no background correction was made for the residual palladium ions that are present in the absence of an electric field. Such a correction is not straightforward because the application of the electric

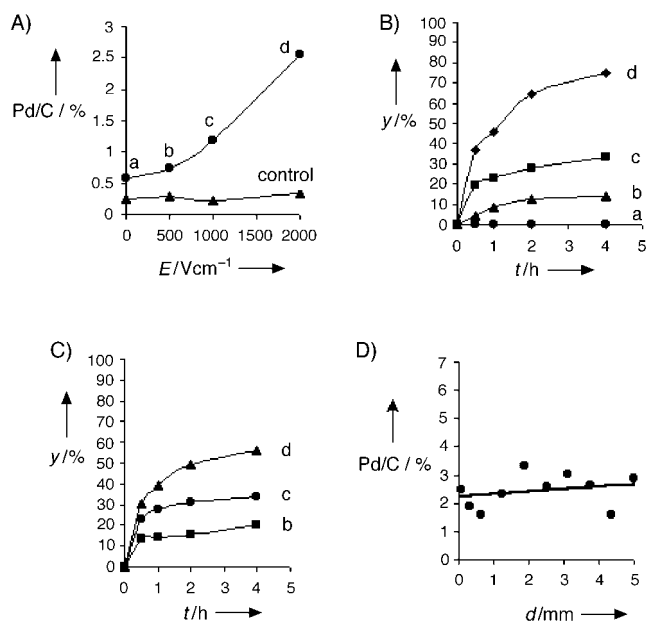


Figure 3. Characterization of four sets of catalysts, prepared by the method of Figure 1, at the following field strengths: a) 0, b) 500, c) 1000, d) 2000 $V\text{cm}^{-1}$. In all cases the duration of exposure to the field was 15 min, the solution was 1 mM PdCl_2 in toluene/acetonitrile (1/1), and the graphite powder amounted to 2.5 wt % of the total dry paper support. The two flat graphite electrodes ($2 \times 2\text{ cm}$) were 0.5 cm apart. Immediately after application of the field the papers were washed exhaustively with acetone then dried and kept in a desiccator. A) The upper curve represents the Pd/C ratio for each catalyst as determined by AAS on samples digested in aqua regia. The control curve corresponds to the theoretical Pd/C percentage ratio for experiments carried out in the absence of graphite (calculated for the same amount of graphite as in the case of the upper curve). Presented are the results of three experiments. The average standard deviation was ± 0.16 for the upper curve and ± 0.04 for the control curve. B) Conversion y (GC analysis) of crotonaldehyde (60 mm in ethanol) to butyraldehyde under 1 atm of hydrogen for the same mass (400 mg) of total dry paper support and catalyst. Presented are the results of three experiments. The average standard deviation was ± 3.68 . C) As for (B), except that the total amount of Pd (determined by AAS) was kept constant at 0.114 mg. Presented are the results of three experiments. The average standard deviation was ± 4.41 . D) Pd content determined at various positions between the anode (leftmost point) and cathode (rightmost point). The field strength was 2.0 kV cm^{-1} .

field appears to substantially deplete this background level. This is evident from the atomic absorption data of the experiment at 500 $V\text{cm}^{-1}$, which show virtually the same loading as in the zero-field experiment but with vastly different catalytic activity (see Figures 3 A and B). A possible explanation may be the conversion of Pd ions adsorbed onto the graphite into catalytically active metal, without substantial additional plating from the surrounding solution under low-field conditions.

To ensure that the palladium was indeed deposited by a bipolar mechanism evenly throughout the volume of the inert matrix, the palladium content was analyzed at various positions between the two electrodes. If the palladium were simply deposited at the cathodic feeder electrode, then the palladium content should exhibit a maximum in the cathodic region and decrease rapidly towards the anode. As shown in Figure 3 D, the palladium is fairly evenly dispersed through-

out; hence, the catalyst was prepared by bipolar electrodeposition.

Evidence for the toposselectivity of the bipolar electrodeposition was obtained by exposing the palladium-derivatized graphite particles to a solution of gold tribromide and reversing the field direction. A transmission electron micrograph of a particle obtained by such a treatment is shown in Figure 4. Energy dispersive X-ray analysis showed that the gold and palladium deposits are located at opposite ends of the particle.

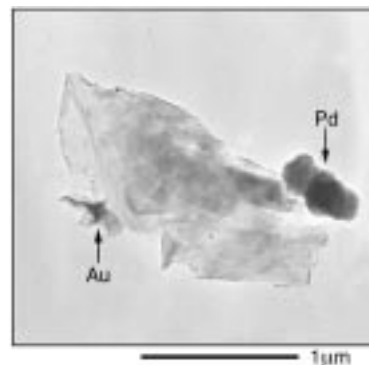


Figure 4. Transmission electron micrograph of an isolated graphite particle with Pd and Au introduced sequentially at opposite ends by bipolar electrodeposition with opposite field directions for each metal. Under the experimental conditions described in Figure 3, the powder was first exposed to 2.0 kV cm^{-1} for 15 min in the presence of 1 mM PdCl_2 in toluene/acetonitrile (1/1) and then washed with acetone and drying. The procedure was then repeated with 0.25 mM AuBr_3 in toluene/acetonitrile with the same field strength and duration but opposite field direction. Energy dispersive X-ray analysis showed the presence of each metal only in the dark areas indicated by the arrows.

Taken together, the above results demonstrate the preparation of a bipolar electrodeposited catalyst (BEC), the catalytic activity of which can be modulated by means of the applied electric field strength. It is well known that various parameters in chemical reductions (type of reductant, temperature, pressure, additives) lead to modulation of catalyst activity and selectivity. Changes in catalyst particle morphology and size, as well as support interactions, are likely the dominant cause. It is therefore expected that in the production of catalysts by electric field mediated reduction, the catalyst activity, and perhaps selectivity will depend on electric field parameters (intensity, time, pulse sequence, and direction). The last parameter is particularly important because it offers the ability to control the position of metal deposition by means of the direction of an electric field vector. This type of microsegregation control on a particulate support would be difficult to obtain by conventional methods and may prove to have important implications for catalytic activity.

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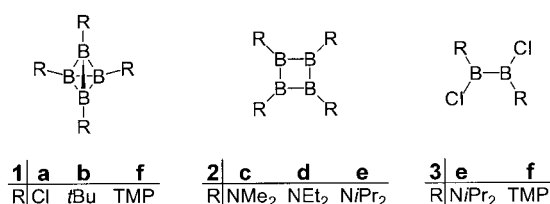
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Blue Tetrakis(diisopropylamino)-cyclo-tetraborane and Yellow Tetrakis(tetramethylpiperidino)tetraborane-tetrahedrane**

Claus-Jürgen Maier, Hans Pritzkow, and Walter Siebert*

Dedicated to Professor Otto J. Scherer on the occasion of his 65th birthday

The chloro^[1] and *tert*-butyl derivatives^[2, 3] of tetraborane(4) (**1**) have a tetrahedral structure (B–B bond lengths in **1b**:^[3] 1.699(6)–1.714(4) Å), whereas an opening of the tetrahedron to a four-membered ring (**2**) is discussed for B₄Cl₄ (**1a**)^[4, 5] and B₄Br₄^[6] in solution. The reaction mixture obtained from di-*tert*-butyldichlorodiborane(4) and Na/K alloy was shown by



Klusik and Berndt to contain **1b** ($\delta(^{11}\text{B}) = 135.7$) and the radical anion $[\text{B}_4\text{tBu}_4]^{+\cdot}$ (**2b⁺**, observed by ESR spectroscopy), for which they postulated a bent ring structure.^[7] Morrison^[8] could not confirm the proposed planar structure

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for $B_4(NMe_2)_4$ (**2c**);^[9] Baudler et al. have mentioned $B_4(NEt_2)_4$ (**2d**).^[10]

To synthesize stable tetraboranes B_4R_4 , we have dehalogenated the sterically hindered diborane(4) derivatives **3e** and **3f**^[11] instead of the RBX_2 compounds.^[3, 10] Here we report the synthesis and structure of the bent ring **2e** and the tetrahedrane **1f**.

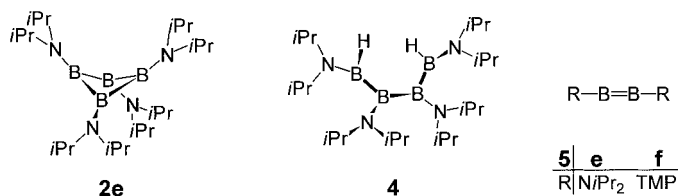
Replacement of two chloride substituents in B_2Cl_4 takes place upon reaction with diisopropylamine or lithium 2,2,6,6-tetramethylpiperidide to provide the colorless compounds **3e** and **3f**, whose constitution was established by spectroscopy and a crystal structure analysis of **3e**.^[12] As is the case for other derivatives,^[13] **3e** has a *gauche* conformation (the torsional angle between the two $B(Cl)NiPr_2$ halves is 91°). The short B–N bond lengths of 1.380(4) Å indicate the existence of a π interaction between the boron atoms and the isopropylamino groups.

Treatment of **3e** with Na/K alloy in hexane (20 °C, 48 h) results in the formation of a gray-green reaction mixture,^[11] from which blue **2e** (18 %), colorless **4** (2 %), and colorless **5e** (3 %) were isolated. The mass spectra of **2e**, **4**, and **5e** each

(av 1.396(3) Å) indicate a π interaction; the nitrogen atoms deviate significantly (0.45 Å) from the corresponding B_3 plane. Thus the atoms N1 and N1' move towards each other, as do N2 and N2'. The inclination of N1 towards N1' (that is, the angle between N1–B1 and B1–B2–B2') and of N2 towards N2' is on average 19.0° .

As the colorless products **4** and **5e** were obtained in only small amounts, they could not be characterized by NMR spectroscopy. However, a crystal structure analysis^[12] confirmed the constitution of **4**, which is similar in structure to $B_4(NMe_2)_6$.^[14] Diborene **5e** and other derivatives (**5**, R = *sec*-Bu₂N, 2,6-dimethylpiperidino) were first identified by Meller and Maringele on the basis of mass spectrometry and ^{11}B NMR spectroscopy as well as trapping reactions.^[15]

To investigate the steric influence of a rigid amino substituent on the formation of the product, we replaced the movable diisopropylamino groups in **3e** by the less flexible TMP ligands. As in the case of **3e**, dehalogenation of **3f** led to a gray-green reaction mixture, from which surprisingly yellow crystals were isolated. Mass spectrometry and ^{11}B NMR spectroscopy ($\delta = 67.5$) suggest the formation of a *cyclo*-tetraborane. However, the yellow color ($\lambda_{max} = 312$ nm, $\epsilon = 15300$) indicates a tetrahedron, in analogy to **1a** and **1b**. The crystal structure analysis^[12] confirmed the presence of tetrahedrane **1f** (Figure 2), in which two oppositely positioned



contain the molecular peak with the correct isotopic distribution. The ^{11}B NMR spectrum of **2e** provides a signal at $\delta = 65$ (identical to the value observed for *cyclo*- $B_6(NMe_2)_6$ ^[4] and *cyclo*- $B_6(NEt_2)_6$ ^[10]), and in the ^1H NMR spectrum a doublet and a septet indicate the presence of equivalent iPr_2N groups in **2e**. For **2e**, which is not planar because of the amino substituents, blue crystals ($\lambda_{max} = 620$ nm, $\epsilon = 40$) were isolated from the turquoise solution in hexane.

