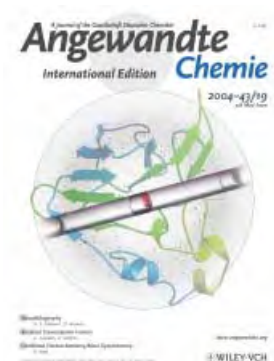


Cover Picture

Bo Zheng, Joshua D. Tice, L. Spencer Roach, and Rustem F. Ismagilov*

Protein crystals can be grown inside nanoliter droplets in a composite polydimethylsiloxane/glass microfluidic device. R. F. Ismagilov and co-workers describe in their Communication on page 2508 ff. how this approach can be used to screen conditions for protein crystallization by both microbatch and vapor-diffusion methods. The quality of the crystals grown in the device was evaluated directly from the X-ray diffraction pattern shown in the background.



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The following Communications are available online (in Wiley InterScience). You can find them, as well as forthcoming Reviews, Highlights, and Essays, at www.angewandte.org, under Early View.

M. P. Johansson,* D. Sundholm, J. Vaara:

Au₃₂: A 24-Carat Golden Fullerene

DOI: 10.1002/anie.200453986

Published online: April 22, 2004

F. Glorius,* N. Spielkamp, S. Holle, R. Goddard, C. W. Lehmann:

Efficient Asymmetric Hydrogenation of Pyridines

DOI: 10.1002/anie.200453942

Published online: April 26, 2004

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Books

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Fabian Gerson, Walter Huber

reviewed by H.-G. Korth

Peptides and Proteins **2470**

Shawn Doonan

reviewed by A. G. Beck-Sickinger

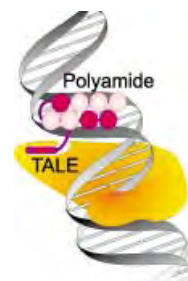
Highlights

Regulation of Gene Expression

K. Schmitz, U. Schepers* — **2472–2475**

Polyamides as Artificial Transcription Factors: Novel Tools for Molecular Medicine?

Misregulation of gene expression often leads to severe defects in the development of an organism or to uncontrolled cell growth. Artificial transcription factors can control the expression of the genes concerned to restore normal cell regulation. Dervan and co-workers have synthesized several DNA-binding polyamides (see picture: TALE (yellow) is a class of homeobox protein) that show potential for the treatment of diseases such as cancer.



Analytical Methods

U. Karst* — **2476–2478**

Electrochemistry/Mass Spectrometry (EC/MS)—A New Tool To Study Drug Metabolism and Reaction Mechanisms

A great combination: The coupling of electrochemistry and mass spectrometry (EC/MS) opens up new possibilities in analytics—not only in biochemistry for the investigation of natural products and metabolites, but also in the screening of

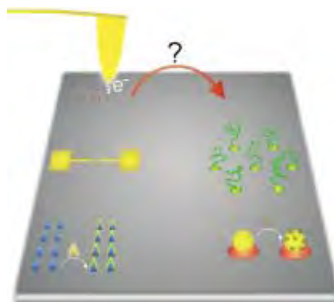
combinatorial libraries and the characterization of transition-metal complexes. The mode of operation, range of application, and perspectives of the current EC/MS methods are the themes of this Highlight.

Reviews

Nanolithography

D. Wouters, U. S. Schubert* 2480–2495

Nanolithography and Nanochemistry: Probe-Related Patterning Techniques and Chemical Modification for Nanometer-Sized Devices



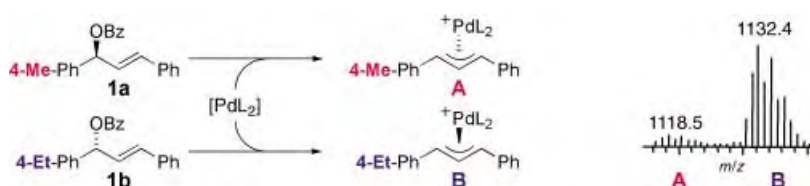
Nanometer-sized devices: Scanning probe based techniques will form the basis for the future production of small devices. This Review describes how oxidative scanning probe lithography can be a versatile platform for creating nanopatterns with (chemical) functionality.

Communications

Enantioselectivity Determination

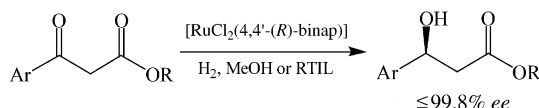
C. Markert, A. Pfaltz* 2498–2500

Screening of Chiral Catalysts and Catalyst Mixtures by Mass Spectrometric Monitoring of Catalytic Intermediates



The **enantioselectivity** of chiral palladium catalysts in the kinetic resolution of allylic esters can be determined by mass spectrometric monitoring of allyl–Pd intermediates **A** and **B**, which are derived from pseudoenantiomeric substrates **1a** and

1b. In contrast to conventional screening methods, which are based on product analysis, simultaneous screening of catalyst mixtures in homogeneous solution is possible.



A family of **chiral diphosphanes** with systematically tunable electronic and steric properties have been used in the asymmetric hydrogenation of β -aryl ketoesters with excellent *ee* values (up to

99.8%) by taking advantage of remarkable 4,4'-substituent effects on binap. These highly enantioselective Ru catalysts have also been effectively immobilized in room-temperature ionic liquids (RTIL).

Enantiomeric Synthesis

A. Hu, H. L. Ngo, W. Lin* 2501–2504

Remarkable 4,4'-Substituent Effects on Binap: Highly Enantioselective Ru Catalysts for Asymmetric Hydrogenation of β -Aryl Ketoesters and Their Immobilization in Room-Temperature Ionic Liquids

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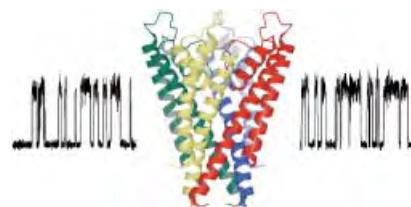
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Ion Channels

F. I. Valiyaveetil, M. Sekedat, T. W. Muir,*
R. MacKinnon* ————— 2504–2507

Semisynthesis of a Functional K⁺ Channel

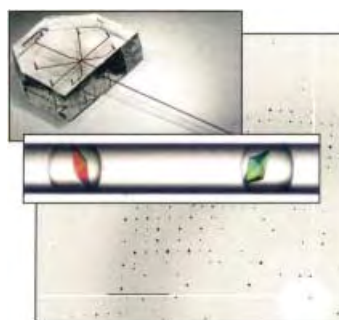
Selective ion-conduction properties in lipid membranes have been found for a semisynthetic KcsA K⁺ channel containing a mutation (A98G) that allows the channel to open. Thus, it is now possible to study ion conduction in a K⁺ channel with chemical modification of the main-chain atoms of the selectivity filter. The picture shows the single-channel responses of the semisynthetic K⁺ channel and its structure.



Crystal Growth

B. Zheng, J. D. Tice, L. S. Roach,
R. F. Ismagilov* ————— 2508–2511

A Droplet-Based, Composite PDMS/Glass Capillary Microfluidic System for Evaluating Protein Crystallization Conditions by Microbatch and Vapor-Diffusion Methods with On-Chip X-Ray Diffraction



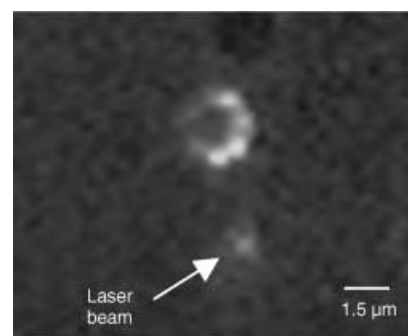
On-chip protein crystallization and diffraction: Proteins can be crystallized in nanoliter volumes by using both microbatch and vapor-diffusion techniques inside composite PDMS/glass microfluidic devices (PDMS = polydimethylsiloxane). The quality of crystals can be assessed directly by on-chip X-ray diffraction (see picture).

Coated Silica Microparticles

G. McNay, F. T. Docherty, D. Graham,
W. E. Smith,* P. Jordan, M. Padgett,
J. Leach, G. Sinclair, P. B. Monaghan,
J. M. Cooper ————— 2512–2514

Visual Observations of SERRS from Single Silver-Coated Silica Microparticles within Optical Tweezers

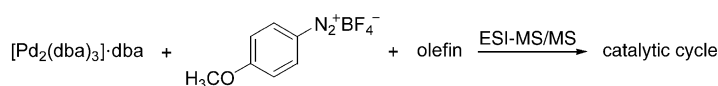
Surface-enhanced resonance Raman scattering (SERRS) is considerably more efficient than Raman spectroscopy, thereby enabling the detection of single molecules. Strong and short-lived SERRS emissions (< 100 ms) from single, partially silver-coated, dye-adsorbed microparticles have been detected within optical tweezers (see picture). Full spectra, recorded from single particles, clearly show that the emission is due to SERRS.



Reactive Intermediates

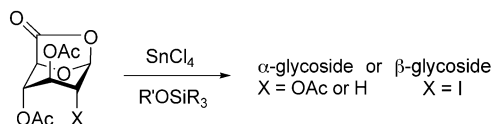
A. A. Sabino, A. H. L. Machado,
C. R. D. Correia,
M. N. Eberlin* ————— 2514–2518

Probing the Mechanism of the Heck Reaction with Arene Diazonium Salts by Electrospray Mass and Tandem Mass Spectrometry



Key ionic intermediates of the Heck reaction of arene diazonium salts have been intercepted and structurally characterized by ESI-MS/MS. The use of electrospray ionization enables the ionic

species to be transferred smoothly to the gas phase. A catalytic cycle for this reaction involving all the Pd cationic species intercepted has been proposed (see scheme).



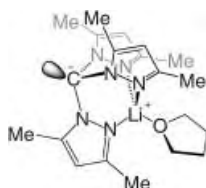
The donor makes the difference: The tin(IV) chloride catalyzed coupling of silyl ethers to donors derived from glucuronic acid has shown that the silyl ethers (see scheme) are significantly better acceptors than the corresponding alcohols or

phenol. The reactions are highly stereoselective and dependant on the donor structure. They provide 1,2-*trans*- and 1,2-*cis*-(deoxy)glycosides in moderate to excellent yields.

Stereoselective Glycoside Synthesis

M. Poláková, N. Pitt, M. Tosin,
P. V. Murphy* — 2518–2521

Glycosidation Reactions of Silyl Ethers with Conformationally Inverted Donors Derived from Glucuronic Acid: Stereoselective Synthesis of Glycosides and 2-Deoxyglycosides

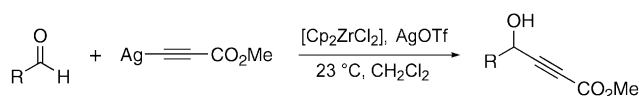


A suitable bridle, created by intramolecular chelation of the Li cation, and lack of π delocalization, yielded the first monomeric lithium organyl compound containing an unassociated tetrahedral carbanionic center (see picture). The zwitterionic structure found in the solid state is preserved in solution. Only weak electrostatic $\text{Li}^+\cdots\text{C}^-$ interactions are apparent; there is no Li–C contact and therefore no “classical” aggregation.

Pyramidal Carbanions

F. Breher,* J. Grunenberg, S. C. Lawrence,
P. Mountford, H. Rüegger — 2521–2524

A Monomeric Organolithium Compound Containing a Free Pyramidal Carbanion in Solution and in the Solid State



The beauty of silver has been demonstrated in the alkylation of functionalized aldehydes and ketones. Silver acetylides of alkynyl propiolates undergo addition to carbonyl compounds

in the presence of $[\text{Cp}_2\text{ZrCl}_2]$ and AgOTf (see scheme) in a reaction that is compatible with both base-sensitive and acid-sensitive functional groups. Tf = trifluoromethanesulfonyl.

Alkylation

S. P. Shahi, K. Koide* — 2525–2527

A Mild Method for the Preparation of γ -Hydroxy- α,β -Acetylenic Esters



Deep penetration by substrates through the size-restricted channels of an apo-ferritin cage results in size-selective olefin hydrogenation at the Pd nanocluster core

(see picture). The encapsulated zero-valent cluster is synthesized in situ by chemical reduction of Pd^{II} ions in the apo-ferritin cage.

Hydrogenation Catalysis

T. Ueno,* M. Suzuki, T. Goto,
T. Matsumoto, K. Nagayama,
Y. Watanabe* — 2527–2530

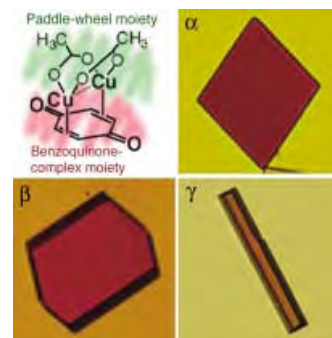
Size-Selective Olefin Hydrogenation by a Pd Nanocluster Provided in an Apo-Ferritin Cage

Supramolecular Chemistry

S. Masaoka, D. Tanaka, Y. Nakanishi,
S. Kitagawa* ————— 2530–2534

Reaction-Temperature-Dependent Supramolecular Isomerism of Coordination Networks Based on the Organometallic Building Block [Cu^I₂(μ₂-BQ)(μ₂-OAc)₂]

Three supramolecular isomers, [Cu₂(μ₄-BQ)(μ₂-OAc)₂]_n (α phase), [Cu₂(μ₃-BQ)(μ₂-OAc)(μ₃-OAc)]_n (β phase), and [Cu₂(μ₂-BQ)(μ₃-OAc)₂]_n (γ phase), were crystallized by using the organometallic building block shown, which has two types of supramolecular synthon. Interestingly, these three supramolecular isomers were crystallized from the same solvent. BQ = benzoquinone.



Protein Synthesis

D. Bang, S. B. H. Kent* — 2534–2538

A One-Pot Total Synthesis of Crambin



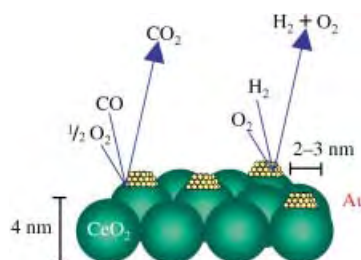
Making up for lost time: The one-pot synthesis of crambin (see structure) with only a single final purification step gave the target protein of exceptional purity in only two days with an overall yield of ≈40%. Three unprotected peptide segments were linked by native chemical ligation, and the polypeptide chain assumed its 3D structure without intermediate purification steps.



Gold Catalysts

S. Carrettin, P. Concepción, A. Corma,*
J. M. López Nieto,
V. F. Puntes ————— 2538–2540

Nanocrystalline CeO₂ Increases the Activity of Au for CO Oxidation by Two Orders of Magnitude

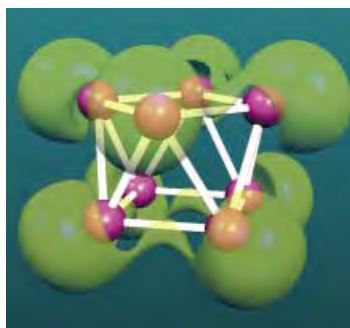


The smaller the better: Depositing gold on nanocrystalline particles of cerium oxide, instead of a regular cerium oxide support, increases the activity of the resulting catalyst by two orders of magnitude for the selective oxidation of CO to CO₂, even in the presence of H₂ (shown schematically).

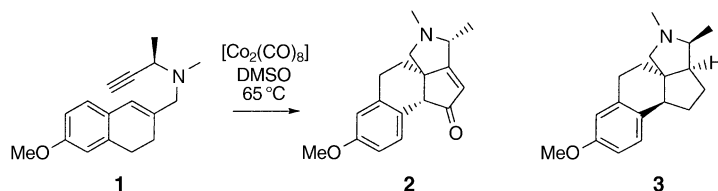
Main-Group Clusters

M. Lindsjö, A. Fischer,
L. Kloo* ————— 2540–2543

Sb₈(GaCl₄)₂: Isolation of a Homopolyatomic Antimony Cation



An almost perfect square-antiprismatic geometry is observed for Sb₈²⁺ (see picture), as predicted by the Wade rules. The title compound, which was synthesized in benzene at room temperature, represents the first homopolyatomic antimony cation and has Sb–Sb bond lengths of 285–300 pm.



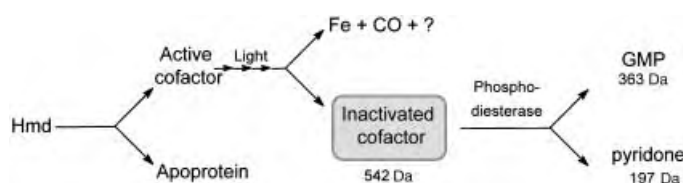
No protecting groups were required in a concise and atom-economical synthesis of the optically active tetracyclic pyrrolidine **3** under mild conditions. A highly diastereoselective Pauson–Khand reaction

(**1**→**2**) was used to construct the compact tetracycle with its quaternary stereocenter. The stereoselectivity of the cycloaddition was affected by the substituent on the propargylic moiety.

Alkaloid Synthesis

B. Jiang,* M. Xu _____ 2543–2546

Highly Enantioselective Construction of Fused Pyrrolidine Systems That Contain a Quaternary Stereocenter: Concise Formal Synthesis of (+)-Conessine



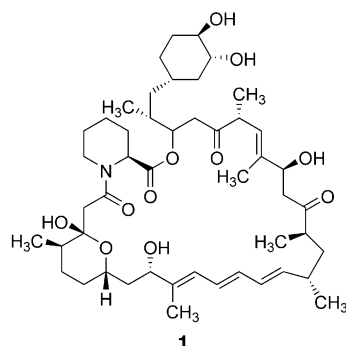
A combination of NMR spectroscopy and mass spectrometry has been crucial in determining the structure of the relatively stable light-inactivated form of the cofactor of the hydrogenase Hmd (see

scheme). These studies have shown that the chromophore bound to GMP through a phosphoester linkage is a pyridone derivative.

Structure Elucidation

S. Shima, E. J. Lyon, M. Sordel-Klippert, M. Kauß, J. Kahnt, R. K. Thauer,* K. Steinbach, X. Xie, L. Verdier, C. Griesinger* _____ 2547–2551

The Cofactor of the Iron–Sulfur Cluster Free Hydrogenase Hmd: Structure of the Light-Inactivation Product

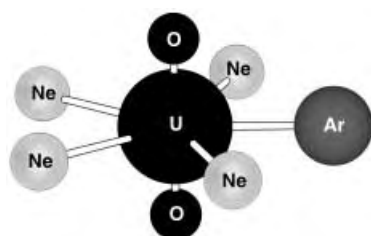


It's a rap: A double recombination strategy has been utilized to uncover the biosynthetic route to the key intermediate in the formation of rapamycin. Removal of a section of the rapamycin gene cluster from *Streptomyces hygroscopicus* produced a strain that generated no rapamycin. Gene complementation proved, surprisingly, that the accumulation of this previously elusive intermediate pre-rapamycin (**1**) depended on the presence of the gene *rapK*.

Natural Product Biosynthesis

M. A. Gregory, S. Gaisser, R. E. Lill, H. Hong, R. M. Sheridan, B. Wilkinson, H. Petkovic, A. J. Weston, I. Carletti, H.-L. Lee, J. Staunton, P. F. Leadlay* _____ 2551–2553

Isolation and Characterization of Pre-rapamycin, the First Macrocyclic Intermediate in the Biosynthesis of the Immunosuppressant Rapamycin by *S. hygroscopicus*



A noble gas and an ignoble cation: A laser-ablated uranium cation reacts with O_2 to give UO_2^+ , which is found to form $[UO_2(Ng)_n]^+$ ($Ng = Ne, Ar, Kr, Xe$) complexes on condensation with excess noble gas. Experiments with mixtures of noble gases lead to five new absorptions for the mixed $[UO_2^+(Ng)_x(Ng')_y]$ complexes and show that the coordination number is five for $Ng = Ar, Kr, \text{ and } Xe$ (see picture).

Noble-Gas Complexes

X. Wang, L. Andrews,* J. Li,* B. E. Bursten _____ 2554–2557

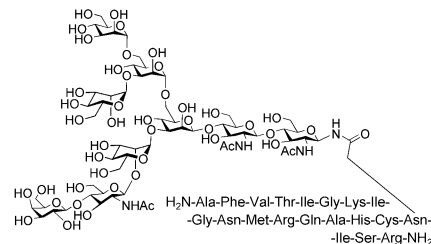
Significant Interactions between Uranium and Noble-Gas Atoms: Coordination of the UO_2^+ Cation by Ne, Ar, Kr, and Xe Atoms

Glycopeptides (1)

M. Mandal, V. Y. Dudkin, X. Geng,
S. J. Danishefsky* — 2557 – 2561

In Pursuit of Carbohydrate-Based HIV
Vaccines, Part 1: The Total Synthesis of
Hybrid-Type gp120 Fragments

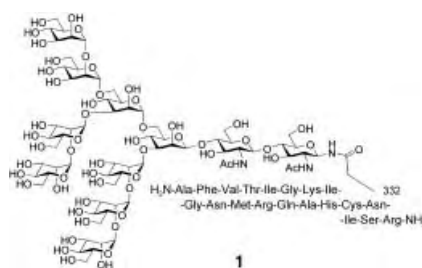
Key features in the successful synthesis of hybrid-type gp-120 fragments (see picture) include β -mannosylations to construct the core trisaccharide and a fragment-coupling strategy to install the “upper” high-mannose domain.



Glycopeptides (2)

X. Geng, V. Y. Dudkin, M. Mandal,
S. J. Danishefsky* — 2562 – 2565

In Pursuit of Carbohydrate-Based HIV
Vaccines, Part 2: The Total Synthesis of
High-Mannose-Type gp120 Fragments—
Evaluation of Strategies Directed to
Maximal Convergence



A “layered approach” and a “block approach” were used to assemble the high-mannose glycan of gp120 glycopeptide fragments (e.g. high-mannose-type conjugates gp120^{316–335} **1**). The glycan was then conjugated with the gp120 peptide segments through direct aspartylation.

Rhodium Redox Chemistry

C. Laporte, F. Breher, J. Geier, J. Harmer,
A. Schweiger,
H. Grützmacher* — 2567 – 2570

Stereochemical Control of the Redox
Potential of Tetracoordinate Rhodium
Complexes

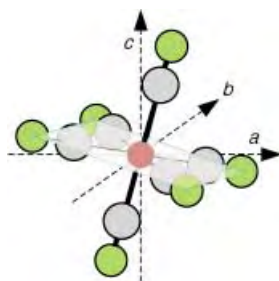
An appetite for reduction: The diastereoisomers of the tetrachelating ligand 1,3-bis[(5*H*-dibenzo[*a,d*]cyclohepten-5-yl)phenylphosphanyl]propane, bis(trpp^{Ph})propane, can be used to control the redox potentials of 16-electron rhodium(I) complexes. Whereas the *R,S*-isomer of the ligand enforces a more planar complex which is difficult to reduce, the *R,R(S,S)*-isomer favors a tetrahedrally distorted structure which is easily reduced to give a stable rhodium(0) complex (see structure, Rh silver, P yellow, C black, H white).



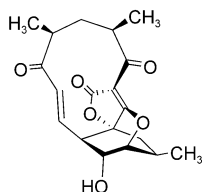
Spin-Density Distribution

F. H. Köhler,*
R. Lescouëzec — 2571 – 2573

Highly Resolved Spin-Density Distribution
in the Prussian-Blue Precursors
Cs₂K[Fe(CN)₆] and Cs₂K[Mn(CN)₆]



In magnetic materials of the Prussian-blue type, what proportion of the unpaired electrons in the simple hexacyanometa-late moves from the metal (red) to the carbon (gray) and nitrogen (green) atoms? Based on the solid-state ¹³C and ¹⁵N NMR spectra, the amount, the sign, and the shape of the spin densities can be distinguished for axial and equatorial CN ligands. There is surprisingly little spin on the N atoms.



Plumbing the depths: Abyssomicin C (structure shown) from ocean floor sediment is a novel antibiotic that inhibits the biosynthesis steps between chorismate and *p*-aminobenzoic acid. Its activity may be explained by the irreversible trapping of the targeted enzymes by a Michael addition. Blocking the biosynthesis of *p*-aminobenzoic acid may be one approach to developing new antibiotics.

Natural Products

B. Bister, D. Bischoff, M. Ströbele, J. Riedlinger, A. Reicke, F. Wolter, A. T. Bull, H. Zähler, H.-P. Fiedler, R. D. Süssmuth* _____ **2574–2576**

Abyssomicin C—A Polycyclic Antibiotic from a Marine *Verrucosipora* Strain as an Inhibitor of the *p*-Aminobenzoic Acid/Tetrahydrofolate Biosynthesis Pathway



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Polyamides as Artificial Transcription Factors: Novel Tools for Molecular Medicine?

Katja Schmitz and Ute Schepers*

Keywords:

drug design · gene expression · polyamides · transcription factors · zinc fingers

Modern medicine requires increasingly elaborate drugs to combat the multifaceted diseases common today. Therefore, chemical research is not only focused on the development of small molecules as pharmacologically active compounds, but also on the development of a variety of larger synthetic compounds that mimic cellular functions. These compounds can range from artificial receptors and their ligands to artificial molecules that specifically recognize duplex DNA. The latter have attracted great interest because of their potential application in the manipulation of gene expression.

In the most diverse organisms, from simple bacteria to higher eukaryotes such as humans, almost all biological functions are controlled individually by the temporal and spatial expression of the genes involved. A variety of developmental and environmental stimuli activate a complex chain of intracellular reactions that mobilize a regulatory molecule from the cytoplasm into the nucleus, where it eventually interacts with a gene to regulate its expression (Figure 1). Such regulatory molecules are either activated transcription factors or proteins that activate transcription factors. The expression of each gene is very sensitive to changes in the composition of transcription factors, thus allowing each cell type to express a unique

and characteristic subset of its genes, and to adjust the amount of the particular genes produced as required. If this

balance is disturbed or interfered with, the misregulation of gene expression can result, which often leads to severe

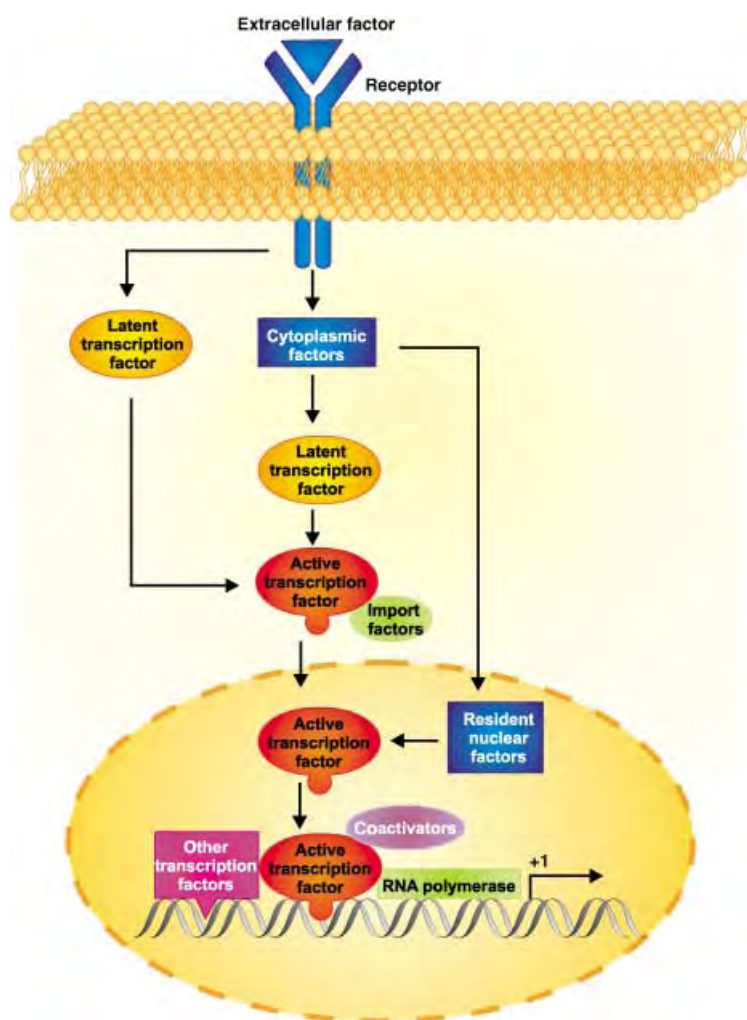


Figure 1. Schematic representation of transcriptional regulation.^[1] Latent transcription factors are usually activated at the cell membrane. The activation might be a response to changes in the concentration of extracellular or intracellular molecules. The activated transcription factors are transported into the nucleus of the cell, where they participate in regulated gene expression. The active factors bind to other transcription factors, coactivators, and RNA polymerase II to initiate transcription.

[*] Dipl.-Chem. K. Schmitz, Dr. U. Schepers
Kekulé-Institut für Organische Chemie und
Biochemie
Universität Bonn
Gerhard Domagk Strasse 1
53121 Bonn (Germany)
Fax: (+49) 228-73-7778
E-mail: schepers@uni-bonn.de

developmental defects or uncontrolled cell growth, for example, as is observed in cancer. Both the transcriptional activation of certain growth-promoting genes (oncogenes) and the transcriptional repression of tumor-suppressor genes trigger the uncontrolled growth of cancer cells. Therefore, intense efforts have been directed toward the development of artificial transcriptional activators and repressors that could regulate the expression of affected genes (tumor suppressors) to restore normal cell regulation and prevent uncontrolled growth.^[1–8]

Natural Transcription Factors

Natural transcription factors usually contain two functional domains, a DNA-binding domain (DBD) and an effector or activation domain (AD). The DNA-binding domain recognizes a particular region of the DNA, whereas the activation domain recruits other proteins of the transcription machinery in the immediate proximity of the DNA to start transcription.^[9] Attempts to control gene expression artificially are often based on the application of this fundamental principle,^[10] which led to the idea of generating novel transcription factors with DNA-binding domains synthesized with the desired DNA-binding specificities and tethered to the appropriate activation domains.

Activation domains are usually modular and retain their function when attached to non-natural DNA-binding domains. Thus, it is possible to synthesize customized libraries of artificial transcription factors for the specific regulation of numerous genes.^[11] This approach differs from many novel methods, such as RNA interference or antisense technology, as it can be used for the up-regulation of gene expression rather than exclusively for its suppression.^[12]

Chemically Induced Artificial Transcription Factors

Some of the first approaches to influence the regulation of transcription were reported by Schreiber and co-workers. They separated the DNA-binding domain from the activation domain and fused both units to the FK-binding protein 12 (FKBP12), which can dimerize in the presence of its synthetic dimeric substrate FK1012, a cell-permeable chemical inducer (CID). CIDs that induce the dimerization of FKBP12 have been used to bring together and covalently link activation domains to the corresponding DNA-binding domain, which results in an up-regulation of gene expression (Figure 2a).^[6,7]

Zinc-Finger Motifs as Modules in Artificial Transcription Factors

Since these initial studies, a variety of other strategies have been used to modulate gene expression, including the exploitation of zinc-finger motifs as DNA-binding modules in artificial transcription factors (Figure 2b).^[10,11] Various proteins for transcriptional regulation have highly selective DNA-binding domains known as zinc fingers, which have fingerlike structures to recognize a specific three-base-pair DNA sequence.^[13]

The great advantage of using zinc fingers in novel transcription factors is their ability to form structured modules. A string of three fingers, each composed of 30 amino acids, produces three DNA-binding domains in a row, each of which recognizes a different sequence of three base pairs. Recently, two research groups connected several modules to generate more than 100 000 artificial zinc-finger proteins with diverse DNA-binding specificities and fused each of them to a transcription activation or repression domain.^[13–15] This was the first description of a method based on the use of transcription factors to induce or suppress a gene function in cells that was applicable to any genome. Analysis of different phenotypes should reveal new modulators of cell growth and development.

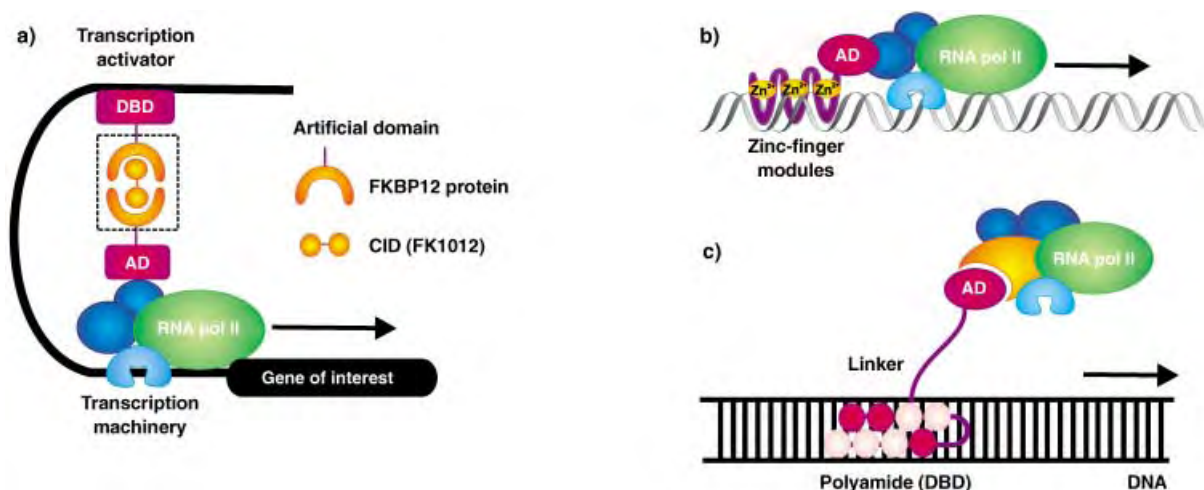


Figure 2. Activation of gene transcription by artificial transcription factors. a) Schematic view of CID-inducible transcriptional activation. The DNA-binding domain (DBD) and the activation domain (AD) are separated and each fused to FKBP12. After addition of FK1012 the two FKBP12 domains can aggregate and link the two parts of the transcription factor. b) Artificial zinc-finger domains that represent the DBD are coupled to a suitable activation domain (AD). c) The artificial DBD is composed of a hairpin pyrrole-imidazole polyamide, which binds to the minor groove. The DNA-binding domain is tethered to the activation domain (AD) by a linker.

However, there are numerous difficulties associated with the application of these artificial transcription factors as therapeutic agents, for example, they must be delivered to the appropriate destination in the body and should not elicit an immune response. Although delivery may be directed by retroviral means, as described by Beerli and Barbas,^[10] this method has risks associated with it, such as the unexpected and unpredictable mutagenesis of the genome, as is often observed in retroviral gene therapy. Therefore, artificial transcription factors based on molecules of low molecular weight may eventually be more suitable as therapeutic agents (Figure 2c).^[11,12]

Polyamides as Modules for Artificial Transcription Factors

To date, attention has been focused mainly on the discovery of smaller organic molecules that interact with DNA in the same way as their natural and semisynthetic counterparts. In so-called artificial transcription factors (ATFs), natural DNA-binding domains are replaced with synthetic variants, such as triplex-forming peptide nucleic acids (PNAs) and hairpin polyamides, which are tethered to an activation domain.^[10,11]

Dervan and co-workers were among the first chemists to synthesize such DNA-binding domains from polyamides.^[16] The design was based on the structure of natural compounds that are known to bind to DNA, whereby they form a kind of triple helix. One of these compounds was the crescent-shaped distamycin, which has a backbone comprising three consecutive amino acids with an *N*-methylpyrrole (Py) group. X-ray crystallographic studies on the distamycin–DNA complex revealed an antiparallel dimerization of the distamycin in an AT-rich region of the minor groove of the DNA. Based on distamycin as a lead structure, Dervan and co-workers further modified the backbone to generate similar antiparallel structures that recognize other sequences in the minor groove of the DNA. Replacement of the pyrrole group with imidazole (Im) or hydroxypyrrole (Hp) allowed recognition of the other DNA bases G and C.

When the antiparallel molecules were bridged with γ -aminobutyric acid (propyl linker), the resulting U-shaped or hairpin polyamide mimicked the antiparallel distamycin dimer. Based on a set of simple pairing rules (Py/Im targets C-G, Py/Hp targets A-T, Hp/Py targets T-A, and Im/Py targets G-C), tailor-made DNA-binding domains could be generated to target a variety of—but not all—DNA sequences tested, with affinities in the nanomolar range. Thus, these artificial transcription factors can compete with their natural counterparts.

The binding of such polyamides often leads to a depletion of the respective transcription factor and to the inhibition of gene expression. The specificity and affinity of the DNA binding can be increased further through the chemical ligation of two hairpin polyamides (tandem hairpins), so that longer sequences can be recognized.^[16] Hairpin monomers with alkyne and azide functionalities can undergo an intracellular 1,3-dipolar cycloaddition reaction at 37°C. The higher affinity of the tandem hairpins might be a result of a cooperative, combinatorial association of the polyamides. This association would mimic the natural phenomenon whereby gene-regulatory proteins cooperate by dimerization or oligomerization of DNA recognition elements, each of which contain four to six base pairs, to target unique adjacent sites in the DNA.^[17]

Polyamides as Artificial Regulators in Developmental Biology

The DNA-binding properties of polyamides have been exploited to generate more elaborate transcription inhibitors by coupling them to DNA-intercalating agents such as acridine. After the polyamide binds to the minor groove, the acridine is oriented towards the major groove, which leads to the deformation of a neighboring transcription-factor binding site.^[18] The question was raised whether such polyamides can also be coupled to transcriptional activator domains to enhance gene expression. It has been shown that a hairpin polyamide tethered to a peptide of an activation domain by a flexible aliphatic

or a rigid poly-L-proline linker is able to recruit the complex transcription machinery to enhance gene expression in a cell-free system.^[19–21] Recently, this method was successfully applied in vitro to imitate a DNA-binding complex that is essential for the correct development of *Drosophila melanogaster*.^[22]

Transcriptional regulators of the homeobox (Hox) family of proteins display a characteristic triple-helical DNA-binding domain (homeodomain), which can be replaced by polyamides. Hox proteins often interact with the TALE (Three Amino Acid Loop Extension) class of Hox proteins to form a Hox–TALE heterodimer for cooperative DNA binding (Figure 3).^[23] Both homeobox proteins interact through a short conserved YPWM-peptide-recognition site that is bound to the N terminus of the homeobox through a variable linker.

If the Hox transcription factor is replaced by a polyamide that imitates a YPWM-peptide-recognition site, the corresponding TALE transcription factor can be recruited to a cognate DNA site. The YPWM polyamide binds to the DNA and simultaneously exposes a bidentate binding site formed of DNA and peptide, which increases the affinity for the TALE transcription factor.

The results indicate a new class of transcriptional regulators that function in concert with natural transcription factors. However, there is a long way to go before we can apply these artificial compounds in therapy. There are still problems concerning the efficiency of cellular uptake and nuclear localization, as well as the accessibility of the minor groove of DNA in the densely packed regions of the genome (chromatin).

Despite some success in applications in vivo, studies by confocal microscopy on the uptake of polyamide–dye conjugates in living cells revealed the predominant localization of the polyamides in the cytoplasm of the cell and not in the nucleus where they are required. However, artificial transcriptional regulators have great potential for novel therapeutic applications in the treatment of various diseases, such as cancer. First experiments with distamycin tethered to the anticancer drug cisplatin revealed its higher efficiency in the cross-linking of DNA than that of free

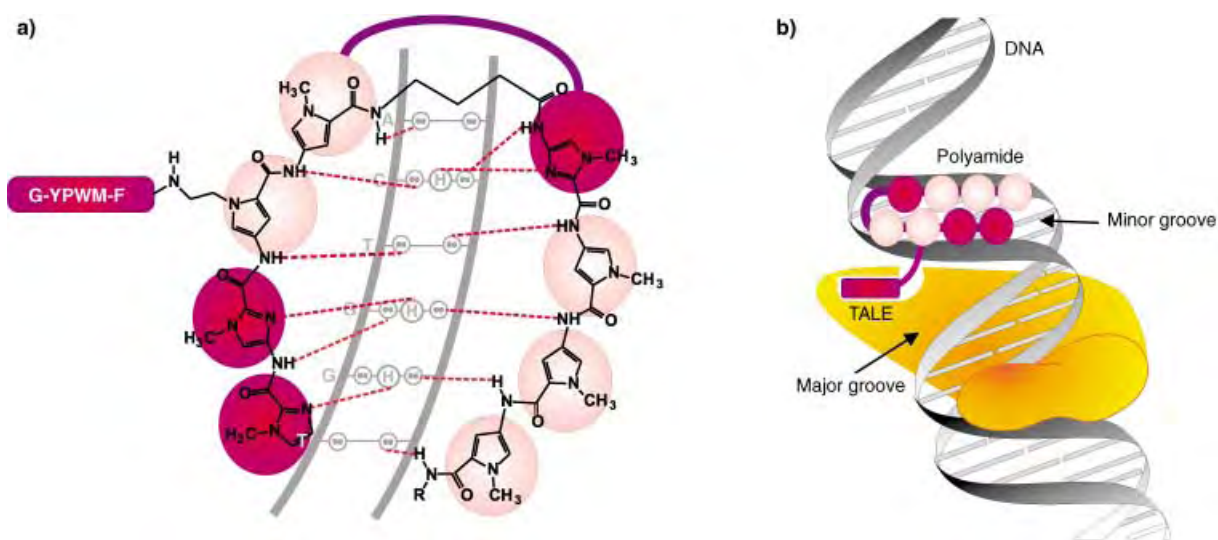


Figure 3. Artificial developmental regulators. a) Structure of the hairpin pyrrole–imidazole polyamide coupled to a recognition peptide (YPWM) for the TALE homeobox protein. b) Recruitment of the TALE homeobox protein to the major groove of the DNA by the peptide-coupled polyamide.

cisplatin, which suggests that polyamides may be used in the future as regulators of gene expression.^[24] The challenge now is to further improve the design and synthesis of non-natural factors for use in biology and medicine.

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Electrochemistry/Mass Spectrometry (EC/MS)—A New Tool To Study Drug Metabolism and Reaction Mechanisms

Uwe Karst*

Keywords:

derivatization · electrochemistry · liquid chromatography · mass spectrometry · metabolism

The possibility of simulating the oxidative metabolism of drugs is only one major reason for the strongly increasing attraction in the on-line coupling between electrochemistry and electrospray mass spectrometry (EC/MS) for both industry and academia. Although this combination has been known from a few publications for a long time and has been covered by recent reviews,^[1] it is now leaving its hibernation phase for good reasons. Some very attractive new applications for EC/MS, partly in combination with liquid chromatography (LC), have recently been published. Herein, the major characteristics of EC/MS are summarized, the most interesting recent applications are discussed, and future perspectives are presented.

In addition to the clear fact that electrospray mass spectrometric (ESI-MS) detection provides a larger wealth of information than electrochemical detection, some other known drawbacks of electrochemical detection are avoided when using EC/MS: As quantification is performed on the basis of the integration of the mass spectrometric signals and not on current measurements, unstable baselines can more easily be avoided and small changes in solvent composition do not automatically lead to quantification problems. This is most

important for coupling with gradient-elution liquid chromatography.

The additional coupling of EC/MS with LC is useful for two different situations. On the one hand, the investigation of the electrochemical conversion of any single substance out of a complex mixture requires a separation. The favored procedure consists first of a reversed-phase LC separation of the individual constituents followed by electrochemical on-line treatment and mass spectrometric detection (Figure 1, route a). In this way, only the reaction products of one single compound are detected at one time. On the other hand, the separation of the oxidation products may provide useful information, for example, on the polarity of the individual products. In this case, a pure starting material is first oxidized electrochemically, and the products are subsequently separated by liquid chromatography and then detected by mass spectrometry (Figure 1, route b). The integration of an additional UV/Vis detector prior to the mass spectrometer is possible in both cases. An interesting aspect is the fact that, in contrast to electrochemical

detection, LC/EC/MS detection is unrestrictedly compatible with gradient elution in LC, thus allowing for improved LC separations to be obtained.

The most widely applied cells for electrochemical detection, for example, thin-layer or wall-jet cells, have the reputation of being contaminated very easily by reaction side products (for example, polymerization products), thus requiring frequent maintenance. This severely hampers the use of electrochemical detection in routine analysis. Pulse techniques, which involve measuring and cleaning steps, are advantageous for increasing the mean time between maintenance intervals. However, a quantitative turnover of the target compound would allow mass spectra to be obtained which contain only a few different peaks and are easy to interpret. This is not possible with pulsed electrochemical techniques. Electrochemical cells with large surface areas, however, are advantageous for combining quantitative turnover with long maintenance intervals, thus rendering the method suitable for the routine analysis of a large number of samples.

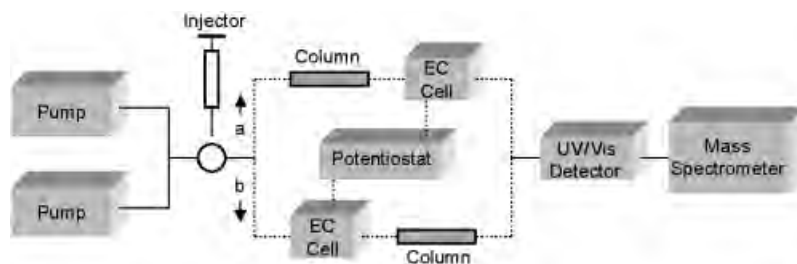


Figure 1. Schematic set-up of LC/EC/MS (route a) and EC/LC/MS (route b), including an optional UV/Vis detector prior to the mass spectrometer.

[*] Prof. Dr. U. Karst
University of Twente
Department of Chemical Analysis and
MESA⁺ Institute for Nanotechnology
P.O. Box 217, 7500 AE Enschede
(The Netherlands)
Fax: (+31) 53-489-4645
E-mail: u.karst@utwente.nl

For these reasons, most of the research groups who are active in this field use commercially available electrodes with an extremely large surface area glassy carbon working electrode, a Pd counter-electrode, and a Pd/H₂ reference electrode. Significant progress is currently being made by academic groups as well as by industry towards making electrodes available which combine a large surface area with other working electrode materials (for example, Pt) and low flow rates.

Bruins and co-workers^[2] have recently described an interesting approach to mimic oxidations catalyzed by cytochrome P450 by using on-line electrochemistry/mass spectrometry. Although not all the reactions catalyzed by cytochrome P450 were observed in the EC/MS system, those initiated by a one-electron oxidation, including dehydrogenation, alcohol oxidation, S and P oxidation, and N dealkylation were found. In contrast, reactions initiated by direct hydrogen abstraction, for example, hydroxylation of unsubstituted aromatic rings and O dealkylation, were not detected because their oxidation potential was too high.^[2] Nevertheless, the method may become an extremely valuable tool in the early phases of drug discovery, as it provides a rapid and simple means to investigate the metabolic stability of possible drug candidates, which may be derived in large numbers from combinatorial substance libraries.

The same research group has used an EC/MS system to rapidly analyze the oxidation products of peptides.^[3] The results of this study confirm earlier literature data obtained using much more laborious methods in which tyrosine-containing peptides were readily oxidized and resulted in the formation of various products, including peptide fragments caused by hydrolysis at the C-terminus of tyrosine (Scheme 1). It became evident in a comparison of phos-

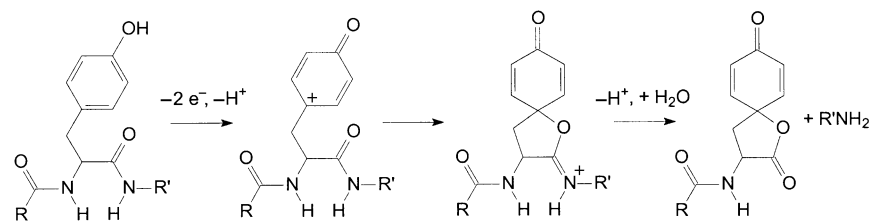
phorylated and non-phosphorylated tyrosine residues that cleavage only occurs at native tyrosine residues. This result allows an easy distinction between phosphorylated and non-phosphorylated peptides. It may be expected that an attractive EC/MS-based protein digestion and peptide-mapping method could be developed from this work in the future.

The application of dedicated derivatizing agents for EC/MS has been introduced by Van Berkel et al.^[4] The oxidation process inherent in the electrospray interface was used to selectively detect the ferrocene-derivatized analytes. In this pioneering article, no additional electrochemical cell was used, and good results were achieved without LC separation of the samples. Applications are presented for the detection of alcohols (with ferrocenoyl azide) and for the detection of diols (with ferroceneboronic acid).^[4] In more recent work, ferrocenoyl azide was used in combination with the same detection technique for the analysis of mixtures of natural products, and the fragmentation pathways were investigated for the derivatives of primary, secondary, and tertiary alcohols.^[5] Karst et al. used ferrocenoyl chloride for the determination of phenols and alcohols. A reversed-phase separation was used, the derivatives were oxidized in an electrochemical cell and an atmospheric pressure chemical ionization (APCI) interface was applied with the corona discharge needle switched off.^[6] It can be expected that these techniques will strongly gain importance for quantitative analysis, as a result of the excellent selectivity and sensitivity; in addition new dedicated derivatizing agents for EC/MS based on ferrocenes and other redox systems will undoubtedly soon be introduced.

Gun et al. investigated the reduction of [(C₅Me₅)₂Mo₂O₅] and related complexes by using on-line EC/MS based on

the conversion in an electrochemical flow cell. The authors succeeded in identifying mono- to tetranuclear organometallic molybdenum oxides.^[7] This work may open up new pathways for the analysis of metal complexes, because the different oxidation states of the analytes can be identified on-line by varying the applied electrochemical potential. As already indicated in earlier work by Amster and co-workers,^[8] the use of high-resolution mass spectrometry may even allow insight into the redox status of metalloproteins to be obtained. The oxidation state of the metal centers was found to be stable in an electrospray interface (both in the positive and negative ion modes). More applications for the analysis of metalloproteins are likely to follow in the near future, because this technique is able to provide information which is not available using any other analytical method.

The large number of EC/MS applications developed within the last few years clearly shows that this technique may now find rapidly increasing application in different scientific areas. The use of EC/MS for metabolic studies and peptide or protein digestion is particularly promising because of the large number of current challenges in bioanalysis. However, it is important that appropriate flow cells suitable for very low flow rates are soon developed to make the technique compatible with micro- and nano-LC separations and minute amounts of available analytes. Different working electrode materials (for example, Pt) in combination with large surface areas would also help to expand the range of applications. The full potential of EC/MS is only just starting to be discovered, and significant new applications in this field can certainly be expected in the near future.



Scheme 1. Electrochemically induced C-terminal peptide cleavage at a tyrosine residue.^[3]

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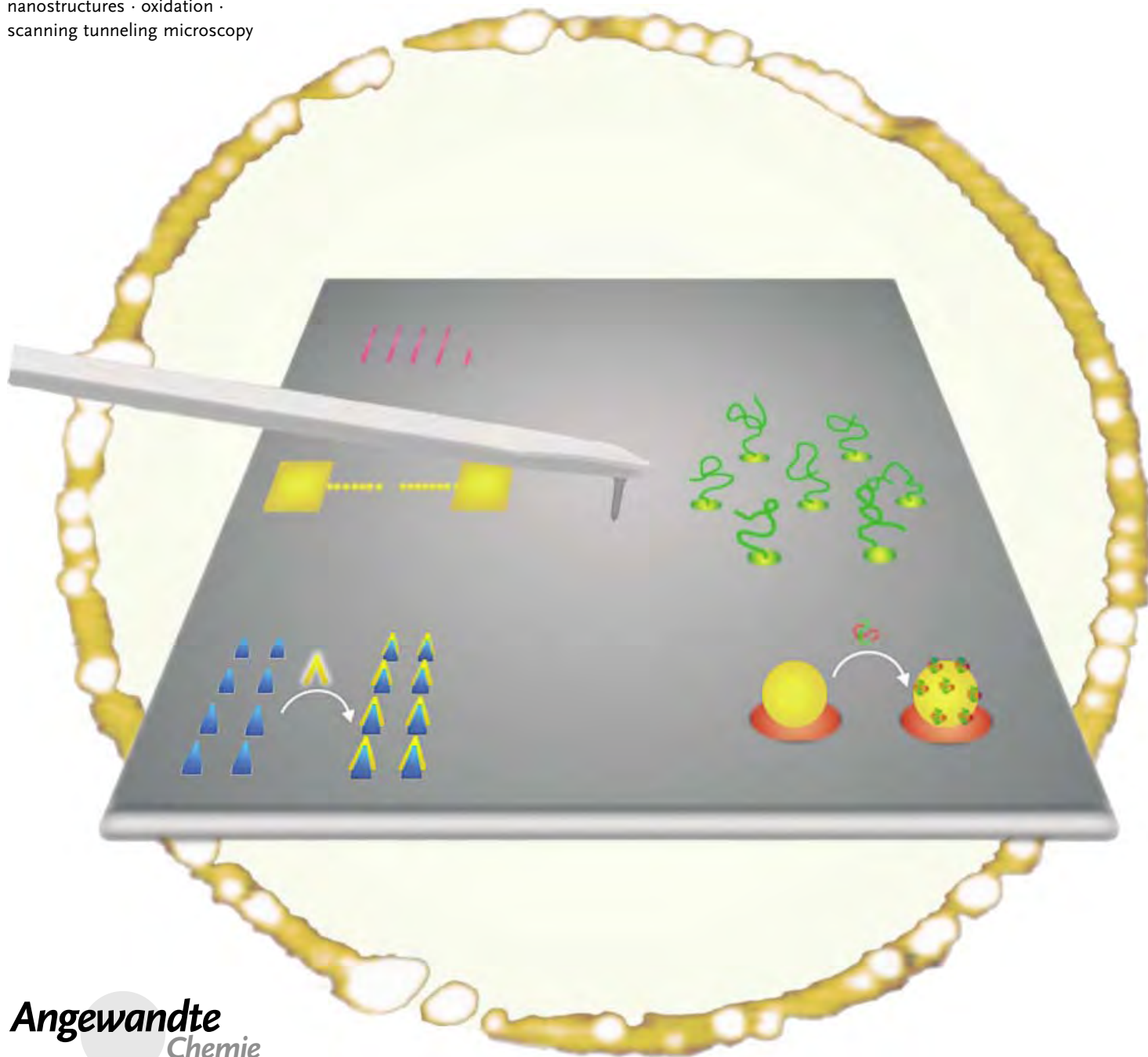
Nanolithography and Nanochemistry: Probe-Related Patterning Techniques and Chemical Modification for Nanometer-Sized Devices

Daan Wouters and Ulrich S. Schubert*

Keywords:

atomic force microscopy · lithography · nanostructures · oxidation · scanning tunneling microscopy

*Dedicated to Professor Dieter Schubert
on the occasion of his 65th birthday*



Angewandte
Chemie

The size regime for devices produced by photolithographic techniques is limited. Therefore, other patterning techniques have been intensively studied to create smaller structures. Scanning-probe-based patterning techniques, such as dip-pen lithography, local force-induced patterning, and local-probe oxidation-based techniques are highly promising because of their relative ease and widespread availability. The latter of these is especially interesting because of the possibility of producing nanopatterns for a broad range of chemical and physical modification and functionalization processes; both the production of nanometer-sized electronic devices and the formation of devices involving (bio)molecular recognition and sensor applications is possible. This Review highlights the development of various scanning probe systems and the possibilities of local oxidation methods, as well as giving an overview of state-of-the-art nanometer-sized devices, and a view of future development.

1. Introduction

Nanotechnology is currently at the center of attention in both the media and in public opinion as well as in governmental and industrial research programs. Large funding programs related to nanotechnology were recently initiated in Europe, Japan, and the USA.^[1,2] This development is reflected by a shift of attention in academic and industrial research centers. The envisioned fields and their potential technological impact on society range from “smart” drugs or sensitive DNA/protein sensors to nanostructured electronic devices with applications in information storage, optical computers, solar cells, and energy storage, as well as novel materials with improved properties such as “intelligent” and self-cleaning or color-changing coatings.^[3–6] It should be noted that many of these envisioned improved properties relate to changes and/or control of surface properties. This directly implies the need for well-defined, controllable, reliable, and affordable surface modification and characterization techniques. In general, two approaches for the creation of nanostructured surfaces are available. The first, the so-called “top-down” approach, epitomizes efforts by physicists and engineers to reduce the size of structures with techniques that are currently available. Most results using this strategy originate from the downscaling of lithographic techniques in semiconductor research. The second “bottom-up” approach has been developed by (bio)chemists, who have considered the self-assembling processes in nature when creating similar patterns that utilize noncovalent molecular interactions such as hydrogen bonding, π – π stacking, and/or metal–ligand coordination.^[7–11] Although the bottom-up approach is clearly interesting, and can lead to unique and interesting structures, it also has some clear disadvantages: The ability of patterning alone is insufficient for successful application in advanced (nano)devices, since besides size and structure, control over both direction and position is also essential. Moreover, the ability to characterize the created

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structures and the feasibility of producing these structures on large areas is of central interest.

As a top-down approach, lithography represents an important surface-patterning technique. Lithography was introduced in 1798 as a printing tool by

Alois Senefelder, who found that ink adsorbed to an image drawn with a greasy fluid onto limestone (lithographic stones, Figure 1) could be used to transfer the image to a piece of

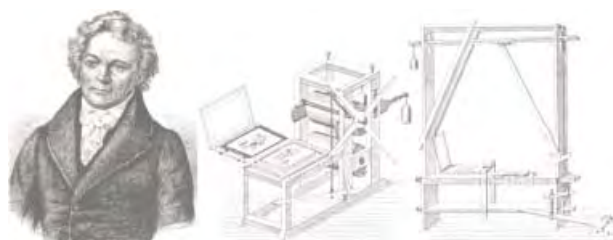


Figure 1. Lithographic stones were introduced by Alois Senefelder.^[12]

paper pressed against the stone.^[12–15] Since then, this printing technique has evolved dramatically and is nowadays not only used for printing but also for the (chemical) modification of substrates on a submicrometer scale, and is often referred to as microcontact printing.^[16–18] The most prominent application of lithography is that of UV-mask lithography, which is widely used in the semiconductor industry. With the development of (computer) chips and their increasing complexity, the requirement for techniques able to create smaller structures is ongoing. Therefore, industry and academia invest intensive

[*] D. Wouters, Prof. Dr. U. S. Schubert
Laboratory of Macromolecular Chemistry and Nanoscience
Eindhoven University of Technology and
the Dutch Polymer Institute (DPI)
P.O. Box 513, 5600 MB, Eindhoven (The Netherlands)
Fax: (+31) 40-247-4186
E-mail: u.s.schubert@tue.nl
Prof. Dr. U. S. Schubert
Center for NanoScience, Ludwig-Maximilians-Universität München
Geschwister-Scholl Platz 1, 80333 München (Germany)

effort in developing UV and deep-UV lithographic techniques, which can provide structural resolution down to 80 nm.^[19] At the same time, particle-, ion-, and electron-beam lithographic techniques have become available for structuring substrates at the 50-nm level. Smaller dimensions are possible, but the costs rise enormously and the addressable substrate sizes decrease enormously. Both UV, particle, and e-beam lithography are well documented, and a large number of recent publications are available.^[20–26] A publication by Grunze et al. is of particular note because it introduces the possibility for chemical lithography using an electron beam. Focused electrons are used to chemically modify the terminal nitro groups of self-assembled monolayers (SAMs) to amines; the subsequent functionalization with carboxylic acid anhydrides was then demonstrated (see Figure 2).^[27,28] Also of note is the preparation of ordered arrays of metallic nanoparticles and metallic rods using the self-assembly of metal-containing block copolymer micelles onto electron-beam-patterned substrates, effectively combining techniques from the bottom-up and the top-down approaches.^[29,30]

As a result of decreasing pattern size, the demand for microscopic characterization techniques has also increased. Since their invention in 1982,^[31] scanning tunneling microscopy (STM) and atomic force microscopy (AFM) have proven to be effective techniques capable of identifying the structure and nature of surfaces and adsorbates on substrates down to molecular and atomic resolution. The application of AFM and STM has not been limited to inorganic semiconductor substrates: also in polymer and supramolecular research has their value also been demonstrated by the elucidation of, for example, polymer phase separation and molecular ordering.^[32–34] In biochemistry, the techniques have been applied for the determination of interaction forces between proteins and receptors.^[35–38] Soon after the invention of AFM and the observation of its ability to modify substrates, the field of scanning-probe-based lithography (SPL) was developed.^[39] The application of probe-based techniques for the modification of substrates has been widespread and a large variety of techniques have evolved, which range from the subtle movement of atoms (using STM),^[40] the formation of local deformations in soft substrates using high-contact force AFM (see Section 2.1) to the local application of “inks” (dip-pen lithography) and the local oxidation of suitable substrates (probe oxidation, see Sections 2.2 and 2.3). With

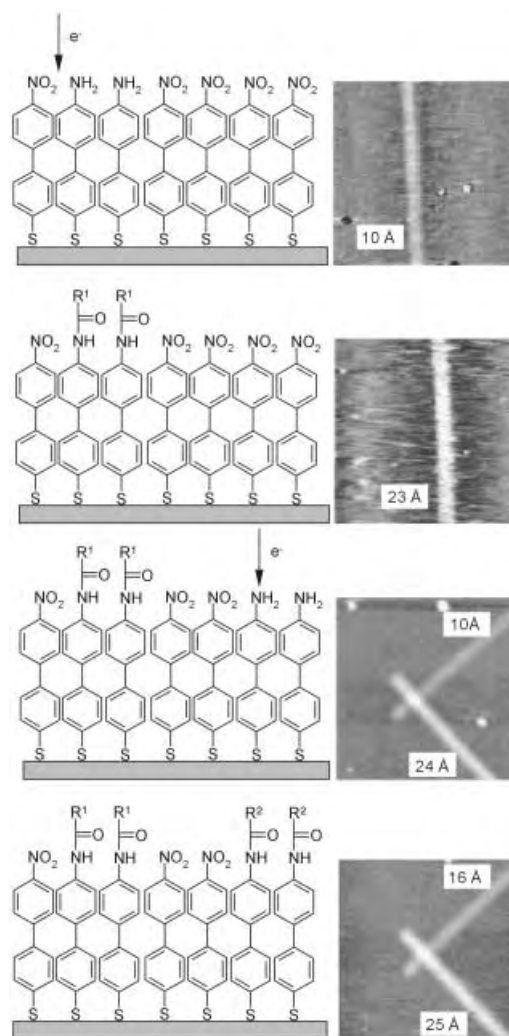


Figure 2. Electron-induced chemical lithography using self-assembled monolayers (SAMs) as a platform for the generation of patterns. Scanning force microscopy images show the selective binding of acetic acid anhydride ($R^1 = \text{CH}_3$) and perfluorobutyric acid ($R^2 = \text{C}_3\text{F}_7$) onto a SAM platform.^[27]

the application of probe-based techniques, control over position and direction is evident. Moreover, due to the very recently developed multi-probe systems (see Section 2.3) and



Daan Wouters studied chemical engineering (1995–2000) at the Eindhoven University of Technology (The Netherlands). During this period he spent 4 months at DSM Desotech (Elgin, USA) on a project regarding UV-curable coatings. He graduated in 2000 under the supervision of Dr. P. C. Thüne (the Surface Science group of Prof. J. W. Niemantsverdriet) working on a surface-science model of the Philips catalyst. Since 2001, he has studied for a PhD with Prof. U. S. Schubert at the same university. His research centers on scanning probe lithography and nano-structured surfaces.



Ulrich S. Schubert was born in 1969 (Tübingen, Germany). He studied chemistry and biochemistry in Frankfurt, Bayreuth, and Richmond (USA). He obtained his PhD (1995) under the supervision of Profs. C. D. Eisenbach (Bayreuth) and G. R. Newkome (Tampa). After postdoctoral training with Prof. J.-M. Lehn (Strasbourg), he obtained his habilitation with Prof. O. Nuyken (TU München) in 1999, followed by a year at the Center for NanoScience (LMU, München). Since June 2000 he has been a Full Professor at the Eindhoven University of Technology (Chair for Macromolecular Chemistry and Nanoscience).

automated scanning probe equipment,^[41] the patterning of large areas has become accessible. Finally, not only patterns on substrates can be created, but also chemical modification reactions on specific substrates are accessible, which allow the combination of techniques from both the top-down and the bottom-up approaches. Bearing this in mind, lithographic techniques based on scanning probe techniques are the method of choice for applications in nanotechnology.^[42]

This Review provides an overview of current probe lithographic techniques and their subsequent (chemical) modification processes, which result in the creation of functional structures. The field of probe lithography can be split into two main areas: chemical and physical surface modification. The latter includes the formation of patterns by the physical movement of material on a substrate (for example, by applying a high contact force or through modifications induced by locally applied heat). Moreover, dip-pen lithography, in which atoms and molecules attached by adhesion to a tip can be transferred to a substrate in a controlled fashion, is also described in this context, since in this process chemical bonds are neither created or broken. The category of chemical modification processes include experiments that use local oxidation processes. This process was first observed in STM experiments, but has also been transferred to AFM and can be applied to conducting (metallic) substrates as well as to thin layers of nonconducting (organic) resists.

For many of the classes in the physical modification category, a number of publications and some applications have been presented, and many good reviews are already available. For this reason, only a short description will be presented here. Moreover, no attempt will be made to present a comprehensive overview; rather a number of typical examples and applications of subsequent chemical modification will be shown. The second part provides examples of the electrical modification of substrates and organic resists using both STM and AFM techniques. Since STM techniques are rather well documented, this Review focuses on the application of conductive AFM tips in the formation of functional nanostructured surfaces.

2. Physical Probe Lithography Techniques

2.1. Mechanical Surface Patterning

Since its invention, the goal of AFM has been to image surfaces without causing surface damage. However, the imaging of soft (organic) materials in contact mode (with relatively hard tips and stiff cantilevers) leads to surface damage. Therefore contact-mode AFM for these kind of samples is usually displaced by noncontact or intermittent contact AFM to reduce tip-induced surface damage. The concept of the controlled mechanical deformation of substrates using standard AFM tips can be transferred to almost any substrate. For example, the controlled application of (soft) tip-crashes in STM led to the formation of patterned substrates. The limiting factor in creating reproducible patterns is the stability of the tip itself, which is prone to deformation and contamination. To prevent excessive defor-

mation, several groups have used diamond or diamond-coated tips. Mechanical patterning (force lithography) has successfully been applied to substrates and films of soft metals such as copper, gold, nickel, and silver.^[43–47] The structures that have been created include thin lines and pits. Figure 3 a)

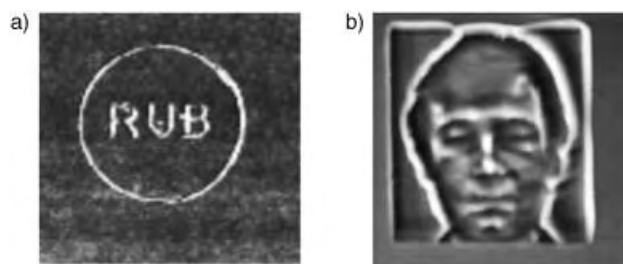


Figure 3. a) AFM image ($2.5 \times 2.5 \mu\text{m}^2$, $z\text{-range} = 10 \text{ nm}$) of a resist (Shipley SP25) patterned with an intermittent-contact SPM. The patterning was performed in vector mode; the dark lines are plowed grooves while the bright lines are embankments due to the displaced resist;^[46] b) AFM image ($2.5 \times 2.6 \mu\text{m}^2$) of a resonance-SPM modification of a polycarbonate film on silicon in which the patterning force has been applied according to greyscale bitmap image.^[48]

mation, several groups have used diamond or diamond-coated tips. Mechanical patterning (force lithography) has successfully been applied to substrates and films of soft metals such as copper, gold, nickel, and silver.^[43–47] The structures that have been created include thin lines and pits. Figure 3 a) shows an example in which a force-induced pattern was inscribed into a photoresist film by high-force intermittent-contact scanning probe microscopy (SPM). High-force AFM has also been applied to polymer substrates and polycarbonate films (see Figure 3b), both in contact as well as intermittent operation.^[46–48] Besides the mechanically induced deformation of substrates and films, scanning probe techniques can also be used for the gentle movement of metallic^[49–51] or latex^[52] nanoparticles into dimers, trimers, linear structures, and letters. The same method has also been applied for the local removal of parts of SAMs and Langmuir–Blodgett (LB) films.^[53] If these experiments are carried out in a solution containing a competing molecule or nanoparticle the free space created by the tip will be filled in situ. Therefore, stable, ordered, mixed monolayers or a special arrangement of nanoparticles can be produced. Liu et al. presented an example of the mixed-monolayer method, where monolayers of 1-octadecanethiol and 11-sulfanyl-1-undecanol on gold were utilized as a substrate and octadecyltrichlorosilane (OTS) as a reagent in subsequent positive and negative pattern-transfer reactions (Figure 4). The same group also demonstrated the possibility of creating holes in SAMs of long-chain alkane thiols on gold surfaces, into which gold nanoparticles with a shell containing free thiols could be deposited.^[54]

Atomic manipulation is perhaps the most appealing form of modification from a scientific viewpoint. In 1990, Eigler and co-workers exhibited the possibility of creating artificial structures by the direct movement of adsorbed atoms on a metal surface in an ultrahigh vacuum (UHV) at low temperatures.^[55] A notable example of a working logic device consisting of molecular cascades developed at IBM was also recently presented by Eigler.^[56] By arranging adsorbed CO molecules on a Cu(111) substrate and using the instability of bent trimer adsorbates, working AND and OR logic circuits

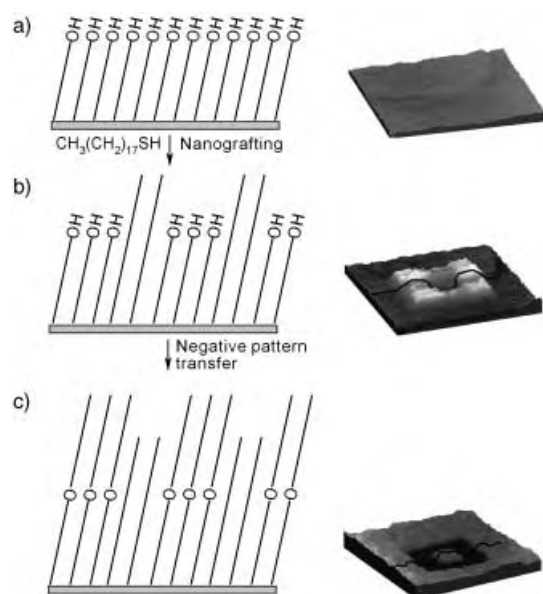


Figure 4. Schematic diagrams and corresponding AFM images ($600 \times 600 \text{ nm}^2$) of a series of experiments for the construction of 3D nanostructures. Nanopatterns of 1-octadecanethiol (b) are grafted into a matrix of 11-sulfanyl-1-undecanol/Au(111) SAMs (a). The hydroxyl end groups within the nanostructure react with octadecyltrichlorosilane. The nanopatterning results in a negative pattern transfer (c).^[53]

were combined into a two-input sorter composed of a setup of 198 molecules on a $9 \times 9 \text{ nm}$ area (Figure 5).

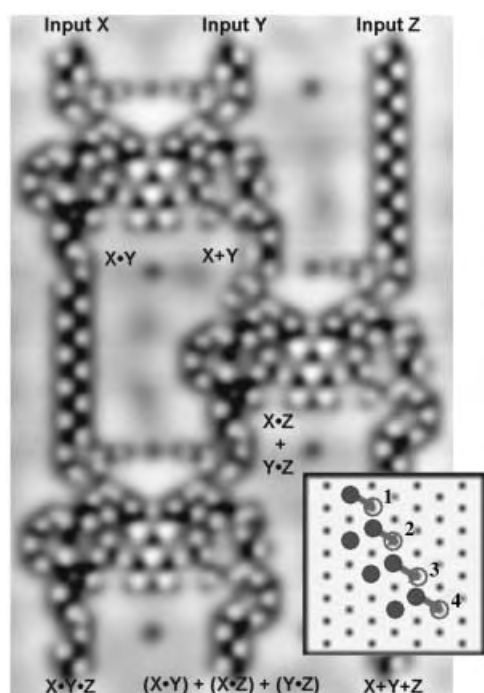


Figure 5. STM image ($12 \times 17 \text{ nm}^2$) of CO molecules adsorbed on a Cu(111) crystal at 5 K arranged into a three-input sorter. By moving a CO molecule with the STM tip (at one or more of the inputs) into an unstable trimer configuration, a molecular cascade is triggered (see inset) that travels down to the output where the STM was used to read out the result of the operation.^[56]

2.2. Dip-Pen Lithography

The group of Mirkin has pioneered a patterning technique that relies on material being transferred from an AFM tip to the surface, known as dip-pen nanolithography (DPN). This material can simply be the constituents of the tip itself (i.e., gold), induced by force or current, or it can be physisorbed material. Material on the tip is transferred to the substrate by capillary forces. Using DPN, Dravid et al.^[57] have patterned silicon substrates with various dyes, whereas Rayment et al.^[58] applied up to fifth-generation dendrimers (G5 DAB, amino terminated) to Si(100) substrates to form several micrometer-long lines with diameters up to 100 nm. The effective obtainable line widths were highly dependent on the utilized writing speed and temperature.^[59] Although studies suggested that the transport of material was dependent on a water meniscus between the tip and the substrate,^[60–66] writing experiments have also been performed at zero relative humidity, which indicates surface diffusion as being a transport factor.^[59,67] Mirkin et al. have used DPN extensively to deposit proteins on surfaces. Recently the formation of a nanoarray of proteins on a gold substrate has been reported.^[68] In this study, proteins (IgG and Lysozyme) were positioned in nanoarrays (islands with diameters of $\approx 200 \text{ nm}$) and subsequently coupled with antibodies (anti-IgG). Figure 6 schematically demonstrates the procedure.

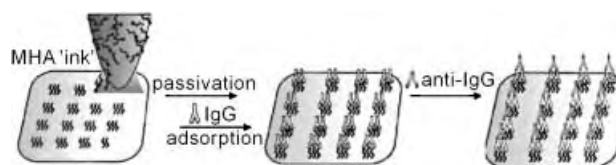


Figure 6. Schematic representation of protein nanoassembly: In the first step, dip-pen lithography was used to create 16-sulfanyl-hexadecanoic acid (HMA) islands on a gold substrate (left). Rabbit-IgG proteins were adsorbed onto these islands (center), followed by coupling with anti-IgG (right). The process could be followed by observing increases in the height profiles of AFM images after each step.^[68]

The same group have utilized oligonucleotides, polystyrene latex particles (with diameters of 190 nm and 1 μm),^[66,69] magnetic Fe_3O_4 nanoparticles,^[70] and metal-precursor-based inks in order to create patterns down to 45 nm. Nanostructures of Al_2O_3 , SiO_2 , and SnO_2 have successfully been prepared from dip-pen depositing solutions of the corresponding MCl_n -salts followed by hydrolysis to the corresponding oxide.^[71] Dip-pen lithography also allows the creation of structures with more than one ink. By switching to another tip, it is possible to functionalize an area next to a previously prepared structure with another ink.^[72] A review by Mirkin et al. supplies a more comprehensive overview on this topic.^[73]

2.3. Thermal Patterning with Multi-Tip Systems

The idea of using thermomechanic patterning for data storage was developed by Mamin and Rugar at IBM

Almaden.^[74] Using a laser diode to heat a tip that is in contact with a poly(methyl methacrylate) (PMMA) film to a temperature higher than the softening point of PMMA creates an indentation in the film that has the shape of the tip. In this way, densities of up to 30 Gbin^{-2} were achieved. Although the size of the features was initially 100 nm , writing and readout was reliable (a system with a single tip traveling a total of 16 km in 145 h reading 200 nm marks with a 400 nm period has been reported).^[75,76] Practical data storage applications were envisioned with the development of sharper tips, higher readout frequency cantilevers, integrated heating, and smoother substrates. Initial tips that had the disadvantage of laser-diode heating were replaced by tips with integrated heating circuits.^[75,77,78] However, mechanical limitations in the resonant frequencies of the cantilevers restricted the obtainable data transfer rates to a few Mbs^{-1} .^[76,79] Patterning speed as well as read-out speed could be significantly increased by the development of multi-tip systems.^[80–88] To the best of our knowledge, the group of Quate (Stanford) first reported the use of a multi-tip system for lithography experiments on amorphous silicon in 1995.^[87] The system had five parallel tips (Figure 7a) and was based on an earlier development by Tortonese. The group fabricated piezoresistive cantilevers that could operate without external sensing requirements due to integrated force sensing. Because the tip arrays did not allow for individual z -movement, the arrays had to be aligned

manually to the substrate. Utilizing this setup, $400 \times 100 \mu\text{m}$ parallel images were obtained in constant-height mode (Figure 7b). In a multiple-tip-scanner setup either an increase in scan size or in scan speed proportional to the number of tips could be realized. The lack of an individual height control limited the applicability of the system for lithographic purposes to a total of two tips operated in parallel. In Figure 7c a lithographic experiment using two parallel-operated tips demonstrates the problems associated with the lack of individual z -control: only one of the two tips was constantly in contact during patterning, while the other tip produced an incomplete pattern.

In 1998, Vettiger and co-workers (IBM, Zürich) reported a 5×5 -tip array with integrated force sensing and its successful application for imaging. For stable operation, the tip array and the substrate had to be precisely levelled. This was realized by detecting the deflection of three cantilevers on three edges of the chip and by control through a setup of actuators on the array and the sample.^[85] Moreover, Quate et al. reported in 1998 a centimeter-scale AFM^[84] that used up to 50 parallel cantilevers with integrated force sensors. Surface areas of $2 \times 2 \text{ mm}$ were imaged at a $0.4 \mu\text{m}$ pixel size in 30 min with 10 parallel tips. An array of 32 tips was used to study 1.28 mm^2 of a diffraction grating and an array of 50 parallel tips was utilized for lithography on hydrogen-passivated Si(100) by electric-field-enhanced oxidation at 15 V . The resulting thin oxide lines were subsequently used as an etching mask resulting in a 1 cm^2 patterned area with lines that were $1.1 \mu\text{m}$ wide.

By combining the original idea of Rugar and that of a multi-tip system, an AFM-based data storage system capable of producing high data density and improved read-out speeds became reality. In 2000, and again in 2003, Vettiger, Binnig and co-workers reported the development of the so-called “Millipede” system (Figure 8).^[81–83] The “Millipede” consists of an array of 1024 tips (32×32) with integrated read/write capabilities using combined effects of contact force and applied heat (Figure 8a). The process of writing bits into thin PMMA films was achieved by heating the tips to 400°C using a current traveling through a highly doped section at the end of the cantilever. This allowed the prestressed lever structure to flex into the polymer (Figure 8b). Erasing large areas of written data required heating the whole medium to 150°C , which causes the PMMA film to undergo thermal reflow. Individual bits could be erased by forming a second pit adjacent to the first pit. Readout of the data was achieved by scanning a warm tip (300°C) over the sample and measuring the heat resistance over the bits: when a tip entered a hole, the cantilever came closer to the PMMA film, thus increasing the heat conductivity.

By combining 1024 tips on a $3 \times 3 \text{ mm}$ chip, patterns with a $12 \mu\text{m}$ pitch and 40 nm data bits corresponding to aerial densities of 400 Gbin^{-2} , were obtained. The first prototypes of 4096-tip arrays (64×64 ; 6.4 mm^2) were already reported^[83] and design parameters are now focused on commercializing the concept. The “Millipede” system will probably be the first real commercial launch of an AFM-based “nanostorage device” as long as issues of cost reduction and long-term operation can be resolved.^[83]

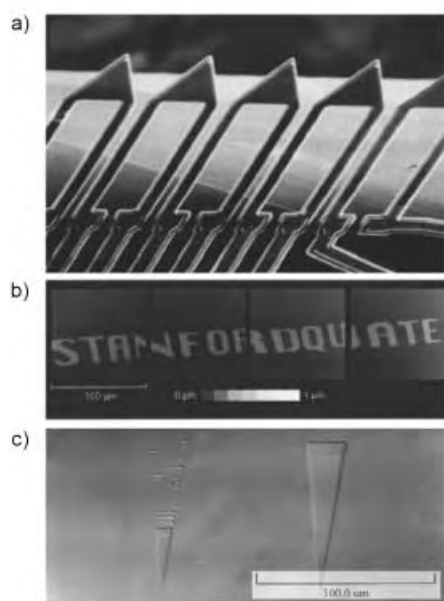


Figure 7. a) SEM image of an assembly combining five AFM tips for parallel operation, as developed by Quate et al; b) an AFM image ($400 \times 100 \mu\text{m}$) of a pattern obtained by the parallel operation of four AFM tips; c) Pattern created by the parallel operation of two AFM tips (by local-probe oxidation of Si, see Section 3). The result demonstrates the limitation of individual z -control: the AFM image on the right (deflection controlled by a laser/detector system) shows the expected pattern, whereas the left tip (assumed to be in contact) performed poorly.^[87]

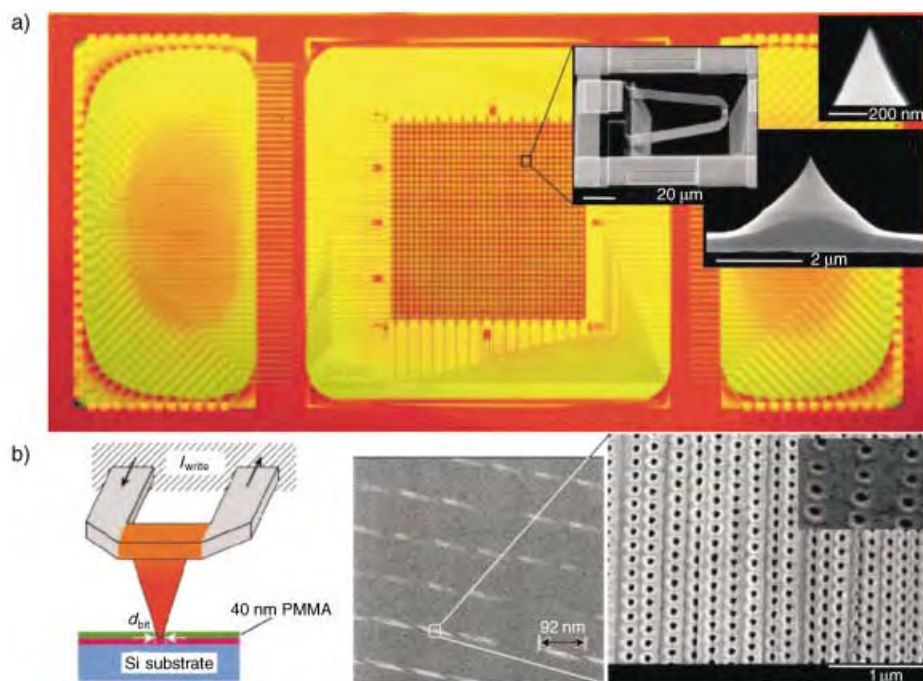


Figure 8. a) The “Millipede” concept developed by IBM consists of an array of 32×32 tips. Each tip can be individually heated by applying an electric current through a highly doped area in the cantilever; b) when the heated tips were pressed against a thin PMMA film, small holes were created. These holes can be used as bits for data-storage applications.^[82]

Research into multi-tip systems has not been limited to storage applications. Progress in parallel dip-pen lithography with an eight-pen nanoplotter was reported by Mirkin and Hong in 2000.^[80] The operating principle is similar to that of the “Millipede”, but in this case an eight-tip array has been used to deposit octadecylthiol on gold in eight identical patterns.^[80] In an inverted approach, Lee demonstrated the application of a tipless cantilever and an array of inverted tips on a substrate for application in force distance measurements.^[89] Difficulties in conventional force measurements involving the placement of addressable active individual molecules on a single tip could be reduced by this approach.

All of the probe-based lithographic techniques described above do not chemically modify the substrate. Functionality is only obtained through the mechanical movement of atoms and molecules. Therefore no chemical modifications are introduced that may be used in subsequent steps. Surface-modification processes using tip-induced force are accompanied by a significant problem in the form of tip wear. This is in contrast to the heat-induced phase changes in thin polymer films (like in the “Millipede” project), where tip wear on the medium-to-long-term time scale (months) has been shown to be small. In contrast to mechanical and/or thermal patterning, dip-pen lithography does not modify the substrate but adds functionality by depositing molecules. These molecules introduce the possibility of secondary modifications using, for example, proteins. Although modification with dip-pen techniques is rather slow, both due to limited ink capacity and relatively slow writing speed, progress can be envisaged through the development of multi-tip approaches.^[80–88]

In addition to the aforementioned techniques, probe oxidation is a promising approach to create structures that can be utilized in subsequent modification steps. In this case, local surface oxidation introduces the chemical (and physical) functionality of the corresponding substrate.

3. Oxidative Probe Lithography Techniques

3.1. Scanning Tunneling Microscopy Patterning

Soon after the invention of scanning tunneling microscopy (STM) by G. Binnig and H. Rohrer in 1982,^[31] researchers observed the ability of STM to modify surfaces, in addition to the effects of pure surface imaging. By 1985 Güntherodt et al. had reported the possibility of creating structures in $\text{Pd}_{81}\text{Si}_{19}$ substrates (Figure 9).^[39] Structure formation was attributed to the polymerization of an adsorbed oil layer originating from the vacuum oil-pumps.

In electrochemical patterning experiments, STM can be used in two distinct emission modes. In the first mode, electrons from the tip (negative bias on the tip) are used to locally oxidize the material below the tip; a type of low-energy (tunneling) electron beam induces local oxidation of the material. In the second method (the field emitting mode), the area below the tip is oxidized by the electric field, while material between the tip (e.g., oxygen or water)

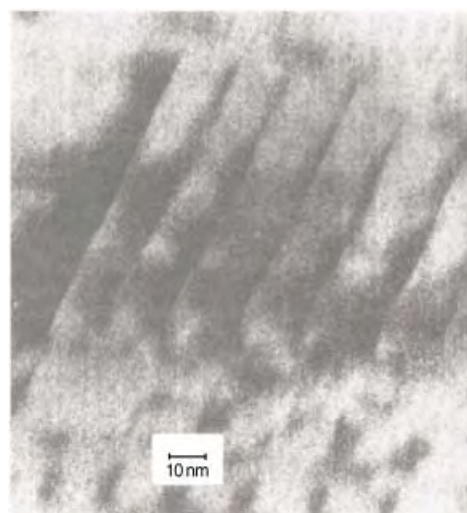


Figure 9. SEM image of freshly drawn lines on glassy $\text{Pd}_{81}\text{Si}_{19}$. The lines were drawn by applying a bias voltage to the STM tip (see text). The effect was ascribed to the local electron-induced polymerization of oil fragments, which originated from the vacuum pump system.^[39]

decomposes and their reaction products oxidize the substrate. A general reaction scheme proposed by Sugimura and Nakagiri describes the anodic reactions [Eq. (1)] and [Eq. (2)] that occur at the substrate surface and the cathodic reaction [Eq. (3)] that occurs at the tip (see also Figure 10):^[101]

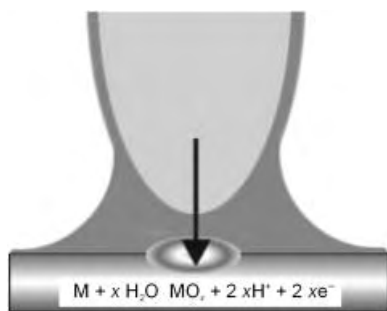
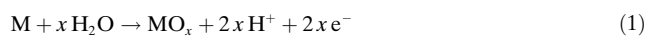


Figure 10. Schematic representation of the tip-induced oxidation of surfaces. The substrate was oxidized by an anodic reaction with the surface water layer. At the tip a cathodic reaction generates gaseous hydrogen.

An example of the field emission method is the direct oxidation of a hydrogen-terminated Si(100) substrate. Currently, this represents the most frequently studied system, together with the direct modification of layered substrates (i.e., HOPG or MoS₂) or metal surfaces (i.e., Ti or Cr); oxidation experiments of organic resists on substrates have been reported less often.

Heinzmann et al. have reported patterning on organic SAMs.^[90,91] In this case, hexadecanethiol and *N*-biphenylthiol on Au(111) and octadecyltrichlorosilane on Si(100) were utilized. A pattern could be etched into the silicon substrate using two wet-etch steps (5% HF, 30% KOH). Earlier experiments by McCord and Pease also described the patterning of PMMA and alkyl halide resists.^[92,93] They also prepared the first working device (a thin-film resistor) by patterning a PMMA resist, followed by a lift-off technique.^[93,94] A comprehensive review covering the early work in STM-induced surface modification was published in 1990 by Shedd and Russell.^[95]

3.2. AFM Probe Lithography

3.2.1. Oxidation of Metallic and Semiconducting Substrates

In the early 1990s, successful lithography experiments with STM led to several groups transferring oxidation techniques to AFM. Field-effect induced oxidation has been applied to Si,^[96–98] Ti,^[87,99] Ta,^[100] and Cr.^[101] Electron-induced oxidation has been applied to organic resists,^[102,103] SAMs,^[104] and LB films.^[105] As early as 1995, the group of Campbell and

Snow reported the application of this technique in order to create one of the first working devices, initially using a silicon tip and cantilever in an experiment oxidizing thin (7 nm) titanium films.^[106] Using a poorly conducting tip no current could be detected, which implied a field-effect induced oxidation of titanium to titanium oxide with adsorbed water. By partly oxidizing thin titanium wires produced by conventional optical techniques, thin titanium wires (10 nm) and metal-oxide junctions were prepared (Figure 11). The *I*–*V* characteristics of a 10 nm constricted titanium wire at room temperature and at 4.2 K were compared to those of a premodified (thick) wire.

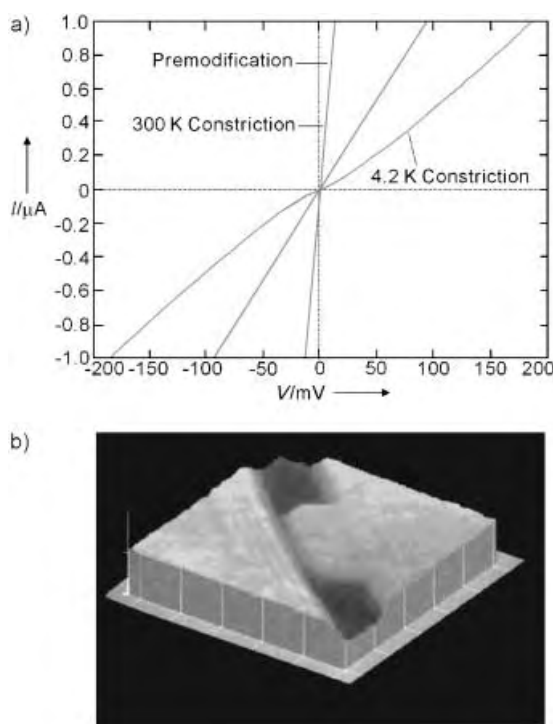


Figure 11. a) *I*–*V* characteristics of a thick Ti wire and a Ti wire with a constriction of < 10 nm, measured at 300 and 4.2 K; b) AFM image of a 120 nm Ti wire with a narrow constriction.^[106]

Also in 1995, a metal-oxide semiconductor field-effect transistor (MOSFET) was produced using AFM lithography to create electrodes at the gate level, with the remaining structure prepared by mask lithography.^[107] The process was confirmed with α -Si films on silicon dioxide, silicon nitride, sapphire, and chromium. Using a tip bias of 12 V (negative at the tip) and patterning speeds of 0.55 $\mu\text{m s}^{-1}$, a MOSFET with an effective channel length of 0.1 μm was formed (with a patterning width of 0.21 μm). With transconductances of 279 ms mm^{-1} , the electron-transport properties of the device were comparable to those produced by electron-beam techniques.

In 1999, Avouris et al. reported the preparation of a single-electron transistor from constricted thin metal wires (Ti and Nb) by means of the local probe oxidation of a thin metal film and a process entitled current induced local

oxidation (CILO).^[108] First a thin metal wire was locally oxidized to constrict the wire to 100–200-nm-wide gaps. Then a transistor was produced by applying a 2 V bias over the constricted metal nanowire while measuring the current over the wire. Using CILO, a 10–50 nm metal-oxide barrier was formed across the constriction of wire in a self-limiting fashion without the need of external control. The experiments suggested that the barrier formation involved current-induced atomic rearrangements and local heating. Using both the probe oxidation and the CILO process, T-shaped junctions of two tunneling barriers with differing widths and a metal island were produced (Figure 12).

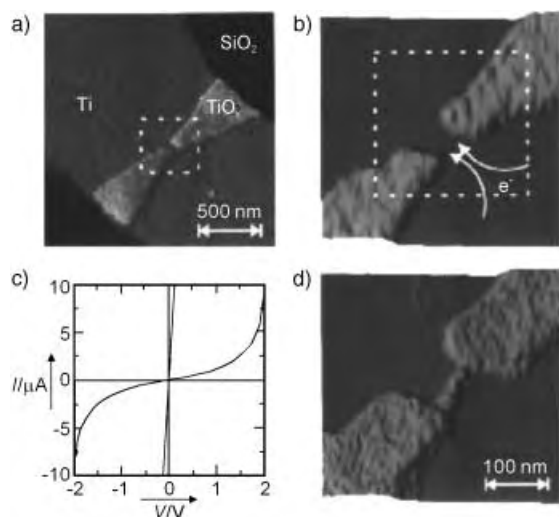


Figure 12. a and b) 1.5 μm thick Ti wire with a narrow gap at the center that was produced by local probe oxidation; c) I – V curves across the device before and after the creation of the barrier; d) current-induced local oxidation (CILO) created a nanometer-scale barrier.^[108]

In 1995, Quate and co-workers demonstrated probe lithography with 40 nm resolution at very high tip speed.^[109] Speeds of 1 mm s^{-1} were obtained on Si substrates coated with thin films of a siloxene, known as “spin-on glass” (SOG). SOG is used as a positive resist, and the etch speed of the underlying silicon depends on the amount of organic content in the SOG, which is reduced by the field-induced oxidation.

The direct oxidation of hydrogen-passivated Si substrates can also be used in the formation of nanostructured functional patterns. In 1995, Sugimura and Nakagiri reported a method in which the created SiO_2 patterns were used as negative resists in an alkaline etching step that resulted in 30 nm steps.^[101] The substrate with the same SiO_2 patterns could also be covered with a gold film. By using electroless plating, gold was deposited on the Si–H surface and not on the insulating silicon oxide patterns. Recently, García et al. determined the linewidth and shape of SiO_2 on silicon.^[110] For this purpose, several high-resolution patterns were produced on silicon wafers with a 2 nm coating of a native oxide by applying 24 V pulses through an n-doped tip; trapezoidal-shaped lines with

a width of 20 nm at the base and 7 nm at the top were produced. Packed patterns of lines with periodicities of 13 nm were also prepared (Figure 13).

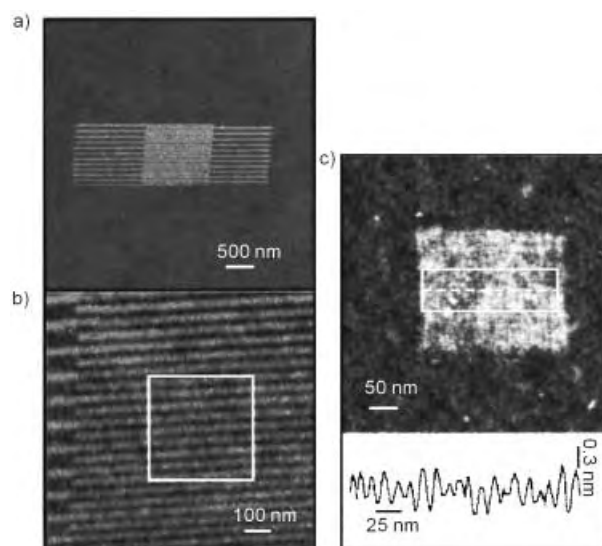


Figure 13. a and b) Local-probe oxidation of Si to SiO_2 by applying a sequence of 21 V pulses for 1 ms to create a set of 23 interdigitated lines placed 43 nm apart; c) a set of 19 lines placed 13 nm apart was created by applying a potential of 24.7 V for 80 μs .^[110]

Lyuksyutov and co-workers have investigated tip–surface interactions during the oxidation of an Si–H surface. They reported an approach for monitoring tip–surface interactions based on power spectral analysis of tip oscillations during scanning probe oxidation.^[111] Utilizing a single-mode harmonic oscillator model, the amplitude, frequency, and damping factor of oscillation were monitored as a function of bias voltage. The results obtained were consistent with the concept that a water meniscus and electrostatic tip–surface interactions dominate contact AFM lithography, that is, a high bias voltage destabilized the water meniscus and the oscillation amplitude was reduced.

3.2.2. Oxidation of Substrates with Organic Resist Layers

The previous examples have described the direct oxidation of Si to SiO_2 . This process can also be achieved with Si samples that are covered with an organic resist. Experiments utilizing resists consisting of (mixed) LB films^[112] and (mixed) SAMs^[113–117] have been reported. In the first case, the application of mixed LB films of hexadecylamine (HAD) and palmitic acid (PA) was reported by Lee et al. Different ratios of the LB components were used and it was concluded that the mixed layers led to significantly thinner pattern widths. Although lines that were approximately three times thinner than those prepared by alternative techniques were observed, there were no comments concerning the shape and condition of the tip that was employed. Liu et al. reported a method to create assemblies of gold nanoparticles on patterned SAMs of OTS on SiO_2/Si (Figure 14).^[113] By locally oxidizing the silicon under the organic resist, they created

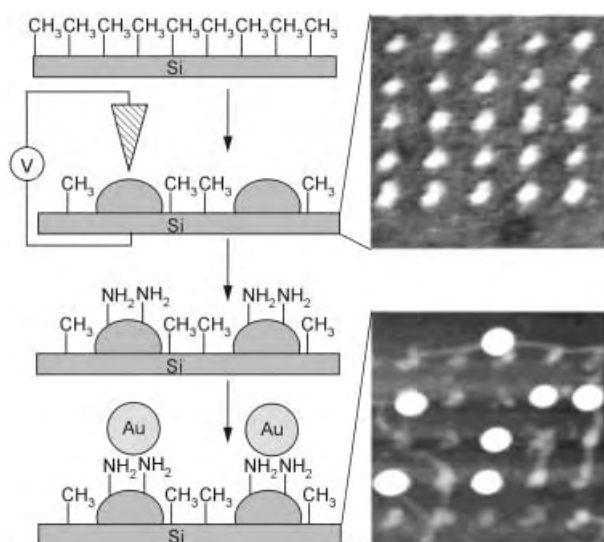


Figure 14. Functionalization method introduced by Liu et al.: In the first step a Si wafer with an OTS resist layer was locally oxidized to create protruding SiO₂ dots (top right). A second silane (APTMS) was then coupled to the dots to introduce amine functionalities exclusively to the oxidized areas. The amine-functionalized areas could be used to bind negatively charged gold nanoparticles (bottom right). If the dots were very close to each other, the amine pattern was not fully covered by gold particles, which was ascribed to mutual repulsion between the gold particles themselves.^[113]

arrays or SiO₂ dots with diameters of 15 nm and a 40 nm spacing. The protruding SiO₂ was used in a subsequent chemical modification step in order to introduce new functionalities. In this step a second silane (aminopropyltrimethoxysilane (APTMS)) reacted with the SiO₂ created by the probe oxidation, whereas the unoxidized OTS resist layer protected the SiO₂ below. The created SiO₂ pattern was transferred and terminal amino groups were introduced to adsorb gold nanoparticles. The successful preparation of a site-selective assembly of 15 nm gold nanoparticles on 100 nm AFM-defined dots with 300 nm spacing was reported. When an array of 30 nm dots with 70 nm spacing was utilized as a template, the site-selective adsorption of gold nanoparticles was incomplete: large portions of the template remained unoccupied. This was attributed to electrostatic repulsion between individual gold nanoparticles. Takai and co-workers also utilized SAMs of OTS on silicon wafers to create SiO₂ nanostructures by local tip-induced oxidation processes.^[114,115] In addition, they also reported a functionalization method by introducing a fluoroalkyl silane (FAS, heptadecafluoro-1,1,2,2-tetrahydro-decyl-1-trimethoxysilane).^[115] Due to the large molecular dipole that is introduced, Kelvin-probe force microscopy could be used to study the created nanopatterns.

In an earlier publication, Takai and Nakagiri reported the successful application of the same technique to create nanostructured gold patterns on surface.^[116] First a silicon wafer with an alkyl silane was locally oxidized to create SiO₂ nanopatterns. The SiO₂ was then removed in a 0.5% HF etching step. Finally, gold was introduced to the patterns in an electroless plating bath. Successful deposition of gold onto the

structures with 100 nm spatial resolution was shown by SEM imaging.

In 2002, Takai and co-workers explored parameter space in the oxidation of Si.^[114] In this study, relationships between the lateral and vertical dimensions of oxide dots, formed on octadecyltrimethoxysilane(ODS)-passivated Si, and the applied bias voltage as well as bias duration time were reported. Based on the results, a stepwise oxidation reaction was proposed: In the first step the organic resist was locally oxidized and/or destroyed. As the SAM layer degrades, anodization of the underlying silicon becomes apparent, as indicated by an observed increase in the lateral force contrast. For the preparation of line structures, a minimum line width of around 20 nm has been found, which is independent of oxidation voltage and probe oxidation speed.

The oxidative removal of SAMs has also been studied for octadecanethiol on gold by Uosaki et al., who reported that thiols could be removed from the gold substrate by applying a 2–3 V positive or negative bias voltage, with both the tip and the substrate placed in toluene containing varying concentrations of water.^[118] The created structures did not consist of bare gold, because only a 1 nm height change could be observed. The remaining nanometer of material was ascribed to adsorbed reaction products of the thiol–gold cleavage, which is consistent with the measured resistivity ($1.7 \times 10^9 \Omega$) for a 1 nm resist on gold.

In addition to the oxidation of Si to SiO₂ and the subsequent functionalization reactions, the oxidation of molybdenum to molybdenum oxide by local probe-oxidation techniques was also reported by Dai and co-workers. This approach represents another successful route for the preparation of devices that then allows subsequent modification steps.^[119] The principle of functionalization in this approach is based on the different solubility of Mo and MoO₃. A thin layer of Mo on a Si substrate was locally oxidized. The produced MoO₃ could easily be removed by dissolving it in water and therefore exposing the bare Si substrate underneath. The exposed silicon was wet-etched by a 30% aqueous solution of KOH at 70°C for 30 s, which yielded V-shaped grooves in the underlying silicon.

Titanium wires were prepared by using a more elaborate functionalization technique.^[119] A thin film of Mo was applied on top of a PMMA film on Si. After the MoO₃ was removed, the PMMA film was locally removed by oxygen plasma etching, which opened a pathway to the silicon wafer for Ti deposition. The remaining Mo film protected the unexposed areas. By this technique, Ti wires (35 nm wide) were prepared (see Figure 15). The same group also suggested the application of chromium and germanium films based on the solubility of the corresponding metal oxides.

3.2.3. Oxidation of Organic Resist Layers: Introducing Chemical Functionality

All of the functionalization processes mentioned so far have been based on the direct oxidation of substrates or the destruction of a thin passive resist layer on an oxidizable substrate. Although complex structures have mainly been prepared using wet etching steps, and in some cases by silane

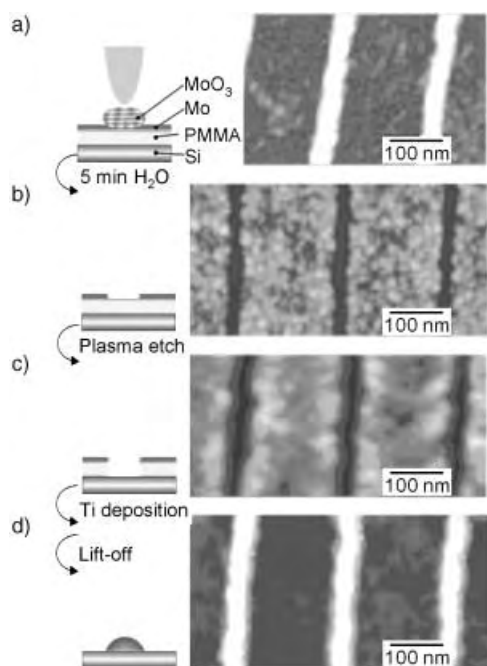


Figure 15. A Mo film (4 nm) was evaporated on top of a PMMA film that was spin-coated onto a p-doped Si substrate. a) The Mo layer was locally oxidized ($7\text{--}11\text{ V}$, $0.4\text{--}1\text{ }\mu\text{m s}^{-1}$) by local-probe oxidation to create a slightly protruding soluble MoO_3 pattern with 35 nm wide lines; b) in the second step, the MoO_3 was dissolved in water to create 25 nm wide gaps that exposed the underlying PMMA film; c) the exposed PMMA film was then etched by exposure to oxygen, to create deep channels; d) in the last step, Ti was evaporated onto the whole substrate. Removal of the PMMA film by a lift-off procedure (using acetone) yielded Ti nanowires on the remaining Si support.^[119]

chemistry, the range of applicable functionalization techniques is limited. A notable exception to destructive functionalization was reported by Sagiv et al., who found that the tip-induced oxidation of organic monolayers introduced surface functionality, which could then be used in a large number of subsequent functionalization reactions with both organic and inorganic substances yielding a range of nanopatterned functional structures.^[120–124] The work involved is summarized in Scheme 1. The procedure was first demonstrated on monolayers of 18-nonadecenyltrichlorosilane (NTS) on a p-doped silicon wafer (see Scheme 1, path 1).^[121] The terminal vinylic end group could be locally oxidized by a conductive AFM tip with an applied bias voltage of approximately 9 V (tip negative). Oxidation was carried out at $5\text{ }\mu\text{m s}^{-1}$ with a variety of conductive tips (boron doped, diamond coated, tungsten, tungsten carbide, copper- and silver coated silicon nitride, and highly doped silicon). Structures were obtained by repeatedly (up to 40 times) scanning the tip along a predefined vector during which no current (above 5 nA) could be detected. The successful selective oxidation of the terminal vinylic end groups to terminal carboxylic acid groups was confirmed by subsequently imaging the area in contact mode with the same probe (without applying a voltage), simultaneously recording height and lateral friction signals. For the unaffected areas the surface remained unchanged, whereas for the oxidized areas the height remained the same

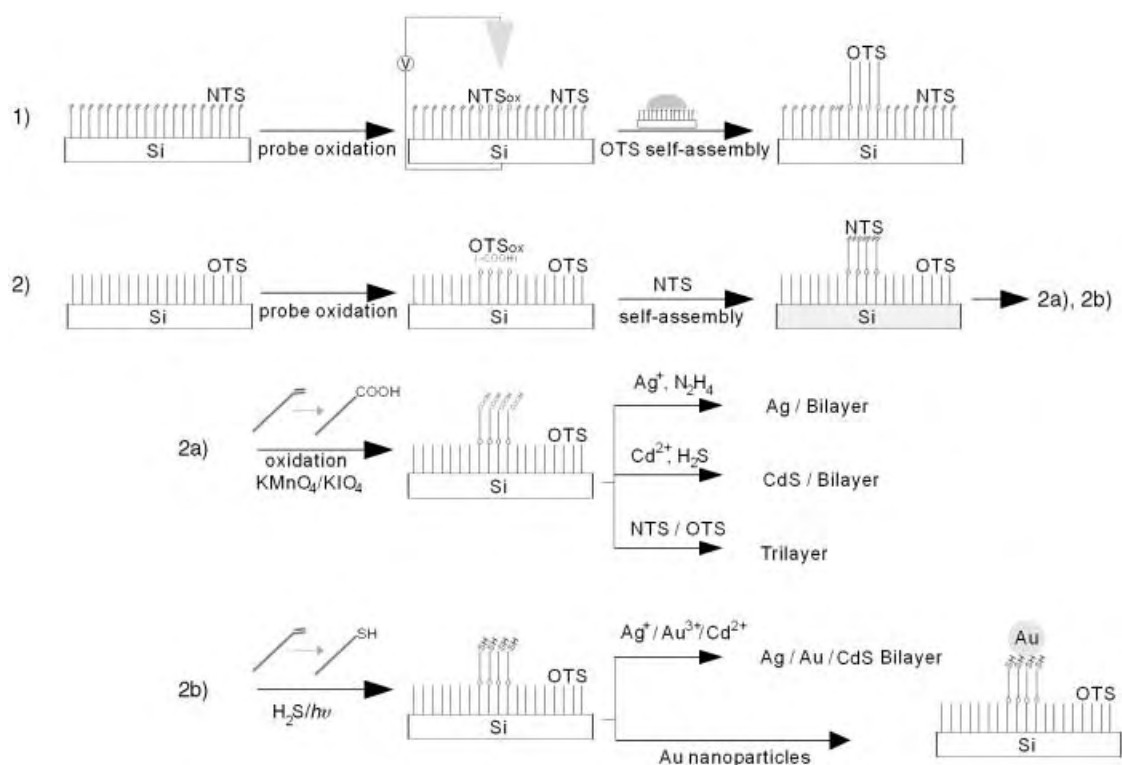
but an increase in friction was observed, which was attributed to changes in local surface polarity. Images of this patterning procedure are displayed in Figures 16 and 17 in which this procedure is used for subsequent modification reactions. Oxidation experiments were verified by partial wet-chemical oxidation: the substrate was partially exposed to a 5 mM solution of KMnO_4 /dicyclohexano-[18]crown-6 and also to electric oxidation of large surface areas by means of a copper grid (connected to a 13 V negative bias voltage). Brewster angle infrared spectroscopy indicated the presence of carboxylic acid groups.

To prevent oxidation of the underlying silicon it is essential that the surface is covered by a densely packed closed monolayer. By controlling the pathway of the tip over the surface, complicated structures with high resolution (9 nm linewidth) could be created. For this procedure both vector-based trajectories as well as bitmap (pixel per pixel) oxidation programs were reported. In their first publication, Sagiv et al. reported the possibility of functionalizing structures using chemical reactions that react exclusively on the oxidized patterns.^[121] The concept was demonstrated by the formation of a second stable monolayer of OTS on top of the oxidized sections of the 18-nonadecenyl layer. The coupling of the second monolayer was followed by contact-mode AFM imaging. Through the coupling of OTS to the oxidized areas, the terminal carboxylic end groups were converted to terminal methyl end groups. This was observed in contact-mode imaging by the disappearance of the friction signal along with a simultaneous increase in the height signal (Figure 16).^[121, 123]

Later publications showed that not only NTS SAMs could be used but also inert SAMs of OTS could locally be oxidized to carboxylic acid end groups (Scheme 1, path 2), thus opening a route for chemical modification.^[123] Noteworthy is the approach in which a monolayer of NTS was attached to locally oxidized OTS (Scheme 1). Thus functionalized patterns have been used as templates for the formation of metallic layers by using wet-oxidation and subsequent adsorption and reduction of metal ions (Scheme 1, path 2a).

The terminal vinyl groups could also be converted to thiol groups by UV irradiation (Hg lamp, 254 nm) for 10 min in an $\text{H}_2\text{S}/\text{Ar}$ (1:1) atmosphere. As a result a stable pattern decorated with thiol end groups placed in well-defined positions was created, and could be used in subsequent functionalization steps. Stable layers of metals could be formed on the templates by reduction of suitable physisorbed metal ions (either tip-induced or by wet chemistry). Application of gold nanoparticles (Au_{55}) to the thiol groups resulted in the formation of gold islands connected by lines that were only two nanoparticles wide (Figure 17).^[120] The method presented by Sagiv et al. for surface patterning and modification has many possibilities for the production of nanometer-sized devices as a result of the large number of available chemical and physical modification steps, for example using silane chemistry (silanes with terminal functionality), the coupling of amines and cationic species, thiol chemistry, and conversion to anhydrides.^[125]

We have performed surface modifications on OTS-functionalized Si wafers using a method similar to that



Scheme 1. Functionalization method developed by Sagiv et al. 1) A monolayer of 18-nonadecyltrichlorosilane (NTS) on a Si wafer was locally oxidized. Chemical functionalities were introduced that were used as templates for subsequent modification steps, such as the attachment of a second (different) chlorosilane; 2) the oxidation procedure was also applied to octadecyltrichlorosilane (OTS) monolayers, introducing similar functionalities. Wafers were prepared with terminal methyl groups (from OTS) patterned with local terminal vinylic functionality end groups. NTS was coupled to local-probed oxidation-induced patterns. The local presence of vinyl groups was utilized in two different pathways: 2a) through chemical oxidation to carboxylic acid functionalities, and 2b) in which the vinyl group was converted to a thiol group. Both functional patterns were used in various subsequent steps including the coupling of gold particles and the formation of metallic silver and cadmium sulfide islands.

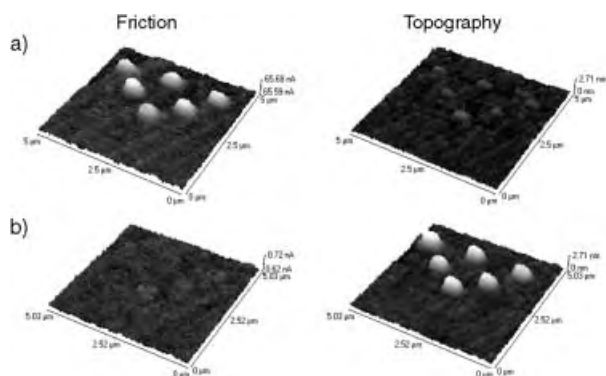


Figure 16. AFM images showing the formation of a NTS-functionalized template structure (see also Scheme 1 a). a) After tip-induced inscription of a pattern of electrooxidized OTS (OTSeo); b) Images of an NTS/OTS bilayer formed by exposure of the patterned OTS monolayer to a 5 mM solution of NTS in bis-cyclohexane (BCH).^[123]

developed by Sagiv et al. By utilizing a number of conductive AFM tips with various coatings (Au, TiN, Pt, and W_2C) and a broad range of stiffnesses ($0.15\text{--}5\text{ N m}^{-1}$), an OTS monolayer was locally oxidized to create templates down to 20 nm resolution, which were then used in a number of different subsequent functionalization steps.^[126,127] Figure 18 shows the

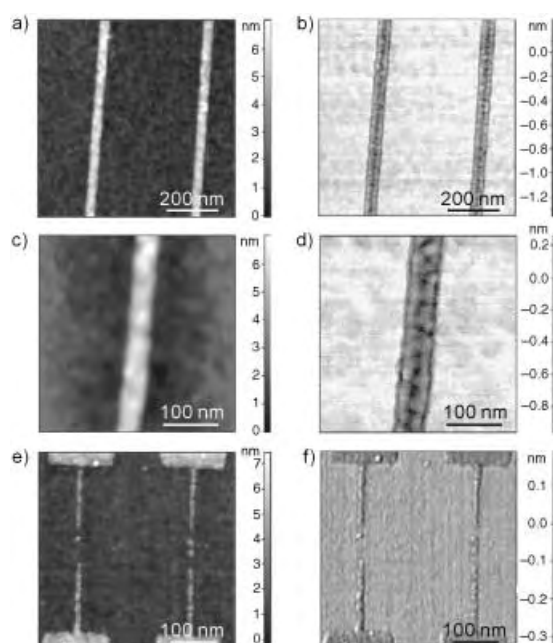


Figure 17. Topography (left) and phase-contrast (right) AFM images showing portions of gold-nanoparticle-coated lines produced by local-probe oxidation (see also Scheme 1, path 2b). Using this method, both wires and contact pads were produced.^[120]

results of the specific adsorption of trimethyloctadecylammonium bromide (TOA). In the first step, the OTS monolayer was locally oxidized to create carboxylic acid groups. The local height remained almost constant after oxidation ($\Delta z_{\text{max}} = 0.5 \text{ nm}$) and when measured in contact mode a clear friction signal originating from the different surface properties of the unmodified OTS and the carboxylic acid groups was observed (Figure 18a and b). Upon adsorption of

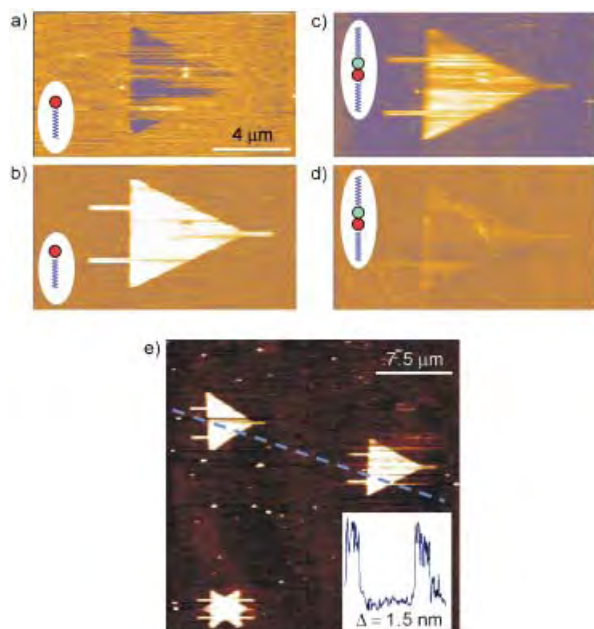


Figure 18. a) Contact-mode height image ($8 \times 14 \mu\text{m}$, z-range 0.5 nm) of an oxidized triangle; b) friction image of the same triangular shape, indicating that successful oxidation had occurred; c) height image ($8 \times 14 \mu\text{m}$, z-range 0.5 nm) after exposing the surface to a solution of trimethyloctadecylammonium bromide (TOA) in water, which causes the height of the triangular area to increase by 2.0 nm and indicates the adsorption of one layer of TOA; d) the corresponding friction image; e) contact-mode height image ($30 \times 30 \mu\text{m}$) of a structure created by a sequential (six-step) method using both oxidation and the adhesion of TOA.

the TOA from an aqueous solution, a height step ($\Delta = 1.5 \text{ nm}$) was observed, which corresponds to the length of the octadecyl chain; simultaneously the friction signal disappeared due to the reformation of an apolar top surface (Figure 18c and d). The created structures were stable when washed with water. The oxidation/adsorption procedure was repeated three times, each time introducing a structure next to the previous one, but never destroying the original pattern (Figure 18e).

Besides the adsorption of quaternary ammonium salts, the procedure has also been demonstrated for positively charged gold nanoparticles ($d = 15 \text{ nm}$, coated with poly-L-lysine; Figure 19). The protein *Candida Antarctica* Lipase B was selected for protein-coupling experiments as it contains thio functionalites on its outer surface. Initial results showed the successful coupling of proteins to a gold template.^[128] Possible applications may be found in nanosensor array devices.

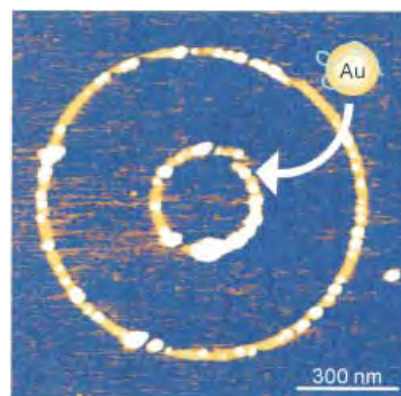


Figure 19. Tapping-mode height image ($1.1 \times 1.1 \mu\text{m}$, z-range 18 nm) of cationic gold nanoparticles adsorbed onto an oxidized structure. The observed height of the particles ranges from 17 to 20 nm .

Successful experiments have also been performed in which functional silanes were coupled to the oxidized patterns. Two silanes, one with a terminal vinyl functionality and one with a terminal methylmethacrylate group (both targets for free radical polymerization reactions) were coupled to the template. In both cases the polymer was grafted to the surface species; the method appears to be promising due to the wide availability of functional silanes.

3.3. Surface Modification by Switching Tip Properties

Ober et al.^[129] introduced a technique called redox probe microscopy (RPM), in which an AFM tip was modified with redox-active materials. When a potential was applied, the tip-surface interaction could be switched.

Two methods of lithographic operation were presented: The first is based on controlling the adhesion between the tip and the particles on the surface, effectively picking them up and positioning them in a pattern (see Figure 20). For this procedure the tip was coated with poly(vinylferrocene) (PVF), which is neutral 0 V , but is oxidized to the ferricenium state when the bias is increased ($+1.0 \text{ V}$), thus becoming highly positively charged. The positively charged tip strongly interacted with negatively charged beads ($15 \mu\text{m}$, sulfonic acid terminated chromatography beads), which could be

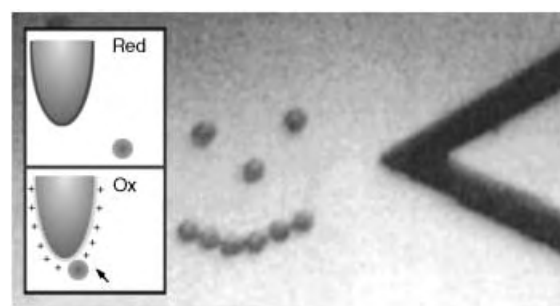


Figure 20. A poly(vinylferrocene)-coated tip in the reduced state is neutral. However, when oxidized (tip bias $+1.0 \text{ V}$) the positively charged tip could be used to pick up negatively charged particles.^[129]

picked up, and released again by reducing the PVF film. The described experiments were repeated with switchable tips consisting of a gold-coated tip modified with a 6-(ferrocenyl-carbonyl)hexanethiol.

In the second method, surface modification was performed by an AFM tip that caused a change in pH value when an electric bias was applied (Figure 21). In this approach, a gold-coated tip was modified with thiomethyl-2,5-hydroquinone (thioQH₂). At positive biases the thioQH₂ is oxidized to the quinone and two protons per molecule are generated. As a result, a film of a pH-sensitive triblock copolymer (MMA-TBMA-MAA) has been patterned.^[129]

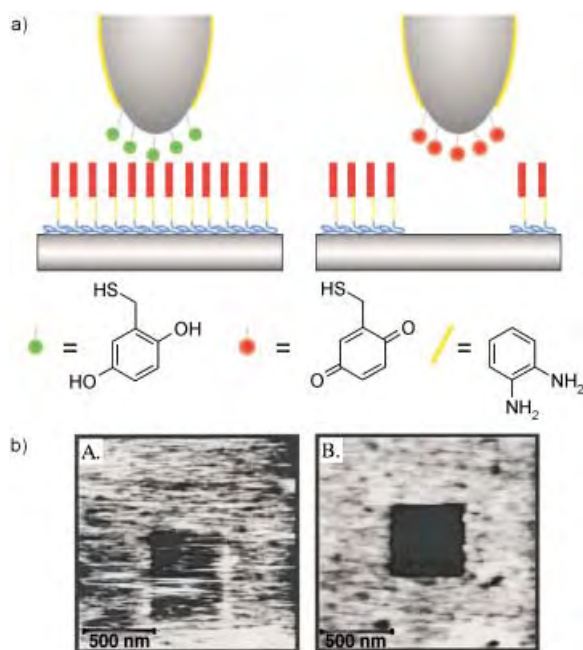


Figure 21. a) The pH lithographic method: The oxidation of thiomethyl-2,5-hydroquinone releases protons that can be used to locally pattern a pH-sensitive block copolymer film of methyl methacrylate (MMA), *tert*-butyl methyl methacrylate (TBMA) and methyl acrylic acid (MAA); b) AFM images of the block copolymer MMA-TBMA-MAA (A, left) showing the effects of tip-induced surface damage due to the repeated scanning (30 min) of a 500 × 500 nm area. Image B (right) shows a similar approach where repeated scanning over an area was performed with the AFM tip cycled between oxidative and reductive modes. Note the enhancement in the generated pattern.^[129]

4. Conclusions and Outlook

UV and UV-mask lithographic processes are currently the most widely employed and studied techniques for the production of sub-micrometer-sized devices. However, no dramatic improvements in the accessible sizes should be expected in the future. Although good progress has been made in field of electron-beam lithography, the conditions remain very demanding, which leads to excessive costs.

With this in mind, scanning-probe-based lithographic techniques seem more feasible. Within this field, research efforts into dip-pen lithographic techniques have led to well-defined systems capable of depositing (biological) materials

onto flat substrates at a relatively high speed. With the development of multi-ink systems, applications into bio-related sensors are envisioned in much the same way as devices already available that are based on microcontact printing. However, dip-pen lithography patterning is limited due to the low availability of suitable ink combinations, the limited stability of the patterning due to diffusion, and the low patterning speed. The patterning speed can be increased dramatically by the introduction of multiple-tip systems, such as those developed by IBM for AFM-based storage systems. The simultaneous operation of 1024 tips allows reasonable reading and writing speeds to be obtained. In combination with automated AFM systems and sample stages, the patterning of large substrates then becomes possible.

Probe-induced oxidative techniques may also become more viable. Such techniques have the advantage of being relatively easy, with respect to the required conditions and equipment. Even more importantly, the method is highly versatile both in applicable substrates as well as in the availability of functionalization routes. Local oxidation can be performed on almost any conducting sample, even on thin organic resists, at high resolution. The technique can be employed to locally oxidize the sample and resist layers, thus inducing changes in physical properties. The obtained systems can be employed in both positive as well as negative development of the pattern by various wet-etching techniques. By this technique, a number of small metallic nanowires and nanometer-sized electronic systems have been prepared. Besides development by etching procedures, local oxidation can also be used for chemical modification steps on organic resists. Chemical functionalities such as thiols, amines, or carboxylic acids can be introduced into defined patterns. Thus, a large number of modification routes are possible, which open up new avenues for the preparation of nanometer-sized functional architectures on surfaces. Applications can be envisaged in various areas, ranging from the defined introduction of biological material (for example, DNA or proteins) for sensor applications, to the coupling of polymerization initiators or catalyst species for mechanistic studies and the preparation of special smart coatings or intelligent polymer brushes.

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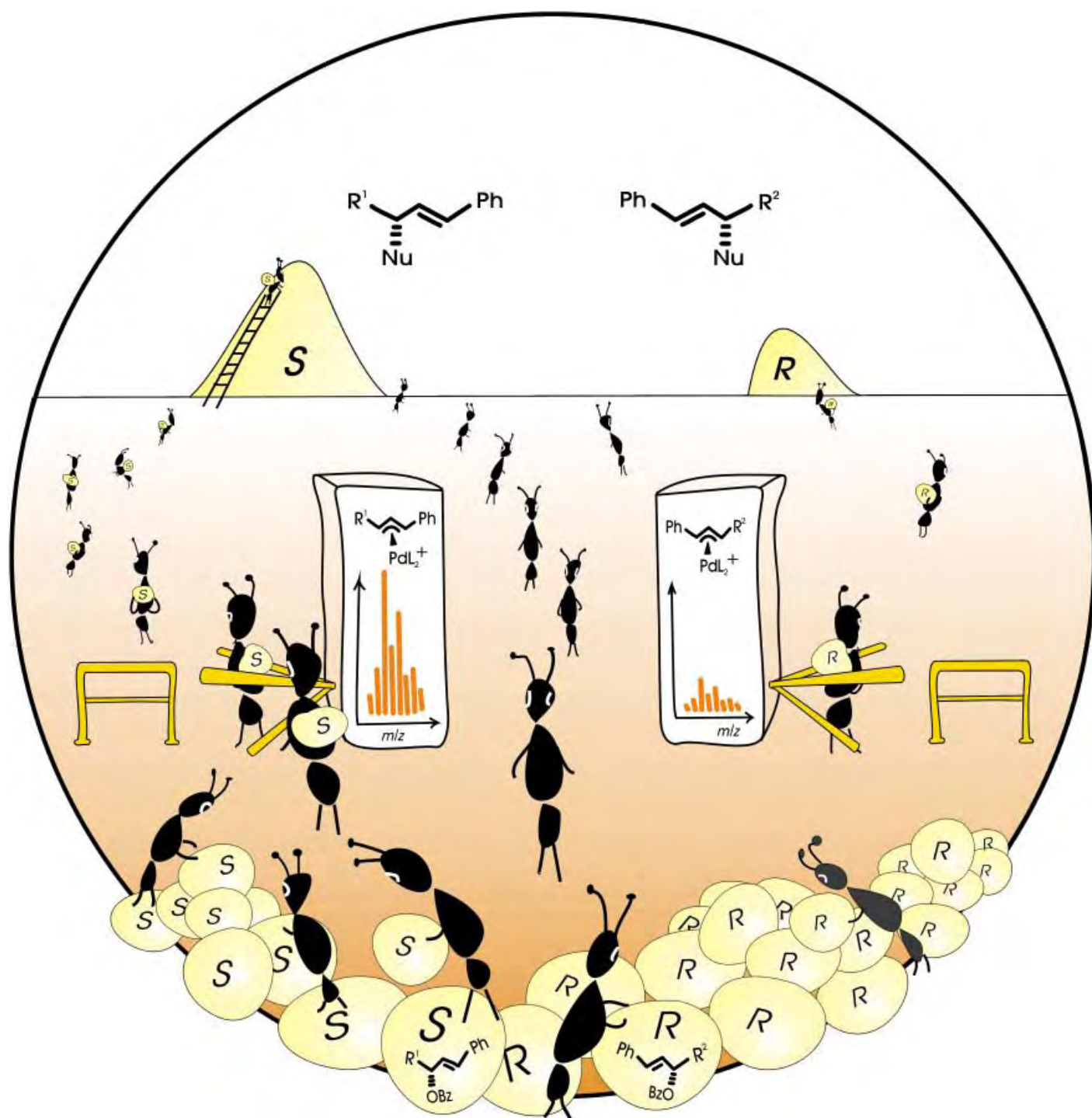
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Communications



The efficiency of chiral Pd catalysts in the kinetic resolution of allylic esters can be readily determined by using pseudoenantiomeric mass-labeled substrates and electrospray mass spectrometry as a tool, as shown by A. Pfaltz and C. Markert on the following pages. Monitoring the Pd-bound allyl intermediates directly provides the intrinsic selectivity of the catalyst.

Enantioselectivity Determination

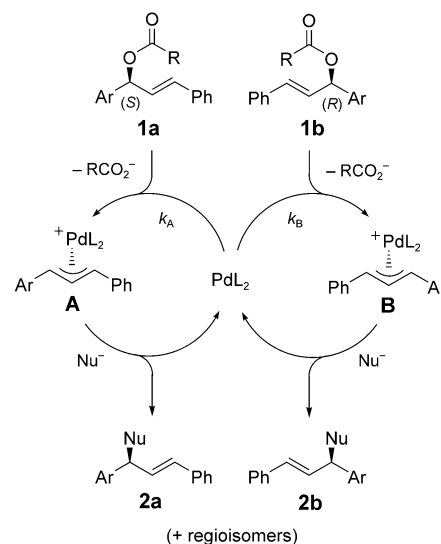
Screening of Chiral Catalysts and Catalyst Mixtures by Mass Spectrometric Monitoring of Catalytic Intermediates**

Christian Markert and Andreas Pfaltz*

Combinatorial methods have become an important focus of research in asymmetric catalysis.^[1,2] During the last few years efficient techniques have been developed for the high-throughput parallel screening of chiral catalysts.^[3] However, parallel screening based on product analysis has potential pitfalls, since the enantioselectivity of a reaction is often lower than the inherent selectivity of the catalyst because of an unselective background reaction, catalytically active impurities, or partial dissociation of a chiral ligand from a metal catalyst. Problems of this kind would be avoided if the catalyst's ability for enantiodiscrimination could be determined directly from examining catalyst–reactant complexes rather than from product analysis. Here we report the realization of this concept for a palladium-catalyzed kinetic resolution of allylic esters by using electrospray mass spectrometry (ESI-MS) as an analytical tool.

Our work was inspired by an ingenious screening method for homogeneous polymerization catalysts developed by Chen:^[4] by using ESI-MS, which is selective for ionic species, he could detect reaction intermediates derived from cationic metal catalysts. In this method the growing polymer chain remains bound to the catalyst during polymerization and so the most effective catalysts carry the longest chains. Thus, the chain length, which can be determined by ESI-MS, is a measure of the activity of a catalyst. Since the signals of the various catalyst intermediates all appear at different m/z values, the screening of catalyst mixtures of different molecular mass is possible. We thought that it should be possible to develop a method of this kind for measuring the inherent enantioselectivity of chiral catalysts directly. The kinetic resolution of allylic esters by palladium-catalyzed allylic substitution^[5] seemed an ideal candidate for this endeavor (Scheme 1).

The first step of the catalytic cycle, formation of Pd–allyl complexes **A** and **B** is fast, while the second step, nucleophilic addition to the allyl system, to give products **2a** and **2b** as well as the corresponding regioisomers (nucleophilic attack at the position next to the Ph substituent), is slower and turnover-limiting. The cationic intermediates **A** and **B** correspond to the resting state of the catalytic cycle and, therefore, should



Scheme 1. Kinetic resolution of allylic esters **1a** and **1b**.

exist in sufficient concentration to be observable by ESI-MS. The ratio **A**:**B** reflects the catalyst's ability to discriminate between the two enantiomeric substrates **1a** and **1b**. Clearly, the two intermediates **A** and **B**, which are derived from the two enantiomers of the substrate, have the same molecular mass and, therefore, can not be distinguished by mass spectrometry. However, if we label **1a** and **1b** with two different alkyl groups at the *para* position of the aryl group (for example, Ar = 4-methylphenyl in **1a** and 4-ethylphenyl in **1b**), then the signals of **A** and **B** would appear at different positions in the mass spectrum and their ratio could be determined by integration. Since the *para*-alkyl substituents are too remote from the reactive part of the molecule to have a notable influence on the reaction, we expected the same selectivity as for the reaction of the parent enantiomers **1a** and **1b** with Ar = phenyl. Mass-labeled enantiomers (or pseudoenantiomers as they are often called) have been used before for determining the enantiomeric purity of chiral products.^[6]

As a first test we treated an equimolar mixture of two pseudoenantiomers with an achiral Pd catalyst and used the anion of diethyl ethylmalonate as the nucleophile. The ESI mass spectrum indeed showed the expected signals corresponding to the allyl intermediates **A** and **B** with the characteristic isotope distribution for palladium (Figure 1). The two signal groups had the same intensity, as anticipated for an achiral catalyst. The reaction with a chiral, enantioselectively pure catalyst derived from ligand **3** showed a strong bias toward one of the two pseudoenantiomers (**A**:**B** = 9:91). The control experiment performed using the same catalyst but inversely labeled pseudoenantiomers (methyl and ethyl groups interchanged) gave a reversed ratio of 91:9.

In the initial phase of the reaction, when the two pseudoenantiomeric substrates are present in equal concentrations, the ratio **A**:**B** is equivalent to the stereoselectivity factor s , which is defined as the ratio of rate constants $k_A:k_B$ of the faster- and slower-reacting substrate enantiomer, respectively. Screening by ESI-MS is extremely fast and requires

[*] Dipl.-Chem. C. Markert, Prof. Dr. A. Pfaltz
Department of Chemistry
University of Basel
St. Johannis-Ring 19, 4056 Basel (Switzerland)
Fax: (+41) 61-267-1103
E-mail: andreas.pfaltz@unibas.ch

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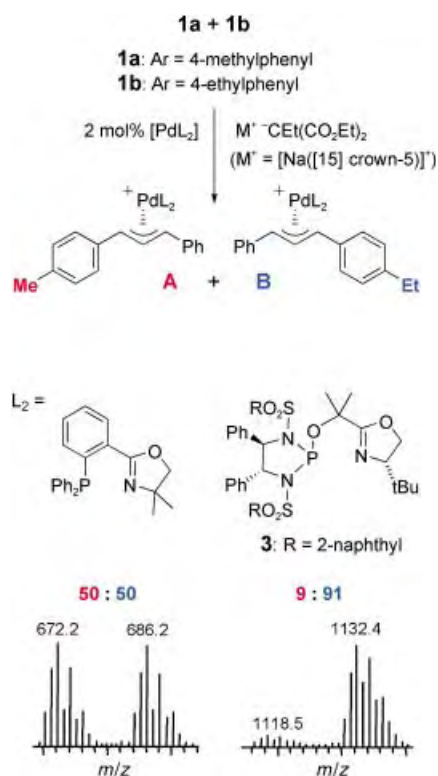
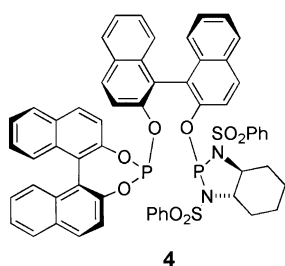


Figure 1. ESI-MS screening of Pd catalysts for the kinetic resolution of **1a** and **1b**.

minimal amounts of substrate compared to conventional screening, which necessitates taking samples at regular time intervals and analyzing them by HPLC on columns with chiral stationary phases. By using our ESI-MS screening technique we were able to test up to ten chiral catalysts per hour, which would have taken several days by conventional methods. We identified several ligands with *s* factors of >20 from a library of 60 ligands comprising mainly P,N ligands previously developed in our group^[7] and commercially available diphosphanes.^[8] An *s* factor of 100 was observed under optimized conditions using the most selective ligand **4**;^[9] this value is



significantly higher than the best selectivities previously reported for this reaction.^[10] In several cases we compared the data from ESI-MS screening with results obtained from kinetic analyses of preparative reactions. Pleasingly, the *s* factors calculated from the curve obtained by plotting the enantiomeric excess of the remaining substrate against conversion closely matched the *s* factors determined by ESI-MS (deviation <10% for *s* factors in the range of 1–20).

The transition states of the first step in the catalytic cycle (**1a**→**A**, **1b**→**B**; Scheme 1) and the second step (**A**→**2a**, **B**→**2b**) have essentially the same geometry. Accordingly, ligands identified by our screening protocol for efficient kinetic resolution should also induce high selectivities in the second step and, therefore, produce high *ee* values in the nucleophilic addition to symmetrically substituted allyl systems (for example, **A**; Ar=Ph). This postulate has been verified in preparative reactions of the racemate **1a/1b** (Ar=Ph).

After establishing a reliable protocol for the screening of single catalysts, we wanted to test if our method could be used for screening mixtures of several Pd catalysts in one reaction. Preliminary experiments gave disappointing results, because rapid exchange of chiral ligands between the different Pd-allyl intermediates took place under the reaction conditions. This strongly affected the relative intensities of the signals corresponding to the two pseudoenantiomers, thus making the results unreliable. However, at lower temperatures (−78°C instead of 23°C) ligand exchange was suppressed while the catalytic reaction was still sufficiently fast. Figure 2 shows the results of a typical experiment carried out under these conditions.

Five different catalyst precursors were combined with the pseudoracemate **1a/1b** (50-fold excess based on the total Pd concentration) at −78°C in toluene. The control spectrum before addition of the nucleophile showed the expected signals corresponding to the five Pd-allyl complexes (Figure 2a). The reaction was initiated by addition of malonate (2 equiv per equiv of Pd). Allyl transfer from Pd to malonate generated the active Pd⁰ catalysts, which reacted with the two pseudoenantiomers to give the corresponding allyl intermediates (Figure 2b). The five catalyst precursors and the ten catalyst complexes derived from the two pseudoenantiomeric substrates all have different molecular masses and, therefore, could all be readily observed and identified in the ESI mass spectrum taken after a reaction time of two minutes. In addition to the selectivity factors, a qualitative reactivity order could be established from the spectrum. Complex **5** shows the lowest reactivity, as evident from the intense signal of the allyl precursor. Complexes **6** and **8** react significantly faster, as demonstrated by the complete consumption of the catalyst precursors. A selectivity order **8**>**7**>**6**~**5**~**9** is derived from the signal ratios, with complex **8** clearly being the most selective catalyst.

The results show that it is indeed possible to obtain reliable selectivity data from catalyst mixtures in homogeneous solution. In principle, there is no restriction on the number of catalysts that can be screened simultaneously, as long as the signals do not overlap. However, the reactivity of the individual catalysts should be on the same order, otherwise the signals corresponding to the least-reactive catalysts become too small. In addition to speed, the fact that the results reflect the inherent enantioselectivity of the catalyst is a big advantage of this method. The same methodology should also be applicable to allylic substitutions starting from *meso* substrates bearing two enantiotopic leaving groups.^[5] There are also other reactions that proceed through ionic catalyst-reactant complexes and, therefore, could be amenable to this screening method.

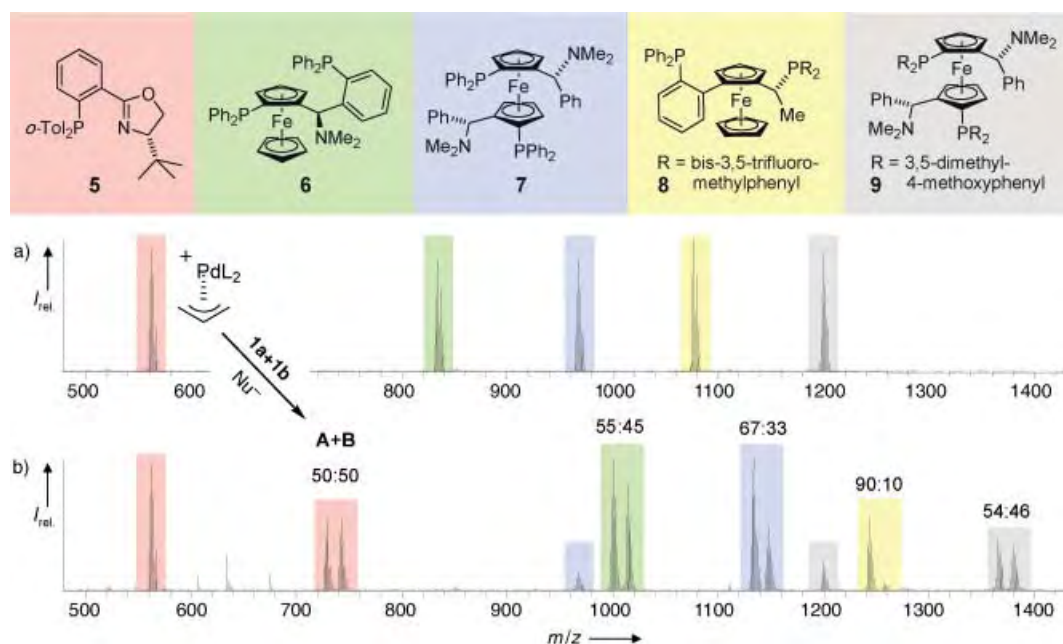


Figure 2. Simultaneous screening of a mixture of five Pd catalysts.

Experimental Section

Substrates **1a** and **1b** were prepared according to reference [11]. In a typical reaction, a precatalyst solution (100 μ L, 2.5 mM in toluene, prepared from equimolar amounts of ligand and $[\text{Pd}(\text{C}_3\text{H}_5)_2(\text{MeCN})_2]\text{OTf}$ (Tf = triflate) was mixed with a solution of **1a** and **1b** (100 μ L, 125 mM in toluene, 2×25 equiv per equiv of Pd). The reaction was started by addition of two equivalents of a nucleophile solution (50 μ L, 10 mM in toluene; prepared from NaH, diethyl ethylmalonate, and [15]crown-5 in THF with subsequent evaporation to dryness). The screening of single catalysts was performed at room temperature, whereas mixtures of complexes were tested at -78°C . After stirring the reaction mixture for 2 min, a sample was taken, diluted to 10^{-5} M (dichloromethane) and analyzed by ESI-MS (MAT Finnigan LCQ). Reactions in Figure 1 were repeated several times with consistent results (relative ESI-MS integrations could be reproduced with deviations of less than $\pm 3\%$).

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Remarkable 4,4'-Substituent Effects on Binap: Highly Enantioselective Ru Catalysts for Asymmetric Hydrogenation of β -Aryl Ketoesters and Their Immobilization in Room-Temperature Ionic Liquids**

Aiguo Hu, Helen L. Ngo, and Wenbin Lin*

Catalytic asymmetric hydrogenation has been established as one of the most efficient strategies for the synthesis of optically active molecules.^[1] The reactivity and enantioselectivity of a transition-metal-based asymmetric catalyst are highly sensitive to the conformational, steric, and electronic properties of the chiral ligand. Numerous chiral ligands, including 1,1'-binaphthalene-2,2'-diylbis(diphenylphosphane) (binap),^[2] 1,2-bis(phosphanyl)benzene (duphos),^[3] 1-[2-(diphenylphosphanyl)ferrocenyl]ethylidicyclohexylphosphane (josiphos),^[4] 1-(*S*)-diphenylphosphanyl-2-(*O*-diphenylphosphanylphenylmethyl)ferrocene (taniaphos),^[5] *P,P'*-1,2-phenylenebis(*endo*-2,5-dialkyl-7-phosphabicyclo[2.2.1]heptanes (pennphos),^[6] 1,1'-di-*tert*-butyl-2,2'-diphospholane (tangphos),^[7] and (3,5-dioxa-4-phosphacyclohepta[2,1-a;3,4-a']-dinaphthalene-4-yl)dimethylamine (monophos),^[8] have been synthesized over the past 30 years to effect a variety of catalytic asymmetric hydrogenation processes. However, many prochiral olefins, ketones, and imines still cannot be reduced in high enantiomeric excesses and with high turnover numbers with the currently available hydrogenation methods. As an example, Noyori et al. reported a highly enantioselective hydrogenation of β -alkyl β -ketoesters by using the Ru–binap system; however, much more inferior *ee* values were obtained for analogous β -aryl ketoester substrates.^[9] Herein, we report the design of a family of highly tunable and enantioselective Ru catalysts for the asymmetric hydrogenation of a wide range of β -aryl ketoesters by taking advantage of the remarkable effects of 4,4'-substituents on binap. These highly enantioselective Ru catalysts have also been effectively immobilized in room temperature ionic liquids (RTILs).

Enantiomerically pure β -aryl β -hydroxy acids, which are obtainable from the asymmetric hydrogenation of β -aryl ketoesters, are important precursors to β -amino acids, β -

hydroxy- α -amino acids and their derivatives, which are building blocks for novel peptides and peptidomimetic drugs.^[10] β -Hydroxy esters are also precursors to a number of important drugs including tomoxetine, a norpinephrine reuptake-inhibiting antidepressant; fluoxetine, a serotonin-reuptake-inhibiting antidepressant; and pinostrobin, a candidate for treatment of estrogen-related tumors.^[11] In spite of the extensive search for chiral ligands for effective asymmetric hydrogenation of β -aryl ketoesters, none of the many chiral diphosphanes examined have shown *ee* values useful for practical applications^[12] and the best system based on chiral bisphosphanite binapo gave high *ee* values only for selected substrates.^[13]

A family of 4,4'-substituted binap derivatives were readily synthesized starting from **1a**^[14] by two different approaches (Scheme 1). In the first, compounds **2–6** were synthesized from **1a** by halogen metathesis, Suzuki coupling, or a Pd-catalyzed phosphonation reaction followed by Ti(OiPr)₄-mediated reduction with triethoxysilane or reduction with phenylsilane.^[15] In the second approach, **1a** was reduced to 4,4'-dibromo-binap (**1**) by Ti(OiPr)₄-mediated reduction with triethoxysilane. Compounds **7–11** were then obtained in good yields by lithiation of **1** with *n*-butyllithium followed by treatment with various electrophiles. Monosubstituted binap derivatives **8b** and **11b** were obtained as minor by-products from the reactions leading to **8** and **11**. All the new compounds were characterized by optical rotation studies; ¹H, ¹³C{¹H}, and ³¹P{¹H} NMR spectroscopic analysis; and mass spectrometric analysis.

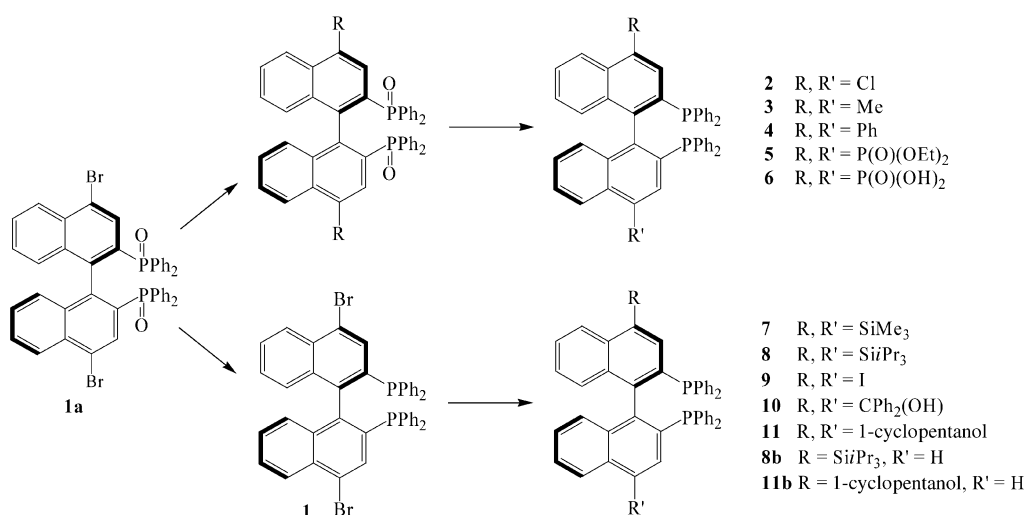
Ruthenium precatalysts were prepared by mixing 4,4'-binap ligands and [(RuCl₂(benzene))₂] in hot DMF and were used for catalysis without further purification. As shown in Table 1, ethyl benzoylacetate was hydrogenated in 85.0% *ee*. Complete conversion was obtained using a Ru–binap catalyst in methanol under a hydrogen pressure of 1400 psi at room temperature for 20 hours.^[9] Although all these Ru–4,4'-binap catalysts showed similar reactivities, their enantioselectivities varied drastically. Electron-withdrawing groups seem to decrease the *ee* values obtained, as exemplified by the trend of **2**(Cl) < **1**(Br) < binap $\leq$ **9**(I). The placing of phenyl groups on the 4,4'-positions also has deleterious effects on the enantiomeric excess. Remarkably, enantioselectivity was drastically enhanced when bulky groups were placed in the 4,4'-positions of BINAP. The combination of the steric demands and electron-donating abilities of the 4,4'-substituents gave the best enantioselectivities, for example, *ee* values of 99.5, 99.3, and 99.2% were obtained for the hydrogenation of ethyl benzoylacetate with Ru catalysts based on chiral ligands **7** (R = R' = TMS; TMS = trimethylsilane), **10** (R = R' = diphenylmethanol), and **11** (R = R' = 1-cyclopentanol), respectively. Compound **8**, which contains electron-donating bulky triisopropylsilyl groups, was obtained in a lower enantiomeric excess probably because of the low solubility of the Ru-catalyst in methanol. Interestingly, the C₁-symmetric ligands **8b** and **11b**, which look like binap on one side and **8** or **11** on the other side, exhibited *ee* values that were between those of binap and **8** or **11**. It is evident from Table 1 that the Ru catalyst based on 4,4'-TMS-binap (**7**) give the highest enantiomeric excess, and was thus chosen to further

[*] Dr. A. Hu,⁺ H. L. Ngo,⁺ Prof. W. Lin
Department of Chemistry, CB no. 3290
University of North Carolina
Chapel Hill, NC 27599 (USA)
Fax: (+1) 919-962-2388
E-mail: wlin@unc.edu

[⁺] Contributed equally to this work.

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Scheme 1. Synthesis of 4,4'-substituted binap derivatives from **1a**.

Table 1: Asymmetric hydrogenation of ethyl benzoylacetate with 4,4'-substituted binap derivatives as the ligand.

Entry	Ligand	4,4'-Substituent groups	<i>ee</i> [%] ^[c]
1	binap	none	85.0
2	1	R = R' = Br	80.8
3	2	R = R' = Cl	77.0
4	3	R = R' = Me	85.0
5	4	R = R' = Ph	71.8
6	5	R = R' = P(O)(OEt) ₂	98.8
7	6	R = R' = P(O)(OH) ₂	97.2
8	7	R = R' = SiMe ₃	99.5
9	8	R = R' = Si ⁱ Pr ₃	98.6
10	9	R = R' = I	86.5
11	10	R = R' = CPh ₂ (OH)	99.3
12	11	R = R' = 1-cyclopentanol	99.2
13	8b	R = Si ⁱ Pr ₃ , R' = H	95.1
14	11b	R = 1-cyclopentanol, R' = H	94.2

[a] All reactions were carried out under 1400 psi of hydrogen gas in methanol at room temperature for 20 hours with 1 mol% catalyst loading. [b] All the reactions were judged to have > 98% conversions based on the integrations of NMR signals for the starting material and product. [c] The *ee* values (%) were determined by gas chromatography (GC) on a Supercio γ-Dex 225 column.

study the scope of the substrate. Polar 4,4'-P(O)(OH)₂-binap (**6**) was also examined so that its performance in RTILs could be evaluated.

A variety of β-aryl ketoesters were successfully hydrogenated with complete conversions and very high *ee* values of 97.8–99.6% by using 1 mol% Ru-catalyst derived from ligand **7** (Table 2). A slight decrease in enantiomeric excess was observed when an electron-withdrawing bulky substituent was placed in the *ortho* position of the aryl group. Such a level of enantioselectivity is the highest observed for the hydrogenation of β-aryl ketoesters. Low conversion (< 10%) and

ee values (< 60%) were observed when the hydrogenation of ethyl benzoylacetate was carried out with 0.1% catalyst loading. Interestingly, however, a much higher conversion (90%) and *ee* value (97.6%) were obtained when the same reaction was carried out in the presence of 0.1 mol% Ru-**7** catalyst and 0.2 mol% AgSbF₆ or AgOTf (OTf = trifluoromethanesulfonate), presumably because of a counterion effect that facilitates the binding of ketoester substrates. The tunable nature of this family of chiral diphosphanes should allow them to be applied to other asymmetric catalytic processes.

Remarkable enhancement of the *ee* values by the 4,4'-substituents on binap can be rationalized by molecular modeling studies (Figure 1). Unlike the analogous β-alkyl ketoesters, the aryl group on the β-aryl ketoesters can form weak π–π stacking interactions with one of the phenyl groups on binap in the disfavored transition state, which will slightly stabilize the disfavored transition state and lead to a deterioration in the enantiomeric excess. In contrast, bulky substituents at the 4,4'-positions will have significant repulsive interactions with the aryl group, cause destabilization of the disfavored transition state, and thus enhance the enantioselectivity drastically.

We have also attempted to immobilize Ru-catalysts based on 4,4'-substituted-binap derivatives in RTILs. RTILs have recently received much attention as alternative reaction media because they are nonvolatile, thermally and oxidatively stable, and weakly coordinating.^[16] In particular, RTILs represent viable alternative solvents for the synthesis of high-value chiral organic compounds by catalytic processes. RTILs have indeed been used as alternative solvents for the asymmetric hydrogenation of a variety of prochiral substrates.^[17] Ideally, the organic products could be separated by extraction with nonpolar solvents and the ionic liquid phase containing the active catalyst could be reused.

As shown in Table 2, Ru-**6** and Ru-**7** catalysts are highly active for catalytic asymmetric hydrogenation of a wide range of β-aryl ketoesters in the homogeneous 1-butyl-3-methyl-

Table 2: Ru-catalyzed asymmetric hydrogenation of β -aryl ketoesters with **6** and **7** as the ligand.

$\text{Ar}-\text{C}(=\text{O})-\text{CH}_2-\text{C}(=\text{O})-\text{OR} \xrightarrow[\text{[RuCl}_2(4,4'-(R)\text{-binap)}]}{\text{H}_2, \text{MeOH, RT}} \text{Ar}-\text{CH}(\text{OH})-\text{CH}_2-\text{C}(=\text{O})-\text{OR}$					
Ar	R	7 MeOH	7 RTIL/MeOH	6 MeOH	6 RTIL/MeOH
Ph	Et	99.5 ^[c]	98.9	97.2	99.3
4'-MeO-Ph	Et	99.5 ^[d]	94.4	94.0	95.9
4'-F-Ph	Me	99.3 ^[e]	98.1	97.1	98.9
4'-Cl-Ph	Me	99.1 ^[e]	97.8	97.6	98.3
2'-Cl-Ph	Me	99.6 ^[f]	98.9	97.2	99.8
4'-CF ₃ -Ph	Me	99.0 ^[e]	98.4	97.2	98.9
3'-CF ₃ -Ph	Me	98.4 ^[f]	98.9	98.4	98.8
2'-CF ₃ -Ph	Me	97.8 ^[e]	96.7	97.3	97.5

[a] All reactions were carried out under 1400 psi of hydrogen gas in methanol at room temperature for 20 hours with 1 mol% catalyst loading. [b] All the reactions were judged to have > 98% conversions based on the integrations of NMR signals for the material and product. [c] The *ee* value (%) was determined by GC using a Superco γ -Dex 225 column. [d] The *ee* value (%) was determined by supercritical fluid chromatography using a Chiralpak AS column. [e] The *ee* values (%) were determined by GC using a Superco β -Dex 120 column. [f] The *ee* values (%) were determined by high-performance liquid chromatography using a Chiralpak AD column.

imidazolium tetrafluoroborate (BMImBF₄)/methanol system. A slight deterioration in *ee* values (1%) was observed for the Ru-**7** catalysts in the RTIL, while an increase (up to 2.6%) was seen for the Ru-**6** catalysts in the RTIL (Table 2). This result is consistent with our earlier observation that the polar nature of asymmetric catalysts can lead to enhanced performances in RTILs.^[17d] Both Ru catalysts were recycled and reused four times, with *ee* values (conversions) of 97.3 (> 98), 93.9 (94), 85.7 (81), and 95.1% (62%) for Ru-**6** and 97.5 (> 98), 86.9 (91), 82.7 (77), and 75.7% (44%) for Ru-**7**. Direct current plasma (DCP) spectroscopic analysis showed that no appreciable leaching of Ru occurred during the extraction of the organic products. We estimated from DCP experiments that less than 0.02 and 0.04% of the Ru catalysts leached into the organic layer for the Ru-**6** and Ru-**7** systems,

respectively. Thus we believe that the deterioration in the conversion rates and *ee* values for the reused catalysts is probably because of the instability of the active Ru-hydride species.

In summary, we have synthesized a family of tunable 4,4'-substituted binap derivatives and applied them to a highly enantioselective hydrogenation of β -aryl ketoesters in both methanol and a RTIL by taking advantage of the 4,4'-substituent effects. The easy tunability of this family of chiral diphosphanes should allow other synthetically useful asymmetric catalysts to be developed.

Experimental Section

Detailed synthetic procedures and spectroscopic data for 4,4'-substituted binap derivatives can be found in the Supporting Information. Typical procedure for the synthesis of the Ru precatalyst: A mixture of [RuCl₂(benzene)]₂ (23 mg, 0.046 mmol) and 4,4'-Br-BINAP (**1**) (78 mg, 0.1 mmol) in anhydrous DMF (2 mL) was heated at 100°C under argon for 30 min and then cooled to 25°C. The solvent was removed under vacuum to give a dark red solid, which was used for asymmetric hydrogenation without purification.

Typical procedure for the asymmetric hydrogenation of β -aryl ketoesters: Ethyl benzoylacetate (47 μ L, 0.25 mmol) and anhydrous methanol (1 mL) were added to the Ru precatalyst (3.0 mg, 2.5 μ mol) previously loaded into a teflon-capped vial in a dry box under argon. The vial was quickly transferred into a stainless-steel autoclave and sealed. After purging the autoclave with hydrogen gas six times, the final pressure of the hydrogen gas was adjusted to 1400 psi. After 20 h, the hydrogen gas pressure was released and water (10 mL) was added. The hydrogenated product was extracted with diethyl ether and passed through a small column of silica gel. The conversions were

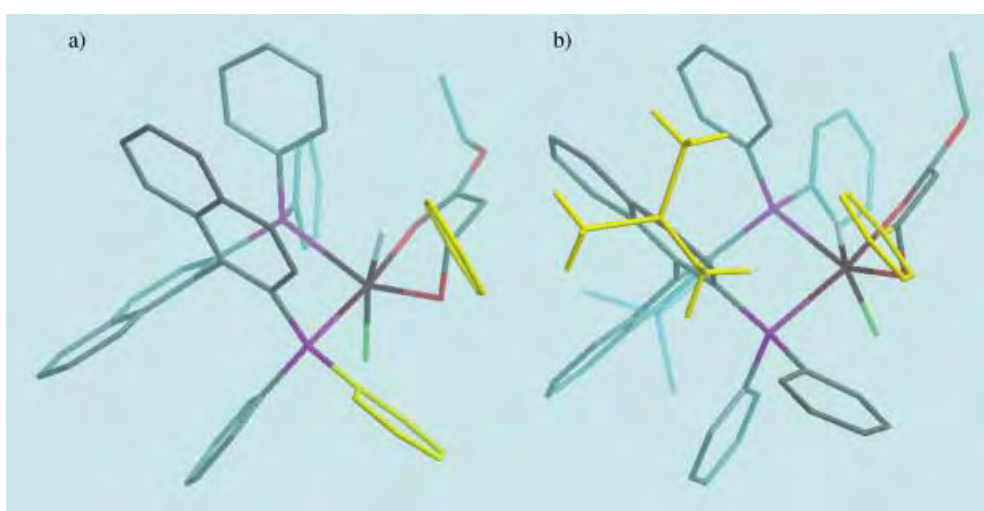


Figure 1. PC Spartan-generated models for the disfavored transition state of a) [Ru((*R*)-binap)(H)(Cl)(ethyl benzoylacetate)] and b) [Ru(4,4'-TMS-(*R*)-binap)(H)(Cl)(ethyl benzoylacetate)]. The groups shown in yellow have dominant interactions.

assessed based on the integration of the peaks of the products and starting materials in the ^1H NMR spectra, while the *ee* values were determined by gas chromatography, high-performance chromatography, or supercritical fluid chromatography.

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Semisynthesis of a Functional K⁺ Channel**

Francis I. Valiyaveetil, Matthew Sekedat, Tom W. Muir,*
and Roderick MacKinnon*

Potassium channels conduct K⁺ ions selectively at very high rates.^[1] They accomplish this task through a structural element in their pore called the selectivity filter. The selectivity filter replaces the water molecules that normally surround a hydrated potassium ion with protein atoms, which are predominantly carbonyl oxygen atoms from the main chain.^[2,3] For this reason, techniques of chemical synthesis rather than conventional mutagenesis are required if we wish to alter the selectivity filter to understand the details of its function.

In a previous study, we produced a semisynthetic KcsA K⁺ channel to enable chemical modifications of the selectivity filter.^[4] This semisynthetic KcsA K⁺ channel contains residues 1 to 125 of the 160-amino-acid protein. Similar to the equivalent recombinant (and truncated) KcsA K⁺ channel, it forms a stable tetramer and binds agitoxin 2.^[5,6] Thus, the semisynthetic and recombinant channels appear to be the same. These truncated channels, however, are not very useful for studies of ion conduction because, in contrast to the full-length KcsA channel, they do not give measurable single-channel currents in planar lipid membranes.^[4] There is a simple reason for the failure: the semisynthetic channel did not include the 35 C-terminal amino acids that apparently are required to open the pore. The most likely explanation is that the probability of the open state becomes too low for single-channel recording methods when the C-terminus is absent.^[4] This problem was not simple to solve, because addition of the missing 35 amino acids brought the length of the synthetic fragment beyond the practical limit for solid-phase peptide synthesis (SPPS).^[7] In this study, we sought to remedy this limitation by identifying mutations within the pore that would

[*] M. Sekedat, Prof. T. W. Muir
The Laboratory of Synthetic Protein Chemistry
The Rockefeller University
Box 223, 1230 York Avenue, New York, NY 10021 (USA)
Fax: (+1) 212-327-7358
E-mail: muirt@mail.rockefeller.edu
F. I. Valiyaveetil, Prof. R. MacKinnon
Howard Hughes Medical Institute
The Laboratory of Molecular Neurobiology and Biophysics
The Rockefeller University
Box 47, 1230 York Avenue, New York, NY 10021 (USA)
Fax: (+1) 212-327-7289
E-mail: mackinn@mail.rockefeller.edu

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favor channel opening even in the absence of the C-terminal residues.

Our rationale for identifying mutations is based on the idea that a gating hinge is important for the conformational change leading to pore opening in K⁺ channels.^[8,9] Closed (KcsA) and opened (MthK) K⁺ channels^[10] are shown in Figure 1a and b, respectively. The major difference in these structures lies in the conformation of the inner helices, which are straight in KcsA and bent in MthK. The fact that the position of the bend (colored red in Figure 1) is conserved as glycine in many K⁺ channels (Figure 1c) implies that it may serve as a hinge point. The gating hinge and its vicinity thus seemed to be a promising target for mutations that might stabilize the open conformation of KcsA.

We introduced glycine or proline residues since they have a tendency to alter the stability of a straight α -helix. Mutant channels with single-site substitutions from position 98 to 104 were expressed in *Escherichia coli*, purified, and reconstituted into lipid vesicles and then planar lipid bilayers. Two mutants, S102P and A98G, exhibited prolonged mean open times compared to wild-type channels in single-channel records (Figure 2a). Under conditions in which the mean open time for the wild-type

channel is 1.6 ms, the S102P and A98G mutants had mean open times of 162 and 8.2 ms, respectively (see the Supporting

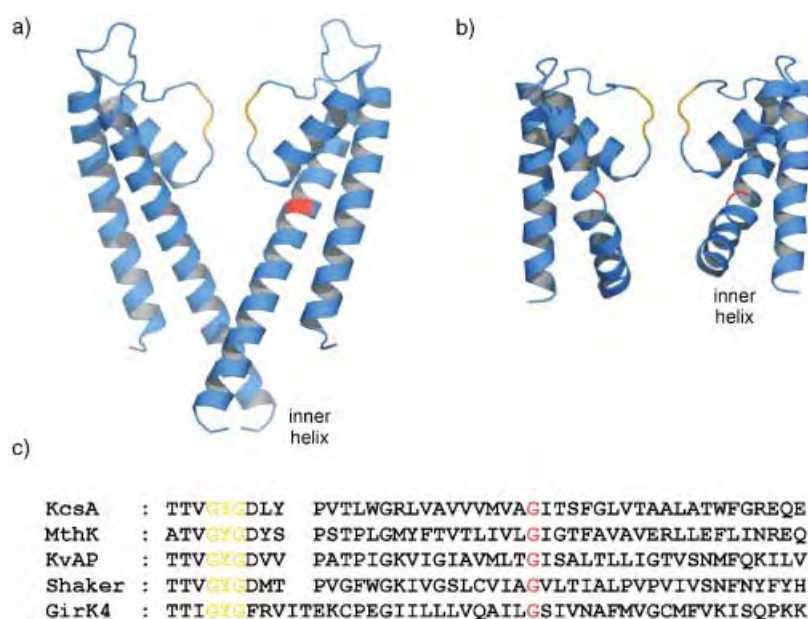


Figure 1. The gating hinge of potassium channels. a) Structure of the KcsA K⁺ channel (pdb code: 1K4C). b) Structure of the MthK K⁺ channel (pdb code: 1LNQ). For clarity, only two subunits of the KcsA and MthK tetramers are shown. c) Sequence alignment of the inner helices of the KcsA (gi1089906), MthK (gi21542150), KvAP (gi38605092), Shaker (gi13432103), and Girk4 (gi976203) K⁺ channels. The conserved GYG sequence in the selectivity filter is colored gold, while the gating hinge glycine is colored red.

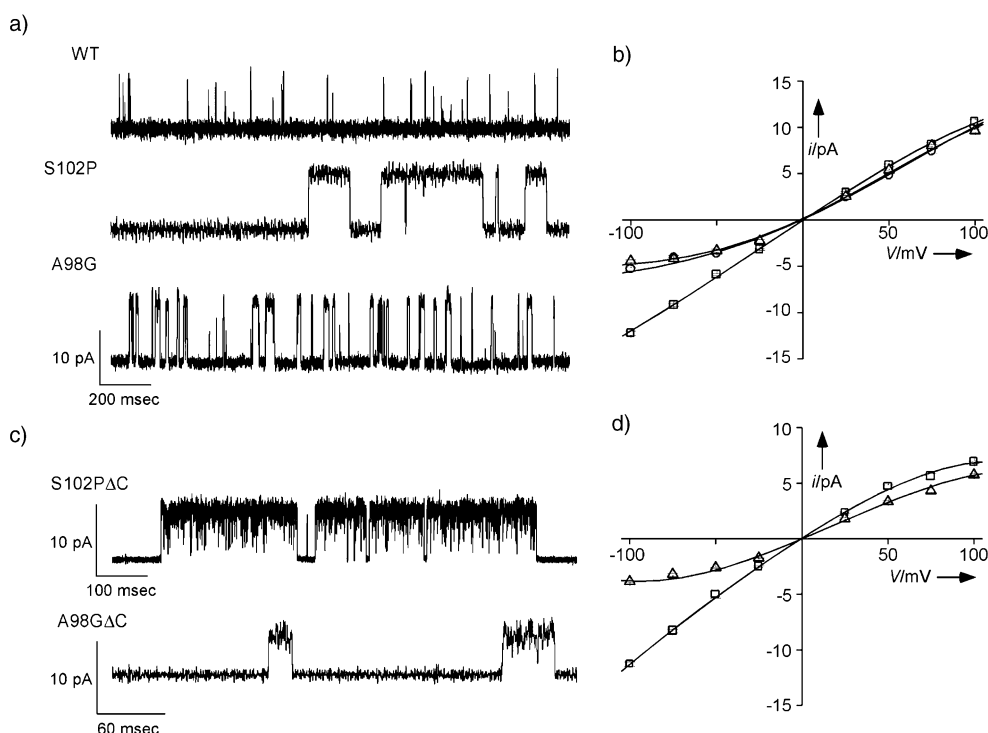


Figure 2. Representative single-channel traces (a) and *i*-*V* curves (b) for full-length wild-type KcsA (○), KcsA-S102P (□), and KcsA-A98G (△). Representative single-channel traces (c) and *i*-*V* curves (d) for the C-terminally truncated KcsA-S102PΔC (□) and KcsA-A98GΔC (△). Single-channel currents were recorded at +100 mV in symmetrical 150 mM K⁺ solutions. Each data point in the *i*-*V* curves represents the average $\pm$ SE ($n \geq 3$) from at least two separate planar lipid membranes. The solid lines in the *i*-*V* plots have no theoretical meaning.

Information). The current–voltage (i – V) curves for wild-type and mutant channels in 150 mM KCl show that A98G is almost indistinguishable from wild type, but that conductance is higher at negative voltages for S102P (Figure 2b).

Since our aim is to identify a truncated KcsA K⁺ channel that will open in planar lipid membranes, we used chymotrypsin to remove amino acids 126 to 160.^[2] Following chymotrypsin cleavage, the truncated channels were purified by gel filtration chromatography, reconstituted, and analyzed in planar lipid membranes. Mutants A98GΔC (ΔC for truncation) and S102PΔC both exhibited single-channel opening (Figure 2c). The much shorter approximate mean open times following truncation of the C terminus (2.90 ms for A98GΔC mutant, Figure 4d) indicate that the C-terminus is still important for gating in these mutants. Nonetheless, the inner helix mutations appear to have shifted the gating equilibrium sufficiently toward the open conformation that single channels are now detectable in the absence of the C-terminal residues. The open-channel properties are also affected by truncation: the ΔC forms of both mutants showed two conductance states of about 60 and 45 pS (A98GΔC) and 70 and 50 pS (S102PΔC), measured as a cord conductance at +100 mV, and the i – V curves showed a reduced conductance at positive voltages compared to the full-length mutants (compare Figure 2b and d). The influence of the C-terminus on conductance may well reflect motions of the inner helices that are too rapid to resolve in the electrophysiological recording system.

Figure 3a outlines the procedure for semisynthesis of the KcsA K⁺ channel.^[4] Of the two mutations identified above, we chose to incorporate A98G into the semisynthetic channel

because it migrates as a stable tetramer in sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE, see the Supporting Information). The semisynthesis consists of two steps: an expressed protein ligation (EPL) reaction for the assembly of the polypeptide, and a folding reaction to form the KcsA tetramer. EPL enables two polypeptides, one recombinant and one synthetic, to be linked through a peptide bond.^[11] The chemistry requires that one of the peptides contain an α -thioester and the other an N-terminal cysteine residue. Position 69 was chosen as the ligation site, which necessitates a cysteine rather than a serine residue (S69C mutant) in the final product. The S69C mutant was found to have similar stability to the wild-type protein, and both migrate as tetramers in SDS–PAGE analysis at room temperature (data not shown). In our previous study, threonine 74 was used as the ligation site,^[4] but the resulting T74C mutant exhibited reduced stability, as judged by SDS–PAGE analysis.

The desired polypeptide was assembled from two fragments: a peptide α -thioester, referred to as the N-peptide, corresponding to residues 1 to 68 of KcsA, and a peptide containing an N-terminal cysteine residue, referred to as the C-peptide, corresponding to residues 69 to 122. The N-peptide was obtained by recombinant expression with a dual-fusion strategy, as described previously.^[4] The C-peptide was obtained by using optimized Boc-based SPPS (Boc = *tert*-butoxycarbonyl) and purified as described previously.^[4] The S69C and A98G mutations were incorporated into the C-peptide during synthesis. Ligation was carried out in the presence of 1% SDS, which was necessary to keep the N- and C-peptides soluble during the course of the reaction. The

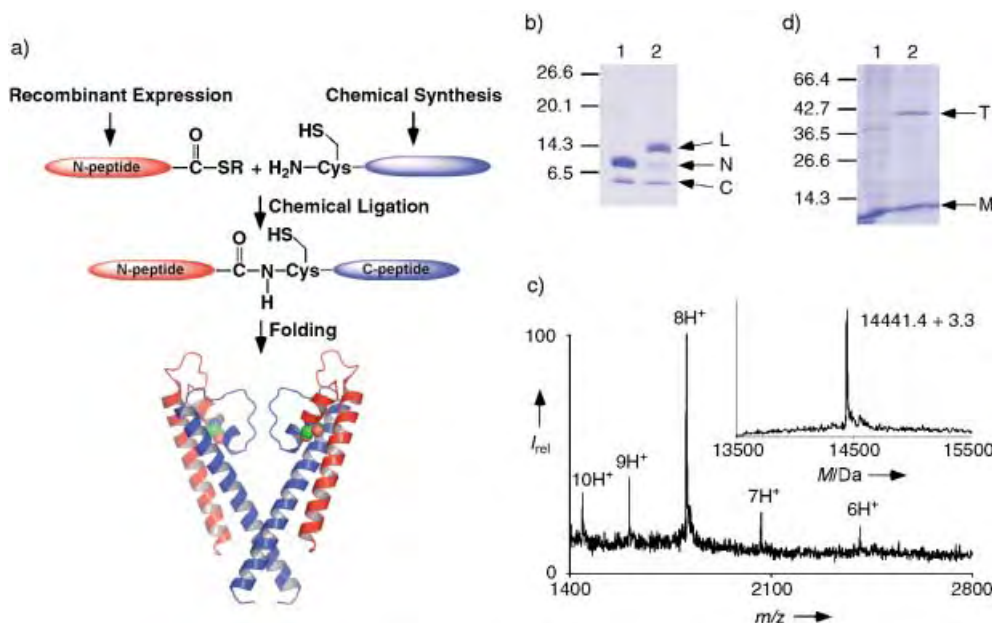


Figure 3. Semisynthesis of a functional K⁺ channel. a) The KcsA polypeptide was assembled by expressed protein ligation of a recombinant N-peptide (residues 1–68, red) and a synthetic C-peptide (residues 69–122, blue). The product was then folded into the native tetramer by using lipid vesicles and purified by affinity and gel-filtration chromatographies. The ligation site, position 69, is depicted in space-filling form. b) SDS–PAGE analysis (15%) of the ligation reaction at 0 min (lane 1) and 24 h (lane 2) showing the C-peptide (C), N-peptide (N), and ligation product (L). c) ES–MS of the purified ligation product (inset: reconstructed spectrum; expected mass 14438.0 Da). d) SDS–PAGE analysis (12%) of the semisynthetic channel before (lane 1) and after (lane 2) addition of lipids. The monomer (M) and the tetramer (T) of semisynthetic KcsA are indicated.

ligation reaction was initiated by the addition of 2% thiophenol and incubation at 37°C. The reaction, progress of which was monitored by SDS-PAGE, was around 50% complete after 4 h and almost complete after 24 h (Figure 3b). Analysis of the ligation product by electrospray mass spectrometry (ES-MS) after purification by reverse-phase high-pressure liquid chromatography (RP-HPLC) yielded a signal that was consistent with the expected mass (Figure 3c).

Unfolded monomeric KcsA can be folded into a tetramer by using lipid vesicles. The ligation product was folded by a published lipid-based folding protocol with minor modifications.^[4,12] Folding was confirmed by identification of the tetramer in SDS-PAGE (Figure 3d). The N-peptide used in the ligation reaction was engineered to carry an N-terminal His₆ tag, which facilitated two-step purification of the final product. In the first step, the folding mixture was passed over a column containing Co²⁺ ions to remove lipid vesicles from the protein, and in the second step gel-filtration chromatography was used to separate the tetramer from unfolded monomer and residual starting materials. In a typical small-scale synthesis, 0.06 mg of purified tetrameric semisynthetic KcsA channel was obtained from the ligation reaction of 0.7 mg of the N-peptide thioester and 1.0 mg of the C-peptide.

Purified semisynthetic KcsA K⁺ channels containing the A98G mutation were reconstituted into lipid vesicles, and single-channel activity was tested in planar lipid membranes; single-channel recordings are shown in Figure 4a. As in the recombinant A98GΔC channel, two conductance states were observed, and the *i*-*V* curve (shown for the higher conductance state) is indistinguishable from that of the recombinant channel (Figure 4b). The histogram of open times has a mean value of 2.8 ms, which compares to a value of 2.9 ms for the recombinant channel (Figure 4c, d). These measurements indicate that the properties of the recombinant and semisynthetic KcsA K⁺ channels are essentially the same.

We have successfully produced a functional semisynthetic K⁺ channel in which the region of greatest interest, the selectivity filter, has been produced by chemical synthesis. These procedures provide a way to study the effects of main-chain chemical modifications. Since K⁺ ions in the filter interact almost exclusively with main-chain carbonyl oxygen atoms, these methods are necessary for the production of channels in which each of the four sites within the filter have been modified. The possibilities for modification are numerous and will be reported in due course.

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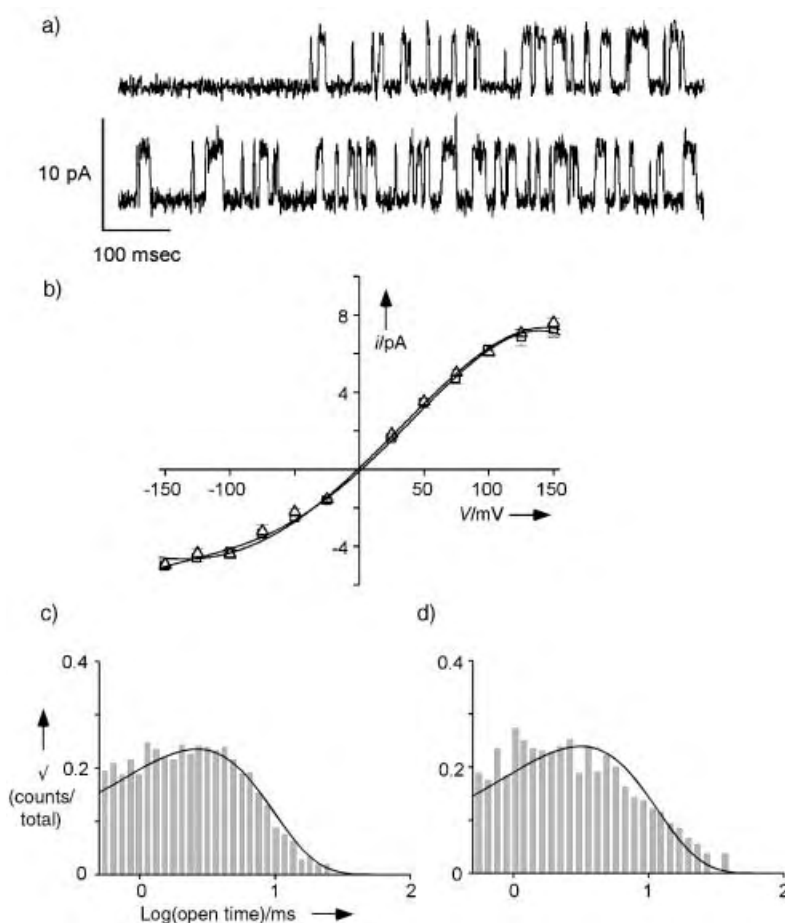


Figure 4. Single-channel activities of semisynthetic KcsA. a) Representative single-channel traces for the semisynthetic channel recorded at +100 mV in symmetrical 150 mM K⁺ solution. b) *i*-*V* curve for the semisynthetic KcsA channel (Δ) and recombinant KcsA-S69C/A98GΔC channel (□). Single-channel currents were recorded in symmetrical 150 mM K⁺ solutions, and each data point represents the average ± SE (*n* ≥ 3) from at least two separate planar lipid membranes. Open-time histograms for semisynthetic KcsA (c) and recombinant KcsA S69CΔC channel (d) at +100 mV in symmetrical 150 mM K⁺ solutions. Solid lines represent single exponential fits to the data (see the Supporting Information).

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A Droplet-Based, Composite PDMS/Glass Capillary Microfluidic System for Evaluating Protein Crystallization Conditions by Microbatch and Vapor-Diffusion Methods with On-Chip X-Ray Diffraction**

Bo Zheng, Joshua D. Tice, L. Spencer Roach, and Rustem F. Ismagilov*

This paper reports a composite microfluidic system for performing protein crystallization trials in nanoliter aqueous droplets inside capillaries suitable for X-ray diffraction and the evaluation of the quality of the crystals directly by on-chip X-ray diffraction. Crystallization conditions can be screened with both microbatch and vapor-diffusion techniques by eliminating evaporation of solutions and by controlling diffusion of water between droplets.

Growing of high-quality crystals of proteins and other macromolecules plays an important role in structural biology. Crystallization conditions are usually identified by performing a large number of trials in which variable ratios of solutions of a protein, precipitants, and additives are pipetted together by hand ($\approx 1 \mu\text{L}$ droplets) or with a robotic dispenser ($\approx 100\text{--}10 \text{ nL}$ droplets).^[1] This process could be improved by a system that minimizes the amount of protein sample consumed, reduces labor, and is simple and economical enough to be implemented in individual laboratories. Microfluidics could serve as a platform for such a system because it allows sophisticated handling of small volumes (nL–pL) of reagents in a potentially simple, inexpensive format.^[2] In addition, microfluidics may provide an opportunity to perform experiments that are difficult to do on larger scale—for example, Hansen et al. have crystallized proteins on a polydimethylsiloxane (PDMS) microfluidic device by free interface diffusion.^[3] Previously, we screened crystallization

conditions^[4] on a PDMS microfluidic chip by using nanoliter droplets^[5] formed in microfluidic channels.

In this work, we rely on the same droplet-based platform, but take three steps that advance this work beyond our previous report. 1) We implemented the microbatch technique, in which we completely eliminated problems of uncontrolled evaporation through PDMS. PDMS is water- and oil-permeable, and the crystallization of proteins in PDMS, at least in our hands, previously required careful control of humidity to limit evaporation. 2) We implemented a controlled vapor-diffusion technique, in which the rate of removal of water from the droplet containing the protein could be easily adjusted. 3) We developed a method for evaluating the quality of crystals by direct on-chip X-ray diffraction. This method eliminates the need for manual handling of crystals that was previously required for the extraction of crystals from the device and subsequent mounting. Manual handling was tedious and had the potential to damage the crystal.

We used composite PDMS/glass capillary microfluidic devices to eliminate the evaporation of solutions during crystallization trials (Figure 1 a, b). Composite microfluidic

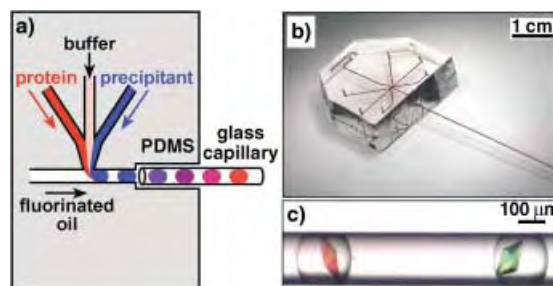


Figure 1. a) A schematic illustration of the method of forming droplets for protein crystallization trials in a PDMS/glass capillary composite microfluidic device. b) A photograph of the composite device. All inlets and channels were filled with $[\text{Fe}(\text{SCN})_x]^{(3-x)+}$ solution for clarity. c) A microphotograph of thaumatin crystals grown inside droplets in a capillary. Droplets were generated by the method illustrated in (a).

devices are well known, especially in capillary electrophoresis.^[6,7] Glass capillaries have been used to grow protein crystals by using the microbatch technique^[8] and several diffusion techniques, such as equilibrium dialysis,^[9] free interface diffusion,^[10] and gel-acupuncture methods.^[11,12] Crystallization of organic molecules has also been performed in glass capillaries.^[13–15] We used the PDMS section of the device to generate droplets that contain protein, additives and precipitants in controlled ratios by using a procedure described previously.^[4] Briefly, we flowed aqueous streams that of solutions of the protein, additives, and precipitants into a flowing stream of fluorinated oil, in which all the aqueous streams formed droplets surrounded and carried by the flow of oil (Figure 1 a). We varied the composition of the droplets by varying the relative flow rates of the aqueous streams. The flow of oil transported droplets into a glass capillary attached to the outlet of the PDMS section. The flow was stopped when the capillary was filled with droplets of desired composition, and the capillary was disconnected, sealed with wax, incu-

[*] Dr. B. Zheng, J. D. Tice, L. S. Roach, Prof. R. F. Ismagilov
Department of Chemistry
The University of Chicago
5735 South Ellis Avenue, Chicago, IL 60637 (USA)
Fax: (+1) 773-702-0805
E-mail: r-ismagilov@uchicago.edu

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

bated at 18°C, and monitored periodically. Crystals formed in the droplets (Figure 1 c) under the conditions similar to those we found by using the previous system.^[4] In contrast to the case in which droplets were incubated in the PDMS channels, droplets and crystals incubated in a sealed glass capillary were stable even in the absence of humidity control, and did not show signs of evaporation over six months. This long-term stability is crucial for protein crystallization on nanoliter scale, because the probability of a nucleation event decreases as the volume of the solution decreases.^[16–18] For example, nucleation rate is proportional to droplet volume for homogeneous nucleation and to the surface area of the droplet for heterogeneous nucleation. We have observed that it takes a longer time to obtain crystals in nL droplets than μL droplets under microbatch conditions. The acceleration of nucleation while still using nanoliter volumes would be desirable for protein crystallization used in high-throughput structural determination.

Vapor diffusion may be used to accelerate the nucleation of protein crystals. During protein crystallization by vapor diffusion, a droplet containing the crystallization mixture is placed in the vicinity of a reservoir that contains a high concentration of salt. Slow diffusion of water from the crystallization solution into the concentrated solution of salt is due to the difference in osmotic pressures of the two solutions. This diffusion leads to a decrease in volume of the crystallization solution, and therefore to a sufficient increase in the supersaturation of the crystallization solution for the nucleation of the protein crystal to occur.

To implement crystallization by vapor diffusion inside capillaries, we wished to generate droplets of the crystallization mixture alternating with droplets that contained a high concentration of salt. We hypothesized that if the oil separating the droplets were water-permeable, crystallization would occur as water diffused from droplets of low concentration to droplets of high concentration until osmotic pressures were equalized. We found that alternating droplets of two sets of aqueous solutions can be easily generated by flowing the two sets of aqueous solutions head-on into a flowing stream of water-immiscible oil (Figure 2a). In the

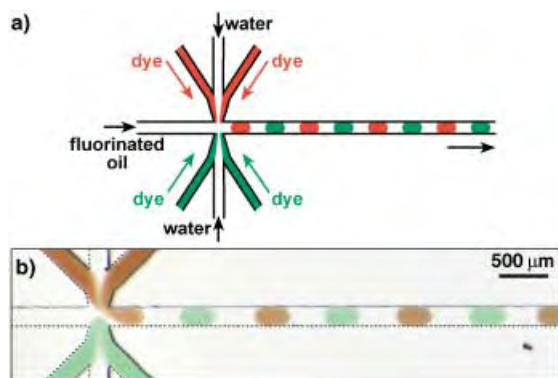


Figure 2. a) A schematic illustration and b) a microphotograph of the microfluidic method of forming alternating droplets in the microfluidic channel. Alternating droplets formed spontaneously when water and aqueous solutions of food dyes (red and green) were continuously flowed into a steady stream of fluorinated oil.

experiment shown in Figure 2b two of the three aqueous streams from the upper set were labeled with a red dye, and two of the three aqueous streams from the lower set were labeled with a green dye. Flowing oil served as the barrier between the two sets of aqueous streams, thus preventing them from coming into contact (Figure 2b). We used fluorescent markers to confirm that there was no cross-contamination between the two sets of solutions.

We used alternating droplets formed with 1.5 M and 0.5 M aqueous solutions of NaCl to measure the rate of transport of water through various oils by measuring the change in size of the droplets (Figure 3a). We found that perfluoroalkanes were impermeable to water, and over a period of several weeks we observed no transport of water between adjacent droplets separated by $\approx 100 \mu\text{m}$ of a perfluoroalkane. This result is consistent with our results in microbatch crystalliza-

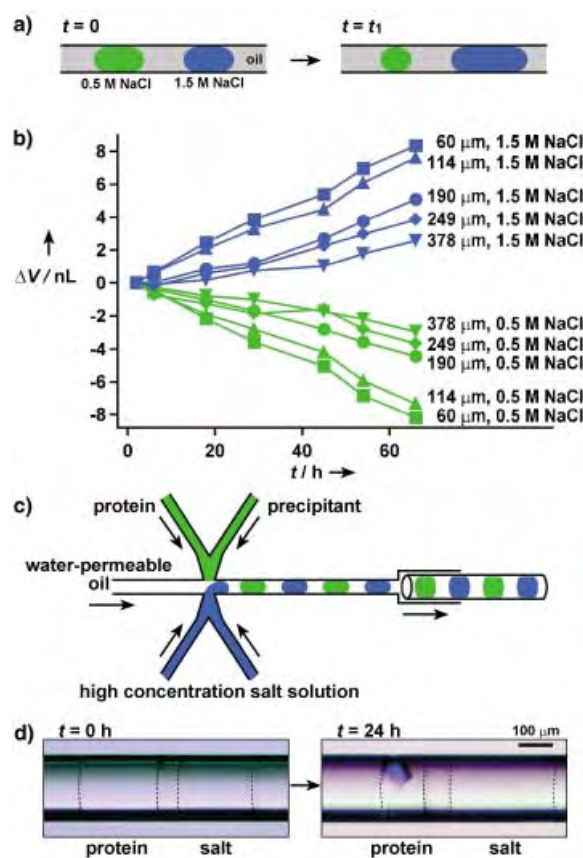


Figure 3. Protein crystallization by vapor diffusion in capillaries: a) A schematic illustration of the principle of vapor diffusion in a capillary. Water diffused through oil from droplets that contained a low concentration of salt to droplets that contained a high concentration of salt. b) A plot of change in the volume of droplets that were initially 25 nL (ΔV) as a function of time (t). Legends on the right side of each curve show the distance between the droplets. The concentrations of NaCl in the droplets are also indicated. c) A schematic illustration of the PDMS/glass composite device for setting up pairs of droplet for protein crystallization under vapor-diffusion conditions in capillaries. d) Two microphotographs that show a pair of droplets immediately after (left) and 24 h after (right) the setup. The droplet of the protein solution yielded a crystal after it had lost about 50% of its water content. Dashed lines were added to show the interface between the aqueous droplets and oil.

tion, in which we used perfluoroalkanes as the oils and did not observe any transport of water between adjacent droplets. We found that polytrifluoropropylmethylsiloxane homopolymer (FMS-121) was permeable to water. A similar oil has been used to absorb water from small droplets to drive the self-assembly of colloids.^[19] To quantify the rate of transport of water through this oil in capillaries, we formed isolated pairs of droplets containing 1.5 M and 0.5 M aqueous solutions of NaCl. To aid visualization we colored 0.5 M NaCl solution with 0.05 M $[\text{Fe}(\text{SCN})_x]^{(3-x)+}$ in 0.3 M KNO_3 . Each pair of droplets in a capillary was separated by ≈ 50 to ≈ 400 μm of oil. Capillaries were stored at 18 °C and monitored. Initially, all droplets were ≈ 25 nL in volume, but the increase in volume of the droplets that contained 1.5 M NaCl and the decrease in volume of the droplets that contained 0.5 M NaCl became noticeable in a few hours (Figure 3b). The initial change of volume was linear with time, and linear changes of up to 30 % were observed over several days (Figure 3b). The rate of transport decreased as osmotic pressure inside the droplets equalized. The observed rate of change in volume is similar to that used in traditional vapor diffusion methods, and can be tuned by controlling the distance between the droplets (Figure 3b). This distance can be controlled by changing the relative flow rates of the aqueous and fluoruous phases.^[20] The control of the evaporation rate of water is important for protein crystallization under vapor-diffusion conditions. An increase in the rate of evaporation of water results in shorter equilibration time, therefore the crystallization trials can be carried out more rapidly.^[21] On the other hand, it has also been proposed that larger, fewer, and better quality crystals are more likely to be obtained by reducing the evaporation rate.^[22] Both approaches could be appropriate depending on a specific protein, and both of them can be implemented in this microfluidic system.

To use this system for the crystallization of proteins by vapor diffusion, one may envision flowing the crystallization solutions through one set of channels, and flowing solutions containing high concentrations of salts through the other set of channels (Figure 3c). For simplicity, Figure 3c shows only two channels for each set of solutions, but we have performed these experiments with up to four channels for each set; the device shown in Figure 1b has three channels for each set of solutions. To screen the crystallization conditions, all concentrations were varied by changing the flow rates of the corresponding aqueous streams.^[4]

To demonstrate crystallization of a protein by vapor diffusion in capillaries, we generated a sequence of droplets that contained lysozyme and precipitants in the first set of droplets, and contained a high-concentration of salt in the second set of droplets (Figure 3c). After 24 hours of incubation, ≈ 50 % of the water in the droplets of lysozyme solution had diffused into salt droplets, thus inducing crystallization (Figure 3d). More than half of the droplets of lysozyme solution yielded crystals after two days. To demonstrate that crystallization is due to the transport of water, a control experiment was performed. The capillary was filled with droplets of lysozyme solution, all at identical concentrations equal to those used in the vapor-diffusion experiment, so that no water transport between droplets could

occur. None of the droplets yielded crystals after an incubation period of one month.

The evaluation of the quality of the crystals is an important part of the screening process. The ultimate test of crystal quality is X-ray diffraction. Crystals obtained from 10–20 nL droplets had dimensions of about 50–200 μm on each side and were large enough for synchrotron diffraction experiments. We have been able to extract crystals grown inside droplets in capillaries simply by cutting the capillary and gently flowing or wicking the liquid out together with the crystal. More importantly, we could obtain diffraction patterns from crystals grown in these thin-walled capillaries without manipulation of the crystals, by subjecting the capillaries with crystals directly to the synchrotron X-ray beam (Figure 4). We have observed scattering patterns from

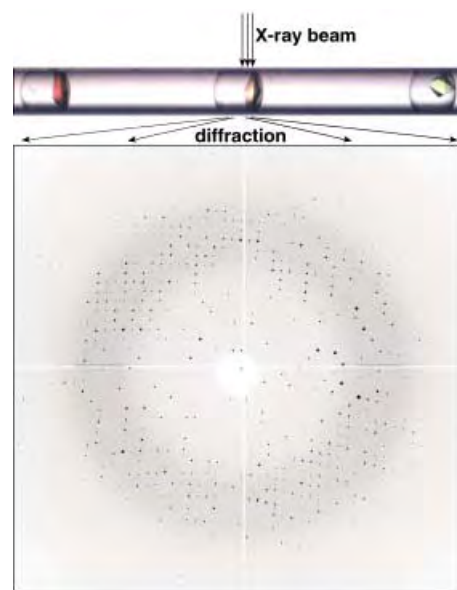


Figure 4. The capillary containing protein crystals (top) can be directly subjected to X-ray diffraction. A diffraction pattern (bottom) of thaumatin crystal from on-chip X-ray diffraction that shows the reflections on the edge of the image at a resolution of 2.0 Å.

the glass and the mother liquor present around the crystal, but this scattering did not obscure the diffraction of crystals. Thaumatin crystals diffracted to ≈ 1.8 Å resolution (Figure 4, diffraction to 2.0 Å is shown). A tetragonal space group with correct unit-cell dimensions could be obtained from a single diffraction image. The diffraction image shown in Figure 4 was refined to unit-cell dimensions $a = 58.45$ Å, $b = 58.45$ Å, $c = 149.02$ Å and $\alpha = \beta = \gamma = 90^\circ$ by using 567 unique reflections; and the literature values are $a = 58.60$ Å, $b = 58.60$ Å, $c = 151.80$ Å and $\alpha = \beta = \gamma = 90^\circ$.^[23] Lysozyme crystals diffracted to ≈ 1.8 Å resolution. The unit-cell dimensions of the crystal derived from the diffraction were $a = 78.99$ Å, $b = 78.99$ Å, $c = 38.40$ Å and $\alpha = \beta = \gamma = 90^\circ$. The reported values are $a = 79.10$ Å, $b = 79.10$ Å, $c = 37.90$ Å and $\alpha = \beta = \gamma = 90^\circ$.^[24]

Exposure of a crystal to synchrotron radiation at room temperature is known to damage the crystal. We observed deterioration of the diffraction patterns for these simple

proteins after 20–50 exposures, each exposure lasting five seconds. From one crystal at room temperature, it would be difficult to obtain a complete set of data required for structural determination, especially for less-stable proteins. Most protein crystals should survive at least a single short exposure to the beam (or two exposures along orthogonal axes) required to obtain a single diffraction frame. As is, this technique would be especially valuable for evaluating and comparing the intrinsic quality of crystals obtained under different crystallization conditions, quality that has not deteriorated through handling and freezing. Freezing of crystals, either extracted from the capillary or directly inside the capillary, should increase their stability sufficiently for structural determination, and we are currently exploring both of these approaches by using a range of cryoprotectants.

In conclusion, we have demonstrated a versatile microfluidic system for screening protein-crystallization conditions in droplets inside capillaries. The system is compatible with both microbatch and vapor-diffusion methods, both of which have advantages and disadvantages.^[25] The quality of crystals obtained in this system can be evaluated directly by X-ray diffraction. Several steps may be taken to improve this system further. We could not obtain capillaries longer than 10 cm, thus limiting us to ≈ 100 experiments per capillary. The cost of capillaries ($\approx \$2$ each) was a less significant limitation. A source of inexpensive long glass or plastic capillaries suitable for X-ray diffraction would make this system even more useful. Fluorocarbons used as carrier fluids in this system can dissolve large amounts of oxygen, and care must be taken to degas the fluids when working with air-sensitive proteins. Once the fluids are deoxygenated, they may be saturated with other gases. Because the capillaries are sealed after the experiment, this method may be useful for long-term crystal growth of proteins^[26,27] that require a controlled environment. An important application of the vapor-diffusion method is to concentrate very low volumes of solutions of proteins. This method may be useful for the crystallization of proteins that cannot be obtained at high concentrations, and may also find applications for concentrating solutions of other samples available in small quantities. Overall, we believe this microfluidic system will be useful for fundamental studies of crystallization phenomena, and will find applications for crystallizing targets ranging from small organic and inorganic molecules to large biological macromolecules.

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Visual Observations of SERRS from Single Silver-Coated Silica Microparticles within Optical Tweezers**

Graeme McNay, Frances T. Docherty, Duncan Graham, W. Ewen Smith,* Pamela Jordan, Miles Padgett, Jonathan Leach, Gavin Sinclair, Paul B. Monaghan, and Jonathan M. Cooper

Surface-enhanced resonance Raman scattering (SERRS) can be up to around 10^{14} times more efficient than Raman spectroscopy thereby enabling the detection of single molecules.^[1–4] Although other techniques such as fluorescence have similar sensitivities, SERRS peaks are narrow, distinct from each other, and very sensitive to molecular structure. In a related field, optical tweezers use the extremely high gradient in the electric field that is produced near the waist of a tightly focused laser beam to trap or manipulate micron-sized organic, inorganic and biological particles. The apparatus required for both SERRS and optical tweezers are similar and comprise a laser light source focused into a sample by using a high magnification microscope objective. A particle in the vicinity of the optical tweezers trap can also be imaged by using the same objective lens making an optical-tweezers setup ideal for the collection of Raman scattering from the trapped particle. By combining optical tweezers and SERRS, we propose a common platform capable of the manipulation and recognition of specific particles and their interactions at the single microparticle level.

Key to the SERRS enhancement is the ability to appropriately coat the surface of the microparticle with metal such that it supports the optical excitation of a surface plasmon. One reason SERRS has not been more widely investigated within optical tweezers is that it is difficult to trap metallic particles.^[5] Herein, we show for the first time that introducing a SERRS active particle to the trapping beam of an optical tweezers system creates intense SERRS emission that can be seen on a video camera. The complete SERRS spectrum can be recorded in 100 ms or less.

In previous work, SERRS spectra have been recorded from trapped silver clusters coated with rhodamine or phenylalanine.^[6] However, even for integration times of 5 seconds, many particles had to cluster together to give a measurable signal. Here we report what we believe to be the first observation of SERRS from dye absorbed on a single silica microbead partially coated in silver and suspended in solution near the focus of an optical-tweezers trap. Our experimental configuration comprised a laser source at 532 nm brought to a focus in the sample plane by using a

$\times 100$ microscope objective. Appropriate filters attenuated the backscattered laser light whilst allowing both white light imaging with a CCD camera and spectral analysis of the wavelength shifted light with a fiber-coupled spectrometer.

The mechanism for optical trapping: Normally optical tweezers rely upon the refraction of light by a transparent object to draw the object to the beam focus. However, for metallic particles, the additional scattering of light from their surface means that they are repelled from the trapping laser. Although it is possible to devise annular^[7] or scanning^[8] beams to trap metal particles, the associated complexity makes the approach restrictive. We use silica spheres partially coated with silver and with an azo dye (3,5-dimethoxy-4-(6'-azobenzotriazolyl)-phenylamine) that has previously been shown to adsorb strongly onto a silver surface.^[9,10] Figure 1 shows transmission electron micrographs of the plain silica spheres and of silica spheres that have been partially coated in silver. The images show that the coated particles have a rough surface with a silver thickness varying up to about 100 nm. The particles resemble the SERRS-active microbeads of Lendl and co-workers.^[11,12] and we have obtained strong

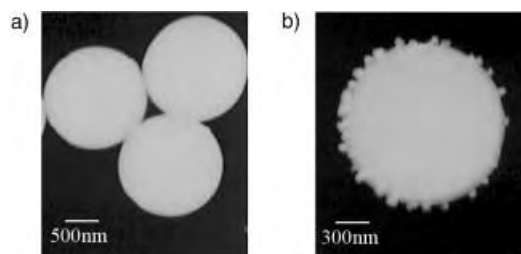


Figure 1. Transmission electron micrographs of a) plain silica microparticles and b) a partially silver-coated silica microparticle.

SERRS spectra from our beads in our standard Raman instrument. However, we also observed that the partial silver coating allowed many of these particles to be trapped in three dimensions within a conventional optical-tweezers setup without recourse to annular or scanning beams.

Observation of SERRS from single particles: Translation of the sample stage allowed a variety of particles to be moved into the focused laser beam and micrometer capture range of the optical trap. As a dye-coated particle approached the laser focus, a bright flash of light was emitted from the particle edge with a spectrum corresponding to a SERRS emission. Interestingly particles with the weakest SERRS were the easiest to trap whilst those with the strongest SERRS were repelled. This is indicative of the amount of silver coating on the particle. In all cases once trapped, the SERRS from the particle ceased, which probably resulted from photobleaching of the dye in the intense laser beam.