The crystal structure analysis shows that **2e** contains a bent B_4 ring (Figure 1, folding angle $B1-B2-B2'/B2-B1'-B2'$ $59.3(1)^\circ$) with nearly identical B–B bond lengths ($B1-B2'$ 1.710(3), $B1-B2$ 1.711(3) Å). The exocyclic B–N bond lengths

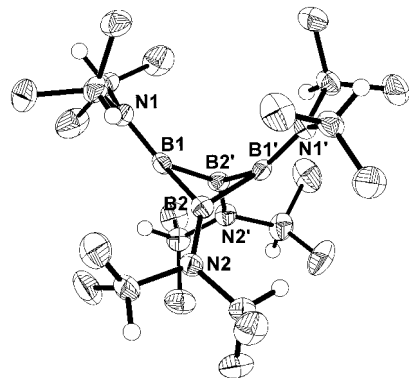


Figure 1. Structure of **2e** in the crystal. Selected bond lengths [Å] and angles [$^\circ$]: B1–B2 1.711(3), B1–B2' 1.710(3), B1–N1 1.397(2), B2–N2 1.396(2); B2–B1–B2' $81.8(1)$, B1–B2–B1' $82.1(1)$, B1–B2–N2 $133.3(2)$, B1'–B2–N2 $137.6(3)$, B2–B1–N1 $138.3(2)$, B2'–B1–N1 $133.0(2)$.

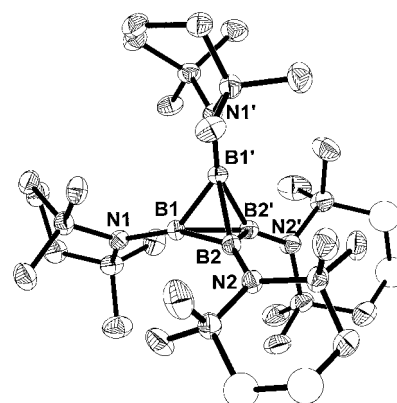


Figure 2. Structure of **1f** in the crystal. Selected bond lengths [Å] and angles [$^\circ$]: B1–B1' 1.695(6), B2–B2' 1.701(7), B1–B2 1.765(5), B1–B2' 1.752(5), B1–N1 1.446(4), B2–N2 1.444(4); B1'–B1–B2 $60.8(2)$, B1'–B1–B2' $61.6(2)$, B2–B1–B2' $57.9(2)$, B2'–B2–B1 $60.7(2)$, B2'–B2–B1' $61.5(2)$, B1–B2–B1' $57.6(2)$.

edges are short ($B1-B1'$ 1.695, $B2-B2'$ 1.701(7) Å) and the others are long ($B1-B2'$ 1.752, $B1-B2$ 1.765(5) Å). The B–N bond lengths of 1.444 and 1.446(4) Å are longer than in **2e**, which indicates a weaker π interaction. We assume that, as is the case for C_4tBu_4 , the “corset effect”^[16] of the bulky TMP ligands causes the complete folding to the tetrabora-tetrahedrane. Theoretical investigations of $B_4(NH_2)_4$ show that the energy difference between the bent structure (D_{2d}) and the tetrahedrane structure (calculated B–B bond lengths: 1.672–1.733 Å) is very low.^[17] For $B_4(NH_2)_4$ the calculations provide an energy difference of 10.8 eV between the HOMO and LUMO for the tetrahedron and 9.8 eV for the D_{2d} structure, which is in qualitative agreement with the observed colors of **1f** and **2e**.

The isolation of **1f** proves for the first time that the TMP-substituted tetrabora-tetrahedrane is more stable than the bent cyclo-tetraborane isomer **2f**.

Experimental Section

3e: B₂Cl₄ (3.01 g, 18.4 mmol) and then diisopropylamine (7.45 g, 73.7 mmol) were condensed into pentane (35 mL) at –105 °C. After the mixture had warmed to room temperature, it was stirred for 15 h and then heated under reflux for 8 h. The ammonium salt (5.1 g) that had formed was removed by filtration, and the filtrate was dried under vacuum to provide colorless **3e** (5.34 g, 99%; m.p. 82 °C). ¹H NMR (200 MHz, C₆D₆, 298 K): δ = 1.00, 1.06 (2 × d, ³J(H,H) = 6.7 Hz, 12H; CHCH₃), 1.31, 1.33 (2 × d, ³J(H,H) = 6.7 Hz, 12H; CHCH₃), 3.2–3.4 (m, 2H; NCHCH₃), 3.66 (sept, ³J(H,H) = 6.7 Hz, 2H; NCHCH₃); ¹H NMR (200 MHz, [D₈]toluene, 345 K): δ = 1.28 (d, ³J(H,H) = 6.7 Hz, 12H; CHCH₃), 1.52 (d, ³J(H,H) = 6.9 Hz, 12H; CHCH₃), 3.60 (sept, ³J(H,H) = 6.9 Hz, 2H; NCHCH₃), 3.86 (sept, ³J(H,H) = 6.7 Hz, 2H; NCHCH₃). Owing to hindered rotation about the B–N and B–B bonds, four doublets, one septet, and one multiplet are observed in the ¹H NMR spectrum measured at 298 K. At 345 K the restriction to rotation is removed, and two doublets and two sharp septets appear. ¹¹B NMR (64 MHz, C₆D₆): δ = 38.1; ¹³C NMR (50 MHz, C₆D₆): δ = 22.1 (CHCH₃), 22.3 (CHCH₃), 23.3 (CHCH₃), 46.5 (NCHCH₃), 54.4 (br, NCHCH₃); high-resolution EI-MS: *m/z* calcd for ¹²C₁₂¹H₂₈¹¹B₂³⁵Cl₂¹⁴N₂: 292.1816, found: 292.1843, Δ*m* = 2.7 mmu; elemental analyses calcd for C₁₂H₂₈B₂Cl₂N₂: C 49.21, H 9.63, N 9.56; found: C 48.73, H 9.41, N 9.29.

3f: B₂Cl₄ (2.20 g, 13.5 mmol) was condensed into a suspension of Li(TMP) (25.0 mmol) in hexane (50 mL) at –100 °C. The mixture was warmed to room temperature within 3 h and then stirred for 15 h. Lithium chloride was removed by filtration and washed with hexane, and the combined filtrate was concentrated to a volume of 10 mL. At –20 °C pale yellow **3f** crystallized (1.27 g, 25%). ¹H NMR (200 MHz, C₆D₆): δ = 1.41 (s, 12H; C(CH₃)₂), 1.49 (s, 12H; C(CH₃)₂), 1.68 (d, 12H; CH₂); ¹¹B NMR (64 MHz, C₆D₆): δ = 38.8; ¹³C NMR (50 MHz, C₆D₆): δ = 15.7 (*p*-CH₂), 30.1, 32.3, 38.0, 39.6 (CCH₃), 33.6, 33.7 (*m*-CH₂), 57.5, 57.6 (NCC); high-resolution EI-MS: *m/z* calcd for ¹²C₁₈¹H₃₆¹¹B₂³⁵Cl₂¹⁴N₂: 372.2442, found: 372.2444, Δ*m* = 0.2 mmu.

2e: To Na/K alloy (1:2.8, ≈ 1 g) in hexane (20 mL) was added dropwise **3e** (350 mg, 1.13 mmol) in hexane (10 mL). After 48 h of stirring the gray-green reaction mixture was filtered, and the residue was washed with hexane (20 mL). The solvent of the green filtrate was evaporated, and the residue was dissolved in a small amount of pentane and kept at –40 °C. Blue crystals of **2e** (45 mg, 18%; m.p. 107–108 °C) and colorless crystals of **4** (≈ 5 mg, 2%) formed. Recondensation of the mother liquor at 20 °C/3 × 10^{–3} Torr provided a colorless liquid (≈ 8 mg, 3%), which according to EI-MS contained **5e** (*m/z* 222) and silicon grease. **2e:** ¹H NMR (200 MHz, C₆D₆): δ = 1.24 (d, ³J(H,H) = 6.7 Hz, 6H; CHCH₃), 3.70 (sept, ³J(H,H) = 6.7 Hz, 1H; NCHCH₃); ¹¹B NMR (64 MHz, C₆D₆): δ = 65 (br); ¹³C NMR (50 MHz, C₆D₆): δ = 24.7 (CHCH₃), 52.7 (NCHCH₃); high-resolution EI-MS: *m/z* calcd for ¹²C₂₄¹H₅₆¹¹B₄¹⁴N₄: 444.4877, found: 444.4876, Δ*m* = 0.1 mmu. **5e:** EI-MS: *m/z* (%): 222 ([M⁺], 61.8), 207 ([M⁺ – CH₃], 22.5), 179 ([M⁺ – C₃H₇], 45.8), 43 ([C₃H₇⁺], 100). **4:** EI-MS *m/z* (%): 446 ([M⁺], 5.8), 333 ([M⁺ – C₆H₁₆BN], 4.4), 222 ([C₁₂H₂₈B₂N₂⁺], 100), 43 ([C₃H₇⁺], 47.5).

1f: To Na/K alloy (1:2.8, ≈ 1 g) in hexane (20 mL) was added dropwise **3f** (330 mg, 0.88 mmol) in hexane (10 mL). After 72 h of stirring the gray-green reaction mixture was filtered, and the residue was washed with hexane. The yellow filtrate was concentrated, and recrystallization from toluene at 5 °C provided yellow **1f** (98 mg, 37%; m.p. 292 °C). ¹H NMR (200 MHz, C₆D₆): δ = 1.55–1.65 (m); ¹¹B NMR (64 MHz, C₆D₆): δ = 67.5; ¹³C NMR (50 MHz, C₆D₆): δ = 17.7 (*p*-CH₂), 33.8 (*m*-CH₂), 39.8 (CCH₃), 54.5 (NCC); high-resolution EI-MS: *m/z* calcd for ¹²C₃₆¹H₇₂¹¹B₄¹⁴N₄: 604.6129, found: 604.6138, Δ*m* = 0.9 mmu.

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- [12] Crystal structure data for **1f**, **2e**, **3e**, and **4**: **1f**: Orthorhombic, space group *Fdd2*, *a* = 20.774(17), *b* = 32.56(2), *c* = 10.852(8) Å, *V* = 7341(10) Å³, *Z* = 8; of 2338 independent reflections, 1767 were observed (*I* > 2σ(*I*)); *R*1 = 0.055, *wR*2 = 0.134. **2e**: Monoclinic, space group *C2/c*, *a* = 18.196(9), *b* = 9.893(9), *c* = 17.739(9) Å, β = 101.24(3)°, *V* = 3132(3) Å³, *Z* = 4; of 2761 independent reflections, 1887 were observed; *R*1 = 0.050, *wR*2 = 0.128. **3e**: Orthorhombic, space group *P2₁nm*, *a* = 6.490(8), *b* = 10.701(15), *c* = 12.92(2) Å, *V* = 897(2) Å³, *Z* = 2; of 2137 independent reflections, 1971 were observed; *R*1 = 0.050, *wR*2 = 0.126. **4**: Monoclinic, space group *P2₁/n*, *a* = 17.941(10), *b* = 9.856(5), *c* = 18.469(9) Å, β = 107.94(4)°, *V* = 3107(3) Å³, *Z* = 4; of 4236 independent reflections, 2379 were observed; *R*1 = 0.069, *wR*2 = 0.207. The measurements were carried out with a four-cycle diffractometer (MoKα, ω scans) at –70 °C or –63 °C (**1f**). The structures were solved by direct methods (SHELXS 86) and refined against *F*² with all measured reflections (SHELXL 97).^[18] Further details on the crystal structure investigation may be obtained from the Fachinformationszentrum Karlsruhe, D-76344 Eggenstein-Leopoldshafen, Germany (fax: (+49) 7247-808-666; e-mail: crysdata@fiz-karlsruhe.de), on quoting the depository numbers CSD-112868 (**1f**), -112869 (**2e**), -112870 (**3e**), and -112872 (**4**).
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Total Synthesis of the CP Molecules CP-263,114 and CP-225,917— Part 1: Synthesis of Key Intermediates and Intelligence Gathering**

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The CP molecules, CP-263,114 (**1**, Scheme 1) and CP-225,917 (**2**), exemplify architectures of unprecedented molecular connectivity and complexity and possess intriguing biological activities. Isolated from an unidentified fungal species by a group at Pfizer (Croton, USA),^[1] these substances (absolute configuration unknown) exhibit impressive cholesterol-lowering properties through inhibition of squalene