When a second particle was brought close to a trapped, now SERRS-inactive particle, a flash of light was observed, this time from the second particle and a SERRS spectrum was recorded. The sequence of photographs in Figure 2 shows the second particle approaching the trapped particle, flashing briefly, and then moving away. Light flashes could be readily observed from numerous individual SERRS-active microspheres and a SERRS spectrum associated with each emission was recorded. Figure 3 shows the spectrum from a single

[*] G. McNay, F. T. Docherty, D. Graham, W. E. Smith
Department of Pure and Applied Chemistry
University of Strathclyde
Glasgow, G1 1XL (UK)
Fax: (+44) 141-552-0876
E-mail: w.e.smith@strath.ac.uk

SERRS active microparticle, compared to that from a colloidal suspension. Figure 4 shows the intensity of the spectral peak at 1369 cm^{-1} as a function of time, which suggests that the duration of these flashes are of the order of 200–300 ms. There are no appreciable changes in the relative intensities of the peaks in the spectrum between each 100 ms accumulation. Thus, the flashes are due to SERRS, which is sufficiently strong to be visualized on a standard (non-intensified) CCD camera.

The trapping beam is extremely tightly focused, giving both a small spot ($< 1\text{ }\mu\text{m}$) and an associated large numerical aperture (1.3). Hence, the excitation volume corresponding to the high light intensity covers a few cubic microns in the vicinity of the beam focus. However, due to the extremely high divergence of the beam, a significant level of illumination light extends over tens of microns. We observe that this light can be sufficient to excite SERRS emissions from a distance of $20\text{ }\mu\text{m}$. Thus, by having a SERRS-active particle with close to the trapping beam, a longer-lived lower-intensity signal is observed on both the CCD camera and spectrometer. Figure 5 shows two such particles that have been excited by

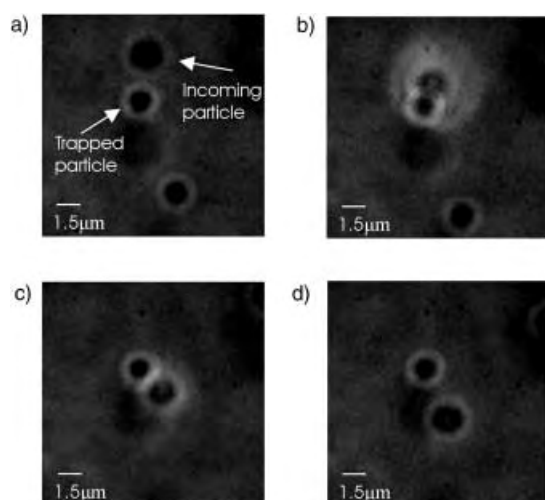


Figure 2. Sequence of images showing a) the approach of a dye coated particle to a trapped particle, b) its contact with a trapped particle and resulting SERRS emission, c) and d) followed by the movement of the particle away from the trap position.

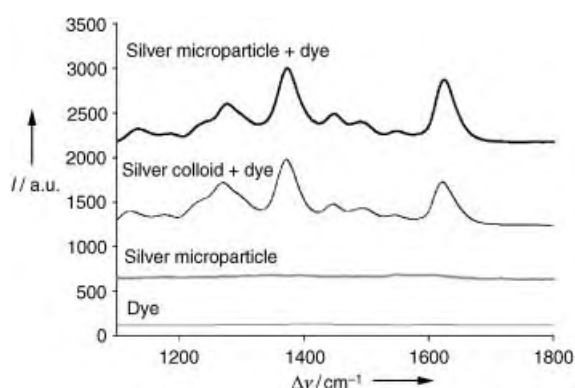


Figure 3. The Raman spectra from the pure azo dye, the plain silver-coated microparticles, the SERRS spectra from the dye adsorbed on aggregated silver colloid, and the dye adsorbed on the silver-coated microparticles. I = intensity, arbitrary units.

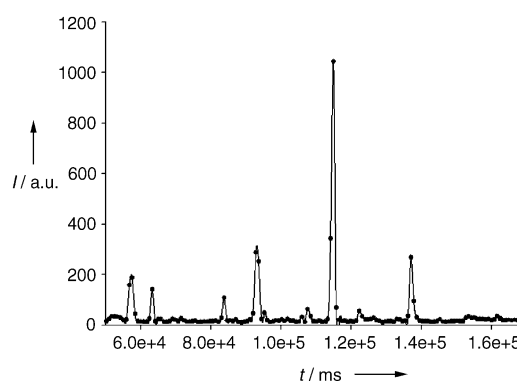


Figure 4. Intensity of the SERRS signal at 1369 cm^{-1} against time (t). Each point in the graph represents 100 ms.

the periphery of the beam. This lower-intensity SERRS was observed for a period of more than ten seconds.

The SERRS emission after touching occurs from a number of points on the particle surface to produce a speckled effect (Figure 5), which suggests that there are strongly enhanced localized optical fields on the particle surface. Markel et al.^[13] have shown by theory and experiment that when clusters of silver colloid particles are excited by

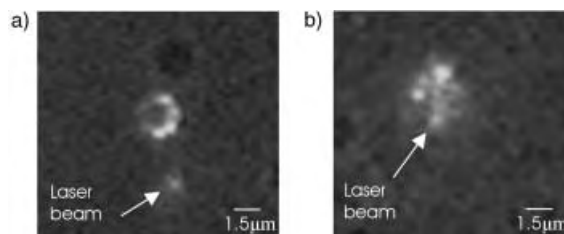


Figure 5. Two examples of the characteristic speckle pattern observed when a single dye-coated particle comes close to the edge of the laser beam. a) A long lived SERRS emission from a particle on the periphery of the beam, and b) a short lived SERRS emission from a particle near the trapping beam.

light tuned to excite a surface plasmon, the intensity distribution in the near-field is heterogeneous with most of the excitation concentrated in hot spots. Their field-intensity distribution forms a similar speckled pattern to that observed in our SERRS emission. Similarly, Hillenbrand and Keilmann^[14] have used a scattering-type scanning near-field optical microscope to measure the optical near-field from gold nanoparticle clusters and found that some particles exhibited enhanced optical near fields, but the highest amplitudes occur in specific location within gaps between particles. With microparticles, it is probable that the local hot spots occur in particular types of topographical environment and are dependent on the coverage, thickness, and surface roughness of the coated particle.

In conclusion, we have detected strong and short-lived SERRS emission ($< 100\text{ ms}$) from single, partially silver-coated, dye-adsorbed microparticles within optical tweezers. Full spectra, recorded from single particles, clearly show that the emission is due to SERRS.

On the periphery of the trapping beam, where the excitation intensity is much lower, the SERRS emission is longer-lived but can still be observed with a standard CCD

camera. The SERRS emission is from individual spots on the surface of the particle rather than the particle as a whole.

The high sensitivity, the ease of molecular recognition and the relatively simple chemistry of SERRS can be combined with the ability to observe, trap, and subsequently manipulate particles. In future this will provide an effective platform on which biologically functionalized beads can be manipulated to give a localized probe, for example, to detect individual binding events.

Experimental Section

Silica beads of 1.5 μm diameter were lightly coated in silver by using Tollen's reagent. The configuration for optical trapping used an infinity-corrected Zeiss Plan-Neofluar oil-immersion objective lens with a numerical aperture of 1.3 and a magnification of $\times 100$. The trapping laser is a frequency doubled commercial Nd:YVO₄ laser (100 mW, $\lambda = 532$ nm). Particles were viewed with a CCD camera protected by a filter to remove light of the laser frequency. The spectra were recorded on a S2000 Ocean Optics spectrometer. To confirm that the spectra recorded in the optical-tweezers setup was from the dye adsorbed on the beads, a SERRS spectrum from the dye adsorbed on silver colloid was acquired. Silver colloid (250 μL), water (250 μL), 1×10^{-7} M dye (10 μL) and 1 M sodium chloride (10 μL) were mixed together and analysed in a cuvette using an acquisition time of 3 seconds.

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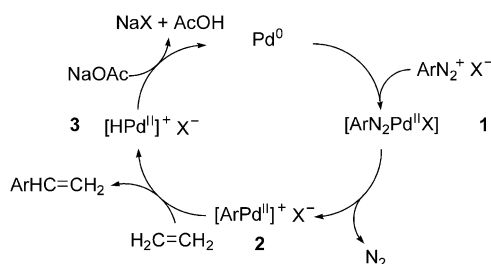
Probing the Mechanism of the Heck Reaction with Arene Diazonium Salts by Electrospray Mass and Tandem Mass Spectrometry**

Adão A. Sabino, Angelo H. L. Machado,
Carlos Roque D. Correia, and Marcos N. Eberlin*

The Heck reaction, with its many creative and effective variations, finds a prominent place among the synthetic tools available in organic synthesis for the construction of C–C bonds.^[1] The Heck reaction has found growing applications mainly because of its rather mild conditions and versatility, and some highly enantioselective versions have been reported.^[2] Despite some impressive demonstrations of its synthetic capability, the Heck reaction still has a number of unaccountable features and unverified mechanistic details.

The phosphane-free version of the Heck reaction also holds great synthetic potential, and is usually more economical, practical, and experimentally simpler than the phosphane version.^[3] One example of the phosphane-free protocol is that in which arene diazonium salts are used as the arylating partner instead of the traditional aryl halides and triflates. This version of the Heck arylation, initially used by Heck and further developed by Matsuda and co-workers,^[4] has been finding increasing application in the synthesis of natural products^[5] and biologically active compounds^[6] but, in contrast to the main-stream halides and triflates, less attention has been given to the understanding of its mechanistic aspects.

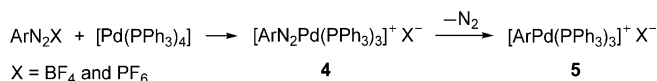
In their initial work,^[4] Matsuda and co-workers proposed the catalytic cycle outlined in Scheme 1 for the Heck reaction with arene diazonium salts. The palladium/aryl diazenide complex **1** was postulated to form from the reaction of arene diazonium salts and $[\text{Pd}(\text{PPh}_3)_4]$ (Scheme 2). Such a reaction



Scheme 1. The catalytic cycle proposed by Matsuda and co-workers for the Heck reaction with arene diazonium salts.

has been proposed to give the palladium/aryl diazenide complex **4**, which undergoes spontaneous nitrogen extrusion to furnish the aryl palladium species **5**.^[7]

The development of electrospray ionization (ESI)^[8,9] has greatly expanded the range of molecules that can be analyzed by mass spectrometry, including those of high polarity, molecular complexity, and mass. ESI is characterized by the gentleness by which gaseous ions are formed, and even



Scheme 2. Oxidative addition pathway to form palladium(II) complex 5.

loosely bonded, supramolecular ionic species survive intact.^[9c,10] ESI-MS is also rapidly becoming the technique of choice for mechanistic studies and high-throughput screening of homogeneous catalysis.^[11] Having recognized the pivotal importance of characterizing the cationic palladium intermediates involved in the catalytic cycle proposed by Matsuda and co-workers (Scheme 1),^[12] we decided to take advantage of the ability of ESI to smoothly transfer ionic species to the gas phase, and to use our experience in the investigation of reaction mechanisms by mass spectrometry,^[13] to probe the mechanism of the Heck reaction. ESI-MS/MS was used to “fish” the palladium intermediates directly from solution to the gas phase, and also to perform their unprecedented structural characterization.

Our investigation began with the oxidative addition step using the arene diazonium salt $4\text{-MeOPhN}_2^+\text{BF}_4^-$ and $[\text{Pd}_2(\text{dba})_3]\cdot\text{dba}$ (dba = dibenzylideneacetone) as the source of palladium. We used ESI-MS to monitor the reaction of $4\text{-MeOPhN}_2^+\text{BF}_4^-$ and $[\text{Pd}_2(\text{dba})_3]\cdot\text{dba}$ in acetonitrile by scanning the first quadrupole (Q1) of a hybrid triple quadrupole linear ion-trap mass spectrometer (Qtrap from Applied Biosystem). The reactant diazonium ion (m/z 135) together with four ionic species could be detected by ESI-MS after one to five minutes of reaction: **6** (m/z 295; all m/z values are reported for ^{106}Pd), **7** (m/z 336), **8** (m/z 488), and **9** (m/z 681; Figure 1a). All the ions display the characteristic isotopic distribution of palladium-containing species.^[14] These species correspond to the proposed ionic intermediate **2** formed by the oxidative addition of the Pd catalyst to the arene diazonium salt (Scheme 1), and differ only in the type and number of ligands coordinated to the central Pd atom. They are, therefore, likely to be present in solution (except **6** which is likely formed by in-source collision-induced dissociation (CID) of **7**) and thus potential intermediates in the catalytic cycle. We also attempted to detect **1** (Scheme 1, Ar = 4-MeOPh) as its cation $[4\text{-MeOPhN}_2\text{Pd}]^+$ (m/z 241) or corresponding acetonitrile complexes. Intermediate **1** is probably too short lived and could not be detected even when using a microreactor for the effective mixing of reactants and fastest possible ESI-MS analysis.^[15]

Interestingly, the composition of the cationic intermediates changes drastically with time: by continuously monitoring these intermediates by ESI-MS we noticed that the ionic composition varies considerably with time. The ionic composition stabilizes after 90 minutes of mixing, with ions **7** and **6** converting almost completely into **8** (Figure 1b), which became by far the most prominent species. Intermediate **9** remained, and a new Pd_2 -containing species **10** (m/z 787) was formed. All these intermediates were then mass-selected for MS/MS structural characterization, and their dissociation chemistry matched their proposed structures. For example, **7** dissociates by three consecutive losses of acetonitrile, fol-

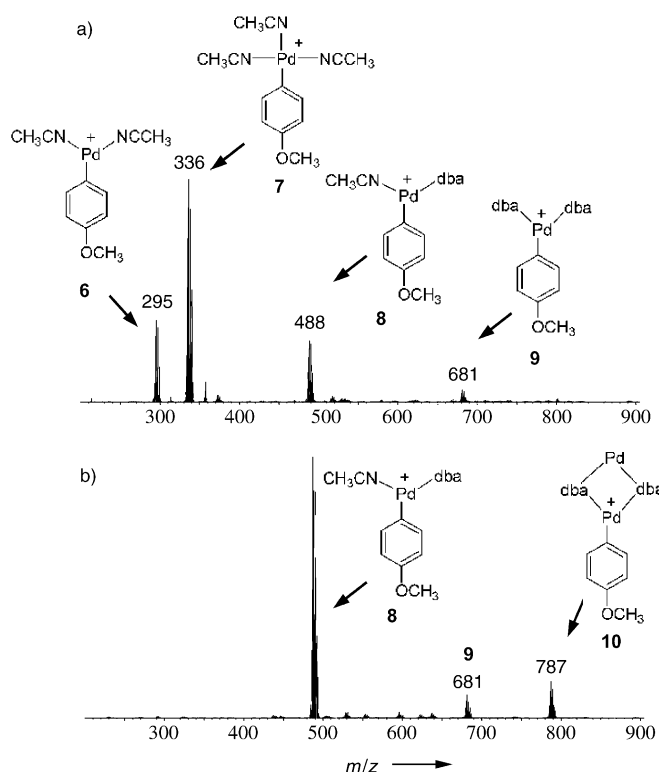


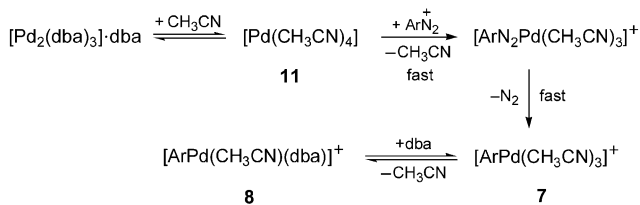
Figure 1. ESI(+)-MS spectrum of a solution (2 nmol L^{-1}) of the arene diazonium salt $4\text{-MeOPhN}_2^+\text{BF}_4^-$ and $[\text{Pd}_2(\text{dba})_3]\cdot\text{dba}$ in acetonitrile after mixing for: a) 5 min and b) 90 min. The reactant diazonium ion (m/z 135) was also detected as the most abundant ion, but spectra were scanned from m/z 200 for clarity.

lowed by loss of formaldehyde. Intermediate **8** dissociates by loss of acetonitrile followed by loss of CO and a neutral loss of a species with mass m/z 102, most probably phenylacetylene from dba.

Thus, an interesting dynamic and time-dependent process involving ligand exchange in **7–10** has been detected, which can be rationalized through a series of equilibrium reactions (Scheme 3). Initially the acetonitrile solvent molecules rapidly replace dba from $[\text{Pd}_2(\text{dba})_3]\cdot\text{dba}$ to form $[\text{Pd}(\text{CH}_3\text{CN})_4]$ (**11**). This palladium(0) complex undergoes an oxidative addition with the arene diazonium salt to form a very unstable intermediate which rapidly forms intermediate **7** (m/z 336) by oxidative extrusion of N_2 .^[7] In the presence of dba complex **7** is slowly converted into **8** (m/z 488), which appears to be the most stable of the cationic Pd intermediates.

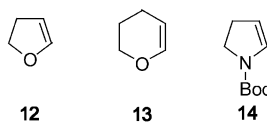
All of the Pd cations **7–10** are potential candidates for the intermediates which add to the olefin in the Heck catalytic cycle (**2** in Scheme 1). However, as the composition of **7–10** varies with time, and to verify which of these intermediates would undergo insertion more efficiently, the model olefins **12**, **13**, and **14** (Boc = *tert*-butoxycarbonyl) were added to the solution of the arene diazonium salt and $[\text{Pd}_2(\text{dba})_3]\cdot\text{dba}$ in acetonitrile after mixing for 5 or 90 minutes.

In the first set of experiments, the olefins were added after mixing $[\text{Pd}_2(\text{dba})_3]\cdot\text{dba}$ and the arene diazonium salt for five minutes. Insertion species were detected in the ESI mass spectrum, but in very low relative abundances and generally



Scheme 3. The time-dependent exchange process for conversion of $[\text{Pd}_2(\text{dba})_3]\cdot\text{dba}$ into intermediate **8**.

involving intermediate **8**. However, after 90 minutes of mixing, olefin insertion products were clearly detected, now with relatively high abundances, and again involving only **8**. For example, three new Pd-containing species were detected



for olefin **12**: **15** (m/z 324), **16** (m/z 517), and **17** (m/z 558; Figure 2). All of these three species correspond to association of **8** with the olefin and are the likely key catalytic intermediates in the Heck reaction (with no loss or loss of one ligand), with the palladium atom bound to the β position of the olefin and the aryl group transferred from the Pd center to the α position of the olefin. Similar results were observed for olefins **13** and **14**. Intermediate **8** is probably the most reactive one and the key intermediate in the olefin-insertion step of the Heck catalytic cycle investigated herein. All the other Pd cations **7**, **9**, and **10** appear to be much less reactive toward olefin insertion.

For structural assignments, **15–17** were mass-selected with Q1 and then submitted to 10 eV CID with nitrogen in q2 in tandem mass spectrometric (MS/MS) experiments. As an example, Figure 3a shows the product-ion mass spectrum for **17** (m/z 558). The dissociation chemistry of **17** is fully consistent with its expected structure: loss of acetonitrile

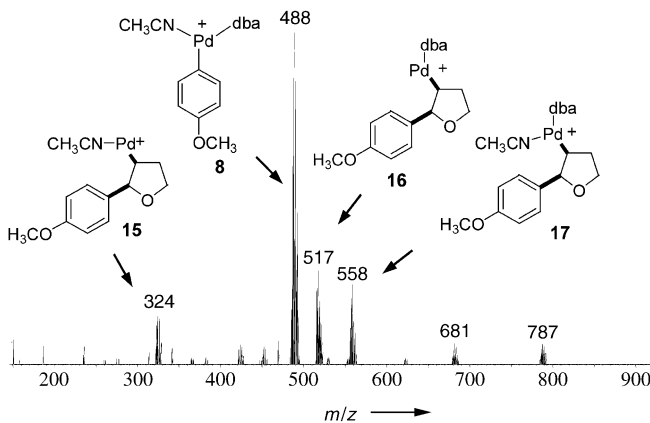


Figure 2. ESI(+)-MS spectrum for a solution (2 nmol L^{-1}) of the arene diazonium salt $4\text{-MeOPhN}_2^+\text{BF}_4^-$ and $[\text{Pd}_2(\text{dba})_3]\cdot\text{dba}$ in acetonitrile after 90 min of mixing and subsequent addition of the olefin **12**.

from the coordination sphere of palladium forms the fragment ion of m/z 517, while further loss of dba forms the main fragment ion of m/z 283. Also enlightening is the fragment ion of m/z 341: its m/z ratio and Pd-isotopic distribution indicates the composition $[\text{HPd}(\text{dba})]^+$, which suggests that a hydride ion has been transferred from the olefin to Pd! This

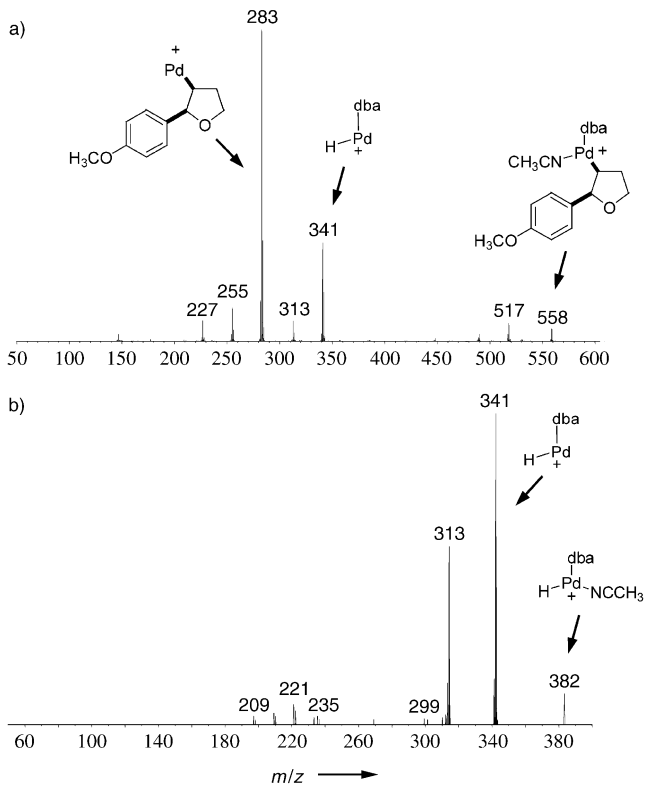


Figure 3. ESI(+) –MS/MS for a) the Heck ionic intermediate **17** (m/z 558) and b) the H-abstraction intermediate [HPd(CH₃CN)(dba)]⁺ (m/z 382).

interesting CID-induced process is equivalent to the last step proposed for the catalytic cycle of the Heck reaction—the hydride elimination step (Scheme 1). The other intermediates **15** and **16** display similar dissociation chemistry as discussed for **17**, including the formation of gaseous $[\text{HPd}(\text{dba})]^+$. Detection of the H-abstraction intermediate $[\text{HPd}(\text{dba})]^+$ in solution was more challenging. However, this intermediate could be detected for olefins **13** and **14** as minor ions associated with acetonitrile and the olefin. One such complex, $[\text{HPd}(\text{CH}_3\text{CN})(\text{dba})]^+$, when mass-selected and subjected to CID (Figure 3b), displayed a characteristic dissociation by loss of acetonitrile that yields $[\text{HPd}(\text{dba})]^+$ (m/z 341) and further loss of CO (m/z 313) from the dba ligand.

The discovery of **8** as the most reactive intermediate suggests that a stepwise addition of reagents in the Heck reaction with diazonium salts would be beneficial, since **8** dominates after mixing 4-MeO-PhN₂⁺BF₄⁻ and [Pd₂(dba)₃].dba for 90 minutes. Hence, [Pd₂(dba)₃].dba and the arene diazonium salt were pre-mixed in catalytic amounts

(in respect to the olefin) in acetonitrile, stirred for 90 minutes to favor formation of **8** followed by addition of stoichiometric amounts of olefin **12** and the arene diazonium salt.^[16] The final Heck product, 2-(4-methoxyphenyl)-2,5-dihydrofuran, was obtained in 80% yield as the only regioisomer after 30 minutes of nitrogen evolution (Scheme 4). This protocol, which features a delay of 90 minutes before addition of the olefin, has become the standard and most-efficient protocol in our laboratory for Heck arylations using arene diazonium salts.

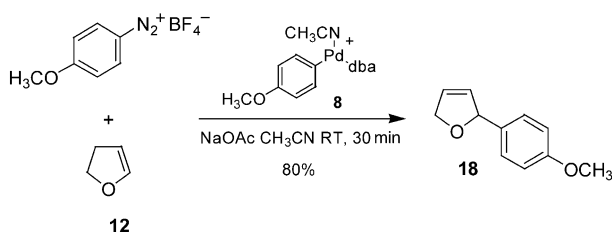
For the first time, therefore, several cationic intermediates of the oxidative addition step of the Heck reaction involving arene diazonium salts have been detected by ESI-MS monitoring and structurally characterized by ESI-MS/MS. A dynamic, time-dependent process with ligand equilibria between several ionic intermediates was also observed for the oxidative addition step. Our results suggest that the most-reactive intermediate for olefin addition is **8**, which dominates

A detailed catalytic cycle for the Heck reaction with arene diazonium salts can be proposed on the basis of the ESI-MS/MS results (Scheme 5, olefin **12** is used as an example).^[16] New aspects of the Heck reaction, such as the role of different solvents and the use of different Pd sources, is currently being investigated by ESI-MS/MS. We are also using tandem mass spectrometric experiments in the Qtrap mass spectrometer^[17] to mass-select ionic intermediates and to explore and compare their intrinsic gas-phase reactivities by performing reactions to probe which of the five ionic intermediates detected in the oxidative addition (**6–10**) are indeed the most reactive toward olefin insertion.

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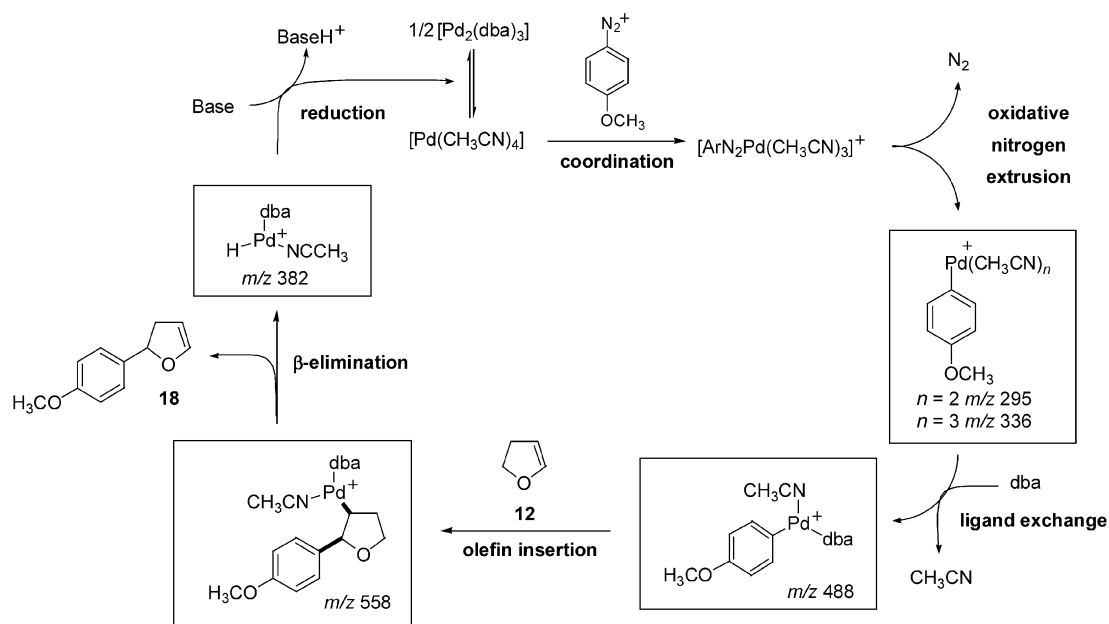
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Keywords: Heck reaction · homogeneous catalysis · mass spectrometry · reaction mechanisms · reactive intermediates



Scheme 4. Heck reaction of **12** with arene diazonium salt 4-MeOPhN₂⁺BF₄[−] promoted by intermediate **8**.

after mixing the arene diazonium salt and [Pd₂(dba)₃]-dba for approximately 90 minutes. Therefore, a delay of 90 minutes before addition of the olefin is beneficial for maximum yield.



Scheme 5. Proposed catalytic cycle for the Heck reaction with arene diazonium salts.

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Stereoselective Glycoside Synthesis

Glycosidation Reactions of Silyl Ethers with Conformationally Inverted Donors Derived from Glucuronic Acid: Stereoselective Synthesis of Glycosides and 2-Deoxyglycosides**

Monika Poláková, Nigel Pitt, Manuela Tosin, and Paul V. Murphy*

Stereoselective glycoside synthesis is of interest because of the biological and medical relevance of oligosaccharides, glycoproteins, glycolipids,^[1] and other carbohydrate derivatives,^[2] and a range of strategies have been developed to produce these compounds.^[3] The 1,6-lactone derivative **2** (see Scheme 1) has potential for use in the synthesis of 1,2-*cis*-glycosides^[4] but its application has been limited because of the low yields obtained from its reaction with alcohols.^[5] We now report that the SnCl₄-catalyzed coupling of silyl ethers^[6] with **2** provides α -*O*-glucuronides in significantly improved yields without loss of stereoselectivity. The methodology has been extended to the related 2-deoxylactones, which give α - or β -glycosides depending on the structure of the donor.

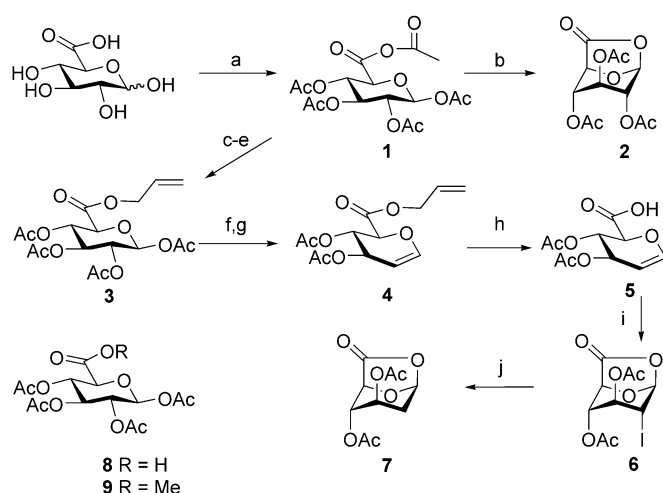
The preparation of **2** was carried out via the mixed anhydride **1** (Scheme 1) by an improved and shorter procedure than that previously described.^[5] The donors **6** and **7** were also prepared, via glycal **4**, because of their potential for the synthesis of 2-deoxyglycosides, which are of biological interest.^[7] Thus, the reaction of the allyl ester **3** with hydrogen bromide in acetic acid gave a glycosyl bromide intermediate

[*] Dr. M. Poláková, Dr. N. Pitt, M. Tosin, Dr. P. V. Murphy
Centre for Synthesis and Chemical Biology
Department of Chemistry
Conway Institute of Biomolecular and Biomedical Research
University College Dublin, Dublin 4 (Ireland)
Fax: (+353) 1-716-2127
E-mail: paul.v.murphy@ucd.ie

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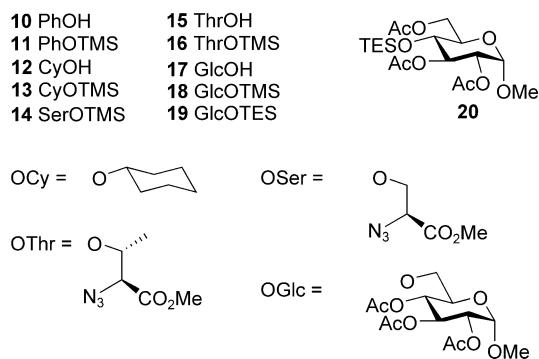
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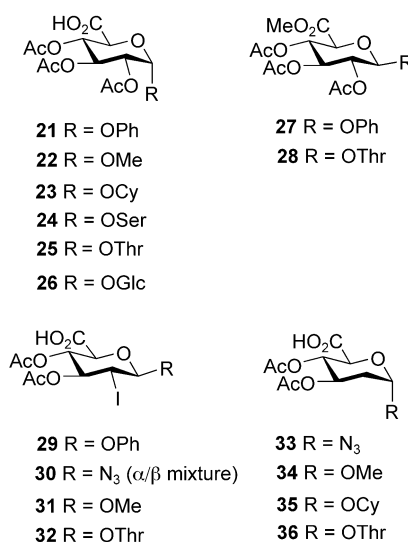
Scheme 1. Synthesis of glycosyl donors. Reagents and conditions: a) Ac_2O , I_2 ; b) SnCl_4 , CH_2Cl_2 ; 65% over two steps; c) H_2O ; d) $(\text{COCl})_2$, DMF; e) allyl alcohol, $\text{C}_5\text{H}_5\text{N}$; 55% over three steps; f) HBr , AcOH ; g) Zn dust, CuSO_4 , AcONa , 50% aq AcOH ; 50% over two steps; h) $[\text{Pd}(\text{PPh}_3)_4]$, pyrrolidine, MeCN , 0°C , 80%; i) NIS , MeCN , 50%; j) Bu_3SnH , AIBN , toluene, 110°C , 15 min, 90%. DMF = *N,N*-dimethylformamide, NIS = *N*-iodosuccinimide, AIBN = azobisisobutyronitrile.

and this was subsequently converted into **4** by treatment with zinc dust, copper(II) sulfate, and sodium acetate in aqueous acetic acid as described previously for the corresponding methyl ester.^[8] The allyl protecting group could be removed from **4**, to leave the glycol intact, by using $[\text{Pd}(\text{PPh}_3)_4]$ and pyrrolidine in acetonitrile to give **5**.^[9] The reaction of **5** with NIS gave the lactone **6**, which was subsequently reduced to give **7** by using $\text{Bu}_3\text{SnH}/\text{AIBN}$.

The results of the glycosidation of these donors using a tin(IV) chloride catalyst^[10] with the acceptors and products shown in Schemes 2 and 3, respectively, are listed in Table 1. Donor **2** gave α -glycosides in a highly stereoselective manner, despite the presence of the 2-*O*-acetyl group, in higher yields and conversions than the carboxylic acid **8**. This methodology was applied to the synthesis of glycosides derived from serine and threonine, with these amino acids being coupled as the azido derivatives (azido acids). An interesting observation was that the disaccharide **26** was obtained in higher yield with



Scheme 2. Acceptors used in the glycosidation reactions. TMS = trimethylsilyl, TES = triethylsilyl, Cy = cyclohexyl.

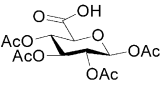
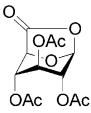
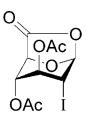
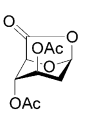
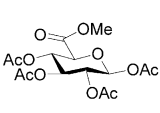


Scheme 3. Glycosidation products from the SnCl_4 -mediated reactions. The reactants and yields are listed in Table 1.

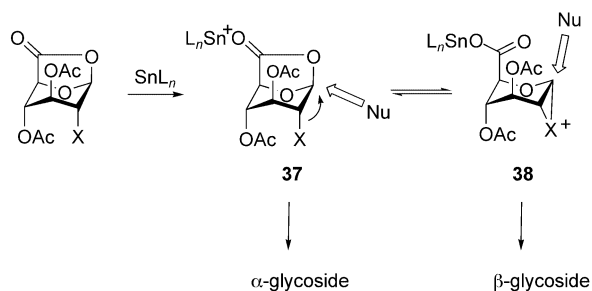
the TES acceptor **19** than with TMS acceptor **18**. The TES derivative **20** gave **26** as a result of acetate migration, presumably mediated by the catalyst, and subsequent glycosidation at the O6 position. The stereoselectivity observed for both **2** and **8** contrasted with that of the methyl ester **9**, which provided β -glucuronides in higher yields than were obtained with phenol or the alcohols. The 2-deoxyglycosyl donor **7** displayed the same behavior as **2** during the glycosidation, giving α anomers with high stereoselectivity, which contrasts the situation with 2-deoxy-2-iodo derivative **6**, which gave β -glycosides.

Possible mechanistic pathways for the glycosidation reactions of the lactones are shown in Scheme 4. Donors **2** and **7** undergo reaction so that inversion of configuration at the C1 position by an $\text{S}_\text{N}2$ process occurs via an intermediate of type **37**. A pathway involving **38** would be expected to provide the β -glycoside for iodide **6**, a fact indicating that the iodine residue is a better participant than the 2-*O*-acetyl group. The lactone **2** is generated in situ from carboxylic acid **8** in a step that accounts for the stereoselectivity observed with **8**; the lower yields obtained result from its lower reactivity because all of its substituents are oriented equatorially and destabilize positive-charge formation during activation of the 1-*O*-Ac group.^[11] The greater reactivity of the donor in the $^1\text{C}_4$ conformation is not unexpected and may be significant given the previously noted low reactivity of glucuronides.^[12] Another conceivable pathway involves **2** or **7** first giving a β -glycoside that rapidly isomerizes to the α anomer. However, although anomerization of β -glucuronic acid derivatives is observed in the presence of SnCl_4 , the rates appear too slow to account for this being the only pathway operating. The concept of using 1,6-anhydro- β -D-glucopyranosides and related compounds has been investigated previously for the synthesis of *O*-glycosides (MeOH as acceptor),^[13] thioglycosides,^[14] and *C*-glycosides;^[15] these reactions gave mixtures or in some cases the pure α - or β -glycoside, for example, a β -glycoside was obtained from a 2-

Table 1: SnCl₄-mediated glycosidation reactions.^[a]

Donor	Acceptor	Donor:acceptor:SnCl ₄	Product ^[b] (yield [%])
	11	1:2.5:0.5	21 α (35)
	13	1:2.5:0.5	23 α (32)
	10	1:2.5:0.5	21 α (64)
	11	1:2.5:0.5	21 α (88)
	TMSOMe	1:0.66:0.33 (10 h)	22 α (62)
	12	1:2.5:0.5	23 α (11)
	13	1:2.5:0.5	23 α (78)
	14	1:1.2:0.5	24 α (51)
	16	1:4:1 (18 h)	25 α (71)
	17	1:4:1 (6 h at RT, 12 h at 40 °C)	26 α (< 10)
	18	1:4:1 (6 h at RT, 12 h at 40 °C)	26 α (44)
	19	1:4:1 (6 h at RT, 12 h at 40 °C)	26 α (58)
	20	1:4:1 (6 h at RT, 12 h at 40 °C)	26 α (56)
	11	1:2.5:0.5	29 β (60)
	TMSN ₃	1:2.5:0.5	30 α (29); 30 β (47)
	TMSOMe	1:2.5:0.5 (5 h)	31 β (39)
	16	1:4:1	32 β (51)
	TMSN ₃	1:2.5:0.5	33 α (66)
	TMSOMe	1:0.66:0.33 (8 h)	34 α (59)
	13	1:2.5:0.5	35 α (57)
	16	1:4:1 (12 h)	36 α (41)
	10	1:2.5:0.5	27 β (66)
	11	1:2.5:0.5	27 β (92)
	15	1:4:1	28 β (< 5)
	16	1:4:1	28 β (75)

[a] All reactions were carried out in CH₂Cl₂ at RT for 15 h unless otherwise stated. [b] ¹H NMR spectroscopy analysis of the crude product was used to determine the ratio of α and β anomers; this was not subsequently altered by purification. When the second anomer is not reported it could not be detected.



Scheme 4. Possible mechanistic pathways to the α- and β-glycosidation products. L = ligand, Nu = nucleophile.

O-acetyl-1,6-anhydromaltose donor.^[16] However, the participation of 2-O-acetyl groups, which normally dominates glycoside synthesis, is not important for **2** in its reaction with a wide range of acceptors. This contrasts with the general behavior of glucuronic acid donors, which have ⁴C₁ ground-state conformations.

There are practical and strategic advantages to using such lactones as described herein. The carboxylic acid is formally protected in the donor and acts as the leaving group in the glycosidation reaction; its release simplifies purification as

the acid can be extracted into aqueous sodium hydrogen carbonate or can be separated from other by-products by solid-phase extraction. In addition, 2-deoxypyranuronic acids synthesized by this route should be more stable to acid than the corresponding 2-deoxypyranosides. There may be potential applications for the glycosyl azido acids as intermediates in glycoconjugate synthesis^[17] and in Staudinger reactions.^[18] It is now required that β-O-glucuronide drug conjugates, formed in animals and humans as part of detoxification, are tested before the parent drug can be approved and their synthesis is often not trivial;^[19] investigation of coupling reactions of the appropriate silyl ethers with donor **9**, similar to the two cases shown herein, may also be worthwhile. These possibilities as well as the potential of these compounds as building blocks for the synthesis of glycosaminoglycan disaccharides^[20] are currently being investigated.

Experimental Section

Glycosidation procedure: The glycosyl donor (0.4 mmol) was dissolved in dry CH₂Cl₂ (5 mL) in an N₂ atmosphere. SnCl₄ (Aldrich) and acceptor were then added, and the reaction mixture was left to stir (see Table 1 for reaction times).

The mixture was then diluted with dichloromethane (10 mL), then saturated aqueous NaHCO₃ solution (15 mL) was added and the resulting mixture was stirred for 30 min. The mixture was filtered through celite, the layers were then separated, and the aqueous layer was acidified with acetic acid. The product was extracted with EtOAc (up to 10 × 10 mL), dried (Na₂SO₄), and filtered. The solvent was then removed to give the glycosidic product. If the glycosidic product was still detected in the aqueous layer then the water was removed by freeze drying and the residue was purified by column chromatography (EtOAc/MeOH gradient elution).

Further experimental details can be found in the Supporting Information.

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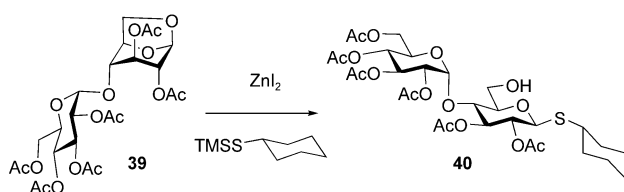
Keywords: carbohydrates · glucuronic acid · glycosides · glycosylation · stereoselective synthesis

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A Monomeric Organolithium Compound Containing a Free Pyramidal Carbanion in Solution and in the Solid State**

Frank Breher,* Jörg Grunenberg, Sally C. Lawrence, Philip Mountford, and Heinz Rüegger

Tris(pyrazolyl)hydroborate ligands are undoubtedly among the most important face-capping, six-electron N-donor ligands in coordination chemistry.^[1] Compared to these anionic species, the isoelectronic, neutral analogues $\text{HC}(\text{R}_x\text{pz})_3$ are considerably less well developed.^[2] However, interest in functionalizing the central methine carbon atom is growing, and such changes to the backbone can dramatically influence the solubility of these ligands and their metal complexes.^[3] Substitution of the methine hydrogen atom is generally accomplished by deprotonation and reaction with a suitable electrophile. Nevertheless, in most cases the interesting organolithium intermediates were not isolated and characterized but were treated in situ to release the desired products.^[3,4] Thus, little information is available on the constitution of such carbanion-containing species.^[5,6]

The type and degree of aggregation of organolithium compounds has stimulated extensive research,^[7] because lithium organyls are of fundamental interest and have important applications in many different fields ranging from synthetic chemistry to technical processes.^[8] In particular, the structural elucidation of unassociated anions is important because any effects due to anion/cation interactions are minimized.^[9] Carbanionic moieties attached to heterocycles or phenyl rings (e.g., $[\text{Ph}_2\text{pyC}]^-$,^[10] $[\text{py}_2\text{CH}]^-$,^[11] and $[\text{Ph}_3\text{C}]^-$ ^[12] with py = pyridyl) have been structurally characterized as their alkali metal compounds. Their main structural feature is the presence of a central *trigonal-planar* carbon atom due to delocalization of the negative charge in the adjacent rings. However, no unassociated alkyl lithium compound containing a “free” *pyramidal* carbanion has been structurally characterized so far. Here we show that a

[*] Dr. F. Breher, Dr. H. Rüegger
Department of Chemistry and Applied Biosciences (D-CHAB)
ETH Hönggerberg
Wolfgang-Pauli Strasse, 8093 Zürich (Switzerland)
Fax: (+41) 1-632-1032
E-mail: breher@inorg.chem.ethz.ch
Dr. J. Grunenberg
Institut für Organische Chemie
Technische Universität Braunschweig
Hagenring 30, 38106 Braunschweig (Germany)
S. C. Lawrence, Dr. P. Mountford
Inorganic Chemistry Laboratory
University of Oxford
South Parks Road, Oxford OX1 3TA (UK)

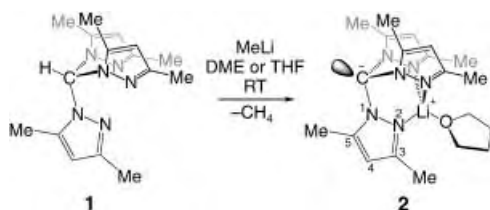
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suitable bridge, created by intramolecular chelation of the lithium cation, and lack of π delocalization yields the first monomeric lithium organyl compound containing a “free” (pseudo)tetrahedral carbanion.

Reaction of tris(3,5-dimethylpyrazolyl)methane, HC(3,5-Me₂pz)₃ (**1**), with methyllithium in DME or THF at ambient temperature afforded a dark red solution. Title compound **2** was isolated as pale red crystals in 87 % yield by crystallization from THF/*n*-hexane (Scheme 1). The crystal-structure



Scheme 1. Synthesis of **2**. DME = dimethoxyethane.

determination^[13] of **2** (Figure 1) verified the formation of a monomeric alkyl lithium compound in which the lithium cation is tetracoordinated by three pyrazole rings and one THF molecule. Compound **2** can formally be described as zwitterion containing a spatially separated anion (C1) and cation (Li1).^[14]

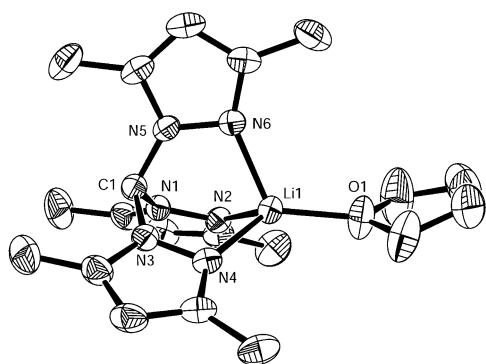


Figure 1. Molecular structure and numbering scheme for **2**; thermal ellipsoids are drawn at the 40 % probability level, and hydrogen atoms have been omitted for clarity. Selected bond lengths [pm] and angles [°] (corresponding values of free pyrazole are given in brackets; superscript integers represent the ring position according to Scheme 1): C1–N1 144.9(2), C1–N3 144.6(2), C1–N5 144.6(2), Li1–N2 202.9(3), Li1–N4 203.4(3), Li1–N6 200.6(3), Li1–O1 190.8(3), N1–N2 137.4(2), N3–N4 137.3(2), N5–N6 136.6(2); N1–C1–N3 109.2(1), N1–C1–N5 109.5(1), N3–C1–N5 109.7(1), O1–Li1–N2 121.9(2), O1–Li1–N4 126.6(1), O1–Li1–N6 118.2(2), N2–Li1–N4 94.7(1), N2–Li1–N6 92.1(1), N4–Li1–N6 95.4(1). Inner ring angles C–N–N at N¹: C11–N1–N2 110.2(1), C21–N3–N4 110.9(1), C31–N5–N6 110.1(1) [109.0]. Average bond lengths within the pyrazole rings: N¹–N² 137.1(2) [136.6], N²–C³ 132.6(2) [132.9], C³–C⁴ 138.7(3) [141.0], C⁴–C⁵ 136.4(3) [136.9], N¹–C⁵ 135.3(2) [135.7].

All N–C1–N angles (109.2–109.7°) are in agreement with a (pseudo)tetrahedral environment at the carbanionic center. The hydrogen atoms of the methyl substituents in the 5-position show no tendency to form nonclassical hydrogen

bonds (i.e., C–H^{δ+}...C^{δ−} contacts). The average bond lengths within the pyrazole rings, the inner ring angles (C–N–N at N¹), and the C1–N distances of about 144.7 pm clearly demonstrate the absence of charge delocalization into the pyrazole rings.^[15] These geometrical features give rise to a localized carbanionic center at C1. However, one would expect an intramolecular electrostatic interaction between the regions of negative and positive charge in **2**. The degree of interaction can be estimated from the C1...Li1 distance of 289 pm, which is approximately 70 pm longer than usually observed in lithium organyl compounds with a direct Li–C bond.^[16] The geometry at the lithium atom can be regarded as a stretched tetrahedron with narrow N–Li1–N angles (average 94.1°) and wide O1–Li1–N angles (average 122.2°). On the basis of equilibrium distance, the Li–N interactions appear to be stronger, as expected. The mean Li–N distance of 202.3 pm lies between those of typical Li–N donor bonds and those of lithium amides.^[10,11] Apparently, the intrinsic rigidity and the steric repulsion of the lone pair of electrons at C1 and the Me substituents in the 5-position inhibit an increase in electrostatic interactions and do not allow a shorter C1...Li1 distance.

Since atom–atom equilibrium distances are not always valid bond-strength descriptors,^[17] we carried out hybrid DFT (B3LYP/6-31 + G(d))^[18] calculations of the compliance constants in **2** to quantify the interatomic interaction strengths and possible crystal-packing effects. The gas-phase geometry was optimized by using the X-ray data as a starting point. Table 1 compares the calculated gas-phase equilibrium values

Table 1: Comparison of selected internal equilibrium coordinates of **2** in the gas phase (theoretical) and solid state (X-ray).^[a]

Bond ^[b]	Distances [pm]		Compliance constants [pm mdyn ^{−1}]
	X-ray	Theoretical	
Li1...C1	289.0	285.2	207.5
Li1–N	200.6–203.4	203.8–204.1	198.1
Li1–O1	190.8	198.3	243.0
C1–N	144.6–144.9	144.8–145.0	25.8
Free pyrazole			
N ¹ –N ²	137.1	138.2	21.9 (17.5)
N ² –C ³	132.6	133.1	13.6 (13.6)
C ³ –C ⁴	138.7	141.4	16.6 (17.3)
C ⁴ –C ⁵	136.4	138.4	14.4 (14.1)
N ¹ –C ⁵	135.3	136.3	15.6 (15.2)

[a] Theoretical (B3LYP/6-31 + G(d)) compliance constants are given as a measure for the interaction strengths (see text for details). [b] Superscript integers represent the ring position (see Scheme 1).

of important internal coordinates with our solid-state data, and points to a realistic description of the electronic structure of **2** by the B3LYP model.

Table 1 also lists the compliance constants for these coordinates.^[19] Since compliance constants measure the displacement of a specific coordinate due to a unit force acting on it, a higher value is associated with a weaker interaction. The large separation between C1 and Li1 leads to a weak (electrostatic) interaction, as measured by the compliance constant of 207.5 pm mdyn^{−1}.^[20] Nevertheless, the Li1–O1

compliance constant ($243.0 \text{ pm mdyn}^{-1}$) measuring the $\text{Li}\cdots\text{THF}$ interaction points to an even weaker interaction, which should result in a pronounced $\text{Li1}\cdots\text{O1}$ displacement on going from the theoretical gas-phase structure to the solid-state geometry. Indeed, the crystal-packing effects appear to be most severe for the $\text{Li1}\cdots\text{O1}$ distance ($\Delta d = 7.4 \text{ pm}$). This is interesting since Li–O contacts are normally regarded as strong interactions. Although the Li–N compliance constant of $198.1 \text{ pm mdyn}^{-1}$ alone points to a moderate bond strength, the synergism of three Li–N contacts leads to an effective encapsulation of the Li ion. Comparing the B3LYP/6-31 + G(d) compliance constants for free pyrazole with those of the pz rings in **2** reveals no major differences in bond strengths, with the exception of the N–N bonds. This bond weakening can be attributed to the increased coordination number at the nitrogen atom. Additionally, one might expect a release of electrons from the pyrazole rings to the N–Li bonds, which should give rise to short N–Li bonds, as observed in the X-ray crystal structure. Altogether, the results are in line with a “localized” methanide carbon atom (a graphical representation is given in the Supporting Information).

In view of the characteristics of **2** in the solid state, that is, a “free” tetrahedral carbanion in a monomeric compound without any significant intermolecular interactions, it seemed worthwhile to investigate its nature in solution. 1D ^1H and ^{13}C NMR spectroscopy showed the expected resonances of intact and equivalent pyrazolyl groups and a methanide carbon atom only slightly shifted to lower frequency, from 81.0 to 73.6 ppm with respect to **1**.^[21] For trigonal-planar carbanions, such signals are generally shifted to higher frequency; for example, the methanide C atoms of $[(\text{Me}_3\text{Si})\text{Ph}_2\text{C}]^-\text{[Li}(\text{tmeda})_2]^+$ ($\text{tmeda} = N,N,N',N'$ -tetramethylethylenediamine) and $(\text{Me}_3\text{Si})\text{Ph}_2\text{CH}$ resonate at 66.0 and 45.9 ppm, respectively.^[22] In contrast to many organolithium compounds, the methanide chemical shift did not show a large temperature dependence, and the absence of detectable coupling to lithium in the entire temperature range suggests weak Li–C interactions if at all. This unassociated character of the carbanion was substantiated by solid-state ^{13}C NMR spectroscopy, which, within experimental error, revealed identical values for the methanide chemical shift in the solid state and in solution. The fate of the cationic part was first addressed by ^7Li and ^1H NMR diffusion measurements, which revealed identical hydrodynamic radii for the cationic and anionic moieties. This result is consistent with either 1) a tight ion pair without direct Li–C interaction or 2) a zwitterionic structure as found in the solid state. These two alternatives were discriminated by recording a high-resolution $^{15}\text{N}, ^1\text{H}$ correlation spectrum (Figure 2), which for one of the two nitrogen atoms revealed a quartet fine structure due to an $I = 3/2$ nucleus (i.e., ^7Li) with a coupling constant of $^1J(^{15}\text{N}, ^7\text{Li}) = 10 \text{ Hz}$.

Furthermore, a $^{15}\text{N}, ^7\text{Li}$ correlation spectrum clearly demonstrated that the lithium cation in **2** is fixed in the N_3 pocket of the tris(pyrazolyl)methanide ligand, fully in agreement with its solid-state structure. Therefore, the title compound is stable towards inversion^[8c,d] due to the rigid encapsulation of the lithium ion, and owing to the absence of lithium–carbon contacts no “classical” aggregation is apparent.^[7]

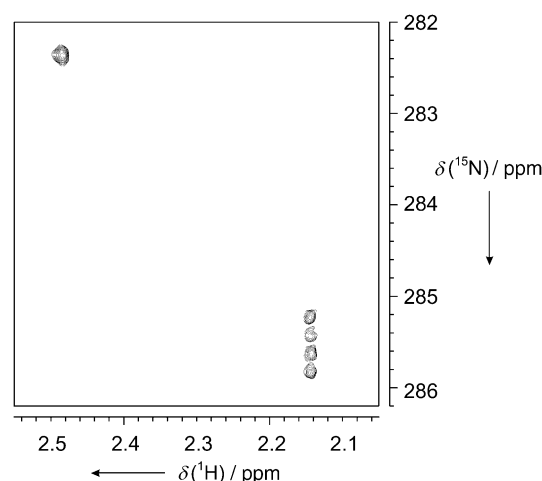


Figure 2. Section of the $^{15}\text{N}, ^1\text{H}$ HMQC spectrum of **2** showing the quartet multiplicity due to the spin of ^7Li .

Experimental Section

All procedures were carried out under argon. Solvents were freshly distilled from sodium/benzophenone (THF, DME) or from sodium/tetraglyme/benzophenone (hexane). Compound **1** was prepared according to literature methods.^[2] NMR spectra were measured on Bruker Avance spectrometers and chemical shifts are given relative to TMS (^1H , ^{13}C), $\text{Li}^+(\text{aq})$ (^7Li), and NH_3 (^{15}N).

2: Methylolithium (7.31 mL of a 1.6 M solution in diethyl ether) was added dropwise to a solution of tris(3,5-dimethylpyrazolyl)methane **1** (3.172 g, 10.63 mmol) in DME (50 mL) at room temperature. Towards the end of addition, a brown solid precipitated from the dark red solution. The reaction mixture was heated at ca. 40°C until the brown precipitate had dissolved. After cooling to room temperature the clear red solution was stirred for 2 h. All volatile components were removed under vacuum. The dark red, oily residue was dissolved in THF (20 mL), layered with *n*-hexane (40 mL), and cooled to -30°C . Pale red, moisture- and air-sensitive crystals of the product were isolated and dried in vacuo. A second crop of the product was isolated from the mother liquor after concentration of the solution and cooling to -30°C . Overall yield: 3.495 g (87%). M.p. (argon, sealed capillary) 184°C (decomp); elemental analysis (%): calcd for $\text{LiC}_{16}\text{H}_{21}\text{N}_6\cdot\text{C}_4\text{H}_8\text{O}$: C 63.1, N 22.7, H 8.1; found: C 63.8, N 22.3, H 7.8; ^1H NMR (500.2 MHz, $[\text{D}_8]\text{THF}$): $\delta = 5.55$ (s, 3H, CH_{pz}), 2.51 (s, 9H, Me), 2.16 ppm (s, 9H, Me); ^7Li NMR (194.4 MHz, $[\text{D}_8]\text{THF}$): $\delta = 9.9$ ppm; ^{13}C NMR (62.9 MHz, $\text{C}_6\text{D}_6/[\text{D}_8]\text{THF}$): $\delta = 144.7$, 144.6 (s, pz C_{quart}), 101.7 (s, pz CH), 73.6 (s, C1), 14.0 (s, Me), 12.5 ppm (s, Me); ^{15}N NMR (50.7 MHz, $[\text{D}_8]\text{THF}$): $\delta = 282.3$, 285.5 ($^1J(^{15}\text{N}, ^7\text{Li}) = 10 \text{ Hz}$); IR (solid, ATR): $\tilde{\nu} = 2920 \text{ m}$ ($\nu(\text{CH})$), 1554 s ($\nu(\text{pyrazole ring})$), 1455 m, 1413 s, 1371 m ($\delta(\text{CH}_2, \text{CH}_3)$), 1319 m, 1270 w, 1204 w, 1095 w ($\nu(\text{CC})$), $\delta(\text{CH}_2, \text{CH}_3)$), 1037 vs ($\nu(\text{COC})$), 971 w, 917 w, 897 m, 864 vs, 786 m, 766 vs, 723 s, 708 m, 691 m, 671 m, 655 cm^{-1} m ($\nu(\text{LiN})$, $\nu(\text{CC})$), $\delta(\text{CC})$); UV/Vis (THF): λ_{max} (lg ϵ) = 278 nm (3.05).

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Keywords: cage compounds · carbanions · lithium · N ligands · zwitterions

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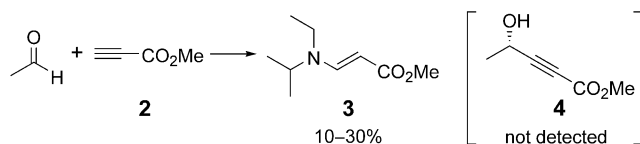
Alkynylation

A Mild Method for the Preparation of γ -Hydroxy- α,β -Acetylenic Esters**

Shatrughan P. Shahi and Kazunori Koide*

The alkynylation of a carbonyl compound is a powerful reaction for forming a carbon–carbon σ bond. Highly functionalized γ -hydroxy- α,β -acetylenic esters $\text{RCH}(\text{OH})\text{C}\equiv\text{C}-\text{CO}_2\text{Me}$ (**A**) are promising synthetic intermediates that can be used as precursors to a variety of complex molecules.^[1] Since the procedure was reported by Midland et al.,^[2] alcohols **A** have mainly been prepared by the treatment of aldehydes with the lithium acetylide $\text{Li}-\text{C}\equiv\text{C}-\text{CO}_2\text{Me}$ (**1**). The acetylide **1** can be generated in situ by the addition of *n*-butyllithium to methyl propiolate (**2**). Because *n*-butyllithium is itself a nucleophile toward aldehydes and ketones, the amount of base used must be controlled carefully. Furthermore, as a result of these strongly basic conditions access to **A** is mostly limited to base-stable compounds. As this widely used method requires a stringent balance in the stoichiometry of the reagents, one of which is moisture sensitive, it is often difficult to obtain satisfactory results. Therefore, a milder and more robust synthetic method with broader scope is needed for the preparation of alcohols **A**.

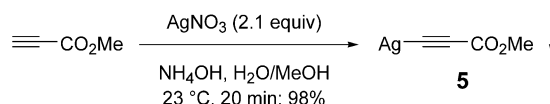
A recent development in the enantioselective addition of a terminal alkyne to an aldehyde, pioneered by Carreira and co-workers, involves the in situ generation of a zinc acetylide in the presence of a tertiary amine.^[3] However, this method did not enable us to couple acetaldehyde with **2** to form the corresponding methyl pentynoate **4** (Scheme 1). Instead, we



Scheme 1. Reagents and conditions: $\text{Zn}(\text{OTf})_2$ (1.0 equiv), $i\text{Pr}_2\text{NEt}$ (1.05 equiv), (–)-*N*-methylephedrine (1.05 equiv), CH_2Cl_2 , 0 $\rightarrow$ 23 $^\circ\text{C}$. Tf = trifluoromethanesulfonyl.

isolated compound **3** as the major product. We attributed this undesired reaction to the facile conjugate addition of the tertiary amine to **2**, followed by loss of one *i*Pr group. These results prompted us to develop alternative reaction conditions under which an aldehyde would undergo condensation with **2** in the absence of a base.

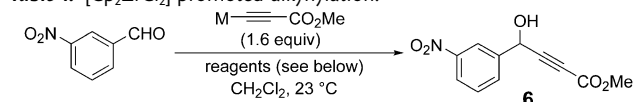
The preparation of metal acetylides $\text{M}-\text{C}\equiv\text{C}-\text{R}$ ($\text{M} = \text{Li}$, Sn , Si , B) often involves the use of a strong base. In contrast, the silver acetylide **5**^[4] can be readily prepared in nearly quantitative yield by a known mild protocol on an ~ 8 -g scale, and this solid material can be stored at ambient temperature in a vial for many months in the dark without any precaution to avoid moisture (Scheme 2).^[5]



Scheme 2. Preparation of the silver acetylide **5**.

Various silver acetylides are known to react with acid chlorides to generate alkyl 4-oxo-2-alkynoates.^[6] However, in the absence of an additive, compound **5** failed to react with 3-nitrobenzaldehyde to afford the alcohol **6** (Table 1, entry 1).

Table 1: $[\text{Cp}_2\text{ZrCl}_2]$ -promoted alkynylation.



Entry	M	Reagents	<i>t</i> [h]	Yield [%] ^[a]
1	Ag	none	30	0
2	Ag	$[\text{Cp}_2\text{ZrCl}_2]$ (1.2 equiv)	30	73
3	Ag	$[\text{Cp}_2\text{ZrCl}_2]$ (1.2 equiv) + AgOTf (0.2 equiv)	5	95
4	H	$[\text{Cp}_2\text{ZrCl}_2]$ (1.2 equiv)	30	0

[a] Yield of isolated product.

After screening several additives, we found that the desired alcohol **6** was produced in 73 % yield after 30 h at 23 $^\circ\text{C}$ in the presence of $[\text{Cp}_2\text{ZrCl}_2]$ (Table 1, entry 2). Although the use of ZnBr_2 , $[\text{Cp}_2\text{TiCl}_2]$, and ZrCl_4 led to the alcohol **6** in 4, ~ 30 , and 51 % yield, respectively, neither $\text{BF}_3\cdot\text{OEt}_2$ nor $\text{Cu}(\text{OTf})_2$ promoted the condensation reaction. Based on these preliminary studies, this alkynylation appears to be facilitated by zirconium reagents. Of the solvents that we employed for the coupling reaction (CH_2Cl_2 , THF, MeCN), the use of CH_2Cl_2 gave the highest yield.

Although we have not characterized any intermediates in the coupling reaction, we hypothesized that $[\text{Cp}_2(\text{Cl})\text{Zr}-\text{C}\equiv\text{C}-\text{CO}_2\text{Me}]$ might be the reactive species.^[7] The observations by Jordan et al. and Suzuki and co-workers that silver salts facilitate the coupling between $[\text{Cp}_2(\text{Cl})\text{Zr}-\text{R}]$ ($\text{R} = \text{CH}_3$ and $\text{R} = \text{alkenyl}$, respectively) and an aldehyde or a ketone prompted us to examine whether our $[\text{Cp}_2\text{ZrCl}_2]$ -promoted coupling could be further accelerated by AgOTf .^[8] Thus, AgOTf (0.2 equiv) was used together with $[\text{Cp}_2\text{ZrCl}_2]$ (1.2 equiv). This modification accelerated the coupling reac-

[*] Dr. S. P. Shahi, Prof. Dr. K. Koide
Department of Chemistry, University of Pittsburgh
219 Parkman Avenue, Pittsburgh, PA 15260 (USA)
Fax: (+1) 412-624-8611
E-mail: koide@pitt.edu

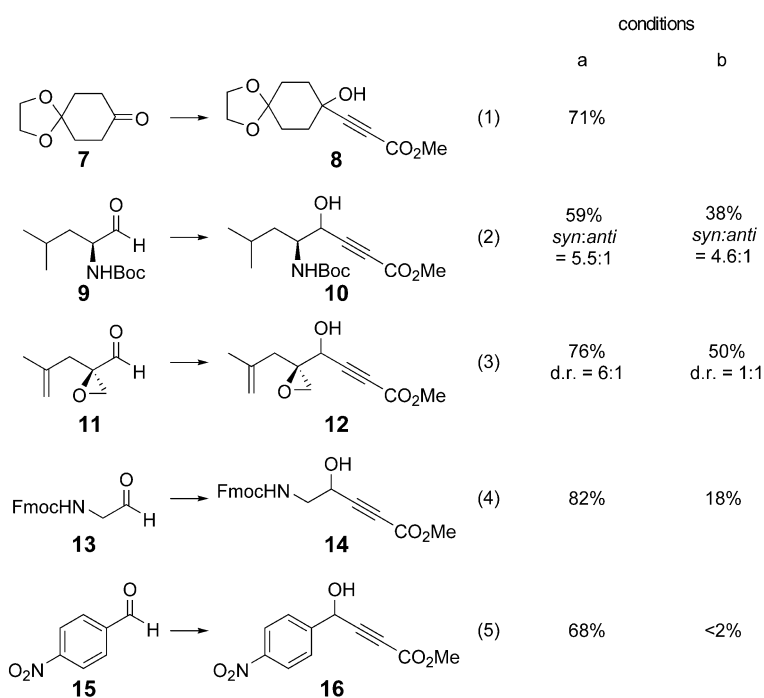
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tion dramatically; the reaction was complete after 5 h (95 % yield) rather than the 30 h required in the absence of AgOTf (Table 1, entry 3). The necessity of the silver acetylide **5** was demonstrated by using **2** in the presence of [Cp₂ZrCl₂], which did not produce the alcohol **6** (Table 1, entry 4).

We then screened a variety of aldehydes to test the scope of the zirconium-promoted alkynylation reaction (Table 2). Both electron-deficient and electron-rich aromatic aldehydes reacted with **5** to form the corresponding alcohols in 76–95 % yield (Table 2, entries 1–3). Cinnamaldehyde reacted in a 1,2-addition fashion to form the corresponding alcohol in 93 % yield (Table 2, entry 4). The reactions of aliphatic aldehydes, including easily enolizable phenylacetaldehyde, also afforded the corresponding alcohols in 53–78 % yield (Table 2, entries 5–8).

This method could be used to couple **5** with the ketone **7**, even in the presence of the cyclic ketal, to afford the corresponding alcohol **8** [Eq. (1), Scheme 3]. To test 1,2-asymmetric induction in this alkynylation method, we prepared the aldehyde **9** according to the published protocol.^[9] We chose this aldehyde because the stereoselectivity in the addition of **1** is known (38 % yield, *syn:anti* = 4.6:1).^[10] The aldehyde **9** was converted into the alcohol **10** under our conditions in 59 % yield with a *syn:anti* ratio of 5.5:1 [Eq. (2), Scheme 3]. This result shows the higher efficiency and comparable degree of 1,2-asymmetric induction of our alkynylation method at room



Scheme 3. Conditions: a) **5** (1.6 equiv), [Cp₂ZrCl₂] (1.2 equiv), AgOTf (0.2 equiv), CH₂Cl₂, 23 °C, 2–7 h; b) **2** (1.6–2 equiv), *n*BuLi or LiN(TMS)₂ (1.4–1.6 equiv), –78 °C, 1 to 30 min. Boc = *tert*-butoxycarbonyl, Fmoc = 9-fluorenylmethoxycarbonyl, TMS = trimethylsilyl.

Table 2: [Cp₂ZrCl₂]/AgOTf-promoted alkynylation of aldehydes.

$\begin{array}{c} \text{RCHO} \\ (1.0 \text{ equiv}) \\ + \\ \text{5} \\ (1.6 \text{ equiv}) \end{array} \xrightarrow[23^\circ\text{C, CH}_2\text{Cl}_2]{\begin{array}{c} [\text{Cp}_2\text{ZrCl}_2] (1.2 \text{ equiv}) \\ \text{AgOTf} (0.2 \text{ equiv}) \end{array}} \begin{array}{c} \text{OH} \\ \\ \text{R}-\text{C}\equiv\text{C}-\text{CO}_2\text{Me} \end{array}$			
Entry	RCHO	<i>t</i> [h]	Yield [%] ^[a]
1		5	95
2		3	76
3	PhCHO	10	84
4		2.5	93
5	CH ₃ CHO	4	53
6		1	78
7	PhCH ₂ CHO	3.5	61
8		4	78

[a] Yield of isolated product.

temperature relative to a widely-used alkynylation method with **1** at –78 °C. A spiroepoxide was also found to be compatible with this Zr-promoted alkynylation. Thus, the alcohol **12** was produced in 77 % yield with a diastereomeric ratio of 6:1 from the aldehyde **11** [Eq. (3), Scheme 3; relative configuration not determined]. In contrast, the treatment of **11** with **1** gave the alcohol **12** in 50 % yield with a diastereomeric ratio of 1:1. The base-sensitive *N*-Fmoc-protected aldehyde **13** was subjected to the same reaction conditions [Eq. (4), Scheme 3]. The corresponding propargylic alcohol **14** was isolated in 82 % yield, whereas the addition of **1** to the same aldehyde afforded the desired alcohol in only 18 % yield. The Zr-promoted alkynylation was also effective for the preparation of the base-sensitive compound **16** from **15** in 68 % yield [Eq. (5), Scheme 3]. In contrast, the coupling of **15** with **1** generated **16** in less than 2 % yield. These examples indicate that the Zr-promoted alkynylation method can be used for substrates that can not be prepared under conventional conditions. The results shown in Equations (2) and (4) also demonstrate that this reaction is compatible with the relatively acidic carbamate protons -NHCO₂R. Although the same products were formed in all these reactions whether AgOTf was present or not, we found that AgOTf accelerated the reactions and led to the formation of the products in higher yields. The quantity of AgOTf used could be reduced to 5 mol % without a decrease in yield (data not shown).

In summary, we have developed a novel method for the coupling of aldehydes or ketones with alkyl propiolates in the absence of a base. Because the reagents involved in this

coupling reaction are easier to handle than *n*-butyllithium and **1**, these reaction conditions can be employed on a small scale (~0.5 mmol) or on a 6 mmol scale without a decrease in yield. We have also demonstrated that various functional groups are compatible with these alkynylation conditions. Additionally, **5**, [Cp₂ZrCl₂], and AgOTf can be stored at room temperature without paying particular attention to the exclusion of moisture. The synthetic use of silver acetylides has been limited until now to only a few reaction types.^[11] This study suggests that the formation of silver acetylides of electron-deficient alkynes is a powerful method for the conversion of non-nucleophilic alkynes into reactive species toward aldehydes and ketones. Studies into the scope and limitation (note: the silver acetylide of the acetylenic ketone Ag-C≡C-C(=O)CH₃ was found to be shock-sensitive) of these reaction conditions with other electrophiles and nucleophiles are in progress in our laboratory.

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Size-Selective Olefin Hydrogenation by a Pd Nanocluster Provided in an Apo-Ferritin Cage**

Takafumi Ueno,* Masako Suzuki, Toshiaki Goto,
Tomoharu Matsumoto, Kuniaki Nagayama, and
Yoshihito Watanabe*

There has been much interest lately in chemical transformations in single capsules of supramolecular assemblies.^[1–4] Well-designed capsule structures could provide increased concentration of substrates in the cavities with high selectivity to allow transformation of the substrates. Proteins are very attractive building components for the construction of self-assembled cage structures.^[1,5–12] For example, Mann and co-workers have reported in their pioneering works the preparation of size-restricted metal oxides and sulfides by using the biosupramolecular cage of ferritin.^[5–7] In addition, protein assemblies of viruses and the chaperonin protein GroEL were also utilized for the encapsulation of metal nanoclusters.^[10–12] However, difficulties still remain in controlling reactions catalyzed by metal clusters in protein cages.

Ferritin is known as an iron-storage protein and comprises 24 subunits that assemble to form a hollow cagelike structure of 12 nm in diameter as shown in Figure 1. Iron atoms are stored as a cluster of ferric oxyhydroxide within a cavity of diameter 8 nm formed by the protein subunits (Figure 1 b).^[13,14] One of the most important consequences of this protein cage is the perforation of the protein shell by small channels that locate at the junctions of the subunits (Figure 1 c and d). The channels are required for the transport of several metal ions and other organic molecules.^[15] In addition, penetration studies of organic molecules have

[*] Dr. T. Ueno
Research Center for Materials Science
Nagoya University
Nagoya 464-8602 (Japan)
Fax: (+81) 52-789-2953
E-mail: taka@mbox.chem.nagoya-u.ac.jp

M. Suzuki, Prof. Dr. Y. Watanabe
Department of Chemistry
Graduate School of Science
Nagoya University
Nagoya 464-8602 (Japan)
Fax: (+81) 52-789-2953
E-mail: yoshi@nucc.cc.nagoya-u.ac.jp

T. Goto
Department of Physics
Graduate School of Science
Nagoya University
Nagoya 464-8602 (Japan)
Dr. T. Matsumoto, Prof. Dr. K. Nagayama
National Institute for Physiological Science
Okazaki 444-8585 (Japan)

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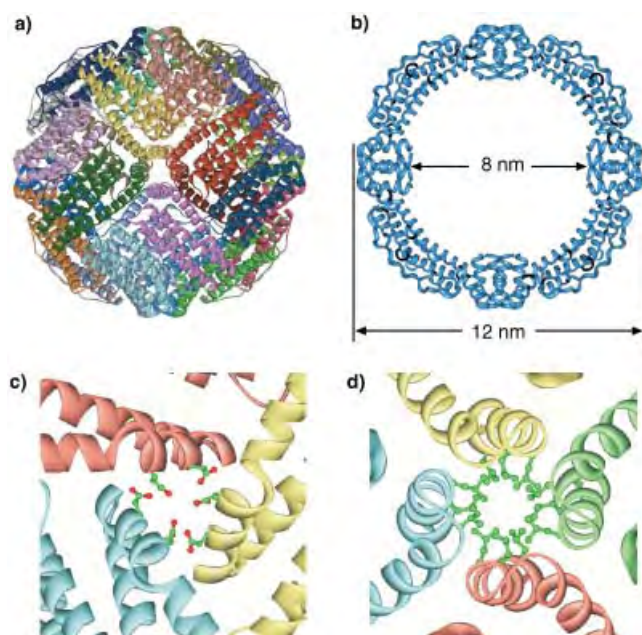
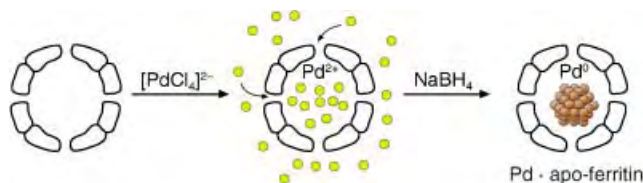


Figure 1. Schematic representation of horse-spleen ferritin: a) the complete 24-meric assembly; b) the inner cavity; c) threefold axis hydrophilic channel; d) fourfold axis hydrophobic channel taken from PDB code: 1IER.^[14]

confirmed the size-selective insertion of them into the holo-ferritin interior through the channels.^[16,17] These results could imply that holo-ferritin could accommodate organic foreign compounds in its cavity even in the presence of metal clusters. Herein we describe a novel strategy for the construction of a size-selective hydrogenation biocatalyst: a Pd nanocluster encapsulated in the apo-ferritin cavity. The Pd cluster is synthesized by *in situ* chemical reduction of Pd^{II} ions concentrated in the interior of apo-ferritin (Scheme 1) and the size selectivity of the olefin hydrogenation is given by the penetration of substrates through the channels.



Scheme 1. Preparation of Pd-apo-ferritin.

Apo-ferritin is treated with 500 equivalents of K₂PdCl₄ to afford a homogeneous pale yellow aqueous solution at pH 8.5 adjusted by 0.01N NaOH (aq). The Pd^{II} ions are reduced by 5 equivalents of NaBH₄ to yield zero-valent Pd clusters (Figure 2a). According to the reduction, the solution spectrum has been changed significantly; there is a broad intense absorption at a longer wavelength (Figure 2a). A similar spectroscopic change was observed for the reduction of Pd^{II} ions in dendrimers.^[18] Under the same conditions, black precipitates were observed in a protein-free control experiment, whereas the solution containing apo-ferritin remained a

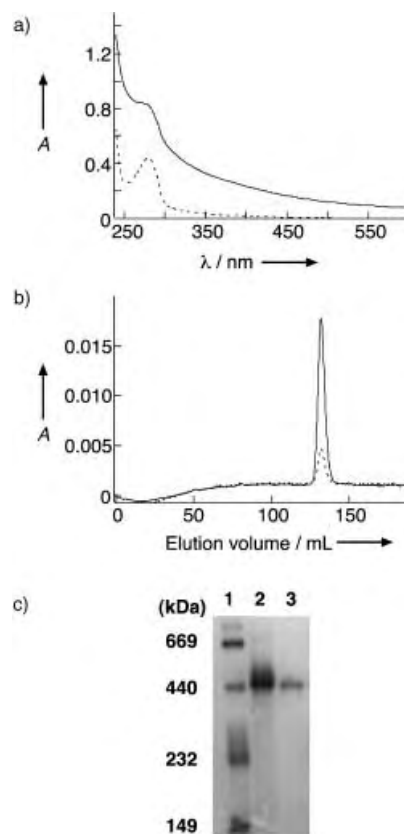


Figure 2. a) UV/Vis spectra of Pd-apo-ferritin (solid line), and apo-ferritin (dashed line); b) size-exclusion chromatography elution profile of Pd-apo-ferritin monitored at 280 nm (solid line) and 400 nm (broken line); c) native PAGE of marker (lane 1), Pd-apo-ferritin (lane 2), apo-ferritin (lane 3). The gel (7.5%) was stained by Coomassie brilliant blue.

clear brown solution. These results suggest specific formation of Pd clusters within the protein cavities. Finally, the Pd-apo-ferritin composite was isolated by size-exclusion chromatography (Superdex G-200). The product elution was monitored either at 280 nm (protein) or at 400 nm (zero-valent Pd cluster), and the coelution of the protein and metallic components through the column is the clear indication of the composite nature of the material (Figure 2b). In addition, a polyacrylamide gel electrophoresis (PAGE) experiment with the Pd-apo-ferritin and apo-ferritin under native conditions also indicates that the protein cage remains unchanged during the reduction as shown in Figure 2c.

The composite materials in aqueous solution were examined by transmission electron microscopy (TEM). The TEM images clearly show that 1) the metal clusters are almost monodispersed particles, 2) their shape is roughly spherical (Figure 3a), and 3) an intact protein shell surrounds the metallic core (Figure 3b). The diameter of the metal particles of Pd-apo-ferritin is 2.0 ± 0.3 nm as shown in the histogram in Figure 3a. We have also confirmed by electron-energy-loss spectroscopy that the metal core contains only palladium but not iron (data not shown).

The catalytic hydrogenation of olefins by Pd-apo-ferritin was evaluated in aqueous medium. The turnover frequencies

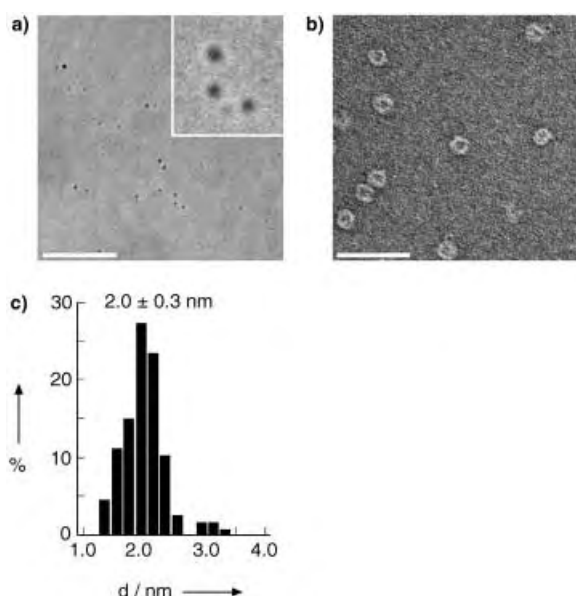


Figure 3. TEM images of the Pd-apo-ferritin: a) An ice-embedded unstained sample and 4× image magnification (inset); b) a sample negatively stained with uranyl acetate; c) histogram of the cluster size. The temperature of the specimen stage was 77 K. The electron microscopes were operated at acceleration potentials of 200 kV for (a) and 300 kV for (b). Scale bars are 50 nm.

(TOF = [product (mol)] per atom of Pd per hour) of hydrogenation for acrylamide derivatives are listed in Table 1. As estimated by atomic-absorption and protein quantitative

Table 1: Hydrogenation activity of Pd-apo-ferritin in water.

Olefins	TOF for Pd-apo-ferritin ^{[a],[b]}	TOF for Pd particles ^{[b],[c]}
CH ₂ =CHCONH ₂ (1)	72 ± 0.7	58 ± 5.9
CH ₂ =CHCOOH (2)	6.3 ± 1.1	12 ± 2.6
CH ₂ =CHCONH- <i>i</i> Pr (3)	51 ± 6.5	15 ± 0.3
CH ₂ =CHCONH- <i>t</i> Bu (4)	31 ± 5.9	6.1 ± 0.6
CH ₂ =CHCO-Gly-OMe (5)	6.3 ± 3.8	28 ± 2.6
CH ₂ =CHCO-D,L-Ala-OMe (6)	Not detected	23 ± 0.3

[a] Hydrogenation reactions catalyzed by Pd-apo-ferritin were carried out at 7 °C (pH 7.5) with 30 μM of Pd. [b] TOFs were calculated on the basis of the ratio of the product to substrate by analyzing the ¹H NMR spectra. [c] The same conditions were used except without apo-ferritin.

analyses each apo-ferritin contains 460 atoms of Pd⁰, thus, the TOF of 72 for **1** implies a TOF of 33000 per Pd-apo-ferritin molecule. The products of these hydrogenation reactions were confirmed by NMR spectroscopy in D₂O solutions. According to the reduction of each substrate by H₂ in the presence of Pd-apo-ferritin, the signals in the region of vinylic protons (5–6 ppm) decreased and concomitant appearance of new signals assignable to the hydrogenation of the C=C double bond in the aliphatic region (1–2 ppm) were observed. These results indicate that the hydrogenation by Pd-apo-ferritin proceeds without forming any other products. After the catalytic reactions, the solution of Pd-apo-ferritin remained clear and the native PAGE results showed no

aggregation of the composites during the reactions. Thus, Pd-apo-ferritin is stable before, during, and after the catalytic reaction. Pd-apo-ferritin also catalyzes the hydrogenation at reasonable rates, for example, the TOF of Pd-apo-ferritin for the *N*-isopropyl acrylamide reduction is almost comparable to that of a dendrimer-encapsulated Pd catalyst in water.^[18] On the other hand, the TOF for acrylic acid (**2**) catalyzed by Pd-apo-ferritin was eight times smaller than that of **3** though TOF of **2** in the absence of apo-ferritin is almost identical to that of **3** (Table 1). When the threefold channels in Pd-apo-ferritin (Figure 1c) were blocked with Tb^{III},^[17,19,20] the TOF of **1** was decreased to 7% of the TOF of **1** by Pd-apo-ferritin without Tb^{III}. Previous diffusion studies have also shown that 4-amino-TEMPO (TEMPO is 2,2,6,6-tetramethyl piperidine-*N*-oxyl) could penetrate through the channels.^[17] These results, therefore, suggest that the anionic threefold hydrophilic channels (Figure 1c) are the pathway for substrates. In comparison with neutral substrates, the TOFs of **5** and **6** by Pd-apo-ferritin are smaller than those of **1**, **3**, and **4** although the electron-deficient alkenes **1**, **5**, and **6** are expected to be reduced faster than **3** and **4** if the electronic substituent effect on the hydrogenation is considered. Actually, the order of TOFs by Pd particle without apo-ferritin was **1** > **5** > **6** > **3** > **4** (Table 1). Molecular-modeling calculations of the substrates indicate that the volumes of the substrates are 54, 97, 110, 106, and 120 Å³ for **1**, **3**, **4**, **5**, and **6** respectively.^[21] The order of the substrate size roughly correlates to the experimental data for the hydrogenation by Pd-apo-ferritin (Table 1). Thus, the substrate size is discriminated by the threefold channels. While we have attempted chiral discrimination in the hydrogenation of racemic **6** at 15 °C, no enantioselectivity in the product was observed.

In conclusion, we have demonstrated in situ synthesis of a Pd nanocluster in the protein cage and size-selective olefin hydrogenation by the Pd-apo-ferritin. The results provide us with a method for the preparation of metal nanoclusters in restricted interiors of a protein assembly. Further design of the ferritin cage and channels to improve the reactivity is currently under progress.

Experimental Section

Horse spleen ferritin was obtained as an 85 mgmL⁻¹ 0.15 M NaCl solution (Sigma). Substrates **4** and **5** were synthesized as reported.^[22]

Preparation of Pd-apo-ferritin: Horse spleen ferritin was demineralized with thioglycolic acid to prepare apo-ferritin by a reported procedure.^[23] The protein concentration was determined by using the reported extinction coefficient at 280 nm.^[24] An apo-ferritin solution (9.3 mg, 0.02 μmol in 0.15 M NaCl/H₂O) was adjusted to pH 8.5 with 0.01 N NaOH, and aliquots of K₂PdCl₄ (40 mM, 250 μL in H₂O) were added and this solution was stirred for 30 min at room temperature. To the resulting solution, a solution of NaBH₄ (50 μmol in H₂O) was added and the mixture was stirred for 30 min at room temperature. The reaction mixture was dialyzed against 0.15 M NaCl/H₂O for 12 h. The protein was isolated by gel chromatography (Superdex 200, eluent: 0.15 M NaCl aq) and the purity was checked by native PAGE. The proportion of Pd atoms to protein was determined by atomic absorption analysis and Bicinchoninate (BCA) method.

Hydrogenation reactions: Hydrogenation reactions were carried out in a 10 mL reaction tube sealed with a septum. A D₂O solution

containing the substrate (6 μmol , 1 mL) was added to the reaction tube and purged with H_2 gas for 15 min. The catalytic reaction was initiated by adding a Pd-apo-ferritin solution (1×10^{-4} μmol , 1 mL) and stirred for 1 h under an H_2 atmosphere with a flow rate of 50 mL min^{-1} at 7°C . After the removal of Pd-apo-ferritin from the resulting solution, the products were identified by ^1H NMR spectroscopy. Pd particles for the blank experiments (Table 1) were prepared by the reaction of K_2PdCl_4 and NaBH_4 in D_2O . The mixtures were used for the hydrogenation reactions without further purification. The enantiomeric excess for the reaction of **6** at 15°C was determined by HPLC analysis by using a DAICEL CHIRALCEL OD column ($0.46 \text{ cm} \times 25 \text{ cm}$) at a flow rate of 0.5 mL min^{-1} (isopropanol/hexane = 5:95).

Transmission electron microscopy: A solution of Pd-apo-ferritin (2 μL) was applied to a copper grid covered with a thin amorphous carbon film, and the excess solution was removed with a filter paper. For the cryo observation, a sample grid was rapidly plunged into liquid propane (-190°C). The images of the ice-embedded and the negatively stained samples were recorded on a JEM-2010HC microscope (200 kV) equipped with a CT-3500 cryoholder and a JEM-4010N microscope (300 kV), respectively.

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Supramolecular Chemistry

Reaction-Temperature-Dependent Supramolecular Isomerism of Coordination Networks Based on the Organometallic Building Block $[\text{Cu}^{\text{I}}_2(\mu_2\text{-BQ})(\mu_2\text{-OAc})_2]$ **

*Shigeyuki Masaoka, Daisuke Tanaka,
Yasunori Nakanishi, and Susumu Kitagawa**

Much effort has been devoted to the design and controlled crystallization of coordination networks based on transition-metal atoms and multifunctional bridging ligands owing to their potential applications such as magnetism, electric conductivity, molecular adsorption, and heterogeneous catalysis. Supramolecular isomerism, the existence of more than one superstructure for given set of components, is of particular importance because the superstructure plays an essential role in determining the properties of crystalline materials.^[1] All phenomena regarding supramolecular isomerism in coordination networks reported so far show crystal structures that contain solvent (guest) molecules to satisfy crystal packing, which result from different stoichiometry of the included solvent.^[2] Therefore, these are categorized as a group of solvent-induced (guest-induced) pseudo-polymorphism. In spite of many investigations, no examples have been reported of supramolecular isomerism with a fixed stoichiometry for all components, which would lie at the very heart of the concept of supramolecular isomerism. Herein we

[*] S. Masaoka, D. Tanaka, Y. Nakanishi, Prof. S. Kitagawa
Department of Synthetic Chemistry and Biological Chemistry
Graduate School of Engineering
Kyoto University
Katsura, Nishikyo-ku, Kyoto 615-8510 (Japan)
Fax: (+81) 75-383-2732
E-mail: kitagawa@sbchem.kyoto-u.ac.jp

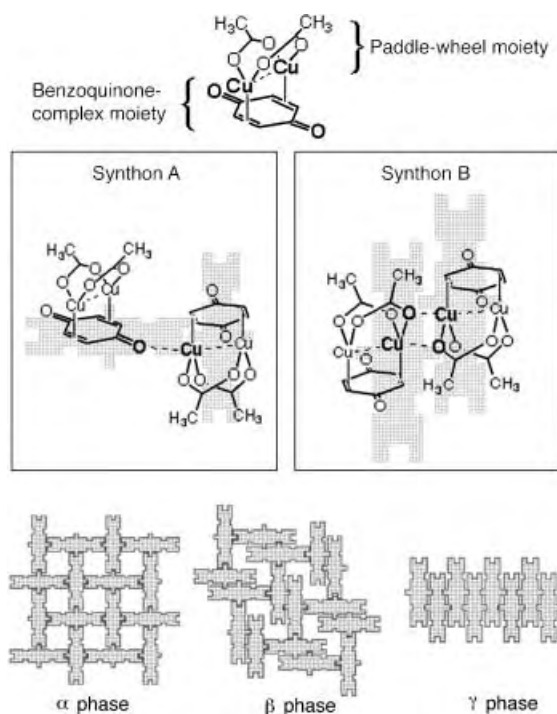
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report three supramolecular isomers without any solvent molecules. Interestingly, these three isomers were crystallized in the same solvent. This result is expected to afford important information for modern crystal engineering.

$[\text{Cu}^{\text{I}}_2(\mu_2\text{-BQ})(\mu_2\text{-OAc})_2]$ (BQ = *p*-benzoquinone) was obtained by the redox reaction between dimetallic copper(II) acetate, $[\text{Cu}^{\text{II}}_2(\text{OAc})_4(\text{H}_2\text{O})_2]$, as oxidant, and hydroquinone (HQ) as reductant.^[3] The use of this organometallic building block is a key to the formation of supramolecular isomers because this complex has two types of supramolecular synthons on coordination (Scheme 1).^[4] Because the building



Scheme 1. Top: two types of supramolecular synthon on coordination between two dicopper building blocks. Bottom: Schematic representation of three supramolecular isomers.

block has two oxygen atoms from the benzoquinone moiety, the lone pairs of the oxygen atoms can coordinate metal ions (synthon A in Scheme 1).^[5] On the other hand, a half of the complex looks like a paddle-wheel structure, and functions as a molecular building block with a mutual metal–oxygen coordinating group (synthon B in Scheme 1). Therefore, the infinite assembly of this organometallic building block could afford a variety of supramolecular isomers owing to competition between the two different intermolecular interactions.

The redox reaction of dimetallic copper(II) acetate, $[\text{Cu}^{\text{II}}_2(\text{OAc})_4(\text{H}_2\text{O})_2]$ (2 g) with hydroquinone (HQ; 2.2 g) in ethanol (100 mL) affords a red solution of the molecular building block $[\text{Cu}^{\text{I}}_2(\mu_2\text{-BQ})(\mu_2\text{-OAc})_2]$. When the solution was left at room temperature, two supramolecular isomers, $[\text{Cu}^{\text{I}}_2(\mu_4\text{-BQ})(\mu_2\text{-OAc})_2]_n$ (α phase) and $[\text{Cu}^{\text{I}}_2(\mu_3\text{-BQ})(\mu_2\text{-OAc})_2]_n$ (β phase), were obtained as thin and thick plates, respectively (Figure 1 a and Figure 2 a). On the other hand, when the reaction temperature was fixed at 60 °C, red

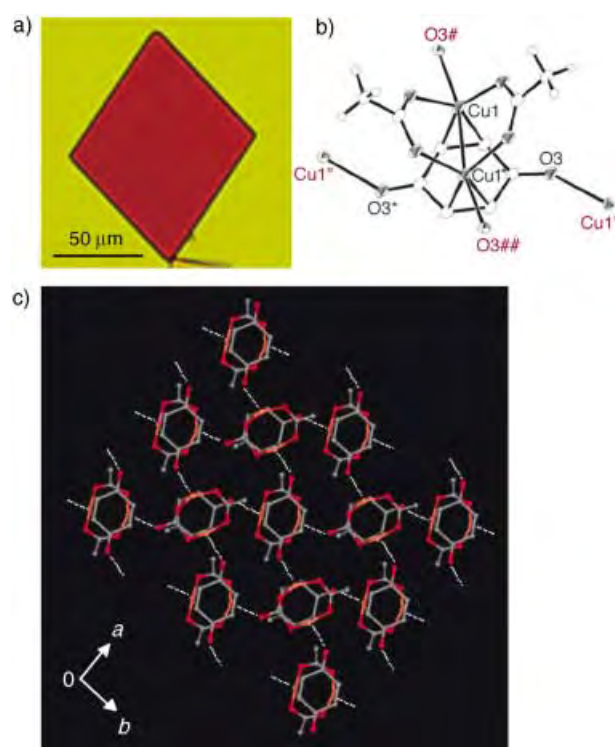


Figure 1. a) Photograph of a single crystal of the α phase. b) Structure of the building block with the nearest-neighbor coordinated atoms in the α phase. The distance of the Cu1–O3# bond is 2.270(3) Å. c) Infinite structure of the two-dimensional sheet of the α phase.

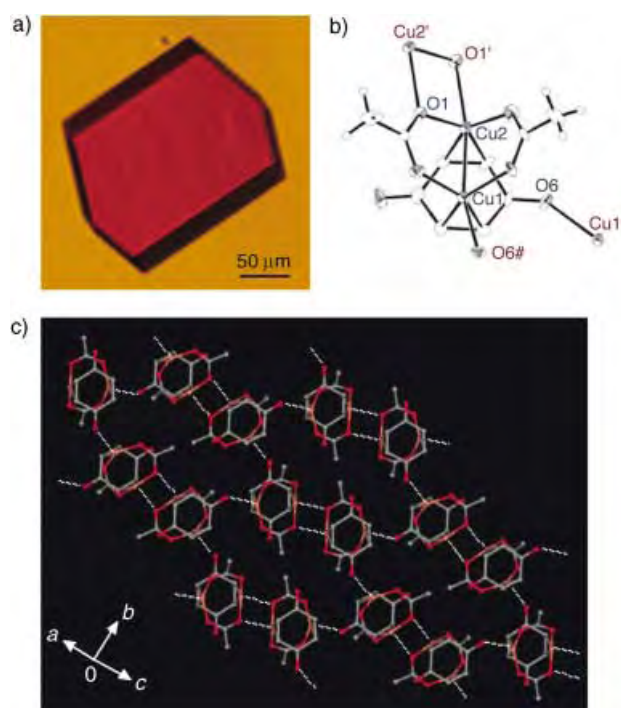


Figure 2. a) Photograph of a single crystal of the β phase. b) Structure of the building block with the nearest-neighbor coordinated atoms in the β phase. The distances of the Cu1–O6# bond and the Cu2–O1' bond are 2.254(3) and 2.278(2) Å, respectively. c) Infinite structure of the two-dimensional sheet of the β phase.

columns of $[\text{Cu}^{\text{I}}_2(\mu_2\text{-BQ})(\mu_3\text{-OAc})_2]_n$ (γ phase) were mainly obtained with red plates of β phase as a minor product (Figure 3a). All compounds were characterized by X-ray crystallography.^[6]

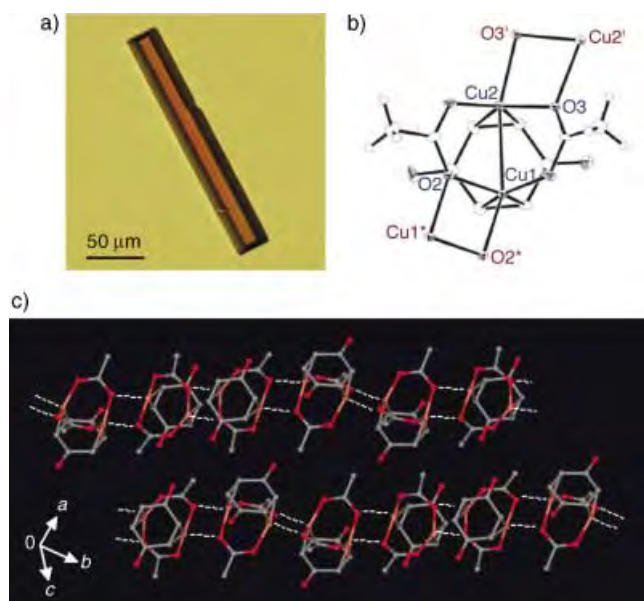


Figure 3. a) Photograph of a single crystal of the γ phase. b) Structure of the building block with the nearest-neighbor coordinated atoms in the γ phase. The distances of the Cu1–O2'' bond and the Cu2–O3' bond are 2.233(4) and 2.227(4) Å, respectively. c) Infinite structure of the one-dimensional chain of the γ phase.

The X-ray diffraction of a red thin plate of the α phase shows orthorhombic space group ($P2_12_12$, no. 18).^[7] The crystal structure of the α phase is shown in Figure 1. The building block $[\text{Cu}^{\text{I}}_2(\text{BQ})(\text{OAc})_2]$ has approximately C_{2v} symmetry in the crystals of the α phase (see Supporting Information). Figure 1b focuses on the building block with the nearest-neighbor coordinated atoms. The oxygen atoms of the BQ coordinate the axial positions of the dicopper cores of the neighboring building blocks (Cu1–O3#: 2.270(3) Å). Consequently, the BQ ligands have μ_4 coordination geometry, resulting in the formation of a 2D sheet (Figure 1c). As shown in Figure 1c, one building block adjoins four other building blocks in the α phase.

Red plates of the β phase have monoclinic space group ($P2_1/n$, no. 14).^[8] The crystal structure of the β phase is shown in Figure 2. The dicopper building block remains approximately C_{2v} symmetry in the crystals of the β phase (see Supporting Information). One of the two axial positions of the dicopper core is occupied by the oxygen atom from the BQ ligand of the neighboring building block, and the other is occupied by the oxygen atom from the AcO⁻ ligand of the neighboring building block (Figure 2b). The Cu1–O6# (BQ) bond length is 2.254(3) Å and that of Cu2–O1' (AcO⁻) bond is 2.278(2) Å. As a result, the benzoquinone ligand functions as a μ_3 bridging ligand, and two AcO⁻ ligands act as μ_2 and μ_3 bridging ligands, thus affording the formula $[\text{Cu}^{\text{I}}_2(\mu_3\text{-BQ})(\mu_2\text{-OAc})(\mu_3\text{-OAc})]_n$. Figure 2c illustrates the infinite structure of

the two-dimensional sheet of the β phase. In β phase, one building block adjoins three other building blocks to form a two-dimensional sheet.

The X-ray diffraction of a red column of γ phase shows orthorhombic space group ($Pccn$, no. 56).^[9] The crystal structure of γ phase is shown in Figure 3. The illustration of the dicopper building block along Cu–Cu axis (see Supporting Information) clearly shows the distortion from the ideal C_{2v} symmetrical structure in the crystals of the γ phase. The Cu–Cu distance of the γ phase (2.7530(9) Å) is longer than those of α and β phases (2.7207(9) and 2.7232(7) Å, respectively). Figure 3b reveals a dicopper unit with the nearest-neighbor coordinated atoms in the crystals of the γ phase. The axial positions of the dicopper core are occupied by the oxygen atoms from the AcO⁻ ligands of the neighboring building blocks (Cu1–O2'': 2.233(4) Å, Cu2–O3': 2.227(4) Å). The two AcO⁻ ligands in γ phase act as μ_3 bridging ligands, forming one-dimensional chain. In the case of γ phase, one building block adjoins two other building blocks.