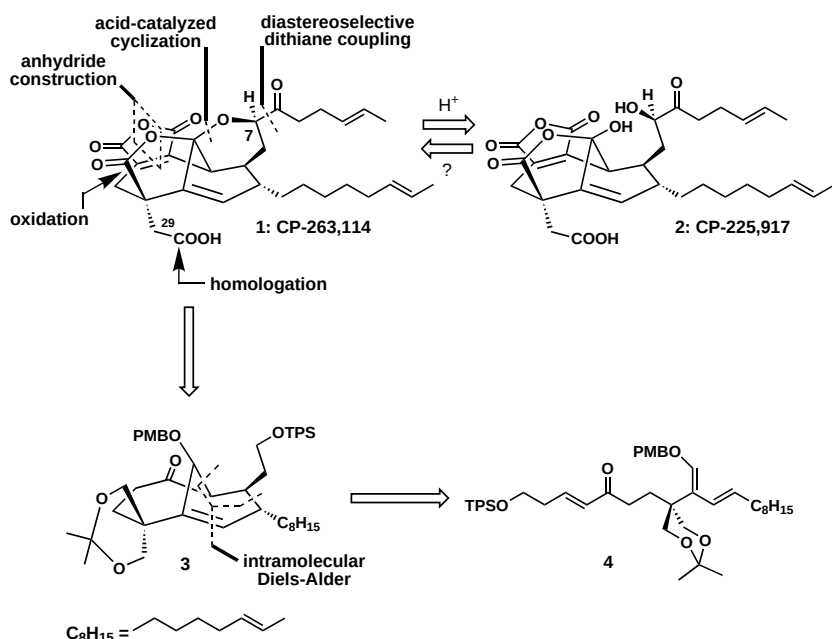
synthase.^[1,2] Furthermore, they inhibit farnesyl transferase, an enzyme implicated in cancer, and as such they stand as leads to potential drug candidates for cancer chemotherapy.^[1,3] The daunting molecular frameworks coupled with the array of sensitive functionalities present within these structures amount to a formidable synthetic challenge to which synthetic chemists have already begun to respond.^[4] In this and the following communication^[5] we recount our studies that led to the successful completion of the total synthesis of the CP molecules (**1** and **2**, racemic), in which a number of novel strategies and cascade reactions were discovered and developed. We begin here with the description of the overall strategy, the construction of a key intermediate (**27**, see Scheme 2), and of two unsuccessful, and yet highly useful in terms of intelligence gathering, attempts to reach the CP molecules **1** and **2**.

The key strategic bond disconnections and retrosynthetic blueprint shown in Scheme 1 serve as a conceptual overview of our synthetic rationale. Because of the known conversion of **2** into **1** under anhydrous acid catalysis^[1] and the inability of **1** to generate **2** under aqueous acidic conditions^[1] it seemed more prudent to target **2**. However, the hope of transforming **1** to **2** under basic conditions (for a mechanistic rationale of this expectation see Scheme 2 in the following communication)^[5] led us to accept that reaching either of the CP molecules was an opportunity to access the other as well. We soon discovered that seemingly logical routes to **1** or **2** from the intermediate compound **3** (Scheme 1) were plagued with countless unpredictable failures, which presumably arose from the unique peculiarities of the CP skeleton and its sensitive functionalities. Herein we report the lessons learned from two such expeditions. Proper implementation of novel chemistry harvested as a result of these synthetic blockades and explorations finally

culminated in a fine-tuned synthetic stratagem capable of effectively addressing these issues.

Diverse model studies aimed at deconvoluting this synthetic maze pointed towards the use of key intermediate **27** as a beachhead from which all further synthetic tactics would diverge (Scheme 2). Notable among the envisioned operations of the forward sequence en route to compound **27** were: 1) the intramolecular Diels–Alder reaction of the prochiral precursor **4** casting the basic elements of the bicyclic core of the CP molecules,^[6] 2) stereoselective fastening of the “upper” side chain by using substrate-directed dithiane chemistry,^[7] 3) the installment of the anhydride moiety onto the periphery of the bicyclic CP skeleton by employing an unprecedented seven-step cascade reaction sequence.^[8]

Scheme 3 summarizes the synthesis of the bicyclic core **17**. Thus, dimethyl malonate (**5**) was converted into aldehyde **6** by a five-step sequence involving: 1) anion formation with NaH and quenching with I(CH₂)₃OTBS (90 %), 2) a second alky-

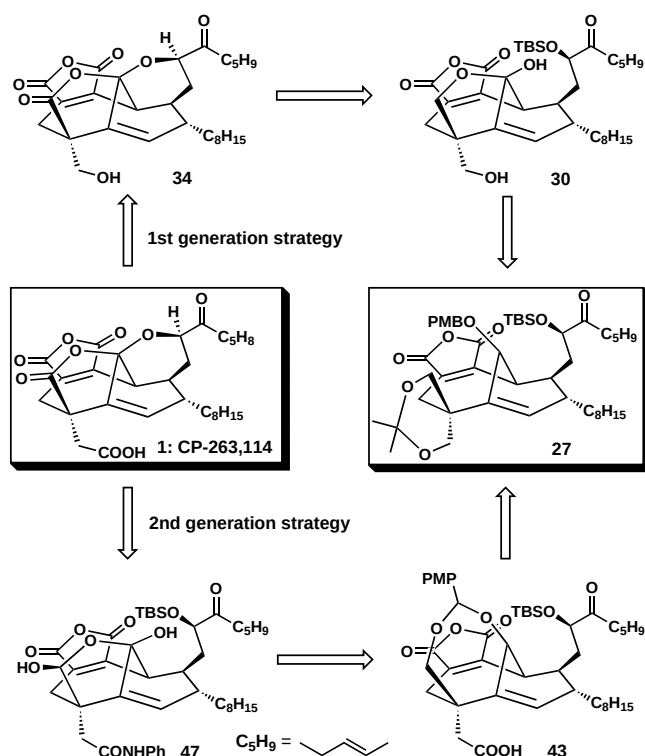


Scheme 1. Structures and retrosynthetic analysis of the CP molecules **1** and **2**. TPS = *tert*-butyldiphenylsilyl, PMB = *p*-methoxybenzyl.

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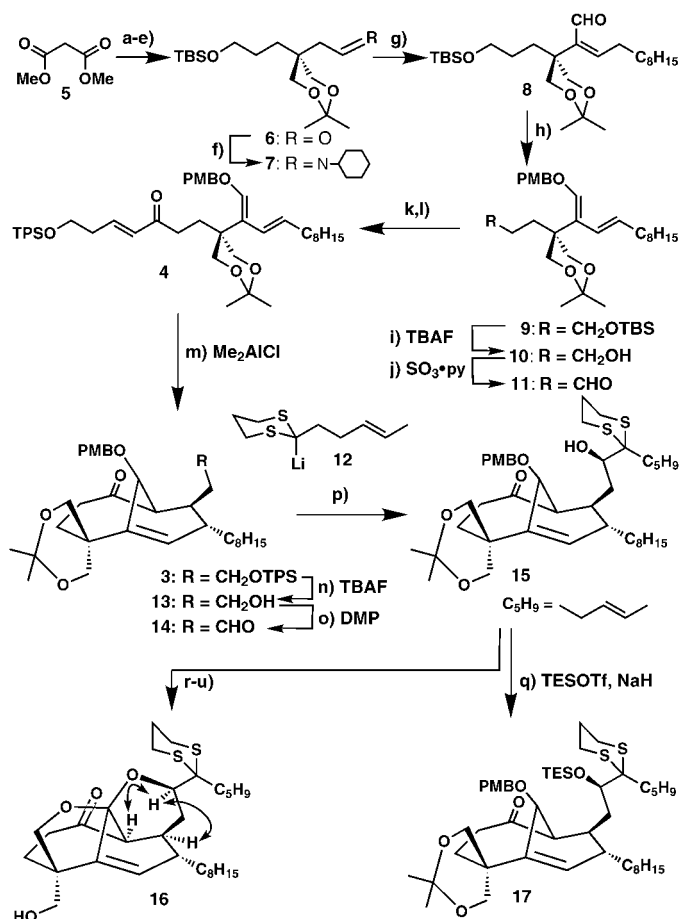
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Scheme 2. Strategies for the first and second generation divergent from the key intermediate **27**. TBS = *tert*-butyldimethylsilyl, PMP = *p*-methoxyphenyl.

lation with NaH and allyl bromide (95%), 3) reduction of both ester groups with LiBH₄ (80%), 4) acetonide formation (82%), and 5) ozonolysis, followed by treatment with Ph₃P (95%). Imine **7** was then formed by combining **6** and cyclohexylamine with the concomitant azeotropic removal of water. Compound **7** was treated with LDA at –20 °C to attain its lithioderivative which was quenched with C₈H₁₅CH₂CHO^[9] to furnish, after acidic workup, α,β -unsaturated aldehyde **8** in 60% overall yield from **6**. Exposure of **8** to KH followed by addition of PMBCl led to **9** as the major product in an approximate 10:1 ratio with its *E,E* isomer and 78% combined yield. Compound **9** was then desilylated by exposure to TBAF, and the resulting alcohol (**10**, 82% yield) was oxidized with SO₃·py in CH₂Cl₂:DMSO to give aldehyde **11** (78% yield). Conversion of this aldehyde (**11**) into the targeted Diels–Alder precursor, enone **4**, required addition (92% yield) of the lithioderivative derived from (*E*)-TPSO(CH₂)₂CH=CHI and *n*BuLi, followed by SO₃·py oxidation (76% yield) of the resulting alcohol.

The intramolecular Diels–Alder reaction of **4** was facilitated by catalytic amounts of Me₂AlCl and ensued at –10 °C to afford the expected cycloadduct **3** in 90% yield (selected spectroscopic data are given in Table 1). The high overall efficiency and facile scale-up of the preceding thirteen steps leading to **3** permitted multi-gram quantities of this key intermediate to be procured for extensive explorations (see below). The next task involved elongation of the “upper” side chain of the molecule, preferably with stereocontrol of the newly installed hydroxyl group, a problem whose solution was not obvious at first glance. To this end, aldehyde **14** was



Scheme 3. Construction of the bicyclic CP core. a) NaH (1.3 equiv), I(CH₂)₃OTBS (1.3 equiv), THF, 80 °C, 12 h, 90%; b) allyl bromide (1.3 equiv), NaH (1.5 equiv), DME, 25 °C, 0.5 h, 95%; c) LiBH₄ (2.0 equiv), THF, 0 → 25 °C, 12 h, 80%; d) Me₂C(OMe)₂ (1.5 equiv), CSA (0.05 equiv), CH₂Cl₂, 25 °C, 0.5 h, 82%; e) O₃, CH₂Cl₂, –20 °C, 1 h; then Ph₃P (1.0 equiv), –78 → 25 °C, 12 h, 95%; f) cyclohexylamine (1.2 equiv), benzene, 80 °C, 1 h; g) LDA (1.1 equiv), Et₂O, –20 °C, 1 h; then C₈H₁₅CHO (1.5 equiv) in Et₂O, –78 → –30 °C, 12 h; then oxalic acid (4.0 equiv), H₂O, 0 °C, 1 h, 60% overall from **6**; h) KH (5.0 equiv), PMBCl (1.5 equiv), DME:HMPA (5:2), 0 °C, 4 h, 78% (*E,E*:*Z,Z,E,E,E* ca. 10:1); i) TBAF (2.0 equiv), THF, 0 °C, 3 h, 82%; j) SO₃·py (3.0 equiv), Et₃N (5.0 equiv), CH₂Cl₂:DMSO (4:1), 25 °C, 0.5 h, 78%; k) (*E*)-TPSO(CH₂)₂CH=CHI (1.5 equiv), *n*BuLi (1.5 equiv), THF, –78 °C, 0.5 h; then **11** in THF, –78 °C, 0.5 h, 92%; l) SO₃·py (4.0 equiv), Et₃N (5 equiv), DMSO:CH₂Cl₂ (1:4), 25 °C, 3 h, 76%; m) Me₂AlCl (0.15 equiv), CH₂Cl₂, –10 °C, 0.5 h, 90%; n) TBAF (2.0 equiv), THF, 25 °C, 93%; o) DMP, NaHCO₃ (5.0 equiv), CH₂Cl₂, 25 °C, 1 h, 92%; p) **12** (1.3 equiv), THF, –78 °C, 8 min, 90% (75% conversion); q) NaH (6.0 equiv), TESOTf (2.0 equiv), THF, 0 → 25 °C, 2 h, 86%; r) DDQ (3.0 equiv), CH₂Cl₂:H₂O (18:1), 25 °C, 0.5 h, 85% (61% conversion); s) PDC (5.0 equiv), CH₂Cl₂, 25 °C, 0.5 h, 88%; t) 80% aq AcOH, 25 °C, 5 h, 91%; u) CH₃SO₃H (0.1 equiv), CH₂Cl₂, 25 °C, 0.5 h, 68%. TES = triethylsilyl, DME = 1,2-dimethoxyethane, CSA = camphorsulfonic acid, LDA = lithium diisopropylamide, PMBCl = *p*-methoxybenzyl chloride; TBAF = tetra-*n*-butylammonium fluoride; DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; PDC = pyridinium dichromate; OTf = trifluoromethanesulfonate; DMP = Dess–Martin periodinane.