Pure α , β , and γ phases were isolated as microcrystals by varying the reaction temperature and concentration. Pure α phase was obtained in a 82 % yield when the reaction temperature was fixed at 30 °C, with 250 mm of $[\text{Cu}^{\text{I}}_2(\text{OAc})_4(\text{H}_2\text{O})_2]$ and 1 M of HQ in ethanol.^[10] When the reaction temperature was fixed at 40 °C, with 50 mm of $[\text{Cu}^{\text{I}}_2(\text{OAc})_4(\text{H}_2\text{O})_2]$ and 200 mm of HQ in ethanol, pure β phase was isolated in a 34 % yield.^[11] When the mixture of 50 mm of $[\text{Cu}^{\text{I}}_2(\text{OAc})_4(\text{H}_2\text{O})_2]$ and 200 mm of HQ in EtOH was heated to reflux, pure γ phase was obtained in an 87 % yield.^[12]

The distortion of the building block in γ phase compared with α and β phases clearly influences spectroscopic properties of these supramolecular isomers. The IR spectra of all isomers show a bathochromic shift of the stretching frequency $\nu(\text{C}=\text{O})$ of the BQ as compared to the free BQ ($\tilde{\nu} = 1662 \text{ cm}^{-1}$), the magnitude of which depends on the type of isomers. In the γ phase, $\nu(\text{C}=\text{O})$ is only slightly shifted ($\tilde{\nu} = 1652 \text{ cm}^{-1}$, $\Delta\tilde{\nu} = 10 \text{ cm}^{-1}$), whereas in the α and β phases the shifts are approximately 30 cm^{-1} (α phase: $\tilde{\nu} = 1632 \text{ cm}^{-1}$, $\Delta\tilde{\nu} = 30 \text{ cm}^{-1}$, β -phase: $\tilde{\nu} = 1629, 1638 \text{ cm}^{-1}$, $\Delta\tilde{\nu} = 33, 24 \text{ cm}^{-1}$). The ¹³C CPMAS NMR spectra also show differences of chemical shifts of three supramolecular isomers (Supporting Information). The chemical shifts of all corresponding carbon atoms in α and β phases are within 1 ppm difference. On the other hand, each carbon atom in the γ phase shows a chemical shift different by 2–3 ppm compared with the corresponding carbon atoms in α and β phases.

Our systematic studies of varying the reaction temperature and concentration in conjunction with supramolecular isomerism provides a map of the crystallization process of the building block $[\text{Cu}^{\text{I}}_2(\mu_2\text{-BQ})(\mu_2\text{-OAc})_2]$ (Figure 4).^[13] High-temperature reaction conditions and a low concentration (50 mm) of the molecular building block results in thermodynamically controlled crystallization such that the γ phase is favored. As the reaction temperature is decreased to 60 °C and with a concentration of the building block of 50 mm, a mixture of β phase and γ phase is obtained, thus indicating that the β phase is kinetically favored compared with the γ phase.^[14] When the temperature is further decreased to 40 °C, only the β phase is obtained. As the temperature is

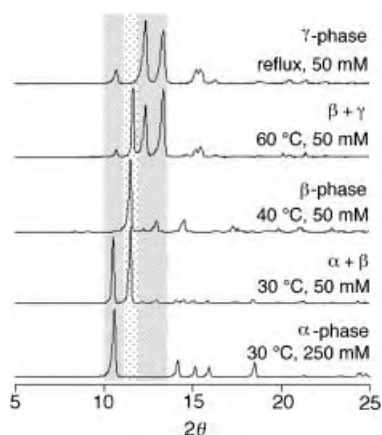


Figure 4. Reaction temperature- and concentration-dependent powder XRD patterns. Top, middle, and bottom lines represent powder XRD patterns of the α , β , and γ phases, respectively. The samples synthesized with a concentration of 50 mM of the building block at 60 °C and 30 °C result in the mixtures of two phases.

fixed at 30 °C and the concentration of the building block is 50 mM, the mixture of α phase and β phase is obtained, thus indicating that the α phase is the most kinetically favored product. A further decrease of temperature leads to disproportionation of the building block into copper(II) acetate and HQ, which prevents the isolation of the α phase at lower temperatures. Kinetically controlled crystallization of the α phase was successfully performed at 30 °C with higher concentrations of the building block (250 mM), which is associated with acceleration of the crystallization process. For this system, the synthon A is preferred to the synthon B in the kinetically controlled crystallization, while the opposite propensity is observed in thermodynamically controlled crystallization, thus resulting in the three supramolecular isomers.

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- [6] X-ray structure determination: All data of the X-ray structure determination of α , β , and γ phases were measured on Rigaku/MSC Mercury CCD diffractometer with graphite-monochromated $\text{MoK}\alpha$ radiation. These structures were solved by direct methods and expanded by using Fourier techniques. The non-hydrogen atoms were refined anisotropically. Refinements were carried out with full-matrix least-square techniques. All calculations were performed with the TEXSAN crystallographic program. CCDC-225749 (α phase), CCDC-225750 (β phase), and CCDC-225751 (γ phase) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).
- [7] Crystal data for α -phase, $\text{C}_{10}\text{H}_{10}\text{Cu}_2\text{O}_6$; $M_r = 353.28$, crystal size $0.20 \times 0.20 \times 0.01$ mm, orthorhombic, space group $P2_12_12_1$ (no. 18), $a = 7.438(3)$, $b = 9.356(3)$, $c = 8.293(3)$ Å, $V = 577.1(3)$ Å³, $Z = 2$, $\rho_{\text{calcd}} = 2.033$ g cm⁻³, $\lambda(\text{MoK}\alpha) = 0.7107$ Å, $F(000) = 352.00$, $\mu(\text{MoK}\alpha) = 37.00$ cm⁻¹, $T = -20^\circ\text{C}$, $2\theta_{\text{max}} = 54.9^\circ$, of the 4987 reflections that were collected, 276 were unique ($R_{\text{int}} = 0.034$); equivalent reflections were merged. For 1252 reflections with $I > 2.00\sigma(I)$, 83 parameters; $R(R_w) = 0.041(0.054)$. The residual electron density (min./max.) is $-0.87/0.93$ e⁻ Å⁻³. The Flack parameter is 0.03(4).
- [8] Crystal data for β phase, $\text{C}_{10}\text{H}_{10}\text{Cu}_2\text{O}_6$; $M_r = 353.28$, crystal size $0.30 \times 0.20 \times 0.03$ mm, monoclinic, space group $P2_1/n$ (no. 14), $a = 10.403(4)$, $b = 7.540(2)$, $c = 15.543(5)$ Å, $\beta = 103.661(7)^\circ$, $V = 1184.8(7)$ Å³, $Z = 4$, $\rho_{\text{calcd}} = 1.980$ g cm⁻³, $\lambda(\text{MoK}\alpha) = 0.7107$ Å, $F(000) = 704.00$, $\mu(\text{MoK}\alpha) = 36.05$ cm⁻¹, $T = -30^\circ\text{C}$, $2\theta_{\text{max}} = 55.0^\circ$, of the 10898 reflections which were collected, 2693 were unique ($R_{\text{int}} = 0.043$); equivalent reflections were merged. For 2407 reflections with $I > 2.00\sigma(I)$, 163 parameters; $R(R_w) = 0.040(0.058)$. The residual electron density (min./max.) is $-0.72/1.00$ e⁻ Å⁻³.
- [9] Crystal data for γ -phase, $\text{C}_{10}\text{H}_{10}\text{Cu}_2\text{O}_6$; $M_r = 353.28$, crystal size $0.40 \times 0.03 \times 0.01$ mm, orthorhombic, space group $Pccn$ (no. 56), $a = 9.422(3)$, $b = 17.301(5)$, $c = 14.416(5)$ Å, $V = 2349(1)$ Å³, $Z = 8$, $\rho_{\text{calcd}} = 1.997$ g cm⁻³, $\lambda(\text{MoK}\alpha) = 0.7107$ Å, $F(000) = 1408.00$, $\mu(\text{MoK}\alpha) = 36.35$ cm⁻¹, $T = -30^\circ\text{C}$, $2\theta_{\text{max}} = 55.0^\circ$, of the 21865 reflections which were collected, 2678 were unique ($R_{\text{int}} = 0.033$); equivalent reflections were merged. For 2033 reflections with $I > 2.00\sigma(I)$, 163 parameters; $R(R_w) = 0.055(0.060)$. The residual electron density (min./max.) is $-0.58/1.03$ e⁻ Å⁻³.
- [10] $[\text{Cu}^{\text{II}}_2(\mu_4\text{-BQ})(\mu_2\text{-OAc})_2]_n$ (α -phase): A solution of HQ (11 g) and copper(II) acetate $[\text{Cu}^{\text{II}}_2(\text{OAc})_4(\text{H}_2\text{O})_2]$ (10 g) in ethanol (100 mL) was stirred at 30 °C for 30 min, and left at 30 °C without a stir. After 3 h red thin plates obtained were collected by filtration, washed with hexane, and dried in vacuo (82% yield). Elemental analysis calcd (%) for $\text{C}_{10}\text{H}_{10}\text{Cu}_2\text{O}_6$: C 34.00, H 2.85; found: C 33.93, H 2.92. An XRPD spectrum of the massive product of α phase fit the profile derived from the crystal structure (see Supporting Information).
- [11] $[\text{Cu}^{\text{II}}_2(\mu_3\text{-BQ})(\mu_2\text{-OAc})(\mu_3\text{-OAc})]_n$ (β phase): A colorless solution of HQ (2.2 g) in ethanol (50 mL) was added to a green solution of copper(II) acetate $[\text{Cu}^{\text{II}}_2(\text{OAc})_4(\text{H}_2\text{O})_2]$ (2 g) in ethanol (50 mL). The mixture was stirred at 40 °C for 1 min, and left at 40 °C without stirring. After 2 h red plates obtained were collected by filtration, washed with hexane, and dried in vacuo (34% yield). Elemental analysis calcd (%) for $\text{C}_{10}\text{H}_{10}\text{Cu}_2\text{O}_6$: C 34.00, H 2.85; found: C 33.74, H 2.97. A powder XRD spectrum of the massive product of β phase fit the profile derived from the crystal structure (see Supporting Information).

- [12] $[\text{Cu}^{\text{I}}_2(\mu_2\text{-BQ})(\mu_3\text{-OAc})_2]_n$ (γ -phase): A solution of HQ (2.2 g) and copper(II) acetate $[\text{Cu}^{\text{II}}_2(\text{OAc})_4(\text{H}_2\text{O})_2]$ (2 g) in ethanol (100 mL) was heated to reflux for 1.5 h to give a red precipitate. This was isolated by filtration, washed with hexane, and dried in vacuo (87% yield). Elemental analysis calcd (%) for $\text{C}_{10}\text{H}_{10}\text{Cu}_2\text{O}_6$: C 34.00, H 2.85; found: C 34.03, H 2.84. A powder XRD spectrum of the massive product of γ phase fit the profile derived from the crystal structure (see Supporting Information).
- [13] Experiment at 60°C: The crystallization at 60°C with a low concentration (50 mM) was performed as follows. A colorless solution of HQ (2.2 g) in ethanol (50 mL) and a green solution of copper(II) acetate $[\text{Cu}^{\text{II}}_2(\text{OAc})_4(\text{H}_2\text{O})_2]$ (2 g) in ethanol (50 mL) were mixed and stirred for 1 min at 60°C, and left without a stir for 2 h at 60°C. The crystalline product obtained was collected by filtration, washed with hexane, and dried in vacuo. A powder XRD spectrum of the product obtained at 60°C corresponded to the mixture of β and γ phases (Figure 4). The other experiments at 30 and 40°C were performed in similar ways to that at 60°C, affording the α , β mixture and the pure β phase, respectively (Figure 4).
- [14] When a solid offers several polymorphs, the metastable phase with the higher solubility tends to be kinetically favored. This empirical rule, known as the Ostwald step rule, is understood by the nucleation kinetics of the solid.^[15] At a given supersaturation ratio, the activation energy of nucleation, which determines the nucleation speed, decreases in proportion as the interfacial tension decreases. Thus, because the solubility is inversely proportional to the interfacial tension, the precipitation of the more soluble phase, or the metastable phase, is therefore kinetically promoted under low temperature condition. For the system described in this paper, the difference of interaction energy between the two supramolecular synthons would cause the difference of stabilities among three crystalline phases.
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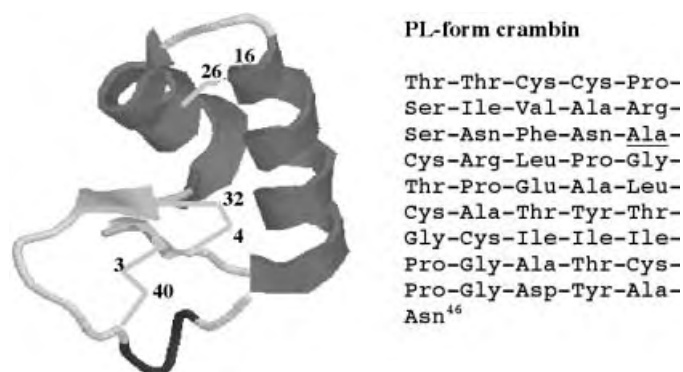


Figure 1. Molecular structure of crambin (PDB accession no. 1AB1) and target amino acid sequence.

crambin has been used as a model system for novel protein NMR techniques^[5] and for computational studies of protein folding.^[6,7] Because of difficulties with recombinant DNA expression of crambin in microbial systems,^[8] presumably arising from its hydrophobic nature, experimental studies on the crambin molecule have been severely limited: no protein engineering of this interesting molecule has been reported.

Our goal is to use crambin as a model system for experimental studies. We envisioned that a complete control of the chemistry of the crambin molecule would enable us to illuminate fundamental principles of protein folding. Recently, we reported an effective total chemical synthesis of [V15A]crambin.^[9] This synthesis used chemical ligation of unprotected peptides in a three-segment strategy to give the full-length 46-residue polypeptide chain that folded efficiently to form native crambin. However, the chemical tactics we used necessitated multiple intermediate purification and lyophilization steps, and was thus arduous and time-consuming. A more effective synthesis with fewer purification steps would facilitate rapid studies of chemical variants of the crambin molecule and would enable site-specific isotopic labeling for solid-state NMR and FT-IR spectroscopy, and other studies.

Here, we report a one-pot synthesis of crambin from three peptide segments that makes use of novel chemical tactics that enable all the ligation steps, and the folding of the full-length polypeptide chain, to be performed without purification or lyophilization of intermediate products. The synthesis makes use of a convergent strategy, with an improved protecting group for the Cys residue at the N-terminal of the middle segment. The resulting synthetic protein is correctly folded, is of exceptional purity, and is obtained rapidly and in high yield.

The synthetic strategy for the one-pot synthesis is shown in Scheme 1. In the one-pot synthesis, only a single, final purification step will be necessary for the production of high-purity crambin. In order to make the one-pot synthesis possible, we investigated and optimized the necessary reactions. In a three-segment ligation strategy (Scheme 1), the N-terminal Cys residue of the middle peptide segment must be protected in order to prevent cyclization caused by intramolecular reaction with the thioester moiety.^[10,11] In our previous synthesis of crambin we used the acetamidomethyl

Protein Synthesis

A One-Pot Total Synthesis of Crambin**

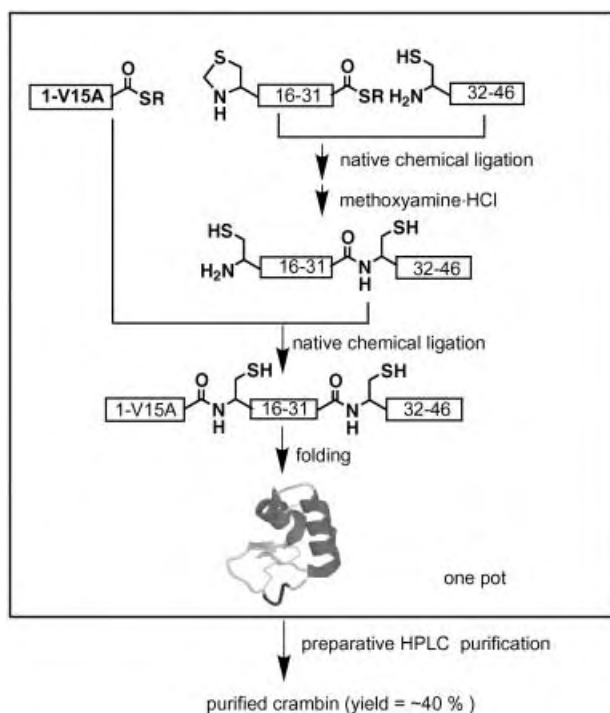
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Crambin (Figure 1) is a small protein isolated from the plant *Crambe abyssinica* that has no known biological function.^[1,2] It contains three disulfide bonds, an α -helix, a β -sheet, and a reverse turn—all features typical of a globular protein molecule—and for that reason crambin has been studied extensively as a model. Ultrahigh resolution (0.54 Å) structures have been obtained by X-ray crystallography.^[3,4] Also,

[*] D. Bang, Prof. S. B. H. Kent
Institute for Biophysical Dynamics
Department of Biochemistry and Molecular Biology
Department of Chemistry, The University of Chicago
Chicago IL 60637 (USA)
Fax: (+1) 773-702-0439
E-mail: skent@uchicago.edu

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Scheme 1. Synthetic strategy for a one-pot synthesis of crambin by ligation of three unprotected peptide segments.

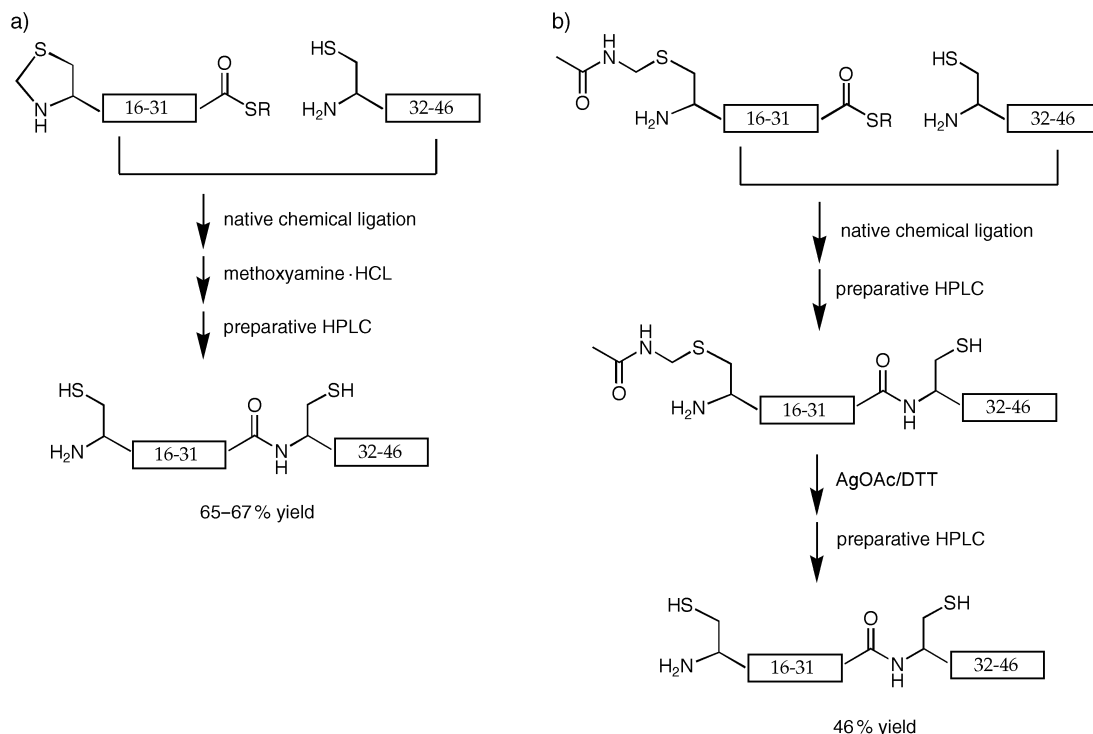
(Acm) group to protect the side-chain thiol of the N-terminal Cys. However, removal of the S-Acm group has proved to be problematic. For crambin we were not able to obtain a yield of

more than 46% for the combined ligation and protection steps (Scheme 2b), even with a highly optimized protocol.^[9]

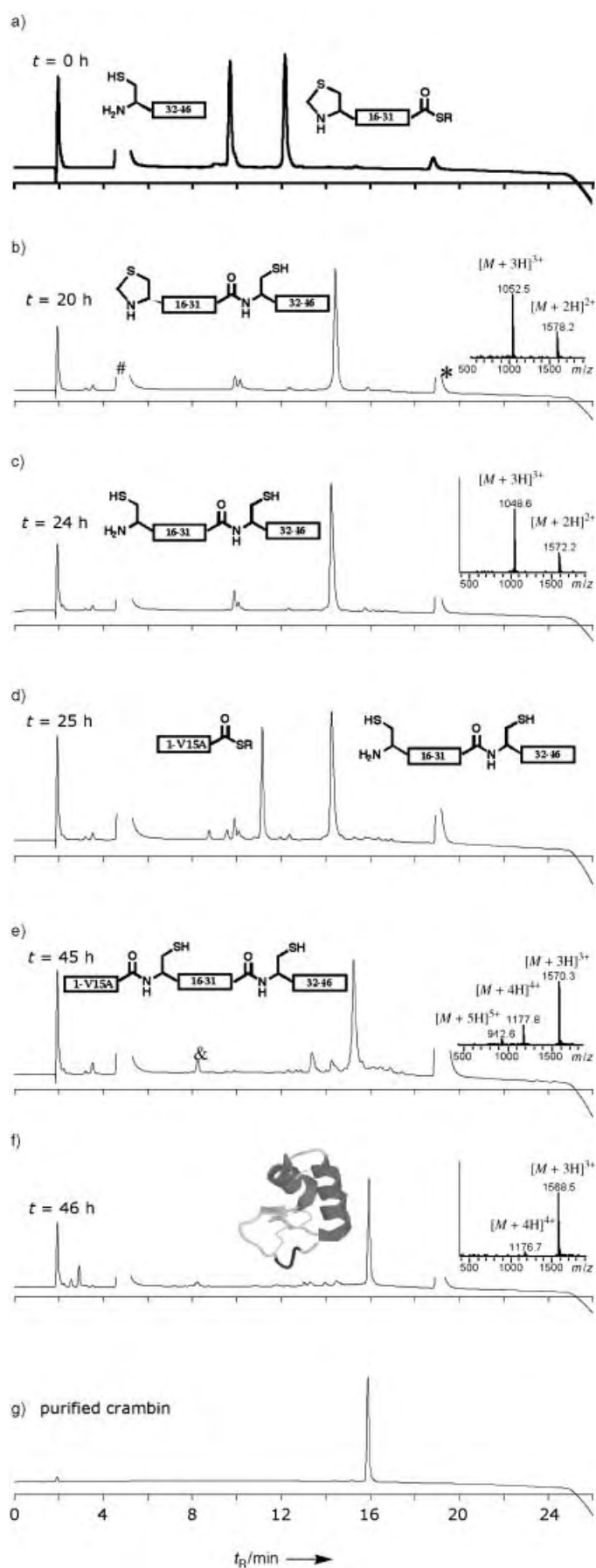
We explored the use of the 1,3-thiazolidine-4-carboxy (Thz) group (Scheme 2a) to protect the N-terminal Cys of the middle peptide segment. Facile conversion of a Thz-peptide to a Cys-peptide,^[12] and use of the Thz group to protect an N-terminal cysteine in native chemical ligation^[13] have been reported. Optimized stepwise solid-phase synthesis^[9] of the segment Cram[Thz¹⁶-31]^αthioester on a 0.4-mmol scale gave a good yield (≈ 50%, ≈ 350 mg) of the purified middle peptide segment.

The ligation of Cram[Thz¹⁶-31]^αthioester and [Cys³²-46]Cram went to completion in 20 h (Figure 2b). Addition of 0.2 M methoxyamine·HCl to the crude ligation mixture reduced the pH to ≈ 4, and reaction for 2 h at ambient temperature resulted in quantitative conversion of the [Thz¹⁶-46]Cram to the desired ligation product [Cys¹⁶-46]Cram (Figure 2c). In order to evaluate the efficiency of this procedure, in preliminary studies we isolated the ligated Cys-peptide product by preparative HPLC. The yields of the recovered product were 65–67% (two trials). Thus, protection of the N-terminal Cys as a Thz residue was much more efficient, both in terms of recovered yield, manipulations required, and time, than with the commonly used Acm group (Scheme 2).

Next, we explored the possibility of carrying out the second ligation reaction directly on the crude product mixture after treatment with methoxyamine·HCl, that is, without purification of the deprotected peptide [Cys¹⁶-46]Cram. The question was whether the thioester moiety would be sensitive



Scheme 2. Comparison of strategies for the protection of an N-terminal Cys residue. a) The strategy based on the thiazolidine resulted in better yield with less manipulation. b) The route based on the Acm group required a preparative-HPLC step before removal of the Acm group and gave a lower overall yield. DTT = dithiothreitol.



to the presence of methoxyamine. Model studies showed that a thioester peptide reacts only very slowly with methoxyamine in pH ≈ 7 buffer to form a small amount of the peptide- α -(*N*-methoxy)carboxamide that does not take part in the ligation reaction. Thus, we conjectured that the second ligation could be conducted by adding to the crude product mixture a slight excess of Cram[1–15] α thioester peptide over [Cys¹⁶-46]Cram. In order to effect the ligation reaction in the same batch, we had to readjust the pH to ≈ 7 , because the conversion of Thz to Cys by methoxyamine-HCl had been carried out at pH ≈ 4 . Several trials were performed to optimize the pH-adjustment procedure (see Experimental Section). Addition of the third peptide segment Cram[1–15] α thioester (in approximately 10% molar excess) to the solution resulting from the first ligation and Thz conversion (Figure 2d) at pH ≈ 7 resulted in quantitative ligation within 20 h (Figure 2e).

We expected that efficient folding of the product 46-residue polypeptide chain could be performed directly in this crude mixture, simply by adjusting the concentration of guanidinium-HCl (Gn-HCl) and the pH, and by addition of suitable redox reagents (if necessary). Thus, we added two volumes of pH 8.5, 0.1 M Tris buffer containing 8 mM Cys-HCl/1 mM cystine^[14] to dilute the Gn-HCl to 2 M and to adjust the pH to ≈ 8 , conditions previously shown^[9] to be optimal for the folding of crambin and formation of disulfide bonds. As anticipated, folding and disulfide bond formation (Figure 2f) gave an essentially quantitative yield of the correctly folded crambin.

Now that we have optimized each reaction, can we carry out a “one-pot” total synthesis of the crambin molecule? Bringing it all together, we performed a one-pot, three-segment ligation and folding on a tens-of-milligrams scale: the first ligation, in pH 7.5 phosphate buffer containing 6 M Gn-HCl; addition of methoxyamine-HCl to convert Thz- to Cys-peptide at pH 3.8; readjustment of the solution pH to 7.3 and addition of the third segment to effect the second ligation; and, addition of two volumes of Tris buffer to dilute the Gn-HCl, followed by addition of cysteine/cystine redox reactants^[14] to fold and form disulfide bonds. The folded crambin molecule was purified directly from the total crude

Figure 2. A one-pot synthesis of crambin. Reactions were monitored by LC-MS. UV profile at $\lambda = 214$ nm and mass data corresponding to major product peaks are shown. The chromatographic separations were performed using a linear gradient (10–50%) of buffer B in buffer A over 20 min (buffer A = 0.1% trifluoroacetic acid (TFA) in water; buffer B = 0.08% TFA in acetonitrile). b) At $t = 20$ h, formation of the first ligation product [Thz¹⁶-46]Cram ($t_r = 14.4$ min) was essentially complete. c) At $t = 24$ h, conversion of Thz- to Cys-peptide ($t_r = 14.2$ min) by methoxyamine-HCl was complete. Note the mass decrease of 12 Da and slightly earlier elution time. d) At $t = 45$ h, formation of the second ligation product Cram[1–46] was essentially complete ($t_r = 15.2$ min, obsd. mass = 4707.7 ± 0.6 Da; calcd. mass = 4708.4 Da.). e) At $t = 46$ h, the folding reaction was essentially complete to give crambin ($t_r = 15.8$ min, observed mass = 4702.7 ± 0.4 Da. calculated mass = 4702.4 Da.). Note that folded crambin eluted later than the linear polypeptide, reflecting the more hydrophobic nature of the protein molecule. # = thiophenol. * = diphenyldisulfide. & = Cram[1-V15A]-NHOCCH₃.

products by preparative HPLC (Figure 2). The optimized crambin synthesis took 48 h total elapsed time and was successfully reproduced three times at different scales (Table 1). Overall yields from starting peptide segments were 35–45%. The covalent and tertiary structures of the synthetic protein were determined by liquid chromatography (LC)-MS (Figure 2g), 2D-NMR (see the Supporting Information), and X-ray crystallography.^[15]

Table 1: Yields from one-pot syntheses of crambin.

Run	Cram[1-V15A] ^a thioester [mg] (mmol)	Cram[Thz ¹⁶ -31] ^a thioester [mg] (mmol)	Cram[32–46] [mg] (mmol)	Purified crambin [mg] (mmol)	Yield [%]
1	50 (0.028) ^[a]	48 (0.026)	41 (0.026)	42 (0.009)	35
2	28 (0.016) ^[a]	28 (0.015)	22 (0.015)	26 (0.0055)	37
3	12 (0.007) ^[a]	11.2 (0.006)	9.5 (0.006)	12.5 (0.0027)	45

[a] Note that ≈ 10 mol% excess Cram[1-V15A]^athioester was required for the second ligation.

This one-pot synthesis of crambin has significant advantages over our previously reported synthesis. Starting from the three purified peptide segments, the previous synthesis took approximately ten days of uninterrupted manipulations, including multiple purifications and lyophilizations to provide purified crambin in $\approx 25\%$ overall yield.^[9] However, the current one-pot synthesis with only one final purification step gave a synthetic crambin product of identical purity in only two days elapsed time, with an improved overall yield of $\approx 40\%$. The one-pot synthesis also vividly demonstrated the synthetic utility of the Thz residue as a reversibly protected form of N-terminal Cys.

In summary, for the first time, we have demonstrated the total synthesis of a protein molecule by the native chemical ligation of three unprotected peptide segments, and the folding of the synthetic polypeptide chain, all in the same reaction mixture without purification of intermediates. This novel one-pot synthesis proved to be a substantially more efficient way of producing the crambin molecule. The synthetic work reported here may serve as a useful model for the synthesis of more complex protein targets. Successful application of this one-pot approach to total synthesis depends on near-quantitative ligation reactions to avoid residual reactants interfering with subsequent steps. In our experience, native chemical ligation reactions proceed in essentially quantitative yield, even with only very modest excesses of one reactant. Carry-over of residual amounts of unreacted segments containing Cys thiol groups could potentially interfere with folding of the full-length polypeptide product. However, reaction with thiols in such residual segments would not normally be expected to be part of a productive folding pathway and will be reversed by the excess thiol reactant used in the folding/disulfide-formation step. For these reasons, using the approach described here we anticipate that it will be straightforward to make molecules containing up to 120–150 amino acids (i.e. from three 40- to 50-residue segments). In principle a similar approach could be used to assemble larger proteins from more than three unprotected peptide building blocks, but at some as-yet undefined point increasing amounts of coproducts will interfere.

Experimental Section

Peptides and peptide-^athioesters were made manually and stepwise by solid-phase peptide synthesis methods based on “in-situ neutralization” Boc chemistry^[16] on -OCH₂-Pam-resins (free “carboxyl peptides”) or on HSCH₂CH₂CO-Leu-OCH₂-Pam-resin^[17] (“thioester peptides”). Detailed procedures for peptide syntheses and for analytical and preparative HPLC were as described.^[9] The first native chemical ligation reaction^[18,19] was performed in 6 M Gn-HCl, 100 mM sodium phosphate, pH 7.5, 1% thiophenol (v/v), at a concentration of 2 mg mL⁻¹ for each peptide. Conversion of Thz- to Cys-peptide was performed by adding 0.2 M methoxyamine-HCl, causing the pH of the reaction mixture to drop to ≈ 4 . Subsequently, 0.5 M NaOH, 6 M Gn-HCl, 100 mM sodium phosphate, 1% thiophenol (v/v) solution was carefully added to the reaction mixture for adjustment to pH 7.0–7.3. The second ligation was performed by adding the peptide Cram[1–15]^a thioester to the reaction mixture at room temperature. The folding reaction was performed by adding two volumes of 0.1 M Tris, pH 8.5 buffer containing 8 mM Cys-HCl/1 mM cystine to give a solution that was pH ≈ 8 and 2 M Gn-HCl.

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Keywords: crambin · native chemical ligation · protein engineering · protein folding

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Gold Catalysts



Nanocrystalline CeO₂ Increases the Activity of Au for CO Oxidation by Two Orders of Magnitude **

Silvio Carrettin, Patricia Concepción, Avelino Corma,*
José M. López Nieto, and Victor F. Puentes

More than twenty years ago it was shown that gold, despite its limited adsorption properties, catalyzes reactions such as CO oxidation,^[1] alkene hydrogenation,^[2] and ethylene hydrochlorination.^[3] However, strong interest in gold for catalysis emerged only recently owing to the seminal work of Haruta et al.^[4] These authors showed that when gold is deposited on a metal-oxide support by a coprecipitation method, the metal exhibits high catalytic activity for CO oxidation at low temperatures. Moreover, they found that the distinctive behavior of the gold catalysts was due to the presence of nanosized Au particles.

In a recent review, Bond and Thompson^[5] showed that, in the case of Au-supported catalysts, factors such as Au particle size, preparation method, pretreatment conditions, and choice of the support play an important role in the CO oxidation activity of the final catalyst. Indeed, when nanoparticles of Au are deposited on TiO₂,^[4b,6a] Co₃O₄,^[4b,6b] ZnO,^[7] Fe₂O₃,^[4b,6a] or MgO,^[8] the catalysts are particularly active for the above-mentioned reaction. In contrast, nanoparticles of Au were much less active when other supports were used.^[5,9] These results suggest that a synergetic effect between the metal oxide support and Au may exist at the interface such that the metal oxide does not simply act as an inert carrier, but intervenes in the catalytic process.

We thought that if gold nanoparticles on the order of 3 to 5 nm were essential for the catalysis (owing to the presence of a large number of surface gold atoms with properties different from those of bulk gold) and if a synergetic mechanism occurs at the gold and metal oxide interface, the characteristics of the metal oxide surface should be of paramount importance. In other words, if the support could be prepared in the form of discrete, well-defined nanoparticles, this should have an

influence on the surface electronic properties and consequently on the gold-support interaction.

Cerium oxide has been reported to be inadequate for preparing active gold catalysts for CO oxidation at low temperatures.^[5] Nonetheless, this oxide support appeared to be interesting for water-gas shift reactions.^[10] It has been reported^[11,12] that coprecipitation of Au and CeO₂ gave CO oxidation catalysts that are active at 100 °C or higher, whereas Au on TiO₂^[4b,6] or Fe₂O₃^[4b,6] is already active below 0 °C. It was therefore clear to us that CeO₂ would be a good candidate to test if its impact on the catalytic activity of gold could be improved by preparing nanocrystals of CeO₂ within a very narrow particle size distribution.

The X-ray diffraction pattern of the support shows that CeO₂ is crystalline with the characteristic diffractogram of fluorite (see the Supporting Information). The CeO₂ particle size was determined from the half-height width of the (111) signal by using the Scherrer equation, and a mean ceria particle size of 3.3 nm was calculated. This value is much smaller than that previously reported for precipitated CeO₂ (15.9 nm).^[13] Transmission electron microscopy (TEM) showed that the support is made up of very regular nanoparticles of about 4 nm. Owing to the small size of the nanoparticles, the BET (Brunner–Emmett–Teller) surface area of the support is very high (180 m² g⁻¹).

Figure 1 shows the TEM analysis of the Au/CeO₂ catalyst. In the image on the right, the Au particles are bright white whereas the rest of the sample appears dark. The observed Au nanoparticles have diameters as low as 1 nm with a mean diameter of approximately 4 nm (see the Supporting Information); they are not agglomerated and are located on the CeO₂ support. The lattice spacing of the crystalline nanoparticles are observed in the HREM (high-resolution electron microscopy) images (see the Supporting Information). To determine the lattice spacing, an inversion of the radius of the dots or rings which appear in the fast Fourier transforms (FFT) of the image was carried out. Thus, the following crystal structures were observed: fcc Au, Cerianite CeO₂, and monoclinic Ce₆O₁₁. The presence of partially reduced CeO₂ could be due to electron irradiation under the high vacuum of the microscope. However, other samples and the analysis do not consistently show the presence of Ce₆O₁₁ as is the case here, suggesting the lower degree of oxidation or strong tendency of the synthesized ceria to be reduced. We also

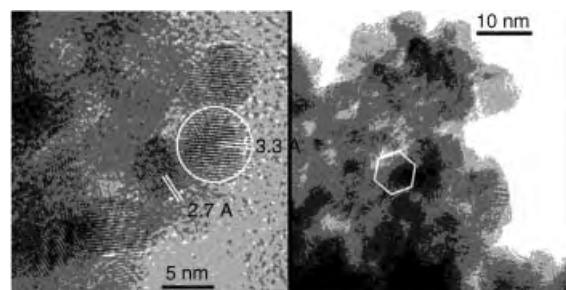


Figure 1. A high-resolution TEM image of the 2.8% Au/CeO₂ sample. Left: the white lines correspond to the (202) Ce₆O₁₁ (3.3 Å) and the (200) CeO₂ (2.7 Å) lattice spacing. Right: a hexagonal faceted (111) Au crystal is indicated.

investigated samples after the catalytic test. Based on TEM, no evolution of the samples (collapse of the mesopores or growth of the Au crystals) was observed.

The resulting catalyst gave 100% conversion of CO at 10°C, and no catalyst deactivation was observed during the 10 h of the run (see the Supporting Information). To compare its activity with that of other previously reported good catalysts, we chose samples of 1.5% Au/TiO₂ and 5% Au/Fe₂O₃ supplied by the World Gold Council.^[14] The specific rates per gram of Au are given in Figure 2. It can be seen that the specific rate with nanocrystalline CeO₂ was almost twice that with the other catalysts. When the reaction was carried out at -10°C, the activity of our Au/CeO₂ catalyst (2.8% Au) was $2.2 \times 10^{-6} \text{ mol CO s}^{-1} \text{ gcat}^{-1}$, which compared very well with that of the best catalyst reported by Tsubota et al.^[6] Using Au/TiO₂ (3.3 wt% Au) they achieved about $3.5 \times 10^{-6} \text{ mol CO s}^{-1} \text{ gcat}^{-1}$ under similar reaction conditions. On the other hand, when we placed Au on a conventional CeO₂ support prepared by precipitation ($S_{\text{BET}} = 70 \text{ m}^2 \text{ g}^{-1}$), the specific rate was almost two orders of magnitude lower when the reaction was carried out at 5°C (CO conversion 1.8%). Therefore, the fact that the CeO₂ support is in the form of nanocrystalline particles appears to be a key factor for

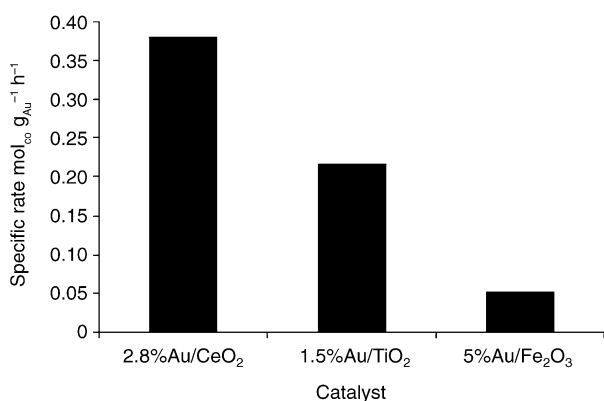


Figure 2. Graph showing the specific rate for CO oxidation on supported gold catalysts. The reference catalysts 1.5% Au/TiO₂ and 5% Au/Fe₂O₃ were supplied by the World Gold Council.^[15] Reaction conditions: the molar ratio for CO/air/He was 0.2:19.8:80, with a contact time (W/F) of $94 \text{ g}_{\text{cat}} \text{ h mol}_{\text{CO}}^{-1}$; reaction temperature 5°C.

achieving high activity in the oxidation of CO to CO₂ when Au is placed on a support.

It is well known the Au active catalysts can selectively oxidize CO to CO₂ in the presence of H₂ at low temperatures.^[15] However, when the reaction temperature is increased to those of interest for fuel cells operation (60°C), the selectivity can be negatively affected.^[15b,16] Our Au/CeO₂ catalyst shows excellent selectivity for CO oxidation in the presence of H₂ at 60°C (Figure 3). As is the case for Au on other active supports,^[17] the CO conversion decreases when H₂ is introduced in the reaction mixture. However, with our catalyst 99.7% of the oxygen was consumed for the oxidation of CO, and only 0.3% of the oxygen reacted with H₂ to give

water. In other words, Au on nanocrystalline particles of CeO₂ is also a very selective catalyst for the oxidation of CO in the presence of H₂.

To conduct infrared (IR) spectroscopy, CO was adsorbed on the 2.8% Au/CeO₂ sample in the IR cell. An intense band with a maximum at 2103 cm⁻¹ together with a broad shoulder on its high-frequency side, with maxima at 2124 and 2133 cm⁻¹, were observed (see the Supporting Information). A low-frequency band at 2103 cm⁻¹ was assigned to CO

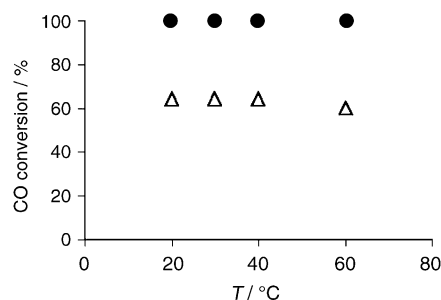


Figure 3. Plot displaying the selective oxidation of CO on 2.8% Au/CeO₂ catalyst in the absence (●) and in the presence (△) of H₂. Reaction conditions: the molar ratio for CO/O₂/He/X was 0.8:0.4:40.4:58.4 (X = H₂, N₂), with a contact time (W/F) of $18.6 \text{ g}_{\text{cat}} \text{ h mol}_{\text{CO}}^{-1}$.

adsorbed on Au⁰ sites in defect positions at the perimeter of the contact area between the Au particles and the support or to metallic Au with a lower degree of coordination.^[18] Bands at 2124–2133 cm⁻¹ were assigned to positively charged gold species.^[19] Furthermore, CO interacting with Ce³⁺ ions of the CeO₂ support also gave a characteristic IR band at 2133 cm⁻¹.^[20] In fact with X-ray photoelectron spectroscopy (XPS) we have observed the presence of Ce³⁺ ions on the catalyst surface.

When O₂ was allowed to react at 25°C with the adsorbed CO (see the Supporting Information) only the IR bands at 2103 and 2124 cm⁻¹ decrease in intensity, and the rate of decrease is faster for the former. As previously indicated, the IR band at 2103 cm⁻¹ is associated to CO adsorbed on Au sites located at the perimeter of the contact area of the metal particle with the support, which can be highly unsaturated.

From the IR adsorption and adsorption–reaction experiments conducted with CO and CO+O₂, it appears that Au and Au^{δ+} species are present in the catalyst and both can intervene in the oxidation process. The high degree of CO adsorption on Au particles located at the perimeter of the contact area between Au (and the cerium oxide support, and the presence of Ce³⁺ ions (i.e. oxygen vacancies) on the surface of the nanocrystalline support that are able to adsorb and activate O₂ may explain the enhanced reactivity of the catalyst.

In conclusion, we have shown that by depositing Au on nanocrystalline particles of cerium oxide it is possible to achieve an increase of two orders of magnitude in the catalytic activity relative to the Au/CeO₂ catalysts prepared by coprecipitation or by Au deposition on a regular cerium oxide support. These results show that the properties of the support can change drastically when the particle size is decreased to the nanosize region, turning a support that was previously considered to be inadequate for preparing Au

catalysts for CO oxidation into a highly active species. Moreover, owing to the high selectivity of Au/CeO₂ for the oxidation of CO in presence of H₂ at higher temperatures, the catalyst may have possibilities for use in fuel cells.

Experimental Section

A colloidal dispersion of CeO₂ nanoparticles was prepared by thermolysis of an acidified Ce(NO₃)₃ solution followed by redispersion, according to a previously described method.^[21] The dispersion was purified and concentrated using an ultra-filtration cell equipped with a 3 KD membrane. The purification was monitored by the residual acidity of the dispersion, determined by an acid titration of the supernatant after ultra-centrifugation at 50000 rpm for 6 h.^[22]

Au was deposited on the nanoparticulated cerium oxide by the following procedure: A solution of HAuCl₄ (0.140 g) in deionized water was brought to pH 10 by the addition of 0.2 M NaOH (7.5 g). Once the pH value was stable the solution was added to a slurry containing CeO₂ (1.260 g) in H₂O (25 mL). After adjusting the slurry to pH 10 with 0.2 M NaOH (1.4 g), the slurry was vigorously stirred for 18 h at room temperature. The solid was then isolated by filtration and washed until free of chloride and dried for 16 h at 100 °C. After this, the catalyst was not calcined. The total Au content of the final catalyst (2.8% Au/CeO₂) was 2.8% as determined by chemical analysis. The catalysts were tested in a quartz reactor (9 mm i.d.), and the products were analyzed by gas chromatography using Porapak and Molecular Sieves columns in combination.

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Main-Group Clusters

Sb₈(GaCl₄)₂: Isolation of a Homopolyatomic Antimony Cation***Martin Lindsjö, Andreas Fischer, and Lars Kloo**

Homopolyatomic species, or clusters, of the majority of the post-transition-group elements are known. However, the bulk part of these are anionic.^[1] For the more-electron-rich main-group elements there is a possibility of forming cationic polyatomic species as well. A series of such polycations has been characterized for the halogen and chalcogen groups as well as for cadmium and mercury.^[2] Within Groups 15–17, well-characterized polycations have been established for all elements but fluorine, phosphorus, arsenic, and antimony.

[*] M. Lindsjö, Dr. A. Fischer, Prof. Dr. L. Kloo
Division of Inorganic Chemistry
Royal Institute of Technology
S-10044 Stockholm (Sweden)
Fax: (+46) 879-09-349
E-mail: larsa@kth.se

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Focusing on Group 15, several bismuth polycations have been synthesized in both the solid state and in solution, for example, Bi_5^{3+} , Bi_8^{2+} , and Bi_9^{5+} as crystalline compounds and Bi_5^{3+} in solution. Based on the general chemical resemblance between antimony and bismuth, it is reasonable to expect that antimony polycations are also stable enough for identification. However, only anionic clusters, such as Sb_7^{3-} ^[3] and Sb_{11}^{3-} ^[4] have been characterized, and no more than circumstantial evidence for the existence of antimony polycations has been reported in the literature.

Several synthetic routes have proved useful for preparing main-group clusters in general and bismuth polycations in particular: synthesis in molten salt mixtures,^[5] stabilization in superacidic media,^[6] and reactions in organic solvents at room temperature.^[7] In the search for antimony analogues, the two first methods were employed. Unfortunately, the results from the molten salt mixture were poor, although Corbett and co-workers found evidence of Sb_2I_4 , a lower antimony iodide displaying direct Sb–Sb bonding.^[8] One serious limitation of this route is the low solubility of antimony metal in liquid SbX_3 or $\text{SbX}_3\text{--AlX}_3$ mixtures ($\text{X} = \text{Cl}, \text{Br}, \text{I}$).^[9]

The experiments in superacid media had a similar outcome. The only published result is the reaction between Sb metal and AsF_5 to form a compound with the empirical formula $\text{Sb}(\text{AsF}_6)$. The cation in this compound is suggested to be polymeric $(\text{Sb}^+)_n$ owing to the diamagnetic behavior.^[10]

No further observations regarding antimony polycations were reported until Ulvenlund et al. described a black product, suggested to be $\text{Sb}_5(\text{GaCl}_4)_3$, which was obtained from the molten salt system $\text{Ga--SbCl}_3\text{--GaCl}_3$.^[11] The proposed composition was based on elemental analysis. No structure determination was performed, but Raman spectroscopy indicated Sb–Sb bonding.

Previously, the same authors had reported a new synthetic route to bismuth polycations: Bi metal could be oxidized by GaCl_3 dissolved in benzene, and $\text{Bi}_5(\text{GaCl}_4)_3$ could be isolated from these solutions.^[7] Unfortunately, this method was, in its original form, unsuccessful for the isolation of antimony polycations, since antimony metal cannot be oxidized by either SbCl_3 or GaCl_3 in benzene.^[12] However, Ulvenlund et al. suggested that gallium metal could be used to reduce SbCl_3 in a $\text{GaCl}_3\text{--benzene}$ solution.

In summary, it is clear that the overall meager results observed with antimony polycations do not compare at all to the success achieved with bismuth polycation synthesis.

In a modification of the synthetic route based on organic solvents, SbCl_3 dissolved in $\text{GaCl}_3\text{--benzene}$ was reduced by a solution of $\text{Ga}^+(\text{GaCl}_4)^-$ in $\text{GaCl}_3\text{--benzene}$. Subsequent extraction of the resulting solution with hexane and mesitylene provided black-red crystals of $\text{Sb}_8(\text{GaCl}_4)_2$ (**1**) together with a black amorphous and a brown polycrystalline phase.^[13]

The crystal structure of **1** is shown in Figures 1 and 2. The compound consists of Sb_8^{2+} polycations with square-antiprismatic geometry (D_{4d} symmetry) and tetrahedral tetrachlorogallate anions. The geometry of the polycation is consistent with the Wade rules. These rules were originally formulated for the topology of boranes but can also be applied to naked post-transition-metal clusters.^[14] The square-antiprismatic geometry of the polycation is also found for the bismuth

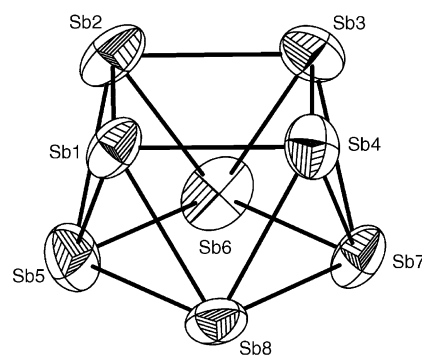


Figure 1. The cation Sb_8^{2+} in the crystal structure of **1**. The thermal ellipsoids are shown at the 70% probability level.

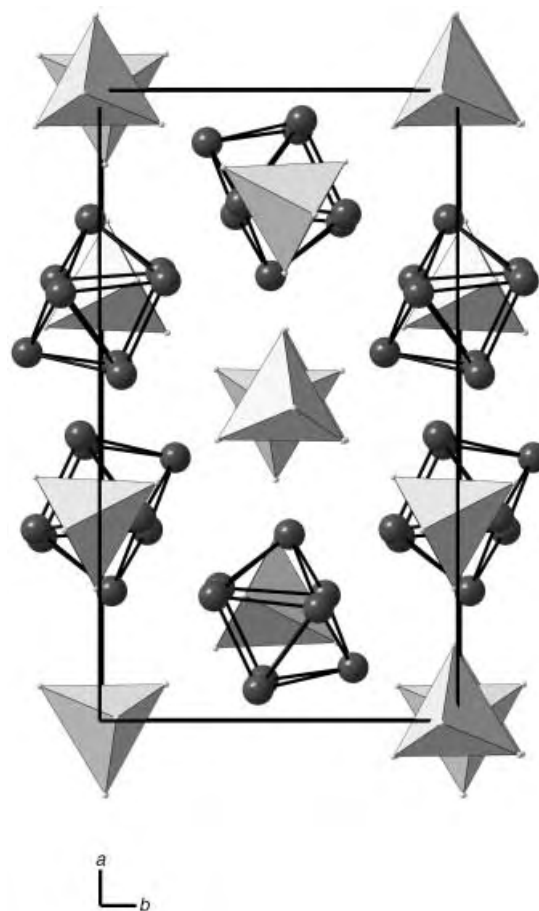


Figure 2. The unit cell of **1** as viewed along the crystallographic c axis.

analogue $\text{Bi}_8(\text{AlCl}_4)_2$.^[15] The Sb–Sb bond lengths in the Sb_8^{2+} polycation are summarized in Table 1, together with corresponding values for Bi_8^{2+} . The closest Sb–Cl bond length in **1** is 343.3 pm; thus, the interaction between cation and anion may be considered to be predominantly electrostatic.

The data in Table 1 demonstrate that there is one important difference between the geometry of Bi_8^{2+} in $\text{Bi}_8(\text{AlCl}_4)_2$ and that of Sb_8^{2+} in **1**: the atom–atom distances between the Sb_4 squares of the Sb_8^{2+} cation are 3.3% longer than those within. In the bismuth cluster cation, on the other hand, the bonds between the two squares are only marginally

Table 1: Summary of the interatomic bond lengths in the polycations Sb_8^{2+} and Bi_8^{2+} .

Compound	Space group	Intrasquare distance [pm] (average [pm])	Intersquare distance [pm] (average [pm])	Intersquare distance Intrasquare distance
$\text{Sb}_8(\text{GaCl}_4)_2$	$Pna2_1$	284.9–288.8 (286.9)	294.0–299.5 (296.5)	1.0331
Sb_8^{2+} (calcd)	–	295.1 (295.1)	304.1 (304.1)	1.0305
$\text{Bi}_8(\text{AlCl}_4)_2$ [15b]	$P6_3/m$	307.8–309.8 (308.6)	309.2–312.3 (311.0)	1.0078
$\text{Bi}_8(\text{GaCl}_4)_2$ [16]	$P6_3$	307.1–312.3 (308.8)	306.7–312.3 (310.0)	1.0038

longer than those within the squares. This difference may indicate a lower degree of delocalization in the individual squares of the Sb cluster. This hypothesis may be investigated by theoretical calculations from which the electron localization function (ELF) in Sb_8^{2+} and Bi_8^{2+} can be evaluated.^[17] However, ELF data display no significant difference in bonding between the two polycations; for both, the intra-square interactions are of localized character while the inter-square ones are more delocalized. The calculated inter-square/intra-square distance ratio for Bi_8^{2+} is 1.0248, that is, significantly larger than the ratio observed in the crystal structures. Thus, the differences in bond lengths may originate from differences in the interactions between cations and anions or between neighboring cluster cations in the crystal structures. For the related cation $\text{Bi}_4\text{Te}_4^{4+}$,^[18] the ELF results indicate an apparent localized character for all bonds, reflecting the fact that this cation has two more bonding cluster electrons than Sb_8^{2+} .

Raman spectra from a single crystal of **1** in the region of 120–500 cm^{-1} revealed four Raman-active vibration modes (Table 2). The band at 343 cm^{-1} is recognized as the symmetric stretch mode of the GaCl_4^- anion.^[19] A second band of this anion should be present at 158 cm^{-1} , but owing to

the theoretical value of 44.4 %. The analysis also shows that no other elements (except Sb, Ga, and Cl) are present.

In conclusion, this work describes the first isolated homopolyatomic antimony cation. The structure has been determined by single-crystal X-ray diffraction, and can be described as consisting of an Sb_8^{2+} polycation with a square antiprismatic shape and two GaCl_4^- counterions. The structure and electronic properties of the cation are similar to those of the previously known Bi_8^{2+} cation. The interaction between the cluster cation and surrounding anions is likely to be predominantly electrostatic, based on the long Sb–Cl distances observed.

Experimental Section

General: GaCl_3 (Aldrich, anhydrous, 99.99 %), SbCl_3 (Alfa, anhydrous, 99.999 %), and gallium metal (Alfa, 99.999 %) were used as received. Benzene (Fluka, 99.5 %), hexane (Merck, 99 %), and mesitylene (Janssen, 99 %) were dried over molecular sieves. Owing to the air and moisture sensitivity of the reactants and products, all synthetic work was performed in a glovebox under an inert atmosphere of nitrogen (< 1 ppm H_2O and O_2). The synthetic route used for **1** was originally developed and optimized to produce high-quality crystals of bismuth polycations.^[16]

1: Gallium metal (0.2 g, 3 mmol) was added to GaCl_3 (1.32 g, 7.5 mmol) in benzene (2 mL). After 24 h, undissolved gallium metal was removed, and the solution was added to a mixture formed by adding SbCl_3 (0.25 g, 1.1 mmol) to a solution of GaCl_3 (1.32 g, 7.5 mmol) in benzene (2 mL). The resulting reaction mixture was left overnight. During this time, a black amorphous precipitate formed, and the solution turned light brown in color. The solution, together with the amorphous material, was extracted with hexane (2 mL), which led to a reduction in the volume of the brown solution. The hexane phase was removed and mesitylene (2 mL) was carefully added on top of the brown solution. The tube was again left overnight. The mesitylene fraction was then removed, and new mesitylene was

added and this time thoroughly mixed with the brown solution by shaking the tube. A brown precipitate formed immediately. The solid phases were washed several times in benzene before drying by evaporation at room temperature. Small, black-red crystals were formed together with a brown powder (probably consisting of small crystals of **1** mixed with GaCl_3) and an uncharacterized, black, amorphous material.

Raman spectroscopy: The Raman spectrum of **1** was obtained using a Renishaw System 1000 spectrometer, equipped with a DMLM Leica microscope and a 25-mW He–Ne laser (633 nm).

Elemental analysis: Energy dispersive X-ray analysis was performed on a JEOL JSM-820 scanning electron microscope. Six points on two different crystals were investigated. Observed average contents (standard deviations): Sb 44(3) atom %, Ga 17(3) atom %, Cl 39(1) atom %; calculated values for **1**: Sb 44.4 %, Ga 11.1 %, Cl 44.4 %.

Quantum chemistry calculations: Ab initio calculations were performed, using the hybrid density function B3LYP, for the Sb_8^{2+} cation with Gaussian98 to evaluate the vibrational spectrum.^[20] Calculations were also carried out for Bi_8^{2+} and $\text{Bi}_4\text{Te}_4^{4+}$ to compare their electronic properties. The Stuttgart quasi-relativistic effective core potentials and valence basis sets,^[21] with one polarization

the very strong band at 180 cm^{-1} , attributable to the polycation, this weak band may be hidden. The positions of the bands assigned to the polycation are in good agreement with the calculated values, and there is almost total agreement between the bands of the Sb_8^{2+} polycation reported herein and those attributed to the proposed Sb_5^{3+} cation in reference [11]. Therefore, it is reasonable to assume that the cationic parts of the products are identical.

Energy-dispersive X-ray analysis on crystals of **1** confirms a high antimony content of 44 atom %, which is very close to

Table 2: Observed and calculated Raman bands ($\geq 120 \text{ cm}^{-1}$) of **1** and their assignments.

Wavenumber [cm^{-1}]	Calcd wavenumber [cm^{-1}]	Calcd relative intensity [%]	" $\text{Sb}_5(\text{GaCl}_4)_3$ " ^[11] [cm^{-1}]	Assignment
343, m				GaCl_4^-
180, s	170	100	183, s	Sb_8^{2+}
171, sh	160	4	174, sh	Sb_8^{2+}
135, sh	130	10	133, sh	Sb_8^{2+}

function added, were used for all atom types. The electron localization functions were calculated with the ToPMod package.^[22]

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Keywords: antimony · cluster compounds · polycations · Raman spectroscopy

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- [13] Crystal structure determination of **1**: orthorhombic, space group $Pna2_1$, $a = 1754.9$ (1), $b = 991.73$ (9), $c = 1285.8$ (1) pm, $V = 2237.8$ (2) $\times 10^6$ pm³, $\rho_{\text{calc}} = 4.15$ g cm⁻³; $2\theta_{\text{max}} = 55.0$, MoK α radiation, $\lambda = 71.073$ pm, $T = 299$ K. The diffraction data were collected on a Bruker-Nonius KappaCCD diffractometer. All atoms were refined using anisotropic temperature parameters. Numerical absorption corrections were applied. The structure was solved with SHELXS97 and refined against F^2 using SHELXL97. Final R values: $R_1 = 0.0286$, $wR_2 = 0.0646$; GOF = 1.088 for 4569 unique reflections and 163 parameters; Flack parameter $x = 0.03$ (2); min./max. residual electron density 0.88/–0.84. Further details on the crystal structure investigation may be obtained from the Fachinformationszentrum Karlsruhe, 76344 Eggenstein-Leopoldshafen, Germany (fax: (+49) 7247-808-666; e-mail: crysdata@fiz-karlsruhe.de), on quoting the depository number CSD-413577.
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Alkaloid Synthesis

Highly Enantioselective Construction of Fused Pyrrolidine Systems That Contain a Quaternary Stereocenter: Concise Formal Synthesis of (+)-Conessine***Biao Jiang* and Min Xu*

The construction of organic molecules with stereogenic quaternary carbon centers by stereoselective reactions is a very challenging task in organic synthesis.^[1] A diverse array of steroidal alkaloids and natural products derived from benzo-hydrindan (**21**) feature quaternary stereocenters in the ring-fused pyrrolidine system.^[2] Although significant progress has been made in the development of strategies for the enantioselective synthesis of such compounds, there remains a need for alternative approaches.

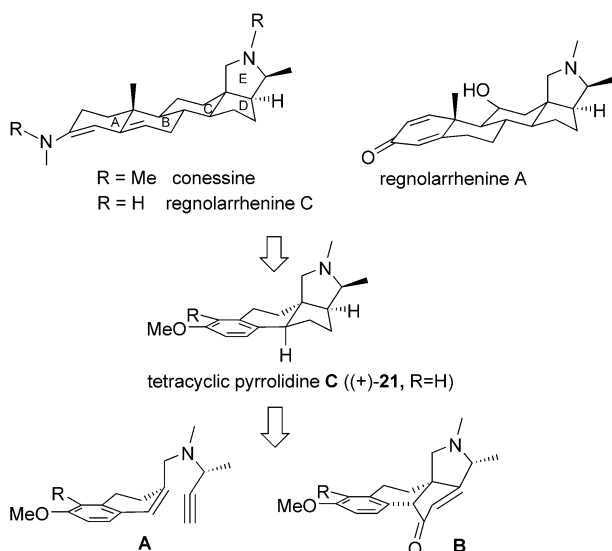
Tetracyclic pyrrolidines **C**, which contain the key BCDE ring system of (+)-conessine (Scheme 1), have four contiguous stereogenic centers, one of which is a quaternary carbon atom. In early reports, the synthesis of racemic steroidal alkaloids from racemic **C** was an efficient pathway.^[3] The

[*] Prof. B. Jiang, M. Xu
State Key Laboratory of Organometallic Chemistry
Shanghai Institute of Organic Chemistry
Chinese Academy of Sciences
354 Fenglin Road, Shanghai 200032 (P. R. China)
Fax: (+86) 21-6416-6268
E-mail: jiangb@mail.sioc.ac.cn

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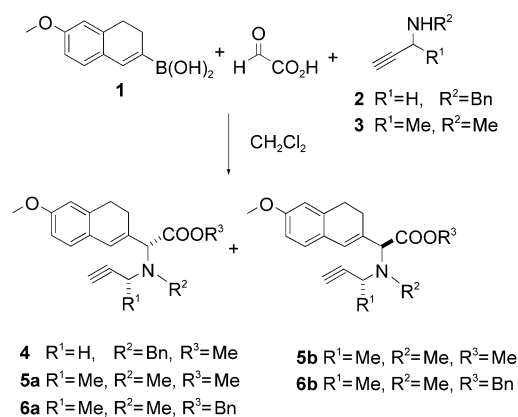


Scheme 1. Structures of the conessine family and retrosynthetic analysis of the BCDE ring system of (+)-conessine.

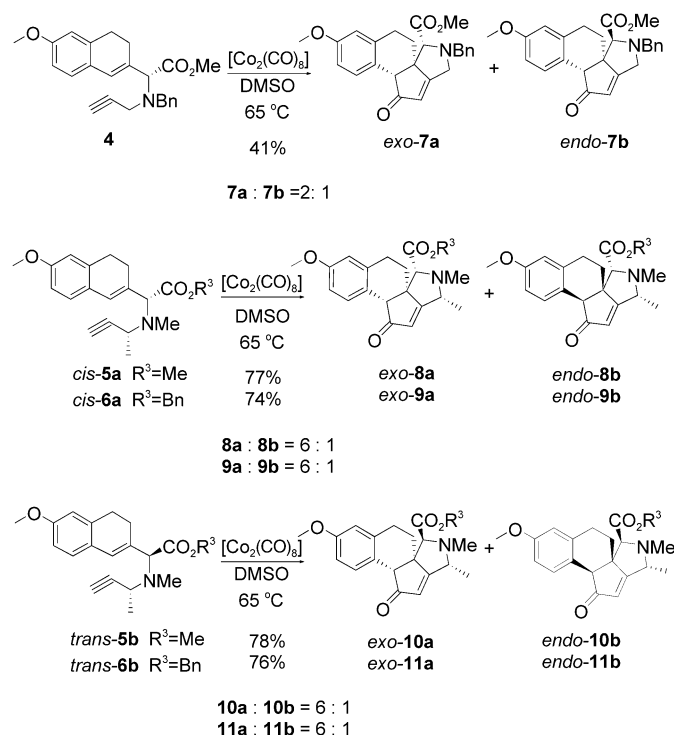
enantioselective synthesis of (+)-**21** was demonstrated by the Meyers research group from a chiral nonracemic lactam in 13 steps. The key transformation was a diastereoselective [3+2] cycloaddition with an azomethine ylide under pressure to construct the pyrrolidine ring system, followed by intramolecular addition of an aryl lithium and an aldol reaction to afford the tetracyclic cyclopentenone skeleton of the tetracyclic pyrrolidine enantioselectively.^[4] Based on our recent studies towards the convergent assembly of the cyclopenta[c]proline ring system in an efficient and stereoselective manner by an intramolecular Pauson–Khand reaction of a chiral enyne amino acid,^[5] it was envisaged that the tetracyclic pyrrolidine **21** of (+)-conessine could be accessed by an asymmetric Pauson–Khand reaction in a single step. The installation of the stereogenic quaternary carbon center would also be possible if the substrates possessed a dihydronaphthalenyl group (Scheme 1).

To check the viability of intramolecular Pauson–Khand reactions of *N*-propargyl dihydronaphthalenyl amino acid derivatives, the model 1,6-enyne **4** was readily prepared in 92 % yield by treating the dihydronaphthalenyl boronic acid **1** with the propargyl amine **2** and glyoxylic acid.^[6] To study the steric effects of the propargylic amine substituent on the efficiency and stereoselectivity of the cyclization, the *cis* and *trans* isomers **5a**, **6a** and **5b**, **6b**, respectively, were prepared from *N*-methyl-*N*-(1-methyl-2-propynyl)amine (**3**) in a ratio of 1:1, and separated by flash chromatography (Scheme 2). With this series of enynes in hand, we proceeded to study their reactivity under typical Pauson–Khand reaction conditions.

Initially, the Pauson–Khand reaction was carried out under catalytic conditions, as described previously.^[5] However, the reaction gave a complex mixture of products. Gratifyingly, the treatment of the enyne **4** with [Co₂(CO)₈] (1.1 equiv) at 65 °C for 6 h in the presence of DMSO (6 equiv) as an oxidant afforded the desired compact tetracyclic pyrrolidine in 41 % yield (Scheme 3). Two diastereomers, *exo*-**7a** and *endo*-**7b**, were isolated in a ratio of 2:1. To



Scheme 2. Synthesis of the 1,6-enyne amino esters **4–6b**.



Scheme 3. Studies on the stereochemical effects of the α methyl group in the propargylic moiety.

examine the influence of the substituent on the propargylic moiety on the stereochemical outcome of the reaction, the enynes **5** and **6** were treated under the same Pauson–Khand reaction conditions. All the reactions proceeded in good yields, and the ratio of diastereomers reached 6:1. It was found that the methyl group α to the N atom in the propargylic moiety played the main role in controlling the stereochemistry of the product. Irrespective of the nature of the ester group and of whether it was oriented *cis* or *trans* with respect to the methyl group α to N, the *exo* products (**8a**, **9a**, **10a**, **11a**) were isolated as the major diastereomers (Scheme 3). The stereochemistry of the products was readily established by NMR spectroscopic studies. The NOESY spectrum of **8a** showed an NOE between H_a and the methyl group α to the N atom in the pyrrolidine ring, thus indicating

a *cis* relationship between this methyl group and the cyclohexyl ring, and confirming that the major product had an *exo* configuration (Figure 1).^[7]

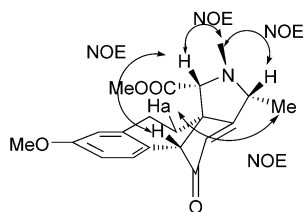
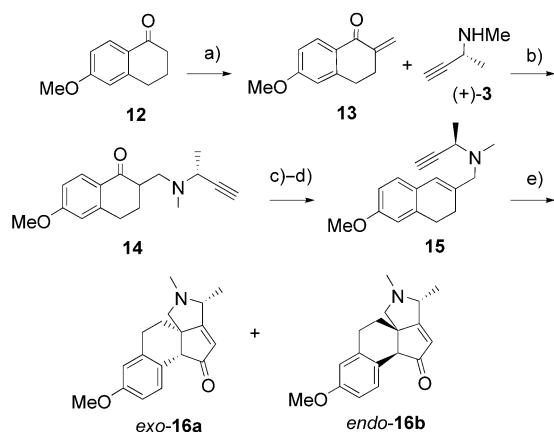


Figure 1. NOEs observed for compound **8a**.

Based on the above results, a practical synthesis of the tetracyclic pyrrolidine (+)-**21** was attempted. Starting from commercially available 6-methoxy-1-tetralone (**12**), the enyne **15** was readily prepared (Scheme 4). A direct methylene-transfer reaction by a general synthetic method produced the



Scheme 4. Synthesis of the key intermediate *exo*-**16a**:

a) $\text{CF}_3\text{CO}_2^- \text{Ph}(\text{CH}_3)\text{NH}_2^+$, $(\text{HCHO})_m$, THF, 65 °C, 4 h, 73 %; b) Al_2O_3 /toluene, room temperature, 24 h, 95 %; c) NaBH_4 , MeOH, 0 °C, 1 h; d) $p\text{-TsOH}\cdot\text{H}_2\text{O}$, benzene, reflux, 91 % (two steps); e) $[\text{Co}_2(\text{CO})_8]$ (1.1 equiv), DMSO (6 equiv), THF, 65 °C, 6 h, **16a/16b** (6:1), 67 %. DMSO = dimethyl sulfoxide, Ts = toluenesulfonyl.

α -methylene enone **13** from **12**.^[8] The Michael addition of (*R*)-(+)-*N*-methyl-*N*-(1-methyl-2-propynyl)amine (**3**) to the enone **13** catalyzed by activated alumina^[9] gave the adduct **14** in 95 % yield. Reduction of the ketone group in **14** with sodium borohydride, followed by dehydration, delivered the required enyne **15** in 91 % yield with 98.2 % *ee* ($[\alpha]_{\text{D}}^{25} = +166.7$ ($c = 1.275$, CHCl_3)). The treatment of the enyne **15** with $[\text{Co}_2(\text{CO})_8]$ in the presence of DMSO as an oxidant at 65 °C for 6 h afforded the desired fused cyclopentenones **16a** ($[\alpha]_{\text{D}}^{25} = +252.6$ ($c = 0.67$, CHCl_3)) and **16b** ($[\alpha]_{\text{D}}^{25} = -306.7$ ($c = 0.60$, CHCl_3)) in a ratio of 6:1 in 67 % yield. The configuration of **16a** was determined by X-ray crystallographic analysis, which further supported the assignment of the *exo* configuration to the major product of the Pauson–Khand reaction (Figure 2).^[10] A possible mechanism for the observed *exo* selectivity is shown in Scheme 5.

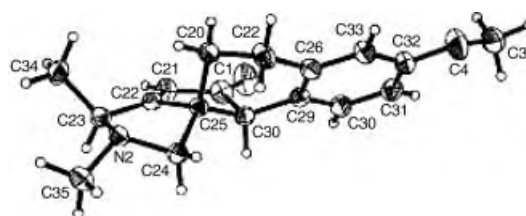
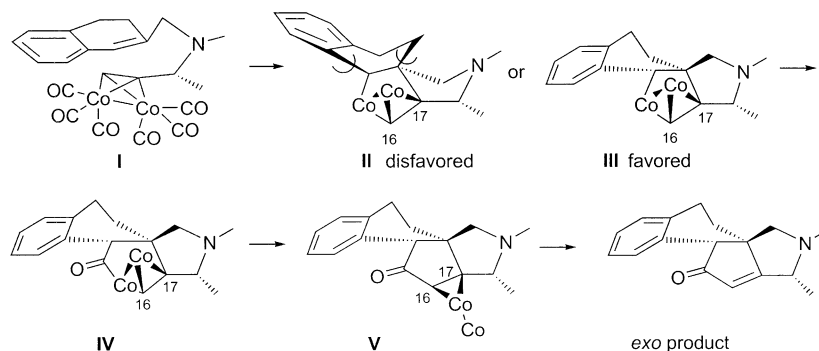


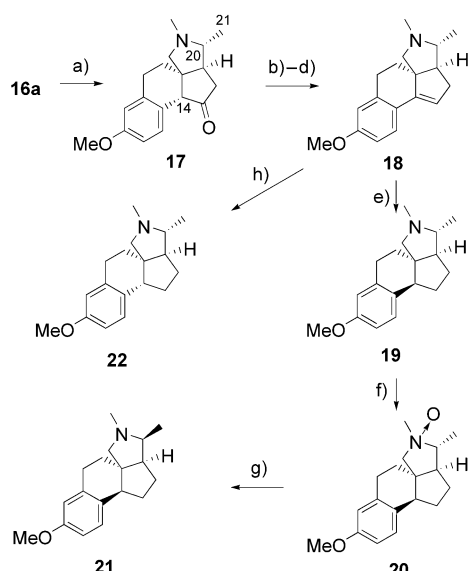
Figure 2. X-ray crystal structure of compound **16a**.

Complex **I** can form two plausible diastereomeric *cis* cobaltacycles **II** and **III** upon alkene insertion into the internal Co–C bond. Because of the bulky tetrahydronaphthalene group on the convex face, the upper side of newly formed *cis* cobaltacycle is quite congested. In the *cis* cobaltacycle **III**, which would lead to the *exo* product, the steric interactions between the methyl group α to the N atom and the C16–C17 bond in the cobaltacycle might be minimized, whereas in **II** a strong steric interaction exists between the methyl group on the *endo* face and the C16–C17 bond. Thus, *exo* selectivity is observed.

At this stage, the scaffold of the tetracyclic pyrrolidine (+)-**21** had been constructed, and the quaternary carbon center had been generated with the desired configuration. Emphasis was then given to the establishment of the correct configuration at C14 and C20 (conessine numbering; Scheme 6). The hydrogenation of the adduct **16a** in the presence of palladium on activated carbon gave the product **17** as a mixture of diastereomers in a ratio of 23:1. The stereoselectivity of the reaction was governed by the relative steric inaccessibility of the concave β face, thus leading to the attack of hydrogen from the α face. We hoped to take advantage of the carbonyl group adjacent to the C14 center and initially attempted to invert the configuration at C14 by the conventional method with DBU (1,8-diazabicyclo[5.4.0]undec-7-ene). However, the inversion product was obtained in less than 35 % yield, and a significant amount of the starting material was recovered (> 50 %). We then attempted to obtain the desired product through hydrogenation of the corresponding olefin **18**. It was anticipated that hydrogenation would occur from the α face on the basis of molecular modeling analysis. The successive treatment of the ketone **17** with sodium borohydride then methanesulfonyl chloride (MsCl) was followed by elimination of methanesulfonic acid by treatment with potassium *tert*-butoxide, to give the olefin **18** in 79 % yield. Unexpectedly, hydrogenation of



Scheme 5. A possible mechanism for the observed *exo* selectivity.



Scheme 6. Completion of the synthesis of the tetracyclic pyrrolidine (+)-**21**: a) Pd/C (10%), MeOH, H₂ (20 atm), 24 h, 92%; b) NaBH₄, MeOH, 0°C, 1 h; c) MsCl (2 equiv), pyridine, 0°C, 3 h; d) KOtBu (10 equiv), THF, room temperature, 24 h, 79% (three steps); e) Et₃SiH (20 equiv), TFA, room temperature, 24 h, 88%; f) MCPBA (2 equiv), CH₂Cl₂, 0°C, 1 h; g) TFAA (2.5 equiv), –30°C; then NaBH(OAc)₃, 93% (two steps); h) Pd/C (10%), H₂ (1 atm), 2 h, 78%.

the olefin **18** in the presence of palladium on activated carbon gave inseparable products in a ratio of 8:1. ¹H NMR spectroscopic analysis revealed that the major product was the undesired product **22** of addition to the β face. This disappointing result led us to investigate other conditions for alkene reduction. To our delight, the treatment of the olefin **18** with triethylsilane^[11] in trifluoroacetic acid (TFA) as the solvent afforded exclusively the required α adduct **19** in 88% yield.

The last challenge was to invert the configuration of the C20 center adjacent to the nitrogen atom. It is well-documented that the Polonovski reaction^[12] is suitable for this transformation. The oxidation of **19** with *m*-chloroperbenzoic acid (MCPBA) gave the *N*-oxide derivative **20**, which was treated directly with trifluoroacetic anhydride (TFAA) at –30°C. Reduction with sodium triacetoxy borohydride then provided (+)-**21** as a single isomer in 93% yield ([α]_D²⁵ = +51 (*c* = 0.21, benzene)). The optical rotation of the product was in agreement with that reported by Meyers and co-workers for the same compound,^[4] and the configuration of the product was further confirmed by X-ray crystallographic analysis.^[13] The steric hindrance at the concave α face of the tetracyclic molecule **20** may explain the excellent stereoselectivity observed.

In summary, we have described a concise and efficient synthesis of the optically active tetracyclic pyrrolidine (+)-**21** under mild conditions. We have demonstrated a highly diastereoselective method for the construction of the compact tetracycle in the fused pyrrolidine system, which bears a quaternary stereocenter. The stereochemistry of the product was affected by the substituent on the starting propargylic amine. No protecting groups were used in the synthesis, and

the entire chiral tetracyclic framework was created in one step by a Pauson–Khand reaction. Thus, the synthesis was efficient in terms of atom economy.

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Keywords: alkaloids · cyclization · enynes · Pauson–Khand reaction · pyrrolidines

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The Cofactor of the Iron–Sulfur Cluster Free Hydrogenase Hmd: Structure of the Light-Inactivation Product**

Seigo Shima, Erica J. Lyon, Melanie Sordel-Klippert, Manuela Kauß, Jörg Kahnt, Rudolf K. Thauer,* Klaus Steinbach, Xiulan Xie, Laurent Verdier, and Christian Griesinger*

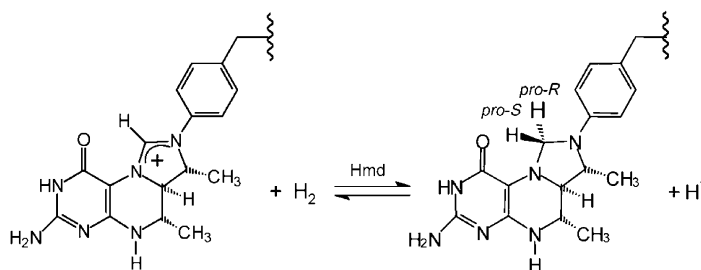
Hydrogenases are enzymes that catalyze reversible reactions that have H_2 as a substrate or product. There are three classes of hydrogenases which appear not to be structurally and phylogenetically related: [NiFe]-hydrogenases, [FeFe]-hydrogenases, and the iron–sulfur cluster free hydrogenase Hmd (H_2 -forming methylenetetrahydromethanopterin dehydrogenase; previously referred to as metal-free hydrogenases).^[1] [NiFe]-hydrogenases are generally found in bacteria and archaea;^[2] they contain a dinuclear [NiFe] center as a prosthetic group which is in long-range electronic interaction with a [4Fe-4S] cluster.^[3–5] [FeFe]-hydrogenases are found mainly in anaerobic Gram-positive bacteria with low guanine/cytosine content and in some anaerobically grown Eucarya;^[6] they contain a dinuclear [FeFe] center covalently bound to a [4Fe-4S] cluster through a bridging cysteine sulfur ligand.^[7,8] The iron–sulfur cluster free hydrogenase Hmd is found only in some methanogenic archaea growing on H_2 and CO_2 .^[9,10] The enzyme catalyzes the reversible reduction of methylenetetrahydromethanopterin with H_2 to methylenetetrahydromethanopterin (Scheme 1), which is an intermediary step in the reduction of CO_2 to methane.^[11,12]

Hmd from *Methanothermobacter marburgensis* was recently shown to harbor a tightly bound iron-containing cofactor, which is required for activity.^[13] The cofactor is released only after unfolding of the enzyme in urea, guanidine-HCl, or methanol in the presence of 2-thioethanol. The Hmd apoprotein is inactive, but becomes active upon addition of cofactor. The cofactor was found to be inactivated upon irradiation with UV-A/blue light.^[14] Light inactivation is associated with the bleaching of the cofactor's color and the release of one iron atom^[14] and most probably two CO molecules.^[15] The active cofactor is also temperature-sensitive:^[13] it is inactivated at 50 °C, with a half-life of only a few minutes. The half-life increases to approximately 60 minutes in the presence of 10 mM 2-thioethanol. Stabilization is most probably a consequence of the binding of the thiol group of 2-thioethanol to the cofactor's iron center.

Attempts to determine the structure or the molecular mass of the active cofactor have until now failed. It was found, however, that a relatively stable product with mass 542 Da is formed upon light inactivation or heat inactivation (Figure 1) which could be purified by HPLC and characterized. Inactivation was much more rapid than the formation of the

product with mass 542 Da, and at least one of the intermediates was seen by HPLC analysis.

The exact mass of the product was determined by FT-ICR MS (ICR = ion-cyclotron resonance) to be 542.1167 ± 0.0003 Da (calculated: $541.1089 + 1.0078$ Da; see the Support-



Scheme 1. Reaction catalyzed by the iron–sulfur cluster free hydrogenase Hmd. Molecular hydrogen reacts with N^5,N^{10} -methylenetetrahydromethanopterin reducing it to N^5,N^{10} -methylenetetrahydromethanopterin and a proton, $\Delta G^\circ = -5.5$ kJ mol⁻¹. A hydride is stereospecifically transferred from H_2 into the *pro-R* site of methylenetetrahydromethanopterin.

ing Information), which is consistent with an elemental composition of $C_{19}H_{23}N_6P_1O_{11}$. Placing the compound in ^2H_2O resulted in the mass increasing by 10 Da when analyzed in the negative mode and by 12 Da when analyzed in the positive mode, which indicates that 11 of the 23 hydrogen atoms were exchangeable with protons of water. When the cofactor was purified from enzyme isolated from cells grown in the presence of $^{15}NH_4Cl$ rather than $^{14}NH_4Cl$, the light-inactivation product with mass 548 Da, thus indicating that the compound with mass 542 Da indeed contained 6 N atoms. The presence of phosphorus was substantiated by the finding of a fragment with mass 79 Da in the ESI mass spectrum (negative mode); this signal corresponds to the molecular mass of PO_3^- . The ^{31}P NMR spectrum obtained with proton decoupling showed only one singlet at $\delta = -6.18$ ppm, which suggested the presence of one P atom. The stoichiometry $H_{23}:P_1$ was confirmed by comparison of the ^1H and ^{31}P NMR spectra obtained using methylphosphonic acid as an internal standard. A fragment with mass 498 Da was always seen in the mass spectra (data not shown), irrespective of whether the samples were prepared in buffers

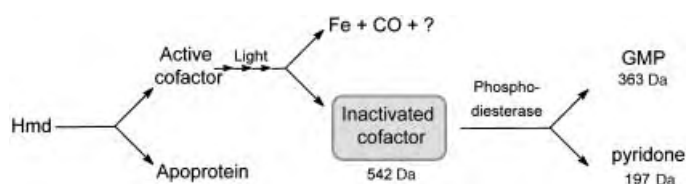


Figure 1. Scheme showing the formation of inactivated cofactor, CO, and other products from active Hmd cofactor by irradiation with light. The active cofactor is released from the holoenzyme after denaturation in the presence of 2-thioethanol. The holoenzyme was found to contain two moles of CO per mole of Fe by FTIR analysis and 2.4 ± 0.2 moles of CO per mole of Fe by chemical analysis.^[15] Upon incubation of the inactivated cofactor (542 Da compound) for 60 min at 37 °C with phosphodiesterase I (0.07 U *Crotalus adamanteus* Venom from Amersham Bioscience, Freiburg, plus 40 μ mol inactivated cofactor in 50 μ L 100 mM Gly/NaOH pH 8.9 containing 100 mM NaCl and 14 mM $MgCl_2$) two main products were formed that are referred to as 363 Da and 197 Da.

containing sodium (23 Da) or lithium (7 Da) ions. The intensity of the signal at 498 Da increased with increasing CID voltage. The mass difference difference of 44 Da (542–498) indicates that the compound with mass 542 Da was partially decarboxylated during the determination of the mass.

A signal at mass 150 Da was found in the ESI mass spectrum of the compound with mass 542 Da (see the Supporting Information). This mass corresponds to the mass of guanine minus one proton. The UV/Vis spectrum of the compound with mass 542 Da resembles in part that of guanosine ($\epsilon_{252} = 13.7 \text{ mM}^{-1} \text{ cm}^{-1}$). The shoulder at 300 nm indicates, however, the presence of a second chromophore. Subtraction of the spectrum of guanosine from that of the compound with mass 542 Da gave a difference spectrum with an absorbance maximum at 300 nm ($\epsilon = 8 \text{ mM}^{-1} \text{ cm}^{-1}$; Figure 2). The compound with mass 542 Da was found to fluoresce with a maximum excitation at 300 nm and emission at 375 nm (Figure 3). These findings suggested that it was composed of a guanosine moiety and a moiety which has a maximum absorption of light at 300 nm.

The observation of a ^{31}P resonance at $\delta = -6.18 \text{ ppm}$ indicated that the product with mass 542 Da could be a phosphodiester. This turned out to be correct. Incubation of the compound with phosphodiesterase at pH 8.9 (optimum pH value of the enzyme) resulted in the formation of two main products: one with a mass of 363 Da and one with a mass of 197 Da (Figure 1) that could be separated by analytical HPLC on a Synergi 4 μ Polar RP 80A column (250 mm $\times$ 4.6 mm) using a linear $\text{H}_2\text{O}/\text{CH}_3\text{OH}$ gradient. The masses add up to 560 Da and indicate that the two products were formed by hydrolysis (18 Da) of the compound with mass 542 Da. The product with mass 363 Da had the same mass, retention time ($R_t = 6 \text{ min}$), and UV/Vis spectrum as GMP. The absorption spectrum of the product with a mass of 197 Da ($R_t = 13 \text{ min}$) was very similar to that shown in the inset of Figure 2 (data not shown).

The UV/Vis spectrum of the compound with mass 542 Da changed when the sample was incubated at pH 8.9 in the presence of phosphodiesterase: the shoulder at 300 nm almost completely disappeared and the spectrum became almost identical to that of GMP (not shown). This finding indicates that after hydrolysis of the compound with mass 542 Da to form GMP and the product with mass 197 Da, the absorbance of the latter species at 300 nm was much lower than when it was still covalently bound.

^1H NMR and ^{13}C NMR spectra of the compound with mass 542 Da containing nitrogen atoms with the natural isotopic distribution and labeled with ^{15}N were recorded in $^2\text{H}_2\text{O}$ and $[\text{D}_6]\text{DMSO}$ to obtain more information on the structure of the inactivated cofactor, especially on the structure of the moiety with mass 197 Da. The 1D ^1H NMR spectrum obtained in $^2\text{H}_2\text{O}$ revealed the presence of 15 protons associated with the compound of mass 542 Da, 3 of which slowly exchanged with $^2\text{H}_2\text{O}$ at pH 7; in DMSO 22 protons associated with the compound of mass 542 Da were seen. The 1D ^{13}C NMR spectrum recorded with proton decoupling in $^2\text{H}_2\text{O}$ revealed the presence of 14 carbon atoms. Five additional carbon atoms were detected by $^{13}\text{C}, ^1\text{H}$ HMBC (C, H -

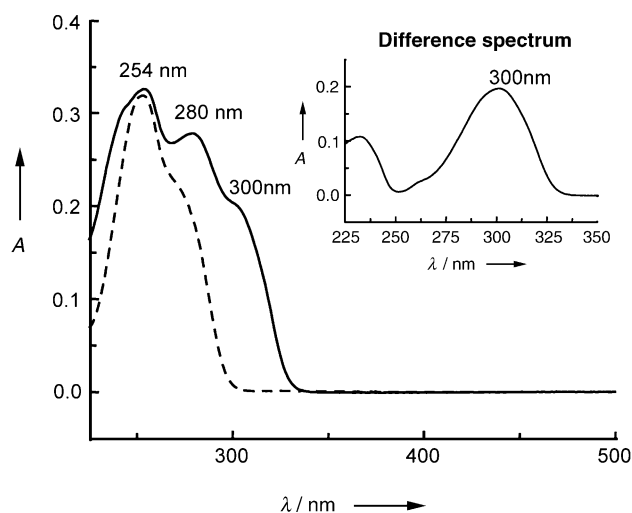


Figure 2. UV/Vis spectrum of the inactivated cofactor and of GMP. The inset shows the difference spectrum: —: inactivated cofactor; ----: GMP. The concentrations of the inactivated cofactor and of GMP were $80 \mu\text{M}$ in 100 mM sodium phosphate at pH 7.0. The path length d was 0.3 cm. The spectra were recorded on a Specord UV/Vis S10 Diode Array (Zeiss, Jena). $\epsilon_{254} = 14 \text{ mM}^{-1} \text{ cm}^{-1}$; $\epsilon_{280} = 12 \text{ mM}^{-1} \text{ cm}^{-1}$; $\epsilon_{300} = 9 \text{ mM}^{-1} \text{ cm}^{-1}$. The cofactor concentration was determined from its phosphate content for the calculation of the extinction coefficients.

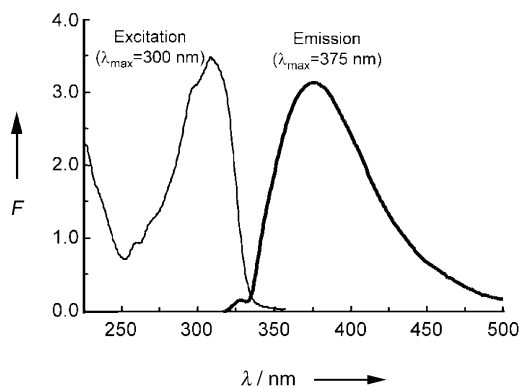
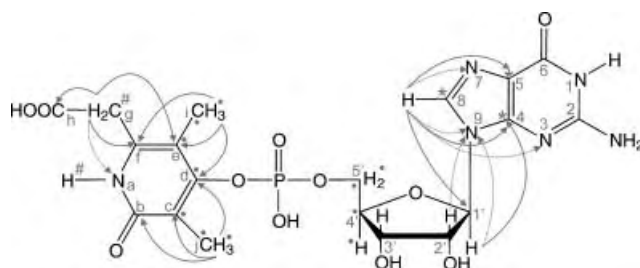


Figure 3. Fluorescence excitation and emission spectra of the inactivated cofactor. The concentration of the compound was $125 \mu\text{M}$ in 100 mM sodium phosphate at pH 7.0. The buffer was used as a reference. The spectra were recorded with a Cary Eclipse Spectrometer (Varian, Darmstadt).

long range correlation) studies.^[16] HSQC^[17] ($^1J_{\text{CH}}$ correlation), HMBC, and HNN^[18] experiments performed on the ^{15}N -labeled compound in DMSO revealed the presence of all the nitrogen atoms (1–3, 7, 9, and a; Scheme 2). All the results together are consistent with the structure shown in Scheme 2, as explained in the following.

The 11 hydrogen atoms in the structure that are exchangeable with protons of water are the protons at positions 1, 2, and 8 of the guanine ring, hydroxy groups at positions 2' and 3' of the ribose, the hydroxy group of the phosphate group, and the g, h, and a protons of the pyridone moiety (Scheme 2). Three of the 11 hydrogen atoms have been shown to exchange only slowly at pH 7 and room temperature; these include the hydrogen atom bound to C(8) of the guanine ring and the two

hydrogen atoms bound to C(g) in the pyridone moiety. The hydrogen atom at C(8) of guanosine is known to exchange slowly and the two hydrogen atoms at C(g) are expected to have similar properties as the two hydrogen atoms at C(2) of malonic acid. The hydrogen atom of the carboxy group C(h) is the only one not seen by NMR spectroscopy; this is because of fast exchange of the carbonic acid protons both in water and in DMSO. The hydrogen atom N(a) was visible in the spectrum recorded in DMSO and displayed a signal intensity of 85 % compared to the reference hydrogen atom H(1'); this is probably because of the existence of tautomeric forms in DMSO. We observed three different signals for H(a) and H(g), whose intensities changed simultaneously over time. The most probable explanation of this result is the presence of



Scheme 2. Structure proposed for the inactivated cofactor. It is a (6-carboxymethyl-3,5-dimethyl-2-pyridone-4-yl)-(5'-guanosyl) phosphate. The arrows indicate heteronuclear connectivities measured in the HMBC spectra (in $^2\text{H}_2\text{O}$ or DMSO), full lines for ^{13}C - ^1H connectivities, and dashed lines for the ^{15}N - ^1H ones. Although a complete COSY spin system was observed for the ribofuranose ring, these connectivities are not shown. The stars indicate extremely broadened signals arising from the presence of iron, the hash indicates two protons H(g) and H(a) that show tautomeric exchanges, and the filled circles indicate the signals correlated to the phosphorus atom (^{13}P - ^1H HMBC correlations or $^J J_{\text{P,C}}$).

tautomeric forms that depend on the water content, which changed over time because of the unavoidable increase in the water content of DMSO samples that are only sealed with parafilm. Only one set of signals each was observed for H(a) and H(g), thus indicating a fast exchange between the previously slowly exchanging tautomeric or hydration forms.

Five of the nineteen carbon resonances in the compound with mass 542 Da (C(4), C(5), C(8), C(g), and C(h)) were very broad in a ^{13}C NMR spectrum recorded in $^2\text{H}_2\text{O}$ with proton decoupling during detection. The line-broadening of the signals corresponding to C(8) and C(g) can be explained by the fact that the hydrogen atoms at these carbon positions had exchanged with the $^2\text{H}_2\text{O}$ solvent and the ^{13}C - ^2H coupling was not decoupled. Indeed, when the same sample was measured directly after dissolution in $^2\text{H}_2\text{O}$ the resonance for C(8) and for C(g) were as narrow as the other ^{13}C resonances. In $^1\text{H}_2\text{O}$, where no exchange of hydrogen atoms with deuterium can occur, the signals corresponding to the C(8) and C(4) atoms were observed in the indirect frequency dimension of an HMBC experiment by a $^3J_{\text{C,H}}$ coupling with hydrogen atom H(1'). In a similar manner, we observed the resonance for the C(8) atom in an HSQC spectrum and the carbon atoms C(4) and C(5) by HMBC (Scheme 2 and Figure 4) in DMSO, where no exchange of protons for deuterium can occur.

The C(h) resonance was broadened in the direct-detection ^{13}C NMR experiment performed in $^2\text{H}_2\text{O}$, probably because of the same effect discussed for the C(g) resonance above. The presence of this carboxy group was first indicated by the finding of a decarboxylation product in the mass spectrum. The resonance of C(h) was observed in an HMBC spectrum obtained in $^1\text{H}_2\text{O}$ or in DMSO through correlation with the hydrogen atoms at C(g). In the case of C(4) and C(5), the explanation for the extremely broadened resonances in the 1D ^{13}C NMR spectrum is less obvious since both carbon atoms are observable in the ^{13}C NMR spectrum of GMP. We found, however, that both signals were extremely broadened when trace amounts of Fe^{II} ions were added to a solution of GMP (1 % Fe^{II} relative to GMP). Such low amounts of iron were probably present in our preparations of the compound with mass 542 Da since it was derived from the iron-containing Hmd cofactor. The signal corresponding to C(8) of GMP was also slightly broadened upon addition of trace amounts of Fe^{II} ions. Thus, the broadening of this signal in the compound with mass 542 Da (see above) is probably not only a consequence of exchange with the solvent.

All the signals of the pyridone moiety were assigned based on through-bond interactions (Scheme 2 and Figure 4). The ^{13}C NMR signals attributed to C(4'), C(5'), C(c), C(d), C(e), and C(i) were split through J couplings with ^{31}P , while for the C(i) H_3 and the C(j) H_3 protons a strong and a weak ^{31}P , ^1H HMBC interaction was observed, respectively (Scheme 2 and Figure 4). The protons of C(g) showed HMBC correlations with N(a) after ^{15}N -labeling of the cofactor. The N(1) and N(2) atoms of the GMP moiety were assigned through HSQC cross peaks with their directly bound protons. In addition, HMBC correlations were observed from H(8) to N(7) and N(9), while HNN correlations were observed from H(8) to N(3) (Scheme 2). The β -D-ribofuranose was clearly assigned using $^3J_{\text{H,H}}$ COSY connectivities, the ^{13}C spectrum, and the NOESY spectrum (see the Supporting Information).

The NMR spectra of the compound with mass 542 Da, the NMR-derived connectivities, and the observation that the hydrolysis product of mass 197 Da has a composition of $\text{C}_9\text{H}_{11}\text{NO}_4$ and five exchangeable protons (see the Supporting Information) indicate that the chromophore bound to GMP through a phosphoester linkage is the pyridone derivative shown in scheme 2. A pyridone structure is also supported by the fluorescence spectrum of the inactivated cofactor (Figure 3). 2-Pyridone derivatives in the tautomeric keto form are known to exhibit room-temperature fluorescence with an excitation maximum near 300 nm and an emission maximum near 370 nm.^[19] As far as we know, a similar structure has not been encountered in any biological system.

It currently remains unclear how the compound with mass 542 Da is involved in iron complexation in the active Hmd cofactor. A possible coordination to N(a) and the carboxy group to yield a six-membered ring can be envisaged from the structure of the pyridone moiety. However, since the active cofactor is stable in the presence of high concentrations of EDTA, there must be additional ligands stabilizing the bidentate iron complex, if it is present. Since the compound with mass 542 Da is formed from the active Hmd cofactor via

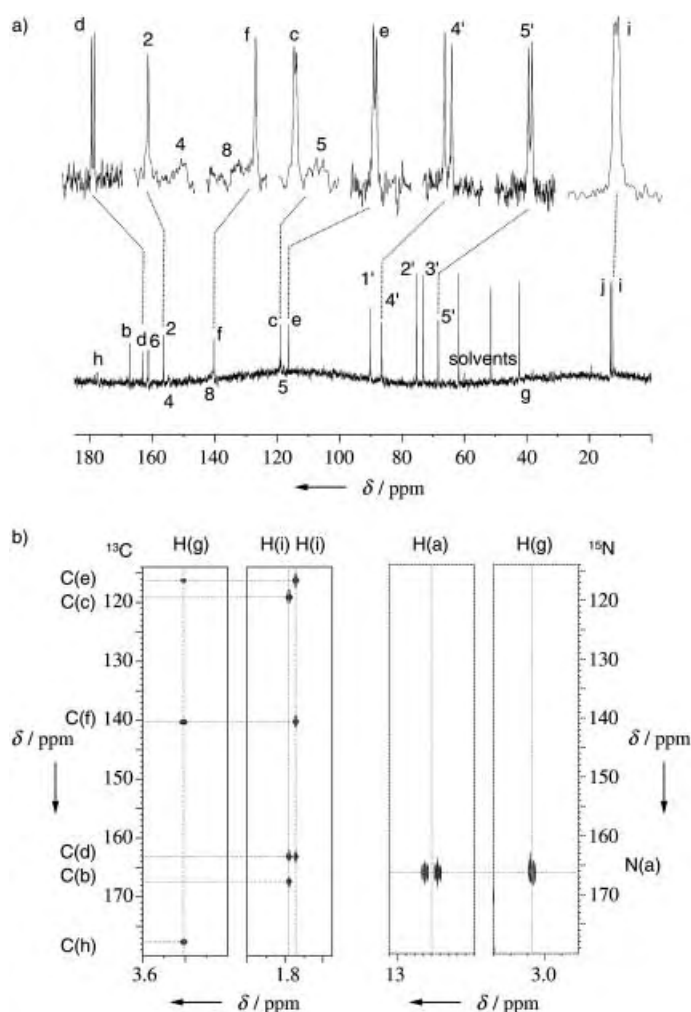


Figure 4. a) 1D ^{13}C decoupled spectrum of the cofactor in $^2\text{H}_2\text{O}$ at 300 K. The lower part shows the full spectrum and the upper part some particular signals obtained using different processing parameters to show the extremely broadened ones. The splittings for the 4', 5', c, d, e, and i signals result from $^J\text{C}_\text{P}$ couplings. The signals 4, 5, 8, g, and h are broadened because of the presence of iron (4, 5, and 8) and/or to chemical exchange (8, h, and g). b) Two dimensional HMBC correlation NMR spectrum in $^2\text{H}_2\text{O}$ at 300 K highlighting the connectivities within the pyridone ring. The two left panels (full lines) show the ^1H - ^{13}C connectivities of (g) and the methyl groups (i) and (j). The right part (dashed lines) shows the ^1H - ^{15}N connectivities of N(a).

intermediates it also has to be considered that the pyridone ring is formed only upon inactivation. We also do not want to exclude a complexation of iron to the pyridone moiety through π binding, such as in cyclopentadienyl- Fe^{II} -carbonyl complexes^[20] or in butadiene- Fe^0 -carbonyl complexes,^[21,22] since there is evidence for the presence of two CO molecules bound to the iron center in the active Hmd cofactor. It is interesting to note that CO is also a ligand to the iron center in [NiFe]- and [FeFe]-hydrogenases.^[2,6,23,24]

Experimental Section

Hmd (subunit molecular mass 38 kDa) was purified from *M. marburgensis* cells (5×100 g, wet mass) grown under nickel-limiting conditions and the active cofactor was extracted and purified

according to the published procedure.^[14] The active cofactor from the enzyme (600 mg, 16 μmol , 300 000 U) was obtained in approximately 25 % yield as determined from its activity after reconstitution with the apoenzyme. The cofactor dissolved in 10 mM ammonium carbonate at pH 9 (125 mL) and 10 mM 2-thioethanol (500 U per mL) was then inactivated at 0°C by exposure to room light for 5 h. This was done in five 100-mL glass vials each containing 25 mL of solution, with 100 % N_2 as the gas phase. Inactivation was continued until activity of less than 1 U mL^{-1} was detected. Once inactivated, each 25-mL sample was passed through a 0.22- μm filter and applied to a 6 mL Resource Q column equilibrated with H_2O . The inactivated cofactor was eluted by washing the column with H_2O (40 mL) and then with 50 mM NaCl (40 mL). Elution was followed through the absorbance at 280 nm. The fractions containing the inactive cofactor from five separate Resource Q columns were combined (3.4 μmol based on the absorbance at 300 nm, 79 % yield) and divided into four fractions, each containing 12.5 mL, and again passed through a 0.22- μm filter. The inactive cofactor was then applied to a Synergi 4 μ Polar RP 80A column (250 mm $\times$ 4.6 mm, Phenomenex, Aschaffenburg) equilibrated with H_2O (pH 4, HCl). The inactive cofactor eluted with 10 % methanol, and the combined fractions yielded 3 μmol of product (87 % yield). The purity was checked by HPLC using the same column and a linear $\text{H}_2\text{O}/\text{CH}_3\text{OH}$ gradient. The combined, purified inactive cofactor was lyophilized and dissolved in H_2O (0.55 mL), almost all of which was required for analysis by NMR spectroscopy.

Spectra were recorded with the following parameters: ^{31}P , ^1H HMBC: number of scans (NS) = 16, defocusing delay: 50 ms, 8192×1024 points in t_2, t_1 ; ^{15}N , ^1H HMQC: NS = 16, 4096×600 points in t_2, t_1 ; ^{15}N , ^1H HMBC: NS = 32, defocusing delay: 30 ms, 4000×600 points in t_2, t_1 ; HNN experiment: NS = 48, defocusing and refocusing delay for the N,N coupling: 40 ms and 120 ms, 4096×600 points in t_2, t_1 ; ^{13}C , ^1H HMBC: NS = 64, defocusing delay: 60 ms, 8192×1024 points in t_2, t_1 ; ^{13}C , ^1H HSQC: NS = 32, 4096×512 points in t_2, t_1 . All spectra were recorded at 600 MHz except for the spectra involving ^{31}P that were recorded on a 700 MHz spectrometer at 283.42 MHz. The ^{13}C spectra were recorded on a 500 MHz spectrometer equipped with a ^{13}C -detecting cryoprobe at 125.721 MHz. δ values for ^{13}C ($^2\text{H}_2\text{O}$), ^1H ($^2\text{H}_2\text{O}$, DMSO), ^{15}N (DMSO), and ^{31}P ($^2\text{H}_2\text{O}$) in ppm: N(1): 148, H(1): –, 10.73, C(2): 156.3, N(2): 73.6, H(2): –, 6.68, N(3): 173.2, C(4): 154.5, C(5): 118.1, C(6): 161.3, N(7): 249.2, C(8): 140.7, H(8): 7.91, 7.98, N(9): 169.8, C(1'): 90.2, H(1'): 5.77, 5.70, C(2'): 75.3, H(2'): 4.86, 4.55, OH(2') and OH(3') (not assigned) [shifts in DMSO: 5.47, 5.56], C(3'): 73.1, H(3'): 4.35, 4.16, C(4'): 86.4, H(4'): 4.21, 3.97, C(5'): 68.3, H(5'/5''): 4.14/4.12, 4.03/3.89, P: –6.18, N(a): 166, H(a): –, 12.60, C(b): 167.3, C(c): 119.1, C(d): 163.1, C(e): 116.2, C(f): 140.2, C(g): 40.2, H(g): 3.44, 3.08, C(h): 177.6, H(COOH) not seen, C(i): 12.4, H(i): 1.78, 1.93; C(j): 13.2, H(j): 1.82, 1.93. Additionally, resonances from contaminants, 2-thioethanol and methanol, were observed.

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Isolation and Characterization of Pre-rapamycin, the First Macrocyclic Intermediate in the Biosynthesis of the Immunosuppressant Rapamycin by *S. hygroscopicus***

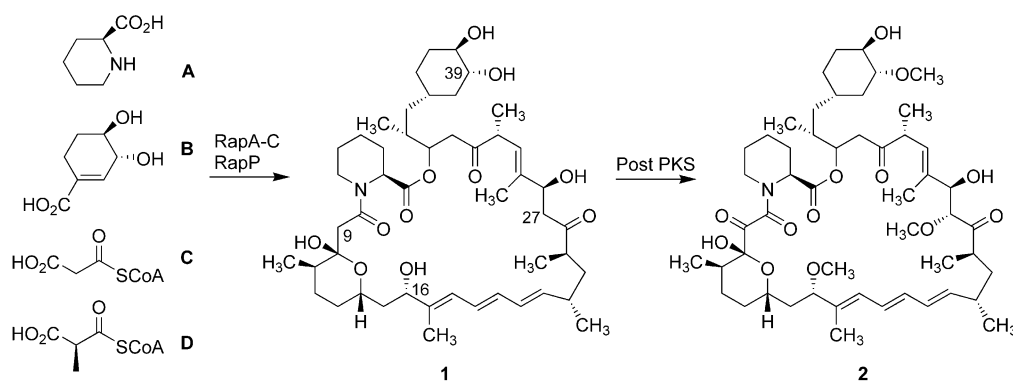
Matthew A. Gregory, Sabine Gaisser, Rachel E. Lill, Hui Hong, Rose M. Sheridan, Barrie Wilkinson, Hrvoje Petkovic, Alison J. Weston, Isabelle Carletti, Huai-Lo Lee, James Staunton, and Peter F. Leadlay*

Rapamycin (**2**, sirolimus) is a lipophilic 31-membered macrocyclic polyketide produced by *Streptomyces hygroscopicus* NRRL5491. It has been shown to have immunosuppressive,^[1] antifungal,^[2] and antitumor activity.^[3] In the commercial form rapamune, rapamycin has found clinical utility for the treatment of organ rejection following renal transplant surgery and the amelioration of some side effects associated with alternative treatments.^[4]

Rapamycin biosynthesis occurs by a mixed type I polyketide synthase (PKS)/nonribosomal peptide synthetase (NRPS) system.^[5] The PKS uses a shikimate-derived 4,5-dihydroxycyclohex-1-enecarboxylic acid starter unit^[6] and carries out a total of 14 successive cycles of polyketide chain extension with 7 propionate and 7 acetate units.^[7] The NRPS component, encoded by the *rapP* gene, then incorporates the L-lysine-derived L-pipecolic acid into the chain, after which the macrocyclic ring is closed.^[8] These latter steps involve transfer of the polyketide acyl chain from the PKS to the amino group of the activated pipecolic acid unit, cyclization, and release of the chain from the RapP protein through attack by a distal hydroxy group at C34 of the polyketide chain. This process yields the first putative enzyme-free intermediate, pre-rapamycin (**1**, 9-deoxo-16-*O*-desmethyl-27-desmethoxy-39-*O*-desmethyrapamycin). Pre-rapamycin (**1**) is believed to undergo further processing steps involving the products of a set of genes downstream of the PKS, including three O-methylations, two hydroxylations, and one ketone-forming oxidation, to yield **2** (Scheme 1).^[9] A mutant with four of these downstream genes deleted has previously been isolated and the compound produced (16-*O*-desmethyl-27-desmethoxyrapamycin) was characterized.^[10] However, the presumed immediate product of the PKS, namely, **1** has never been identified.

Herein we report the isolation and structural characterization of the key intermediate, pre-rapamycin (**1**). The region of the rapamycin cluster from *rapQ* to *rapI*, which encodes the processing genes, was removed by using a double recombination strategy (Figure 1).^[11] LCMS analysis of culture extracts of this mutant (*S. hygroscopicus* MG2–10) did not

[*] Dr. M. A. Gregory, Dr. H.-L. Lee, Prof. P. F. Leadlay
Department of Biochemistry and
Cambridge Centre for Molecular Recognition
80 Tennis Court Road, Cambridge CB2 1GA (UK)
Fax: (+44) 1223-766-091
E-mail: pfl10@mole.bio.cam.ac.uk



Scheme 1. Biosynthesis of pre-rapamycin (**1**) and rapamycin (**2**) from the precursors, pipecolic acid (**A**), dihydroxycyclohex-1-enecarboxylic acid (**B**), malonyl-CoA (**C**), and methylmalonyl-CoA (**D**).

show the rapamycin parent ion. In addition, exhaustive analysis of culture extracts revealed no additional compounds with a chromophore ($\lambda_{\text{max}} = 277 \text{ nm}$) typical of the triene of **2** and related compounds. Complementation of this mutant, using integrative expression plasmids based on pSET152,^[11] was attempted separately with each of the individual late-processing genes that had been removed: *rapK*, *rapI*, *rapJ*, *rapM*, *rapQ*, *rapN/O*, and *rapL*. Analysis of extracts where the culture was complemented by *rapK* only revealed accumulation of a new compound (m/z 841.5) that displayed a chromophore typical for the triene of **2**. High-resolution mass determination was consistent with the molecular formula

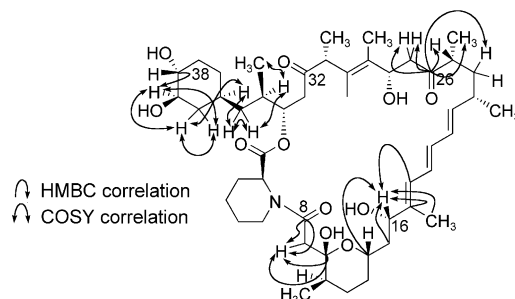


Figure 1. Region of the rapamycin cluster removed in *S. hygroscopicus* MG2-10.

$\text{C}_{48}\text{H}_{75}\text{NO}_{11}$. The fragmentation pathways of **2** and related compounds have been investigated by using electrospray ion-trap mass spectrometry.^[12] Compound **1** was investigated using tandem LCMS experiments and, in addition, high-resolution mass analyses of the fragmentation pathways were used to confirm the molecular formula proposed for each fragment. These data were fully consistent with the proposed fragmentation scheme (see the Supporting Information) for the putative first product of the rapamycin PKS, namely pre-rapamycin (**1**).

Compound **1** was isolated (8.5 mg) with a high degree of purity by preparative chromatography for NMR analysis.^[13] The key structural differences between **1** and the fully processed **2** are the absence of a keto group at C9, the lack of a methoxy group at C27, and no *O*-methylation at the C16-OH and C39-OH groups. A range of 2D NMR experiments, namely, ^1H - ^1H COSY, TOCSY, HMQC, and HMBC, were performed to aid the elucidation of the ^1H and ^{13}C NMR spectra of **1**. The key correlations that contributed to the

assignment of the altered parts of the molecule are depicted in Scheme 2. HMBC correlations from the C10 ketal and the C8 amide carbon atoms ($\delta_{\text{C}} = 98.9, 171.6 \text{ ppm}$) to an isolated methylene group ($\delta_{\text{H}} = 2.46, 3.23 \text{ ppm}$) led to the identification of H9a and H9b. HMBC correlations from C14, C15, C17, C18, and C44 (C17-methyl), to a methine atom resonating at $\delta_{\text{H}} = 3.95 \text{ ppm}$ allowed the assignment of H16. The triene fragment was readily assigned from sequential COSY correlations from H44 (C17-methyl) along the spin system to H46 (C25-methyl). HMBC correlations from the C26 ketone to protons in this triene-containing fragment (H24, H25) and to a pair of doublet of doublet proton resonances ($\delta_{\text{H}} = 2.42, 2.89 \text{ ppm}$) enabled assignment of the methylene protons H27a and H27b. The fragment H35 to H39 could be built up from COSY correlations. H39, which is geminal to a hydroxy group,



Scheme 2. Key 2D-NMR correlations for pre-rapamycin (**1**).

was assigned as resonating at $\delta_{\text{H}} = 3.24 \text{ ppm}$. An HMBC correlation from H39 to C40 ($\delta_{\text{C}} = 75.9 \text{ ppm}$) confirmed the presence of the C39,C40-diol. Careful examination of the complicated 2D NMR data confirmed that the assignment of the remaining proton and carbon atoms was consistent with the structure proposed for **1**.

The NMR spectra of rapamycins are complicated by the existence of rotamers.^[15] Acquiring the NMR data at 10°C made it possible to assign one of the rotamers, although the spectra were still complicated by the resonances of the second rotamer (for example, H9a, H9b). The highly complex nature of the ^1H NMR spectrum made the elucidation of coupling constants difficult, and for the most part impossible. It is,

however, reasonable to assume that the stereochemistry of **1** is likely to be the same as that of **2** since it is established during the biosynthesis of the polyketide chain.

The gene *rapK* was originally proposed to encode a pteridine-dependent dioxygenase^[9] involved in the oxidation of a putative C9 hydroxy group to a ketone.^[16] Aside from the proposed function, the evidence from the present study suggests, surprisingly, that *rapK* is required for the accumulation of **1**, the immediate product of the PKS. It is interesting to note that there are close homologues of *rapK* in the FK506(*fkbO*),^[17] FK520(*fkbO*),^[18] and *hyg(orf5)*^[19] clusters, all of which might be assumed to fulfil similar functions. Further work is being undertaken in our laboratory to elucidate the function of the *rapK* gene and confirm that it is essential for accumulation of **1**.

Rapamycin (**2**) is a powerful immunosuppressive drug, which has been the subject of much study during the past decade. A mutant of *S. hygroscopicus* NRRL5491, which is specifically blocked in the biosynthesis of **2**, has been shown to lead to accumulation of **1**, whose structure was confirmed by NMR and MS methods. The availability of **1**, with its array of functional groups, opens the way to systematic use of both enzymatic and chemical modification to delineate more precisely the final steps of rapamycin biosynthesis and to create semisynthetic derivatives with interesting and potentially novel bioactivity.

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Significant Interactions between Uranium and Noble-Gas Atoms: Coordination of the UO_2^+ Cation by Ne, Ar, Kr, and Xe Atoms**

Xuefeng Wang, Lester Andrews,* Jun Li,* and Bruce E. Bursten

We recently reported the existence of direct bonding interactions between uranium atoms and noble-gas (Ng) atoms^[1] in the matrix-isolated neutral CUO molecule from the reaction of laser-ablated U atoms with CO and isolated in solid noble-gas matrices.^[2] We have since demonstrated that CUO forms complexes with four or more noble-gas atoms bonded to the uranium atom of CUO.^[3,4] The discovery of U–Ng bonding depended on the remarkable energetic closeness of two very different electronic states of CUO with distinct vibrational frequencies, and we have demonstrated that the binding of three or four Ar, Kr, or Xe atoms to CUO causes a changeover in the electronic ground state of the $\text{CUO}(\text{Ng})_n$ complexes.^[5,6]

The discovery of actinide–noble-gas bonding adds to the recent renaissance in noble-gas chemistry,^[7,8] including the HArF molecule of Räsänen and co-workers,^[9] which is kinetically stable in solid argon, and the $[\text{AuXe}_4]^{2+}$ ion of Seidel and Seppelt,^[10] which is stable enough to be characterized by X-ray crystallography. The U–Ng bonds in the $\text{CUO}(\text{Ng})_n$ complexes are weak: The U–Ng binding energy ranges from about 4 kcal mol^{−1} for Ar to about 7 kcal mol^{−1} for Xe, values that are at the threshold of what can be called a bond. In contrast, the Au–Xe bonds in $[\text{AuXe}_4]^{2+}$ are strong: Calculations estimate the Au–Xe binding energy in $[\text{AuXe}_4]^{2+}$ as 57 kcal mol^{−1} per bond.^[11,12]

The U–Ng bonds in our systems and the Au–Xe bonds in $[\text{AuXe}_4]^{2+}$ are formed from Lewis acid–base interactions in which electron density in the Ng lone pairs is donated into

vacant orbitals on the metal center. We anticipated that part of the strong interaction between the Au and Xe atoms is due to charge polarization effects facilitated by the positive charge of Au^{2+} , and we predicted that the positively charged UO_2^{2+} ion, which is isoelectronic with CUO, might form stronger U–Ng bonds.^[1] Herein we report that U^{V} monocation, UO_2^+ , forms a variety of noble-gas complexes in solid noble-gas matrices, and our calculations indicate that the U–Ng bonding in these complexes is stronger than the U–Ng bonds that involve neutral CUO. The UO_2^+ ion has previously been isolated and characterized by a 980.1 cm^{−1} antisymmetric U–O stretching frequency in solid neon.^[13]

The laser-ablation matrix-isolation method used to prepare uranium dioxide molecules has been described previously.^[13–15] Laser-ablated uranium atoms insert into dioxygen to form UO_2 during condensation with excess noble gas at 4 K. Other product molecules include UO and UO_3 . In addition laser-ablation produces U^+ cations, which react in a similar fashion to form the linear, centrosymmetric UO_2^+ cation.^[13] These uranium-bearing species are identified and characterized from matrix-infrared spectra by using $^{16}\text{O}_2$, $^{18}\text{O}_2$, and $^{16}\text{O}_2$, $^{16}\text{O}^{18}\text{O}$, $^{18}\text{O}_2$ mixtures, from comparison to frequencies observed by trapping uranium-oxide vapor species in solid argon,^[16] and to frequencies calculated by using relativistic density functional theory (DFT).

The UO_2^+ cation exhibits a strong, sharp U–O stretching mode absorption at 980.1 cm^{−1} in solid neon, at 952.3 cm^{−1} in solid argon, at 940.6 cm^{−1} in solid krypton, and at 929.0 cm^{−1} in solid xenon.^[13,14,17] These bands reveal the 1.0527 ± 0.0001 $^{16}\text{O}/^{18}\text{O}$ isotopic frequency ratio characteristic of the antisymmetric stretching mode of a linear O–U–O linkage. Our calculated antisymmetric stretching frequency of isolated UO_2^+ is 1007 cm^{−1} at the CCSD(T) level. The matrix shifts from this yet-to-be-observed gas-phase frequency are caused by progressively stronger bonding interactions and ion-induced dipole interactions between U and the heavier, more polarizable noble gas (Ng) atoms. How can we determine the number of Ng atoms that bind intimately to UO_2^+ in the first coordination sphere? We prepare these $[(\text{UO}_2)(\text{Ng})_n]^+$ complexes by using mixtures of a lighter noble gas host (Ng) doped with heavier noble-gas guest (Ng') atoms and determine the number of mixed $[(\text{UO}_2)(\text{Ng})_n(\text{Ng}')_m]^+$ complexes prepared from their perturbations on the UO_2^+ infrared spectrum. Here we warm the solid samples to allow successive substitution of heavier Ng' for lighter Ng in the first coordination sphere and to determine the number of new absorption bands formed.

The infrared spectra for UO_2^+ in pure neon, argon, and xenon, and in mixtures of the noble gases are compared in Figure 1. Spectrum A shows the characteristic 980.1 cm^{−1} UO_2^+ absorption in pure neon. The weaker O_4^- band^[18] decreases on annealing while the UO_2^+ absorption increases. Experiments were done with 0.1, 0.3, and 3% Ar added to 0.01% O_2 /neon samples and spectra B, C, and D evolve for successive annealing cycles into the 10–15 K range. Note the appearance of just five new absorptions at 975.1, 970.3, 965.5, 961.0 and 957.1 cm^{−1}, marked with arrows, that approach the

[*] Dr. X. Wang, Prof. Dr. L. Andrews
Department of Chemistry, University of Virginia
McCormick Road, P. O. Box 400319
Charlottesville, VA 22904-4319 (USA)
Fax: (+1) 434-924-3710
E-mail: lsa@virginia.edu

Dr. J. Li
William R. Wiley Environmental Molecular Sciences Laboratory
Pacific Northwest National Laboratory
Richland, WA 99352 (USA)
Fax: (+1) 509-376-0420
E-mail: jun.li@pnl.gov

Prof. Dr. B. E. Bursten
Department of Chemistry
The Ohio State University
Columbus, OH 43210 (USA)

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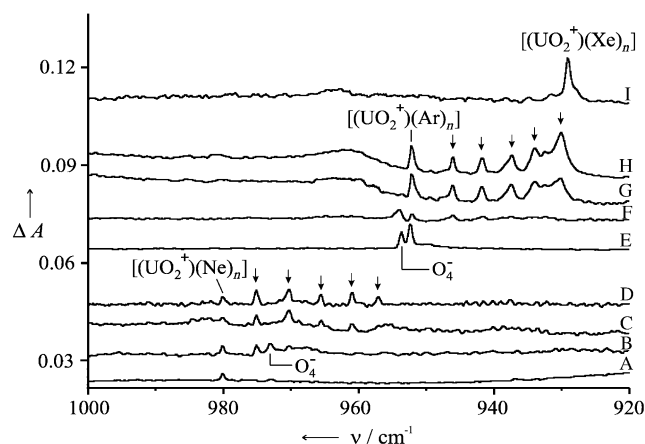


Figure 1. Infrared spectra in the 1000–900 cm^{−1} region for UO₂⁺ in various solid noble-gas environments at 4 K. Samples were co-deposited with laser-ablated uranium. A) Neon with 0.01 % O₂ after annealing to 10 K. B) Neon with 0.1 % O₂ and 0.3 % Ar. C) After annealing to 14 K. D) After annealing to 14.5 K. E) Argon with 0.4 % O₂ after annealing to 20 K. F) Argon with 0.1 % and 6 % Xe. G) After annealing to 40 K. H) After annealing to 45 K. I) Xenon with 0.2 % O₂ after annealing to 40 K. Arrows denote new product absorptions.

952.3 cm^{−1} absorption of UO₂⁺ in pure argon^[13] illustrated in spectrum E.

Xenon was allowed to interact with UO₂⁺ in excess argon. The 952.3 cm^{−1} [(UO₂)(Ar)_n]⁺ band was observed as before, and the O₄[−] band shifted to 953.7 cm^{−1}.^[19] Annealing and increasing the Xe concentration evolved a set of five new absorptions at 946.1, 941.5, 937.2, 933.8 and 930.6 cm^{−1}, spectra F, G, and H, which terminate before the pure xenon absorption at 929.0 cm^{−1} shown in spectrum I. A similar experiment with ¹⁸O₂ gave five new analogous bands shifted to lower frequencies with ¹⁶O/¹⁸O ratios of 1.0528 ± 0.0001.

Infrared spectra were recorded for UO₂⁺ in neon doped with Kr and with Xe and in argon doped with Kr, and the spectra are similar to those shown in Figure 1. Infrared absorptions for UO₂⁺ in various noble gas environments are presented in Table 1.

Relativistic DFT calculations were performed for [(UO₂)(Ng)_n]⁺ (Ng = Ne, Ar, Kr, Xe; n = 0–7), [(UO₂)(Ne)₄(Ng')]⁺ and [(UO₂)(Ne)(Ng')₄]⁺ (Ng' = Ar, Kr, Xe) complexes by using the Amsterdam Density Functional (ADF) program^[20] with the ZORA Hamiltonian,^[21] the PW91 functional,^[22,23] and triple-zeta plus two polarization functions (TZ2P) basis sets for U and Ng.^[24] To calibrate these results

Table 1: Infrared absorptions for UO₂⁺ in various solid noble-gas environments.

Host	ν[cm ^{−1}]	Guest	ν[cm ^{−1}]
Ne	980.1	Ar ^[a]	975.1, 970.3, 965.5, 961.0, 957.1
Ne	980.1	Kr ^[b]	972.6, 967.3, 964.6, 959.8
Ne	980.1	Xe ^[c]	970.0, 960.0, 952.5, 940.0
Ar	952.3	Kr ^[d]	949.4, 947.3, 945.5, 943.3, 941.6
Ar	952.3	Xe ^[e]	946.0, 914.5, 937.5, 933.8, 930.6 ^[f]

[a] Ar (0.1, 0.3, 3 %). [b] Kr (0.3, 1, 2 %). [c] Xe (0.3, 1, 3 %). [d] Kr (5, 8, 12 %). [e] Xe (2, 3, 6, 8 %). [f] Frequencies, ± 0.2 cm^{−1}.

additional ab initio CCSD(T) calculations were carried out for UO₂⁺ and the model complexes [(UO₂)(Ng)]⁺ (Ng = Ne, Ar, Kr, Xe) by using the MOLPRO program^[25] with the Hay–Martin effective core potentials and basis sets for U^[26] and the Stuttgart pseudopotentials and basis sets for the O and Ng atoms.^[27] UO₂⁺ is an f¹ complex with a ²Φ_u ground state in the absence of spin–orbit coupling. The ground states of all the noble-gas complexes of UO₂⁺ are derived from this same state; unlike CUO, there is no significant change in the ground state upon coordination of Ng atoms. The calculated ground-state geometries, total binding energies (−ΔE for UO₂⁺ + nNg → [(UO₂)(Ng)_n]⁺), and vibrational frequencies are listed in Table 2. Note that the optimized U–O and U–Ng

Table 2: Predicted geometry parameters, binding energies, and stretching vibrational frequencies of UO₂⁺, [(UO₂)(Ng)]⁺, and [(UO₂)(Ng)_n]⁺ (Ng = Ne, Ar, Kr, Xe).^[a,b]

	U–O	U–Ng	E _B	ν _s	ν _{as}	ν _{as} (exp)
UO ₂ ⁺	1.773(1.789)	–	0.0	911	987	–
UO ₂ (Ne) ⁺	1.773(1.790)	3.08(2.83)	2.6(3.4)	909	986	–
UO ₂ (Ar) ⁺	1.778(1.792)	3.04(3.06)	7.0(7.9)	902	979	–
UO ₂ (Kr) ⁺	1.778(1.793)	3.14(3.18)	9.5(9.2)	900	976	–
UO ₂ (Xe) ⁺	1.781(1.794)	3.28(3.36)	12.7(12.1)	895	971	–
UO ₂ (Ne) ₆ ⁺	1.776	3.24	13.5	906	985	980.1
UO ₂ (Ar) ₅ ⁺	1.788	3.15	28.2	884	958	952.3
UO ₂ (Kr) ₅ ⁺	1.791	3.29	35.4	878	953	940.6
UO ₂ (Xe) ₅ ⁺	1.794	3.49	42.9	869	943	929.0

[a] The bond lengths are in Å, binding energies in kcal mol^{−1}, and frequencies in cm^{−1}. [b] The optimized CCSD(T) U–O bond lengths and binding energies are listed in parenthesis for comparison.

(Ng = Ne, Ar, Kr, Xe) distances and binding energies of UO₂⁺ and [(UO₂)(Ng)]⁺ from the PW91 DFT calculations are in reasonably good agreement with those from the CCSD(T) calculations, and the calculated frequencies also agree well with the experiments.

The total DFT binding energies for four series of Ng complexes are shown Figure 2. We see that the n = 6 complex is most stable for Ne, while the n = 5 complexes appear to be

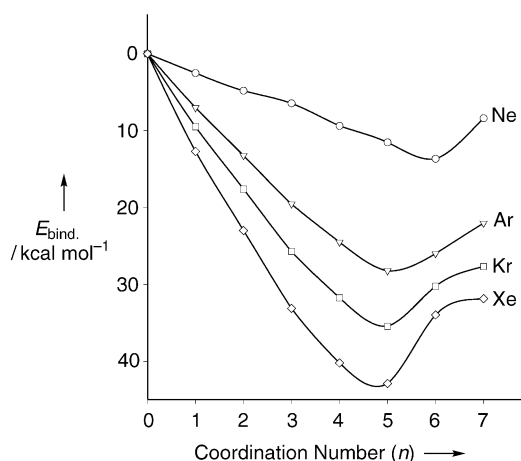


Figure 2. Total binding energy curves calculated for [(UO₂)(Ng)_n]⁺ (Ng = Ne, Ar, Kr, Xe) complexes by using relativistic density functional theory.

the most stable for Ar, Kr, and Xe. Our calculations indicate that all the ground states of these $[(\text{UO}_2)(\text{Ng})_n]^+$ species are derived from the $\text{UO}_2^+ {}^2\Phi_u$ state. Even though the states derived from the ${}^2\Delta_u$ state of UO_2^+ are only $\approx 2 \text{ kcal mol}^{-1}$ higher in energy, spin-orbit coupling will favor ${}^2\Phi_u$ as the ground state. Note also that at the scalar-relativistic DFT level, replacement of six Ne atoms with five Ar atoms lowers the energy by 15 kcal mol^{-1} , replacement of Ar with Kr lowers the energy by another 7 kcal mol^{-1} , and replacement of Kr with Xe lowers the energy by an additional 7 kcal mol^{-1} . The calculated binding energies of $[(\text{UO}_2)(\text{Ne})_4(\text{Ng}')^+]$ ($\text{Ng}' = \text{Ar, Kr, Xe}$) are larger than the binding energy of $[(\text{UO}_2)(\text{Ne})_6]^+$, thus indicating that replacing two Ne atoms in the first coordination sphere with one heavier Ng atom is energetically beneficial. This observation explains why only 0.3% Ar (or Kr or Xe) in neon leads to the formation of complexes with heavier Ng' atoms. Furthermore, as shown by the calculations on the $[(\text{UO}_2)(\text{Ne})_4(\text{Ng}')^+]$ and $[(\text{UO}_2)(\text{Ne})(\text{Ng}')_4]^+$ complexes, substitution of Ne atoms in $[(\text{UO}_2)(\text{Ne})_6]^+$ by heavier Ng' atom(s) is energetically favorable. The theoretically optimized structure for the $[(\text{UO}_2)(\text{Ne})_4(\text{Ar})]^+$ complex is illustrated in Figure 3.

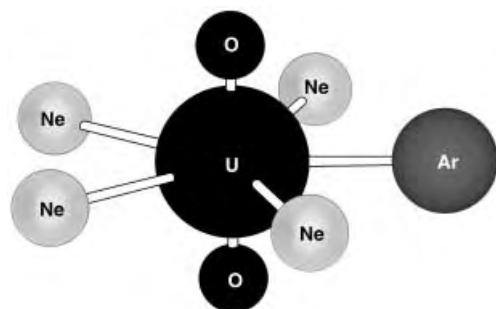


Figure 3. Structure optimized for the $[(\text{UO}_2)(\text{Ne})_4(\text{Ar})]^+$ cation complex by using relativistic density functional theory.

Several chemical comparisons can be made for the observed and calculated frequencies. First, the predicted strong infrared active antisymmetric stretching frequencies shift from 987 cm^{-1} for ${}^2\Phi_u \text{ UO}_2^+$ to 985 cm^{-1} for $[(\text{UO}_2)(\text{Ne})_6]^+$, and to 958 , 953 , and 943 cm^{-1} for $[(\text{UO}_2)(\text{Ng})_5]^+$ ($\text{Ng} = \text{Ar, Kr, Xe}$), in good agreement with experimental data. The calculated frequency shifts for substituting two Ne atoms with one Ar, Kr, and Xe atom in the first coordination shell of UO_2^+ are 8 , 13 , and 14 cm^{-1} , in accord with the experimentally determined shifts of 5.0 , 7.4 , and 10.1 cm^{-1} . Shifts of 5.6 , 7.8 , and 10.7 cm^{-1} in the U–O stretching mode have been observed for the analogous $(\text{CUO})(\text{Ne})_{n-1}(\text{Ng}')$ complexes.^[5,6] On the other hand, smaller 2.9 and 6.3 cm^{-1} shifts are found for $[(\text{UO}_2)(\text{Ar})_{n-1}(\text{Ng}')^+]$ with Kr and Xe substitution, similar to what is observed for $(\text{CUO})(\text{Ar})_{n-1}(\text{Ng}')$.^[3,4]

The combination of experimental and theoretical results presented here provide strong evidence that UO_2^+ coordinates five heavy noble-gas atoms in forming complexes. In each of the mixed noble-gas experiments, five new IR bands are observed, which we propose are due to the stepwise

formation of $[(\text{UO}_2)(\text{Ng})_{5-n}(\text{Ng}')_n]^+$ ($n = 1, 2, 3, 4, 5$) complexes.^[28] The calculations predict maximum stability for UO_2^+ coordinated by five heavy Ng atoms, and the calculated frequency shifts for the five-coordinate complexes are in very good agreement with the observed shifts.

Based on a comparison of the binding energies, the U–Ng bonds in the Ng complexes of UO_2^+ are about twice as strong as those we reported for CUO, largely because of the combination of electron-donation and ion-induced dipole interactions. The calculated binding energies are also much larger than those for neutral $[(\text{UO}_2)(\text{Ng})_n]$.^[29] The interactions of noble-gas atoms with the uranyl ion, UO_2^{2+} , may be strong enough to form stable and isolable complexes by analogy to $[\text{AuXe}_4]^{2+}$.

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Glycopeptides (1)

**In Pursuit of Carbohydrate-Based HIV Vaccines,
Part 1: The Total Synthesis of Hybrid-Type gp120
Fragments*****Mihirbaran Mandal, Vadim Y. Dudkin, Xudong Geng,
and Samuel J. Danishefsky**

Notwithstanding enormous scientific effort, the development of a vaccine against HIV has, thus far, proven to be elusive.^[1] To date, commonly utilized vaccine formulations have been

unable to elicit potent and broadly neutralizing immune responses.^[2] The very high rate of viral variation is often cited as the major impediment to vaccine design. Another serious complication is the very low immunogenicity of the protein domain of the viral surface envelope protein gp120. This phenomenon could be explained by a number of factors.^[3] Extensive glycosylation of this envelope protein, gp120, is arguably the most effective viral defense mechanism.^[4,5] Indeed, gp120 is typically modified with 24 carbohydrate motifs, which render most of its polypeptide architecture virtually inaccessible to the immune system.^[6]

Our thought was that these glycans could themselves serve as targets for an anti-HIV vaccine. In favor of exploring such a course is the fact that some of the glycans are highly conserved, and are located on the outer side of the gp120 trimer—a positioning that could well enhance their accessibility. Thus, if a suitable construct, capable of eliciting a focused immune response to gp120 glycans could be designed and synthesized, a key element of the HIV defense system would have been targeted, and an effective vaccine could be at hand. Indeed, some agents that bind gp120 glycans (e.g. the dendritic cell receptor DC-SIGN and the bacterial protein cyanovirin-N) are actually presently known.^[7,8]

Further support for this line of attack arose from investigations on the epitope structure of 2g12, one of the most potent broadly neutralizing antibodies known to date.^[9,10] Alanine scanning mutagenesis demonstrated that the presence of a number of glycan-carrying asparagines in gp120 is essential to antibody binding.^[11] It was initially suggested that glycans at Asn295 and 332 may form the antibody epitope. In a separate account, the nature of the glycans crucial to 2g12 binding was elucidated. Experiments with a range of mannosidases and endoglycosidases prompted the conclusion that hybrid or high-mannose-type glycans on the gp120 comprise the 2g12 epitope-forming carbohydrates.^[12] These findings provide a conceptual launching point for designing an HIV vaccine.

Even in the face of spectacular advances in the field of biotechnology,^[13] it is our judgment that, at the present time, chemical synthesis constitutes the *only* prospect for building strictly homogeneous glycopeptides of suitable complexity in the required amounts and with the structural diversity needed to support such a vaccine-targeting discovery program.^[14–16] Herein, we describe the first synthesis of a hybrid-type nonasaccharide and its incorporation into HIV gp120^{316–335} N-linked glycopeptides.

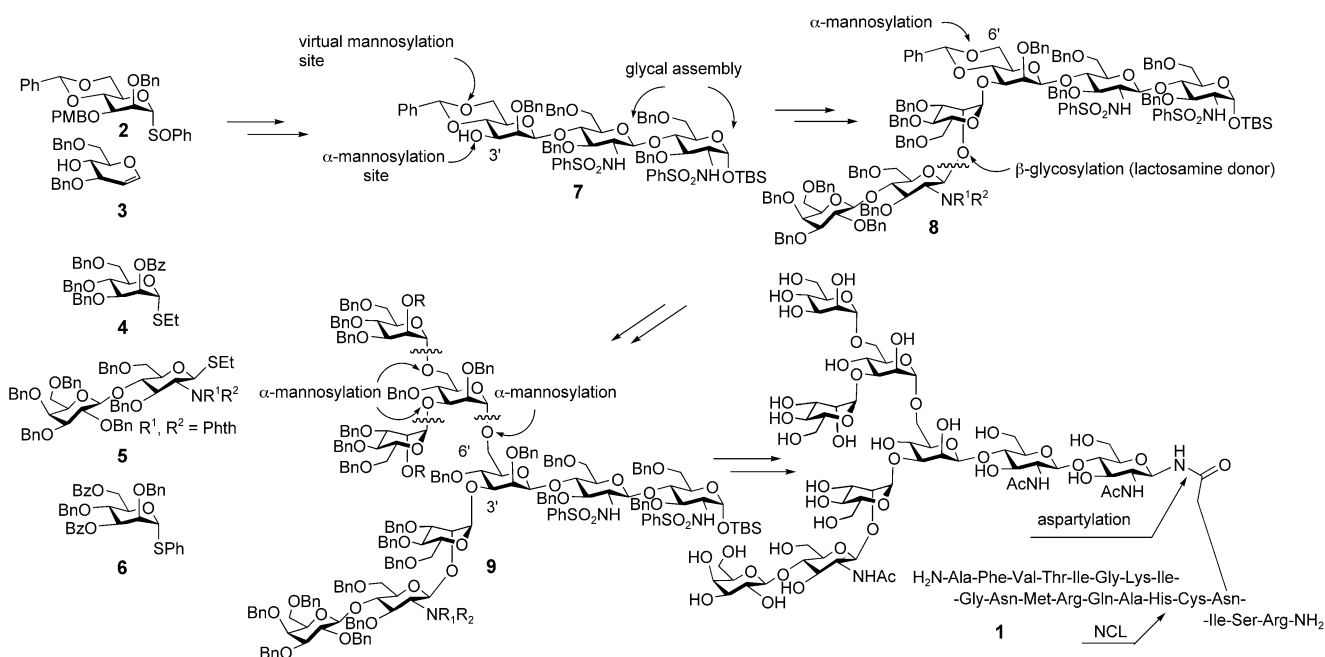
We commence by setting forth the essentials of the underlying logic of the strategy. Synthesis of the glycan (Scheme 1) would start with the preparation of trisaccharide **7**, which contains “virtual” and “in-place” acceptor points on the terminal mannose unit. The plan takes recourse to glycal assembly methods that we developed in the past.^[17,18] Applicability of the methods to the problem at hand gained enormously from the very powerful β -mannosylation procedure introduced by Crich and co-workers.^[19,20] The “lower” (C3') branching mannose would first be installed, followed by glycosylation with a lactosamine donor (see **7**→**8**). Opening of the benzylidene ring would then provide the opportunity to install the “upper” high-mannose cluster at C6' (**8**→**9**).

[*] Dr. M. Mandal, Dr. V. Y. Dudkin, Dr. X. Geng, Prof. S. J. Danishefsky
Laboratory for Bioorganic Chemistry
Sloan–Kettering Institute for Cancer Research
1275 York Ave, New York, NY 10021 (USA)
Fax: (+1) 212-772-8691
E-mail: s-danishefsky@ski.mskcc.org

Prof. S. J. Danishefsky
Department of Chemistry
Columbia University
Havemeyer Hall 3000 Broadway, New York, NY 10027 (USA)

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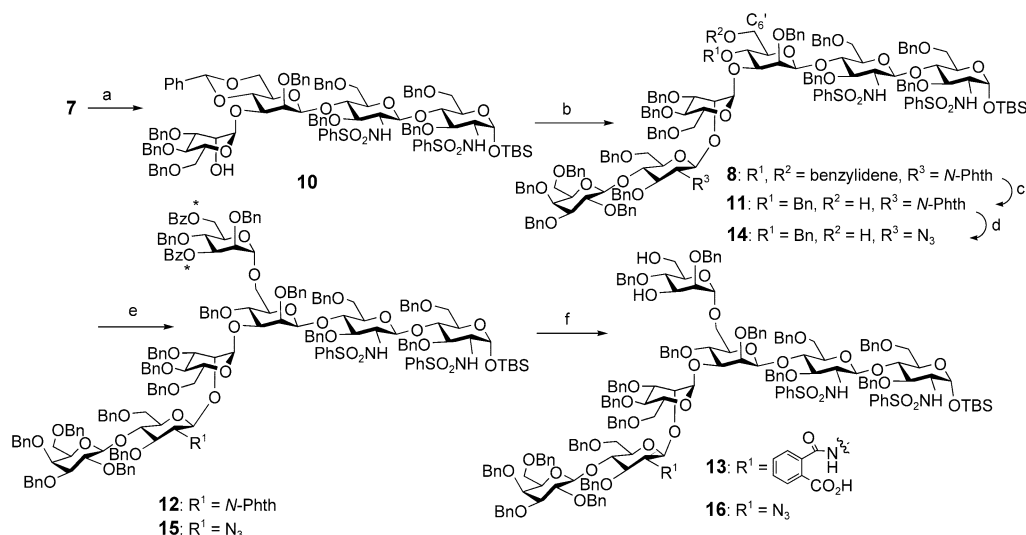
Supporting information for this article is available on the WWW under <http://www.angewandte.org> or from the author.



Scheme 1. Synthetic strategy for the preparation of glycopeptide **1**. PMB = *p*-methoxybenzyl; TBS = *tert*-butyldimethylsilyl; Phth = phthaloyl. NCL = native chemical ligation.

We had earlier documented the use of an amination-aspartylation native chemical ligation (NCL) sequence (drawing from earlier findings by the groups of Kochetkov,^[21] Lansbury,^[22] and Kent and Muir^[23]) for the synthesis of large N-linked glycopeptides of high complexity.^[24–26] The 331 Cys adjacent to the aspartylation site (332) in gp120 could provide a convenient disconnection point for NCL. Accordingly, we sought initially to prepare a glycan pentapeptide construct expecting to use it in conjugation with the pentadecapeptide thioester (Scheme 1).

In the event, the progression began with the trisaccharide acceptor **7**,^[27] which was prepared from 3,6-dibenzylglucal **3** and the sulfoxide donor **2**.^[20] Glycosylation of **7** with donor **4** by using the Sinaý radical activation conditions^[28] proceeded uneventfully to afford the tetrasaccharide in 80% yield. Debenzoylation provided alcohol **10** and set the stage for coupling with the lactosamine thio donor **5** (Scheme 2). Hexasaccharide **8** was obtained in 60% yield, thereby completing the synthesis of the “lower” part of the glycan (projecting from C3' of the initial trisaccharide) in an efficient



Scheme 2. Synthesis of heptasaccharides **13** and **16**; a) **1**, $(\text{BrC}_6\text{H}_4)_3\text{NSbCl}_6$, MeCN, 80%; **2**, NaOMe/MeOH, 89%; b) **5**, $(\text{BrC}_6\text{H}_4)_3\text{NSbCl}_6$, MeCN, 60%; c) $\text{BH}_3\cdot\text{THF}$, Bu_2BOTf , CH_2Cl_2 , 75%; d) **1**, $\text{NH}_2\text{CH}_2\text{CH}_2\text{NH}_2$, toluene, 90°C; **2**, TfN_3 , MeOH/ CH_2Cl_2 , 50%; e) **6**, $(\text{BrC}_6\text{H}_4)_3\text{NSbCl}_6$, MeCN, 58% for **12**, and 48% for **15**; f) NaOMe/MeOH, 62% for **16**. Tf = trifluoromethanesulfonyl.

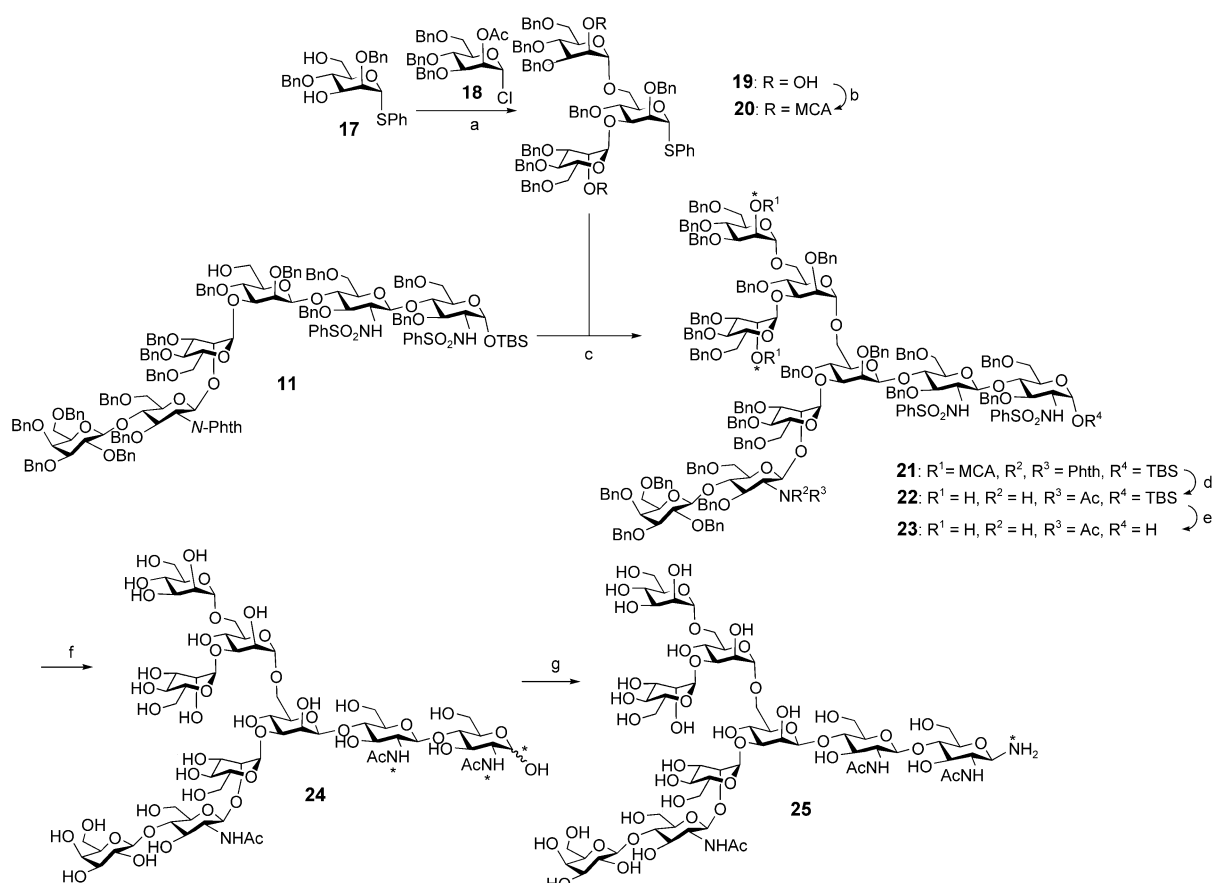
manner. To incorporate the high mannose segment, the primary hydroxy group (C6') in **8** was liberated by regioselective opening of the 4,6-benzylidene acetal with Bu₂BOTf/BH₃,^[29] and the resulting acceptor, **11**, was glycosylated with diester thio donor **6** to give **12** in 58% yield.

Saponification of the two benzoates in **12** (see asterisks) followed by dimannosylation of the corresponding diol would have completed the nonasaccharide assembly. However, this seemingly routine deprotection step, when conducted in the multifaceted setting of **12**, turned out to be problematic. In particular we found that the cleavage of the second benzoate group in **12** was very slow, and further complicated by concurrent hydrolysis of the phthalimide group (giving **13**). To circumvent this problem, the phthalimide group of **11** was replaced by an azide function to give **14**,^[30] which was coupled with **6** to provide heptasaccharide **15** in 48% yield.

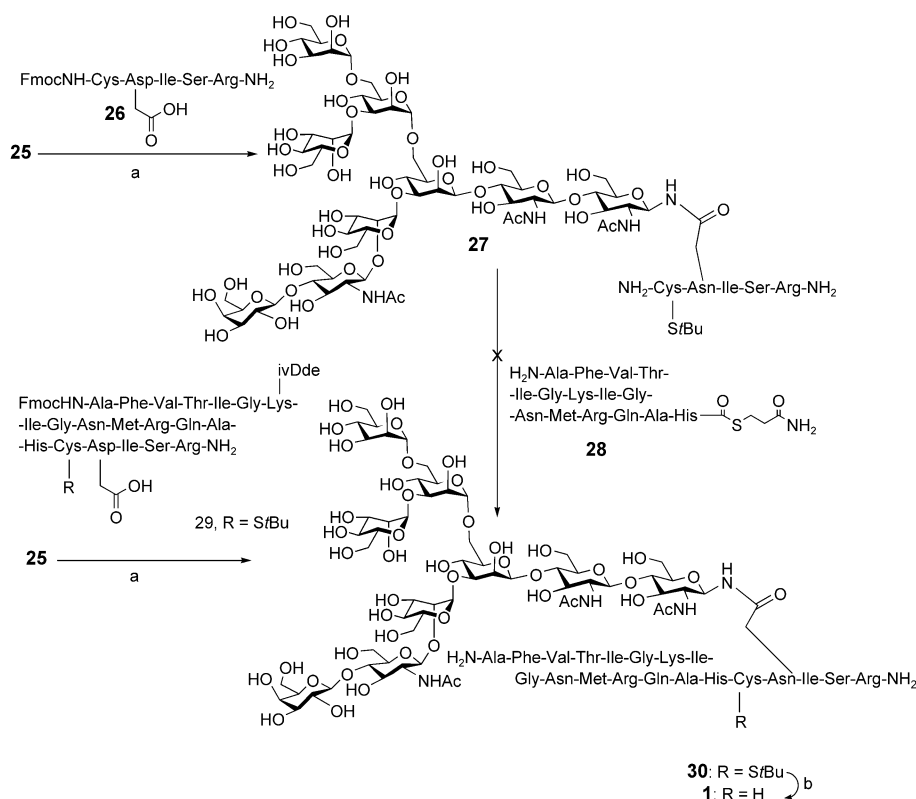
Benzoate deprotection in the presence of excess NaOMe now proceeded smoothly in 62% yield. Although the phthalimide instability problem had been overcome by recourse to an azide function at the level of **11**, we decided to explore a still more convergent approach, featuring condensation of a preassembled trimannose block with the primary hydroxy group in **11**, retaining the intact phthalimide group (Scheme 3).

The required trisaccharide was prepared from thiomannoside diol **17** and chloride **18** in 65% yield following the removal of the two acetates. The resulting alcohols were reprotected as their monochloroacetates in 79% yield, anticipating that the MCA protecting groups could be selectively removed without affecting phthalimide group. In this way, clean access to the required branching point acceptor sites (see asterisks in **21**) would have been provided.

After optimization of the coupling conditions, it was found that the glycosylation of hexamer acceptor **11** with trisaccharide donor **20** proceeded in 78% yield to afford the nonasaccharide **21**. The MCA protecting groups were then quantitatively removed by thiourea. This step was followed by conversion (see structure **22**) of the phthalimide group into the required acetamide function and liberation of anomeric hydroxy site with TBAF/AcOH. Compound **23**, bearing the free reducing end, was then subjected to global deprotection with sodium in liquid ammonia. At this stage we exploited the remarkable stability of the anomeric reducing end (see asterisk) to a massive excess of sodium—ammonia, which was reported earlier by our group.^[31–33] The free amines in the product were then acetylated with acetic anhydride in saturated NaHCO₃ (see asterisks) to afford the glycan **24** bearing the anomeric OH (see asterisk) in 70% yield.



Scheme 3. Synthesis of free glycosyl amine **25**; a) 1. AgOTf, DTBP, CH₂Cl₂, 2. NaOMe/MeOH, 65%; b) (ClCH₂CO)₂O, py, CH₂Cl₂, 79%; c) (BrC₆H₄)₃NSbCl₆, MeCN, 78%; d) 1. NH₂CH₂CH₂NH₂, *n*-BuOH/toluene, 90 °C; 2. Ac₂O, py; 3. NaOMe/MeOH, 69%; e) TBAF/AcOH, 89%; f) 1. Na/NH₃, −78 °C, 2. NaHCO₃, Ac₂O, 70%; g) NH₄HCO₃/H₂O. DTBP = 2,6-di-*tert*-butylpyridine; MCA = monochloroacetate; TBAF = tetra-*n*-butylammonium fluoride.



Scheme 4. Synthesis of glycopeptide; a) 1. HATU, DIPEA, DMSO; 2. NH_2NH_2 , piperidine, DMF, 30% for **27** and 20% for **30**; b) MES-Na, DMF, DIPEA, H_2O , 95%. HATU = *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate; DIPEA = *N,N*-diisopropylethylamine; DMSO = dimethyl sulfoxide; DMF = *N,N*-dimethylformamide; Fmoc = 9-fluorenylmethyloxycarbonyl; ivDde = 4,4-dimethyl-2,6-dioxocyclohex-1-ylidene-3-methylbutyl.

Compound **25** bearing the free anomeric amine, (see asterisk) was obtained by following the Kochetkov amination procedure^[21] and was used for peptide coupling without further purification.

Coupling of **25**, with excess pentapeptide **26**, under previously developed conditions^[24] was followed by cleavage of the Fmoc group to afford glycopeptide **27** (30% yield for two steps). Unfortunately, despite numerous attempts, we were unable to produce **1** by native chemical ligation of **27** and **28** (Scheme 4).^[34] This unexpected failure obliged us to investigate the possibility of direct coupling of the non-asaccharide amine **25** and eicosapeptide **29**. The latter was prepared by using automated Fmoc synthesis.^[35] We were pleased to find that conjugation of such large substrates is in fact feasible, though not yet efficient. In the event, glycopeptide **30** was obtained in 20% yield following the removal of Fmoc and ivDde groups from the resulting glycopeptide.

Deprotection of the SiBu blocking the sulfhydryl moiety of Cys in **30** was the only step remaining for the preparation of **1**. Cleavage of this disulfide bond proved to be unexpectedly challenging. All attempts to cleave the disulfide of **30** by using MES-Na (2-sulfanylanethanesulfonic acid, sodium salt),^[36,37] DTT (dithiothreitol),^[38–40] or TCEP (tris(2-carboxyethyl)-phosphane hydrochloride)^[41–43] as reducing agents in phosphate or guanidine buffer either led to no reaction or provided complex mixtures.

Finally, we attempted to carry out the reduction in organic solvent. When the glycopeptide **30** was stirred with MES-Na in DMF with diisopropylethylamine as base at room temperature, the reduction proceeded in quantitative conversion and the corresponding product **1** was isolated in homogeneous form (NMR and LC-MS analyses^[44]) in 95% yield after purification by HPLC. Full characterizations are provided in the Supporting Information.

In summary, we have, for the first time, synthesized mature hybrid type HIV gp120 glycopeptide fragments.^[45] This project is now at the stage of optimal conjugation to various immunogens and en route to detailed exploration of the immunological issues discussed above.

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Keywords: antigens · carbohydrates · glycoconjugates · glycopeptides · synthetic methods

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- [45] In the following paper in this issue, we describe our efforts for the synthesis of high-mannose-type HIV gp120 glycopeptides fragments: X. Geng, V. Y. Dudkin, M. Mandal, S. J. Danishefsky, *Angew. Chem.* **2004**, 116, 2616–2619; *Angew. Chem. Int. Ed.* **2004**, 43, 2562–2565.

Glycopeptides (2)

In Pursuit of Carbohydrate-Based HIV Vaccines, Part 2: The Total Synthesis of High-Mannose-Type gp120 Fragments—Evaluation of Strategies Directed to Maximal Convergence**

Xudong Geng, Vadim Y. Dudkin, Mihirbaran Mandal, and Samuel J. Danishefsky*

There are strong grounds to suppose that some selected glycosylation patterns of the HIV viral protein gp120 can themselves serve as epitopes for potent, broadly neutralizing antibodies (e.g. 2g12).^[1,2] The epitopes in question may comprise several hybrid or high-mannose-type glycans at particular asparagine loci (Asn295, 332, 339, 386, and 392). The 2g12 antibody has been shown to recognize a cluster of α 1 $\rightarrow$ 2 linked mannose residues on the HIV surface. Another argument in favor of the high-mannose-type glycan cluster epitope was reported by Burton, Wilson, and co-workers.^[3] These workers described a structure of 2g12 cocrystallized with the high-mannose-type reducing oligosaccharide Man₉-GlcNAc₂. The crystal structure demonstrated that the antibody may bind up to four individual high-mannose glycans simultaneously, thus favoring a very high affinity recognition. Accordingly, a synthetic construct that is able to elicit a strong immune response to a conserved cluster of gp120 high-mannose glycans could potentially emerge as a valuable candidate for incorporation into an HIV vaccine. In the preceding paper,^[4] we related a strategy for the construction of a hybrid type gp120 glycopeptide construct.

Herein we describe the synthesis of gp120 fragments comprising one of key asparagine sites (332) modified with a fully synthetic high-mannose glycan. Although the nonmannose section of the molecule was previously prepared and tested in binding with cyanovirin-N,^[5–7] no total chemical

synthesis of any Man₉GlcNAc₂ containing glycopeptides has been reported.^[8]

In our route to the glycan portion of the glycopeptide, we utilized, as proposed earlier, trisaccharide **2**,^[9] which already encompasses the synthetically difficult β -mannosidic linkage, as well as differentiated C3 and C6 access points (see asterisks) for the subsequent introduction of the nonsymmetrical mannose branching pattern.

From this point onward, two strategies for progression to the octamannose motif presented themselves. One strategy would start with two consecutive mannosylations of the 3-OH and 6-OH groups of **2**, employing mannoside donors **3** and **4**, respectively, to complete the first “mannose layer”. In turn, the second “layer” of three mannose units would be introduced by triple mannosylation of the pentasaccharide triol acceptor with mannoside donor **3**, providing the Man-6 octasaccharide. Saponification of the esters followed by the introduction of another trimannose layer should provide the desired Man-9 undecamer glycan (Scheme 1; “layered approach”).

Alternatively, one would construct the “upper” pentamannose **5** and “lower” trimannose **6** building blocks separately, followed by coupling them with the key trisaccharide **2** at the “real” (C3) and the “virtual” (C6) acceptor sites (see asterisks), thus reaching the undecamer **7b** in a highly convergent fashion (Scheme 1; “block approach”).

With the glycan matrix assembled, the next phases of the program would involve global deprotection^[10] followed by amination at the anomeric site.^[11] We initially envisioned that a small (penta)peptide would be introduced by aspartylation.^[12,13] Finally, native chemical ligation (NCL) would complete the synthesis of **1** (Scheme 1), paving the way for conjugation to a carrier immunogen en route to fashioning a testable vaccine.^[14,15]

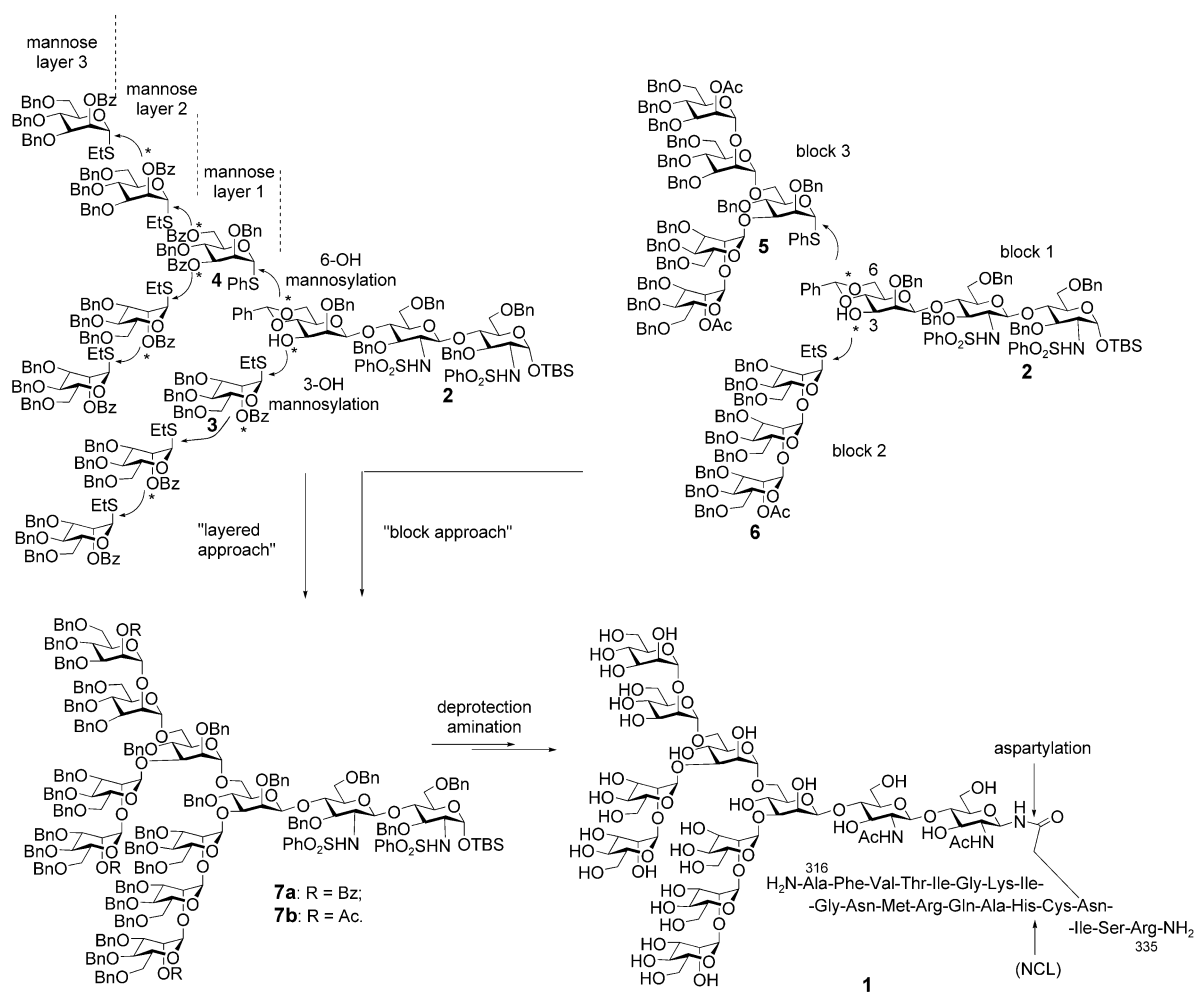
The “layered” approach was explored first (Scheme 2). Glycosylation of the 3-OH group of trisaccharide **2** with ethylthiomannoside donor **3** under the Sinaÿ radical activation conditions^[16] gave tetrasaccharide **8** bearing the benzylidene group spanning C4 and C6. The acetal linkage was opened in a reductive fashion to afford tetrasaccharide **9**. The primary hydroxy group of **9** was in turn mannosylated with phenylthiomannoside **4**. Saponification of the resulting pentasaccharide **10** exposed the three required acceptor sites (see asterisks). Trimannosylation of **11** delivered octasaccharide **12** in high yield (55%). This protocol (saponification followed by trimannosylation) was repeated to synthesize the desired protected undecasaccharide **7a**.

Having demonstrated that the protected undecasaccharide could be assembled by the “layered approach” in an efficient manner (7 steps, 11% overall yield), we explored a still more convergent “block approach” (Scheme 3). Pentasaccharide block **5** was assembled efficiently through two consecutive dimannosylation reactions starting from phenylthiol mannoside **14** and chloromannose donor **15**. The “lower” two trisaccharide “blocks” were joined by a MeOTf-mediated glycosylation to afford hexasaccharide **18** efficiently. Reduction of **18** released the primary hydroxy group to give **19**, which was then subjected to a 6+5 glycosylation with donor **5**. Following the examination of

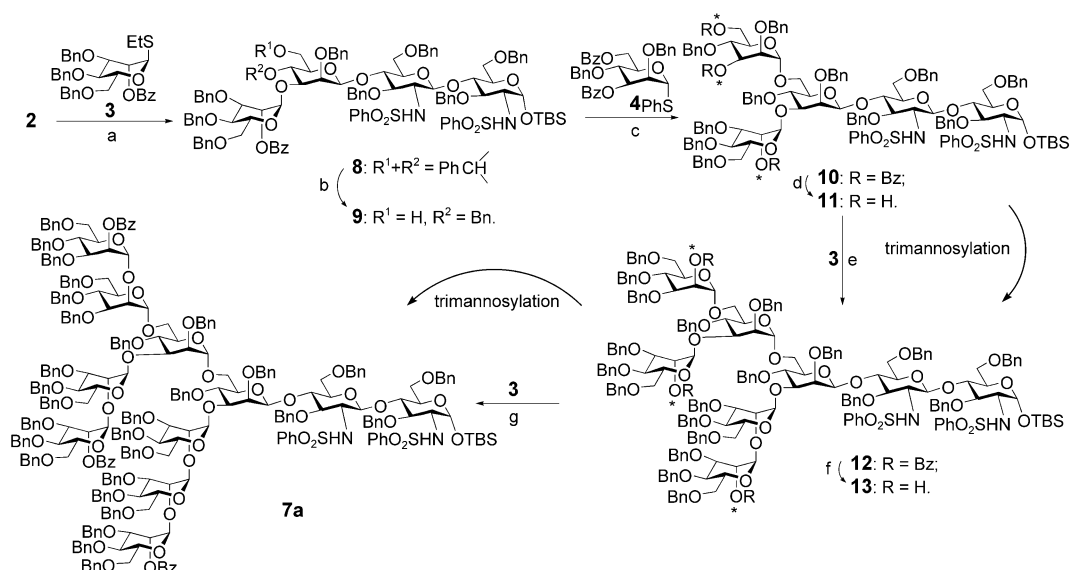
[*] Dr. X. Geng, Dr. V. Y. Dudkin, Dr. M. Mandal, Prof. S. J. Danishefsky
Laboratory for Bioorganic Chemistry
Sloan-Kettering Institute for Cancer Research
1275 York Avenue, New York, NY 10021 (USA)
Fax: (+1) 212-772-8691
E-mail: s-danishefsky@ski.mskcc.org
and
Department of Chemistry
Columbia University
Havemeyer Hall, 3000 Broadway, New York, NY 10027 (USA)

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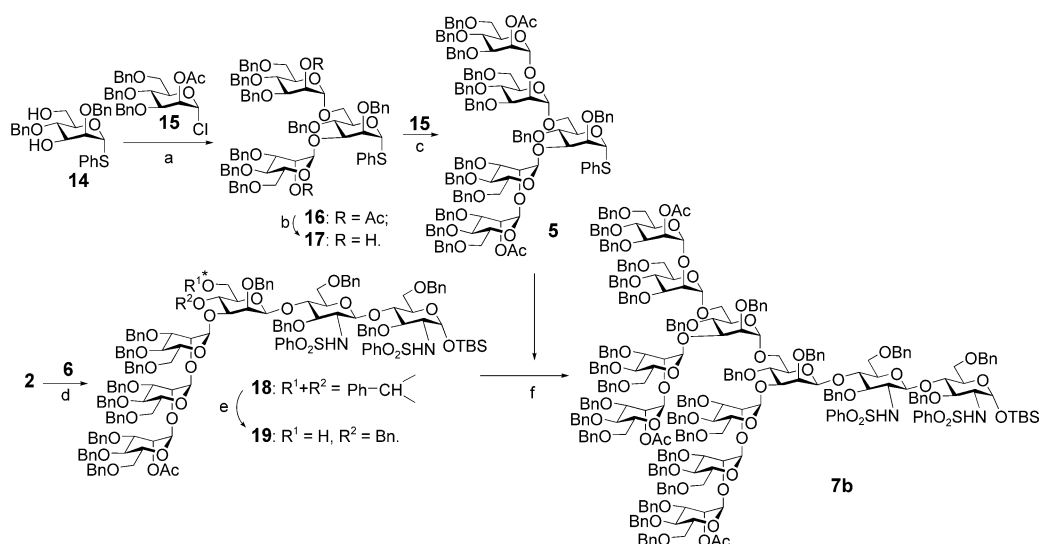
Supporting information for this article is available on the WWW under <http://www.angewandte.org> or from the author.



Scheme 1. Synthetic strategy for the assembly of the gp120 glycopeptide fragments. TBS = *tert*-butyldimethylsilyl.



Scheme 2. Synthesis of Undecasaccharide **7a** through the "layered" approach. a) **3**, (BrC₆H₄)₃NSbCl₆, CH₃CN, 4 h, 78%; b) BH₃, Bu₂BOTf, THF, 0°C, 7 h, 90%; c) **4**, (BrC₆H₄)₃NSbCl₆, CH₃CN, 4 h, 74%; d) NaOMe, MeOH, 12 h, 91%; e) **3**, (BrC₆H₄)₃NSbCl₆, CH₃CN, 12 h, 55%; f) NaOMe, MeOH, 12 h, 84%; g) **3**, (BrC₆H₄)₃NSbCl₆, CH₃CN, 12 h, 51%. Tf = trifluoromethanesulfonyl.

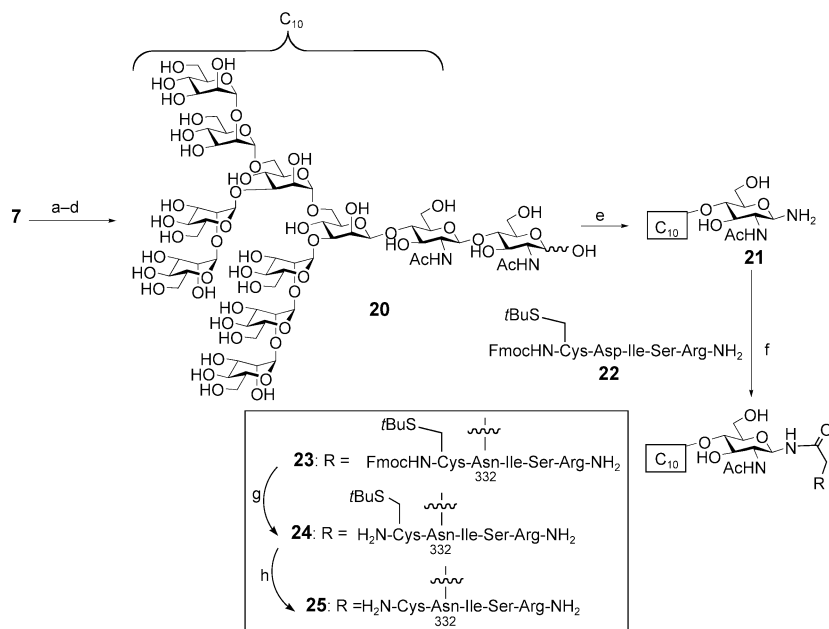


Scheme 3. Synthesis of undecasaccharide **7b** through “block” approach. a) **15**, AgOTf, DTBP, CH₂Cl₂, –10°C→RT, 18 h; b) NaOMe, MeOH, 10 h, 50% for two steps; c) **15**, AgOTf, DTBP, CH₂Cl₂, –10°C to RT, 18 h; 87%; d) MeOTf, DTBP, CH₂Cl₂, –40°C to RT, 12 h, 70%; e) BH₃, Bu₂BOTf, THF, 0°C, 7 h, 86%; f) **5**, (BrC₆H₄)₃NSbCl₆, CH₃CN, 10 h, 63% (85% based on recovered **19**). DTBP = 2,6-di-*tert*-butylpyridine.

several protocols for coupling, it was found that the Sinaÿ radical activating conditions worked best,^[16] delivering the desired undecasaccharide **7b** in 63% yield (85% based on recovered acceptor **19**). At least in this endeavor, the ultimately convergent “block approach” was indeed shown to be more concise, bringing forward the protected high-mannose **7b** in 51% yield over three steps (starting from **2**).

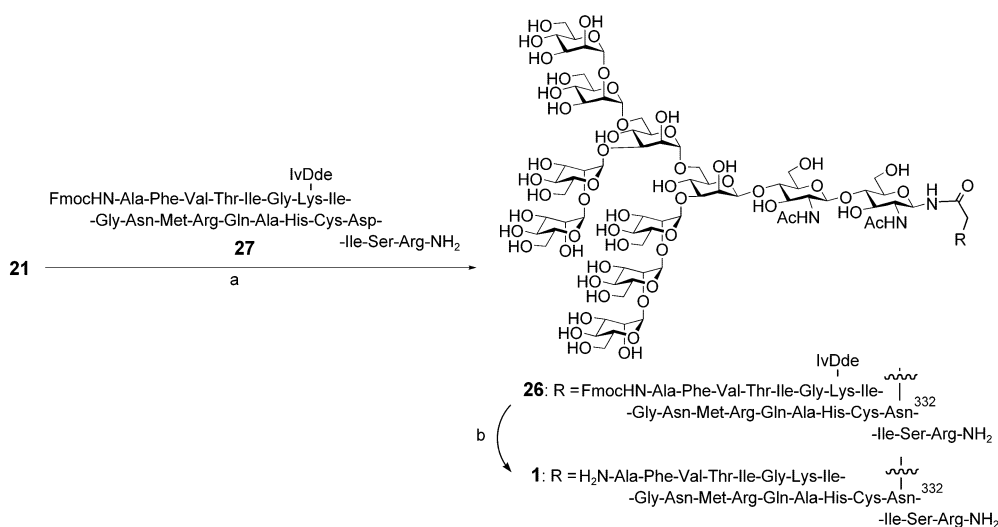
With the protected oligosaccharide in hand, we proceeded to the next phase, global deprotection (Scheme 4). Deacetylation, desilylation, and reduction (dissolving metal) of **7** afforded free glycan **20**.^[10] The latter was advanced to glycosylamine **21** through Kochetkov amination.^[11] This compound was first coupled with gp120^{331–335} pentapeptide segment **22** (bearing Asp with the protected cysteine thiol and the N-terminal amino groups). Following removal of the Fmoc group and reduction of the disulfide, gp120^{331–335} pentapeptide–high-mannose glycan conjugate **25** was in hand.

Our initial expectation was to utilize native chemical ligation to complete the assembly of the gp120^{316–335} peptide–high-mannose glycan conjugate. However, NCL with **24** (thiol on Cys protected) or **25** (free thiol on Cys) failed to deliver the desired gp120^{316–335} peptide–high-mannose glycan conjugate after several attempts. We recall that in the preceding manuscript,^[4] a similar breakdown was noted with the same polypeptide elements and a related complex glycan. Together, these cases underscore unexpected limitations in the applicability of NCL in such highly ornate settings.



Scheme 4. Synthesis of gp120 glycosylated fragments **25**. a) NaOMe/MeOH, 12 h, 96%; b) TBAF, HAc, THF, 0°C, 1 h, 98%; c) Na/NH₃(L), –78°C, 2 h; d) Ac₂O, NaHCO₃ (sat. aqueous solution), 87% for two steps; e) NH₄HCO₃ (sat. aqueous solution), 2 d, 40°C; f) **22**, HATU, DIPEA, DMSO, 7 h; g) piperidine, DMF, 15 min, 24% from **20**; h) HSCH₂CH₂SO₃Na, TCEP, 3 days, 60%. Fmoc = 9-fluorenylmethyloxycarbonyl. TBAF = tetra-*n*-butylammonium fluoride; HATU = O-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate; DIPEA = diisopropylethylamine; DMSO = dimethyl sulfoxide; DMF = *N,N*-dimethylformamide; TCEP = tris(2-carboxyethyl)phosphane hydrochloride.

Fortunately, as reported earlier,^[4] direct coupling with a gp120^{316–335} eicosapeptide fragment **27** (bearing Asp at 332 with protected Lys and the N-terminus amino groups) was feasible (Scheme 5). The desired conjugate gp120^{316–335} peptide–high-mannose glycan, **1**, was isolated following aspartyl-



Scheme 5. Synthesis of gp120 glycosylated fragments **1**. a) **27**, HATU, DIPEA, DMSO, 7 h; b) N₂H₄, piperidine, DMF, 15 min, 16% from **20**. IvDde = 4,4-dimethyl-2,6-dioxocyclohex-1-ylidene-3-methylbutyl.

lation and deprotection in 16% yield over 3 steps. Compound **1** was purified by reverse-phase HPLC and ¹H NMR spectroscopic analysis and MS data^[17] are consistent with the desired structure as a homogeneous entity. Full characterizations are provided in the Supporting Information.

In summary, we have reported the first chemical synthesis of gp120 glycopeptide fragments (high-mannose-type conjugate gp120^{316–335} **1** and gp120^{331–335} **25**). The glycan was assembled through two efficient methods, that is, a “layered approach” and a “block approach”, and then conjugated with gp120 peptide segments through direct aspartylation. In combination with the preceding manuscript,^[4] our total synthesis program provides direct access to mimics of the epitope of broadly neutralizing antibody 2g12, that is, high-mannose and hybrid-type gp120 glycopeptide fragments. With the organic synthesis phase complete, the project has entered the immunogen conjugation phase, en route to a thorough evaluation of the immunological issues discussed earlier.^[4]

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Keywords: antigens · glycoconjugates · glycopeptides · mannosylation · total synthesis

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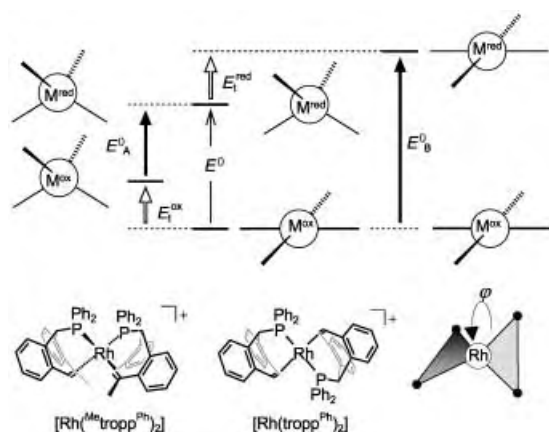
Rhodium Redox Chemistry

Stereochemical Control of the Redox Potential of Tetracoordinate Rhodium Complexes**

Cécile Laporte, Frank Breher, Jens Geier, Jeffrey Harmer, Arthur Schweiger, and Hansjörg Grützmacher*

Dedicated to Professor Helmut Werner on the occasion of his 70th birthday

Understanding the factors which control the redox potential of transition-metal complexes is of fundamental importance for the design of functionality. In classical experiments, the redox potential of $\text{Cu}^{\text{III/I}}$ or Ni^{II} complexes^[2] was shifted substantially to less negative values (i.e. anodic $|E_{\text{A}}^{\text{red}}| < |E^{\text{red}}|$ in Scheme 1) by enforcing tetrahedral structures on the



Scheme 1. Schematic representation of approaches for the stereochemical control over transition-metal-complex redox potentials. Left: Destabilization of the oxidized form $|E_{\text{A}}^{\text{ox}}| < |E^{\text{ox}}|$; right: destabilization of the reduced form $|E_{\text{B}}^{\text{red}}| > |E^{\text{red}}|$.

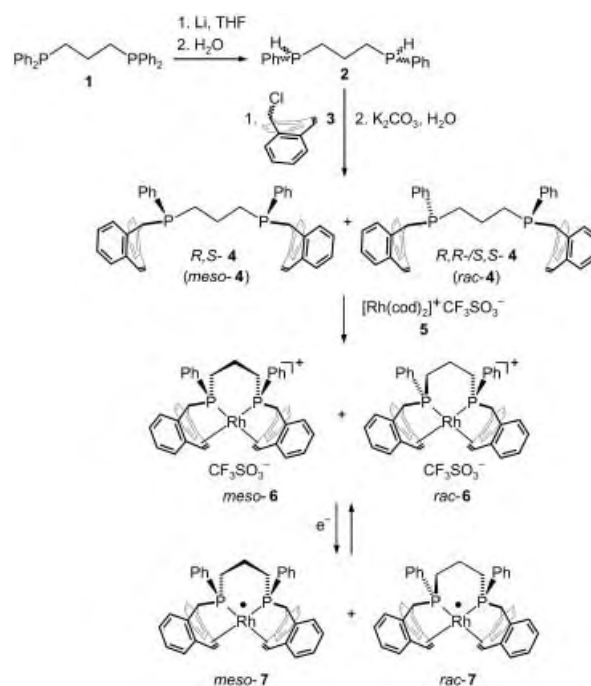
usually planar M^{II} complexes which are destabilized by the torsion energy E_{t}^{ox} . Tetrahedral structures are preferred for the reduced M^{I} complexes. To our knowledge, this concept has not been systematically applied to complexes of the transition metals from the fifth and sixth periods. For such an investigation the redox couple of tetracoordinate $\text{d}^8\text{Rh}^{\text{I}}/\text{d}^9\text{Rh}^{\text{0}}$ complexes are ideally suited because 16-valence-electron-configured d^8 rhodium(I) complexes strongly favor

planar structures while the reduced 17-valence-electron-configured d^9 rhodium(0) complexes prefer a distorted tetrahedral form.^[2]

Longato et al.^[3] and our group^[4] have prepared strongly tetrahedrally distorted tetracoordinate 16-electron rhodium complexes, such as $[\text{Rh}(\text{dppf})_2]^+$ ($\varphi = 49.7^\circ$; $\text{dppf} = 1,1'$ -bis-(diphenylphosphanyl)ferrocene) and $[\text{Rh}(\text{Me-tropp}^{\text{Ph}})_2]^+$ ($\text{tropp}^{\text{Ph}} = 5$ -diphenylphosphanyl-5*H*-dibenzo[*a,d*]cycloheptene; $\varphi = 42.0^\circ$; where φ is the intersection of the planes spanned by the rhodium atom and the two donor atoms of each bischelate ligand) but a significant anodic shift of the reduction potentials was not observed.^[5] The EPR spectra of these rhodium(0) complexes clearly show that the unpaired electron is predominantly located at the metal center.^[3,6]

We therefore thought of another experiment allowing the control the redox potential. A destabilization of the structure of the reduced species in a redox couple by the energy $E_{\text{t}}^{\text{red}}$ should lead to a cathodic shift of the reduction potential, that is, $|E_{\text{B}}^{\text{red}}| > |E^{\text{red}}|$ (Scheme 1; right). For this experiment we used the stereoisomers of a ligand system; one enforcing an unfavorable coordination sphere for the reduced metal complex, and the other one allowing the coordination sphere to adapt to the more favorable geometry on reduction of the metal center. Structure models indicated, that the diastereomers of the tetrachelating bis(tropp) ligand **4** may fulfill these requirements. This approach allows a direct comparison of the stereochemical influence on the redox potential while electronic influences (i.e. different donor/acceptor properties) are minimized.

The synthesis of **4** is presented in Scheme 2. Treating 1,3-bis(diphenylphosphanyl)propane (**1**) with finely divided lithium in THF leads to cleavage of one P–Ph bond in each PPh₂ group and after hydrolysis furnished **2** as a mixture of



Scheme 2. Syntheses of the stereoisomers of the bis(tropp) ligands **4** and the corresponding rhodium(I)/(0) complexes **6** and **7**.

[*] Dr. C. Laporte, Dr. F. Breher, J. Geier, Dr. J. Harmer, Prof. Dr. A. Schweiger, Prof. Dr. H. Grützmacher
Department of Chemistry and Applied Biosciences
ETH-Hönggerberg
8093 Zürich (Switzerland)
Fax: (41) 163-31-032
E-mail: gruetzmacher@inorg.chem.ethz.ch

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isomers.^[7] Reaction with 5-chloro-5*H*-dibenzo[*a,d*]cycloheptene (trop-Cl, **3**) gives after alkaline work-up the desired bis(tropp) compounds *R,S*-**4** (*meso*-**4**) and the racemic mixture *R,R*/*S,S*-**4** (*rac*-**4** in the following) in satisfactory yield ($\approx 70\%$). The diastereomers, *meso*-**4** and *rac*-**4**, could not be separated and were treated with $[\text{Rh}(\text{cod})_2]^+\text{CF}_3\text{SO}_3^-$ (COD = cyclooctadiene) to yield almost quantitatively the corresponding mixture of diastereomeric rhodium(I) complexes *meso*-**6** and *rac*-**6** as a microcrystalline red powder. Pure *rac*-**6** crystallizes from an approximately 0.02 M solution in THF, while the other diastereomer, *meso*-**6**, is obtained by layering the mother liquor with *n*-hexane (contaminated with about 10% *rac*-**6**).

The structures of *rac*-**6** and *meso*-**6** were determined by X-ray structure analyses (Figure 1 a and b and Table 1).^[8]

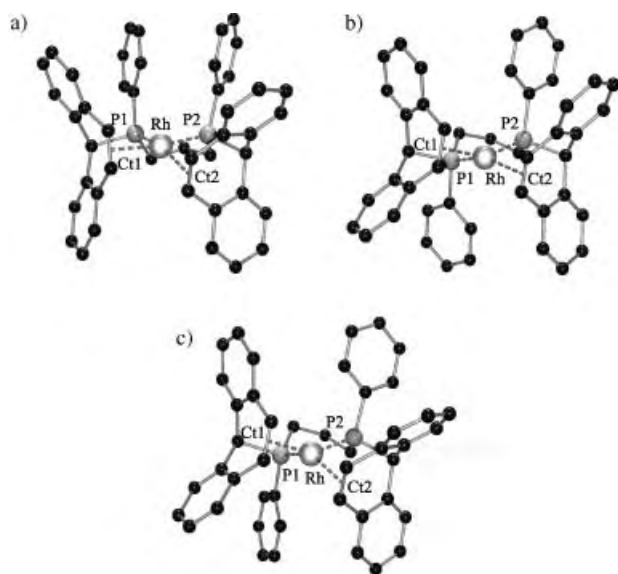


Figure 1. Structures of the cation in *meso*-**6** (a), the *R,R*-configured cation in *rac*-**6** (b), the *R,R*-configured enantiomer in *rac*-**7** (c).

As planned, the coordination sphere in the *rac*-**6** cation is significantly distorted towards a tetrahedral geometry ($\varphi \approx 30^\circ$). The six-membered Rh-P-(CH₂)₃-P ring has a twist conformation. In the *meso*-**6** cation, however, this ring adopts the expected chair conformation with the two phenyl groups at P1 and P2 in the axial positions and the sterically demanding dibenzo[*a,d*]cycloheptenyl (trop) units in the equatorial positions. This arrangement permits a metal coordination sphere which is closer to square planar ($\varphi \approx 19^\circ$).^[9]

Table 1: Selected structural data for *meso*-**6**, *rac*-**6**, and *rac*-**7**.

Compound	Rh-P [Å]	Rh-Ct [Å] ^[a]	C=C _{trop} [Å]	P1-Rh-P2 [°]	P1-Rh-Ct1 [°]	P2-Rh-Ct2 [°] ^[a]	Ct-Rh-Ct [°] ^[a]	φ [°] ^[b]
<i>meso</i> - 6	2.242	2.199	1.373	87.1	89.9	90.3	94.5	18.6
<i>rac</i> - 6	2.240	2.182	1.383	86.3	91.4	90.9	97.5	26.8
<i>rac</i> - 6 ^[c]	2.209	2.203	1.395	88.0	91.6	91.5	98.1	32.9
<i>rac</i> - 7	2.231	2.084	1.413	88.9	92.0	92.0	102.7	42.7

[a] Ct is the center of the coordinated C=C_{trop} bond. [b] φ is the angle between the planes P1-Rh-Ct1 and P2-Rh-Ct2 and is a measure of the tetrahedral distortion. [c] Crystals of *rac*-**6** contain two independent molecules (one molecule of each enantiomer) per asymmetric unit.

In the cyclic voltammogram of pure *rac*-**6** in THF/0.1 M $[\text{nBu}_4\text{N}]^+\text{PF}_6^-$ only two reversible redox waves are observed ($E_{1/2}^1 = -1.11$ V; $E_{1/2}^2 = -1.42$ V (Figure 2a)).

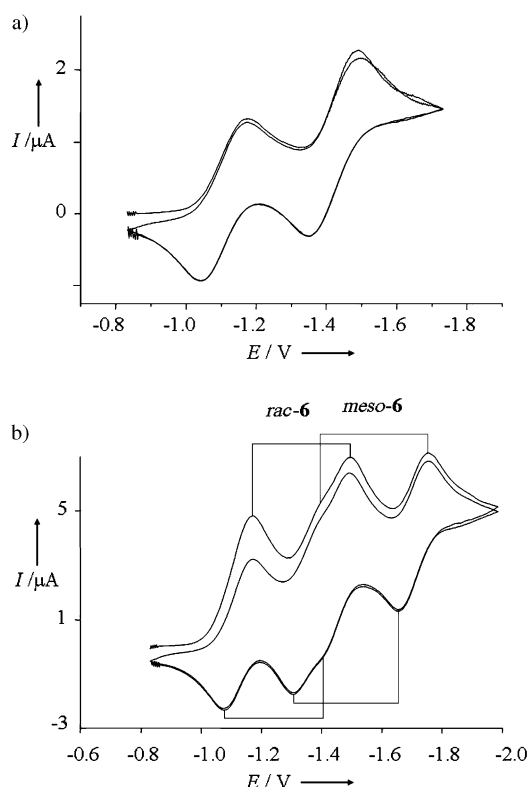


Figure 2. Cyclic voltammograms of a) pure *rac*-**6**, b) a mixture of *rac*-**6** and *meso*-**6**; 0.1 M $[\text{nBu}_4\text{N}]^+\text{PF}_6^-$, THF, $T = 298$ K, Pt working electrode, versus Ag/AgCl, scan rate = 100 mV s⁻¹.

A mixture of *rac*-**6** with *meso*-**6** in THF shows a cyclic voltammogram with four separated redox waves (Figure 2b). Clearly, *rac*-**6** is much easier to reduce than *meso*-**6** (*rac*-**6**: $E_{1/2}^1 = -1.12$ V, $E_{1/2}^2 = -1.44$ V; *meso*-**6**: $E_{1/2}^1 = -1.36$ V, $E_{1/2}^2 = -1.72$ V). The same features and almost identical redox potentials are obtained in a cyclic voltammogram taken in CH₂Cl₂, which shows that solvent effects are not responsible for the different redox potentials. The slight cathodic shift of $E_{1/2}^1$ by about 100 mV observed for *rac*-**6** in comparison to $[\text{Rh}(\text{tropp})_2]^+$ (see Scheme 1) is probably caused by replacing one P-phenyl group in the tropp^{Ph} ligand by the alkyl chain in the bis(tropp) ligand.^[10]

On a preparative scale, *rac*-**6** can be reduced to give pure *rac*-[Rh{bis(tropp)}]⁰ (*rac*-**7**) with sodium or sodium naph-

thalenide, $[\text{Na}^+(\text{naph})^-]$ in THF. Alternatively, the neutral paramagnetic complexes *rac-7/meso-7* are obtained by a comproportion reaction of *rac-6/meso-6* with the fully reduced 18-valence-electron sodium rhodates $[\text{Na}(\text{thf})_n]^+ \text{-} \text{rac/meso-}[\text{Rh}[\text{bis}(\text{tropp})]]^-$ (*rac-8/meso-8*).

The result of an X-ray structure analysis^[8] for *rac-7*, which is only the third example of the still rare mononuclear rhodium(0) complexes, is shown in Figure 1c. As previously observed,^[3,5] the Rh–Ct bonds (Ct = midpoint of the coordinated C=C bond) is shorter and the coordinated C=C_{trap} bond slightly longer in *rac-7* than in *rac-6*. The twist conformation of the central six-membered chelate ring is retained, however, the distortion towards a tetrahedron is more pronounced ($\varphi \approx 43^\circ$) in *rac-7* and equals the one observed in *cis-}[\text{Rh}(\text{tropp}^{\text{Ph}})_2]^0 ($\varphi \approx 43^\circ$).^[5]*

Crystals of the other diastereomer *meso-7* suitable for an X-ray analysis could not be obtained. However, information concerning the structure of *meso-7* were obtained from continuous wave (CW) X-band EPR spectra in a THF solution. The EPR spectrum of pure *rac-7* (Figure 3a) consists

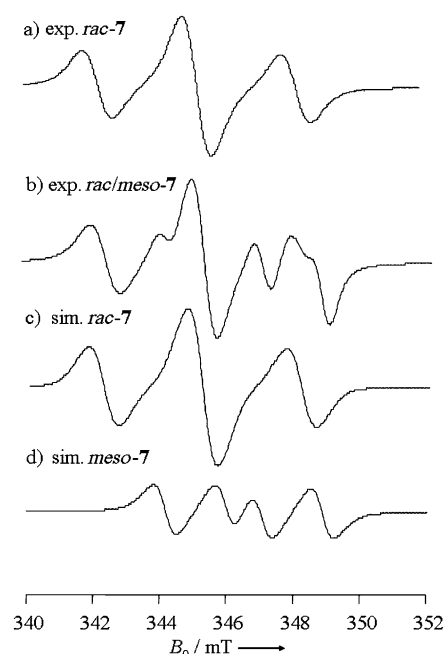


Figure 3. CW X-band EPR spectrum of a) pure *rac-7*, b) a mixture of *meso-7* and *rac-7* in THF, c) the simulated spectrum of *rac-7*, d) and the simulated spectrum of *meso-7*. Experimental conditions: room temperature, modulation frequency 100 kHz, modulation amplitude 0.05 mT, microwave frequency 9.76 GHz.

of a triplet (intensity ratio 1:2:1, and $g=2.0208$) with a ^{31}P hyperfine coupling of $A_{\text{iso}}=84$ MHz corresponding to two equivalent phosphorus nuclei as seen in the solid state structure.

Subtraction of the spectrum of *rac-7* from a spectrum of the *rac-7/meso-7* mixture (Figure 3b) allows the EPR parameters for *meso-7* to be determined (Figure 3c, d).^[11] In contrast to *rac-7*, the *meso*-diastereomer has a lower symmetry and the hyperfine couplings with two inequivalent phosphorus nuclei lead to the observation of a doublet of

doublets with $g=2.0137$, $A_{\text{iso}}^1=81.8$ MHz and $A_{\text{iso}}^2=51.4$ MHz. A straightforward explanation for this observation is that the d^9 valence-electron *meso-7* radical is unable to adapt to the preferred tetrahedral structure because of the rigid chair conformation of the central Rh–P–(CH₂)₃–P chelate ring. As a consequence, one Rh–P bond elongates which also lowers the energy of the SOMO.^[12] However, this stabilization is less effective than the distortion towards a tetrahedron and as a net result, a significant cathodic shift for the potential of the $[\text{meso-7}]^+ / [\text{meso-7}]^0$ redox couple is observed.

While it seems to be difficult to influence the redox couples of tetracoordinate $d^8\text{M}/d^9\text{M}$ centers ($\text{M} = \text{Rh}, \text{Ir}, \text{Pt}$) using the classical approach for the 4th period metals, that is, destabilizing the oxidized form (d^8 complex) by distortion towards a tetrahedron, the experiments reported herein (in combination with preliminary results for $\text{M} = \text{Ir}$ and Pt)^[13] show that the redox potentials of late-transition-metal complexes can be controlled effectively by destabilization of the reduced complex (d^9 complex) by enforcing a more planar structure.

Experimental Section

In the following, short descriptions of the syntheses and selected NMR resonances of diagnostic value are given. ^1H and ^{13}C NMR spectra are referenced relative to TMS, ^{31}P NMR spectra to H_3PO_4 , and ^{103}Rh NMR spectra to $\Xi=3.16$ MHz. For full details, see the Supporting Information.

***rac-4/meso-4*:** A solution of **2** (1 g, 3.8 mmol) in toluene (50 mL) was added to a solution of **3** (1.74 g, 7.6 mmol) in toluene (50 mL). After stirring for 2 h at RT, an aqueous solution of K_2CO_3 (40 mL; 1M) was added and the reaction mixture was stirred for 14 h at RT, and then heated under reflux until the organic phase became yellow. The organic layer was separated, the solvent was removed under vacuum, and the residue was recrystallized from ethanol (30 mL) to afford 1.6 g (66%) of a 1:1 mixture of *meso-4/rac-4* as a white air-sensitive solid. M.p. (mixture): 162–166°C. Only some of the NMR signals of the *rac-4/meso-4* mixture and could be assigned to one isomer: ^1H NMR (500.2 MHz, C_6D_6 , 298 K): $\delta = 6.92$ (2d, 4H, =CH), 6.86 (2d, 4H, =CH), 4.09 (d, 2H, $^2J_{\text{PH}}=5.7$ Hz, $\text{CHP}(\text{rac})$), 4.08 (d, 2H, $^2J_{\text{PH}}=5.7$ Hz, $\text{CHP}(\text{meso})$), 1.89 (m, 2H, $\text{PCHH-CH}_2\text{-CHHP}(\text{rac})$), 1.80 (m, 2H, $\text{PCHH-CH}_2\text{-CHHP}(\text{meso})$), 1.40 (2m, 4H, $\text{PCHH-CH}_2\text{-CHHP}(\text{meso} + \text{rac})$), 1.23 (2m, 3H, $\text{PCH}_2\text{-CH}_2\text{-CH}_2\text{P}(\text{meso} + \text{rac})$), 0.97 ppm (m, 1H, $\text{PCH}_2\text{-CHH-CH}_2\text{P}(\text{meso})$); ^{13}C NMR (125.7 MHz, C_6D_6 , 298 K): $\delta = 130.4$ (2d, $J_{\text{PC}}=1.4$ Hz, =CH), 130.1 (2d, $J_{\text{PC}}=1.4$ Hz, =CH), 60.8 (2d, $J_{\text{PC}}=19.4$ Hz, CHP), 29.3–28.9 (4d, $J_{\text{PC}}=19.4$ Hz, PCH_2), 23.5 ppm (2t, $J_{\text{PC}}=19.6$ Hz, $J_{\text{PC}}=18.8$ Hz, $\text{PCH}_2\text{-CH}_2\text{-CH}_2\text{P}$); ^{31}P NMR (202.5 MHz, CDCl_3 , 298 K): $\delta = -22.6$ (s, *rac*), -22.8 ppm (s, *meso*).

***rac-6/meso-6*:** The phosphanes *rac-4/meso-4* (137 mg, 0.2 mmol) in CH_2Cl_2 (5 mL) were treated with a solution of $[\text{Rh}(\text{cod})_2]\text{OTf}$ (**5**; 100 mg, 0.2 mmol; $\text{Tf} = \text{CF}_3\text{SO}_2$) in CH_2Cl_2 (5 mL). After stirring for 20 min, the solution was concentrated to 20% of its volume and hexane was added to afford *rac-6* and *meso-6* as an orange-red air stable microcrystalline powder (164 mg, 92%). The diastereomers were separated by fractional crystallization from THF; *rac-6* crystallized first from a 0.02M solution and *meso-6* crystallized after addition of *n*-hexane to the mother liquor in about 90% purity. *rac-6*: M.p. > 250°C. ^1H NMR (400.1 MHz, CD_2Cl_2 , 298 K): $\delta = 7.31$ (m, 4H, =CH), 5.09 (m, 2H, CHP), 2.36 (m, 2H, $\text{PCHH-CH}_2\text{-CHHP}$), 1.83 (m, 2H, $\text{PCHH-CH}_2\text{-CHHP}$), 0.82 ppm (m, 2H, $\text{PCH}_2\text{-CH}_2\text{-CH}_2\text{P}$); ^{13}C NMR (100.7 MHz, CD_2Cl_2 , 298 K): $\delta = 103.3$ (m, $^2J_{\text{PC}} + ^2J_{\text{P,C}} = 5.1$ Hz, $J_{\text{Rh,C}} = 6.3$ Hz, =CH), 92.4 (m, $^2J_{\text{PC}} + ^2J_{\text{P,C}} = 10.8$ Hz, $J_{\text{Rh,C}} = 5.0$ Hz, =CH), 52.9 (m, $J_{\text{PC}} + ^3J_{\text{PC}} = 18.1$ Hz, CHP), 17.3 (m, PCH_2 -

CH₂-CH₂P), 16.0 ppm (m, ¹J_{PC} + ³J_{PC} = 38.7 Hz, PCH₂-CH₂-CH₂P); ³¹P NMR (162.0 MHz, CD₂Cl₂): δ = 80.3 ppm (d, ¹J_{Rh,P} = 170 Hz); ¹⁰³Rh (12.7 MHz, CD₂Cl₂): δ = -330 ppm (t); *meso*-6: M.p. > 250 °C. ¹H NMR (400.13 MHz, CD₂Cl₂, 298 K): δ = 6.70 (m, 2H, =CH), 6.57 (m, 2H, =CH), 4.72 (m, 2H, CHP), 2.50 (m, 2H, PCHH-CH₂-CHHP), 2.19 (m, 1H, PCH₂-CHH-CH₂P), 1.86 (m, 2H, PCHH-CH₂-CHHP), 0.89 ppm (m, 1H, PCH₂-CHH-CH₂P); ¹³C NMR (100.7 MHz, CD₂Cl₂, 298 K): δ = 84.6 (m, ²J_{PC} + ²J_{P,C} = 10.0 Hz, =CH), 79.3 (m, ²J_{PC} + ²J_{P,C} = 13.7 Hz, =CH), 51.2 (m, ¹J_{PC} + ³J_{P,C} = 24.4 Hz, ²J_{PC} = 9.0 Hz, CHP), 21.2 (m, ²J_{PC} + ⁴J_{P,C} = 37.2 Hz, PCH₂-CH₂-CH₂P), 20.5 ppm (m, PCH₂-CH₂-CH₂P); ³¹P NMR (162.0 MHz, CD₂Cl₂, 298 K): δ = 72.0 ppm (d, ¹J_{Rh,P} = 163 Hz); ¹⁰³Rh NMR (12.7 MHz, CD₂Cl₂, 298 K): δ = -576 ppm (t).

rac-8/*meso*-8: Sodium (3 mg, 0.13 mmol) was added to a suspension of the complexes *rac*-6 and *meso*-6 (50 mg, 0.056 mmol) in THF (5 mL). The reaction mixture was sonicated for 12 h at 25 °C, and subsequently the resulting deep red reaction mixture was filtered. The filtrate was evaporated to dryness and washed several times with hexane leading to a deep red highly air-sensitive powder (45 mg, 67 %). *rac*-8: ¹H NMR (400.1 MHz, [D₈]THF, 298 K): δ = 4.35 (m, 2H, CHP), 4.20 (m, 2H, =CH), 2.83 (m, 2H, =CH), 1.40 (m, 2H, PCHH-CH₂-CHHP), 1.01 (m, 2H, PCH₂-CH₂-CH₂P), 0.81 ppm (m, 2H, PCHH-CH₂-CHHP); ¹³C NMR (100.7 MHz, [D₈]THF, 298 K): δ = 57.2 (m, CHP), 53.7 (m, =CH), 40.3 (d, ²J_{PC} or ²J_{Rh,C} = 11.1 Hz, =CH), 25.6 (m, PCH₂-CH₂-CH₂P), 21.5 ppm (m, PCH₂-CH₂-CH₂P); ³¹P NMR (121.5 MHz, [D₈]THF, 298 K): δ = 81.1 ppm (d, ¹J_{P,Rh} = 179 Hz); ¹⁰³Rh NMR (12.7 MHz, [D₈]THF, 298 K): δ = -681 ppm (t). *meso*-8: ³¹P NMR (121.5 MHz, [D₈]THF, 298 K): δ = 88.1 ppm (br).

rac-7: A solution of sodium-naphthalenide in THF (0.056 M, 0.056 mmol, 1 mL) was added to a suspension of *rac*-6 (50 mg, 0.056 mmol) in THF (2 mL). The deep green solution was filtered. After removing the solvent from the filtrate under reduced pressure, a deep green highly air-sensitive powder was obtained, which was washed several times with hexane (40 mg, 93 %). EPR (THF, 298 K): *g*_{iso} = 2.017 [t, *A*_{iso} (³¹P) = 84 MHz].

rac-7/*meso*-7: A 1:1 mixture of the compounds *rac*-8 and *meso*-8 (29 mg, 0.024 mmol) were mixed in THF (2 mL) with a 1:1 mixture of *rac*-6 and *meso*-6 (20 mg, 0.022 mmol). An immediate color change from red to deep green was observed. After removing the solvent under reduced pressure, the product was isolated as highly air-sensitive deep green powder (40 mg, 93 %). *meso*-7: EPR (THF, 298 K): *g*_{iso} = 2.0145 [dd, *A*_{iso}¹ (³¹P) = 81.8 MHz, *A*_{iso}² (³¹P) = 51.4 MHz].