generated in two steps from **3** by a sequence entailing desilylation (TBAF, →**13**, 93% yield) and oxidation of the primary alcohol (Dess–Martin periodinane,^[10] 92% yield). Treatment of **14** with lithiodithiane **12** (prepared from the corresponding dithiane and *n*BuLi) furnished the hydroxy

Table 1. Selected physical properties of compounds **3**, **27**, **34**, and **48**.

3: R_f = 0.61 (silica gel, ethyl acetate:hexane 1:2); IR (film) $\bar{\nu}_{\max}$ = 2973, 2931, 2843, 1704, 1614, 1515, 1455, 1418, 1372, 1303, 1250, 1200, 1111, 1035, 967, 823, 702 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3): δ = 7.68–7.64 (m, 4H), 7.43–7.33 (m, 6H), 7.14 (d, J = 8.5 Hz, 1H), 6.82 (d, J = 8.5 Hz, 1H), 5.50–5.38 (m, 2H), 5.37–5.32 (m, 1H), 4.45 (d, J = 11.0 Hz, 1H), 4.27 (brd, J = 11.5 Hz, 1H), 4.17 (d, J = 11.0 Hz, 1H), 4.10–4.07 (m, 1H), 4.02 (d, J = 12.0 Hz, 1H), 4.00 (d, J = 12.0 Hz, 1H), 3.79–3.74 (m, 1H), 3.78 (s, 3H), 3.74–3.65 (m, 1H), 3.49 (brd, J = 11.5 Hz, 1H), 2.79 (brs, 1H), 2.31 (dd, J = 13.0, 11.5 Hz, 1H), 2.26–2.13 (m, 2H), 2.10–1.90 (m, 3H), 1.85–1.77 (m, 1H), 1.70–1.60 (m, 7H), 1.58–1.48 (m, 1H), 1.48–1.10 (m, 14H), 1.02 (s, 9H); ^{13}C NMR (150 MHz, CDCl_3): δ = 213.5, 159.2, 142.1, 135.6 (2C), 135.5 (2C), 133.9, 133.7, 131.4, 129.8, 129.7 (4C), 129.3, 127.6 (4C), 124.8, 113.8 (2C), 97.6, 77.5, 71.0, 67.2, 65.1, 61.4, 58.7, 55.2, 41.5, 41.1, 39.9, 38.2, 36.5, 32.7, 32.6, 29.6, 29.3, 29.2, 27.6, 26.9, 26.8 (3C), 20.4, 19.1, 17.9; HRMS (FAB): calcd for $\text{C}_{49}\text{H}_{66}\text{O}_6\text{SiNa}^+$ (M + Na^+): 801.4526, found: 801.4539

27: R_f = 0.49 (silica gel, ethyl acetate:hexane 1:4); m.p. 77–78 °C (cyclohexane); IR (film) $\bar{\nu}_{\max}$ = 2991, 2920, 2861, 1761, 1714, 1655, 1508, 1461, 1379, 1250, 1191, 1091, 1038, 968, 915, 838, 591 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3): δ = 7.23 (d, J = 8.7 Hz, 2H), 6.87 (d, J = 8.7 Hz, 2H), 5.45–5.38 (m, 4H), 5.30 (brs, 1H), 4.65 (dd, J = 9.5, 2.5 Hz, 1H), 4.58 (d, J = 11.0 Hz, 1H), 4.39 (d, J = 11.0 Hz, 1H), 4.27 (brd, J = 11.3 Hz, 1H), 4.23 (brs, 1H), 4.00 (d, J = 11.3 Hz, 1H), 3.93 (d, J = 11.0 Hz, 1H), 3.80 (s, 3H), 3.29 (brd, J = 11.0 Hz, 1H), 3.26 (d, J = 16.5 Hz, 1H), 3.12 (brs, 1H), 2.90 (dd, J = 16.5, 3.1 Hz, 1H), 2.83 (dt, J = 18.0, 7.5 Hz, 1H), 2.60 (dt, J = 18.0, 7.0 Hz, 1H), 2.25 (m, 2H), 2.01–1.92 (m, 3H), 1.68–1.48 (m, 11H), 1.44 (s, 3H), 1.41 (s, 3H), 1.38–1.15 (m, 6H), 0.93 (s, 9H), 0.16 (s, 3H), 0.13 (s, 3H); ^{13}C NMR (125 MHz, CDCl_3): δ = 210.3, 166.2, 165.9, 159.5, 145.6, 142.3, 142.1, 131.4, 129.9, 129.3 (2C), 129.1, 128.9, 125.5, 124.7, 114.1 (2C), 98.0, 77.1, 74.6, 71.3, 68.1, 66.0, 55.3, 46.0, 42.4, 42.3, 41.5, 38.7, 38.5, 32.9, 32.5, 29.6, 29.4, 29.1, 28.2, 26.3, 26.2, 25.8 (3C), 20.9, 17.9 (3C), –4.2, –4.5; HRMS (FAB): calcd for $\text{C}_{47}\text{H}_{60}\text{O}_9\text{SiCs}^+$ (M + Cs^+): 937.3687, found: 937.3669

34: R_f = 0.48 (silica gel, ethyl acetate:hexane 1:2); ^1H NMR (500 MHz, CDCl_3): δ = 5.79 (d, J = 1.6 Hz, 1H), 5.50–5.30 (m, 4H), 4.53 (t, J = 8.0 Hz, 1H), 4.08 (s, 2H), 3.51 (s, 1H), 3.11 (brd, J = 19.5 Hz, 1H), 2.86 (dd, J = 19.5, 2.0 Hz, 1H), 2.71–2.60 (m, 2H), 2.56–2.51 (m, 1H), 2.38–2.21 (m, 5H), 2.06 (dd, J = 14.0, 10.0 Hz, 1H), 1.97–1.88 (m, 2H), 1.65–1.59 (m, 6H), 1.35–1.15 (m, 8H); ^{13}C NMR (150 MHz, CDCl_3): δ = 207.2, 175.3, 164.4, 164.2, 141.1, 140.9, 138.0, 132.2, 131.0, 129.1, 126.3, 125.0, 105.3, 75.3, 62.5, 51.6, 43.6, 43.2, 38.5, 38.4, 36.0, 35.6, 35.2, 32.3, 29.7, 29.3, 28.9, 26.8, 25.9, 17.9; HRMS (FAB): calcd for $\text{C}_{30}\text{H}_{36}\text{O}_8\text{Cs}^+$ (M + Cs^+): 657.1465, found: 657.1448

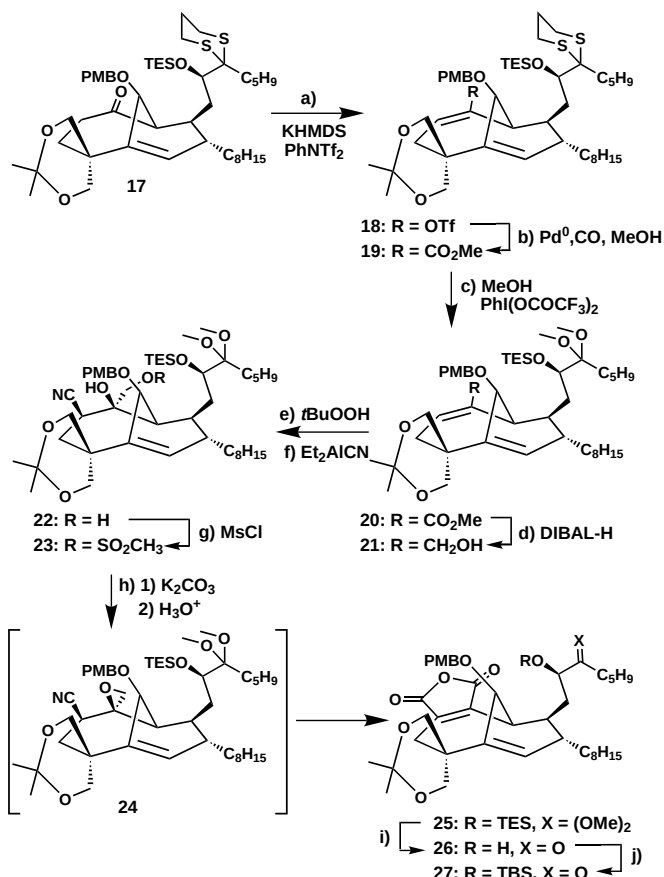
48: R_f = 0.47 (silica gel, ethyl acetate:hexane 1:1); IR (film) $\bar{\nu}_{\max}$ = 3387, 2975, 2915, 2847, 1768, 1713, 1661, 1607, 1484, 1437, 1161, 1092, 1072, 967, 932, 838 cm^{-1} ; ^1H NMR (600 MHz, CDCl_3): δ = 7.40 (dd, J = 7.6, 1.3 Hz, 1H), 7.30 (td, J = 6.8, 1.3 Hz, 1H), 7.19 (t, J = 7.6 Hz, 1H), 7.01 (d, J = 6.8 Hz, 1H), 5.48–5.32 (m, 4H), 4.55 (dd, J = 10.5, 3.1 Hz, 1H), 4.39 (d, J = 12.0 Hz, 1H), 4.15 (brd, J = 9.2 Hz, 1H), 3.76 (d, J = 9.2 Hz, 1H), 3.18 (d, J = 19.8 Hz, 1H), 2.72 (s, 2H), 2.69–2.51 (m, 2H), 2.40–2.05 (m, 3H), 2.00–1.90 (m, 3H), 1.80–1.50 (m, 9H), 1.45–1.20 (m, 5H), 0.88 (s, 9H), 0.13 (s, 3H), 0.08 (s, 3H); ^{13}C NMR (150 MHz, CDCl_3): δ = 212.5, 176.9, 164.9, 164.6, 150.0, 145.5, 136.2, 132.3, 131.3, 129.8, 128.6, 126.8, 125.5, 124.8, 124.1, 119.2, 108.6, 77.8, 76.0, 73.7, 48.0, 44.3, 41.7, 40.1, 37.6, 37.4, 35.7, 32.5, 30.4, 29.6, 29.3, 27.5, 26.1, 25.7 (3C), 23.0, 17.9 (3C), 14.2, –4.5, –4.9; HRMS (FAB): calcd for $\text{C}_{43}\text{H}_{57}\text{NO}_9\text{SiCs}^+$ (M + Cs^+): 892.2857, found: 892.2874

compound **15** (90% based on about 75% conversion) contaminated with small amounts of its C-7 diastereoisomer (approximate ratio 11:1).

The stereochemistry of the major component (namely **15**) of this mixture was deduced from NOE studies on the cyclized product **16** obtained from **15** through the sequence indicated in Scheme 3, and later confirmed by X-ray crystallographic analysis of a crystalline descendant (see Figure 1). This fortuitous selectivity may be a consequence of significant shielding of one face of the aldehyde by the neighboring CP

skeleton. Not surprisingly, the sterically hindered ketone group in **14** did not interfere with the side-chain addition. Finally, silylation of **15** with TESOTf and NaH furnished the desired compound **17** in 86% yield.

Installation of the maleic anhydride moiety onto the bicyclic core **17** proceeded in accordance with our recently reported protocol.^[8] Thus, enolate formation with KHMDS followed by quenching with PhNTf₂ converted **17** into enol triflate **18** (Scheme 4) in 95% yield. The carboxymethylation



Scheme 4. Installment of the maleic anhydride moiety: synthesis of key intermediate **27**. a) PhNTf₂ (1.5 equiv), KHMDS (2.0 equiv), THF, 0 °C, 10 min, 95%; b) Pd(OAc)₂ (0.06 equiv), Ph₃P (0.12 equiv), MeOH (40 equiv), Et₃N (2.0 equiv), DMF, CO (1 atm), 25 °C, 10 min, then **18**, 50 °C, 40 min, 78%; c) PhI(OCOCH₃)₂ (2.0 equiv), CaCO₃ (20 equiv), MeOH, 25 °C, 6 min, 81%; d) DIBAL-H (3.0 equiv), toluene, –78 °C, 95%; e) [V(O)(acac)₃] (0.2 equiv), *t*BuOOH (1.4 equiv), benzene, 25 °C, 0.5 h, 83%; β : α ca. 10:1; f) Et₂AlCN (5.0 equiv), toluene, 0 °C, 15 min; then 25 °C, 1 h, 73%; g) MsCl (3.0 equiv), Et₃N (10 equiv), THF, 0 °C, 5 min; h) 1) K₂CO₃ (20 equiv), MeOH, 1 h, 2) Et₂O, 10% aq oxalic acid, air, 0.5 h, 56% overall from **22**; i) 1) 90% aq AcOH, 25 °C, 1.5 h, 2) Me₂C(OMe)₂ (1.5 equiv), CSA (0.05 equiv), CH₂Cl₂, 25 °C, 30 min., 77% overall; j) TBSOTf (2.0 equiv), 2,6-lutidine (10 equiv), CH₂Cl₂, –20 → 0 °C, 1 h, 90%; Ms = methanesulfonyl, Tf = trifluoromethanesulfonyl; KHMDS = potassium bis(trimethylsilyl)amide; DIBAL-H = diisobutyl aluminum hydride.

of **18** with CO and methanol was catalyzed by Pd(OAc)₂ in the presence of Et₃N, and led to the α,β -unsaturated methyl ester **19** in 78% yield. Subsequent experiences, whose description will have to await the full account of this work, dictated the exchange of the dithiane group for a dimethoxy ketal at this

stage. Thus, exposure of **19** to $\text{PhI}(\text{OCOCF}_3)_2$ and CaCO_3 in methanol according to the procedure of Stork and Zhao^[11] led to compound **20** (81 %), which was reduced with DIBAL-H to afford allylic alcohol **21** (95 %). Vanadium-assisted epoxidation of **21**^[12] led to the corresponding β -epoxide (83 % yield, $\beta:\alpha$ approximately 10:1), whose opening with Et_2AlCN (Nagata reagent)^[13] in toluene led smoothly to cyano diol **22** (73 %). The anomalous stereochemical outcome of this opening (proven by NOE studies) may be attributed to the special environment of the epoxide moiety within the CP skeleton.^[41] Exposure of **22** to $\text{MsCl}/\text{Et}_3\text{N}$, followed by treatment of the resulting mesylate **23** with K_2CO_3 in methanol, and oxalic acid workup, gave maleic anhydride **25** via cyano epoxide **24** (56 % overall yield from **22**). The synthesis of the desired synthetic intermediate **27** was brought about by the sequential use of aqueous acetic acid (to remove the TES group) and dimesoxypropane/camphorsulfonic acid (to protect the resulting 1,3-diol) to furnish the hydroxy ketone **26** (77 % overall yield) whose silylation with TBSOTf proceeded smoothly in the presence of 2,6-lutidine (**27**, 90 % yield). The X-ray crystallographic analysis of crystalline **27** (see ORTEP drawing in Figure 1) confirmed its structure and those of its predecessors.

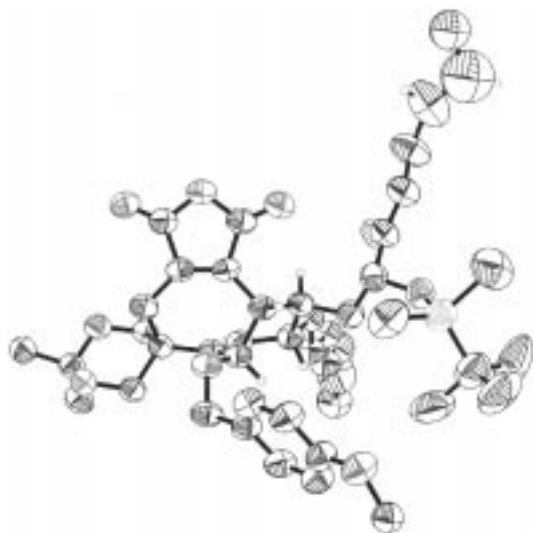
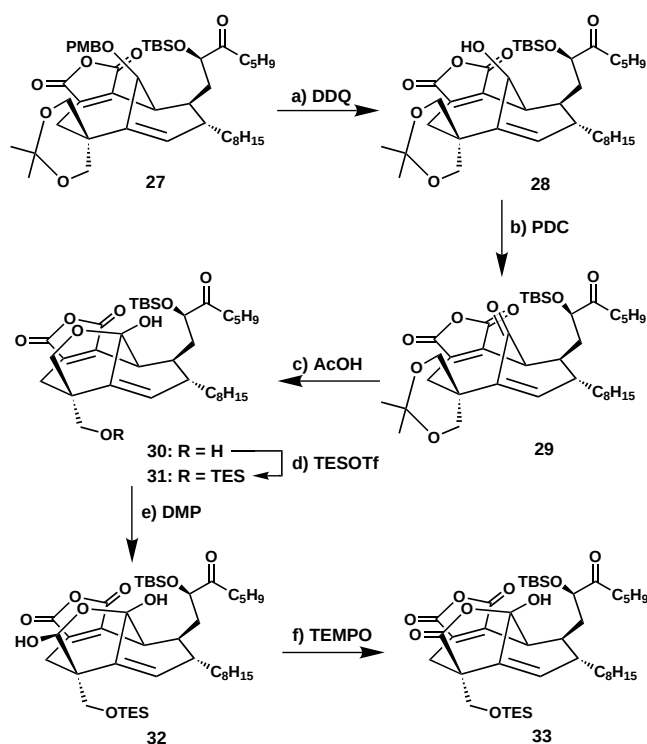


Figure 1. Structure of **27** in the crystal.

One of our first strategies towards the CP molecules was set forth on the notion that intermediate **34** (see Scheme 7) might be amenable to a late-stage one-carbon homologation to provide **1** directly. The synthesis of, and chemical explorations with intermediate **34** follow (Schemes 5 and 7).

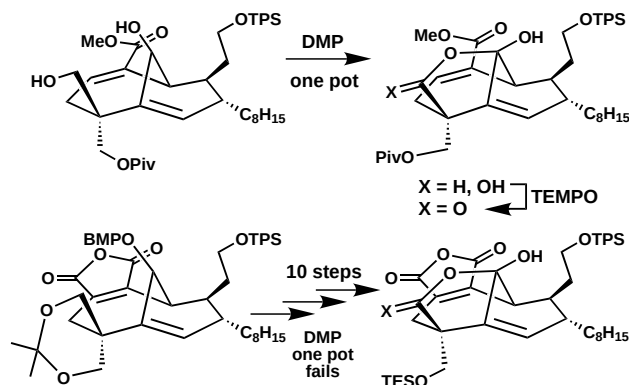
The construction of the γ -hydroxylactone functionality within the CP framework required a delicate balance of chemical manipulations and was finally accomplished through the sequence depicted in Scheme 5. The PMB group of **27** was cleaved by the action of DDQ (64% based on about 75% conversion) to afford hydroxy compound **28**, which was smoothly oxidized to enone **29** with PDC in 89% yield. Acetic acid induced removal of the acetone group then liberated the hydroxymethyl groups, one of which immediately cyclized with the bridgehead carbonyl functionality by virtue of its proximity to it, leading to hemiketal **30** in 70% yield. The



Scheme 5. Construction of the γ -hydroxylactone functionality: synthesis of key intermediate **33**. Reagents and conditions: a) DDQ (2.0 equiv), $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$ (18:1), 25 °C, 1 h, 64% based on approximately 75% conversion; b) PDC (3.0 equiv), CH_2Cl_2 , 25 °C, 2 h, 89%; c) 80% aq AcOH, 25 °C, 6 h, 82% based on 85% conversion; d) TESOTf (1.5 equiv), 2,6-lutidine (10 equiv), 0 °C, 2 h, 92%; e) DMP (5.0 equiv), benzene, 80 °C, 25 min, 63%; f) $\text{PhI}(\text{OAc})_2$ (30 equiv), TEMPO (30 equiv), CH_3CN , 25 °C, 1.5 h, 74%. TEMPO = 2,2,6,6-tetramethyl-1-piperidinoxyl.

remaining hydroxyl group on the concave face of the molecule was then temporarily masked as a TES ether, furnishing **31** (92% yield).

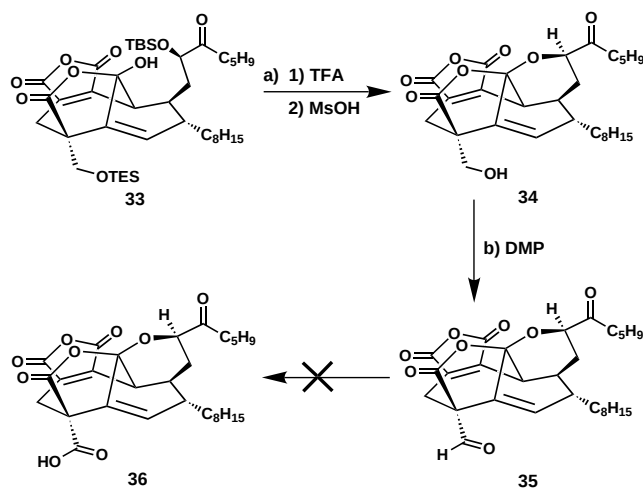
At this juncture it is instructive to reflect on our previous encounters with the challenge of constructing the γ -hydroxylactone moiety of the CP molecules in the presence of the fused maleic anhydride system (Scheme 6). We reported recently our difficulties in dealing with this problem.^[14] Utilizing a rather circuitous ten-step sequence based on careful protecting group manipulations we were indeed able to produce the γ -hydroxylactone moiety from a suitable 1,4-



Scheme 6. Summary of observations regarding the γ -hydroxylactone formation.^[14]

diol system. Only in certain model systems that did not contain the maleic anhydride functionality were we able to effect a four-step domino DMP oxidation reaction to create γ -hydroxylactols that could then be readily converted into the coveted γ -hydroxylactones. Thus, a distinguishing feature of the present strategy resides in the design and execution of a variant of this tandem DMP oxidation protocol, which triumphs even in the presence of the maleic anhydride moiety. Considering that the key step of this cascade is the ring-chain tautomerization^[15] of a requisite hemiketal, logic dictated that temperature elevation in a suitable solvent may be sufficient to coax the hemiketal into baring the latent hydroxyketone long enough for the required additional oxidation step to take place. Subsequent hydration and ring closure of the resulting aldehyde functionality would complete the sequence and furnish the corresponding γ -hydroxylactol. In the event, we were pleased to observe formation of γ -hydroxylactol **32** (Scheme 5) in 63 % yield and as a single stereoisomer upon oxidation of **31** with DMP in refluxing benzene. In accordance with our model studies,^[14] TEMPO-mediated oxidation^[16] of lactol **32** to the desired γ -hydroxylactone **33** proceeded smoothly and in 74 % yield.