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Spin-Density Distribution

Highly Resolved Spin-Density Distribution in the Prussian-Blue Precursors $\text{Cs}_2\text{K}[\text{Fe}(\text{CN})_6]$ and $\text{Cs}_2\text{K}[\text{Mn}(\text{CN})_6]$ **

Frank H. Köhler* and Rodrigue Lescouëzec

Understanding the properties of magnetic materials at the atomic level is essential for the design of new materials. This is particularly true for the chemistry-oriented area of molecule-derived magnetic materials, because the macroscopic magnetic properties are the result of interactions between the spin sources of the molecular building blocks. So far, the most versatile and successful building blocks are paramagnetic transition-metal complexes, and there is general agreement that the magnetic interactions in the materials derived from them is mediated by bridging ligands, that is, by superexchange.

The ions $[\text{Fe}(\text{CN})_6]^{3-}$ ($S = 1/2$) and $[\text{Mn}(\text{CN})_6]^{3-}$ ($S = 1$) are prominent building blocks of magnetic materials. They feature in Prussian-blue-type magnets,^[1] whose general formula is $\text{A}_x\text{M}'_x[\text{M}(\text{CN})_6]_y(\text{H}_2\text{O})_z$ (M , M' = transition metal; A = optional alkali ion) and whose transition temperature to bulk magnetism can be adjusted by varying M and M' .^[1c] Also, both ions have recently been used for the assembly of multiple-spin clusters,^[2] ferromagnetic coordination polymers,^[3] and “hybrid materials” exhibiting, for instance, chirality or conducting properties in addition to magnetism.^[4] For all these compounds a fundamental question is: How do the unpaired electrons get from $[\text{M}(\text{CN})_6]^{3-}$ to the adjacent building blocks? The answer can be given based on the fraction of an unpaired electron, also known as spin density, at the atoms of the bridging CN ligand. This report demonstrates that the spin delocalization within $[\text{Fe}(\text{CN})_6]^{3-}$ and $[\text{Mn}(\text{CN})_6]^{3-}$ can be studied in great detail by using ^{13}C and ^{15}N solid-state NMR spectroscopy.

The magic angle spinning (MAS) NMR spectra of microcrystalline samples of isotopically enriched $[\text{Fe}(\text{CN})_6]^{3-}$ and $[\text{Mn}(\text{CN})_6]^{3-}$ are reproduced in Figures 1 and 2, respectively. In them the ^{13}C NMR signal patterns are shifted up to -8900 ppm, whereas the ^{15}N NMR signal patterns appear up to 2200 ppm at the opposite frequency side. Herzfeld–Berger analysis^[5] of the spinning sideband patterns gave the isotropic signal shifts at the absolute temperature T , δ_T^{iso} , which are collected in Table 1. The NMR results are related to the spin densities of the respective nucleus N , $\rho(\text{N})$ (in atomic units), through Equation (1).^[6]

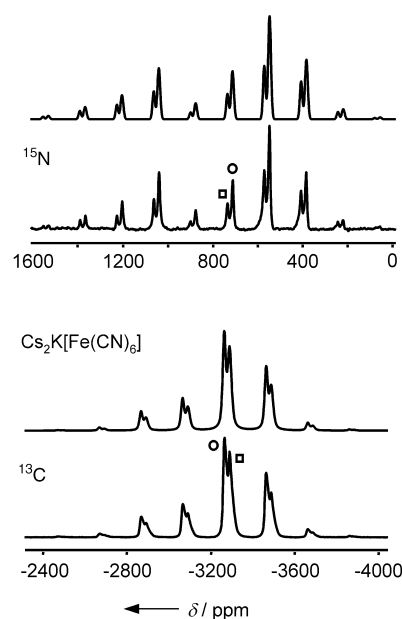


Figure 1. MAS NMR spectra of $\text{Cs}_2\text{K}[\text{Fe}(\text{CN})_6]$ at 326.4 K. Rotational frequencies of 15 kHz and 5 kHz for ^{13}C and ^{15}N , respectively. In each pair of spectra the upper one has been simulated with the data given in Table 1. The axial and equatorial isotropic signals are labeled with $\square$ and $\circ$, respectively.

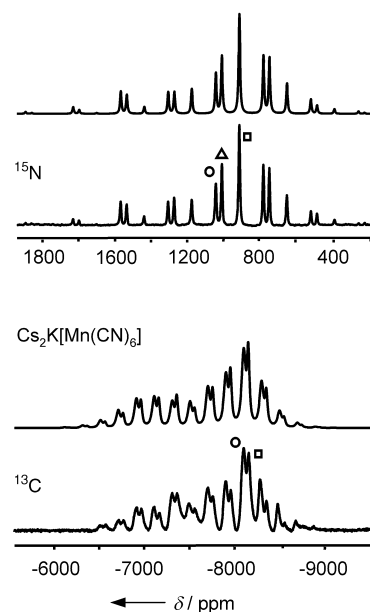


Figure 2. MAS NMR spectra of $\text{Cs}_2\text{K}[\text{Mn}(\text{CN})_6]$ at 326.4 K. Rotational frequencies of 15 kHz and 8 kHz for ^{13}C and ^{15}N , respectively. In each pair of spectra the upper one has been simulated with the data given in Table 1. The (pseudo)axial and (pseudo)equatorial isotropic signals are labeled with $\square$ and $\circ$ (plus Δ), respectively.

$$\rho(\text{N}) = \frac{9kT a_0^3}{\mu_0 g_{\text{av}}^2 \beta_c^2 (S+1)} \delta_T^{\text{con}}(\text{N}) \quad (1)$$

Here k is the Boltzmann constant, μ_0 is the vacuum permeability, g_{av} is the mean electron g factor, β_c is the Bohr magneton, a_0 is the Bohr radius, S is the electron spin

[*] Prof. Dr. F. H. Köhler, Dr. R. Lescouëzec
Chemie Department, Technische Universität München
85747 Garching (Germany)
Fax: (+49) 89-2891-3762
E-mail: f.h.koehler@lrz.tu-muenchen.de

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Table 1: Solid-state NMR data^[a] and spin densities^[b] of Prussian-blue precursors.

	Cs ₂ K[Fe(CN) ₆]		Cs ₂ K[Mn(CN) ₆]		
	Site of cyano group		Site of cyano group		
	axial	equatorial	1 (axial)	2 (equatorial)	3 (equatorial)
$\delta_T^{\text{iso}}(^{13}\text{C})$	−3269	−3295	−7760	−7709	−7709
intensity/width	1/1300	2/1300	1/2400	1/4000	1/4000
$\delta_{298}^{\text{con}}(^{13}\text{C})$	−3491	−3431	−8482	−8231	−8231
$\rho(^{13}\text{C})$	−0.02583	−0.02537	−0.04705	−0.04566	−0.04566
$\delta_{298,xx}^{\text{para}}[\text{C}]$	−4022	−3998	−9223	−9194	−9194
$\delta_{298,yy}^{\text{para}}[\text{C}]$	−4096	−4204	−9532	−9612	−9612
$\delta_{298,zz}^{\text{para}}[\text{C}]$	−3067	−3069	−7188	−7059	−7059
$\delta_{298}^{\text{con}}(^{15}\text{N})$	727	704	896	992	1026
intensity/width	1/400	2/400	1/300	1/300	1/300
$\delta_{298}^{\text{con}}(^{15}\text{N})$	873.1	857.1	1039.2	1189.2	1226.5
$\rho(^{15}\text{N})$	0.00646	0.00639	0.00576	0.00660	0.00680
$\delta_{298,xx}^{\text{para}}[\text{N}]$	370	333	883	756	727
$\delta_{298,yy}^{\text{para}}[\text{N}]$	231	261	413	450	422
$\delta_{298,zz}^{\text{para}}[\text{N}]$	2063	1978	1922	2289	2481

[a] $T = 326.4$ K, δ in ppm, width in Hz. [b] ρ in au. [c] $|\delta_{zz} - \delta_{\text{iso}}| \geq |\delta_{yy} - \delta_{\text{iso}}| \geq |\delta_{xx} - \delta_{\text{iso}}|$.

quantum number, and $\delta_T^{\text{con}}(\text{N})$ is the Fermi contact shift of nucleus N at the temperature T .

The resolution of the spin densities at both the C and the N atoms of the cyano groups is unusually high. It is possible to distinguish differences of 5×10^{-5} au, which corresponds to a signal shift difference of 7 ppm in the MAS NMR spectrum of Cs₂K[Fe(CN)₆] in Figure 1. Therefore, in this compound even a small distortion of the spin-density distribution toward axial and equatorial ligand sites can be detected. For Cs₂K[Mn(CN)₆] the symmetry is still lower. This follows from the different spin densities at the N atoms (6.60×10^{-3} au and 6.80×10^{-3} au, respectively) of what would be equatorial CN ligands at lower resolution. Corresponding spin densities at the C atoms could not be resolved, although the greater width of the ¹³C NMR signals of the equatorial CN groups points to two overlapping signals (Table 1). More generally, comparison of the spectra in Figures 1 and 2 shows that the resolution of the ¹⁵N data is better than that of the ¹³C data. One would expect that the distortion of the spin-density distribution corresponds to the crystallographic nonequivalence of the CN groups of [M(CN)₆]^{3−}. However, for [Fe(CN)₆]^{3−} conflicting results have been reported,^[7] while for [Mn(CN)₆]^{3−} only axial symmetry has been mentioned.^[7d,8] It follows that for the title compounds the resolution of the NMR data is better than that of the diffraction data.

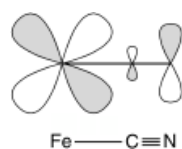


Figure 3. π -Type orbital contributions of a $\text{M}-\text{C}\equiv\text{N}$ fragment qualitatively representing the transfer of positive spin density from the metal to the CN ligand.

All of the ¹³C and ¹⁵N NMR signals appear at low and high frequencies (Figure 1 and 2), respectively, and hence the spin density at the C atoms is negative, while it is positive at the N atoms. This proves that the spin delocalization proceeds through π -type orbitals (Figure 3) with large and small contributions of the N and C atomic orbitals, respectively.^[9] Polarization of the $\text{M}-\text{C}$

and $\text{C}-\text{N}$ bonding electrons through spin at the M and N atoms leads to negative spin density at the C atoms, which overcompensates the small positive spin density resulting from the delocalization shown in Figure 3. In Cs₂K[Fe(CN)₆] the spin density at the six C atoms corresponds to (−)15.3 % of the unpaired electron, while (−)27.8 % is found for Cs₂K[Mn(CN)₆] (see Table 1). The ratio of the spin densities is close to what one expects for $S = 1/2$ and $S = 1$ species, respectively. Surprisingly, at the N atoms the spin density is virtually the same for both compounds (3.8 % and 3.9 %, respectively), giving rise to speculations whether in Prussian blues the efficiency of spin transfer beyond the N atoms varies or not.

We are not aware of any experimental method providing this sort of information. A polarized neutron diffraction study of Cs₂K[Fe(CN)₆]^[10] gave good results for the spin density at iron, but it was less precise for the C and N atoms, claiming (−)5 % and 6 % of an unpaired electron, respectively. So it is no wonder that those numbers differ from ours. Early static solid-state ¹³C and ¹⁴N NMR studies gave only broad and featureless signals,^[11] and the ¹³C NMR signals Takeda et al.^[12] obtained recently from static samples of cobalt and manganese hexacyanoferrates were very broad as well.

The analysis of the signal patterns in Figures 1 and 2 revealed considerable signal shift anisotropies, which pointed to nonspherical distributions of the spin densities about the C and N atoms. Spectrum simulation showed that the spin distribution about these atoms has axial symmetry for [Fe(CN)₆]^{3−} and nearly so for [Mn(CN)₆]^{3−}. This applies to all nonequivalent CN groups, while the individual values differ. Therefore, the principal shift values at 298 K, $\delta_{298,xx}^{\text{para}}$, $\delta_{298,yy}^{\text{para}}$, and $\delta_{298,zz}^{\text{para}}$, given in Table 1 are due to the unpaired electrons. There is a striking difference between the shift anisotropies: For the C atoms $|\delta_{298,zz}^{\text{para}}| < |\delta_{298,xx}^{\text{para}}| \cong |\delta_{298,yy}^{\text{para}}|$ holds, whereas for the N atoms we find $|\delta_{298,zz}^{\text{para}}| > |\delta_{298,xx}^{\text{para}}| \cong |\delta_{298,yy}^{\text{para}}|$. If the shift anisotropies are visually represented by spheroids, this means that they would be oblate and prolate at C and N, respectively. We suggest that the orientation of the spheroids relative to the $\text{M}-\text{C}\equiv\text{N}$ axis is as shown in Figure 4. This would correspond more closely to the expectation that the induction of spin at the C atoms involves polarization of electrons in σ -type orbitals parallel to the $\text{M}-\text{C}\equiv\text{N}$ axis, while direct spin transfer to the N atoms involves π -type orbitals perpendicular to that axis.



Figure 4. Sketch of the anisotropic spin distribution about the C and N atoms of the $\text{Fe}-\text{C}\equiv\text{N}$ fragment as derived from the NMR data.

In conclusion, by using solid-state NMR spectroscopy, we have determined quantitatively small spin densities on the CN ligands of [Fe(CN)₆]^{3−}

and $[\text{Mn}(\text{CN})_6]^{3-}$. Because CN ligands are responsible for the interaction in magnetic materials derived from these complexes, it is enlightening that the spin distribution in different directions of the crystal lattice can be distinguished as well. Finally, the shape of the spin distribution about the C and N atoms can be derived and related to the spin delocalization mechanisms engaged. It is expected that in the same way many related compounds having suitable electron spin relaxation times can be investigated in great detail.

Experimental Section

Samples of $\text{Cs}_2\text{K}[\text{Fe}(\text{CN})_6]$ and $\text{Cs}_2\text{K}[\text{Mn}(\text{CN})_6]$ were prepared according to the literature^[7d,13] by using K^{13}CN and KC^{15}N , each 99 % enriched. The powders used for packing the 4-mm ZrO_2 rotors in a glove box were obtained from large single crystals. Nickelocene was added to the powders for measuring the temperature inside the rotor.^[14] The spectra were recorded with a Bruker Avance 300 spectrometer. For spectrum analysis and simulation the respective programs HBA and Wsolids^[15] were used. The isotropic signal shifts at the measuring temperature T , δ_T^{iso} , were determined relative to external adamantane and ammonium nitrate. The δ_T^{iso} values and the anisotropic signal shifts were converted to paramagnetic shifts at 298 K by subtracting the respective signal shifts of $\text{Cs}_2\text{K}[\text{Co}(\text{CN})_6]$ and by considering the Curie law, $\delta^{\text{para}} \propto 1/T$. For obtaining the contact shifts, $\delta_{298}^{\text{con}}$, the dipolar shifts were subtracted from the δ^{para} values (see the Supporting Information for details). The contact shifts were converted to the spin densities, ρ , by using Equation (1).

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Keywords: cyanides · magnetic properties · NMR spectroscopy · spin-density distribution

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Abyssomicin C—A Polycyclic Antibiotic from a Marine *Verrucosipora* Strain as an Inhibitor of the *p*-Aminobenzoic Acid/Tetrahydrofolate Biosynthesis Pathway**

Bojan Bister, Daniel Bischoff, Markus Ströbele, Julia Riedlinger, Andreas Reicke, Falko Wolter, Alan T. Bull, Hans Zähner, Hans-Peter Fiedler, and Roderich D. Süssmuth*

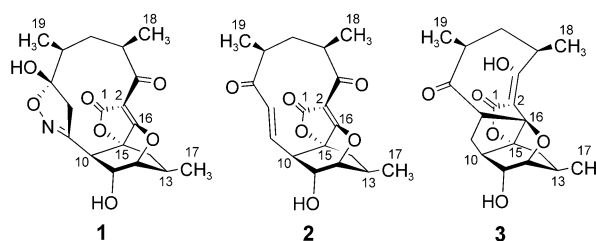
Dedicated to Günther Jung and Louis Moroder

For many multiresistant Gram-positive bacteria, for example, methicillin-resistant *Staphylococcus aureus* strains (MRSA), vancomycin- or teicoplanin-type glycopeptide antibiotics are the sole remaining means of treatment. As a consequence, efforts of researchers from various disciplines have been directed towards the discovery and development of novel antibiotics. In this process of drug finding, nature has always been an important source, and the cultivation of microorganisms collected from previously unexplored habitats may possibly reveal lead structures for new antibiotics. Similarly, the targeting of biosynthetic processes in the bacterial cell other than those previously investigated might also lead to the discovery of new antibiotics. In this context we have directed our attention to rare actinomycetes from the deep-sea plain and screened them for inhibitors of *p*-aminobenzoate (*p*ABA) biosynthesis.

The biosynthesis of *p*ABA is part of the biosynthesis of tetrahydrofolate (THF).^[1] Among the few but prominent inhibitors of THF biosynthesis are sulfonamides and trimethoprim. The biosynthesis of *p*ABA is a very attractive target since it is found in many microorganisms but not in humans.

In the present contribution we describe the structure elucidation of new polycyclic polyketide-type antibiotics, which we have named abyssomicins. Interestingly, the abyssomicins fulfill the criteria mentioned above: they are produced by the rare actinomycete *Verrucosipora* and are inhibitors of the *p*ABA biosynthetic pathway. To our knowledge, the abyssomicins are the first known substances derived from a bacterial source that inhibit the biosynthesis of *p*ABA.

The actinomycete *Verrucosipora* strain AB 18-032 was isolated from a sediment sample collected in the Japanese Sea at a depth of 289 m. By using an agar-plate diffusion assay,^[2] we found that the inhibitory effects of abyssomicins, which could be depleted upon addition of *p*ABA, were localized in the bacterial biosynthesis pathway between chorismate and *p*ABA. In the subsequent HPLC/diode array analysis three compounds were detected which could not be assigned to known metabolites by means of our HPLC-UV/Vis Database.^[15] Compounds **1–3** (Scheme 1) were purified by sub-



Scheme 1. Structural formulae of abyssomicins **1** (**1**), **C** (**2**), and **D** (**3**) showing the *R* configuration at C(11).

sequent size-exclusion and adsorption chromatography and preparative reversed-phase HPLC, and further analyzed by mass spectrometry, NMR spectroscopy, and X-ray structure determination.

The high-resolution ESI-FTICR mass spectra of the Na adducts of **1**, **2**, and **3** showed masses of 400.13654, 369.13079, and 371.14663 Da, respectively, corresponding to the molecular formulas $C_{19}H_{23}NO_7$ (**1**) [$(M+Na)^+_{\text{theor}} = 400.13667$, $\Delta m = 0.34$ ppm], $C_{19}H_{22}O_6$ (**2**) [$(M+Na)^+_{\text{theor}} = 369.13085$, $\Delta m = 0.20$ ppm], and $C_{19}H_{24}O_6$ (**3**) [$(M+Na)^+_{\text{theor}} = 371.14650$, $\Delta m = 0.32$ ppm]. From 1D and 2D NMR spectra the partial structural motif shown in Figure 1 was determined for all three compounds, indicating that compounds **1–3** indeed belong to the same family. A second partial fragment of **2** was identified as bearing a Michael system: that is, a *trans* double bond ($J = 13.5$ Hz) in conjugation with a ketone. Database searches of the molecular formulae of **1–3** as derived from FTICR-MS combined with the structural motif determined from NMR experiments (Figure 1) did not result

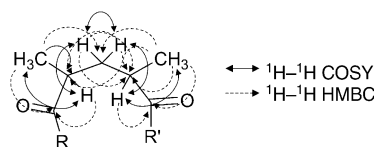


Figure 1. Characteristic structural motif of abyssomicins **1–3** determined by NMR experiments

[*] B. Bister, Dr. D. Bischoff, F. Wolter, Dr. R. D. Süssmuth
Institut für Organische Chemie, Universität Tübingen
Auf der Morgenstelle 18, 72076 Tübingen (Germany)
Fax: (+49) 707-129-5560
E-mail: roderich.suessmuth@uni-tuebingen.de

Dr. M. Ströbele
Institut für Anorganische Chemie, Universität Tübingen
Auf der Morgenstelle 18, 72076 Tübingen (Germany)

J. Riedlinger, A. Reicke, Prof. Dr. H.-P. Fiedler
Mikrobiologisches Institut, Universität Tübingen
Auf der Morgenstelle 28, 72076 Tübingen (Germany)

Prof. Dr. A. T. Bull
Research School of Biosciences, University of Kent
Canterbury CT2 7NJ (UK)

Prof. Dr. H. Zähner
Kistlerweg 7, 3006 Bern (Switzerland)

[**] The work of R.D.S. has been supported by an Emmy Noether Fellowship for young investigators of the Deutsche Forschungsgemeinschaft (DFG) (SU 239/2-1) and the DFG-Forschergruppe (SU 239/1-1 and 1-2). We thank Prof. H.-J. Meyer for support in X-ray analyses and Prof. M. E. Maier for helpful discussions.

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

in matches with any known compound from the DNP database^[3] or from the CAS-online database.^[4]

For further structure determination we focused on both the evaluation of NMR data and crystallization experiments. We obtained single crystals of all three compounds (**1–3**), and X-ray structure determination revealed the relative configurations shown in Figure 2. NMR analyses performed on

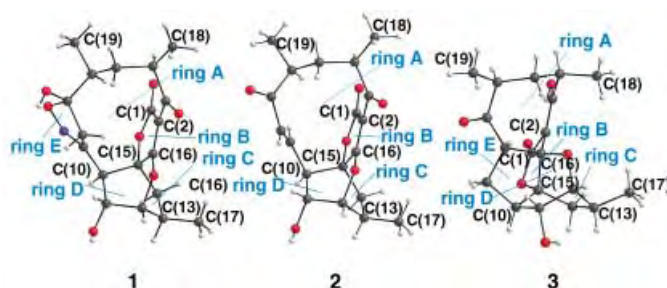


Figure 2. X-ray structures of abyssomicins B (**1**), C (**2**), and D (**3**) showing the relative configuration. The ring systems are assigned alphabetically.

compound **2** (Table 1) confirmed the X-ray data. The absolute stereochemistry of compound **3** was determined by both the Mosher method^[5,6] and the Helmchen method.^[7,8] Both methods consistently suggest an *R* configuration at C(11) of ring D. On the basis of structural analogy, the same configurations were assumed for the analogous positions in compounds **1** and **2**. Thus the absolute configurations were deduced for **1–3**.

Table 1: ¹H and ¹³C NMR shifts of abyssomicin C.^[a]

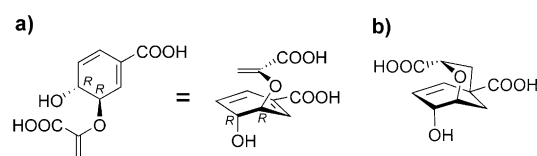
No.	$\delta(^1\text{H})$ [ppm]	Multiplicity and coupling constants [Hz]	$\delta(^{13}\text{C})$ [ppm]
1	—	—	173.8
2	—	—	106.7
3	—	—	202.8
4	—	—	45.3
5	2.01 (a) 1.44 (b)	$m, {}^2J_{5a,5b} = 14.1; {}^3J_{5a,4} = 11.2; {}^3J_{5a,6} = 10.1$ $m, {}^2J_{5b,5a} = 14.1; {}^3J_{5b,4} = 2.7; {}^3J_{5b,6} = 1.6$	42.3
6	—	—	50.3
7	—	—	208.4
8	6.55	$d, {}^3J_{8,9} = 13.5$	137.1
9	5.98	$dd, {}^3J_{9,8} = 13.5; {}^3J_{9,10} = 9.5$	137.3
10	2.99	$dd, {}^3J_{10,9} = 9.5; {}^3J_{10,11} = 6.1$	51.5
11	5.06 4.59 (OH)	$dd, {}^3J_{11,10} = 6.1; {}^3J_{11,12} = 3.3$ —	76.0 —
12	4.57	$d, {}^3J_{12,11} = 3.3; {}^3J_{12,13} = \text{n.d.}^{[b]}$	88.9
13	2.73	$\text{n.d.}^{[b]}$	28.1
14	1.26 (a) 2.69 (b)	$dd, {}^2J_{14a,14b} = 12.4; {}^3J_{14a,13} = 4.8$ $dd, {}^2J_{14b,14a} = 12.4; {}^3J_{14b,13} = \text{n.d.}^{[b]}$	39.6
15	—	—	81.1
16	—	—	189.8
17	1.17	$d, {}^3J_{17,13} = 7.0$	21.5
18	1.09	$d, {}^3J_{18,4} = 6.7$	19.3
19	1.11	$d, {}^3J_{19,6} = 7.2$	23.0

[a] $c = 20.5 \text{ mg mL}^{-1}$, [D₄]methanol, 298 K. [b] n.d. = not determined.

Compounds **1–3** are previously unknown natural products and were named abyssomicins B, C (**1**, **2**), and D (**3**) (from “abyss”, referring to the site of strain habitat). In antibacterial testing, however, only abyssomicin C (**2**) showed antibiotic activity against Gram-positive bacteria including pathogenic *Staphylococcus aureus* strains.^[2] The minimal inhibition concentrations against methicillin-resistant *S. aureus* (MRSA) and a multiresistant including vancomycin-resistant *S. aureus* strain were $4 \mu\text{g mL}^{-1}$ and $13 \mu\text{g mL}^{-1}$, respectively.^[2]

Considering their low molecular masses, abyssomicins contain an unusually large number of ring systems (four in the case of **2** and five for **1** and **3**), quaternary carbons, and stereocenters. The tetrone acid motif of ring B indicates a likely relationship to tetrocarcin-type antibiotics.^[9] Further structural features characteristic of all three compounds are two methyl groups (C(18), C(19)) of ring A, and the oxabicyclooctane ring system of rings C and D.

The structures of the abyssomicins could be simply catalogued with many other newly discovered antibiotics. However, from the described structures we see several interesting details that suggest a possible mode of action and cast a different light on these compounds. A closer consideration of the oxabicyclooctane system (C and D rings) in **1–3** revealed striking similarities to the solution conformation of chorismate^[10,11] as well as to synthetic transition-state analogues of chorismate mutase inhibitors (Scheme 2).^[12,13]



Scheme 2. a) Diaxial conformation of chorismate in aqueous solution.^[10] b) Synthetic transition-state analogue for chorismate mutase inhibition.^[13]

Furthermore, the fact that the Michael system (C(7)–C(9)) adjacent to the oxabicyclooctane system of abyssomicin C (**2**) is missing in the antibiotically inactive abyssomicins B (**1**) and D (**3**) is a strong indication for its significance for antibiotic activity. Currently, our hypothesis is that in bacterial metabolism of the abyssomicins, abyssomicin D is derived from abyssomicin C by Michael addition of a hydride equivalent (with NADH as a putative hydrogen source). Abyssomicin B might be the result of a Michael addition of hydroxylamine (as yet of unknown origin) followed by oxidation, leading to an unusual five-membered heterocycle. We therefore speculate that the Michael system is directly involved in the mechanism of action of abyssomicin C, and that nucleophilic amino acid side chains from the targeted enzyme are covalently trapped. Thus, subsequent biosynthesis steps from the branch-point metabolite chorismate especially to *p*ABA are inhibited. Viewed in this way, abyssomicin C would represent a substrate mimetic that traps the enzyme by reaction with a Michael system.

We assume that abyssomicins are formed by the polyketide biosynthesis pathway in analogy to other tetrocarcin-related antibiotics.^[14] Further investigations on the abyssomi-

cins with regard to the biosynthesis, the exact determination of the targeted enzyme(s), and the mode of action are currently in progress.

Experimental Section

LC-MS experiments were performed on a Bruker Esquire 3000plus (Bruker-Daltonics, Bremen, Germany) coupled to an Agilent 1100 HPLC system (Agilent, Waldbronn, Germany). FTICR-ESI mass spectra were recorded on an APEX II FTICR mass spectrometer (4.7 T, Bruker-Daltonics, Bremen, Germany). NMR experiments were recorded on an AMX600 NMR spectrometer (Bruker, Karlsruhe, Germany) equipped with a 5-mm triple-resonance probehead with z gradients. Single-crystal X-ray data were collected on an STOE IPDS 1 system with monochromated $\text{MoK}\alpha$ radiation. CCDC 226556–226558 (1–3) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk). The Mosher method^[5,6] and the Helmchen method^[7,8] for the determination of the absolute stereochemistry were performed in each case with 10 mg of abyssomicin D. Further physico-chemical data are given in the Supporting Information.

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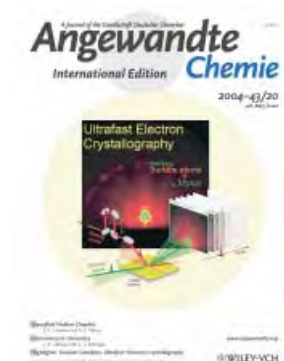
Keywords: antibiotics · metabolism · Michael addition · natural products

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Cover Picture

Franco Vigliotti, Songye Chen, Chong-Yu Ruan, Vladimir A. Lobastov, and Ahmed H. Zewail*

Surface structural dynamics are determined with unprecedented spatial and temporal resolution by ultrafast electron crystallography. The dynamics are initiated by a femtosecond laser pulse, and transient surface structures are imaged by an ultrashort electron pulse: A diffraction snapshot is obtained at a given time, δt , and allows the real-time observation of surface structural dynamics with atomic-scale resolution. For more details see the Communication by A. H. Zewail and co-workers on page 2705 ff.



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D. H. Paik, D.-S. Yang, I.-R. Lee, A. H. Zewail*:

The Transition State of Thermal Organic Reactions: Direct Observation in Real Time

DOI: 10.1002/anie.200453962

Published online: April 28, 2004

W. U. Dittmer, A. Reuter, F. C. Simmel*:

A DNA-Based Machine That Can Cyclically Bind and Release Thrombin

DOI: 10.1002/anie.200353537

Published online: April 28, 2004

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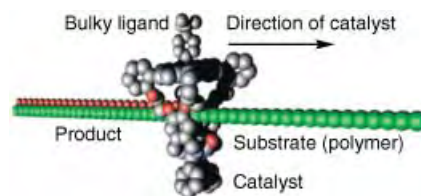
Highlights

Supramolecular Chemistry

P. R. Carlier* _____ 2602 – 2605

Threading the Needle:
Mimicking Natural Toroidal Catalysts

Can a hollow catalyst process a polymeric substrate by “threading the needle?” Nature’s DNA-processing enzymes effortlessly use this pseudorotaxane mechanism, but until recently this topology has not been realized in a synthetic supramolecular system. However, there is now compelling evidence that a toroidal metallophyrin does just that in the

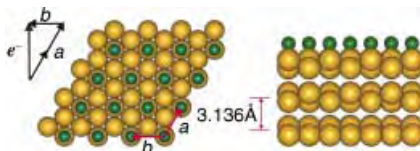


epoxidation of polybutadiene (see picture).

Electron Crystallography

J. M. Thomas* _____ 2606 – 2610

Ultrafast Electron Crystallography:
The Dawn of a New Era



Submonolayer sensitivity, spatial resolutions of 0.02 Å, and time resolutions of

less than 1 ps are key features of the latest developments in electron crystallography, a method that allows the determination of the structures of solid surfaces (see picture). The road to this powerful technique and a comparison with complementary structure-determination techniques are discussed.

Essays

Structure Elucidation

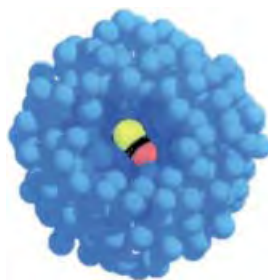
J. A. Labinger,*
S. J. Weininger* _____ 2612–2619

Controversy in Chemistry: What Counts as Evidence?—Two Studies in Molecular Structure

Hindsight is a wonderful thing: Controversies are always difficult to characterize while they are in progress; however, as shown for the cases of stereochemistry and bond-stretch isomerization, it is much easier once all the evidence has been amassed. These two examples have demonstrated how important it is to

consider all the experimental results before coming to a conclusion. The second example shows above all that some evidence is more equal than others—crystal-structure analysis was considered at all stages to be the most reliable.

Helium droplets as a matrix: Helium droplets (see picture) have unusual properties that are very useful in spectroscopy and in the study of chemical reactions of molecules and complexes. Single molecules (e.g. OSC; S yellow, C black, O red) can be embedded, and homo- and heteronuclear clusters can be formed in their interiors. This unique matrix technique opens the way for a variety of new possible applications in chemistry and physics.

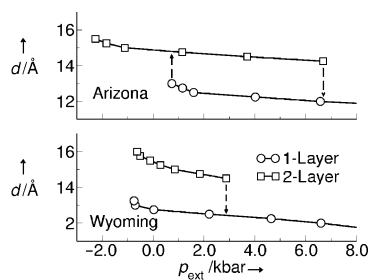


Reviews

Matrix Techniques

J. P. Toennies,* A. F. Vilesov 2622–2648

Superfluid Helium Droplets: A Uniquely Cold Nanomatrix for Molecules and Molecular Complexes



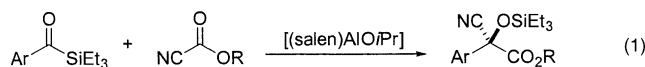
Feats of clay: Molecular simulations are used to study the mechanism of clay-swelling hysteresis. A free-energy barrier separating stable layered hydrates is found to be the origin of this hysteresis. The simulations are also used to predict that changing the external pressure on a clay mineral layer can induce hysteresis (see picture).

Communications

Clay Minerals

T. J. Tambach,* P. G. Bolhuis,
B. Smit _____ 2650–2652

A Molecular Mechanism of Hysteresis in Clay Swelling



An enantiopure (salen)aluminum alkoxide complex catalyzes the asymmetric coupling of acylsilanes and cyanoformate esters [Eq. (1), R = Bn, Et]. The reactions afford unsymmetrical, fully protected

malonic acid derivatives that may be elaborated to nonnatural β -amino acid derivatives, and represent the first asymmetric catalytic reactions involving protected cyanohydrin anions.

Cyanohydrin Anions

D. A. Nicewicz, C. M. Yates,
J. S. Johnson* _____ 2652–2655

Catalytic Asymmetric Acylation of (Silyloxy)nitrile Anions

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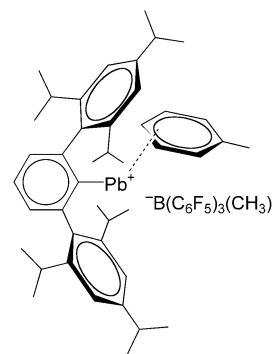
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Low-Coordinate Species

S. Hino, M. Brynda, A. D. Phillips,
P. P. Power* 2655 – 2658

Synthesis and Characterization of a
Quasi-One-Coordinate Lead Cation

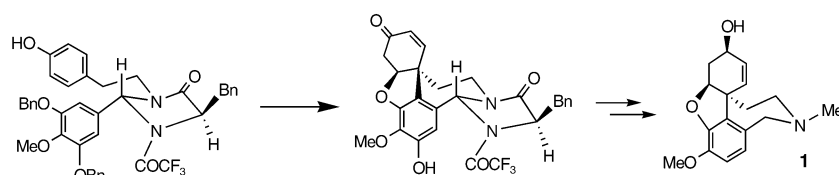
Easily lead: The demethylation of $[\text{PbMe}(\text{2,6-Trip}_2\text{C}_6\text{H}_3)]$ ($\text{Trip} = 2,4,6\text{-iPr}_3\text{C}_6\text{H}_2$) with tris(perfluorophenyl)borane in toluene affords $[\text{Pb}(\text{2,6-Trip}_2\text{C}_6\text{H}_3) \cdot (\eta^2\text{-MeC}_6\text{H}_5)][\text{B}(\text{Me})(\text{C}_6\text{F}_5)_3]$ (see picture), in which the lead center is singly bound to the 2,6-Trip₂C₆H₃ moiety and also weakly solvated by toluene.



Natural Product Synthesis

S. Kodama, Y. Hamashima, K. Nishide,
M. Node* 2659 – 2661

Total Synthesis of (–)-Galanthamine by
Remote Asymmetric Induction



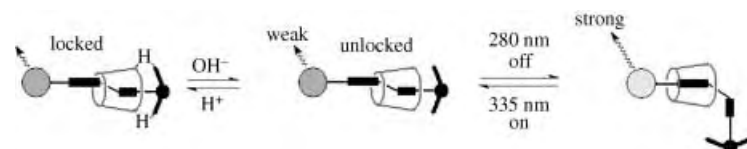
A pivotal intramolecular Michael addition to form a fused 5,7,5 ring system and the skeleton of (–)-galanthamine (**1**) was completely controlled by a remote chiral imidazolidinone auxiliary derived from D-

phenylalanine (see scheme). This total synthesis of the allylic alcohol **1** avoids the corresponding enone narwedine, a highly allergenic intermediate in previous syntheses of **1**.

Molecular Devices

Q.-C. Wang, D.-H. Qu, J. Ren, K. Chen,
H. Tian* 2661 – 2665

A Lockable Light-Driven Molecular Shuttle
with a Fluorescent Signal



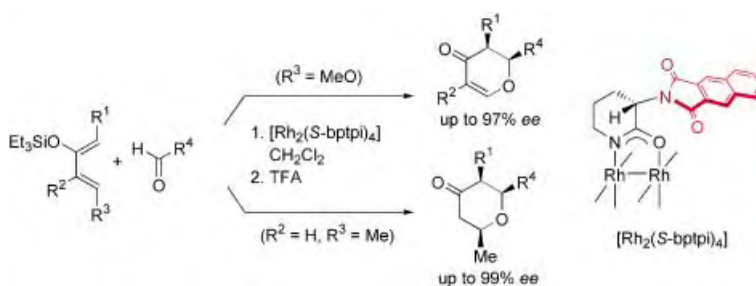
A turn on! A lockable photodriven molecular shuttle is based on a [2]rotaxane that consists of an α -cyclodextrin threaded by a stilbene derivative. The fluorescent signals originate from the naphthalimide stopper and indicate the position of the

shuttle. Once unlocked, the device can be turned on by irradiation at 335 nm and turned off by irradiation at 280 nm. The shuttle can be locked or unlocked by the addition acid or alkali, respectively (see picture).

Cycloadditions

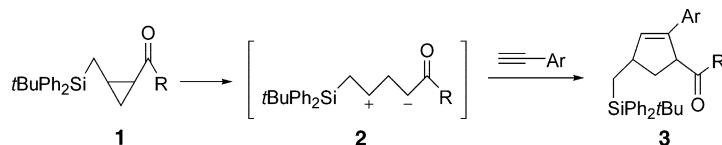
M. Anada, T. Washio, N. Shimada,
S. Kitagaki, M. Nakajima, M. Shiro,
S. Hashimoto* 2665 – 2668

A New Dirhodium(II) Carboxamidate
Complex as a Chiral Lewis Acid Catalyst
for Enantioselective Hetero-Diels–Alder
Reactions



Excellent enantioselectivities (up to 99% *ee*) and turnover numbers (up to 48 000) were attained in the hetero-Diels–

Alder reaction of a diverse range of aldehydes and activated dienes catalyzed by $[\text{Rh}_2(\text{S-bptpi})_4]$ (see scheme).



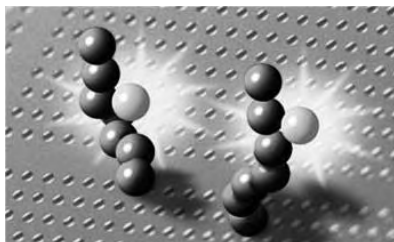
Push–pull substitution of cyclopropanes can make these compounds synthetic equivalents of 1,3-dipolar synthons **2**, suitable for formal [3+2] addition reactions with aryl acetylenes. Upon treatment

with TiCl_4 , *tert*-butyldiphenylsilylmethyl-substituted cyclopropyl ketones **1** reacted with aryl acetylenes to furnish cyclopentene derivatives **3** regio- and stereoselectively. $\text{R} = n\text{Bu}$, *t*Bu, Ph.

Cyclopropane Reagents

V. K. Yadav,* V. Sriramurthy 2669–2671

Formal [3+2] Addition of Acceptor-Substituted Cyclopropylmethylsilanes with Aryl Acetylenes



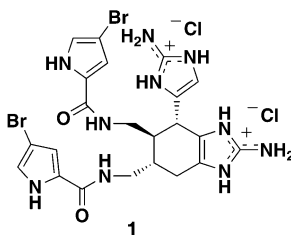
Thousands of kinase assays at one stroke are possible by using high-content peptide microarrays. Such experiments deliver information regarding peptidic kinase substrates and accelerate the development of high-throughput screening assays. Moreover, knowledge-based selection of human sequences allows the reliable detection of downstream targets of protein kinases, as demonstrated for 3-phosphoinositide-dependent protein kinase.

Automated Synthesis

M. Schutkowski,* U. Reimer, S. Panse, L. Dong, J. M. Lizcano, D. R. Alessi, J. Schneider-Mergener* 2671–2674

High-Content Peptide Microarrays for Deciphering Kinase Specificity and Biology

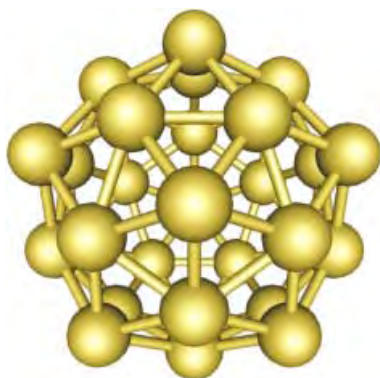
Microwave-induced magic: There is a widely held conviction that the antiviral marine alkaloid ageliferin **1** arises biosynthetically from a Diels–Alder reaction which, although possible, has yet to materialize in the laboratory. A total synthesis of **1** from scep trin is now reported that has led to a new hypothesis for how **1** and other dimeric pyrrole-imidazole alkaloids might be formed in nature.



Natural Product Synthesis

P. S. Baran,* D. P. O'Malley, A. L. Zografos 2674–2677

Sceptrin as a Potential Biosynthetic Precursor to Complex Pyrrole–Imidazole Alkaloids: The Total Synthesis of Ageliferin



Golden balls! Relativistic quantum chemical theory suggests the existence of an interesting and surprising gold cluster. The all-gold fullerene Au_{32} (see picture) is structurally very similar to the familiar buckminsterfullerene C_{60} , as it is approximately the same size and hollow. Au_{32} is stabilized by relativistic effects and spherical aromaticity; the magnetic shielding at the center of the fullerene has the highest predicted value.

Gold Fullerenes

M. P. Johansson,* D. Sundholm, J. Vaara 2678–2681

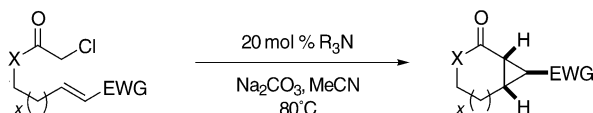
Au_{32} : A 24-Carat Golden Fullerene



Intramolecular Cyclization

N. Bremeyer, S. C. Smith, S. V. Ley,
M. J. Gaunt* ————— 2681 – 2684

An Intramolecular Organocatalytic
Cyclopropanation Reaction



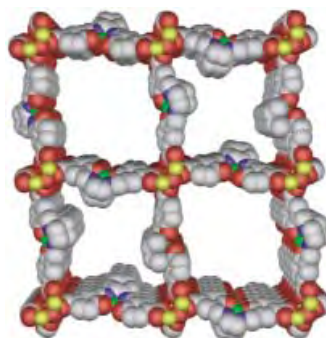
Nucleophilic tertiary amines catalyze an intramolecular cyclopropanation reaction to give $[n.1.0]$ bicycloalkanes in good yield (see scheme). The reaction is tolerant of a wide range of functional groups and is highly diastereoselective. Furthermore,

when a catalytic amount of a chiral cinchona alkaloid derivative is used the reaction produces cyclopropanes with enantiomeric excess of 94%. EWG = electron withdrawing group.

Crystal Engineering

R. Kitaura, G. Onoyama, H. Sakamoto,
R. Matsuda, S.-i. Noro,
S. Kitagawa* ————— 2684 – 2687

Immobilization of a Metallo Schiff Base
into a Microporous Coordination Polymer



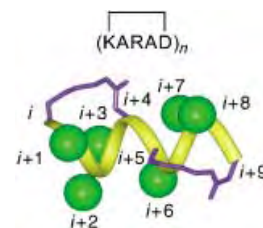
Wall decoration: Coordinatively unsaturated metal centers (Cu^{II} , Co^{II} , and Ni^{II}) can be embedded in the pore wall of a microporous coordination polymer containing Schiff base type metalloligands $[\text{M}(\text{salphdc})]^{2-}$ ($\text{H}_4\text{salphdc} = N,N'$ -*o*-phenylenebis(salicylideneimine-5,5'-dicarboxylic acid)). These coordination polymers possess large 1D channels with dimensions of approximately $14 \times 14 \text{ \AA}^2$ (see structure).

Helical Structures

N. E. Shepherd, G. Abbenante,
D. P. Fairlie* ————— 2687 – 2690

Consecutive Cyclic Pentapeptide Modules
Form Short α -Helices that are Very Stable
to Water and Denaturants

A generic approach to mimicking α -helices has been achieved by using sequences of consecutive macrocyclic pentapeptides (for example, $\text{cyclo}(1 \rightarrow 5)\text{-Ac-[KARAD]}_n\text{-NH}_2$) to form 3-turn (see picture) and 4-turn α -helices, which have high conformational stability in water and are resistant to protein-denaturing conditions (65°C , 8 M guanidine-HCl, trypsin digestion).

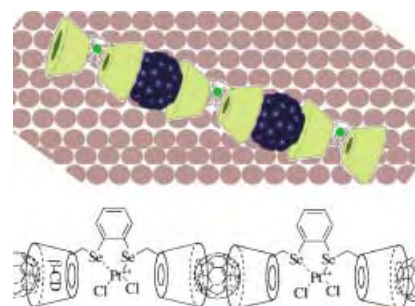


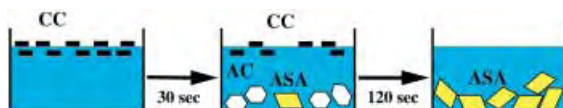
Supramolecular Chemistry

Y. Liu,* H. Wang, P. Liang,
H.-Y. Zhang ————— 2690 – 2694

Water-Soluble Supramolecular Fullerene
Assembly Mediated by Metallobridged
 β -Cyclodextrins

A coordinated metal center aids water solubility of a supramolecular fullerene assembly. Two cyclodextrins rings are bridged back-to-back in one unit, and these units form an end-to-end intermolecular inclusion complex with fullerene to produce an intermolecular polyfullerene (see picture). Under the right conditions the assembly acts as an efficient photo-driven DNA-cleavage reagent.





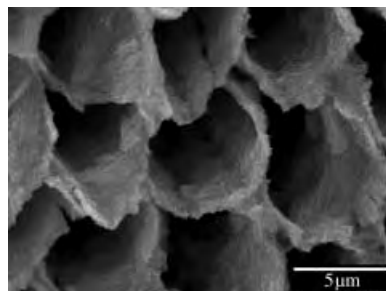
Blink and you will miss it! The structure of carbasalatum calcium (CC) has been determined by synchrotron powder diffractometry. By scrutinizing crystal dissolution and nucleation, it was found that the degradation of CC in water involves

acetylsalicylatum calcium (AC) as a furtive species that quickly exchanges its acetylsalicylic acid (ASA) ligands with water (see scheme). The crystal structure of AC is also reported.

Structure Elucidation

P. Ochsenbein,* M. Bonin, O. Masson, D. Loyaux, G. Chapuis, K. J. Schenk _____ **2694 – 2697**

Direct Evidence for a Furtive State in the Degradation of Carbasalatum Calcium



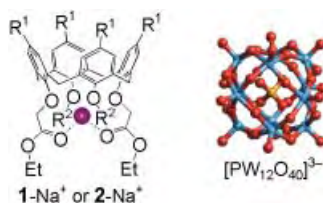
Dissolution of nanosized crystallites may be inhibited and even suppressed due to their small size. Nanoparticles with size distribution similar to that of critical dissolution pits (see picture) can be kinetically stabilized against further dissolution even in undersaturated solutions. In biological systems, this behavior confers remarkable self-preservation on biominerals in the fluctuating milieu.

Biominerals

R. Tang, L. Wang, C. A. Orme, T. Bonstein, P. J. Bush, G. H. Nancollas* _____ **2697 – 2701**

Dissolution at the Nanoscale: Self-Preservation of Biominerals

Amphiphilic pore walls facilitate reversible sorption of short-chain alcohols by the 1D microporous organic–inorganic hybrid $[1\text{-Na}]_3[\text{PW}_{12}\text{O}_{40}]$ assembled from calix[4]-arene- Na^+ complex **1**- Na^+ ($\text{R}^1 = \text{R}^2 = \text{H}$) and the polyoxotungstate anion $[\text{PW}_{12}\text{O}_{40}]^{3-}$. The analogous assembly formed with **2**- Na^+ ($\text{R}^1 = t\text{Bu}$, $\text{R}^2 = \text{CH}_2\text{CO}_2\text{Et}$) has a 3D microporous architecture.

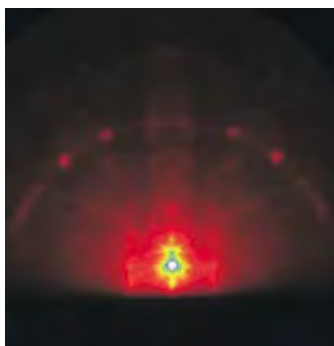


Hybrid Materials

Y. Ishii, Y. Takenaka, K. Konishi* _____ **2702 – 2705**

Porous Organic–Inorganic Assemblies Constructed from Keggin Polyoxometalate Anions and Calix[4]arene- Na^+ Complexes: Structures and Guest-Sorption Profiles

A formidable contender to X-ray diffraction is ultrafast electron crystallography. Whereas the former is more suited to investigate the bulk of the substrate, the time, length, and sensitivity scales of electron crystallography provide powerful and complementary information on atomic-scale structural dynamics at the surface (see diffraction image of GaAs crystal).



Electron Diffraction

F. Vigliotti, S. Chen, C.-Y. Ruan, V. A. Lobastov, A. H. Zewail* _____ **2705 – 2709**

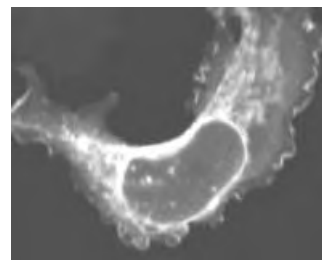
Ultrafast Electron Crystallography of Surface Structural Dynamics with Atomic-Scale Resolution

Fluorescent Probes

R. Reents, M. Wagner, J. Kuhlmann,*
H. Waldmann* _____ 2711–2714

Synthesis and Application of
Fluorescence-Labeled Ras-Proteins for
Live-Cell Imaging

Seeing is believing! Semisynthetic Ras-proteins with a fluorophore introduced at an appropriate site have been developed as probes for live-cell imaging experiments. They are processed by the cellular machinery, and their intracellular location can then be determined by confocal laser fluorescence microscopy.

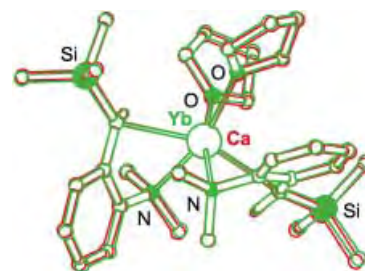


Stereoselective Polymerization

S. Harder* _____ 2714–2718

The Chemistry of Ca^{II} and Yb^{III}:
Astoundingly Similar But Not Equal!

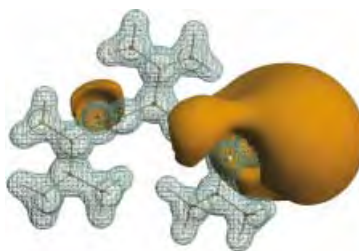
Not a red/green stereographic, but a plot showing the striking similarity between a Ca and Yb complex. The molecular structures, and the NMR and IR spectra of these Ca/Yb benzyl complexes are nearly identical. However, their reactivity in styrene polymerization is completely different: an internally coordinated dibenzyl-terbium(III) complex produces polystyrene of high syndiotacticity ($r=94.9\%$, $rr=90.0\%$).



Electron Density Studies

B. Dittrich,* T. Koritsánszky,
P. Luger _____ 2718–2721

A Simple Approach to Nonspherical
Electron Densities by Using Invariants



The transfer of multipole parameters from theoretically calculated pseudoatoms (invariants) allows the derivation of non-spherical electron densities and related properties even from low-resolution X-ray data, the electrostatic potential of tri(L-valine) is obtained in this way (see picture).



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Threading the Needle: Mimicking Natural Toroidal Catalysts**

Paul R. Carlier*

Keywords:

epoxidation · homogeneous catalysis · porphyrinoids · rotaxanes · supramolecular chemistry

One major aim of supramolecular chemistry is to create simplified analogues of enzymes and receptors that mimic the exquisite specificity and selectivity of their natural counterparts.^[1] The beginning of the 21st century has witnessed a series of exciting developments in this field, which include the development of supramolecular catalysts that exhibit high catalytic efficiencies, allosteric regulation, and enzyme-like topologies. Knowing that the hydrolytic ring-opening of epoxides is second-order in [(salen)Co] catalyst (salen is the 2,2'-[1,2-ethanediylbis(nitrilomethylidene)]bisphenol dianion), Jacobsen and Ready prepared cyclic oligomeric [(salen)Co] catalysts.^[2,3] These oligomeric catalysts offer dramatic rate enhancements in the asymmetric ring-opening of cyclohexene oxide compared to a monomeric catalyst, as a consequence of the reduced entropic penalty for bimetallic cooperativity.^[2,3] A related dimeric [(salen)Cr] catalyst was prepared by Mirkin et al., that allows allosteric regulation of the cooperative bimetallic catalysis.^[4] In this case the innovation is linkage of the [(salen)Cr] units by means of a sulfide/phosphane-rhodium chelate; sulfide ligand exchange on the rhodium atoms alters the distance between the catalytic chromium sites, thereby modulating the efficiency of the bimetallic catalysis.

An allosterically regulated catalyst for phosphate diester hydrolysis has been prepared by linking three hexapeptides to a trivalent scaffold.^[5] In the presence of zinc(II) species, the catalyst selectively hydrolyzes 2-hydroxypropyl-*p*-nitrophenylphosphate; in the absence of zinc(II) species, the catalyst selectively hydrolyzes oligomeric RNAs. Allosteric regulation of bimetallic cooperativity can also be controlled photochemically. The *cis*-isomer of the bis(barium) complex of azobis(benzo[18]crown-6) was shown to have higher catalytic activity than the *trans*-isomer in the hydrolysis of trifluoroacetyl anilides; switching between these isomers is effected by irradiation at 370 and 480 nm.^[6] To simulate the protective topology of cytochrome P450, Nguyen et al. embedded a metalloporphyrin in a molecular square.^[7] The resulting encapsulated catalyst shows a 10-fold enhancement in turnover number (relative to a control catalyst), which was attributed to steric prevention of deactivating μ -oxo formation. A cytochrome P450 model with a completely different topology appeared in a recent article by Rowan and Nolte and co-workers.^[8] They described a toroidal supramolecular epoxidation catalyst that exhaustively functionalizes a polymeric substrate by "threading the needle" (Figure 1).

This topology of substrate–catalyst interaction is characteristic of a number of DNA-processing enzymes, which include λ -exonuclease^[9] and T4 DNA polymerase holoenzyme.^[10] However, Rowan, Nolte, and co-workers are the first to achieve this catalysis topology in a purely synthetic system.

The work depends on several recent key developments by the Rowan and

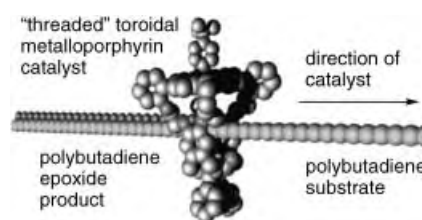


Figure 1. Proposed pseudorotaxane mechanism for epoxidation of polybutadiene by a toroidal metalloporphyrin catalyst. Modified with permission from ref. [8].

Nolte groups. The toroidal catalyst **1** used in the study consists of a metalloporphyrin with a binding pocket. The corresponding porphyrin **2** was first reported by Rowan and co-workers (Figure 2);^[11] the binding pocket that is essential to the design of the toroidal catalyst was created by means of a remarkable U-shaped glycoluril "clip" that had been developed a year earlier by Nolte et al.^[12] Rowan and co-workers verified that the binding pocket of the porphyrin host **2** strongly bound viologen (**4**), and used the standard technique of end-capping with bulky "stoppers" to transform this [2]pseudorotaxane into the permanently threaded [2]rotaxane.^[11] In 2000, Rowan, Nolte, et al. reported that **1** served as a cytochrome P450 analog, catalyzing epoxidation of alkenes by NaOCl.^[13]

Since the metalloporphyrin catalyst **1** has both an *exo*- and an *endo*-face, both simple *exo* (bimolecular) and *endo* (host–guest) epoxidation manifolds could operate. How could the geometry of substrate–catalyst interaction be controlled? One final innovation set the stage for the present work. Axial S- and N-donor ligands are known to increase the activity of iron–porphyrin and man-

[*] Prof. Dr. P. R. Carlier
Department of Chemistry
Virginia Tech
Blacksburg, VA 24061-0212 (USA)
Fax: (+1) 425-984-8099
E-mail: pcarlier@vt.edu

[**] I thank Professors Rowan and Nolte for providing original artwork.

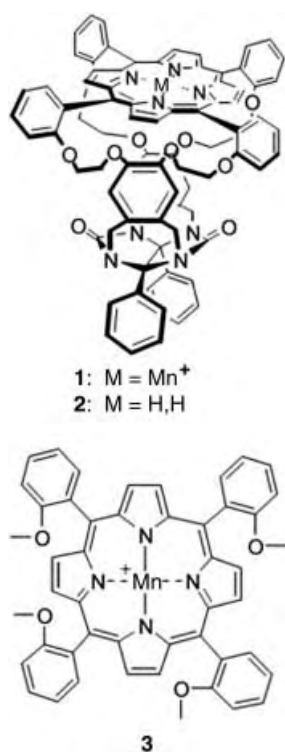


Figure 2. Toroidal metalloporphyrin epoxidation catalyst **1**, parent porphyrin **2**, and control epoxidation catalyst **3**. Modified with permission from ref. [8].

ganese–porphyrin epoxidation catalysts, presumably by facilitating formation of the putative M^V -oxo intermediate. Since the binding cavity of **1** has considerable affinity for organic guests, small donor ligands, such as pyridine (py) prefer to bind to the *endo*-face, forcing the oxo unit to go outside, thereby ensuring an *exo*-epoxidation manifold (Figure 3). However, if the axial donor ligand is too large to fit in the cavity, it has no choice but to bind to the *exo*-face. In the

presence of a large excess of 4-*tert*-butylpyridine (tbp), the *endo*-epoxidation manifold would be expected to predominate (Figure 3).

With all the pieces in place, the key experiment could be attempted. Could the toroidal metalloporphyrin **1** be used to catalyze the epoxidation of a polymeric substrate by a “thread-the-needle” pseudorotaxane mechanism? Using >98% *cis*-polybutadiene (**5**) as substrate, PhIO as the stoichiometric oxidant, and tbpy (500 equivalents) as the axial ligand, epoxidation reactions were carried out using **1** and the control, nontoroidal metalloporphyrin **3** (Figure 2 and Figure 4).

Not unexpectedly, the sterically more hindered toroidal catalyst **1** had a slightly lower turnover frequency (TOF) than **3**. Two observations at this point suggested that catalysis by **1** occurred within the cavity. First, whereas epoxidation by **3** transformed **5** to **6** with moderate stereoselectivity (*cis:trans* = 78:22), epoxidation with **1** as the catalyst occurs with considerable stereochemical leakage (*cis:trans* = 20:80). This leakage to the sterically less demanding *trans*-epoxide might be expected if catalysis occurred in the constricted cavity of **1**. Second, addition of viologen (**4**) a high-affinity guest for **2**,^[11] caused a 40% reduction in the TOF for epoxidation with **1**, but slightly increased the epoxidation TOF of **3**. These divergent results are consistent with the idea that **4** inhibits the formation of the active *endo*- Mn^V -oxo catalyst in **1**, and/or the threading of **5** through the cavity of **1**. However, the failure of the high-affinity guest **4** to completely inhibit **1** suggests

that despite the high concentration of bulky axial ligand tbpy (500 equivalents relative to **1**), some epoxidation of **5** by an *exo*-mechanism could be occurring (Figure 5; mechanism (a)).

To assess the rate of the *exo*-epoxidation reaction of **5** with **1**, a “blocked” [2]rotaxane analogue of **1** was prepared. In this case the cavity of the catalyst is permanently occupied by an end-capped viologen guest, so any epoxidation of **5** by this catalyst must occur by a bimolecular *exo*-mechanism. Since the blocked [2]rotaxane metalloporphyrin analogue had only 20% of the catalytic activity of **1**, it was estimated that for the epoxidation of **5** with **1**, 80% of the reaction occurs inside the cavity (Table 1, entries 1 and 2).

An *endo*-epoxidation manifold might appear to logically require a pseudorotaxane mechanism (Figure 1). However, an alternative possibility is that the polymeric substrate folds, and inserts a loop into the cavity (Figure 5, mechanism (b)). Though this possibility seems unlikely on steric grounds, to decisively rule it out, a novel polybutadiene designed to initiate pseudorotaxane formation was prepared, which allowed synthesis of a catalyst–substrate rotaxane. Polymer **7** (Figure 4) features a polybutadiene region consisting of 50–100 monomers, capped by two 4,4'-bipyridyl units, which has an affinity for the cavity of **1**. Combination of **7** with two equivalents of **1** thus forms a [3]pseudorotaxane, that can be converted into the permanently threaded [3]rotaxane **8** using the standard pyridine-alkylation capping method (Figure 6).

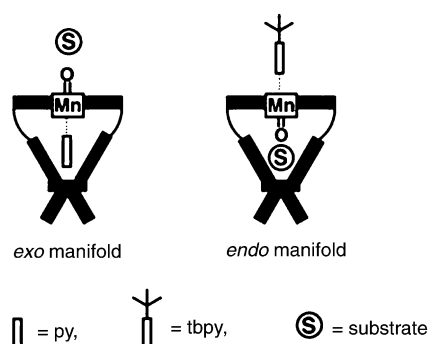


Figure 3. *Exo*- and *endo*- manifolds for epoxidation with **1**. Modified with permission from ref. [8].

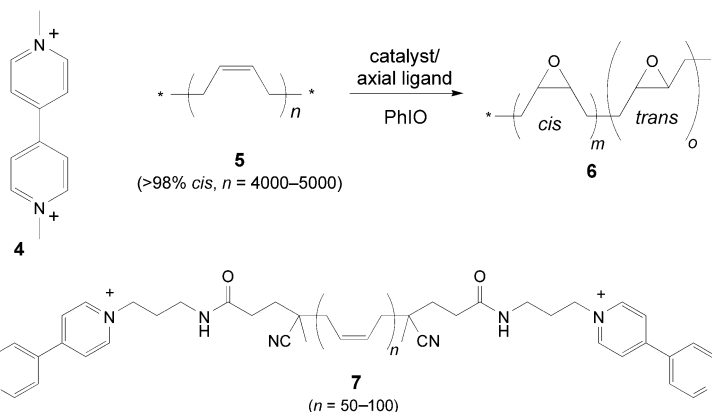


Figure 4. Toroidal catalyst guest **4**, substrates (**5**, **7**), and product **6**.

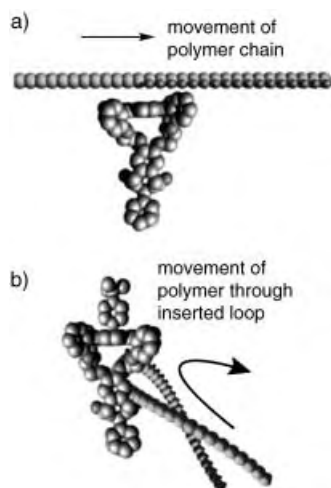


Figure 5. Alternatives to a pseudorotaxane mechanism for epoxidation of **5** with **1**: a) *exo*-mechanism, b) loop-insertion mechanism. Modified with permission from ref. [8].

At low concentrations designed to disfavor bimolecular epoxidation pathways, PhIO was added to the topologically linked catalyst–substrate rotaxane **8**. As hoped, transformation to the polyepoxide rotaxane occurred, which demonstrates the viability of the rotaxane mechanism and suggests that a loop insertion mechanism is not required for epoxidation of **5** with **1**. Under similar conditions, conversion of **8** into the polyepoxide occurred nearly twice as fast as epoxidation of **7** with **1** (Table 1, entries 3 and 4). Thus, as would be expected, the enforced rotaxane topology of **8** confers a kinetic advantage relative to the fluxional pseudorotaxane interaction of **1** and **7**. Finally, the operation of a significant *exo*-bimolecular manifold for the conversion of **8** into the polyepoxide rotaxane was ruled out by the observation that under these conditions, **7** is not epoxidized by **8**.

Table 1: Catalytic activities of selected manganese(III) porphyrins in the presence of tbpy in CDCl₃ in the oxidation of polyenes using PhIO.

Entry	Catalyst	Substrate	[C=C] [mM] ^[a]	[C=C]/[cat.]	TOF [h ⁻¹] ^[b]
1	1	5	8	42	15
2	“blocked” [2]rotaxane analogue of 1	5	8	42	3
3	1	7	8	43	7
4 ^[c]	8	8	6	37	12

[a] Concentration of double bonds in the substrate (concentration = [substrate] × number of double bonds per substrate molecule). [b] Reaction performed in CDCl₃ in the presence of tbpy (500 equivalents relative to catalyst) using PhIO as the stoichiometric oxidant; Turnover frequencies were calculated by ¹H NMR spectroscopy at 30% conversion. [c] In **8** the catalyst and substrate are topologically linked.

With a pseudorotaxane mechanism for epoxidation of **5** with **1** now established, another question emerges: does this exhaustive epoxidation occur by a sequentially processive mechanism (depicted in Figure 1), or by a random, sliding process (not shown)? The DNA enzyme λ-exonuclease is highly processive: it binds a free end of double-stranded DNA and remains bound to the remaining single-strand as it sequentially cleaves around 3000 nucleotides.^[9] To achieve a sequential processive mechanism in the present case would require extremely high rates of epoxidation and Mn^{III} reoxidation; if these constraints were not met, Brownian “sliding” of the toroid along the polymer should lead to random epoxidation. However, in a two phase aqueous–organic system, the solubility of **1** and **5** in organic solvents, coupled with the high water solubility of the polyepoxide product **6** might favor operation of a sequential processive mechanism. Tantalizingly, Rowan, Nolte, and co-workers observe significantly increased TOF values in two-phase epoxidation reactions.^[8]

Could the use of toroidal catalysts, and the operation of a sequential proc-

essive mechanism enable post-polymerization transformation of functional polymers into new block copolymer structures? Will synthetic toroidal catalysts prove useful for in vitro and in vivo transformations of biopolymers? The studies of Rowan and Nolte described herein have set the stage for these and other investigations.

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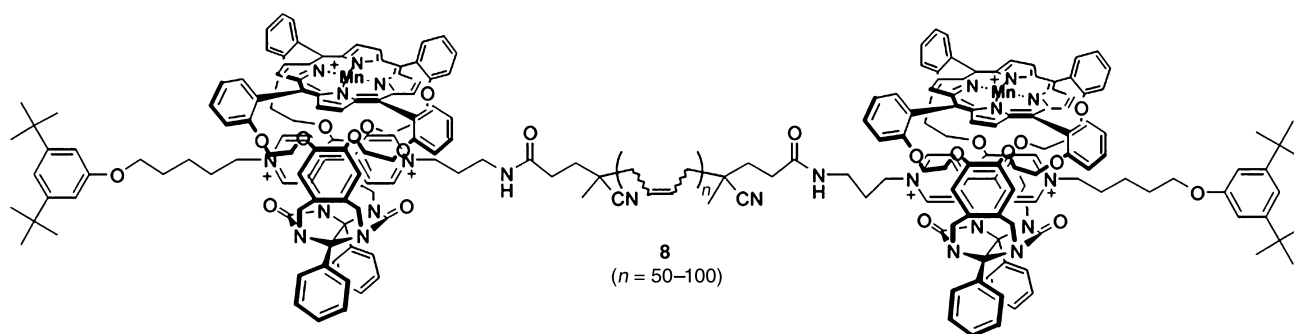


Figure 6. Topologically-linked catalyst–substrate rotaxane **8**. Modified with permission from ref. [8].

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Ultrafast Electron Crystallography: The Dawn of a New Era

John Meurig Thomas*

Keywords:

dynamic structures · electron crystallography ·
electron diffraction · femtochemistry ·
surface structures

Introduction

In 1991, Williamson and Zewail^[1] took femtochemistry into the realm of diffraction, in particular into ultrafast electron diffraction (UED). The merit in doing so centered largely on the fact that electron diffraction entails the scattering of electrons off *all* the atoms and atom–atom pairs in the molecular assembly under investigation, in contrast to spectroscopy in which the probe is tuned to specific transitions in the molecule. In other words, an electron beam is sensitive to all species in its path and is hence potentially capable of uncovering the entire landscape of individual structures to which spectroscopy may be blind. UED utilizes a femtosecond (fs) laser pulse to initiate a desired chemical reaction; however, the subsequent laser pulses used in femtospectroscopy to probe the progress of the reaction are replaced with ultrashort pulses of electrons. Diffraction patterns are then recorded in real time to determine the changing internuclear distances of the molecular species under investigation.

When the paper by Williamson and Zewail appeared, it was soon appreciated^[2] that, provided the fiendishly difficult^[3] experimental requirements to

perfect UED could be met, one could foresee a new era in charting the course of the dynamics of structural change. It was also appreciated that UED would open up very many more systems that could be investigated at the quantum limits of resolution than could be probed by femtospectroscopy alone. In his Nobel lecture,^[4] Zewail envisaged UED as a major new direction for his post-Nobel research endeavors and he and his colleagues have developed three, progressively more sophisticated, generations of UED apparatuses, admirably illustrated in a recent comprehensive review.^[5]

UED is an extraordinarily powerful technique for a variety of reasons. First, electron-scattering cross-sections are higher than those of X-ray scattering from molecules by a factor of approximately six orders of magnitude. Second, because of the short penetration depth of the electron (arising from the strong electron–specimen interaction), it is well suited for the study of tenuous gases, of thin condensed samples, and it is particularly sensitive to surface features (see below). Third, electrons are less damaging to specimens, *per useful scattering event*, than are X-rays. Fourth, by recording differences in diffraction patterns (at a given evolved time as against zero (or “negative”) time) UED achieves unprecedented sensitivity in detecting chemical changes—values as low as 1% are measurable (Figure 1). Last, notwithstanding the complexity of the various components that need to be orchestrated to work in unison in UED,^[3] the diffractometer is of ‘tabletop’ scale, unlike, for example, a synchrotron, which in principle could

ultimately address many of the problems that may be studied by UED.

Consideration is currently being paid by some molecular biologists to build a ‘free-electron’ synchrotron source, which they argue could attain femtosecond resolution in the structural elucidation of the sequence of molecular processes undergone by substances such as bacteriorhodospin (a component of the purple membrane). Quite apart from the likelihood that specimen damage by such intense X-ray pulses may prove critical, the cost implications far exceed those pertaining to the ‘tabletop’, sophisticated apparatus of Zewail.

A Résumé of the Achievements of UED to Date

The exceptional levels of sensitivity and spatio-temporal resolution achievable by UED have already resulted in the elucidation of a wide diversity of chemical phenomena, all involving reactants and products isolated in the gas phase.^[5] The quintessential point about UED—in contrast to what gas-phase, steady-state electron diffraction (initiated by Mark and Wierl^[6] and subsequently extended by other groups) has achieved—is that it probes transient structures as well as the states and processes in which they participate. These may encompass reactive intermediates (e.g. carbenes), pseudorotary transition states, spin states, radiationless processes, non-equilibrium geometries, conformations on complex energy landscapes, and reaction pathways. Some examples are considered below:

[*] Prof. Dr. Sir J. M. Thomas
Davy Faraday Laboratory of the
Royal Institution
21 Albemarle Street, London W1S 4BS (UK)
and
Department of Materials Science
University of Cambridge
Pembroke Street, Cambridge CB2 3QZ (UK)
Fax: (+44) 1223-334-567
E-mail: jmt@ri.ac.uk

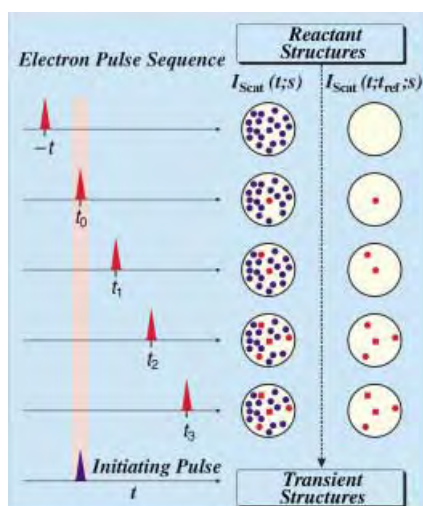


Figure 1. The concept of UED. An ultrafast initiation pulse (blue signal) triggers the reaction, and a second ultrashort electron pulse (red signal) probes the resulting structural change. The electron pulse can be timed to arrive before the initiating pulse (negative time), thus probing the parent (filled blue circles) or after the laser pulse (positive time), now probing the transient structures also (filled red circles and squares). The time at which the light and electron pulses arrive simultaneously at the molecular sample is the zero of time (t_0). With increasing time lapse after the initiation pulse, the transient species undergoes a population change (indicated by the growth of the filled red units at the expense of the blue filled circles), and a structural change (shown as the transformation of red circles into red squares). The difference between each time-resolved diffraction pattern and a reference pattern at t_{ref} (chosen at negative or at positive time), determined by the diffraction-difference method, allows the extraction of this small population of time-evolving structures embedded in the large background signal of unreacted parents. This dramatically enhances the significance of the contribution by the transient species in the diffraction-difference patterns, $I_{\text{scat}}(t; t_{\text{ref}}; s)$. The small residual contribution of unreacted parents to the diffraction-difference pattern is not shown here for sake of simplicity.

a) Pyridine: Upon excitation, several possible reaction pathways are open

to the molecule, including valence isomerization, fragmentation, and ring opening. UED uncovered a hitherto-unknown ring-opened diradical intermediate structure resulting from the scission of the C–N bond, thereby shedding new light on a longstanding enigma concerning the reactivity of aromatic molecules. Moreover, the ring-opened diradical structure was isolated from a veritable zoo of other entities.

b) Haloethanes (e.g., $\text{C}_2\text{F}_4\text{I}_2$): UED greatly extended the evidence for the nature of their decomposition, which was first acquired by picosecond photofragment spectroscopy.^[7] Following excitation, the first I atom is lost on the femtosecond timescale to form the transient $\text{C}_2\text{F}_4\text{I}$ radical intermediate. The question immediately arises as to whether the structure of this radical is classical or nonclassical. UED leaves no scintilla of doubt that it is indeed classical (Figure 2a).

c) $[\text{Fe}(\text{CO})_5]$: Among the largest family of organometallic carbonyl complexes, $[\text{Fe}(\text{CO})_5]$ stands out as prominently as does *Drosophila* in the living world: It is one of the most extensively studied molecular systems. It may photodissociate into five distinct products, $[\text{Fe}(\text{CO})_x]$ ($x = 4, 3, 2, 1, 0$), depending on the excitation wavelength used. In all these reactions, $[\text{Fe}(\text{CO})_4]$ is considered a primary intermediate and serves as a kind of conduit for various ensuing reactions, such as decomposition, recombination with CO, and coordination with solvent molecules.^[8] Elucidating the nature of $[\text{Fe}(\text{CO})_4]$, including its electronic states and the corresponding molecular geometries, is therefore important in the context of understanding the role of intermediates in the photolysis of transition-metal car-

bonyl complexes. The molecular structure was unambiguously determined by UED (Figure 2b). The technique clearly showed that the first major product is the transient $[\text{Fe}(\text{CO})_4]$, and that this is formed in the singlet ($^1\text{A}_1$) state, rather than in the ground triplet ($^3\text{B}_2$) state.

Ultrafast Electron Crystallography (UEC)

In recent publications,^[9,10] which are unmistakably landmarks in the general domain of time-resolved structural studies, Zewail and co-workers reported the use of their so-called fourth-generation apparatus for UED to determine transient structures of molecules and surfaces and phase transitions, and have thereby entered the altogether new realm of ultrafast electron crystallography.

Before embarking on a discussion of the methodology and promise of UEC, it is instructive first to recall how the structures of solid surfaces (encompassing adsorbed phases and the reconstituted pristine solids) are determined at present. It is also prudent to outline the various meanings nowadays associated with the term electron crystallography in the general determination of the structures of both inorganic solids and the various macromolecular entities that are ubiquitous in molecular biology. All these methods of structure determination (other than UEC in the sense used by Zewail) arrive at *static* structures.

The technique known as LEED (low-energy electron diffraction), of ancient lineage,^[11] generally requires ordered two-dimensional (meshes) of either the adsorbate and/or the underlying solid (substratum) before it can be applied. It is suited for the study of well-defined, single-crystal (typically

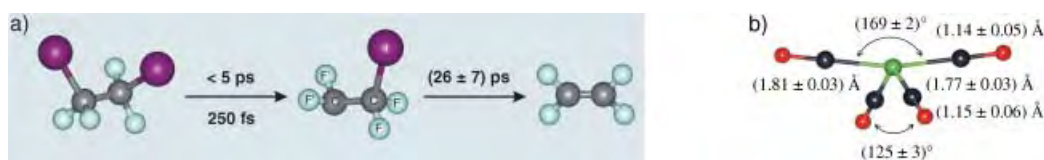


Figure 2. a) The elimination reaction of $\text{C}_2\text{F}_4\text{I}_2$ was determined by UED on the given timescales as described in reference [5]. The bond lengths and angles of all three species were determined and compared with those calculated ab initio and DFT methods. b) The refined molecular structure of $[\text{Fe}(\text{CO})_4]$ in the $^1\text{A}_1$ state, determined by UED.^[5,8]

$10 \times 10 \text{ nm}^2$) discs and it is most usefully applied to the determination of elemental or simple binary solids as well as those on which relatively small adsorbed organic species such as benzene, ethene, and ethynylidyne ($\text{CH}_3\text{-C}\equiv$) are adsorbed. The spatial resolution in directions parallel to the surface is approximately $0.05 \text{ \AA}$ and perpendicular to it about $0.02 \text{ \AA}$.

In an electron microscope, minute specimens (of substantially less than $100 \times 100 \text{ nm}^2$ in area) are often amenable to total structural determination at atomic resolution by the technique of convergent beam electron diffraction (CBED).^[12,13] In this case, a focused beam of monoenergetic, high-energy (typically 100–300 keV) electrons, often a few nm in diameter, is used to select the area from which the diffraction is formed. The high convergence angle gives rise to diffraction discs rather than spots in the back focal plane of the objective lens, and the contrast seen within each disk can be regarded as a 'map' of diffracted intensity as a function of orientation for each set of planes (reflections). This degree of detail confers upon CBED the power to elucidate the symmetry of crystals and their orientation and, importantly, enables the structure factors to be determined with such accuracy that charge-density maps may be calculated.^[12]

An extra advantage of this method is that when specimens contain heavy elements (so that, normally, low-order reflections suffer from multiple scattering effects), so-called high-order Laue zones may be recorded. In these Laue zones, electron scattering is kinematic (or quasi-kinematic), thus enabling the conventional procedures used in X-ray crystallography (in which kinematic conditions apply) to be performed on electron diffraction intensity data.

Electron crystallography has been used to determine the structure of many organic materials and of viruses.^[14–16] The method entails using the amplitudes from the intensities of the electron diffraction spots and the extraction of the phases of the corresponding reflections from the high-resolution image.^[17]

Just as the arrival of exceptionally sensitive two-dimensional charge coupled device (CCD) cameras has played a crucial role in the progressive evolution

of Zewail's UED work, so also has the availability of slow-scan CCD cameras and the concomitant revolution in digital processing transformed the field of conventional (static) electron crystallography, particularly in the hands of Terasaki and co-workers.^[16] A high-resolution transmission electron micrograph of an ultrathin specimen—to eliminate multiple scattering of electrons—contains all the required information (i.e. the relative phases and the amplitudes of the diffracted beams) for an ab initio solution of the crystal structure. Crystal structure factors are obtained from a set of (calculated) Fourier diffractograms of high-resolution images recorded along a few zone axes. In essence, these diffractograms contain not only amplitudes of diffracted peaks but also their relative phases, and so the structure of the thin crystal is solvable (provided enough reflections have been collected in the diffractogram of a series of zone axes).^[18]

This extended prelude to the discussion of the breakthrough by Zewail and co-workers in UEC underlines the fact that all preceding methods of electron crystallography yield only static structures. Likewise, all the high-resolution (transmission and scanning transmission) electron microscopic imaging procedures reported to date have either provided entirely static pictures or, when in situ methods^[19,20] are employed, have achieved temporal resolutions in the order of seconds or minutes. In their most recent papers, Zewail and co-workers^[9,10,21] demonstrated the feasibility of determining structures at solid surfaces with submonolayer sensitivity and time resolutions of less than 1 ps.

Two fascinating sets of experiments were performed by using the setup shown in Figure 3. In one experiment, (111) surfaces of silicon were exposed to either hydrogen, chlorine, or trifluoriodomethane, and the changes in bond lengths caused by these adsorbed species could be determined to within approximately $0.01 \text{ \AA}$. Typically, the optical initiating pulse is 120 fs. (At a wavelength of 266 nm, the penetration depth is 4 nm, but with pulses of IR radiation (800 nm) it is $7 \mu\text{m}$.) Repetition rates of 1 kHz were used, and a diffraction pattern could be recorded in 1–5 s (compared to 100 min for the third-generation apparatus for UED).

Zewail and co-workers^[9] studied the phase transition of silicon at high-energy fluences. Irradiation of the crystalline silicon with IR pulses for a few hours, just below the damage threshold, resulted in amorphous silicon—the loss of crystallinity was apparent from replacement of Bragg diffraction spots by smooth rings. The amorphous-to-liquid phase transition occurs in approximately 10 ps, but the amorphous state is recovered on a much different timescale. Interestingly, the liquid phase persists for about 100 ps, which shows that surface layers probed by the electrons remain very hot as they are unable to dissipate the excess thermal energy.

Exciting as the results presented in this recent paper undoubtedly are, perhaps the most exciting prospects of all are latent in the comments made by Zewail and co-workers in their final paragraph: Among other statements, they proclaim that it will be possible to probe, by means of UEC, the structural dynamics of macromolecular assemblies and the catalytic reactions of the type outlined by Zhou and me last year.^[22] As far as the first of these is concerned, a recent UED study by Zewail and co-workers^[23] showed far-from-equilibrium structures that reflect large amplitude torsional motions in processes such as the ring opening of cyclooctatriene. Based on these results, it is almost certainly the case that such torsional modes play a key role in the biological function of macromolecules (see reference [24]).

Using the same setup shown in Figure 3 (see also Figure 1 of reference [10]), Zewail and co-workers turned their attention to the dynamics of the (111) surface of chlorine-terminated gallium arsenide. From the changes (i.e. shift in position, width, and intensity) of particular Bragg diffraction peaks, they discovered that *compression* and *expansion* of the surface layers occur on the $-0.01 \text{ \AA}$ to $+0.02 \text{ \AA}$ scale, respectively, and that the transient temperature (calculated from known Debye–Waller factors) reaches a maximum value of 1565 K in 7 ps. Remarkably, the temperature evolution precedes the lattice expansion by 13 ps—in other words, it takes this long for the structural expansion to occur after the rise in temperature. Intriguing

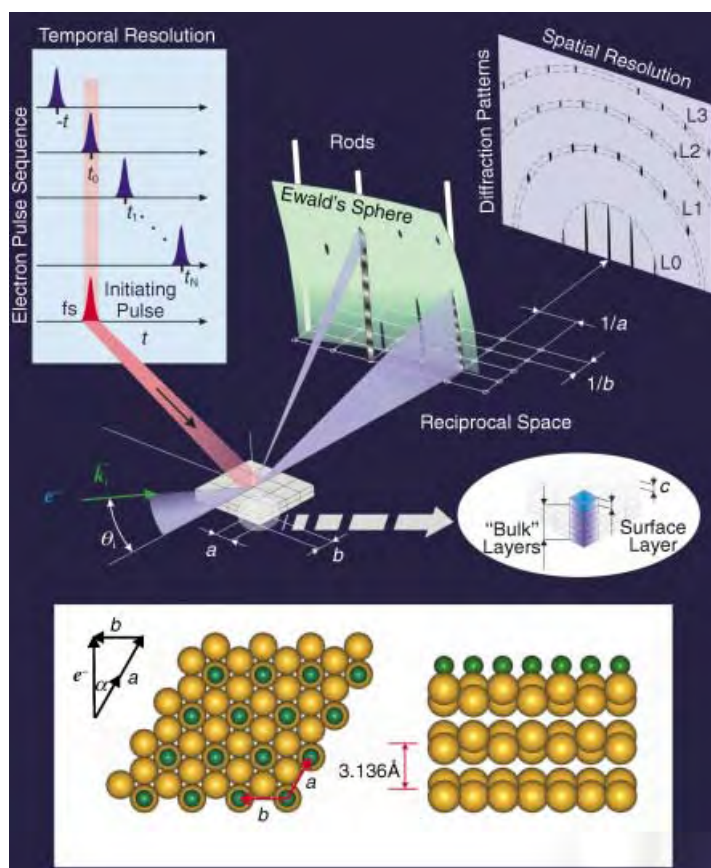


Figure 3. The concept of UEC and the generic structure of the studied crystals ((111) surface of silicon with adsorbate). For simplicity, only a few selected reciprocal lattice rods are shown; each circle in the plane represents a rod. Both the Ewald sphere and Laue zones (L0, L1, ...) are displayed (see text). The electron interacts with the crystal surface at an incidence angle of ϕ_i and an azimuthal angle α in the [2,1] zone axis. Inset, bottom: structure and distance between adjacent bilayers.^[9]

ingly, the temperature jump to 1565 ± 83 K just exceeds the melting point (1513 K); however, the peak temperature does not last long enough for the crystallinity to be lost.

What is particularly advantageous about this study (with grazing-incidence 30-keV electrons) is that only the outermost (surface) layers of the crystal are probed. (With such electron beams, the penetration depth is approximately 7 Å, whereas in femtosecond X-ray crystallography^[25,26] a heating pulse typically has a μm -scale penetration depth, so that it cannot unambiguously probe the dynamics of surface atoms of solids.)

As far as the suggestions in reference [22] are concerned, it is highly probable that the UEC will detect and characterize the superoxide ion (O_2^-) and similar transient species at the surfaces of selective oxidation catalysts. (This species has been identified under

static conditions by high-resolution electron-energy-loss spectroscopy at single-crystal surfaces.^[27]) However, it remains to be seen whether the heating up of the solid surfaces, unavoidably inflicted by the laser and electron pulses that are an intrinsic feature of the UEC technique, will itself open up (as has been witnessed in other femtosecond pulse studies of catalysts^[28]) a reaction pathway that is different from the thermally mediated one which is the initial object of the dynamic structural study of solid catalysts.

Most recently, Zewail and co-workers reported another significant advance in the study of nanometer-scale macromolecular structures on hydrophobic and hydrophilic surfaces.^[29] In particular, they directly determined the structure and dynamics of interfacial water on a hydrophilic surface (chlorine-terminated silicon) with atomic-scale reso-

lution. The success of UEC in studying assemblies on solid substrates opens up new directions for mapping out structural dynamics of macromolecules with unprecedented sensitivity and resolution, both spatial and temporal.

The very meaning of electron crystallography has been fundamentally changed now that it has been unambiguously demonstrated to be capable of the ultimate (quantum) limits of spatial-temporal resolution of surface phenomena. Already many recent speculations on “time-resolved chemistry”^[30,31] have been radically transformed by what is reported and promised in the current papers of Zewail and co-workers.^[9,10,29]

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Controversy in Chemistry: What Counts as Evidence?—Two Studies in Molecular Structure**

Jay A. Labinger* and Steve J. Weininger*

Keywords:

history of science · isomers · stereochemistry · structure elucidation

An Overview

“The facts speak for themselves.” Well ... in science as in life, sometimes yes but often no. The very process of turning scientific data into “facts” can itself be contentious, so that even at this level the “facts” may be far from universally agreed upon. Even when they are, assessing their larger significance is an interpretive process that frequently leads to disagreement or worse. In brief, the normal practice of science provides ample opportunity for controversy; indeed, it is the rare scientist who has not followed, if not participated in, a vigorous scientific controversy that has stretched out for months or even years.

Precisely because controversies are part and parcel of normal science, our colleagues in science studies—historians, philosophers, and sociologists of science—have turned increasingly to the study of controversies for what they reveal about the processes of science.^[1] Among those colleagues there is a growing consensus that controversy is not only characteristic of science but essential to its development. As practicing chemists we are not surprised, shocked, or embarrassed at the role that

controversy has played in the growth of our discipline. As chemical educators, however, we are disturbed at the way in which this aspect of science has been hidden from our students and even from our peers. It's almost as though there were a “conspiracy” to conceal the fact that chemistry is an open and dynamic network of investigations, and to present it instead as a closed, static body of facts. The naive student or lay person is often led to envisage science as an imperturbable machine that regularly ejects neatly sealed parcels of imperishable truth, a vision that does not serve them well.^[2]

In what follows we explain why this bland, sanitized picture of science does a disservice to students, scientists, and science itself, and lead up to one possible corrective that we have used: a series of case studies of chemical controversies. The incentive to assemble and coordinate the material described in this and subsequent articles came through developing a course for students majoring in science or engineering.^[3] However, the gaps that this material seeks to fill are widespread not only within the university but in the larger educated community as well.

We should point out that a number of articles related to the theme of controversy in chemistry, written by chemists for an audience of chemists, have appeared recently, several in this journal. A few examples are: a recent book by Jerome Berson that covers a variety of chemical/philosophical controversies;^[4] a survey of literary responses to Fritz Haber and his role in chemical warfare;^[5] an assessment of credit due for the explanation of the structure of ferrocene;^[6] and an examination of the role and power of Ock-

ham's Razor in chemistry.^[7] The last of these articles has been cited in about 30 research reports, which has encouraged us in our belief that attention to such considerations can indeed have a positive impact on scientific practice.

Real versus Idealized Science

There has been a long-standing concern in science policy circles over the small proportion of students who choose science as a profession, as well as over the low level of scientific literacy among the general public. These issues are unquestionably large in scope, yet we believe that *in part* they have a similar origin, namely the way in which science is often taught. Several decades of research have made apparent the discrepancy between the skeletal fiction taught to students as “the scientific method” and actual scientific practice. This discrepancy is not news to practicing scientists, yet the representation of science often fails to match the reality. Thomas Kuhn's characterization of science pedagogy, written 45 years ago, is still appropriate:

“Except in their occasional introductions, science textbooks do not describe the sorts of problems that the professional may be asked to solve and the variety of techniques available for their solution ... Even the most faintly liberal educational theory must view this pedagogic technique as anathema ... and yet we must recognize ... [that] education in the natural sciences ... remains a dogmatic initiation in a pre-established tradition that the student is not equipped to evaluate.”^[8]

[*] Dr. J. A. Labinger
Beckman Institute
California Institute of Technology
139-74, Pasadena, CA 91125 (USA)
Fax: (+1) 626-449-4159
E-mail: jal@its.caltech.edu
Prof. S. J. Weininger
Worcester Polytechnic Institute
Worcester, MA 01609-2280 (USA)
Fax: (+1) 508-831-5933
E-mail: stevejw@wpi.edu

[**] Controversy in Chemistry Part I.

While “facts” are surely central to any discussion of science, context-free presentations which suppress the reality that “science is a complex and messy business”^[9] turn many away from science altogether, often at an early stage. As Nobel laureate Dudley Herschbach has noted,

“Many students [in introductory science courses] have told me about a disheartening syndrome: the questions and problems seem to have only one right answer, to be found by some canonical procedure. The student who does not quickly grasp the “right” way, or finds it uncongenial, is soon likely to become alienated from science ... Nothing could be further from what actual frontier science is like.”^[10]

One of us (S.W.) has felt this same unease, fed by extensive experience teaching basic organic chemistry. He sees many capable and industrious students become frustrated by their apparent inability to grasp concepts that are typically presented as not only logical but inescapable. Had those concepts been represented as the product of long, collective struggles within the chemical community, and subject to revision or even rejection, many students might have been spared disaffection and self-doubt.

Of those students who remain in science, many continue to hold a rather distorted idea of what it is really like. One of us (J.L.) first realized this while reading the student evaluations of an advanced course he taught on catalysis. In courses that cover primarily recent and still ongoing work, he likes to present much of the material in case-study mode. That is, he might describe some of the first experiments on a given topic, explain how they led to a certain conclusion, then show how later work changed the interpretation, and so on. A number of students don't seem to care for this at all. “It's too confusing,” one course evaluation read, “why can't you just give us the facts?” This response was rather surprising, especially from graduate students who would shortly be going out into the real world and engaging in precisely that sort of process

themselves. But perhaps it *isn't* so surprising, if most science education portrays science itself as the unproblematic generation of “facts.”

Some readers may feel that the best course of action with respect to many of these controversies would be to ignore them, because bringing them into the limelight would only promote antiscientific attitudes. While our sample is admittedly small, the reactions of our students do not at all support that view. In fact, the discovery that science is a human enterprise has encouraged some students to believe that ordinary mortals like themselves have a place in science. Furthermore, the realization that chemistry is in fact a dynamic, fluid, and occasionally contentious enterprise has made it more appealing for them as a career choice, and we harbor the (perhaps immodest) hope that it will even make them better scientists. As one student wrote,

“I have come to realize that this whole course has changed my outlook on science. I had always assumed that it was straightforward research in black and white that led to definitive answers. I realize in reading all of these articles that it's not as cut and dried ... This has also taught me to see that there is more to science than meets the eye. Although I found it difficult not to take sides on a particular issue that I felt strongly about, I have learned that a critical eye is very necessary in analyzing scientific work.”

In our view the problem of science literacy is one that transcends the distinction between scientists and non-scientists. Scientists may have a greater knowledge of scientific facts and techniques, but it is not clear whether they enter their careers having a greater sense of the complex reality of science than nonscientists do.

Case Studies of Controversies: A Window on Real Chemistry

An often prescribed antidote for the problems just discussed is the inclusion of historical material in the education of

scientists. Over a decade ago the American Association for the Advancement of Science advised the following:

“Science courses should place science in its historical perspective. Liberally educated students—the science major and the non-major alike—should complete their science courses with an appreciation of science as part of an intellectual, social, and cultural tradition ... Science courses must convey these aspects of science by stressing its ethical, social, economic, and political dimensions.”^[11]

However, attempts to implement such recommendations encounter difficulties. To begin with, there seems to be a widely held belief in the science studies community that the history of chemistry can yield very few general insights into the fundamental workings of science, a supposition also held by many scientists themselves. For example, the report from the AAAS cites ten “significant discoveries and changes that exemplify the evolution and impact of scientific knowledge” that it recommends for introducing historical perspective into science teaching. Of these, only one, conservation of matter, even partly falls into the domain of chemistry.

The challenge then is to use historical material in a way that does justice to the multifaceted reality of chemistry. That certainly cannot be accomplished by augmenting the standard parade of chemical facts and algorithms with an equally uninspiring parade of names and dates. Rather, the aim must be to emphasize the relevance of chemical developments to larger intellectual issues so that

“... the history of chemistry is no longer seen as a single main line of advance, with regrettable detours like that through the phlogiston theory. Past controversies must be seen as involving choice between live options; theories must be judged according to the information available, and the canons agreed upon, at the time they were proposed.”^[12]

Our approach to answering this challenge has been to assemble a set of case studies, in this and forthcoming articles, of controversies that have claimed and often continue to claim the attention of both chemists and non-chemists. They span a period from the 18th century to contemporary times, and demonstrate that chemical controversy is a special characteristic of neither the past nor the present. We feel it is particularly important to avoid presenting historical issues as somehow qualitatively different from contemporary ones—as the old saying goes, history begins with yesterday. Also, we strive to do justice to the technical and contextual aspects alike, and even to question the neat division between science and context. Beyond these goals, it is our hope to recapture the sense of urgency and even drama that permeated those events and made them seem critical to the future development of chemistry and, at times, even the world. These disputes generated extensive investigations and strong passions on the part of many intelligent men and women—it can only benefit our understanding of chemistry to find out why.

The nature of evidence and its proper interpretation has always been a major preoccupation of all modern sciences, and chemistry is no exception. In the history of our discipline these problems have generated many intense controversies. Here we explore two cases, well separated in time and *apparently* quite different in character as well—but perhaps not quite so different as appearances might suggest. First we consider the disputes surrounding the birth of stereochemistry, which are not only of major historical interest but also raise issues that are still with us today. Then we turn to a much more recent controversy, the case of bond-stretch isomerism.

Case 1: The Nature and Interpretation of Evidence in the Birth of Stereoisomerism

Justus Liebig's analytical method for determining C and H (1830), Edward Frankland's enunciation of the valency concept (1852), and the reform of atomic weights that took place in the 1860s

provided the requisite underpinnings for the structural theory of organic compounds. Grounded in the assumed quadrivalency of the carbon atom and its chain-forming abilities, structural theory allowed the chemist to envisage all the constitutional isomers that could correspond to a given molecular formula. For example, when in 1864 Aleksandr Mikhailovich Butlerov synthesized the previously unknown $C_4H_{10}O$ isomer tertiary butyl alcohol, he recognized it as a striking confirmation of the structural theory.

By the 1870s the theory was in the ascendant in the major European chemical centers, especially Germany and Britain. As powerful as it was, the theory could only rely on the results of elemental analysis and chemical reactions to verify its conclusions, which were symbolized in structural formulas. And for many chemists, those formulas represented the limit of what chemical methods could reveal about chemical structures. There were few experimental means available to chemists at that time; in particular, there were no techniques that gave any direct insight into the spatial arrangement of atoms in molecules. So the question posed itself: to what (if any!) extent should inferences from chemical methods, particularly the existence or nonexistence of isomers, be taken as acceptable evidence concerning structure?

One might envision the dilemma of structural chemistry by thinking about a sealed power supply which is represented by a circuit diagram (the structural formula). The circuit diagram indicates clearly the connections among the components of the power supply (in our case, the atoms) but it doesn't claim to show how they are actually positioned with respect to one another. To gain any information of that sort the power supply (for us, the molecule) has to be broken apart, which might very well lead to a scrambling of the components. Indeed, the recognition that molecules were dynamic objects undergoing rapid internal motions led many to ask whether it would *ever* be possible to arrive at three-dimensional molecular structures.

The question was of course open to speculation, and some indeed did speculate about molecular arrangements. But that could be unhealthy for one's

professional reputation. For many chemists of this period “speculation” was a term of derogation—it raised the specter of the much-despised *Naturphilosophie*. The prevailing atmosphere among chemists in the 1860s and 1870s was one in which optimism and caution (a sense of progress and an awareness of limits) were in delicate equilibrium. That equilibrium was soon to be disturbed.

The year 1874 saw the simultaneous and independent publication of two seminal papers, by Joseph Achille Le Bel and Jacobus Henricus van't Hoff, that came to be the founding documents of stereochemistry.^[13] Grounded in different traditions and adopting different approaches, the French and the Dutch chemist nonetheless agreed on one salient point: that the cause of optical activity in organic compounds was molecular asymmetry. Furthermore, they also concurred that the presence of a carbon atom tetrahedrally connected to four different substituents would be a sufficient cause of asymmetry. Le Bel, whose approach was geometrical and general, seems to have been somewhat ambivalent about whether optical activity either proved or required the presence of a tetrahedral atom.^[14] van't Hoff by contrast was a strong realist, both ontologically (he believed in the physical existence of atoms and molecules) and epistemologically (he believed that the three-dimensional arrangements of atoms within molecules could be known). Given the kind of evidence available, van't Hoff had to rely on inductive arguments. He pointed out that all naturally occurring, optically active substances whose structures were seemingly secure contained an asymmetric carbon atom and cited, for example, lactic, malic, and aspartic acids as well as a variety of sugars. van't Hoff further noted that if the asymmetry is destroyed (for example, on conversion of malic into maleic acid), the optical activity disappears.