Two tasks remained for the success of this strategy, namely internal ketalization and a one-carbon homologation of the carboxylic acid side chain. Thus, treatment of **33** first with aqueous trifluoroacetic acid effected global deprotection, furnishing the corresponding triol, which upon exposure to $\text{CH}_3\text{SO}_3\text{H}$ ^[1] in anhydrous CHCl_3 led to the desired hydroxy ketal **34** (50 % overall yield from **33**; Scheme 7). Arrival at **34** brought us within one carbon atom and two oxidation states



Scheme 7. Synthesis of the labile aldehyde **35**: a dead end. a) 1) CH_2Cl_2 :TFA: H_2O (40:4:1), 25 °C, 2 h; 2) $\text{CH}_3\text{SO}_3\text{H}$ (1.0 equiv), CHCl_3 , 25 °C, 24 h, 50 % overall; b) DMP (5.0 equiv), NaHCO_3 (10 equiv), CH_2Cl_2 , 25 °C, 2 h, 95 %. TFA = trifluoroacetic acid.

away from the natural product CP-263,114 (**1**). Oxidation of **34** with DMP proceeded smoothly to provide aldehyde **35** (95 %), which was now poised for further oxidation followed by an Arndt–Eistert homologation.^[17] Unfortunately, however, our unrelenting efforts to effect this oxidation were thwarted by the fragile nature of aldehyde **35** coupled with the

apparent extreme instability of the presumed carboxylic acid **36**. The unexpected instability of acid **36** may be a result of decarboxylative decomposition pathways (see Figure 2 for a mechanistic rationale) and has been confirmed to be a general phenomenon in such systems by relevant model studies.^[18] Several other alternative maneuvers to utilize aldehyde **35** as a starting point for homologation also failed.

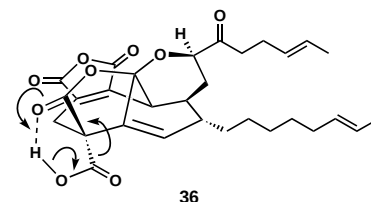
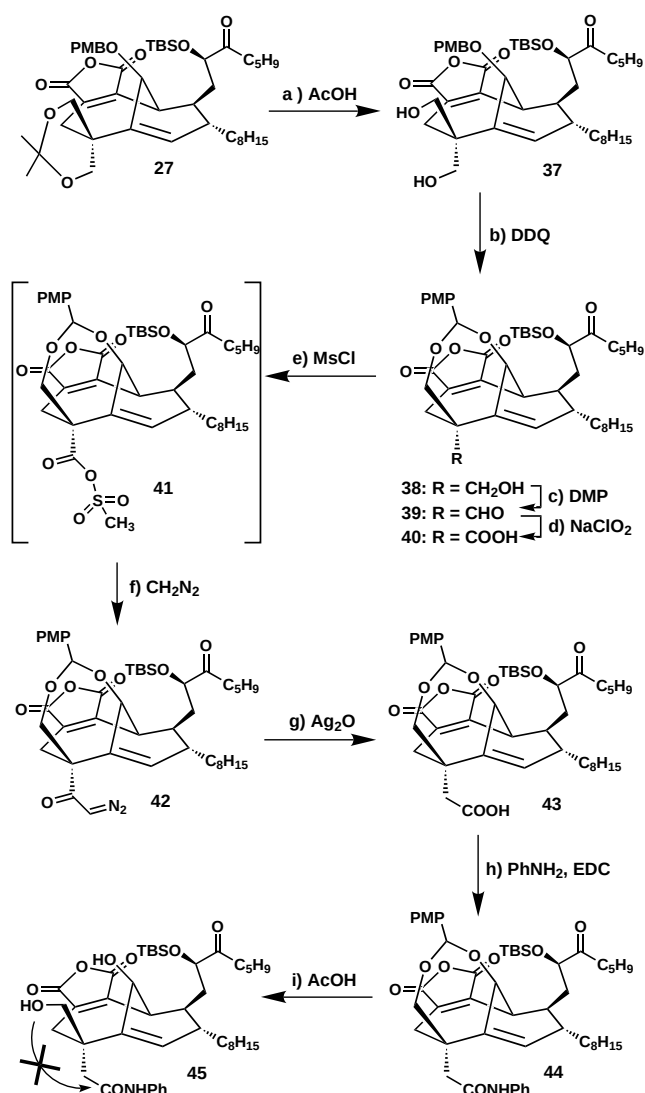


Figure 2. Possible explanation for the unstable nature of the elusive carboxylic acid **36** (intramolecularly assisted decarboxylation, decomposition).

After numerous attempts to alter this strategy so as to avoid these unstable species failed we were able to devise and pursue a novel second generation approach, results from which paved the way for the eventual total synthesis of the CP molecules. The reigning theme of this strategy (see Scheme 2) was the enlistment of an amide functionality to disguise and deactivate the electrophilic center of a homologated carboxylic acid. In doing so we could elude the problems associated with a late-stage homologation (see above) and, subsequently, be faced with what we perceived to be the comparably facile task of amide hydrolysis.

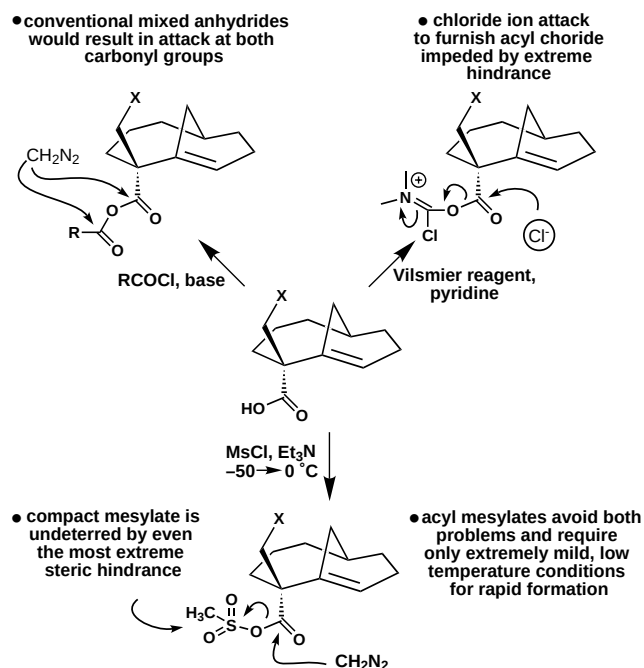
Our second generation approach to the CP molecules (Scheme 8) was launched from the key synthon **27** by removal of the isopropylidene group (aqueous acetic acid, 85 %) to give diol **37** followed by DDQ-mediated^[19] formation of the seven-membered benzylidene acetal **38** in 57 % yield. The hydroxy compound **38** was then oxidized to the corresponding carboxylic acid **40** through the intermediacy of aldehyde **39** by the sequential action of DMP (90 %) and NaClO_2 (80 %). In order to execute the venerable Arndt–Eistert homologation protocol we required a mild method for the activation of hindered carboxylic acids such as **40**, since acid chloride formation with these systems was extremely inefficient or did not work at all. We rationalized that an acyl mesylate might provide a unique solution to the dilemma of activating sterically crowded acids for reasons illustrated in Scheme 9.

In the event carboxylic acid **40** was smoothly transformed into diazoketone **42** via the acyl mesylate **41** upon treatment with $\text{MsCl}/\text{Et}_3\text{N}$ at 0 °C, followed by addition of excess CH_2N_2 as a dried ethereal solution.^[20] To conclude the homologation a Wolff rearrangement took place at 120 °C in 1 minute in $\text{DMF}:\text{H}_2\text{O}$ (2:1) in the presence of excess Ag_2O to furnish the homologated acid **43** in 38 % overall yield from **40**. Amide bond formation between carboxylic acid **43** and aniline in the presence of EDC/4-DMAP proceeded smoothly to produce anilide **44** in 85 % yield. Acid-induced hydrolysis of the bezylidene acetal **44** led to diol **45** (89 %), which, as predicted, was found to be resistant to cyclization.^[21]

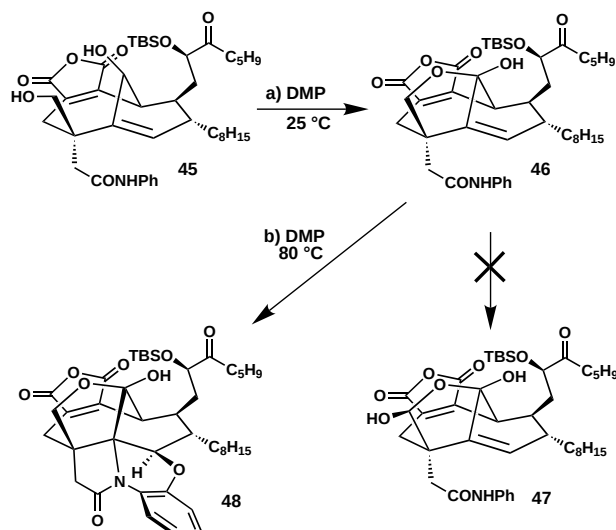


Scheme 8. Synthesis of diol-amide **45**: a stable intermediate resistant to intramolecular cyclization. a) 90% aq AcOH, 25 °C, 5 h, 85%; b) DDQ (2.0 equiv), fluorobenzene, 25 °C, 2.5 h, 57%; c) DMP (3.0 equiv), NaHCO₃ (10 equiv), CH₂Cl₂, 25 °C, 1 h, 90%; d) NaClO₂ (3.0 equiv), NaH₂PO₄ (1.5 equiv), 2-methyl-2-butene (50 equiv), *t*BuOH:H₂O (2:1), 25 °C, 20 min, 80%; e) MsCl (5.0 equiv), Et₃N (10 equiv), THF, 0 °C, 5 min; f) CH₂N₂ (excess), Et₂O/THF, 0→25 °C, 1 h; g) Ag₂O (5.0 equiv), DMF:H₂O (2:1), 120 °C, 1 min, 38% overall from **40**; h) PhNH₂ (1.5 equiv), EDC (3.0 equiv), 4-DMAP (1.0 equiv), CH₂Cl₂, 25 °C, 1 h, 85%; i) 80% aq AcOH, 25 °C, 1.5 h, 89%. 4-DMAP = 4-*N,N*-dimethylaminopyridine, EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride.

With diol **45** in hand, the stage was now set for the application of the DMP cascade oxidation sequence to install the γ -hydroxylactone functionality (Scheme 10). Admittedly, we had little, if any, intuition of the surprise that was soon awaiting us. When diol **45** was submitted to oxidation with DMP in benzene at room temperature, indeed rapid formation of hemiketal **46** was observed (90%). Further oxidation in refluxing benzene, however, furnished a compound which was clearly not the desired γ -hydroxylactol **47**. After rigorous spectroscopic analysis coupled with mechanistic reasoning (see Scheme 11) the structure was elucidated to be the novel polycycle **48**. With support from appropriate models, we offer the following speculative mechanism for the formation of **48**



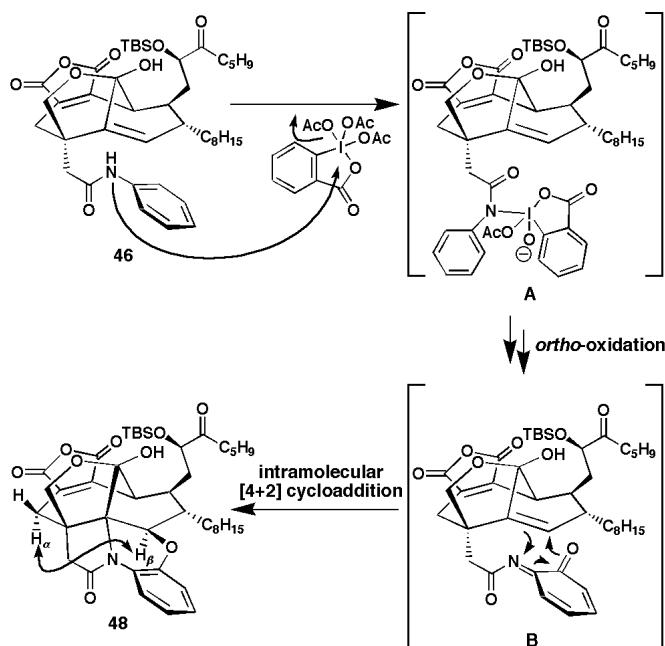
Scheme 9. Comparison of the use of acyl mesylates with conventional methods for activation of hindered acids.



Scheme 10. Serendipitous formation of polycycle **48**. a) Dess–Martin periodinane (2.0 equiv), benzene, 25 °C, 40 min, 90%; b) Dess–Martin periodinane (5.0 equiv), benzene, 80 °C, 20 min, 45%.

from **46**. Thus, the amide NH group of **46** engages the electrophilic DMP reagent to furnish intermediate **A** as shown in Scheme 11. *ortho*-Oxidation of **A** through intra- or intermolecular modes would furnish intermediate **B**, which is poised for an intramolecular [4+2] cycloaddition with the adjacent bridgehead double bond, thereby permitting rearomatization and resulting in fusion to the unprecedented structure **48**. The potential that this serendipitous discovery may hold in a general synthetic context is currently under investigation.

Although these strategies failed to deliver the targeted CP molecules, they provided enough reconnaissance information



Scheme 11. Proposed mechanism for the formation of polycycle **48**. The NOE between H_α and H_β confirmed the indicated stereochemistry.

to form the foundation from which a revised synthetic strategy could be formulated. The coalescence of these two unique strategies into a final approach capable of addressing all of the aforementioned pitfalls and culminating in the total synthesis of the CP molecules (**1** and **2**) is reported in the following communication.^[5]

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German version: *Angew. Chem.* **1999**, *111*, 1774–1781

Keywords: domino reactions • natural products • polycycles • total synthesis

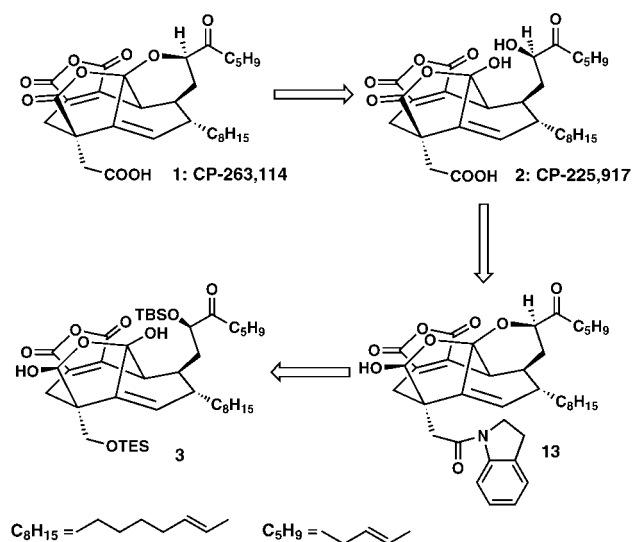
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- [22] Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as Supplementary publication no. CCDC-118962. Copies of the data can be obtained free of charge on application to CCDC, 12 Union road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccc.cam.ac.uk).

**Total Synthesis of the CP Molecules
CP-225,917 and CP-263,114—
Part 2: Evolution of the Final Strategy****

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In the preceding communication^[1] we described our findings from two unique strategies aimed at the total synthesis of the CP molecules **1** and **2**.^[2, 3] The current retrosynthetic analysis (Scheme 1) is predicated on the basic strategic



Scheme 1. Final retrosynthetic analysis of the CP molecules **1** and **2**. TBS = *tert*-butyldimethylsilyl, TES = triethylsilyl.

considerations and chemistry reported earlier from these laboratories.^[1, 4, 8] In addition, extensive model studies, whose disclosure will have to await the full account of this work, led us to conclude that a plan that targeted CP-263,114 (**1**) first would be most prudent. The virtues of such a scheme would

include a decreased reliance on protecting group chemistry, greater stability and ease of handling intermediates with the pyran motif,^[5] and a rare opportunity to attempt the rather daring challenge of converting the seemingly robust CP-263,114 (**1**) into its hydrated counterpart CP-225,917 (**2**). Indoline amide **14** was targeted as an ideal precursor for the CP molecules by virtue of its facile accessibility from **3**^[1] and because of the relative ease with which this special amide can be hydrolyzed in a two-step procedure.^[6]

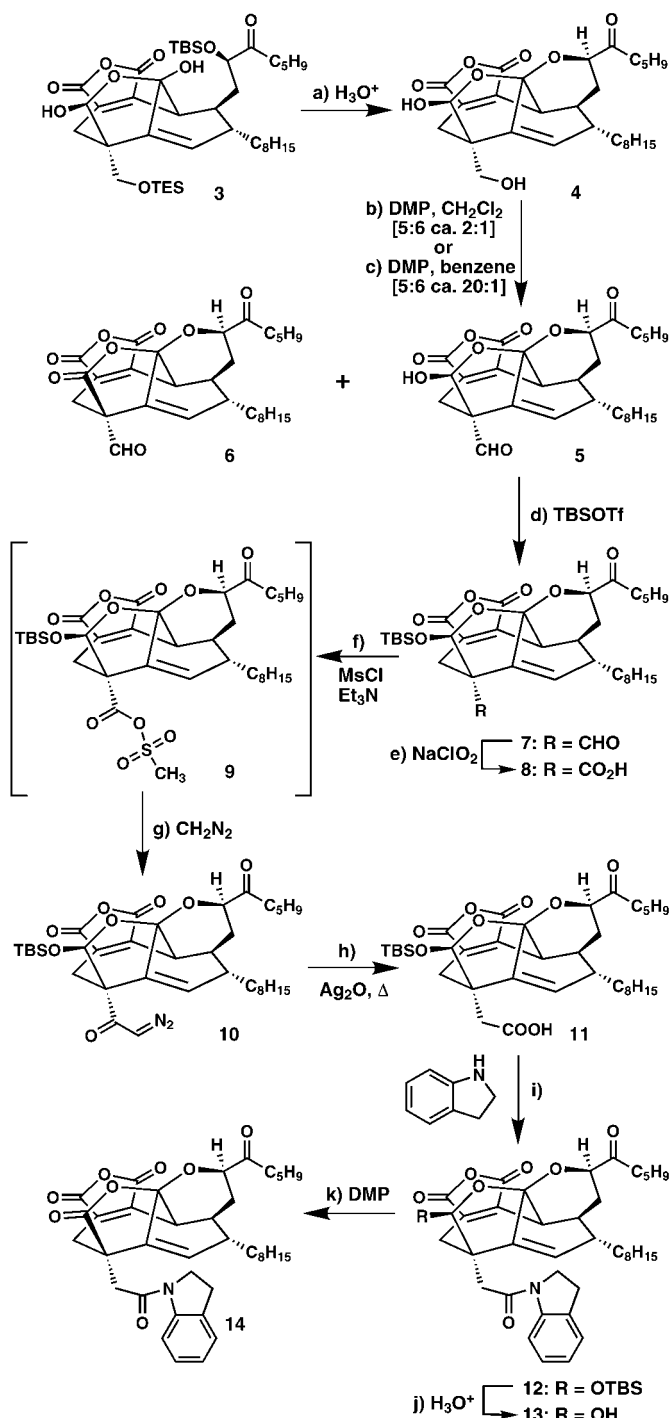
Thus, sequential treatment of **3** with aqueous TFA/CH₂Cl₂ to remove both silicon groups followed by exposure to CH₃SO₃H^[2] in dry CHCl₃ led to pyranlactol **4** (Scheme 2; see Table 1 for selected physical data). A key observation was made during the selective oxidation of diol **4** to aldehyde **5**. When diol **4** was oxidized with DMP^[7] in CH₂Cl₂ at ambient temperature the aldehyde lactol **5** was isolated as the major product along with significant quantities of lactone **6** (**5**:**6** approximate ratio 2:1, 85% combined yield). The fact that DMP was sufficient to effect this transformation, which was normally only possible using TEMPO,^[1, 8] indicated to us that not only was the lactolpyran behaving as a normal lactol but also that it would be plausible to employ the simple DMP protocol to reach the amide **14** (see below). Over the course of this work we have taken notice of the ability to fine-tune the reactivity of DMP by mere solvent alteration. Thus, the undesired, yet highly informative, lactonealdehyde **6** could essentially be eliminated (**5**:**6** approximate ratio >20:1, 90% combined yield) by carrying out the oxidation in benzene at 25 °C.

The lactol **5** was shielded from the ensuing homologation procedure (potential ring closure) by protection as the TBS-ether **7** (TBSOTf, 2,6-lutidine, 85% yield). NaClO₂ oxidation of **7** proceeded smoothly to produce acid **8** in 90% yield. The intimidating task of converting the sterically congested (neopentyl, concave face) carboxylic acid **8** into diazoketone **10** was easily accomplished via the acyl mesylate **9** (prepared in situ with MsCl/ Et₃N at 0 °C) with excess CH₂N₂ at 0 °C.^[1] The diazoketone so obtained was immediately dissolved in DMF:H₂O (2:1) and heated to 120 °C in the presence of excess Ag₂O for one minute to generate the homologated acid **11** in 35% overall yield from **8**. Combining carboxylic acid **11** and indoline in the presence of EDC and 4-DMAP provided amide **12** (85% yield). Removal of the pendant TBS group, assisted by TFA, revealed lactol **13** (95% yield), which could easily be oxidized to lactone **14** in 80% yield by using DMP for reasons alluded to above.

At this point in our synthesis it was critical to find reliable conditions for the counterintuitive^[2] conversion of CP-263,114 (**1**) into CP-225,917 (**2**). By utilizing a small amount of natural **1** we were able to explore conditions to accomplish this conversion. We rationalized that LiOH might be efficaciously exploited for this purpose by virtue of its unique nucleophilicity and solubility profile.^[10] In the event we were able to cleanly effect the desired transformation (**1** to **2**, Scheme 3) in over 90% yield with no significant decomposition or epimerization at C-7. A conceivable mechanistic rationale for this stunning cascade reaction sequence, which involves no less than four anions residing in the same molecule and a dizzying number of distinct steps occurring in the same reaction vessel,

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Scheme 2. Arrival at the key intermediate **14**. a) 1) CH_2Cl_2 :TFA: H_2O (40:4:1), 25 °C, 2 h; 2) $\text{CH}_3\text{SO}_3\text{H}$ (0.3 equiv), CHCl_3 , 25 °C, 2 h, 83% overall; b) DMP (5.0 equiv), CH_2Cl_2 , 25 °C, 1 h, 85%, **5:6** ca. 5:1 ratio; c) DMP (5.0 equiv), benzene, 25 °C, 2 h, 90%, **5:6** ca. >20:1 ratio; d) TBSOTf (20 equiv), 2,6-lutidine (50 equiv), CH_2Cl_2 , 0 → 25 °C, 3 h, 85%; e) NaClO_2 (3.0 equiv), NaH_2PO_4 (1.5 equiv), 2-methyl-2-butene (50 equiv), $t\text{BuOH}:\text{H}_2\text{O}$ (2:1), 25 °C, 10 min, 90%; f) MsCl (5.0 equiv), Et_3N (10 equiv), THF, 0 °C, 5 min; g) CH_2N_2 (100 equiv), $\text{Et}_2\text{O}:\text{THF}$, 0 °C, 45 min; h) Ag_2O (5.0 equiv), $\text{DMF}:\text{H}_2\text{O}$, (2:1) 120 °C, 1 min, 35% overall from **8**; i) indoline (1.5 equiv), EDC (3.0 equiv), 4-DMAP (1.0 equiv), CH_2Cl_2 , 25 °C, 1.0 h, 85%; j) CH_2Cl_2 :TFA: H_2O (40:4:1), 25 °C, 1.5 h, 95%; k) DMP (20 equiv), NaHCO_3 (50 equiv), CH_2Cl_2 , 25 °C, 24 h, 80%. TFA = trifluoroacetic acid, DMP = Dess–Martin periodinane, OTf = trifluoromethanesulfonate, Ms = methanesulfonyl, EDC = 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide hydrochloride, 4-DMAP = 4-*N,N*-dimethylaminopyridine.