Recognizing that inductive arguments are very vulnerable to a single disconfirming case, van't Hoff sought to confront those head on. He focused particularly on accounting for those cases in which compounds known to possess asymmetrically substituted carbon atoms were nonetheless optically

inactive, and offered three possible explanations:

- The compound in question is actually an equimolar mixture of optical isomers that cannot be resolved.
- The inherent optical activity of the compound is small or not easily measured because of solubility problems.
- The presence of an asymmetrically substituted carbon atom may not be sufficient for optical activity, which might also depend on the “character” of the groups attached to the carbon atom (*meso* compounds would constitute a clear case, although van't Hoff gave no examples).

van't Hoff also ventured to make a number of predictions based upon his hypothesis—for example, that optically active amyl alcohol must have the structure $\text{C}_2\text{H}_5(\text{CH}_3)\text{CHCH}_2\text{OH}$ and that 2-butanol was the simplest optically active alcohol. (Chancel's claim to have isolated an optically active propyl alcohol was dismissed as arising from contamination by an impurity. As in so many chemical controversies, questions of sample purity were always present.)^[13b]

van't Hoff's assertions were disputable on at least three grounds: the relevance of evidence put forward to support them; the compatibility of his theory with current ideas about the nature of molecules; and the style of his argument. He thus became the lightning rod in the ensuing controversy. Considering the opposition that his hypotheses eventually aroused it is noteworthy that it took several years before lightning struck. When it did it was accompanied by quite a thunderclap, Hermann Kolbe's notorious diatribe in which he depicted van't Hoff as a fantasist having “no taste for exact chemical research”, who had spun out his theories on the “chemical Parnassus” whence he had arrived by “Pegasus”.^[15] van't Hoff, who was rather an unknown at this time, was shrewd enough to realize that the immoderate nature of Kolbe's attack would actually serve to publicize his hitherto overlooked hypotheses, and he actually reprinted Kolbe's tirade in the 1877 and later German editions of his pamphlet.

This incident was unfortunate in several ways. For one thing, it gave the impression that Kolbe was “an obscurantist and a fool”, which he assuredly was not.^[16] More consequentially, it left the impression among many later observers that van't Hoff's opponents had no credible grounds for opposing his proposition. That was not the case.

One source of contention was the type of evidence deemed admissible. Charles Gerhardt, the mid-19th century French chemist who contributed greatly to the acceptance of modern atomic weights and the development of notions of valence, wanted to restrict chemists to the use of chemical evidence alone; in his eyes, data derived from such sources as optical rotation measurements were irrelevant to chemistry.^[17]

Gerhardt himself eventually relented on this point, and by the last quarter of the 19th-century many chemists were prepared to accept the significance of physical measurements for chemical problems. Indeed, several of van't Hoff's most trenchant critics argued that his proposals were flawed *precisely* because they disagreed with current physical theory.^[18] For example, physics provided no basis for postulating directed forces such as those that supposedly emanated tetrahedrally from the carbon atom. Marcelin Berthelot criticized stereochemical theory from another angle, for failing to take into account the evidence from physics that pointed to the dynamic character of molecules. This criticism was supported by Victor Meyer's observation that many asymmetric compounds containing hydrogen at the asymmetric carbon atom, such as $\text{CH}_3\text{CH}(\text{Br})\text{NO}_2$, had never been resolved. Meyer attributed this failure to a very rapid interchange of isomeric forms resulting from the vibrational mobility of the light H atom.^[19]

In pointing out the difficulty of incorporating dynamic considerations into structural hypotheses, critics such as Berthelot drew attention to one of the enduring problems of chemical theory.^[20] van't Hoff himself had noted that 19th-century chemical theory was incapable of satisfactorily representing either the positions or the intramolecular motions of atoms within molecules. However, as one historian has perceptively observed, “[van't Hoff's] proposal

for a tetrahedral carbon atom solved the first problem at the expense of the second.”^[21] The attempt to juggle these two incompatible demands resulted in a theoretical flexibility that bordered on self-contradiction. The dilemma is nicely illustrated by Alfred Stewart's treatment of static and dynamic stereochemistry. He begins his exposition of the tetrahedral carbon atom and optical activity in this way:

“The atoms of a molecule may be supposed to be in a state of continual vibration, but this vibration merely carries them to and fro about some fixed points, so that at any time two atoms are within a certain distance of one another, and their movements never withdraw them from each other to any appreciable extent. It is therefore most convenient to neglect these atomic motions, and deal with the molecule as if it were a series of atoms whose positions with regard to one another vary only during the course of intramolecular reactions.”^[22]

Later on, in the context of an introduction to steric hindrance, Stewart summarizes A. W. Hofmann's work on the reaction of tertiary amines with alkyl iodides, and notes that Hofmann's and related results can be explained by assuming “that in any molecule each atom vibrates about a certain centre, and that the amplitude of vibration is not negligible in comparison with the size of the molecule itself.”^[23] No mention is made of any possible conflict between this hypothesis and the one put forth at the start of the book.

Given that there were serious objections to the basic postulates of stereochemical theory, some of which could not be refuted by its defenders, why did it nonetheless survive and attract ever greater numbers of adherents? Certainly the theory's ability to explain observations that were essentially inexplicable in any other convincing manner was a primary reason, especially since isomerism was a central concern of organic chemists. And there were numerous experimental results that were in accord with prediction; for example, no compound lacking an asymmetrically substituted carbon atom showed optical activity or could be resolved into antipodes. (There were reports of optical

activity in symmetric compounds such as styrene, which were eventually disproved. On the other hand, many asymmetric compounds could not be resolved, but these failures were put down to experimental difficulties.)

In addition, stereochemical theory could be extrapolated in new and fruitful directions. van't Hoff had already shown how the proposal of a tetrahedral carbon atom could be used to rationalize isomerism in unsaturated compounds,^[13b] but it was Johannes Wislicenus who systematically applied this model to instances of geometrical isomerism in alkenes, and extended it to speculations about the mechanisms of organic additions and eliminations. This turned out to be “a bridge too far”; through an unfortunate choice of a supposedly paradigmatic addition reaction Wislicenus made a number of incorrect predictions about the stereochemical outcomes of various reactions, for which he was severely and justly criticized by Arthur Michael.^[24] Stereochemistry prospered nonetheless.

What conclusions might be drawn from this recounting? One inescapable conclusion is that, despite Karl Popper, an apparently fruitful hypothesis will not be done in by empirical disconfirmations and wrong predictions alone. For one thing, the theory can always be adjusted and unknown factors used as an excuse for any discrepancies. Arnold Eiloart described just such tactics by the adherents of stereochemistry:

“This discussion [of the catalyzed interconversion of maleic and fumaric acids] illustrates the elasticity of stereochemical theory with regard to these transformations—an elasticity that makes it impossible to crush the theory by the pressure of facts bearing on isolated points, but indicates at the same time a vagueness which only future experiments can diminish. Meanwhile the contest between Wislicenus and his opponents continues.”^[25]

As we indicated at the beginning of this article, clashes over the nature and relevance of evidence were very much to the fore in this controversy. At least as important were the interpretations drawn from that evidence. It may be

difficult for us today to realize how contentious the atomic theory was in the 19th century, especially in its physical (that is, realist) form.^[26] Many (although by no means all) stereochemical skeptics were of an older generation,^[27] whose concerns about “speculation” and “hypotheses” were really concerns about the character and status of chemistry as a science.

The numerous 19th century quarrels about induction versus hypothesis were more about rhetoric than about how chemistry was actually done.^[28] But such verbal volleys should never be dismissed as “mere” rhetoric, because they provide invaluable insight into chemists' understanding of their science and of their self-understanding. In fact, an increasing number of them began to publicly laud the value of hypotheses, among whom were some prominent stereochemists. In defending his assumptions against the criticisms of Wilhelm Lossen, Wislicenus asserted that “a speculative discussion ... could bring more clarity than the certain, admittedly long, path of empirical research.”^[29] van't Hoff chose the theme “Imagination in Science” for his inaugural address at the University of Amsterdam.^[30]

“Speculation” is no longer an occasion for dispute in chemistry, and a great deal of the “vagueness” in stereochemical theory has been removed with the aid of numerous and sophisticated theoretical and physical tools. It might thus be fair to ask whether the issues raised in this controversy have been laid to rest. Our answer would be—not quite. For example, on the theoretical side there have been intense discussions about whether it is meaningful to speak of the chirality of isolated molecules.^[31] In the experimental realm, despite the abundance of methodology, we still often surprise ourselves—as we see in the next case.

Case 2: Bond-Stretch Isomerism—Some Evidence is More Equal than Others

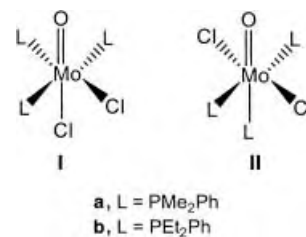
We jump forward in time, about a century, to the bond-stretch isomerism controversy. At first glance this story might not seem in any way comparable to the preceding one, particularly in

terms of significance—interest was pretty much limited to inorganic chemists, with virtually no awareness in the rest of the chemistry community, let alone the scientific community at large. Nonetheless, we feel there are some important lessons here, concerning the nature of acceptable evidence and the settling of controversies thereby, that intriguingly resonate with the earlier story.

The controversy basically turns on the question of whether two different isomers of a molecule, which differ essentially *only* in the length of one of the bonds, might both be sufficiently stable (thermodynamically and kinetically) to be isolated. This is an issue of potentially wide-ranging scope,^[32,33] but we focus here only on its application to a particular class of species, metal–oxo (or metal–sulfur, in a couple of examples) coordination complexes. A detailed history was published a few years ago by Parkin;^[34] a much briefer summary follows.

In 1970 Butcher and Chatt reported a series of oxomolybdenum complexes, all giving acceptable elemental analyses for the formula $[\text{MoOX}_2\text{L}_3]$, which were either blue or green depending on the phosphane ligand L and the halide (or pseudohalide) ligand X and which were assumed to be geometric isomers.^[35] In one case, for $\text{L} = \text{PMe}_2\text{Ph}$ and $\text{X} = \text{Cl}$, both blue and green products could be isolated, depending upon the conditions. The two species in solution exhibited identical electronic and ^1H NMR spectra (solutions of the green compound rapidly changed to blue) but did show different IR bands (for crystalline samples as Nujol mulls), at 943 and 954 cm^{-1} for the green and blue species, respectively.

The crystal structure analysis of the blue isomer revealed that it had the *cis,mer* configuration **Ia**; initially it was suggested that the green isomer was *trans,mer* **II**.^[36] However, the crystal structure of green $[\text{MoOCl}_2(\text{PET}_2\text{Ph})_3]$



(suitable green crystals of the PMe_2Ph analogue were not obtained), determined the next year, showed the same *cis,mer* configuration (**1b**) as blue $[\text{MoOCl}_2(\text{PMe}_2\text{Ph})_3]$.^[37] Comparing the two molecular structures, the Chatt research group found that the rotational orientation of the organic groups on the phosphane ligands was quite different and that the $\text{Mo}=\text{O}$ bond length was considerably shorter for blue **1a** than for green **1b** (1.676(7) versus 1.801(9) Å), with smaller differences in the $\text{Mo}-\text{Cl}$ bond lengths and some bond angles. They proposed that this represented “a new type of isomerism, involving two equilibrium arrangements of ligands, which differ in the distortions of the highly strained coordination polyhedron of the metal”—in other words, that nonbonded interactions involving the phosphane ligand substituents were primarily responsible—for which they offered the term “distortional isomerism.”

Interest in this phenomenon was not really fired until Wiegardt and co-workers reported in 1985 that salts of the cationic complex $[\text{CnWOC}_2\text{L}_3]^+$ ($\text{Cn} = \kappa^3\text{-}N,N',N''\text{-trimethyl-1,4,7-triazacyclononane}$) could be crystallized in two different forms, again one blue and one green.^[38] In contrast to $[\text{MoOCl}_2(\text{PMe}_2\text{Ph})_3]$, the two forms each appeared to be indefinitely stable in solution (acetonitrile), although addition of water to the green solution caused an immediate color change to blue. The IR spectra differed only in the position of the peak assigned to $\text{W}=\text{O}$ stretching (980 and 960 cm^{-1} for blue and green, respectively). The elemental analyses agreed well with each other and the nominal formula. Most intriguingly, the molecular structures of the two apparent isomers were identical in all respects except for the $\text{W}=\text{O}$ bond lengths (blue, 1.72(2); green, 1.89(2) Å) and, to a lesser degree (possibly below statistical significance), the length of the $\text{W}-\text{N}$ bond *trans* to O (blue, 2.37(2); green, 2.32(2) Å). This finding appeared to exclude the interpretation by Chatt et al., as there are no differences in ligand orientation, steric effects, packing, or anything of that nature—just a difference in bond length. A number of other examples (see Parkin's review^[34] for specifics) from several different laboratories around the same time also

suggested that the $\text{M}=\text{O}$ bond length, not the ligand orientation, was central to this form of isomerism.

Two papers by Hoffmann and co-workers appeared in 1988, which reported calculations that suggested that it might indeed be possible to have a double minimum along a coordinate that essentially represents one bond length.^[39,40] They proposed the term “bond-stretch isomerism” as a more appropriate descriptor for this behavior, based on earlier work on quite different classes of molecules.

By this point, with a number of apparently proven examples and a plausible theoretical framework in hand, bond-stretch isomerism began to be seriously discussed at meetings, as well as in reviews, monographs, and even textbooks—although often hedged with a degree of residual doubt. For example:

“To our knowledge this is the only suggestion that molecules of any kind have two stable structures with substantially different bond distances. Therefore, if distortional isomerism is a real phenomenon and not an artifact of some kind, it has significant implications beyond metal–ligand multiple bonding.”^[41]

However, in 1991 Parkin and co-workers showed that the crystallographic support for bond-stretch isomerism, at least in the $[\text{MoOCl}_2\text{L}_3]$ class of complexes, was indeed an artifact.^[42] Crystals of green $[\text{MoOCl}_2(\text{PMe}_2\text{Ph})_3]$ (which had previously been found in both blue and green forms) contained two independent molecules in the asymmetric unit, with quite different $\text{Mo}=\text{O}$ bond lengths (1.698(8) and 1.866(7) Å). On the other hand, the crystals exhibited only a single IR band in the $\text{Mo}=\text{O}$ stretching region. Crystallographic analysis on a crystal obtained from a different batch yielded two different $\text{Mo}=\text{O}$ bond lengths (1.772(12), 2.154(8) Å). Clearly these could not all be real, stable molecules.

The anomaly results from contamination with $[\text{MoCl}_3\text{L}_3]$, which can co-crystallize with $[\text{MoOCl}_2\text{L}_3]$ in almost any proportion. Since substitution of $[\text{MoCl}_3\text{L}_3]$ for $[\text{MoOCl}_2\text{L}_3]$ effectively

puts a Cl ligand where an O atom is supposed to be, the calculated structure (based on the assumption that one is dealing with pure $[\text{MoOCl}_2\text{L}_3]$) will have an apparently long $\text{Mo}=\text{O}$ bond length, as $\text{Mo}-\text{Cl}$ is longer than $\text{Mo}=\text{O}$. Since the electron density of Cl is much higher than that of O, even relatively small amounts of contamination can cause a significant perturbation. Furthermore, contamination of blue $[\text{MoOCl}_2\text{L}_3]$ by yellow $[\text{MoCl}_3\text{L}_3]$ gives a green sample; also the contaminant is paramagnetic, so it will not show up in the NMR spectrum except as broad, substantially shifted signals—which were observed, once they were looked for. A series of samples of $[\text{MoOCl}_2\text{L}_3]$ deliberately contaminated with increasing amounts of $[\text{MoCl}_3\text{L}_3]$ showed a steady increase in the apparent $\text{Mo}=\text{O}$ bond length, along with an apparent deepening of the green color.

At about the same time, Enemark and co-workers reexamined the complexes prepared by both the Chatt and Wiegardt research groups by chromatographic and spectroscopic methods, and found decisive evidence for mixtures.^[43,44] Higher-level theoretical work also indicated that the proposed mechanism(s) would *not* produce energy differences, along a single stretching coordinate, sufficiently large to permit two isomers to exist as stable species.^[45]

General consensus that the controversy was over was quickly reached,^[46–48] thus making it appear that this was a rather straightforward case with little in the way of take-home lessons, besides perhaps being more careful with crystallographic results. In fact, though, we believe the controversy represents another illustration of the fundamental issue here—what counts as evidence—and in particular the dominant status that is (unconsciously?) granted to crystallographic evidence in structural investigations. Here is a typical quote (from a book written by spectroscopists!):

“X-ray crystallography is the ultimate arbiter of chemical structure ... Spectra and chemical reactions alone can never tell you the structure of a compound. All they can do is give you pieces of information.”^[49]

At the same time, routine crystallography has become so automated—"black-boxed", to apply a term from science studies^[50]—that there is a tendency to accept the semi-automated process leading to the structure solution without question, in the absence of any apparent difficulty. As noted above, there is also a tendency to ignore discordant findings from methods considered less reliable, such as spectroscopy, in the face of an "ultimate" answer from crystallography. So crystallography becomes a particularly privileged form of evidence—results are difficult to challenge on either intrinsic or extrinsic grounds—while all other forms are heavily discounted or even ignored.

We can see this playing out over the course of the bond-stretch story. Hints that something might be wrong were available from the very beginning. For example, in the original work by Butcher and Chatt, the identical electronic and NMR spectra of the two "isomers" as well as the higher Cl content for the green form might have argued against simple isomerism. In the tungsten case reported by Wieghardt and co-workers, differing electronic spectra for the "isomers" can (in retrospect) clearly be seen to be a result of superposition, while the color change on addition of water seems much more indicative of a chemical reaction than an isomerization. However, none of these anomalies raised any concern about possible crystallographic errors—even among trained crystallographers. Only a clear red flag *within* crystallography—four different apparent Mo=O bond lengths for a single complex—triggered Parkin's systematic reinvestigation.

Even after the controversy seemed to be settled to the satisfaction of most of the community, we can still see the effects of privileging evidence. Note that the differing IR bands assigned to Mo=O stretching were *not* accounted for by the contamination explanation, as the contaminant contains no Mo=O bond! Commentators either ignored this^[42] or attributed it to an error in the initial report.^[43] This brings us full circle in the consideration of crystallography as privileged evidence. Initially, contrary evidence from any other source had little or no impact on the acceptance of crystallographic evidence supporting bond-

stretch isomers, while the IR findings appeared to be generally consistent with the crystallography. As soon as the crystallography was found to point the other way, the IR evidence was no longer consistent; but even though unexplained, it was completely discounted.

The IR problem was resolved for the [MoOCl₂L₃] system only later, when Gibson, McPartlin and co-workers^[51] found that blue **1a** can be obtained in two different crystalline forms that exhibit the two different IR stretching bands reported by Chatt. The IR differences disappear in solution, so this is a consequence of crystal packing—a familiar phenomenon. On the other hand, the complex [NbSCl₃(PMe₃)₃] has been obtained in two crystalline forms, orange-yellow and green, that exhibit different apparent Nb=S bond lengths and stretching frequencies; the latter difference persists in solution, apparently ruling out an explanation based on a solid-state effect.^[52,53] However, this residual unexplained discrepancy does not attract much, if any, attention (it isn't even mentioned in the recent review cited above^[33]). In the presence of crystallographic results, no "lower" form of evidence counts for much of anything.

One might well be tempted to view this story as being of a quite different nature from the preceding one. In Kuhnian terms, the birth of stereochemistry looks much more like a fundamental, even revolutionary development in the history of chemistry, as opposed to the apparently puzzle-solving, normal science of the birth and demise of bond-stretch isomerism. Such a conclusion, however, depends heavily (and unfairly) on hindsight. While it was in progress, the controversy over bond-stretch isomerism seemed to have considerable potential as a fundamental change in chemical understanding. Also, of course, it is very difficult to characterize a controversy while one is in the middle of it. All one can really do then is consider the evidence—and as we have tried to show, the question of which evidence merits consideration was central to *both* cases.

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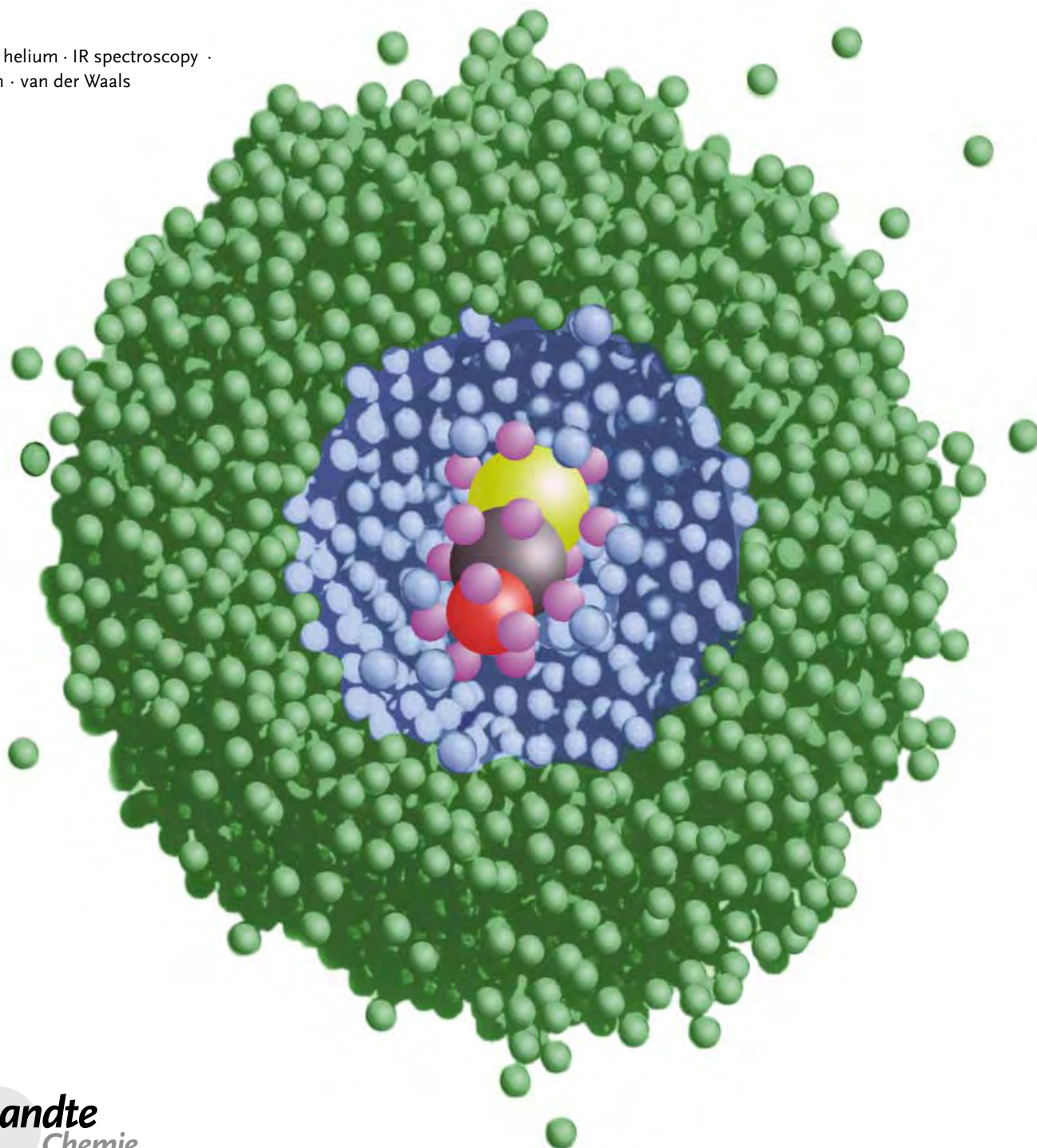
Matrix Techniques

Superfluid Helium Droplets: A Uniquely Cold Nanomatrix for Molecules and Molecular Complexes

J. Peter Toennies and Andrey F. Vilesov*

Keywords:

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Chemie

Herein, recent experiments on the spectroscopy and chemical reactions of molecules and complexes embedded in helium droplets are reviewed. In the droplets, a high spectroscopic resolution, which is comparable to the gas phase is achieved, while an isothermal low-temperature environment is maintained by evaporative cooling at $T=0.37$ K (^4He droplets) or 0.15 K (^3He droplets), lower than possible in most solid matrices. Thus the helium-droplet technique combines the benefits of both the gas phase and the classical matrix-isolation techniques. Most important, the superfluid helium facilitates binary encounters, and absorbs the released binding energy upon recombination. Thus the droplet can be viewed as an isothermal nanoscopic reactor, which isolates single molecules, clusters, or even a single reactive encounter at ultralow temperatures.

1. Introduction

Since its discovery in 1954^[1] cryogenic matrix isolation has become a powerful experimental technique, which is particularly useful in studies of transient species, such as atoms, molecular radicals, and ions.^[2–6] Seeded supersonic beams, another celebrated technique in the arsenal of the physical chemist, have also been used to cool free molecules, and to produce free molecular complexes and clusters.^[7,8] Recently a combination of the two techniques led to the development of a new experimental approach, the isolation of species in ultracold ($T=0.37$ – 0.15 K) helium droplets, which are made up of 10^3 to 10^8 helium atoms.^[9–13] The fluid helium drops can readily pick up atoms and molecules and can form complexes from the species embedded in their interiors, or on their surfaces, thereby providing unique experimental opportunities. Extensive experiments conducted in the last 10 years have demonstrated helium droplets to be the ultimate matrix. They are characterized by extremely low temperatures, little matrix broadening, and offer unique possibilities to synthesize new molecular complexes.

1.1. Comparison of Seeded Molecular Beams and other Cryomatrices with Helium Droplets

The most detailed information on free molecules with a spectral resolution approaching the fundamental limits is achieved by cooling in seeded molecular beams.^[7,8,14] Because of the limited number of collisions available during the gas expansion, however, the vibrational degrees of freedom of larger molecules and clusters are not cooled as efficiently as the rotational degrees of freedom,^[15] thus hot bands may complicate the spectrum. The easy preparation of atomic and molecular clusters in seeded beams is definitely among the most important advantages of this technique,^[16–19] but the cluster sizes are difficult to control owing to the complicated formation kinetics, and it is even more difficult to produce predefined multicomponent clusters made up of more than

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two or three entities. Another related technique designed for studying chemical reactions at temperatures down to 13 K involves injecting the reactants into the flow of an inert gas expanded through a large convergent–divergent Laval nozzle.^[20] Other techniques, such as the use of a low temperature (1 K) gas cell filled with a helium buffer gas^[21,22] or sympathetic cooling in laser traps^[23] have up to now not found widespread use, partly because of difficulties in preventing undesired condensation.

Matrix isolation in crystals of rare gases or hydrogen permits solute species to be cooled to the sub-Kelvin range ($T \leq 1$ K),^[2] although temperatures in the range of 4–10 K are more common. Table 1 summarizes the properties of different cryocrystals and compares them with the corresponding data for a liquid-helium matrix. The strength of the interaction of the matrix species with the foreign species can be gauged from the mutual interaction of the matrix particles. This interaction is characterized by the intermolecular potential well depth (ϵ) and well position (R_m). The decrease in ϵ is paralleled by a decrease in the dipole polarizability, which in turn determines the extent to which the frequency of the foreign species is shifted by the matrix environment.^[30,31] The columns 5–7 in Table 1 provide insight into quantum effects. The de Boer parameter [Eq. (1)] can be regarded as the ratio of the

$$A^* = h R_0^{-1} (m \epsilon)^{-1/2} \quad (1)$$

de Broglie wave length of relative motion to the distance of closest approach R_0 ($R_0 \approx 2^{-1/6} R_m$).

[*] Prof. Dr. J. P. Toennies
Max Planck Institute for Fluid Dynamics (Strömungsforschung)
Göttingen (Germany)
Fax: (+49) 551-517-6607
E-mail: jtoenni@gwdg.de
Prof. Dr. A. F. Vilessov
Department of Chemistry
University of Southern California, Los Angeles (USA)

Table 1: Comparison of some important properties of commonly used matrix materials, including liquid helium.^[a]

Matrix species	ε [K] ^[b]	R_m [Å] ^[b]	α [Å ³] ^[c]	Λ^*	V_0/R_0^3 ^[d]	T_{tp} [K] ^[e]
Xe	283	4.37	4.01	0.06	0.933	161
Kr	201	4.01	2.48	0.11	0.951	116
Ar	143	3.76	1.64	0.20	0.976	83.8
Ne	43.3	3.09	0.397	0.62	1.09	24.6
H ₂	34.8	3.44	0.803	1.97	1.32	14.0
⁴ He	11.0	2.97	0.205	2.87	1.87	no t.p.
³ He	11.0	2.97	0.205	3.31	2.82	no t.p.

[a] ε and R_m are the well depth and well position of the intermolecular potential between the matrix particles, α is the dipole polarizability, Λ^* is the de Boer parameter (see text), V_0/R_0^3 is the ratio of the measured volume per atom at $T=0$ K and the volume expected for a classical solid, T_{tp} is the triple-point temperature. [b] All values are from ref. [24] except for H₂ (ref. [25]), for which the values are orientation averaged, [c] from ref. [26]. [d] V_0 values are from ref. [27, 28]. [e] from ref. [29].

Another measure of the zero-point energy is the increase in the actual volume V_0 occupied by the particles in the condensed phase at 0 K, in relation to the volume they would occupy in a classical lattice (which, for a given lattice type is proportional to R_0^3). The large increases in both Λ^* and V_0/R_0^3 in going from the heavy rare gases to H₂, ⁴He, and ³He are thus attributed to an increase in the zero-point energy and the resulting increase in the delocalization of the matrix particles. In the case of helium this quantum effect is largest and explains why it is the only substance, which remains liquid at saturated vapor pressures even down to absolute zero.

This remarkable difference between the helium isotopes and a classical rare gas, such as argon, is seen on comparing their phase diagrams (Figure 1). From the ⁴He phase diagram another unique property is apparent. Instead of solidifying below 2.2 K it undergoes a unique phase transition to a superfluid state called HeII. The transition temperature for the bosonic (nuclear spin $I=0$) ⁴He isotope is $T=2.18$ K. The fermionic ($I=1/2$) ³He isotope becomes superfluid at a much lower temperature, $T=3 \times 10^{-3}$ K (which is not relevant for the present Review). Superfluid ⁴He exhibits a range of unusual phenomena, such as a vanishingly small viscosity, a very high heat conductivity (30-times greater than copper) and many other bizarre effects, such as the He fountain, film

flow and creep, and quantized vortices.^[32, 33] The fundamental differences in the behavior of classical liquids or solids and the quantum liquid helium, respectively, determines the extraordinary differences in their properties as spectroscopic matrices.

Isolation in a solid matrix usually involves a co-deposition of the matrix and solute species. Once the matrix is prepared isolated species may be ionized or photodissociated to produce ions or radicals, respectively. Thus matrix isolation provides a versatile tool to study a wide variety of these transient species.^[2–5] Usually it is possible to obtain a rather large optical density of isolated species and maintain them for long times of several hours and even days which facilitates the straightforward application of the Fourier transform infrared and other optical spectroscopic techniques, such as Raman scattering. The relatively strong interaction with the matrix surrounding, which, on the one hand, localizes the solute species and provides stability within the matrix, often results, however, in considerable inhomogeneous broadening of the spectral lines owing to the number of different sites which the solute molecules can occupy. A solute molecule usually does not perfectly fit into a void within the matrix crystal, which invariably leads to a range of configurations of its nearest-neighbor matrix environment. Moreover the mobility of the particles in a solid matrix is low and ill defined, which makes it difficult to control experiments involving two or more species.^[34] Thus, the assignment of the spectral features arising from complexes is often ambiguous.^[34, 35] Among the cryosolids, hydrogen is the most delocalized one, thus use of a solid hydrogen matrix minimizes to some extent inhomogeneous effects inherent to a solid matrix. Indeed some molecules, such as the spherical CH₄ molecule, embedded in crystalline para-hydrogen, demonstrate unique phenomena, such as a rotationally resolved spectrum and extremely narrow lines in the infrared spectrum.^[36, 37] However, in general, a solid hydrogen matrix bears more resemblance to the matrices of the heavier noble gases.

The situation is quite different for a molecule inside liquid helium. Because of their large quantum mechanical delocalization the helium atoms in the vicinity of the molecule are able to adopt a unique and largely delocalized configuration corresponding to the lowest (ground) quantum mechanical



J. Peter Toennies obtained his Ph.D. in Physical Chemistry at Brown University, Providence, USA. He then moved to the Physics Department at Bonn University and habilitated in the group of Prof. Wolfgang Paul in 1965. In 1969 he became a Director at the Max Planck Institute for Fluid Dynamics (Strömungsforschung), where he is now an Emeritus Professor. The study of rotational and vibrational inelastic as well as reactive scattering in crossed molecular beam experiments has been one of the major areas of research. High-resolution scattering of

helium atoms from surfaces has been developed in his laboratory for measurements of surface phonon dispersion curves, low-energy adsorbate vibrations, and diffusion. Currently both large and small quantum-fluid clusters of H₂, ³He, and ⁴He are under investigation.



Andrey F. Vilesov obtained his Ph.D. in Physics at S. Petersburg State University, St. Petersburg, Russia. He then moved to the Max Planck Institute for Fluid Dynamics (Strömungsforschung), Göttingen and habilitated in the group of Prof. J. Peter Toennies in 1999. Since 2000 he has been a Professor of Chemistry at the University of Southern California, Los Angeles, USA. His early research was devoted to the spectroscopic study of the chemical kinetics and energy transfer processes in molecular beams. More recently, his work has concentrated on

spectroscopy of molecules and molecular clusters in helium droplets and the study of quantum fluids on an atomic scale.

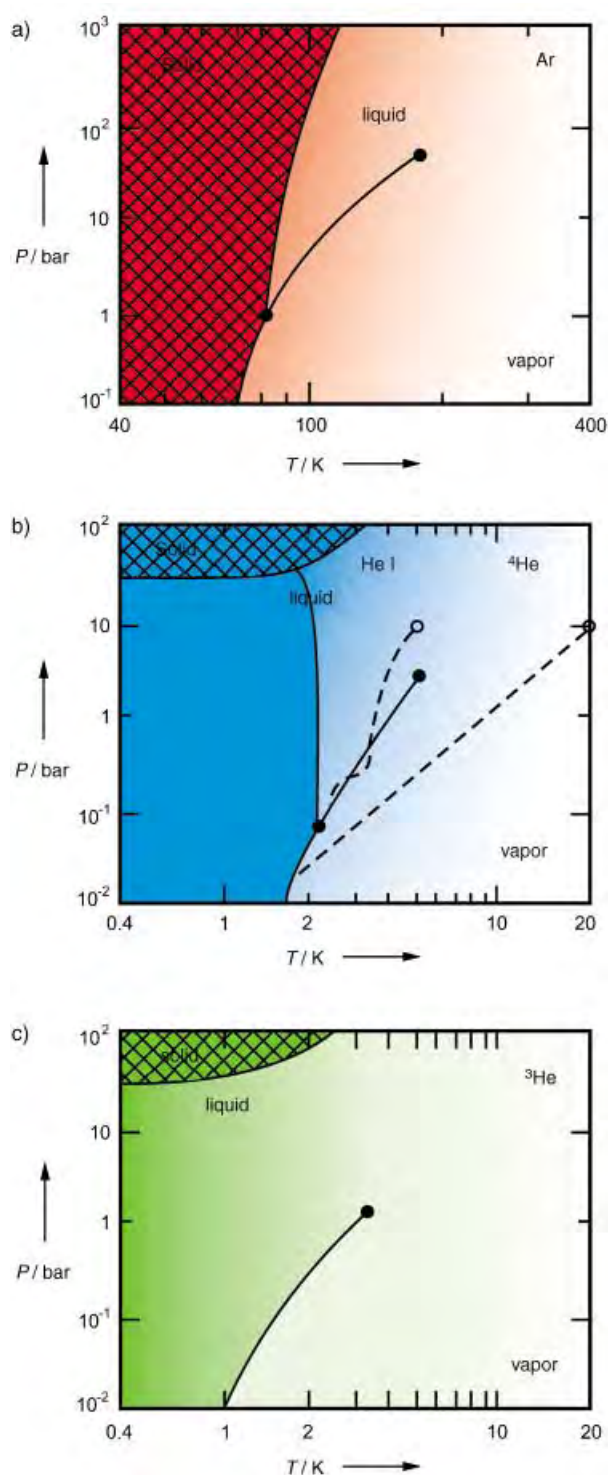


Figure 1. The pressure–temperature phase diagrams of the classical noble gas Ar (a) and the quantum liquids ^4He (b) and ^3He (c). Dashed lines on the ^4He diagram show typical supercritical (upper) and subcritical (lower) expansion isentropes (see Section 3.2).

state. Thus, in comparison to a molecule in a solid matrix, which is often described as a molecule in a “crystal field”, liquid helium gently adapts itself to the solute giving rise to a void perfectly tailored to a particular molecule. Moreover, helium is an ideal matrix material since it is completely

transparent in the entire spectral range from the far IR to the vacuum UV. On the other hand, in its superfluid state molecules may move unhindered in the helium liquid, thus remaining highly mobile even close to absolute zero. It is this high mobility which in the past has prevented the use of liquid helium as a spectroscopic matrix in the bulk phase. Many attempts to isolate molecules have failed because of an uncontrolled precipitation of the solute species.^[39–41] With the advent of helium-droplet beam technology it has become possible to isolate single molecules or certain numbers of the molecules inside a finite sized free helium droplet (Figure 2).

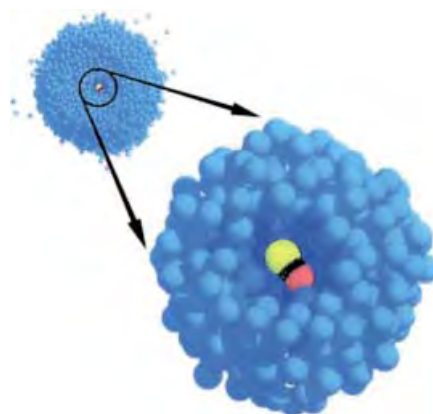


Figure 2. Computer simulation showing an OCS molecule inside a ^4He droplet made up of 500 atoms; ^4He blue, O red, S yellow, C black.^[38]

The confinement of single molecules inside or on the surface of the free droplets avoids any problems with their aggregation or precipitation on container walls.^[9] At the same time the droplets provide a number of additional unique experimental opportunities. The droplet may be viewed as a “personal” nanocryostat for each individual molecule, “powered” by the evaporative cooling of the droplet material. The technological trade-off is that the experiments involve molecular beams of droplets inside a high-vacuum apparatus. Thus the lifetime of a single-droplet nanocryostat is only the typical time of their flight through the apparatus which is in the range of a few milliseconds. In addition, the optical density in the beam is far too low to apply directly most absorption spectroscopic methods, calling for the application of laser light sources and special techniques for detection.

2. Structure and Thermodynamics of Helium Droplets: “The Ideal Nanocryostat and Nanocalorimeter”

Long before the first spectroscopic experiments with He droplets, they had been extensively studied theoretically, mostly by nuclear physicists, as a model for finite-sized many-body quantum systems. The theoretical methods and results are extensively discussed in several recent reviews.^[42–46] Despite their weak interatomic interactions ^4He atoms form stable clusters for all number sizes N_4 and the binding energy per ^4He atom is predicted to increase monotonically from

1.3×10^{-3} K^[47] for the dimer to the bulk-phase value of 7.2 K for $N_4 \geq 10^4$ ($1 \text{ K} \triangleq 0.69 \text{ cm}^{-1} \triangleq 8.6 \times 10^{-5} \text{ eV} \triangleq 2 \text{ cal mol}^{-1}$).^[48–51] Large He clusters with $N_4 \geq 10^3$ ^4He atoms will be referred to as droplets. Large clusters and droplets of ^3He are also stable although small clusters with less than about 30 atoms are not.^[52] Since the interatomic potentials are the same in both isotopes this difference reflects merely the lower mass of ^3He compared to ^4He . In the ground state the droplets are expected to be spherical and, assuming a uniform density and a sharp outer edge as in the liquid-drop model, their radii are given by $R_0 = 2.22 N^{1/3} \text{ \AA}$ for ^4He and $R_0 = 2.44 N^{1/3} \text{ \AA}$ for ^3He droplets.^[53] In the center of the droplet the particle density (PD) is close to the bulk-liquid value of $0.022 \text{ atoms \AA}^{-3}$. Theory predicts the thickness of the surface region, where the density falls from 90 % to 10 % of its bulk value (Figure 3a),^[51,57] to be about 6 $\text{\AA}$ (^4He) or 8 $\text{\AA}$ (^3He) in good agreement with experiments.^[57,58] According to the liquid-drop model^[53] elementary excitations of a ^4He droplet can be classified as either volume or surface vibrational modes. The volume vibrational modes are so high in energy that at the low temperatures of the droplets

they are not excited. The surface vibrational modes are an order of magnitude lower in energy and have no influence on the embedded chromophore molecule (Figure 4).^[56] The residual excitations of the surface modes play an important

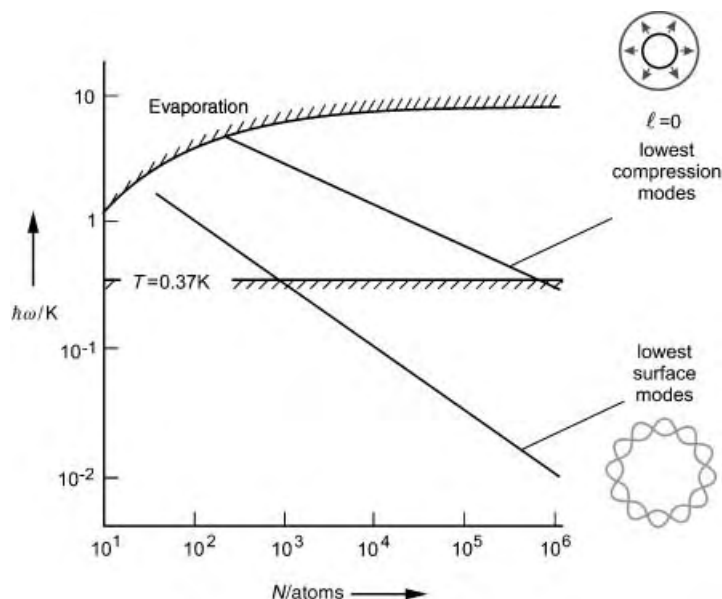


Figure 4. Dependence of the lowest fundamental frequencies of the compressional and surface vibrations and the evaporation energy for a single ^4He atom of a pure ^4He cluster on cluster size N .^[56] The typical shapes of the lowest volume compressional and surface excitations are shown schematically. At the experimental droplet temperature of $T = 0.37 \text{ K}$ (horizontal line) only the surface vibrations are excited for droplets with $N_4 = 10^3$ – 10^6 atoms.

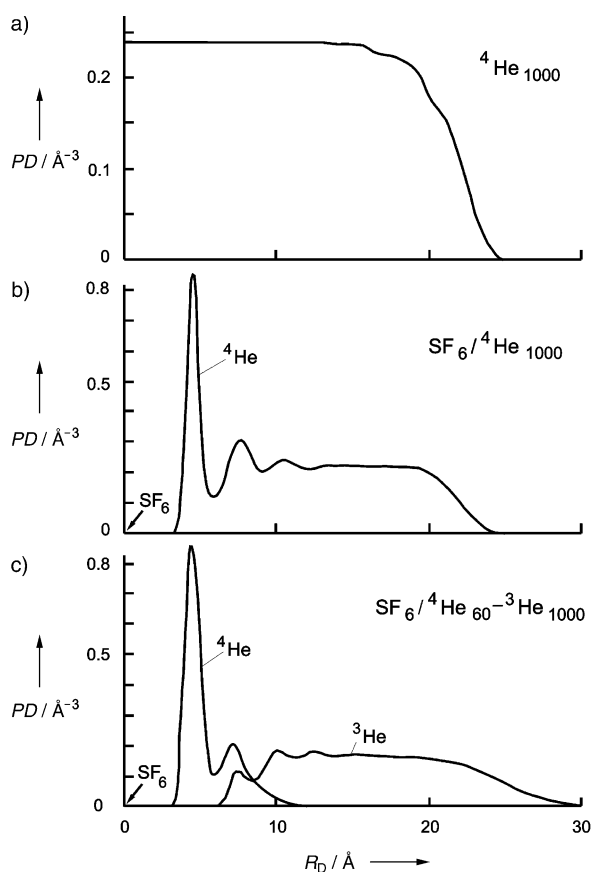


Figure 3. Results of the density functional calculations of the dependence of particle density (PD) on droplet radius (R_0): a) a neat helium ^4He droplet of $N_4 = 1000$ atoms,^[51] b) a ^4He droplet of $N_4 = 1000$ atoms doped with a single SF_6 molecule,^[54] c) a mixed ^4He – ^3He droplet of $N_3 = 1000$ ^3He atoms and $N_4 = 64$ ^4He atoms doped with a single SF_6 molecule.^[55]

role in determining the rate of evaporative cooling of the droplets propagating in the vacuum apparatus (Section 3).^[53,59] The microcanonical thermodynamic properties of helium droplets have recently been analyzed in great detail.^[60]

The dependence of the energy of the volume modes on an effective droplet wavenumber resembles closely the sharp dispersion curves of the elementary excitations of the bulk ^4He liquid with the characteristic “maxon” maximum and the “roton” minimum at $Q \approx 2 \text{ \AA}^{-1}$. The roton provides a “gap”^[32] in the excitations of about 8.5 K, in analogy to the “gap” in the electronic density of states of a superconductor. In a finite sized droplet the smooth dispersion curve is replaced by discrete energies.^[49,61,62] The gradual appearance of the roton minimum in calculations for a ^4He cluster having $N_4 \geq 100$ atoms was interpreted as an indication of the onset of superfluidity.^[61] Other theoretical indications of superfluidity in even small clusters with 64 atoms are derived from calculations of anomalies in the heat capacity and the reduction in the effective moment of inertia compared to the value for classical rigid rotation.^[63]

Theory also provides values for the chemical potential μ (the binding energy to the entire droplet) of different impurities placed in the center of the droplets (Table 2). Negative μ values indicates a decrease in the free energy for the complete immersion of the particle, which may be referred to as “heliophilic”. Extensive experimental^[9,12,64]

Table 2: Interactions of atoms and molecules in ^4He .^[m]

X	ε [K]	R_m [Å]	E_b [K]	μ [K]
H	0.773 ^[a]	3.46 ^[a]	no bonding state	+75 ^[b]
Na	1.76 ^[c]	6.44 ^[c]	–	+49 ^[d]
K	1.29 ^[c]	7.31 ^[c]	–	+68 ^[d]
Rb	1.17 ^[c]	7.54 ^[c]	–	+69 ^[d]
Cs	1.00 ^[c]	7.95 ^[c]	–	+84 ^[d]
^4He	11.0 ^[e]	2.97 ^[e]	-10^{-3} ^[e]	–7.2 ^[f]
Ne	20.8 ^[g]	3.043 ^[g]	–3.74 ^[g]	–39 ^[d]
Ar	29.6 ^[g]	3.50 ^[g]	–10.0 ^[g]	–195 ^[d]
Kr	30.5 ^[g]	3.69 ^[g]	–	–243 ^[d]
Xe	29.4 ^[g]	3.97 ^[g]	–11.1 ^[g]	–313 ^[d]
H ₂	13.7 ^[h]	3.39 ^[h]	–0.043 ^[h]	–20 ^[b]
SF ₆	41, 64, 86 ^[i]	4.4, 4.2, 3.8 ^[i]	–38.3 ^[i]	–601 ^[d]
OCS	40, 65, 38 ^[i]	4.5, 3.4, 4.8 ^[i]	–28 ^[i]	< –250 ^[k]
Na ⁺	400 ^[l]	2.4 ^[l]	–	< –5600 ^[l]

[a] Ref. [70]. [b] Ref. [71]. [c] Ref. [72]. [d] Ref. [66]. [e] Ref. [47]. [f] Ref. [53]. [g] Ref. [73]. [h] Ref. [74, 75]. [i] Along C₄, C₃, and C₂ coordinated directions, respectively.^[76] [j] At the S-atom end, at the waist, and at the O-atom end of the OCS molecule, respectively.^[77] [k] Ref. [78]. [l] Ref. [79]. [m] ε well depth, R_m well position of the He–X potential, binding energy E_b (with respect to the zero-point energy). The chemical potential μ of the species X in bulk helium is indicated in the last column

and theoretical^[42,65,66] results indicate that in fact all closed-shell atoms and molecules investigated to date are heliophilic. The rather flat effective potential of the droplet near its center allows considerable delocalization of a solute in the radial direction,^[56] predicted to be about 1 nm for SF₆ in a droplet having $R_0 = 3$ nm.^[67] The relatively strong van der Waals attraction exerted by the impurity molecule causes a considerable radial localization of the He atoms in the immediate vicinity of the impurity.^[42,54,55,68,69] The calculated density profile of He atoms near SF₆ reveals a number of compressed shells of He atoms (Figure 3b).^[54] For SF₆ the first and second solvation shells were found to consist of 22 and 50 atoms with a factor of 4 and 1.3 larger peak effective density than in the bulk, respectively. Calculations with anisotropic potentials show in addition, a strong angular anisotropy of the density profile for SF₆ and OCS^[42,68,69,78,80] but a nearly isotropic profile for the more spherical molecules, such as HF,^[81] HCN^[82] and CO.^[83] Despite the strong localization of the first He shell around SF₆ and OCS molecules, path integral Monte Carlo calculations reveal that the He atoms in the first shell are still able to participate to a considerable extent in multiparticle permutational exchanges with the surrounding atoms of the bulk phase.^[42,84] As first proposed by Feynman in 1954 such exchange processes are a characteristic microscopic feature of a superfluid.^[85] However, in the vicinity of stronger interacting impurities, such as benzene^[86,87] or ions^[79,88,89] some of the nearest He atoms may be so strongly localized that exchanges are completely suppressed.

“Heliophobic” particles having positive μ values, such as ^3He atoms,^[90] alkali-metal atoms^[66,91,92] and their small clusters^[93–95] are expected to reside on the surface of the droplet. Near such an impurity the droplet surface is distorted forming a type of a dimple.^[93] Using a dopant-He-pair potential a dimensionless parameter λ has been proposed,^[96] which links the gain in energy from the interaction of the

impurity atom with the helium solvent and the energy needed to create a helium cavity [Eq. (2)] where σ is the surface

$$\lambda = 2^{-1/6} \sigma^{-1} \rho \varepsilon R_m \quad (2)$$

tension of the liquid, ρ is the number density, and ε is the well depth and R_m is the equilibrium bond length of the corresponding solvent–solute particle potential (Table 2).

Atoms with values of λ less than 1.9 reside on the surface, whereas dopants with λ greater than 1.9 are expected to be solvated.^[96,97] Electrons, which are also heliophobic,^[98,99] can be accommodated in a metastable state inside large bubble-like voids about 34 Å in diameter.^[100] Provided the droplets are sufficiently large ($N_4 > 10^5$ atoms) the lifetimes of these metastable states are between 10^{-3} to 0.2 s, depending on the droplet size.^[101,102]

Pure and doped ^3He droplets are in many respects qualitatively very similar to ^4He droplets, when their 25 % lower particle density is taken into account.^[52] Density functional calculations of mixed doped ^4He – ^3He droplets,^[103] show the separation of a ^4He -rich fraction close to the impurity, which is surrounded by a shell of ^3He atoms extending out to the surface (Figure 3c).^[55] As a result of the lower evaporation temperature of the outer ^3He shell the central ^4He core also has been shown to have the same reduced lower temperature of 0.15 K as the pure ^3He droplets.^[104] This property of mixed droplets has made possible spectroscopic studies at two temperatures 0.37 K (pure ^4He droplets) and at 0.15 K (mixed ^4He – ^3He droplets).^[105,106]

3. Experimental Methods

Presumably there have been many proposals in the past to use the optical spectroscopy of foreign species as a microscopic probe for liquid helium, after all, spectroscopic techniques have found widespread applications in other areas of condensed-matter physics. However, until recently, these studies were not feasible owing to the extremely low equilibrium solubility of single molecules of any foreign species in liquid helium. Impurities quickly coagulate to form clusters that either rise to the surface or fall to the bottom of the reaction vessel.^[39–41] Contemporary methods of immersing foreign atoms into bulk liquid helium usually involve laser-induced ablation of metals into the liquid.^[107–109] This method makes it possible to obtain rather large transient concentrations of “metal atoms” in the liquid, however, to date the successful implantation of species other than metals, in particular of intact molecules, into bulk liquid helium has not been possible. One recent experimental development involves first embedding the foreign species inside large helium droplets formed in a pulsed beam and then directing the beam at the surface of the helium bath.^[110]

3.1. Droplet Sources

Macroscopic droplets of helium, which appear as a fog upon rapid Joule–Thomson cooling of the gas, were first

observed about 100 years ago.^[111] More recently helium fogs have been generated by a piezoelectric transducer, placed few millimeters below the surface of liquid helium.^[112] Other experiments involve laser-^[113] or magnetic-field-assisted^[114] levitation of single very large droplets, about 10 μm to 2 cm in diameter in vacuum. Large positively charged helium droplets have been produced by field ionization of a liquid in an electrostatic-spray experiment.^[115] Another recent approach is the formation of helium droplets by a pulsed expansion of cold helium gas into bath helium cryostat.^[110] Although of considerable interest these methods have not yet been used for matrix isolation purposes.

At present all molecular spectra in liquid helium have been obtained in helium droplets produced in free jet expansions, mostly of gaseous He but also in a few cases in expansions of the liquid. Helium-droplet production in gas expansions was first demonstrated by Becker et al. in 1961.^[116] Beams containing either ^4He and ^3He droplets or mixed droplets with between several hundred and 10^4 atoms are readily produced today by expanding the gas at a high source pressure $P_0 = 20\text{--}100$ bar and a low source temperature $T_0 = 20\text{--}5$ K, through a tiny nozzle of several microns diameter or a narrow thin-walled orifice into vacuum (Figure 5 a).^[9,11,117,118]

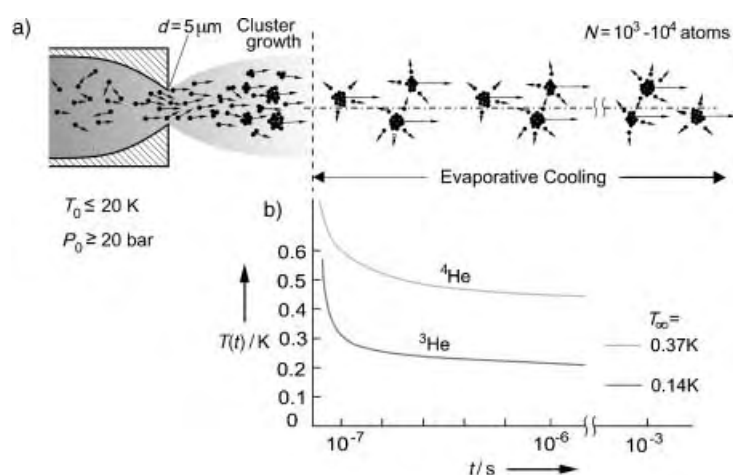


Figure 5. a) Schematic representation of the processes leading to the formation and subsequent cooling of helium droplets in a gas expansion. b) Calculated dependence of the droplet temperature on time for ^4He and ^3He droplets after they have left the cluster-growth region in the expansion and are traveling in vacuum.^[53,59]

The properties of droplets formed in continuous beams obtained by expansion through a 5- μm diameter orifice are now well characterized.^[57,58,119] Recently pulsed (30–50 microsecond) beams of helium droplets with comparable properties have been developed with 1000-times larger peak droplet intensities.^[120]

3.2. Helium Droplets from Free Jet Gas Expansions

In the usual mode of operation, referred to as a “subcritical” expansion, the initial state in the source is well within the gaseous region of the phase diagram.^[117] The

expanding helium gas in the jet is cooled by the adiabatic change in state along isentropes in the phase diagram to the final ambient temperatures and pressures well beyond the vapor–liquid phase transition (see the lower “trajectory” in Figure 1 b). As a result of the cooling an extensive condensation to droplets begins to occur in this region (Figure 5 a). In the further course of the expansion, at distances of about 1000 orifice diameters from the source, collisions among the expanding atoms gradually cease and the beam consists of a mixture of He atoms and droplets.^[117,118] In this vacuum environment the droplets then undergo further cooling by evaporation. Figure 5 b presents the results of calculations of the droplet temperature in the final stages of evaporative cooling (based on the liquid-drop model).^[53,59] Since the initial very rapid rate of evaporation ($\approx 10^{10} \text{ K s}^{-1}$ ^[121]) decreases exponentially with time, the temperature of the ^4He droplets levels out at $T \approx 0.4 \text{ K}$ for times larger than about 10^{-4} s , in accord with experimental results.^[104,122] Similar calculations predict $T \approx 0.15 \text{ K}$ for ^3He droplets,^[53,59] which correlates with the lower (bulk) binding energy per ^3He atom ($E_b = 2.7 \text{ K}$) compared to ^4He ($E_b = 7.2 \text{ K}$). Because of the very rapid initial rate of evaporation it has not been possible, despite several attempts, to significantly increase the droplet temperatures, for example, by exposing them to many successive collisions^[123] or by laser heating.^[124] Essentially they possess a very large effective heat capacity providing for a nearly perfect thermostatic control.

Depending on the source temperature the velocities of the expanded droplets lie in the range of $200\text{--}400 \text{ m s}^{-1}$ and as a result of a unique quantum effect affecting collisions between the He atoms^[47] have a sharp velocity distribution ($\Delta v/v \approx 0.01\text{--}0.03$).^[117] The intensity of a continuous beam of droplets with a mean size of 5×10^4 ^4He atoms ($P_0 = 20 \text{ bar}$, $T_0 = 10 \text{ K}$, 5- μm nozzle) has been measured to be 2×10^{14} droplets $\text{sr}^{-1} \text{ s}^{-1}$.^[128] Alternatively, in the “supercritical” regime the initial state is in the liquid region of the phase diagram and the expansion isentrope crosses the coexistence line from the liquid side (see the upper trajectory in Figure 1 b). In this case a jet of liquid HeI passes through the nozzle, which subsequently breaks up into large droplets (see middle image in Figure 6). These droplets will also cool by evaporation to the same temperatures as the smaller droplets. Under supercritical source conditions, a bimodal droplet size distribution has been observed consisting of large droplets with $\bar{N}_d > 10^6$ (Figure 6; $\bar{N}_d$ = mean number size) and smaller droplets with $\bar{N}_d \approx 10^4\text{--}10^5$.^[117,118] Recently very intense beams of even larger (1–3 μm) droplets containing about $10^{10}\text{--}10^{12}$ atoms have been produced by expanding pressurized normal or superfluid liquid helium at $T_0 = 4.2\text{--}1.6 \text{ K}$ into a vacuum through a micron-sized orifice (upper image in Figure 6).^[127]

3.3. Droplet Sizes and Size Distributions

Both the number size distributions and the scattering cross sections of droplets produced under the different expansion conditions have been investigated by deflecting the droplets through the impact of atoms from a secondary

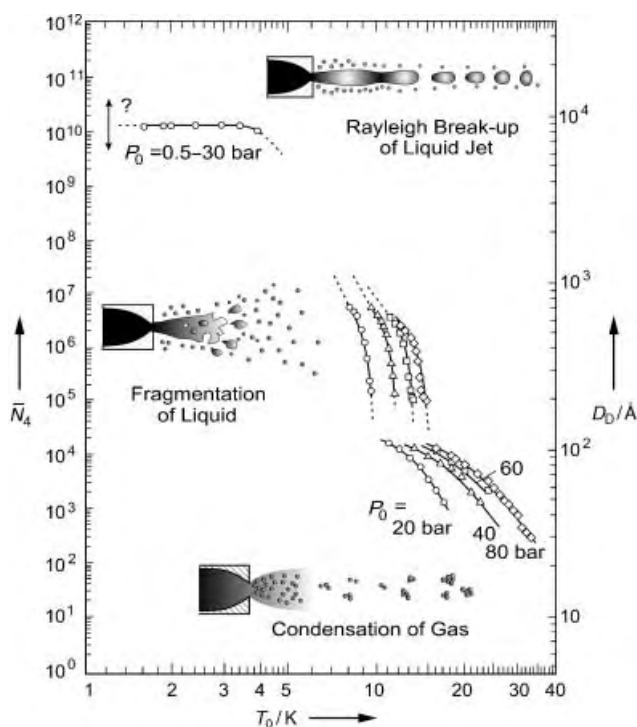


Figure 6. Overview of the mean number size ($\bar{N}_4$) and liquid-droplet diameters (D_0) of ^4He droplets formed in different types of continuous modes of jet operation. The smallest clusters are obtained in subcritical expansions (see bottom image and Figure 5) involving the adiabatic expansion of a gas with subsequent condensation.^[57,119] Larger droplets with $\bar{N}_4 > 10^4$ atoms are produced by expanding liquid helium in supercritical expansions (middle image). After leaving the orifice the liquid fragments into large droplets as a result of cavitation.^[125,126] Even larger droplets with about 10^{10} atoms are formed in expansions of the normal or superfluid liquid at source temperatures below about 4.2 K (top image). Rayleigh oscillations tend to break up the liquid into a series of nearly uniform sized droplets.^[127] Droplets with $10^4 \leq \bar{N} \leq 10^5$ can also be obtained using a pulsed jet source (not shown).^[120]

beam ($\bar{N}_4 = 10^4$ atoms).^[57,58,119] Larger droplets ($\bar{N}_4 > 10^5$ atoms) have been analyzed by first embedding single electrons and then deflecting the charged droplets in an

electric field.^[125] Figure 6 shows the dependence of the mean droplet size and liquid drop diameters on the source stagnation conditions P_0 and T_0 , measured for 2–5- μm diameter orifices in the different modes of operation.^[126] Droplets having sizes in the range of 10^4 – 10^5 atoms have also been obtained in pulsed droplet beams.^[120] Simultaneous measurements of the number sizes by deflection scattering and of the total scattering cross sections of droplets in continuous beams have been used to determine the radial density distributions in ^4He ^[57] and ^3He ^[58] droplets (see also Section 2).

3.4. Apparatus for Spectroscopy

The apparatus developed at the Max Planck Institute for Fluid Dynamics (Strömungsforschung) in Göttingen for spectroscopic studies is shown schematically in Figure 7.^[122,129,130] The apparatus makes use of the facile pick-up of foreign species by the droplets as they pass through a chamber filled with the vapor of the foreign substance.^[11,131,132] After the droplets have picked up one or more molecules they are exposed to the laser beam, which enters the apparatus coaxially and in the opposite direction to the propagating beam of droplets, providing an optimal overlap of the two beams. The energy deposited in the droplets following resonant absorption of a laser photon leads to the evaporative loss of several hundred He atoms.^[129] The decrease in the overall size of the droplet reduces the electron-impact cross section for ionization and the mass spectrometer signal shows a distinct dip at resonance, which is called a “depletion” signal. For example, one IR photon at 2000 cm^{-1} corresponds to an energy of 2800 K and assuming a heat of evaporation of 7 K leads to the loss of about $2800/7 = 400$ He atoms. For a typical droplet with $\bar{N}_4 = 5 \times 10^3$ this leads to a 5% depletion dip in the mass spectrometer signal.^[133] In the IR spectral region resonance detection by depletion is the only technique presently available. In the visible spectral range laser-induced fluorescence of embedded molecules can also be used and in many cases it has been found to provide superior signal-to-noise ratios.^[13]

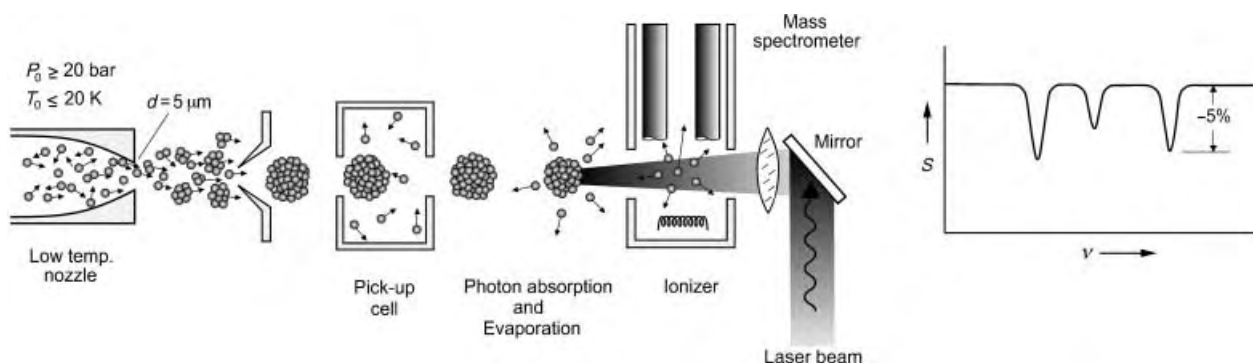


Figure 7. Schematic representation of the laser-depletion apparatus used for the pick-up and depletion spectroscopy of molecules inside helium droplets.^[9] After the adsorption of a laser photon the droplet size decreases as a result of evaporative loss of helium atoms as the photon energy deposited in the chromophore relaxes and temporarily heats the droplet. The absorption signal is detected as a decrease (D_{rel}) of the mass spectrometer signal S .

The apparatus in Figure 7 has proven to be universally applicable and with some modifications has been employed for spectroscopic investigations of a wide range of embedded particles and clusters (see refs. [9,12,13]). One modification of the experimental apparatus introduced by the groups of Scoles at Princeton^[12] and Miller at Chapel Hill^[134] is shown in Figure 8. Instead of a continuous-flow cryostat, a compressor-driven refrigeration unit is used. In this way a local supply of liquid helium is not required, the lowest temperatures which can be reached are about 15 K,^[12] and down to 4 K if modern powerful cryogenerators are employed.^[135] For detection, a liquid-helium-cooled bolometer^[12] is used instead of a mass spectrometer to monitor the laser-induced depletion of the beam intensity. The laser beam enters nearly perpendicular to the droplet beam and is directed into a multipass cell, which in the Chapel Hill apparatus has two parallel plane mirrors.^[136,137] Electrodes have also been added (Figure 8) to the Chapel Hill apparatus to apply large DC electric Stark fields to the interaction region (up to 40 kV cm^{-1}). Some experiments using electric fields will be described in Section 4.3.

Recently the possibility to perform experiments with pulsed helium-droplet beams became available.^[120] The beam is pulsed by a modified commercially available pulsed valve attached to the tip of a cryostat similar to the continuous-flow cryostat used in the Göttingen apparatus (Figure 7). Figure 9 compares the laser-induced fluorescence (LIF) spectrum measured with a pulsed dye laser for a continuous droplet beam (Figure 9a) and for a pulsed droplet beam (Figure 9b). The signal and the signal-to-noise ratio are more than a factor of 100 greater with the pulsed nozzle. The pulsed droplet beams are ideally suited to be used with other pulsed techniques, such as pulsed depletion spectroscopy in the visible and IR ranges, laser photolysis,

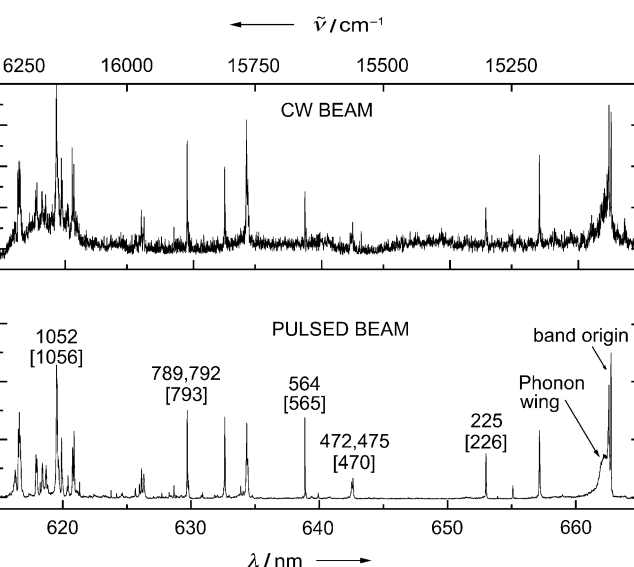


Figure 9. Comparison of the LIF spectra of phthalocyanine molecules embedded in helium droplets produced in continuous-wave (cw) nozzle (a) and in helium droplets produced with a pulsed nozzle (b).^[120] In both cases a pulsed dye laser (10 μs , 70 Hz) was used and operating conditions were optimized to achieve the best possible signal. The vibronic transition frequencies relative to the band origin are given in cm^{-1} ; the numbers in square brackets are the frequencies of the free molecules (see Section 5).^[138]

laser ablation, and time-of-flight (TOF) mass spectroscopy. Interesting perspectives to the field are expected to come from this new development.

3.5. Doping of Helium Droplets

As shown in Figures 7 and 8 the droplets are doped by passing the droplet beam through a pick-up cell filled with the vapor of the chromophore. This technique was first introduced and used for spectroscopic experiments with heavier

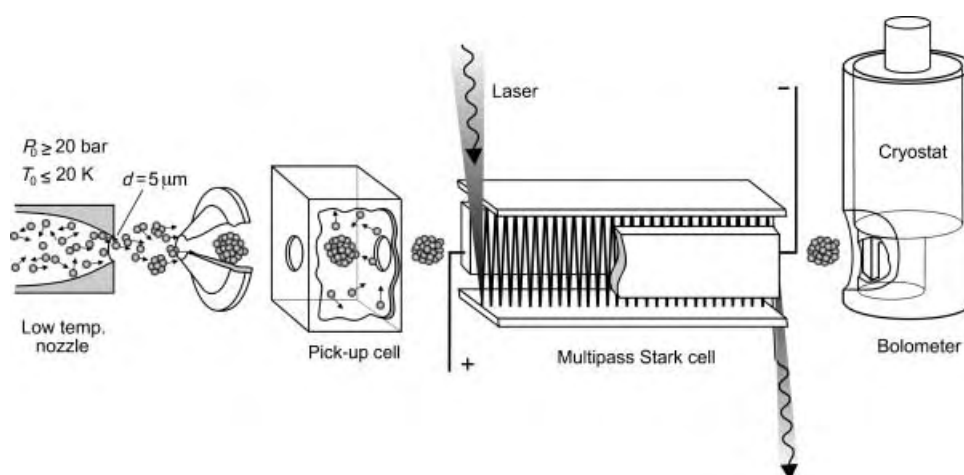


Figure 8. Schematic representation of the bolometer-based laser-depletion apparatus.^[134] The laser beam enters the apparatus from the side into a multipass cell, where it is reflected by two parallel mirrors so that it crosses the droplet beam about 20 times to increase the absorption. At right angles to the mirrors two electric field plates are used to apply a large DC electric field for pendular state experiments (see Figure 15).

noble-gas clusters by Scoles et al.^[139] Initially it was not clear whether this technique could be used for helium droplets, because early experiments in the group of Gspann^[140] were interpreted as indicating that because of their superfluid state He droplets are transparent for foreign species, such as Xe and Cs atoms. Only later did careful mass spectroscopic experiments^[132] in Göttingen demonstrated that He droplets have the unique ability to pick up any species with which they collide.

Low-vapor-pressure species, such as metals or large organic molecules, are sublimated in a heated cell. Since the pick-up cross section is close to the geometrical cross sections of the droplet, that is, typically of the order of 5000 Å² or larger (see Figure 6) a vapor pressure of about 10⁻⁶–10⁻⁵ mbar is sufficient for picking up of a single particle. Therefore, even species with low volatility, for example, amino acids^[141] or refractory compounds and metals may be successfully embedded. As mentioned in the previous Section pulsed droplet beams^[120] open up the possibility to use laser ablation and other pulsed techniques to pick-up low volatility species, such as larger biomolecules, or unstable transient species, such as radicals and ions.^[142] Recently, laser ablation of copper has been used to dope helium droplets obtained in the pulsed nozzle beam expansion over the liquid helium surface.^[110] Laser ablation of Mg close to the continuous helium droplet source, was used to form helium droplets doped with Mg atoms and Mg⁺ ions.^[143]

The pick-up process leads to a decrease in the initial droplet size, a result of the kinetic energy and the internal energy of the impacting molecule, as well as its chemical potential, all of which are transferred to the droplet and lead to a subsequent rapid evaporation of He atoms. As a rule of thumb 1 eV of heat released corresponds to the evaporation of about 1600 ⁴He atoms or 4300 ³He atoms from the droplets. Droplet size measurements before and after pick-up of SF₆ molecules by droplets consisting of $\bar{N}_4 \approx 10^4$ atoms reveal that approximately 600 atoms are evaporated for each captured molecule.^[131] Thus these losses set a limit of about 1000 atoms on the minimum size of the droplets which may be used. If the decrease of the droplet size upon pick-up can be neglected, that is, in the approximation of the constant pick-up cross section, the probability for pick-up of k particles is given by a Poisson distribution [Eq. (3)], where z is the

$$P_k(z) = \frac{(z)^k}{k!} \exp(-z) \quad (3)$$

average number of collisions which lead to capture of the particles by the droplet.^[9]

Owing to the superfluid state of the droplets, the k embedded species are free to move within the droplet and within a short time (presumably $\tau \approx 10^{-8} \times 10^{-10}$ s) coagulate to form a single large complex or in some cases several smaller complexes.^[131] Figure 10 illustrates the Poisson dependence of the depletion signal on the SF₆ pressure in the pick-up cell, as measured for absorption bands corresponding to different number of molecules in the (SF₆) _{k} complex.^[130] Solid curves are fits according to Equation (3) with a pick-up cross section of 3900 Å². The good agreement

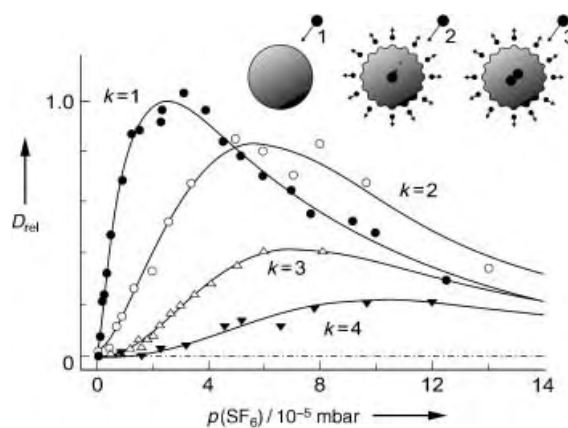


Figure 10. Dependence of the depletion signal D_{rel} on the SF₆ pressure in the pick-up cell, as measured for the absorption bands for (SF₆) _{k} complexes with different k values.^[130] Solid curves are fits according to Equation (3). The mean droplet size is $\bar{N}_4 = 4.1 \times 10^3$ atoms. The insert shows a schematic representation of the pick up, coagulation, and evaporative cooling processes.

of the experiment and the fit justifies the assumption of a constant pick-up cross section, which is usually a good approximation if a small number species are embedded. The Poisson dependence of the pick-up probabilities is widely used to assign spectral features in the spectra to complexes of a particular size. An assignment of the size of the complex has also been possible in several cases in which the spectral features of each cluster with a given number of constituents are sufficiently well separated to allow their direct identification (see Section 4.1).

With this technique the spectroscopy of pure clusters, such as (SF₆)₂^[130,144] (HCN)₂₋₇,^[145,146] or mixed clusters, such as OCS-(H₂)₁₋₁₆,^[148-151] Mg₃-HCN,^[152] HF-Ar₁₋₅,^[153] or tetracene-Ar₁₋₅^[154] complexes have been investigated. Large metal clusters of Ag, Eu with up to 200 atoms^[155-157] and Mg with 2000 atoms^[158,159] have been produced by sequential pick-up of many single atoms by large droplets. For producing heterocomplexes two or more successive pick-up cells are employed.^[151,153,160,161] The use of several well-defined pick-up zones has enabled the tailoring of the structure of the formed complexes by an appropriate choice of the pick-up sequence.^[147,153] For example, the composition of van der Waals complexes of several Ar atoms with flat molecules, such as tetracene^[147] or Mg-phthalocyanine,^[162] has been shown to depend on the pick-up sequence (Figure 11). If a pure Ar cluster is first formed in the initial pick-up cell, it remains intact after the addition of the chromophore molecule in a second cell and the intact Ar _{n} cluster can attach itself to one of the faces of the chromophore. If the pick-up sequence is reversed then the individual solitary Ar atoms attach with equal probabilities to both sides of the molecule.

Another unique property of the droplets stems from the interplay between the very low temperature and the ability of a molecule to rotate freely inside superfluid helium droplets (see Section 4.1).^[122] Thus weak anisotropic electrostatic or van der Waals forces may determine the approach geometry of two molecules to give rise to unusual structures of the

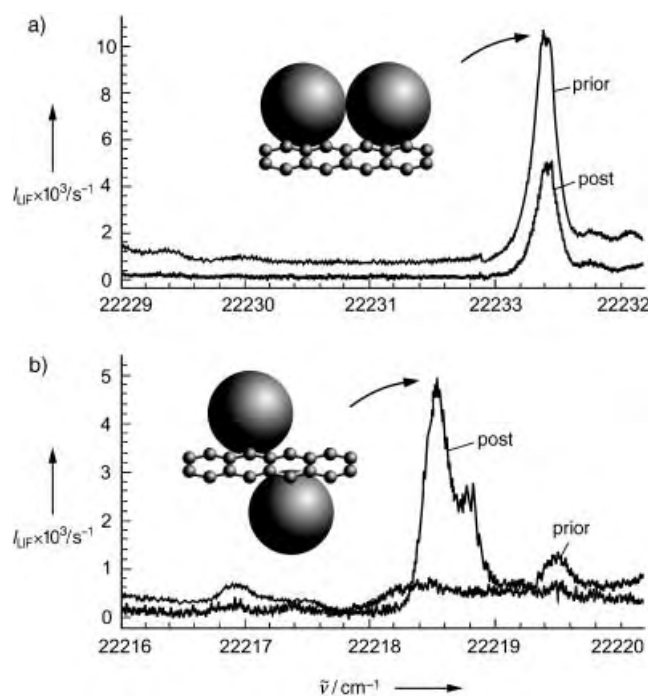


Figure 11. Comparison of high-resolution spectra of tetracene–Ar₂ complexes measured for different arrangements of the pick-up gases. a) “Prior” indicates that the Ar atoms were picked up prior to the pick-up of tetracene. The Ar atoms first form dimers, which then attach to the tetracene (shown). b) The part of the spectrum for configurations involving single Ar atoms at opposite faces of the chromophore. This configuration is dominantly produced if tetracene is first picked up and Ar added afterwards (“post”); I_{LIF} = laser-induced-fluorescence intensity.^[147]

clusters. One of the most striking examples of this is the formation of long-chain complexes with up to 10 polar molecules of HCN.^[145] (see Section 5.3)

4. IR Spectra

4.1. Free Rotations of Molecules and Clusters in Helium

The first experimental evidence for a unique behavior of ⁴He droplets as a spectroscopic matrix was reported by Scoles et al. in 1992.^[144,163] They observed several absorption lines for SF₆ with a half-width of about 0.25 cm^{−1} which is much less than in other rare gas clusters. Subsequent experiments in Göttingen revealed the expected P-, Q-, and R-branches of SF₆.^[122,129] The more recent high resolution IR spectra of SF₆^[104,105,122,123,129] and later OCS,^[133,164] in ⁴He droplets (Figure 12) exhibit a well resolved fine structure, which can be assigned to the rotational lines of the fundamental vibrational transition. In contrast, rotational lines in classical liquids^[165] and solids are rarely resolved and then only for simple molecules, such as HF,^[166,167] which in their interaction with the surroundings are nearly spherical and, moreover, have large rotational constants. In the droplets the spectral line spacings of relatively heavy molecular rotators, such as OCS, are significantly reduced compared to the free mole-

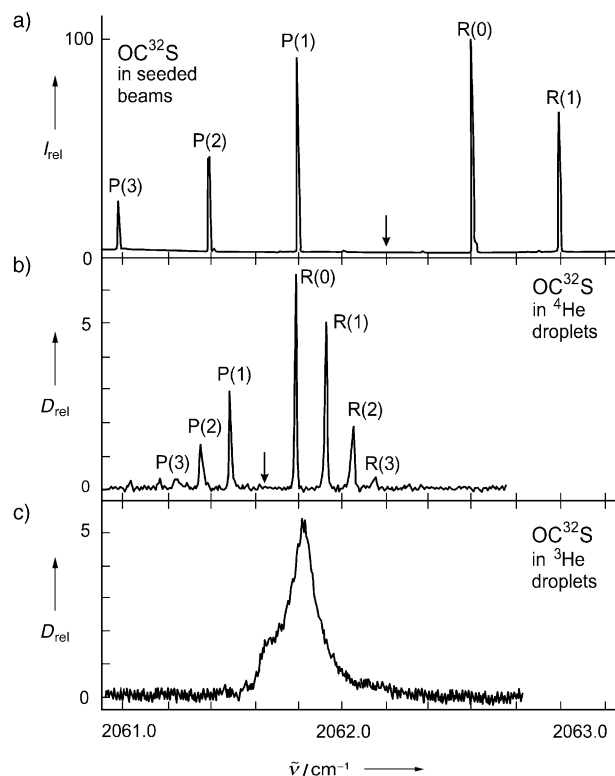


Figure 12. Comparison of the rotational spectra of OC³²S in different matrices: a) the free molecule in an Ar seeded beam,^[168] b) in pure ⁴He droplets with $\bar{N} = 6000$ atoms,^[133] c) in pure ³He droplets with $\bar{N} = 1.2 \times 10^4$ atoms.^[106] The various lines correspond to different transitions with the rotational quantum number j , which are coupled to the OCS asymmetric stretch vibration. The P-branch corresponds to $j \rightarrow j-1$ and R-branch to $j \rightarrow j+1$ transitions. The P- and R-lines are labeled by the initial j value. Note the Q-branch is missing (position indicated by arrows) both for the free molecule and in the He droplets and the reduced spacing of the lines in the He droplet, due to the larger effective moment of inertia.

cule. The rotational energies for a linear molecule are approximately given by Equation (4) where B is the rota-

$$E_{\text{rot}} = B j(j+1) \quad (4)$$

tional constant $B = h^2/8\pi^2 I$, where I is the moment of inertia of the molecule and j is its rotational quantum number. The closer spacing of the rotational levels of OCS in a ⁴He droplet indicates a considerably larger effective moment of inertia of the OCS molecule in this medium (Figure 12b). In recent years many examples have been found in which the spectra of the embedded molecule or cluster in virtually all other respects can be described by the same Hamiltonian as that of the free molecule. Since the line intensities follow a Boltzmann distribution, the temperature within the droplets can be determined, this gives values of 0.37 K in the ⁴He droplets and of 0.15 K in mixed droplets.^[104,133] The good agreement of the experimental temperature with earlier theoretical predictions indicates that despite the weak coupling with the matrix the rotational internal degrees of freedom are fully equilibrated with the helium bath.

Up to Spring 2003 more than 22 different molecules and about 33 bimolecular van der Waals complexes with, in each case, between two and seven attached ligands^[9,12,42] have been studied in helium droplets. In all systems sharp rotational lines were found. For some representative molecules, Table 3 lists the rotational constants of the embedded single molecules and their free-molecule values as well as the line shifts of the band origin. The results for glyoxal and formic acid are the only examples of asymmetric top molecules for which the rotational constants for all three axes have been analyzed (Table 3). Glyoxal is the only system for which the rotational structure could be resolved in the visible spectrum ($S_1 \leftarrow S_0$ transition). Thus an analysis of the spectra also provides information on the rotational constants of the excited S_1 state, which differ by up to 25 % from the ground state.

The molecules in Table 3 have been listed in order of decreasing magnitude of the rotational constant (increasing moments of inertia). Noteworthy is that the ratio of the free-molecule to the embedded-molecule rotational constants has a tendency to increase as the rotational constant decreases.

Table 3: Comparison of the rotational constants of free molecules (free) with measurements in ^4He droplets (He) and the frequencies and shift of the vibrational origin.

Molecule	Mode	Axis	Rotational constant			$\nu_{\text{gas}} [\text{cm}^{-1}]$	$\nu_{\text{gas}} - \nu_{\text{He}} [\text{cm}^{-1}]$	Ref.
			Free [cm^{-1}]	He [cm^{-1}]	Ratio free/He			
HF	ν_1	b	19.787	19.47	1.016	3961.42	+2.22	[169]
HCOOH	ν_2	a	2.576	1.381	1.86	2942.06	-2.36	[170]
(CHO) $_2$ S_0	—	a	1.844	0.643	2.93	—	—	[171]
(CHO) $_2$ S_1	—	a	1.964	0.782	2.61	—	—	
HCN	ν_1	b	1.478	1.204	1.23	3311.46	+0.26	[137, 172]
HCCH	ν_3	b	1.1766	1.042	1.13	3294.84	-0.13	[173]
HCOOH	ν_2	b	0.402	0.297	1.35	2942.06	-2.36	[170]
CO $_2$	(02 ^{0} 1)	b	0.39	0.154	2.53	3612.84	+0.42	[174]
HCOOH	ν_2	c	0.347	0.1996	1.74	2942.06	-2.36	[170]
CH $_3$ CCH	2 ν_1	b	0.2851	0.0741	3.85	6568.17	+0.21	[175]
OCS	ν_1	b	0.2029	0.0732	2.78	2062.20	+0.56	[133]
(CHO) $_2$ S_0	—	b	0.160	0.0722	2.22	—	—	[171]
(CHO) $_2$ S_1	—	b	0.155	0.055	2.81	—	—	
(CHO) $_2$ S_0	—	c	0.147	0.0706	2.03	—	—	[171]
(CHO) $_2$ S_1	—	c	0.144	0.0916	2.79	—	—	
CF $_3$ CCH	2 ν_1	b	0.0959	0.0355	2.7	6557.87	+0.37	[175]
SF $_6$	ν_3	b	0.0911	0.034	2.68	947.97	+1.41	[122]
(CH $_3$) $_3$ SiCCH	2 ν_1	b	0.0654	0.0144	4.54	6520.31	-0.87	[175]

Thus, for heavy molecules, such as SF $_6$, OCS, and the substituted acetylenic derivatives, the moments of inertia are between 2.5- and 4.5-times larger, whereas for HF, H $_2$ O, and some other small light molecules the ratio is about unity (no increase). There are, however, some notable exceptions especially for the asymmetric top molecules for which the A constant (a axis) and the C constant of formic acid are respectively too large or too small. Figure 13 shows a plot of the rotational constants as a function of the corresponding gas-phase values for heavy and light molecules, in helium droplets.^[134] The slope of close to unity for light molecules is explained by the energetic fast rotational motion and the small anisotropy of these molecules, both of which enable a

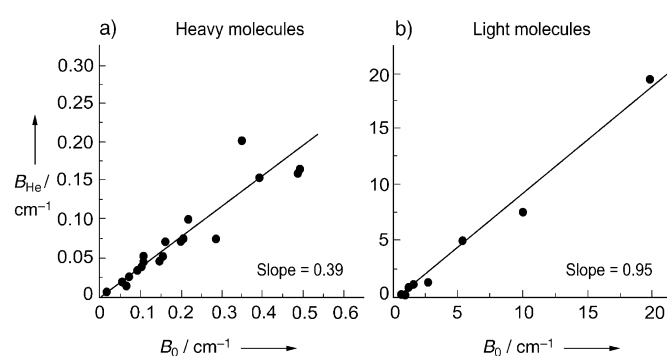


Figure 13. The experimentally available rotational constants in helium droplets (B_{He}) and the gas-phase (B_0) for heavy molecules (a) and light molecules (b).^[134]

decoupling from the hindrance imposed by the surrounding helium bath. On the other hand, the average slope of about 0.4 found for the heavy systems, indicates an average factor of 2.5 increase in the moments of inertia, which is attributed to a stronger coupling of the molecular rotation to the helium bath.

Homogeneous clusters which have been studied in the IR region include (SF $_6$) $_n$ ^[130,144] ($n=2-4$), (HCCCN) $_2$,^[136] (CH $_3$ OH) $_n$ $n=2-5$,^[176] (CH $_3$ CN) $_n$ $n=2-6$,^[176] (HCN) $_n$ $n=2-7$,^[145,146] (HF) $_n$ $n=2-8$,^[177-179] and (H $_2$ O) $_n$ $n=2-6$.^[180-182] The number of heterogeneous van der Waals complexes studied is even larger and includes such systems as HCN-H $_2$ (para-hydrogen (pH $_2$), ortho-hydrogen (oH $_2$), para-deuterium (pD $_2$), ortho-deuterium (oD $_2$), HD),^[183,184] HCN-C $_2$ H $_2$,^[185] HCN-Mg $_n$ ($n=1-3$),^[152,186] HF-H $_2$ (pH $_2$, oH $_2$, pD $_2$, oD $_2$, HD),^[187,188] and HF-Ar $_n$ ($n=1-6$) (Ne,Kr),^[189] and OCS-(H $_2$) $_n$ ($n=1-17$).^[148-151] In most of these systems rotational resolution has been achieved. Often the spectra for the different sized clusters were sufficiently well separated so that they could be identified by their dependence on pressure and analyzed in terms of their spectroscopic constants. The largest clusters for which the rotational bands could be resolved are OCS-(oD $_2$) $_n$ $n=1-17$ ^[148] but this was only found inside the colder (0.15 K) mixed ^4He - ^3He droplets. The resolution in the case of the OCS-(pH $_2$) $_n$ clusters was not as good but, despite some overlap, their spectra could be resolved up to about $n=8$. As expected for these heavy complexes the moments of inertia were generally greater than for the free clusters by factors ranging up to about nine.^[150]

The detailed mechanism responsible for the decrease in the rotational constants of molecules in the helium matrix has been extensively discussed in the literature.^[42,80,190-193] The

most advanced and instructive theoretical calculations involve quantum mechanical simulation methods based either on the diffusion Monte Carlo (DMC) or path integral Monte Carlo (PIMC) techniques.^[42,68,191] For molecules such as SF₆ and OCS these calculations indicate that the relatively strong attractive van der Waals interactions impair the superfluid behavior of the first shell of surrounding ⁴He atoms. According to Feynman,^[194,195] the unhindered exchange, which is provided by many possible permutation cycles of the ⁴He bosons, is one of the outstanding characteristics of a superfluid. The PIMC simulations not only confirm this behavior but reveal, moreover, in the region closest to the molecule, where there is an apparent local accumulation of density, that the length of the permutation chains is considerably shortened.^[42,68,69,82,84] This so-called local nonsuperfluid part is partially dragged along by the rotating molecule or, in other words, is said to “adiabatically follow” the rotations. The simulated rotational constants are in good agreement with experimental results for SF₆,^[42,69,84] OCS,^[42,68] and HCN.^[82] The very recent high-resolution spectra of free OCS-(He)_{1–20} clusters^[196–198] confirmed that the anomalous rotational constants are determined by short-range effects.^[199] On completion of the first He solvation shell around the molecule the rotational constants approach the value found in He droplets.

The spectral resolution is frequently sufficient to determine the value of the centrifugal distortion coefficient *D* in addition to the rotational constant. As first reported for SF₆,^[122] and since confirmed for many other molecules, the value of *D* in the droplets is typically three to four orders of magnitude larger than for the free molecule. In analogy to a simple diatomic harmonic oscillator for which $D = 4B^3/\omega^2$, where ω is the harmonic oscillator frequency^[200] this result suggests that additional bonds, presumably those between the molecule and the attached He atoms, may be involved. These bonds are expected to be sufficiently weak with low frequency ω to explain the observations.^[133] At present, however, no completely satisfactory theory is available to explain these findings although some efforts in this direction have been undertaken.^[201]

The close relation between the superfluidity of the droplets and free molecular rotations has also been demonstrated experimentally. In non-superfluid ³He droplets the rotational spectrum of the OCS chromophore (see Figure 12c) is considerably broadened and a clear rotational structure is no longer seen.^[106] This occurrence has been attributed to the different elementary excitations of the non-superfluid ³He liquids, which are essentially similar to those of the classical liquids.^[165]

4.2. Vibrational Line Shifts and Broadening

Table 3 also lists the line shifts of the vibrational frequencies of a number of molecules which have been measured in the IR region in helium droplets with $\bar{N}_4 = 10^3$ – 10^4 . Most of the molecules exhibit small red shifts of less than $\Delta\nu = 2\text{ cm}^{-1}$. This is consistent with the predominant contribution to the vibrational shifts coming from the attractive

electrostatic induction and dispersive interactions between the chromophore and helium bath. The lack of splitting in the high-resolution vibrational spectra indicates that the liquid-helium environment does not influence the symmetry of the vibrations and that unlike in a solid matrix there are no local site effects. This situation is especially clearly seen for the triple degenerate ν_3 vibrations of SF₆^[104,105,122,123] and some other molecules, such as OCS,^[133,164] for which the experimental line widths set an upper limit on any possible splitting of only 150 MHz.^[133,164] On the other hand, the large-amplitude bending modes in the ground-state of NH₃,^[202] and in the electronically excited S₁ states of pentacene,^[154] and glyoxal^[203] are blue shifted and broadened ($\delta\nu = 1$ – 10 cm^{-1}) indicating an increase in the repulsive interactions in the excited vibrational state. Information on the vibrational frequencies in the electronically excited S₁ state comes from vibronic spectra in the visible and near UV regions (Section 5).

Calculations of the vibrational or vibronic line shifts in helium requires a knowledge of the interaction potential including the coupling to the internal degrees of the molecule in both the ground and excited states. This information is still difficult to obtain with the desired precision of about 0.1 cm^{-1} . Recently a few full-scale calculations of the vibrational shifts have become available for simple molecules, such as HF,^[81] CO,^[83] and OCS.^[78]

The change in the matrix-induced line shift of SF₆ with average droplet size ($\bar{N}_4$) has been studied (Figure 14).^[105,123] The matrix shift was found to increase by only 120 MHz as $\bar{N}_4$ increases from 400 to 1000. The excluded-volume theory for the matrix shift^[31,204,205] predicts that with respect to its bulk value the line shift should increase as $\bar{N}^{-1}$. For droplets larger than $\bar{N}_4 \approx 1000$ the matrix shift remains nearly constant, which indicates a transition to properties resembling those of the bulk phase. Surprisingly, in this size range there is a small decrease of the frequency ($\Delta\nu \approx 20\text{ MHz}$) with increasing $\bar{N}_4$.^[123] This anomalous shift has been shown to be entirely consistent with the theoretical predicted shell structure of ⁴He atoms (see Figure 4) around the embedded molecule.^[55,69,206]

For small clusters of $\bar{N}_4 < 1000$ atoms, the inhomogeneous broadening of the spectra with tails extending towards higher frequencies can be largely explained by the droplet size dependence of the matrix shift (see Figure 14). For OCS this broadening amounts to about 300 MHz for $\bar{N}_4 = 1000$ but decreases to about 150 MHz for $\bar{N}_4 = 6000$.^[133] For other molecules the broadening is expected to be roughly proportional to the matrix-induced line shift. The precise origin of the additional line broadening in helium droplets is still the subject of research. Vibrational relaxation of the molecule in the matrix is one possible mechanism. For polyatomic molecules, the efficiency of the vibrational relaxation is expected to increase with the increased energy of vibrational excitation, a result of the higher density of states of the vibrational modes. This situation correlates with the experimental observation that overtone transitions are at least twice as broad as the fundamentals^[175] and vibrational bands in the 3- μm region^[12] are broader than in the 10 and 5- μm region. On the other hand, for diatomic molecules, such as HF, the vibrational relaxation is observed to be very slow^[169]

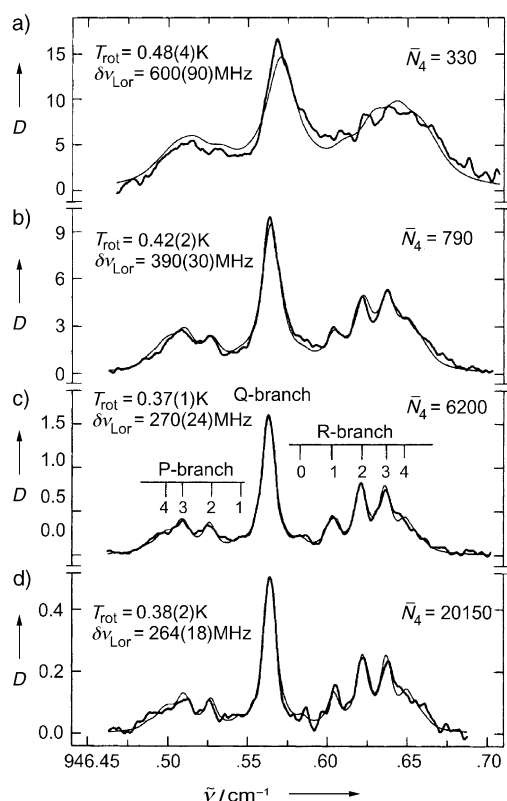


Figure 14. a–d) The depletion spectra (in %) of SF_6 in ^4He droplets of four different mean sizes.^[123] The simulations of the spectra are shown as thin solid lines and give the rotational temperatures T_{rot} and the widths $\delta\nu_{\text{Lor}}$ (full width at half maximum height; FWHM) of the Lorentzian line profiles. The numbers in parentheses are the single standard deviations. The assignment to individual rotational lines of the P- and R-branches is indicated in spectrum (c).

and the broadening is primarily due to fast rotational relaxation. There is also some evidence that thermal effects can also explain part of the broadening. For example, the OCS lines narrow to a half-width of only 50 MHz inside mixed ^4He – ^3He droplets for which the temperature is reduced from 0.37 K to 0.15 K.^[104,122] A similar effect has been found for large $\text{OCS}-(\text{oD}_2)_{15-17}$ clusters.^[148] Another source of broadening is related to the possibility of the molecule as a whole moving unhindered in the superfluid environment. This phenomenon, which is considered to be a necessary condition for superfluidity, was first predicted by Landau in 1941.^[207,208] Since the overall attraction to the helium droplet decreases towards the periphery, the embedded molecule is expected to undergo harmonic vibrations, with some coupling to its rotations. Despite some exploratory theories^[56,67] and some indirect evidence^[67] the details of this fascinating phenomenon are still not fully understood. Highly vibronically excited NO_2 molecules have recently been studied in helium droplets.^[209] The results show a variety of rather broad (7 cm^{-1}) bands, which all have similar shifts from the gas-phase values, which is consistent with quantum chaotic behavior in these highly excited states of NO_2 .

4.3. Elimination of the Rotational Structure

For the identification of chemical species the rotational spectra usually plays a minor role and the set of vibrational frequencies is much more important. Thus in many cases it is desirable to completely eliminate the rotational lines since for large molecules they tend to broaden the band contour and lead to a decrease in the effective spectral resolution and peak-absorption cross section of a vibrational band. In the case of polar molecules helium droplets provide a unique environment for achieving this goal in a straightforward way by the application of an electric Stark field. Figure 15a shows

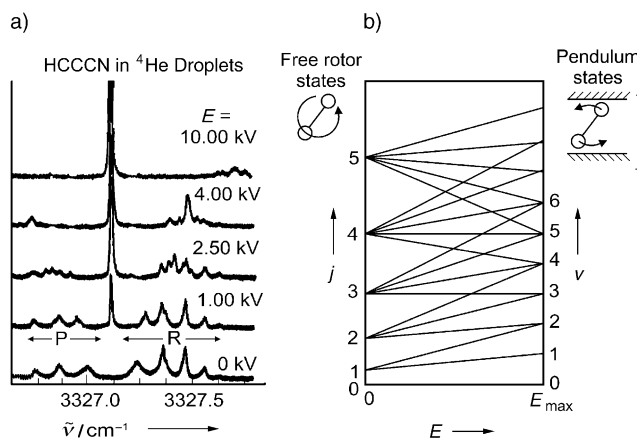


Figure 15. a) Spectrum of cyanoacetylene (HCCCN) in ^4He droplets^[136] as a function of the voltage applied to the electric field plates in the laser interaction region (see Figure 8). b) The rotational energy levels at zero field are gradually transformed into pendular-state levels with increasing electric field.^[1210]

the spectrum of cyanoacetylene (HCCCN) in ^4He droplets^[136] as a function of the strength of the electric field applied in the laser interaction region (see Figure 8). The lower spectrum exhibits clearly resolved P- and R-lines, typical for a parallel vibrational transition. With increasing electrical field the rotational structure of the spectrum collapses into a narrow band close to the vibrational band origin. The physical origin of this effect is illustrated in Figure 15b, which shows that with increasing electric field strength the molecular dipole becomes partly oriented along the field direction. This situation leads to a gradual transformation of the rotational motion into a hindered vibrational motion of the molecule with respect to the field direction.^[1210]

Another more universal technique, which can also be applied to nonpolar molecules, is to embed the molecules inside non-superfluid ^3He droplets. As a result of the now strong collisional interactions with the non-superfluid nearly classical-liquid ^3He environment, transitions between the rotational levels can readily occur and the rotational spectrum is collapsed into a single line (see Figure 12c).^[106]

The use of an applied electric field also makes it possible to orient the embedded molecule with respect to the direction of a polarized IR laser. Since the angle between the permanent dipole moment of the molecule and its transition dipole moment depends on the structure of the molecule this

effect could be used to assign the bands of cytosine in helium droplets to the three different tautomers.^[211]

5. Electronic Excitation Spectra

Many of the aspects discussed in the previous Section in connection with rotational–vibrational spectroscopy apply also to electronic spectroscopy^[13] with, however, some important differences. Whereas the state of helium is usually conserved upon the rotational–vibrational excitation of the embedded molecule, the helium degrees of freedom may be excited in the case of the more “violent” electronic transition. This occurrence is related to the large change in the repulsive interaction between the molecule and the helium atoms upon electronic excitation, which results in a considerable change in the equilibrium configuration of the helium solvation shell. The electronic transitions to the first excited singlet states ($S_1 \leftarrow S_0$) for a number of organic molecules, such as glyoxal,^[171,203] polyaromatics,^[154,212,213] indoles,^[214] and porphyrins^[213,215] in helium droplets have been investigated. In most cases the spectra were detected by LIF except for glyoxal, naphthalene, *N*-acetyl tryptophan amide (NATA), and C_{60} for which the depletion technique was used (Section 3.4). The spectra usually exhibit a sharp transition to the vibrational ground state 0_0^0 of the electronically excited state (zero phonon line). This band is accompanied by clearly resolved vibronic bands on the high-energy side. The excellent resolution obtained in helium droplets is illustrated in Figure 16 where the C_{60} spectrum^[216] is compared with spectra obtained in a neon matrix^[217] and in a seeded beam.^[218] The resolution of the droplet spectrum is much better than in the neon matrices and comparable to the seeded-beam spectrum. Surprisingly most of the lines show no appreciable shift compared to the free molecule.