Table 1. Selected physical properties of compounds **4**, **11**, and **14**.

4: R_f = 0.36 (silica gel, ethyl acetate:hexane 1:1); IR (film): $\tilde{\nu}_{\text{max}}$ = 3383, 2973, 2916, 2847, 1762, 1716, 1648, 1508, 1630, 1517, 1464, 1374, 1260, 1100, 1018, 958, 804 cm^{-1} ; ^1H NMR (600 MHz, CDCl_3): δ = 5.55 (d, J = 1.5 Hz, 1H), 5.45–5.28 (m, 5H), 4.40 (dd, J = 7.7, 4.2 Hz, 1H), 4.13 (d, J = 11.2 Hz, 1H), 4.00 (d, J = 11.2 Hz, 1H), 3.72–3.68 (brs, 2H), 3.16 (d, J = 1.8 Hz, 1H), 2.89 (d, J = 21.4 Hz, 1H), 2.70–2.57 (m, 2H), 2.51 (d, J = 21.4 Hz, 1H), 2.42–2.08 (m, 6H), 2.04–1.86 (m, 3H), 1.66–1.48 (m, 6H), 1.36–1.16 (m, 5H), 1.12–0.92 (m, 2H); ^{13}C NMR (125 MHz, CDCl_3): δ = 210.2, 165.4, 165.0, 143.3, 141.5, 139.5, 131.1, 130.5, 129.5, 125.9, 124.9, 103.5, 99.5, 75.8, 62.8, 55.3, 43.0, 41.7, 38.9, 38.1, 36.5, 35.6, 32.3, 32.0, 29.3, 27.4, 26.3, 17.9 (2C); HRMS (FAB): calcd for $\text{C}_{30}\text{H}_{38}\text{O}_8\text{Na}$ ($M+\text{Na}^+$): 549.2464, found: 549.2474

11: R_f = 0.47 (silica gel, ethyl acetate:hexane 1:1); IR (film): $\tilde{\nu}_{\text{max}}$ = 3371, 2973, 2917, 2846, 2672, 1764, 1714, 1648, 1618, 1461, 1440, 1385, 1258, 1227, 1101, 1076, 924, 838 cm^{-1} ; ^1H NMR (600 MHz, CDCl_3): δ = 5.53 (s, 1H), 5.44–5.36 (m, 4H), 4.32 (dd, J = 8.0, 4.2 Hz, 1H), 3.35 (dd, J = 21.8, 1.5 Hz, 1H), 3.13 (s, 1H), 3.12 (d, J = 17.8 Hz, 1H), 3.06–2.99 (m, 1H), 2.74 (d, J = 17.8 Hz, 1H), 2.55 (m, 2H), 2.42–2.35 (m, 1H), 2.32 (dd, J = 21.8, 2.0 Hz, 1H), 2.30–2.10 (m, 5H), 1.96–1.90 (m, 2H), 1.81–1.42 (m, 9H), 1.40–1.10 (m, 6H), 0.90 (s, 9H), 0.18 (s, 3H), 0.14 (s, 3H); ^{13}C NMR (150 MHz, CDCl_3): δ = 210.9, 172.4, 165.6, 165.5, 143.2, 141.7, 135.2, 131.1, 129.8, 129.0, 125.9, 124.9, 109.3, 103.1, 75.0, 65.8, 52.2, 43.0, 41.4, 40.5, 38.1, 36.5, 35.7, 35.5, 32.3, 29.7, 29.3, 28.9, 27.5, 26.3, 25.6, 18.0, 17.9, 17.8, –4.2, –5.7 (2C); HRMS (FAB): calcd for $\text{C}_{37}\text{H}_{52}\text{NO}_8\text{SiNa}$ ($M+\text{Na}^+$): 892.2857, found: 892.2874

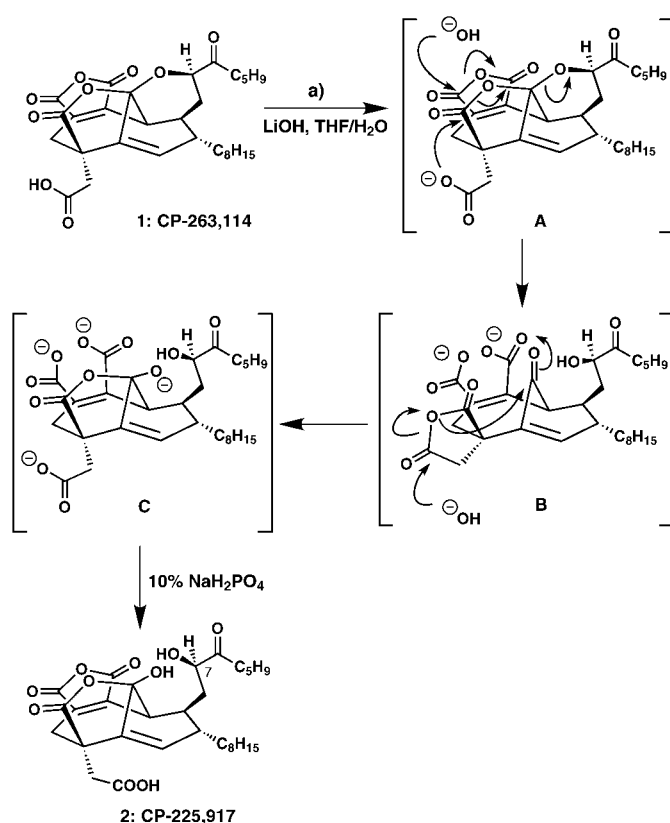
14: R_f = 0.70 (silica gel, ethyl acetate:hexane 1:1); IR (film): $\tilde{\nu}_{\text{max}}$ = 2971, 2915, 2847, 1763, 1714, 1648, 1618, 1461, 1440, 1385, 1258, 1227, 1101, 1076, 924, 838 cm^{-1} ; ^1H NMR (600 MHz, CDCl_3): δ = 8.19 (d, J = 7.9 Hz, 1H), 7.18 (d, J = 7.4 Hz, 1H), 7.15 (t, J = 7.9 Hz, 1H), 7.02 (t, J = 7.4 Hz, 1H), 5.59 (s, 1H), 5.53–5.32 (m, 4H), 4.57 (t, J = 8.0 Hz, 1H), 4.15–4.0 (m, 2H), 3.53 (s, 1H), 3.32 (d, J = 16.8 Hz, 1H), 3.30–3.20 (m, 1H), 3.06 (brd, J = 19.4 Hz, 1H), 3.03 (d, J = 16.8 Hz, 1H), 2.89–2.75 (m, 2H), 2.55–2.45 (m, 1H), 2.40–2.10 (m, 4H), 1.97–1.86 (m, 1H), 1.69–1.51 (m, 13H), 1.35–1.15 (m, 5H); ^{13}C NMR (150 MHz, CDCl_3): δ = 208.8, 175.8, 165.6, 164.6, 164.4, 142.3, 141.9, 140.7, 140.3, 131.1, 130.7, 129.5, 128.2, 127.5, 125.9, 125.0, 124.5, 124.2, 117.4, 104.7, 75.8, 47.9, 47.8, 43.5, 42.6, 40.3, 40.0, 38.3, 36.3, 35.6, 34.6, 32.3 (2C), 29.3, 28.9, 28.1, 26.9, 26.1, 17.9; HR-MS (FAB): calcd for $\text{C}_{39}\text{H}_{43}\text{NO}_8\text{Cs}$ ($M+\text{Cs}^+$): 786.204, found: 786.2068

appears in Scheme 3.^[11] This cascade may have important implications in a general sense, including future total syntheses of the CP molecules, their possible biosynthetic origin, and their mechanism of action. In addition, it opens up new paths for the design and construction of analogues.

Since the danger of intramolecular attack by the newly installed hydroxyl group upon the electrophilic carbonyl group of the homologated carboxylic acid had now passed, amide **14** was transformed to indole **15** (Scheme 4) by the oxidative action of excess *p*-chloroanil^[9] (toluene, Δ , 75% yield based on 50% conversion), thus regenerating the electrophilicity at the carbonyl center and rendering this moiety susceptible to mild base hydrolysis.

Application of the above unprecedented protocol to indole amide **15** (Scheme 4) succeeded in furnishing CP-225,917 (**2**, racemic, 72% yield),^[12] thus verifying the relative configuration at C-7.^[2] Furthermore, direct treatment with methanesulphonic acid in CDCl_3 over the course of 36 h resulted in an essentially quantitative conversion into CP-263,114 (**1**, 90% yield of isolated product, racemic).^[12]

The journey to the CP molecules required an unrelenting quest through a synthetic labyrinth endowed with countless obstacles, yet filled with numerous hidden treasures. When taken together with the discovery of novel cascade reactions,



Scheme 3. Mechanistic analysis of the LiOH-induced cascade reaction. a) LiOH (10 equiv), THF:H₂O (4:1), 25 °C, 1 h, 90 %.

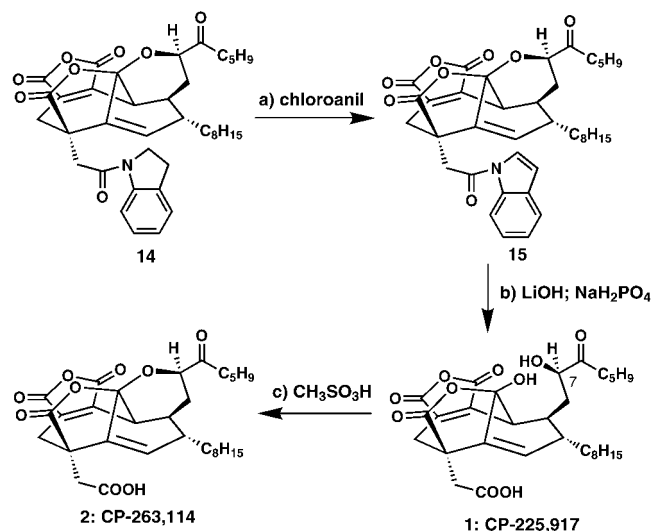
new synthetic technologies and unprecedented synthetic tactics, the current total syntheses are both a tribute to the innate complexity of these molecules and a triumph of modern organic synthesis over them. An asymmetric variant of the current synthesis, explorations to broaden the scope of newly discovered reactions, and the rational design of novel CP analogues for chemical biology studies are in progress.

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Scheme 4. Final stages of the total synthesis of CP-225,917 (**2**) and CP-263,114 (**1**). a) *p*-chloroanil (10 equiv), toluene, 110 °C, 2.5 h, 70 % based on 50 % conversion; b) LiOH (10 equiv), THF:H₂O (4:1), 25 °C, 3 h, then 10 % NaH₂PO₄, 10 min, 72 %; c) CH₃SO₃H (1.0 equiv), CDCl₃, 25 °C, 36 h, 90 %.

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- [12] Synthetic **1** and **2** exhibited identical chromatographic and spectroscopic data to those of authentic samples.