Another especially spectacular demonstration of the remarkable opportunities for spectroscopy of large molecules is provided by the recent highly resolved LIF spectrum of the large (38 atom) planar 3,4,9,10-perylenetetracarboxylic di-

anhydride (PTCDA) molecule shown in Figure 17a.^[13,222] PTCDA films are widely used as organic semiconductors in light emitting devices. Despite its technical importance its electronic spectrum could not be resolved vibronically either

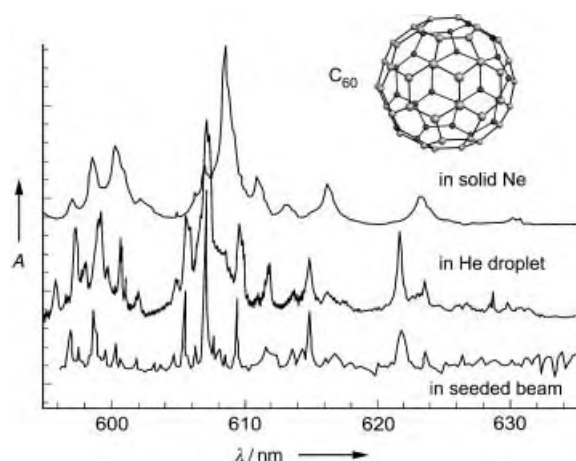


Figure 16. Visible absorption spectra of C_{60} in solid Ne,^[217] in helium droplets ($N = 2 \times 10^4$),^[216] and in a helium-seeded pulsed molecular beam.^[218]

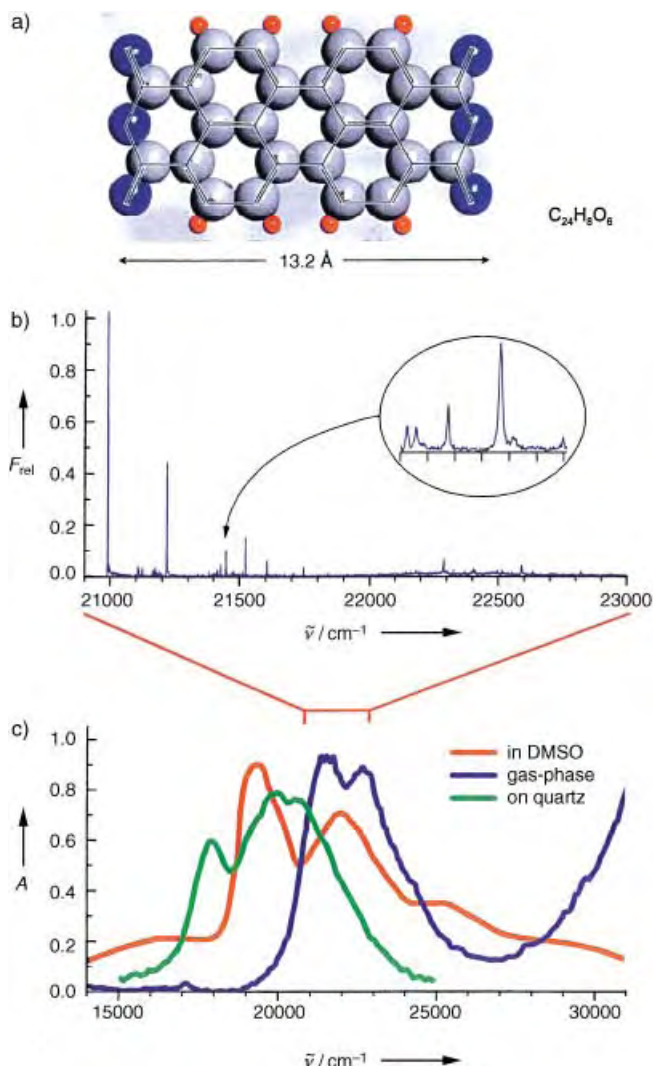


Figure 17. Comparison of the spectra of PTCDA (a); the LIF spectrum in helium droplets (b)^[222] in the gas-phase, in dimethyl sulfide (DMSO),^[219] and on a quartz surface (c).^[220] The most intense line at about 21 000 cm^{-1} is the zero-phonon transition and the structure at higher frequencies are vibrationally resolved $S_1(v') \leftarrow S_0(v'' = 0)$ transitions.

in the gas phase (see Figure 17), nor in a film or in solution.^[219] In particular, charge-transfer collective excitonic transitions in films or solution could not be separated from the single-molecule spectrum because of extensive broadening (Figure 17c). In helium droplets (Figure 17b) the molecular transitions are clearly resolved (line-width $< 1 \text{ cm}^{-1}$) and the broad ($\delta\nu \approx 500 \text{ cm}^{-1}$) charge-transfer excitations of PTCDA aggregates are completely suppressed. The narrow vibrational lines in Figure 17b, resemble the vibrational structure, found in theoretical calculations^[220] and Raman spectra of the PTCDA films.^[221]

The information on the vibrational frequencies in the S_1 excited state provided by vibronic spectra complement the vibrational information for the ground state from the IR spectra. The large number of vibrational levels, which can be excited is illustrated for phthalocyanine in Figure 9. At the low temperatures of $T=0.37$ K in helium droplets, the molecules are initially in the ground vibrational state, which eliminates hot bands, which would otherwise complicate the assignment of the spectra considerably. As can be seen in Figure 9 the vibrational frequencies obtained from the vibronic spectra in helium droplets^[120,154,213] are in good agreement (± 5 – 10 cm^{-1}) with the available corresponding frequencies obtained in the gas phase (Figure 9b).

In most molecules the 0_0^0 origin and each of the vibronic bands have in addition to the narrow zero phonon lines (ZPL) peaked phonon wings (PWs) on the high-frequency side.^[154,203,213,214] In Table 4 the $S_1 \leftarrow S_0$ ZPL electronic origins

Table 4: The zero-phonon line (ZPL) origins of the $S_1 \leftarrow S_0$ transitions of some molecules in helium droplets.

Molecule	Frequency in He [cm^{-1}] ^[a]	Frequency shift in He [cm^{-1}]	Number of the ZPLs	Ref.
glyoxal	21942.8	−30.6	1	[171]
naphthalene	32035	+15	1	[223]
indole	35282	+43	3	[214]
3-methylindole	34943	+61	3	[214]
NATA ^[b]	35014	+59	4	[214]
tryptophan	34960.8	+55	1	[141]
tryptamine	34959.6	+44.1	3	[214]
tyrosine	35538.0	+47.8	1	[141]
tetracene	22293.4	−103	2	[154]
pentacene	18545.0	−104	1	[154]
PTCDA ^[c]	21217.5	^[d]	1	[13]
porphin	16310	−10	1	[213]
phthalocyanine	15088.9	−43	1	[213]
Mg phthalocyanine	15556.2	−56	1	[224]
C_{60}	15666	-14 ± 4 ^[e]	1	[216]
BaO ($A^1\Sigma^+ - X^1\Sigma^+$)	16722.3	+40.3	1	[161]

[a] The typical errors are about 1 cm^{-1} because of uncertainties in the laser calibration. [b] NATA, *N*-acetyl-L-tryptophanamide $\text{C}_{13}\text{H}_{15}\text{N}_3\text{O}_2$. [c] PTCDA, 3,4,9,10-perylenetetracarboxylicdianhydride. [d] Gas-phase spectrum is unknown. [e] Small differences in the shifts are found for the different vibronic bands.

for 16 mostly organic or biologically interesting molecules in helium droplets are listed. Compared to the free molecules, the ZPL are shifted either to the red or blue by about 10 – 100 cm^{-1} , which is about 1% of the transition energy and in this respect quite similar to the relative shift of the vibrational transitions (see Table 3) discussed in Section 4.2. The ZPL shifts in the droplets are a factor of 10 – 100 smaller than in conventional matrices.

The PWs are attributed to compressional volume vibrations of the helium environment, which are simultaneously excited upon the electronic excitation of the molecule. The ratio of the total intensity of the ZPL to that in the PW is usually less than 10% at low laser fluency (1 mm^2 beam, 1 μJ per 10 ns pulse). At a larger laser fluency of about 5 mJ per

pulse the PW may, however, appear to be even stronger than the ZPL as the ZPL-transitions become power saturated. The PW of a single glyoxal molecule in a ^4He droplet (Figure 18a)^[203] was found to be separated from the ZPL by a

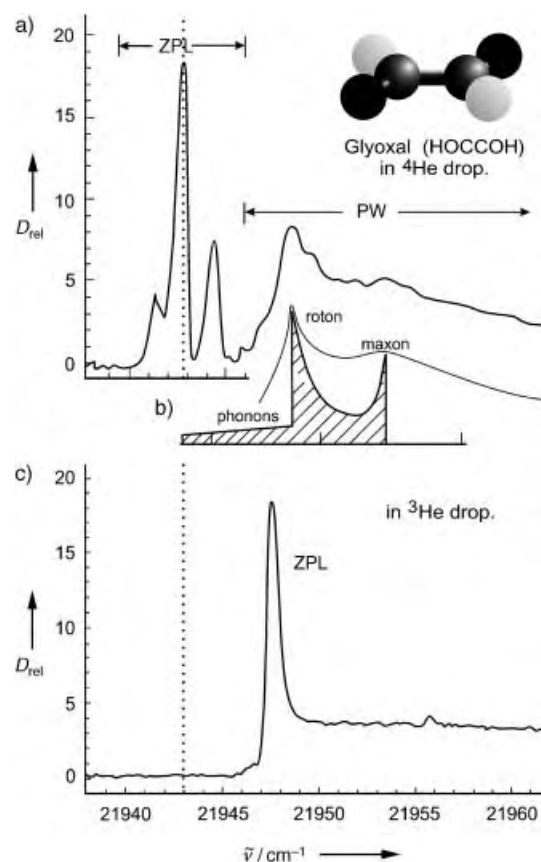


Figure 18. Depletion spectrum (in %) of the band origin of the $S_1 \leftarrow S_0$ transition of glyoxal ($\text{C}_2\text{H}_2\text{O}_2$) in a) a ^4He droplet ($\bar{N}=5 \times 10^3$) and c) a ^3He droplet ($\bar{N}=5 \times 10^3$) showing the zero-phonon line (ZPL) and the phonon wing (PW).^[226] b) The density of states of elementary excitations in liquid helium (^4He) is shown by the shaded region and the smooth line curve shows the results of a theoretical simulation. The gap between the ZPL and the PW and the shape of the PW are clear evidence that the ^4He droplets are superfluid.^[203] This result is confirmed by the spectrum in ^3He which lacks any gap between the ZPL and PW droplets.^[226]

distinct gap of about 5 cm^{-1} . A theory developed from the Huang–Rhys model^[225] for the PWs of impurities in solids, predicts that the PW intensity is proportional to the density of the accessible phonon states weighted by a smooth transition-probability factor.^[203] With this theory the PW in glyoxal could be nicely fitted on the basis of the density of states of the dispersion curve of the elementary excitations of bulk superfluid helium (see Figure 18b).^[203] Thus, the gap between the ZPL and the maximum in the PW spectrum corresponds directly to the energy of the roton in bulk liquid helium. Since the sharp phonon–roton dispersion curve and the roton gap are unique characteristics of the superfluid state of helium, this experiment, reported in 1996, provided the first experimental evidence that ^4He droplets are superfluid. This

interpretation of the glyoxal spectra has been corroborated by the recently measured spectrum of glyoxal in pure non-superfluid ^3He droplets (Figure 18c) where the gap between the ZPL and the PW is completely lacking.^[226] This result is consistent with the difference in the elementary excitations of liquid ^4He and fermionic ^3He , which occur because the fermionic ^3He has a broad additional particle-hole excitation branch.

It is gratifying to see however that the ZPL in the ^3He droplets is nearly as sharp as in ^4He and not broadened as found for the vibrational transitions (shown in Figure 12c). Thus high-resolution electronic spectra can also be expected in the colder ^3He environment. The difference in the wavelengths of the two ZPLs in Figure 18a and c is explained by the 25% smaller particle density in the ^3He droplets than in ^4He .

At high resolution the small peaks next to the glyoxal ZPL seen in Figure 18a could be resolved as shown in Figure 19 to

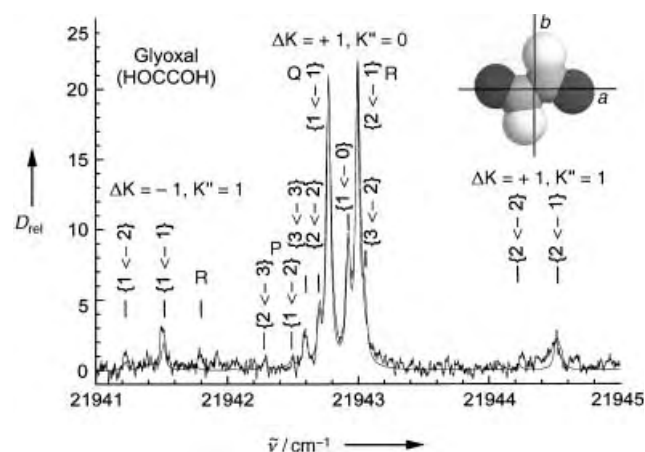


Figure 19. Rotationally resolved depletion spectrum of the $S_1 \leftarrow S_0$ 0_0^0 zero-phonon line of glyoxal in ^4He droplets ($\bar{N}_d = 2 \times 10^3$).^[171] The thin curve is a best fit calculated for a free asymmetric top convoluted with a Lorentzian. The $j \leftarrow j'$ assignments of the transitions are given for the most prominent lines. Inset: the structure of glyoxal and the location of the a and b axis, the c axis is perpendicular to the plane of the drawing.

reveal well-resolved rotational lines.^[171] From an analysis of these spectra large differences of up to 25% in the rotational constants between the electronic and ground states were established. These differences are attributed to the change in the electronic wave function of the excited state.^[171]

Because the interaction between the chromophore molecule and the helium atoms is much stronger than that between two helium atoms, the symmetry of the environment is expected to fully adapt itself to that of the chromophore molecules, even for very asymmetric species, such as amino acid molecules.^[141] This property of the helium matrix makes it possible to use the sharp ZPL transitions in the electronic spectra to interrogate small changes in the molecular structure, which are usually associated with small spectral shifts. A particular interesting demonstration of the great potential of He droplets as an advantageous cryomatrix is

provided by the $S_1 \leftarrow S_0$ spectrum of the amino acids tryptophan (Figure 20) and tyrosine.^[141] The tryptophan spectrum shows a number of lines, which have been assigned to the different possible structural conformers of this floppy mole-

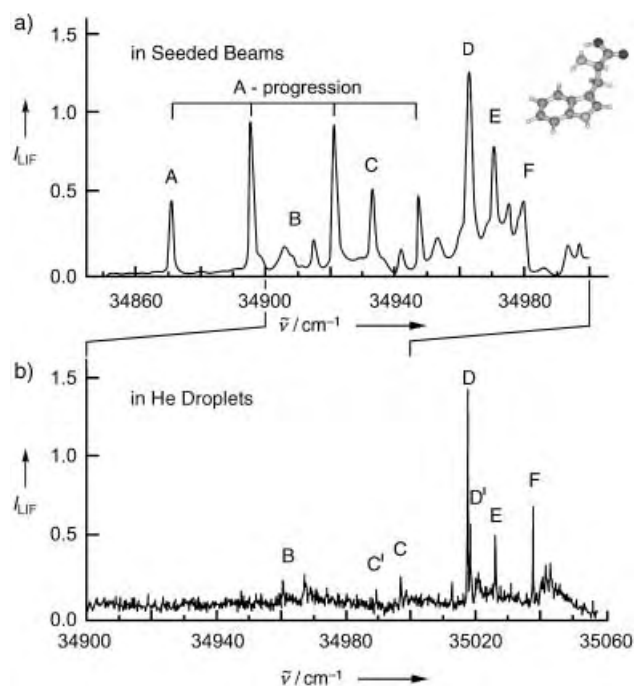


Figure 20. Comparison of the LIF spectrum of tryptophan ($\text{C}_{11}\text{H}_{12}\text{N}_2\text{O}_2$) in a) a seeded beam^[227] b) in He droplets ($\bar{N} = 8 \times 10^3$).^[141] Inset: the structure of one of the ten known conformers of tryptophan.^[228]

cule, some of which surprisingly, are missing in the helium-droplet spectra (Figure 20). This result suggests that some of the conformers are frozen out in the helium matrix. These spectral studies also demonstrate another advantage, mentioned earlier, namely, that only low vapor pressures of about 10^{-5} mbar are required. For this reason these sensitive biological molecules could be sublimed at sufficiently low temperatures where decomposition plays a minor role.

Another very recent important development is the successful measurement of the dispersed fluorescence (DF) spectrum of a large organic molecule, in this case phthalocyanine.^[229] Figure 21 shows an overview of these experiments. Figure 21b–d show sections of the fluorescence emission spectrum found when the molecule is excited at the three different frequencies indicated by the arrows in the LIF spectrum in Figure 21a. The region shown is somewhat larger than that in Figure 9. Whereas in the seeded-beam experiments the emission lines exhibited a red shift of up to 60 cm^{-1} with increasing excitation wavenumber, in the droplets the spectra consist of two sets of sharp lines, separated by 10.3 cm^{-1} with frequencies which are completely independent of the excitation energy. Since the energy-dependent shift observed in the free molecules is attributed to an incomplete vibrational relaxation, the droplet spectra suggest a very efficient relaxation into two well-defined

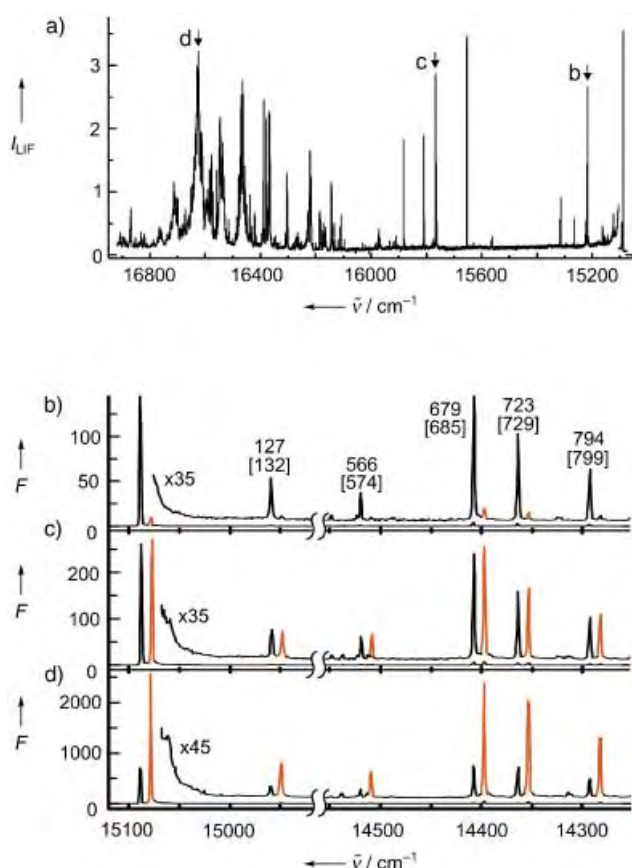


Figure 21. a) LIF excitation and b–d) emission spectra of the $S_1 \leftarrow S_0$ and $S_2 \leftarrow S_0$ electronic transitions of base-free phthalocyanine in ^4He droplets ($N_4 = 4 \times 10^4$); F = fluorescence signal in counts per s. The emission spectra in (b), (c), and (d) correspond to excitation at the frequencies indicated in (a). With increasing excitation frequency the red-shifted line in emission increases in intensity and provides evidence for a second emission channel not found for the free molecule.^[229]

quantum states of the helium-bath/chromophore. The decrease in the intensity of the main spectrum and the increase of the red-shifted replica with increasing excitation energy suggests the presence of a barrier which must be overcome before the quantum states associated with the red-shifted spectrum can be accessed. A similar model was previously introduced to explain anomalies in pump-probe experiments on tetracene, where a similar splitting was observed in absorption.^[124] These new experiments reveal another remarkably unique property of the helium-droplet matrix: the ability to efficiently relax highly excited complex molecules, while at the same time preserving sharp spectral features seen otherwise only in the gas phase.

6. Taylor-Made Clusters

As mentioned in Section 3.5 the very low temperatures and the high mobility of the embedded species makes it possible to form clusters of heliophilic species of virtually any desired composition. In recent years these possibilities have

been used in specially designed experiments to provide either more detailed information on the clusters or to create clusters with novel structures.

6.1. The Determination of the Structures of Small van der Waals Complexes

One of the shortcomings of the helium-droplet matrix is the large and as yet little understood increase in the moments of inertia compared to the free molecules (see Section 4.1). This situation is unfortunate since the experimental moments of inertia may provide important information on the structures of the molecules or complexes under investigation. In a recent study this problem could be circumvented by using isotopic substitution^[149] as demonstrated for the OCS-H_2 van der Waals complex. In Figure 22 the IR spectrum of the bare OCS molecule (a) is compared with that measured with an added para- H_2 ($p\text{H}_2$) molecule (b). In addition to a few lines from the bare OCS molecules the well-resolved OCS-pH_2 spectrum has at least 11 additional lines. These could be fitted very well by assuming an OCS-H_2 complex with the H_2 molecule attached to a “waist” (equatorial) site thereby forming an asymmetric top in which the H_2 molecule rotates freely around the OCS molecule. As observed for the bare OCS molecule the moments of inertia were found to be much

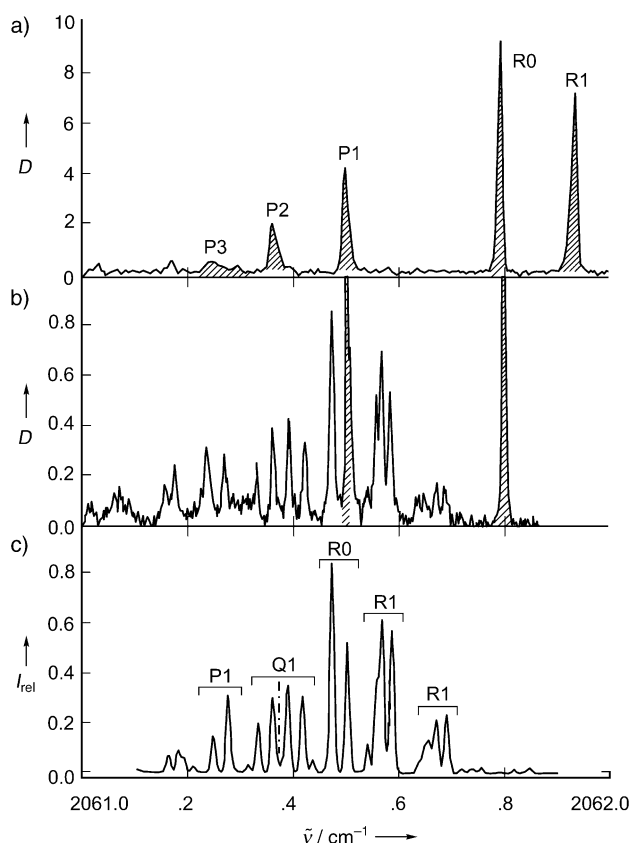


Figure 22. a) IR spectrum of pure OCS^{32}S in a ^4He droplet ($N_4 = 3 \times 10^3$),^[133] b) IR spectrum of OCS with an attached $p\text{H}_2$ molecule inside a mixed $^4\text{He-}^3\text{He}$ droplet,^[149] c) a best-fit simulation (0.15 K) based on the same Hamiltonian for a free asymmetric top molecule.^[149]

larger than expected for the free complex. However, by also measuring the spectra for HD and D₂, which were equally well resolved, the dependence of the moments of inertia on the mass of the attached hydrogen molecule could be ascertained. By assuming the same structure for all three isotopomers the differences in the moments of inertia were used, through Kraitchman's equations,^[230] to determine the structural parameters. After these results were reported the free OCS–H₂ (HD, D₂) van der Waals complexes were studied by high-resolution IR spectroscopy in a pulsed free jet expansion^[231] making a comparison with the helium-droplet analysis possible. The line shifts for all three complexes in the droplets were found to be the same for pH₂, oH₂, oD₂, pD₂, and HD and are only about 0.61 cm^{−1} compared to the corresponding free complexes.^[231] Even more surprising the structure obtained from the Kraitchman analysis agrees remarkably well (within the mutual errors) with the structure determined for the free complex. This unexpected result suggests a strategy for structural determinations based on the rotational spectra, which avoids the complications arising from the enhanced moments of inertia.

6.2. Spectroscopy of Taylor-Made Quantum-Fluid Clusters

Helium droplets also offer a unique opportunity to serve as hosts for other quantum-fluid clusters. For example, pure ³He droplets doped on average with less than one OCS molecule were passed through a second pick-up chamber filled to different pressures with ⁴He gas, which allowed different numbers of ⁴He atoms to be added to each droplet.^[106] Upon pick-up the ⁴He atoms diffuse inside the ³He droplet, where, because of their greater mass and hence lower zero-point energy and resulting stronger binding, they are able to replace the ³He atoms adjacent to the central OCS molecule.^[106] The IR spectra in a pure ³He droplet without ⁴He, has only a single collapsed peak (Figure 12c). With increasing numbers of ⁴He atoms the position of the central maximum in the spectra is shifted slightly to the blue. With more than $\bar{N}=40$ atoms, additional peaks appear, and after the addition of about 60 ⁴He atoms the rotational spectrum with a distinct P(1)-, R(0)-, and a R(1)-line appears. Since, as discussed in Section 4.1, the appearance of resolved rotational lines is a manifestation of superfluidity this experiment provides the first experimental evidence for the actual number of atoms needed for superfluidity and, moreover, that even small systems with about 60 atoms can support superfluidity. These experiments have stimulated considerable discussion and some controversy concerning the implications of these microscopic manifestations of superfluidity and how they relate to the more familiar macroscopic superfluid phenomena.^[232]

Other recent experiments were devoted to the search for new superfluids.^[148] In 1972 Ginzburg and Sobyenin predicted^[233] that, since at low temperatures para-H₂ molecules are spinless bosons, these molecules might undergo a transition to the superfluid state. Their predicted transition temperature of $T_\lambda = 6$ K is far below the triple point of 13.8 K implying that extensive supercooling is required to prevent

solidification before superfluidity can set in. The most recent theories predict the transition at even lower temperatures, between 2 and 3 K.^[234,235] Later in 1991 Ceperley et al.^[236] predicted that small para-hydrogen clusters having less than 18 molecules should remain fluid at $T=0$ and exhibit superfluid properties below about 2 K. To achieve similar experimental conditions clusters of pH₂ and oD₂ with up to about $n=20$ hydrogen molecules were formed around an OCS chromophore in a series of experiments involving pure ⁴He, pure ³He, and mixed ⁴He–³He droplets. In each case the hydrogen molecules were added by multiple pick-up in a way similar to that described for ⁴He.^[148] Luckily the resolution was sufficient to resolve the rotational bands and the vibrational shift for each added H₂(D₂) was large enough to separate the IR spectra for each value of n . As shown in Figure 23 for larger clusters, $n=14$ –17, in the pure ⁴He droplets ($T=0.37$ K), both systems exhibited Q-branches indicating that rotations around the OCS symmetry axis are excited. Even in the much colder ⁴He–³He droplets ($T=0.15$ K) the Q-branch together

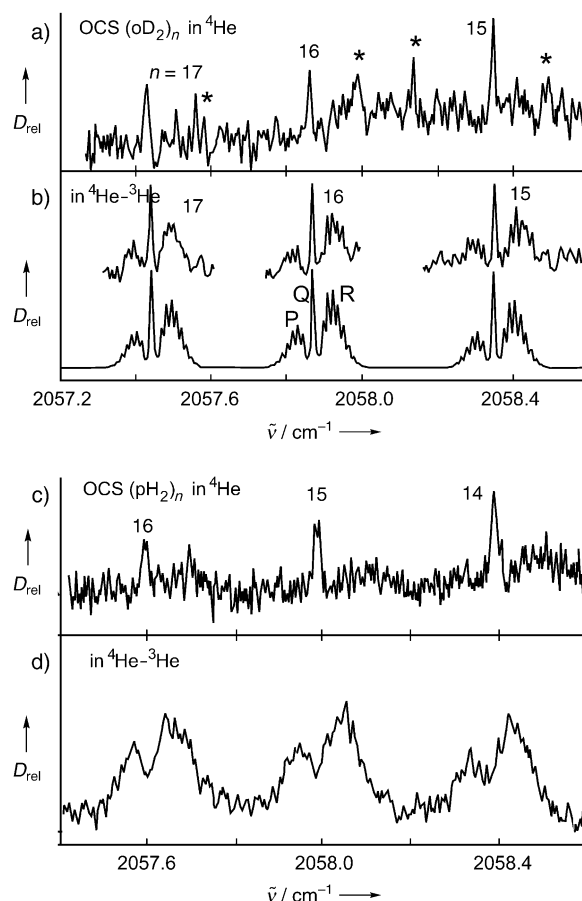


Figure 23. a, b) Comparison of the IR spectra measured for three different OCS–(oD₂)_n clusters in helium droplets with $n=15$, 16, and 17 in pure ⁴He droplets ($\bar{N}_4=8\times 10^3$; $T=0.37$ K) and in mixed ⁴He–³He droplets with 10^4 ³He atoms and 500 ⁴He atoms ($T=0.15$ K).^[148] c, d) Comparison of IR spectra of OCS–(pH₂)_n clusters with $n=14$, 15, and 16 in the same droplet environments. The asterisks in (a) denote peaks resulting from the inclusion of pD₂ impurities. The bottom part of (b) shows best-fit simulations based on a free symmetric top Hamiltonian with spectral resolution of $\Delta\nu=0.01$ cm^{−1}.

with the resolved P- and R-branches were observed in the spectra of the oD_2 clusters. Surprisingly, in the case of the pH_2 complexes the Q-branch was no longer observed in the colder mixed droplets. Since these clusters are expected to have a prolate symmetry structure the lack of a Q-branch indicates that the angular momentum along the OCS axis is no longer excited. This observation could only be explained by a large decrease in the moment of inertia of the pH_2 complexes much below the expected classical value. As a result, the rotational energy constant is so large that the axial rotations are no longer excited at the low local temperatures. This dramatic decrease in the moment of inertia, which was first found in nuclei,^[237] is considered to be a manifestation of superfluidity. Although oD_2 molecules are also bosons, they have a large nuclear spin multiplicity and therefore are, to a large extent, not indistinguishable. Consequently the superfluidity of oD_2 is expected to set in at even lower temperatures. Thus, these experiments provide the first evidence for superfluidity in a liquid other than helium. This interpretation has recently been supported by PIMC calculations of $\text{OCS-(H}_2\text{)}_{17}$ clusters.^[191]

6.3. Complexes of Polar Molecules

An interplay between the very low temperature and the ability of a molecule to rotate freely in liquid helium have important consequences for molecular complex formation. Thus even the weak anisotropic electrostatic or van der Waals forces between molecules may be sufficient to orient the molecules as they come together and give rise to metastable complexes with unusual structures.^[145, 154, 182] One of the most striking examples of this type of self-organization is the chain structure of the complexes of polar molecules, such as HCN and cyanoacetylene.^[136, 145] Figure 24 shows the pendular-state IR spectrum of cyanoacetylene complexes in helium droplets in the presence of an electric field (see Figure 15). The pendular-state lines are assigned to hydrogen-bonded C–H stretch transitions of the linear $(\text{HCCCN})_n$ complexes, in agreement with theoretical calculations.^[136] Figure 25 illustrates the mechanism responsible for the formation of polar molecular chains in helium droplets. Upon pick-up, the additional molecule interacts with the electrical field of the dipole moment of the polar chain formed by prior pick-ups. As the molecule comes closer to the polar chain the gradient of the field emanating from the chain attracts the molecule to one end of the chain, where on further approach it is locked into position by strong electrostatic forces. Thus, despite the fact that the global minimum of the potential energy surface corresponds to either cyclic or branched structures, which are indeed observed for complexes formed in seeded beams at somewhat higher temperatures,^[238] the ultralow temperatures and the mediating effect of the liquid favor the formation of large metastable chains with up to 10 constituent molecules. To date, the fraction of molecules forming chains relative to branched and cyclic structures and which molecules will predominantly give rise to chain complexes is not completely clear. For example, the IR spectra of acetonitrile complexes $(\text{CH}_3\text{CN})_4$ have been assigned to nonpolar square D_{2d}

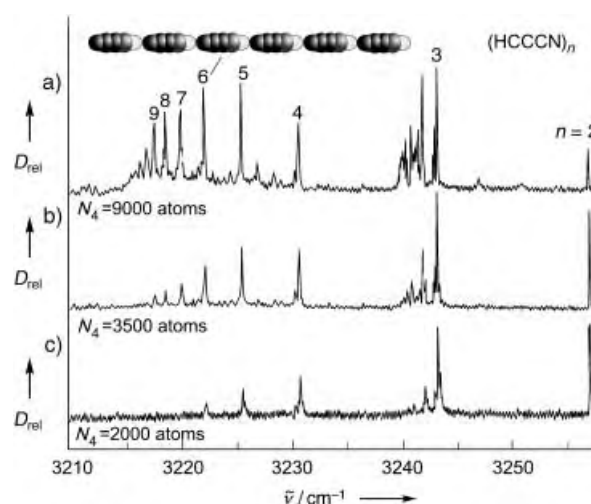


Figure 24. Comparison of pendular state IR spectra of cyanoacetylene (HCCCN) clusters in three different ^4He droplets with a) $\bar{N}_4 = 9 \times 10^3$, b) $\bar{N}_4 = 3.5 \times 10^3$, and c) $\bar{N}_4 = 2 \times 10^3$.^[136] The spectra are of the C–H stretch vibrations at one end of the linear cluster chains. The longest chains with 12 monomer units and a length of 8 nm are found in the largest droplets (a) with a diameter of 9.2 nm.

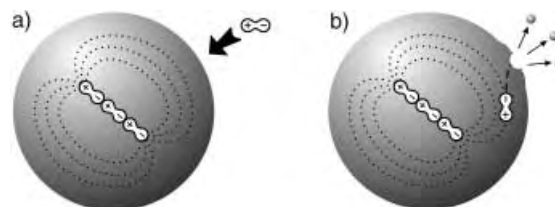


Figure 25. Schematic representation of the mechanism leading to the formation of chains of polar molecules in ^4He droplets. a) A fourth molecule approaches a droplet containing a chain with three molecules. b) On entering the droplet some ^4He atoms are displaced and the molecule is aligned and attracted by the electric field of the trimer chain.

complexes.^[176] Although this structure is different from the S_4 structure, which corresponds to the global surface minimum as observed in seeded beams,^[176] there is no indication of chain formation in this case, despite the large permanent dipole moment of acetonitrile ($\mu = 3.4$ D).

6.4. Chemical Reactions in Helium Droplets

Helium droplets may also provide new opportunities to explore chemical reactivity at ultralow temperatures. Of fundamental interest is the low-temperature limit of a reaction rate, which is defined by quantum mechanical tunneling.^[239, 240] The same weak-orienting electrostatic or van der Waals forces, which govern the formation of novel complexes as described in the previous Section, may make it possible to steer reaction pathways. If the long-range interaction favors a geometry corresponding to a high potential barrier to reaction, such that the reaction is hindered, then the reaction energy could be stored in the form of highly metastable collision complexes. The stored energy might be

released by laser excitation or after complete evaporation of the helium droplets following laser-pulse heating or possibly by intentional collisions with He atoms in a gas cell or in frontal collisions with a wall. Undesired reaction pathways may also be blocked by the preformation of complexes with inert species. Also, small barriers may be modified by pre-attaching an inert atom or molecule to one of the reactants.

Of particular interest in this context are complexes consisting of a radical attached to a molecule. These can be viewed as reaction-entrance-channel precursors and their spectroscopy can provide valuable information on the potential energy surfaces near the transition-state region. Such free complexes, for example, OH-CO ,^[241] OH-CH_4 ,^[242] and OH-H_2 ,^[243,244] have been studied recently in seeded molecular beams. In general, however, it is quite difficult to stabilize the free complexes in the shallow van der Waals minimum as they tend to react instead. At the other extreme of solid matrices the photodissociation of molecules is widely used to prepare cold radicals.^[2] In this case the control over the formation of complexes is impaired by the inhomogeneous distribution of the reactants, strong matrix-interaction effects, and the ill-defined mobility of the photofragments.

Exploration of the new experimental opportunities offered by helium droplets is just now beginning. At present it is not clear which free radicals are heliophobic and which are heliophilic. Early theoretical studies indicate, for example, that hydrogen atoms are heliophobic.^[245] Thus clearly as a first step a versatile method has to be found to embed radicals in helium droplets. To be used for helium-droplet experiments the source of radicals has to be very clean since any undissociated or precursor species will also be picked up by the droplets. The atoms of most metals can be picked-up in a straightforward way by passing a beam of helium droplets through a high-temperature oven.^[92,94,152,156,161] Some radicals, such as propargyl, may be produced by efficient pyrolysis of precursor molecules.^[246] Photodissociation, which is usually used to produce radicals in a solid matrix, has not yet been applied to particles embedded in helium droplets. The short duty cycle of pulsed lasers usually used in experiments can be compensated by the use of the recently developed pulsed droplet-beam sources.^[120]

To date, only a few examples of chemical reactions in helium droplets have been reported. The early mass-spectrometer studies^[64,131] with electron-beam ionization revealed a rich ion-molecule reaction chemistry. Ionization of doped helium droplets ($\bar{N}_4 < 10^5$) leads, in addition to the usual He_n^+ fragments,^[117,118,247-249] also to bare ionic fragments of the embedded neutral species, which was first studied in detail for SF_6 .^[64] The ionization mechanism involves the primary creation of a positive He^+ hole in the droplet.^[117,118,247-249] This hole migrates within the droplet until it is localized by ionizing the embedded impurity molecule to which it is attracted by induction forces. In many cases the extent of fragmentation of single molecules is greatly reduced compared to the free molecules.^[64,247-249] Also the fragmentation of embedded clusters is greatly simplified compared to the same free clusters, as has been found for noble-gas clusters formed inside ^4He droplets.^[131,247-249] Surprisingly, up to about 30 He atoms have been found to be attached to the ionized

impurity atoms but not to the polyatomic ion fragments of the noble gases,^[131,247-249] or to Li^+ ions.^[250-252] For small clusters ($N_4 < 1000$) the energy released in the charge-transfer ionization may be sufficient to completely destroy the droplet thus leaving behind either the bare ions (Ag ,^[155] tetracene^[124]), or possibly one predominate ion fragment (tryptophan^[141]) or, in other cases, protonated ion fragments (complexes, such as methanol clusters, acetonitrile clusters^[176]).

In a recent study of ion-molecule reactions a number of different ion products from reactions of the nascent He^+ ion with embedded D_2 , N_2 , and CH_4 were observed in the mass spectra after electron impact ionization.^[253] The secondary reactions $\text{N}_2^+ + \text{D}_2 \rightarrow \text{N}_2\text{D}^+ + \text{D}$ and the reaction $\text{CH}_4^+ + \text{D}_2 \rightarrow \text{CH}_4\text{D}^+ + \text{D}$ and $\text{CH}_3^+ + \text{D}_2 \rightarrow \text{CH}_3\text{D}_2^+$ could also be identified on the basis of the dependence of the product-ion signals on the composition and pressure of the embedded gas mixture.

Heliophobic sodium atoms have been observed to recombine on the surface of helium droplets to form Na_2 and Na_3 molecules.^[92,94,160,254-256] Upon reaction, the weakly bound triplet-state dimers and the quartet-state trimers have a greater tendency to remain attached to the droplet (see Figure 26), whereas their much more strongly bound singlet and doublet counterparts, are detached from the droplet owing to the larger thermal agitation induced when the

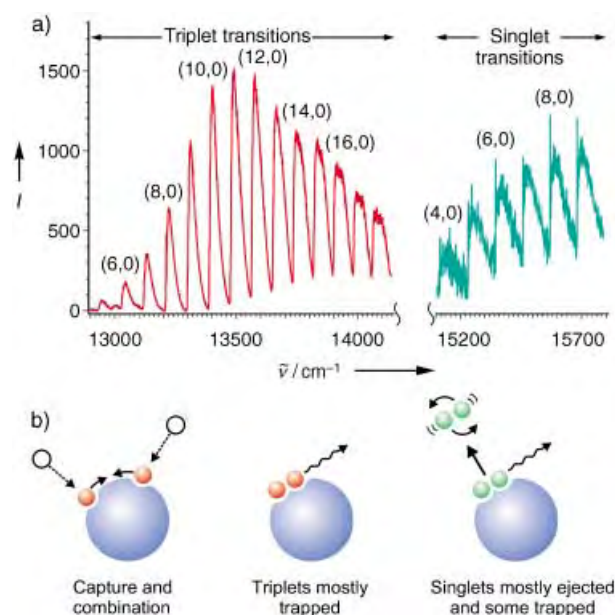
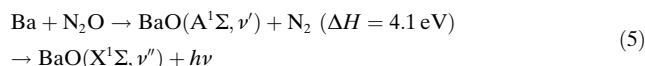


Figure 26. Alkali-metal dimers created by recombination at the surface^[10,92,94,160] of liquid helium droplets are formed in both their triplet and singlet states, resulting in distinct electronic spectra (a), shown here for sodium dimers on droplets of about 10^4 helium atoms (the singlet line intensities have been multiplied by a factor 3). Formation of a strongly bound singlet dimer releases a large amount of energy, which results in extensive evaporation and significant probability for desorption of the alkali-metal molecule. In contrast triplet dimers are weakly bound by van der Waals interaction and are therefore preferentially trapped on the surface (b). Their higher spectral intensity confirms the enrichment of the high-spin species above their statistical weight. The excitation spectra for both species show a progression of vibrational bands. The singlet bands show zero-phonon lines and phonon wings analogous to those seen in Figure 18.

binding energy is released to the surface of the helium droplets (Figure 26). The enhancement of the triplet Na_2 molecules has facilitated the spectroscopy of the triplet-transition manifold.^[92,94,160] For the Na trimer this technique enabled the isolation of the quartet state for the first time. Following excitation to higher lying quartet states an interesting emission spectrum was observed into a dissociation channel with either electronically excited atoms or trimers.^[254–256] Formation of sodium clusters having up to 13 atoms,^[257] and potassium clusters with up to six atoms^[258] in or on helium droplets has been recently detected mass spectrometrically. Although the mechanisms for their formation and the alignment of the magnetic moments still need clarification, these experiments open up interesting new opportunities for the future.

The aggregation of atoms and molecules to form large clusters, with up to several-thousand atoms as demonstrated for Mg,^[158,159] and discussed in Section 3.5 is, of course, also an example of a recombination reaction. The extremely low temperatures required for the formation of these large alkaline-earth metal clusters has facilitated the observation of interesting unexpected anomalies in the filling of their electronic shells.^[158]

So far only one bimolecular chemical reaction involving neutral species has been studied. For this pilot experiment the highly exothermic chemiluminescent reaction [Eq. (5)] was chosen.



Previously, this reaction had been extensively studied in crossed-beam experiments and also on the surfaces of Ar and Ne clusters.^[259,260] It has the advantage that the intense chemiluminescent emission serves not only to indicate that the reaction has occurred but also identifies the products and their internal state distributions. Figure 27b shows the BaO^* chemiluminescence spectrum in helium droplets compared to results for the same reaction on Ar clusters Figure 27a.^[259,260] The spectrum found with Ar clusters was assigned to two components: In the range of 400–600 nm, a broad apparently structureless maximum, while between 600–700 nm, a series of sharp peaks are observed. The former corresponds to ejected rotationally and vibrationally hot $\text{BaO}(\text{A}^1\Sigma^+)$ product molecules which emit light immediately after leaving the cluster. The sharp peaks are attributed to the fluorescence from $\text{BaO}(\text{A}^1\Sigma^+)$ molecules, which remain attached to the droplet long enough so that their internal rotational and vibrational degrees of freedom are completely relaxed before the emission can occur. In helium droplets^[161] the same components are observed, but the latter bands, are much narrower, both because of the lower temperature and the lack of inhomogeneous broadening (Figure 27b). Surprisingly the vibrational progression seen in the emission spectrum is in nearly perfect agreement with the spectrum expected for an internally relaxed, electronically excited, free BaO product molecule.

Another remarkable effect was also discovered in the course of these experiments. If about 15 Xe atoms are first

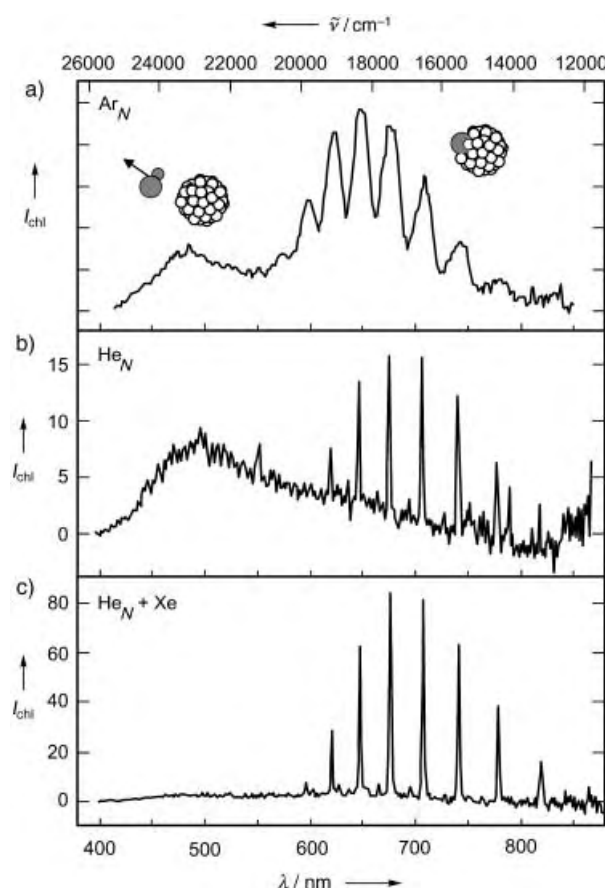


Figure 27. Comparison of the chemiluminescent (chl) emission spectrum of $\text{BaO}(\text{A}^1\Sigma^+\nu' = 0 \rightarrow \text{X}^1\Sigma^+\nu'')$ on a) Ar clusters ($N_{\text{Ar}} = 2 \times 10^3$),^[259] b) pure ^4He droplets ($N_{\text{He}} = 2 \times 10^5$),^[161] and c) inside pure ^4He droplets which are first doped with about 15 Xe atoms.^[161] The images in (a) illustrate the processes responsible for the spectra above and below $\lambda \approx 570 \text{ nm}$.

added to the droplet, the hot channel is almost completely suppressed and virtually all the emission is in a vibrational progression of sharp lines (Figure 27c). This result was interpreted as indicating that none of the products was able to leave the droplet before emitting light. Since the heliophilic Xe atoms formed a large cluster inside the droplet, it exerted a sufficiently strong force on the heliophobic Ba atoms on the surface to draw them into the interior where they reacted with the previously embedded N_2O molecules. Figure 28 shows a schematic diagram illustrating the various steps in the reaction when catalyzed by the internal Xe cluster.

6.5. Deposition of Taylor-Made Clusters

Surface deposition of the clusters formed in helium droplets was recently demonstrated in one of our laboratories. The aim was to form polymetallic clusters with between 10 and 1000 atoms on an oxide surface.^[261] Small metal clusters having diameters of 1–5 nm (with 10–1000 atoms) often demonstrate new catalytic properties and can be highly selective in forming only the desired products of chemical

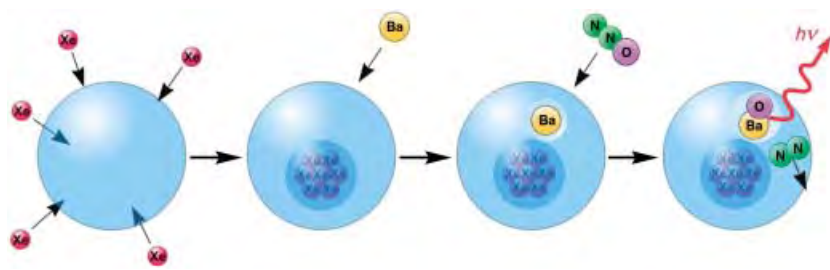


Figure 28. Schematic representation of the sequence of events occurring within the ^4He droplets as they pass through the apparatus.^[161] Ba atoms arriving at the surface are first drawn into the interior by the previously embedded Xe atoms. The heliophilic N_2O molecules penetrate into the interior of the droplet where they are attached to the Ba atom and react on contact forming a highly excited BaO ($A^1\Sigma^+$) molecule with about 2 eV rotational–vibrational energy. This energy is rapidly dissipated by the evaporation of about 3200 He atoms from the droplet leaving the BaO molecule in the $v'=0$ state. Finally, the BaO molecules radiate to the ground state after which the surrounding bubble collapses. The ultimate fate of the product N_2 molecules remains unclear.

reactions or carry out novel low-temperature reactions.^[262–265] In particular, bimetallic clusters, are known to have higher selectivity and are less likely to be poisoned by reaction products than are single-component clusters. The current lack of control of the composition of metal clusters, which is required for many applications, necessitates the development of more versatile experimental methods. Present experimental methods are often based on the laser-ablation technique,^[265] which is difficult to extend towards composite clusters. The helium-droplet technique ensures “soft landing” of the clusters through the transfer of the collision energy to the evaporation of helium atoms. Helium droplets provide a rather large rate of deposition, sufficient for surface catalysis experiments. A disadvantage is a Poisson distribution of the metal clusters formed which has a width of about $N^{1/2}$. Gold clusters and mixed gold–silver clusters of about 20 atoms have been formed in helium droplets and subsequently deposited, providing a flux of about $1.2 \times 10^{11} \text{ atoms cm}^{-2} \text{ s}^{-1}$ ($\Omega = 10^{-4} \text{ sterad}$).^[261]

7. Summary and Outlook

In the six years since our previous Review^[9] a tremendous amount of progress has been made in exploring the many new possibilities for unique types of experiments made accessible by utilizing helium droplets as a spectroscopic matrix. A large number of experiments in the IR region have confirmed the free rotations of molecules in the ultracold, gentle superfluid liquid-helium environment. Theory has made extensive progress in better understanding the reduction in the rotational energy constants, which is especially pronounced in heavy anisotropic molecules.^[42, 191, 192] The computational path integral Monte Carlo technique has now been applied to a large number of these molecules including OCS,^[42, 68, 266] HCN,^[82] SF_6 ,^[42] C_6H_6 (benzene),^[86, 87] and $(\text{HCN})_n$ clusters.^[192] Hopefully, in the near future it will be possible to reliably predict the changes in the rotational constants so that structural information on the bare molecules can be obtained. In the IR region two techniques have now been demonstrated

for accessing the vibrational levels, either by collapsing the rotational spectra inside non-superfluid ^3He droplets or by applying simultaneously a strong electric field in the absorption region. Both techniques also hold great promise for studying large clusters and biomolecules.

The advantages of helium droplets for vibronic spectroscopy in the visible region had already been demonstrated in 1998 for a number of systems. Several large molecules, such as PTCDA ($\text{C}_{24}\text{H}_8\text{O}_6$)^[267] and the phthalocyanines^[213, 229] have now been successfully studied. In addition to extensive information on the vibrational modes of these large molecules, these experiments revealed unexpected fine structures ($\delta\nu \approx 1\text{--}3 \text{ cm}^{-1}$).^[213] Very recently similar fine structures could also be observed in phthalocyanine in highly resolved emission spectra.^[229] These structures are interpreted in terms of bound states of one or several

helium bath atoms attached to the chromophore or in terms of discrete quantum levels of attached helium atoms. These new developments point the way to a wide range of fascinating new experiments, which can be expected to help unravel some of the remaining mysteries concerning the small residual coupling of bath atoms to the chromophores. In connection with both IR and visible spectra an important remaining challenge for theory is the understanding of the small line shifts so that in the future the true line positions of the free molecules can be predicted with precision.

Perhaps the most spectacular new developments over the last six years have come from experiments which make full use of the possibilities to fabricate and create complexes or clusters of desired composition. Such tailor-made clusters have been used for experiments not possible otherwise. Examples, which were discussed, are $\text{OCS}-(\text{H}_2)_n$ or $\text{OCS}-(\text{D}_2)_n$ ($n \approx 1\text{--}17$) which provided, through anomalies in their resolved rotational spectra, the first evidence for superfluidity of pH_2 ,^[148, 191] the formation of chains of polar molecules, such as $(\text{HCN})_n$ ($n = 1\text{--}10$),^[145] the creation of huge metal clusters with up to 2000 atoms which have lead to new insight into electronic shell effects,^[158] or the recent demonstration of the use of helium droplets as disposable shields for the soft landing of metal clusters on surfaces.^[261] The soft landing of tailor-made clusters opens up the possibility of creating novel surface-deposited catalysts. Another especially impressive demonstration is provided by an experiment on a chemiluminescent reaction, where it was possible to steer the reaction between a heliophilic and a heliophobic reactant away from the surface into the interior of the droplets.^[161] In the future there should be no problem to create polymetallic clusters, mixed semiconductor clusters, chains of alkali halides, complexes of metal atoms, or clusters with attached biological molecules. Not to mention the vast number of different complexes of biomolecules which can be synthesized. Photochemistry of such complexes and photosynthesis of new species are other possible areas of future research.

Additional new avenues of future research can be expected based on recently introduced helium-droplet sources. The new pulsed droplet beams are ideally matched to

pulsed lasers.^[120] Thus not only is the sensitivity greatly enhanced but also a broader range of laser light sources become available. Another new source in which liquid helium at $T_0 \leq 4$ K is squirted into vacuum has been shown to produce huge droplets containing more than 10^9 atoms.^[127] These droplets can accommodate much larger molecules or clusters. Moreover, heterogeneous broadening, which is attributed to the effects of the surface, can be greatly reduced and the temperatures of these giant droplets may be controlled.

We hope that we have demonstrated that helium droplets do indeed provide the “ultimate spectroscopic matrix” as proclaimed by our colleagues Giacinto Scoles and Kevin Lehmann in 1998.^[268] Clearly this unique matrix promises to continue to provide many opportunities for innovative applications in many areas of physics and chemistry. Additional information on helium droplet spectroscopy can be found in a recent special issue of the *Journal of Chemical Physics*,^[11–13,45,269] a recent *Physics Today* article^[10] as well as our earlier reviews.^[9,193]

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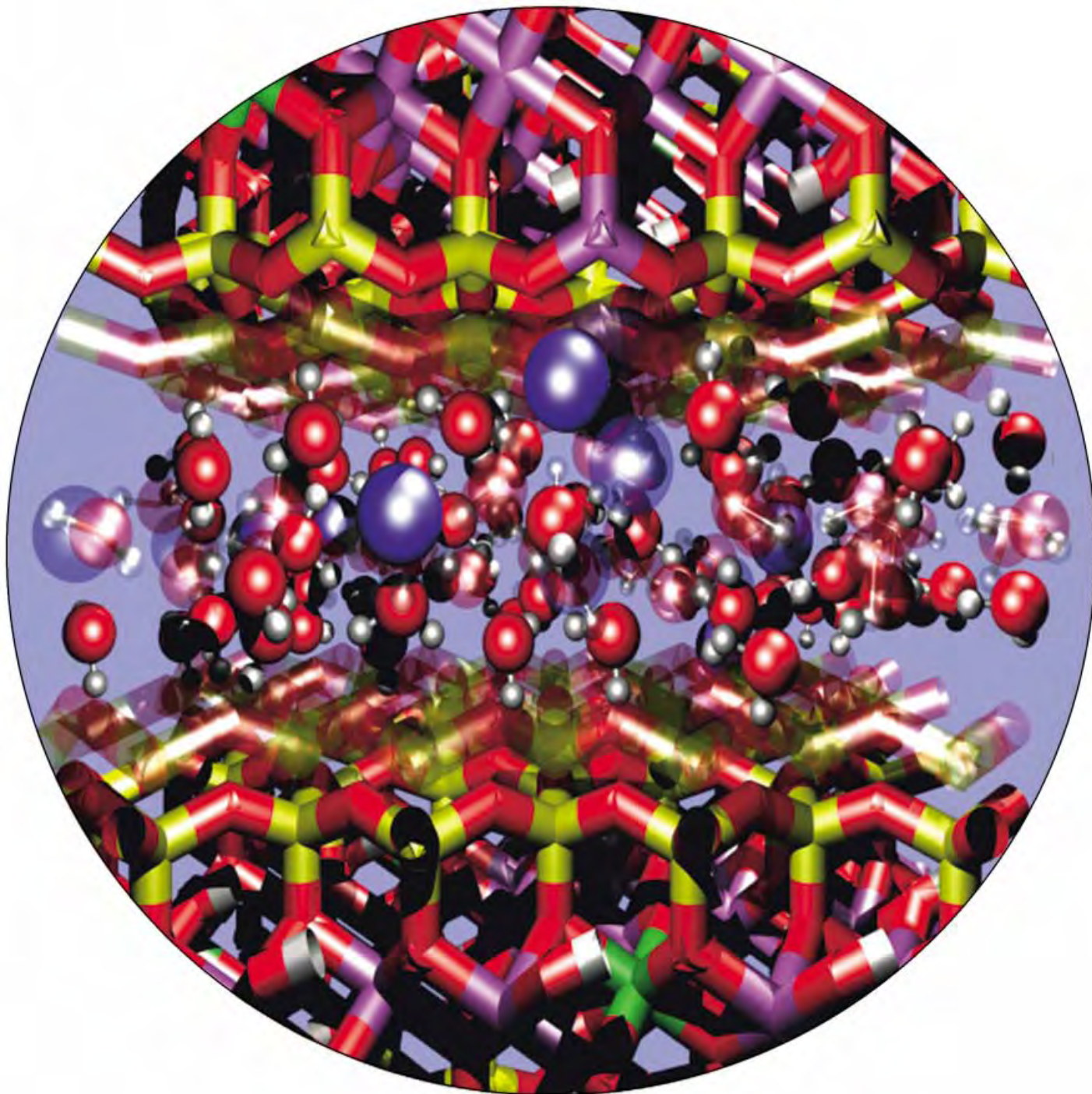
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Communications



Swelling and shrinking in clay minerals, such as the NA-montmorillonite shown, occurs in a stepwise fashion and by different pathways. Through simulations it has been shown that a free-energy barrier separating stable layered hydrates is the cause of this hysteresis. For more information see the Communication by T. J. Tambach et al. on the following page.

A Molecular Mechanism of Hysteresis in Clay Swelling**

Tim J. Tambach,* Peter G. Bolhuis, and Berend Smit

Clay minerals are an important class of materials because they are extensively present in many soils and sediments.^[1] They are also used in many very different applications, such as construction material, additives to mud drilling, or catalysis.^[2] In these applications it is important to control the swelling properties of clay minerals. For example, the extreme swelling capacity of these materials can cause severe difficulties for construction on clay soils.^[3] Depending on their structure, certain clay minerals are capable of swelling as a function of the relative humidity,^[4–9] salt concentration,^[10,11] or temperature.^[12,13] From a fundamental point of view the swelling behavior is only partially understood. Experiments showed that swelling proceeds stepwise, forming layers of water molecules (layered hydrates) between the clay-mineral platelets.^[5–13] Interestingly, this swelling shows hysteresis; the adsorption and swelling proceeds differently from desorption and shrinking.^[4,6,8–10,12–14] In the literature there is little consensus about the molecular origin of this hysteresis.^[15] Explanations are given in terms of structural rearrangements in the clay^[8,16] or changes in interactions between layers upon expansion or contraction.^[13,17] In this work, we use molecular simulation to study the mechanism of Na-montmorillonite swelling hysteresis in detail. In particular, we demonstrate that this hysteresis has a thermodynamic origin and its molecular mechanism is a free-energy barrier separating the transition between stable layered hydrates. Our simulations also predict how swelling hysteresis depends on the external pressure.

Before discussing the mechanism of hysteresis in detail, we should establish that we can reproduce the experimental system sufficiently accurately. In our simulations we use the TIP4P water model and we describe the clay–water and clay–clay interactions with Lennard–Jones and Coulomb interactions. We used clay mineral structures modeling Arizona^[18] and Wyoming^[19] montmorillonite, which have a different layer charge and swelling properties.^[7] To mimic the experimental setup, we allow the number of water molecules to vary in our simulations. For a given clay mineral layer spacing,

we impose the temperature and the chemical potential of water corresponding to a particular water (partial) pressure. Special techniques are essential for these simulations to ensure that we can sample the high-density water phase correctly. Details on the simulation techniques and models can be found in the Supporting Information and in references [18] and [19]. In Table 1 we compare the stable states

Table 1: Comparison of the simulation results with experimental measurements for Wyoming (W) and Arizona (A) montmorillonite.^[a]

	Basal spacing [Å]		Water content [g g ^{−1}]	
	Sim.	Exp.	Sim.	Exp.
1-layer (W)	12.75	12.49 ^[b]	0.122	0.11–0.13 ^[b]
2-layer (W)	15.50	15.55 ^[b]	0.231	0.19 ^[b]
1-layer (A)	12.75	12.5 ^[c]	0.135	–
2-layer (A)	15.00	15.3 ^[c]	0.232	–

[a] The stable one-layer (RH = 25 %) and two-layer (RH = 67 %) states correspond to those basal spacings for which the free energy has a (local) minimum at the given external pressure (1 atm). [b] From Cases et al.^[9] [c] From Chipera et al.^[6]

obtained from our molecular simulations with the experimentally observed stable states for Arizona^[6] and Wyoming^[9] Na-montmorillonite. These results show that our water–clay model can accurately reproduce both the basal spacing as well as the water content. The computed basal spacings are also in agreement with computer simulations.^[14,19,20]

Experimentally, adsorption and desorption experiments can be carried out by changing the temperature or the relative humidity (RH), related to the water chemical potential (μ). Figure 1 shows the free energy as a function of the basal

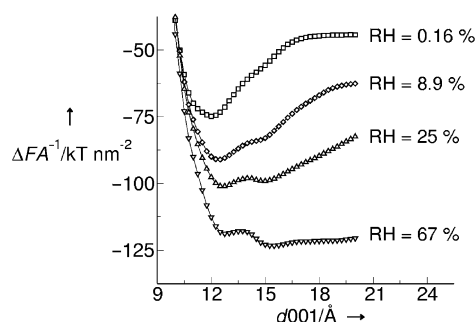


Figure 1. Free energy (ΔF) per unit area of the clay layers (A) of the Wyoming clay–water system as a function of the basal spacing (d_{001}) for various values of the RH; as a reference we used the free energy of the dehydrated clay.

spacing of the clay mineral for various values of the relative humidity. The minima in the free-energy curves are related to the packing of the water molecules and therefore indicate layer formation between the clay-mineral platelets. At low relative humidity the one-layer state has the lowest free energy. If we increase the humidity, we observe that above a particular value of the relative humidity the two-layer state has the lowest free energy. Figure 2 shows the relative free energies of the stable states as a function of the relative humidity or, equivalently, the chemical potential. Without

[*] Dr. T. J. Tambach, Dr. P. G. Bolhuis, Prof. Dr. B. Smit
Department of Chemical Engineering
University of Amsterdam
Nieuwe Achtergracht 166, 1018 WV Amsterdam (The Netherlands)
Fax: (+31) 20-525-5604
E-mail: tambach@science.uva.nl

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

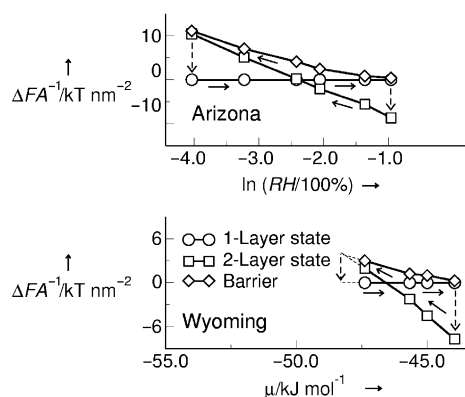


Figure 2. ΔF of the two-layer hydrate and the free-energy barrier separating the stable states, relative to the free energy of the one-layer hydrate. For both Arizona and Wyoming montmorillonite, the free energy is shown per A, as a function of the chemical potential (μ) or, equivalently, the RH. We used the experimental equation of state to convert the chemical potential to the relative humidity. The arrows indicate the hysteresis loop. Note that outside the hysteresis loop there is only one minimum and hence the relative free energies of the two-layer hydrate and the barrier cannot be defined.

hysteresis the system would jump from the one-layer to the two-layer state and vice versa at exactly the point where the two free energies are equal. However, Figure 1 also shows that the two stable states are separated by a maximum in the free energy. To swell or shrink, the system has to overcome the free-energy barrier. For swelling, this barrier is the difference between free energies of the maximum and the one-layer stable state, and for desorption this barrier is the difference between the free energies of the maximum and the two-layer stable state. Figure 2 shows that one has to decrease or increase the relative humidity significantly beyond the point of equal free energies before these barriers disappear.

According to our simulations, the free-energy barrier alone explains the existence of hysteresis in an adsorption/desorption experiment. The free-energy barrier we calculate in our simulation is the barrier of swelling the entire clay system at once. Since this barrier is proportional to the total area of the clay-mineral platelets, the total free-energy barrier should be very large and, in a perfect crystal, the system can only jump if this barrier has (almost) disappeared. This fact is also observed when we simulate a swelling experiment; the jumps in the swelling and shrinking curves correspond to those relative humidity values for which the barrier has disappeared.^[14] Figure 2 also shows that the hysteresis loop depends on the type of clay mineral. Compared to Wyoming, the hysteresis loop for Arizona montmorillonite shifts to lower values of relative humidity.

Our simulations therefore do not support the hypothesis that clay swelling hysteresis is caused by irreversible changes in the clay mineral structure.^[8,16] However, this does not imply that defects or local changes in the structure of the clay mineral have no influence on the hysteresis. In our mechanism defects can induce “nucleation” sites from which the new stable state “grows” into the system and therefore result in a hysteresis loop that is significantly smaller than one would observe in an ideal system.

Relatively little is known about the swelling behavior of clay minerals at high external pressures, representing conditions in sedimentary basins.^[21] We can use our simulations to investigate the effect of external pressure on the hysteresis loop. Figure 3 indicates that one can also observe a hysteresis

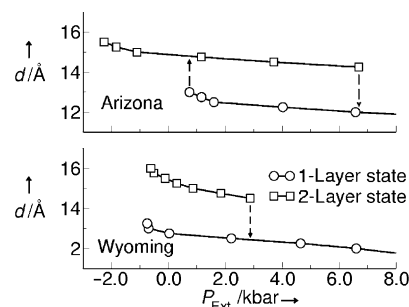


Figure 3. Basal spacing (d_{001}) of the one- and two-layer hydrate as a function of the external pressure (P_{Ext}) for Wyoming and Arizona montmorillonite. In these simulations we impose the temperature ($T = 298 \text{ K}$) and the chemical potential ($\mu = -45.0 \text{ kJ mol}^{-1}$) or, equivalently, the relative humidity ($RH = 67\%$); the external pressure is an additional thermodynamic variable imposed on the system.

loop by changing the external pressure. By increasing the external pressure, one can induce desorption by changing the clay mineral from a two-layer into a one-layer state. Interestingly, because of hysteresis, a subsequent decrease in pressure does not recover the original two-layer hydrate for Wyoming montmorillonite. It would be interesting if experiments can confirm swelling hysteresis as a function of the external pressure.

In summary, our simulations show that clay swelling hysteresis has a thermodynamic origin. The molecular mechanism for this hysteresis is a free-energy barrier, which separates stable layered hydrates. This barrier determines the swelling and shrinking of clay minerals. We also show that applying external pressure on clay minerals can induce hysteresis.

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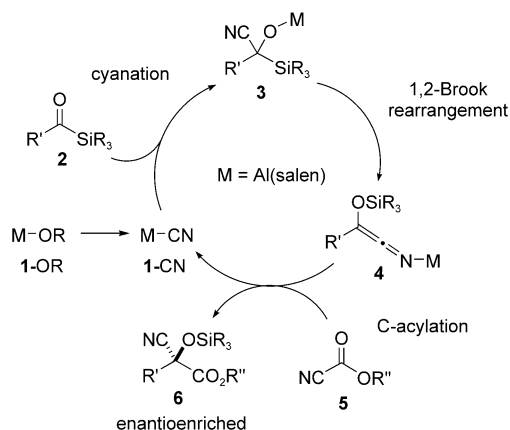
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Catalytic Asymmetric Acylation of (Silyloxy)nitrile Anions**

David A. Nicewicz, Christopher M. Yates, and Jeffrey S. Johnson*

Anions of protected cyanohydrins are useful intermediates in the formation of carbon–carbon bonds.^[1–4] These stabilized carbanions offer considerable potential for asymmetric synthesis, but stereoselective transformations involving them are surprisingly rare.^[5–8] One barrier to enantioselective catalysis is the apparent requirement of one equivalent of a strong base to generate the key carbanionic species. As an alternative, we considered the possibility that protected cyanohydrin anions might be generated by the reaction of chiral metal cyanide complexes with acylsilanes. This hypothesis was predicated on the observation of the facile Brook rearrangement of acylsilanes in the presence of alkali metal cyanides.^[9–12] Here we describe the successful development of asymmetric cyanation/1,2-Brook rearrangement/C-acylation reactions of acylsilanes mediated by (salen)aluminum alkoxides (Scheme 1). These represent, to the best of our knowledge,



Scheme 1. Catalytic asymmetric acylation of (silyloxy)nitrile anions.

[*] D. A. Nicewicz, C. M. Yates, Prof. J. S. Johnson
Department of Chemistry
University of North Carolina, Chapel Hill
Chapel Hill, NC 27599-3290 (USA)
Fax: (+1) 919-962-2388
E-mail: jsj@unc.edu

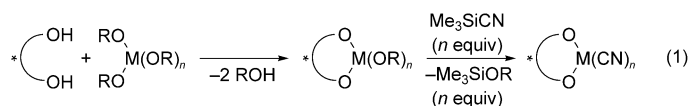
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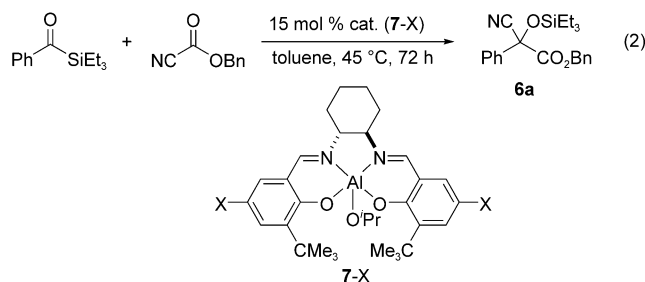
the first catalytic asymmetric reactions of protected cyanohydrin anions.

In an attempt to render the acylation of (silyloxy)nitrile anions^[13] enantioselective, we chose to investigate a variety of chiral metal cyanide sources. We speculated that such complexes might be conveniently prepared according to Equation (1). Ligand exchange between a chiral diol and a



metal alkoxide should result in loss of two equivalents of alcohol and formation of a new, chiral metal alkoxide. Upon treatment with the appropriate amount of Me₃SiCN, chiral metal cyanide complexes were expected.

Evaluation of 96 metal–ligand complexes prepared by the described protocol revealed the Jacobsen (salen)aluminum complexes^[14] as promising candidates for effecting the catalyzed acylation with good enantiocontrol [Eq. (2)].^[15]



Optimization experiments, the results of which are summarized in Table 1, demonstrated that: 1) The reaction may be conducted with the metal alkoxide in the absence of Me₃SiCN as activator; the observation of the mixed carbonate *i*PrO-C(O)OBn by ¹H NMR spectroscopy suggests that the metal alkoxide reacts with benzyl cyanoformate^[16] to form a

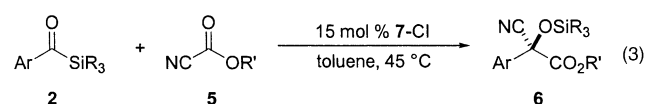
Table 1: Optimization of catalyzed reactions of acylsilanes and cyanoformate esters [Eq. (2)].^[a]

Entry	X in 7-X	<i>c</i> ₀ (PhC(O)SiEt ₃) [M]	<i>ee</i> ^[b] [%]	Conv. [%] ^[c]
1	CMe ₃	2.5	26	> 95
2	CMe ₃	1.5	34	> 95
3	CMe ₃	0.5	46	80
4	NO ₂	2.5	44	> 95
5	NO ₂	1.5	54	90
6	NO ₂	0.5	54	30
7	Cl	2.5	56	> 95
8	Cl	1.5	62	> 95
9	Cl	0.5	67	> 95
10	Cl	0.05	79	> 95
11	OMe	0.05	70	> 95
12	NMe ₂	0.05	46	> 95

[a] PhC(O)SiEt₃ (1.0 equiv), BnO₂CCN (2.0 equiv). [b] Determined by CSP-SFC. [c] Determined by ¹H NMR spectroscopy.

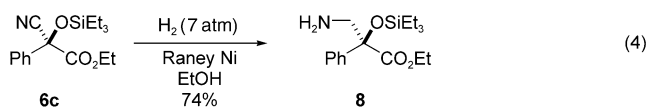
catalytically active (cyanido)aluminum complex (1-OR → 1-CN, Scheme 1). 2) The enantioselectivity is subject to significant effects of remote substituents on the ligand, with a *para*-chlorine substituent delivering optimal results (79 % *ee*, entry 10). 3) The enantioselectivity is strongly dependent on concentration: in dilute solutions, more highly enantioenriched acylated products are formed.

With good levels of enantiocontrol achieved using complex 7-Cl, we investigated the scope of the reaction. Acylsilanes bearing a variety of aryl substituents (Ar) with either triethylsilyl or *tert*-butyldimethylsilyl groups (SiR₃) were treated with either benzyl or ethyl (R') cyanoformate in the presence of catalytic amounts of 7-Cl to give the desired enantioenriched cyano ester products 6 [Eq. (3)]. The results



of these experiments are summarized in Table 2. Varying the silyl group led to a significant reduction in selectivity (entries 1 and 2). Conversely, variation of the cyanoformate made little or no difference on either yield or enantioselectivity (see entries 1 vs. 3 and 7 vs. 8). Electron-donating substituents on the aryl ring of the acylsilane (entries 4 and 6) provided good levels of enantioinduction (up to 82 % *ee*, entry 6), while the enantioenrichment with their electron-withdrawing counterparts (entries 5, 7, 8, and 10) was only moderate (61–64 % *ee*). The only exception was 4-FC₆H₄C(O)SiEt₃ (78 % *ee*, entry 9). Overall, the substrates examined gave moderate to good enantioselectivities with good to excellent yields. An air-stable aluminum oxo complex derived from the *p*-Cl-salen ligand, [(Cl-salen)Al(O)Al(salen-Cl)]^[14c] effected the formation of 6a with identical selectivity (80 % *ee*) as complex 7-Cl (entry 1). Alkyl acylsilanes (MeC(O)SiMe₃, *i*PrC(O)SiEt₃) were unreactive under (salen)Al catalysis, in contrast to catalysis by KCN/[18]crown-6.^[13]

The products of the asymmetric cyanation/Brook rearrangement/C-acylation sequence are fully substituted malonic acid derivatives. Preliminary experiments suggest that the dense functionality may be selectively manipulated: exposure of adduct 6c to Raney Ni/H₂ results in reduction of the nitrile group to afford the protected β-amino-α-hydroxy-α-phenyl acid derivative 8 in 74 % yield [Eq. (4)].



In summary, a new catalytic asymmetric cyanation/1,2-Brook rearrangement/C-acylation reaction of acylsilanes with cyanoformates was developed. Access to chiral (silyloxy)nitrile anions is facilitated by metal cyanide-promoted Brook rearrangement reactions of acylsilanes. The asymmetric acylsilane/cyanoformate coupling provides compounds (6)

Table 2: Catalytic asymmetric cyanation/Brook rearrangement/C-acylation of acylsilanes [Eq. (3)].^[a]

Entry	Ar	SiR ₃	R'	Product	Yield [%] ^[b]	ee [%] ^[c]	
1	Ph	SiEt ₃	Bn		6a	83	79 ^[d]
2	Ph	Si ^t BuMe ₂	Bn		6b	82 ^[e]	64 ^[d]
3	Ph	SiEt ₃	Et		6c	93 ^[f]	77 ^[d,g]
4	4-MeC ₆ H ₄	SiEt ₃	Bn		6d	79	80 ^[h]
5	2-naphthyl	SiEt ₃	Bn		6e	90	62 ^[h]
6	4-MeOC ₆ H ₄	SiEt ₃	Bn		6f	84 ^[i]	82 ^[h]
7	4-ClC ₆ H ₄	SiEt ₃	Bn		6g	87	64 ^[h,j]
8	4-ClC ₆ H ₄	SiEt ₃	Et		6h	87	61 ^[d,g]
9	4-FC ₆ H ₄	SiEt ₃	Bn		6i	81	78 ^[h,j]
10	4-NCC ₆ H ₄	SiEt ₃	Bn		6j	70	64 ^[h]

[a] ArC(O)SiR₃ (1.0 equiv, 0.05 M), NCCO₂R' (2.0 equiv) for 72 h unless otherwise noted. [b] Yield of analytically pure material; average of at least two experiments. [c] Determined by CSP-SFC analysis of the adduct, unless otherwise noted. [d] Absolute configuration determined by correlation to a compound of known configuration. See the Supporting Information for details. [e] 20 mol% catalyst used. [f] *c*₀(**2**) = 0.025 M. [g] Determined by CSP-SFC analysis after reduction of the nitrile and coupling to (*S*)-mandelic acid. See Supporting Information for details. [h] Absolute configuration assigned by analogy. [i] *c*₀(**2**) = 0.1 M. [j] Determined by CSP-SFC analysis after reduction of the nitrile and protection as the *N*-Boc carbamate. See the Supporting Information for details.

that may be considered formally as products of R₃Si–CN addition to α -keto esters, a transformation that has yet to be rendered asymmetric. The interception of intermediates like **4** (Scheme 1) in other stereoselective bond constructions will be the focus of future work.

Experimental Section

Synthesis of 6a (representative procedure): A flame-dried Schlenk tube equipped with a magnetic stir bar was charged with the aluminum complex **7-Cl** (19.4 mg, 0.033 mmol, 0.15 equiv) under Ar. Acylsilane (60 mg, 0.27 mmol, 1.0 equiv), benzyl cyanofornate (88 mg, 0.54 mmol, 2.0 equiv), and toluene (5.4 mL, 0.05 M) were all added to the Schlenk tube under Ar, and the tube was sealed and heated to 45 °C. After 72 h the flask was cooled to 25 °C, the solvent was removed under vacuum, and the crude product was purified by flash chromatography with 95:5 petroleum ether/EtOAc to afford 85.3 mg (83%) of **6a** as a colorless oil in 79% ee as determined by CSP-SFC analysis (CSP = chiral stationary phase, SFC = supercritical fluid chromatography; Chiralpak OD, 0.3% MeOH, 0.5 mL min^{−1}, 10 atm, 40 °C, 240 nm, *t*_{R(major)} = 37.0, *t*_{R(minor)} = 41.5 min). Analytical

data for **6a**: [α]_D²⁵ = +4.2 (*c* = 1.97, CH₂Cl₂); IR (thin film): $\tilde{\nu}$ = 3067, 3035, 2958, 2878, 2245, 1766, 1588, 1451, 1241, 1152, 1003, 835 cm^{−1}; ¹H NMR (400 MHz, CDCl₃): δ = 7.63–7.61 (m, 2H), 7.38–7.35 (m, 3H), 7.29–7.26 (m, 3H), 7.19–7.17 (m, 2H), 5.17 (s, 2H), 0.93 (t, *J* = 7.6 Hz, 9H), 0.70 ppm (q, *J* = 7.6 Hz, 6H); ¹³C NMR (100 MHz, CDCl₃): δ = 167.1, 134.5, 129.9, 128.9, 128.8, 128.7, 128.2, 125.7, 118.3, 75.1, 68.9, 6.8, 5.4 ppm; TLC (95:5 hexanes/EtOAc): *R*_f = 0.35; elemental analysis calcd for C₂₂H₂₇NO₃Si: C 69.25, H 7.13, N 3.67; found: C 69.37, H 7.28, N 3.60.

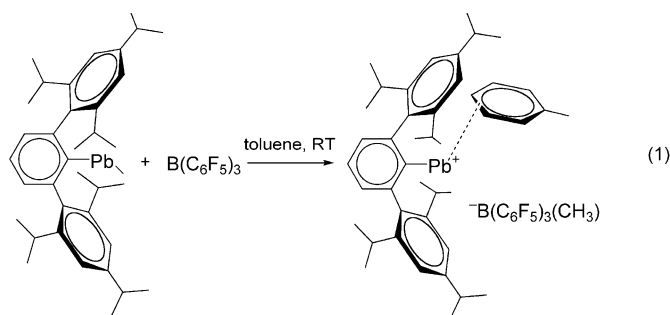
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the metal cation is η^5 bonded to five carbon atoms of the cyclopentadienyl ring.^[1–3,17] Parallel, recent work has shown that bulky terphenyl ligands can stabilize a range of low-coordinate neutral and anionic species with unusual or previously unknown bonding.^[27] We were therefore anxious to test the effectiveness of these useful monodentate ligands in stabilizing cationic Group 14 species with unusually low coordination numbers. We chose to focus initially on the synthesis of a one-coordinate lead cation because $[\text{Ar}^*\text{Pb}]^+$, ($\text{Ar}^* = 2,6\text{-Trip}_2\text{-C}_6\text{H}_3$; $\text{Trip} = \text{C}_6\text{H}_2\text{-2,4,6-}i\text{Pr}_3$), is isoelectronic to the neutral Ar^*Tl compound which has a one-coordinate thallium center.^[28] We now show that weakly solvated, quasi-one-coordinate lead cations can be prepared by the reaction of Ar^*PbMe with $\text{B}(\text{C}_6\text{F}_5)_3$ to give the salt $[\text{Ar}^*\text{Pb-}\eta^2\text{-PhMe}][\text{MeB}(\text{C}_6\text{F}_5)_3]$ (**1**) as shown in Equation (1). Furthermore we



Low-Coordinate Species

Synthesis and Characterization of a Quasi-One-Coordinate Lead Cation**

Shirley Hino, Marcin Brynda, Andrew D. Phillips, and Philip P. Power*

There has been intense, recent interest in the synthesis and properties of heavier Group-14-element (Si–Pb) analogues of carbocations.^[1–26] However, they have proven difficult to isolate in the absence of stabilization by further coordination of the Group 14 element. Many have been intramolecularly stabilized by solvation of the cationic center by either unsaturated moieties,^[25,26] or by N-, O-, S-, or P-centered Lewis base donors.^[8,10,13,14,18,19,21,24] Others have been intermolecularly stabilized through coordination with various solvent molecules.^[5–7,9,12,20] Nonetheless, a number of groups have shown that essentially free, uncomplexed silicon, germanium, and tin species can be isolated and structurally characterized.^[11,15,18,20,21,23] For the heaviest Group 14 elements, tin and lead, work had been focused primarily on derivatized cyclopentadienyl half-sandwich complexes, where

show that the weakly complexed toluene in **1** can be readily displaced by pyridine to afford the salt $[\text{Ar}^*\text{Pb}(\text{py})_2][\text{MeB}(\text{C}_6\text{F}_5)_3]$ (**2**).

The reaction of Ar^*PbMe with 1 equiv of $\text{B}(\text{C}_6\text{F}_5)_3$ ^[29] in toluene yielded an orange oil. Upon recrystallization from hexane, the salt **1** was obtained as red-orange needles. Single-crystal X-ray crystallography^[30] showed that **1** (Figure 1) consisted of an $[\text{Ar}^*\text{Pb}]^+$ ion as well as a $[\text{MeB}(\text{C}_6\text{F}_5)_3]^-$ counterion. There are no close interactions ($\leq 3.964 \text{ \AA}$) between the lead and the anion. The metal is bound to the

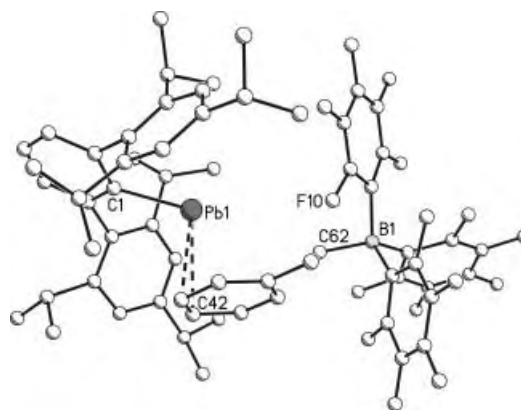


Figure 1. Molecular structure of **1**; hydrogen atoms are not shown for clarity. Selected bond lengths [$\text{\AA}$] and angles [$^\circ$]: Pb1–C1 2.250(7), Pb1–C37 2.832(10), Pb1–C42 2.907(9), Pb–(centroid) 2.827, Pb1–F10A 3.963; C1–Pb1–C37 99.3(2), C1–Pb1–(centroid) 127.0(2), Pb1–C37–C42 79.4(2), Pb1–C37–C38 86.1(2).

[*] S. Hino, Dr. M. Brynda, Dr. A. D. Phillips, Prof. P. P. Power
Department of Chemistry, One Shields Avenue
University of California, Davis, CA 95616 (USA)
Fax: (+1) 530-752-8995
E-mail: pppower@ucdavis.edu

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Ar* ligand through the C1-ipso carbon atom and interacts with the solvent toluene through the C37 and C42 carbon atoms. The Pb–C1 bond length is 2.250(7) Å, and this is marginally shorter than the corresponding bond length (2.272(9) Å) in the precursor Ar*PbMe.^[31] The Pb–toluene centroid distance is 2.827 Å and the C1–Pb1–centroid angle is 127.0°. However, the Pb–C interactions involving the toluene-ring carbon atoms span a wide range from 2.832–3.438 Å, and the closest involve atoms C37 (2.832(10) Å) and C42 (2.907(9) Å), which suggests that the Pb–toluene interaction is best described as being of η^2 type. The closest contact between the cationic lead center and [B(CH₃)(C₆F₅)₃][–] is 3.963(6) Å for Pb1...F10A. This separation is substantially longer than the Pb^{II}–F bond lengths found in complexes such as [PbF(AsF₆)] or [Pb(HF)(AsF₆)₂], which have Pb–F bond lengths in the ranges 2.272(8) to 3.071(9) and 2.48(4) to 3.06(3) Å, respectively.^[32] The ²⁰⁷Pb NMR spectrum of **1** in C₆D₆ displayed a broad signal, far downfield at 8974 ppm.^[32] The ¹⁹F NMR spectrum displayed three distinct signals characteristic of [MeB(C₆F₅)₃][–] due to the *ortho*, *meta*, and *para* F atoms of the C₆F₅ groups. These signals appear at similar shifts to those observed in transition-metal^[33] and main-group-metal^[9] salts that have [MeB(C₆F₅)₃][–] as a counterion. DFT calculations^[34] on the model species [PhPb–C₆H₆]⁺ or [PhPb–PhMe]⁺ afforded an energy of interaction versus aryl ring distance plot (Figure 2), which had a

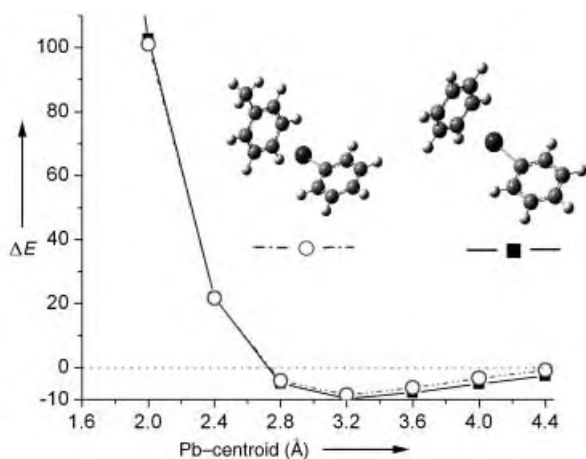


Figure 2. Calculated interaction energies between [PhPb]⁺ and benzene or toluene. Interaction energies (ΔE) are given in kcal mol^{–1}.

minimum near 3.2 Å (compared with $\approx$ 2.85 Å in the crystal structure). The calculated maximum interaction energy (8–9 kcal mol^{–1}) suggests significant dissociation in solution. However, the actual energy may be significantly lower due to the greater bulk of the ligand in **1** and the fact that, at the experimental distance near 2.85 Å, the interaction energy is calculated to be $\approx$ 5 kcal mol^{–1}.

The reaction of **1** in toluene at 25 °C with 2 equiv of pyridine [Eq. (2)] results in an immediate color change from orange to pale yellow. X-ray quality crystals of **2** were isolated after recrystallization from hexane.^[30] The structure of **2** (Figure 3) consists of well-separated cations and anions. The lead center in the cation is bound to Ar* and two pyridine

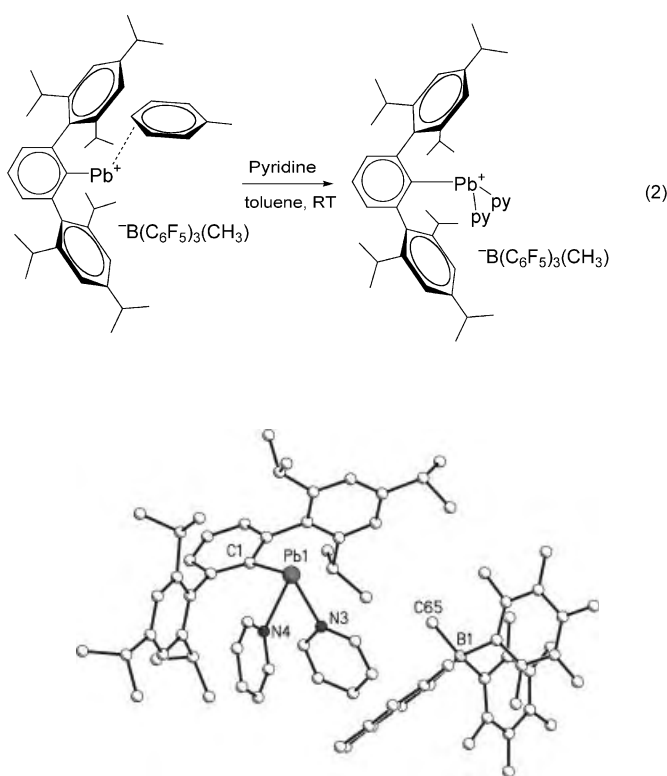


Figure 3. Molecular structure of **2**; hydrogen atoms are not shown for clarity. Selected bond lengths [Å] and angles [°]: C1–Pb1 2.313(5), Pb1–N3 2.409(5), Pb1–N4 2.512(5); C1–Pb1–N3 90.39(2), C1–Pb1–N4 106.67(2), N3–Pb1–N4 80.10(2).

groups to produce a pyramidal metal coordination. Clearly, pyridine has readily displaced the coordinated toluene to afford the salt [Ar*Pb(py)₂][MeB(C₆F₅)₃] (**2**). The Pb–C bond length is lengthened to 2.313(5) Å and the two Pb–N separations are 2.409(5) and 2.512(5) Å. The pyramidal coordination geometry at the Pb center is indicated by the angles C1–Pb1–N3, C1–Pb1–N4, and N3–Pb1–N4 (90.39(2), 106.67(2), and 80.10(2)°, respectively; sum of angles = 277.16°). The ²⁰⁷Pb NMR spectrum of **2** in C₆D₆ displayed a signal at 4764 ppm—about 4000 ppm upfield of **1**.

The long Pb–C(toluene) bond lengths and low-field ²⁰⁷Pb NMR chemical shift observed for **1** support the view that it is primarily a one-coordinate [Ar*Pb]⁺ ion that is loosely coordinated to a solvent toluene molecule. The Pb–C(η^2 -PhMe) distances may be compared with the Pb–C separations found in the salts [(η^5 -C₅Me₅)Pb]⁺X[–] (X = BF₄[–] or CF₃SO₃[–]),^[1] which range from 2.512 to 2.598 Å. The Pb–centroid length for [(η^5 -C₅Me₅)Pb]⁺ was found to be near 2.27 Å.^[17] Thus, the Pb–centroid separation in **1** is over 0.5 Å longer than those in the above salts.^[17,35] It is also notable that within the toluene ring in **1**, the C–C bonds average 1.38 Å, and the crucial C37–C42 (1.370(2) Å) bond is not elongated relative to free toluene.^[36] The downfield ²⁰⁷Pb NMR chemical shift of 8974 ppm may be compared to the –4961 and –5041 ppm observed for [(η^5 -C₅Me₅)Pb]CF₃SO₃ and [(η^5 -C₅Me₅)Pb]BF₄, respectively.^[1] The chemical shift for compound **1** is thus more than 13 500 ppm downfield of these [(η^5 -C₅Me₅)Pb]⁺ salts. This very large difference is consistent with

a lower effective coordination number in **1** and weak coordination of PhMe to lead. It is notable that the ^{207}Pb NMR chemical shift of the two-coordinate precursor Ar^*PbMe (7420 ppm)^[31] also lies upfield of that of **1**, which further supports the weak η^2 -solvation of Pb by toluene.

The $[\text{Ar}^*\text{Pb}]^+$ fragment is isoelectronic to $[\text{Ar}^*\text{Tl}]$, the structure of which has been already described.^[28] In $[\text{Ar}^*\text{Tl}]$, the thallium center is monocoordinate and the Tl–C(ipso) bond length is 2.34(1) Å,^[28] which is ≈ 0.09 Å longer than the Pb–C bond in **1**. The shorter value for Pb–C1 is probably due to the cationic character of $[\text{Ar}^*\text{Pb}]^+$; however, since Pb and Tl have similar covalent radii,^[37] the lack of any increase in the Pb–C(ipso) distance upon interaction with toluene is consistent with weak solvation. In $[\text{Ar}^*\text{Pb}(\text{py})_2]^+$, the large size of the aryl substituent is reflected in the over 15° difference in bond angles of N3–Pb1–C(ipso) (90.39(2)°) and N4–Pb1–C(ipso) (106.67(2)°) and the very narrow N3–Pb1–N4 angle (80.10(2)°). The Pb–C(ipso) bond distance increases to 2.313(5) Å (compared with 2.251(7) Å in **1**), which is consistent with the higher lead coordination number. The Pb–N bond lengths (2.409(5) and 2.512(5) Å) are similar to those seen in $[\text{Pb}(\text{Br})\text{Ar}^*]\cdot\text{py}$ (2.502(4) Å),^[31] and are much shorter than those found in the PbBr_2 complexes $[\{(4\text{-MeH}_4\text{C}_5\text{N})_2\cdot\text{PbBr}_2\}_n]$ ^[38] and $[\{(3\text{-MeH}_4\text{C}_5\text{N})_2\cdot\text{PbBr}_2\}_n]$ (both 2.60(2) Å),^[38] and in the plumbocene–pyridine complexes $[\text{Pb}(\eta^5\text{-C}_5\text{H}_5)_2]\cdot\text{TMEDA}$ and $[\text{Pb}(\eta^5\text{-C}_5\text{H}_5)_2]\cdot 4,4'\text{-Me}_2\text{bpy}$ (Pb–N 2.702–2.879 Å; bpy = bipyridyl),^[39] which have higher effective coordination numbers at their Pb centers. The ^{207}Pb NMR chemical shift of **2** is at ≈ 4000 ppm higher field compared to **1**, which is consistent with an increase in coordination number from one to three.

In summary, the plumbyl cation **1**, which contains a quasi-one-coordinate lead center, was obtained through methanide-ion capture using $\text{B}(\text{C}_6\text{F}_5)_3$. The structural and spectroscopic data show that it is stabilized by a weak interaction with a toluene molecule that can be readily displaced by pyridine.

Experimental Section

All manipulations were carried out under anaerobic and anhydrous conditions.

1: The compound Ar^*PbMe ^[31] (0.704 g, 1.0 mmol) was dissolved in toluene (20 mL) and added dropwise to a solution of $\text{B}(\text{C}_6\text{F}_5)_3$ (0.518 g, 1.0 mmol) in toluene (20 mL) at $\approx 0^\circ\text{C}$ with constant stirring. The reaction mixture, which was initially red, became orange after about 4 h. The reaction was allowed to warm to room temperature and stirred overnight. The toluene was removed under reduced pressure and the orange oil was extracted with hexane (50 mL). After filtering through celite, the volume of the orange solution was reduced to initiate crystallization and stored in a freezer at -20°C for 2 days to give product **1** as orange crystals (0.682 g, 52%); m.p. 228–236°C; ^1H NMR (C_6D_6 , 300 K): $\delta = 0.632$ (s, 3 H, B–CH₃), 1.019 (d, 12 H, *o*-CH(CH₃)₂, $^3J_{\text{HH}} = 6.6$ Hz), 1.059 (d, 12 H, *o*-CH(CH₃)₂, $^3J_{\text{HH}} = 6.8$ Hz), 1.211 (d, 12 H, *p*-CH(CH₃)₂, $^3J_{\text{HH}} = 7.2$ Hz), 2.104 (s, 3 H, *C*₆H₅–CH₃), 2.753 (sept, *p*-CH(CH₃)₂, $^3J_{\text{HH}} = 6.8$ Hz), 2.913 (sept, *o*-CH(CH₃)₂, $^3J_{\text{HH}} = 6.8$ Hz), 6.997 (t, 1 H, *p*-C₆H₅–CH₃, $^3J_{\text{HH}} = 7.5$ Hz), $\delta = 7.017$ (d, 2 H, *o*-C₆H₅–CH₃, $^3J_{\text{HH}} = 7.4$ Hz), 7.107 (t, 2 H, *m*-C₆H₅–CH₃, $^3J_{\text{HH}} = 7.5$ Hz), 7.263 (s, 4 H, *m*-Trip), 7.911 (d, 2 H, *m*-C₆H₃, $^3J_{\text{HH}} = 7.2$ Hz), 8.508 ppm (t, 1 H, *p*-C₆H₃, $^3J_{\text{HH}} = 7.6$ Hz); $^{13}\text{C}\{^1\text{H}\}$ NMR (C_6D_6 , 300 K): $\delta = 14.29$ ($[\text{H}_3\text{CB}(\text{C}_6\text{F}_5)_3]^+$, CH₃), 21.36 (CH₃–C₆H₅), 23.69 (*o*-CH(CH₃)₂), 23.85 (*o*-CH(CH₃)₂), 24.92 (*p*-CH(CH₃)₂), 30.53

(*o*-CH(CH₃)₂), 34.65 (*p*-CH(CH₃)₂), 122.72 (*m*-Trip), 125.53 (*p*-C₆H₅–CH₃), 125.63 (*p*-C₆H₃), 128.48 (*m*-C₆H₅–CH₃), 129.14 ($[\text{H}_3\text{CB}(\text{C}_6\text{F}_5)_3]^+$, ipso-C), 129.42 (*o*-C₆H₅–CH₃), 133.89 (ipso-Trip), 138.29 ($[\text{H}_3\text{CB}(\text{C}_6\text{F}_5)_3]^+$, *m*-C, *p*-C), 142.24 (*m*-C₆H₃), 145.18 (*o*-Trip), 148.33 ($[\text{H}_3\text{CB}(\text{C}_6\text{F}_5)_3]^+$, *o*-C), 149.54 (*p*-C₆H₃), 152.34 (*o*-C₆H₃), 356.57 ppm (ipso-C₆H₃); $^{207}\text{Pb}\{^1\text{H}\}$ NMR (C_6D_6 , 300 K): $\delta = 8974$ ppm; $^{11}\text{B}\{^1\text{H}\}$ NMR (C_6D_6 , 300 K): $\delta = -11.62$ ppm; $^{19}\text{F}\{^1\text{H}\}$ NMR (C_6D_6 , 300 K): $\delta = -104.45$ (t, 6 F, $^3J_{\text{FF}} = 24.4$ Hz), -102.13 (s br, 3 F), -69.27 ppm (d, 6 F, $^3J_{\text{FF}} = 20.8$ Hz).

2: **1** (1.40 g, 1.07 mmol), was dissolved in toluene (25 mL). Pyridine (0.2 mL, 2.48 mmol) was added to the orange solution while stirring. The reaction mixture, which immediately became pale yellow on the addition of pyridine, was then stirred overnight. The volatile solvent was then removed and the residue was extracted with hexanes (50 mL). The solution was filtered through celite and concentrated to induce crystallization (15 mL) and stored in a freezer at -20°C for 2 days to afford **2** as colorless crystals (0.615 g, 40%); m.p. 210–215°C; ^1H NMR (C_6D_6 , 300 K): $\delta = 0.529$ (s, 3 H, B–CH₃), 0.856 (d, 12 H, *p*-CH(CH₃)₂, $^3J_{\text{HH}} = 6.8$ Hz); 1.030 (d, 12 H, *o*-CH(CH₃)₂, $^3J_{\text{HH}} = 6.4$ Hz), 1.169 (d, 12 H, *o*-CH(CH₃)₂, $^3J_{\text{HH}} = 7.2$ Hz), 2.696 (sept, *p*-CH(CH₃)₂, $^3J_{\text{HH}} = 6.8$ Hz), 2.887 (sept, *o*-CH(CH₃)₂, $^3J_{\text{HH}} = 7.28$ Hz), 6.584 (t, 4 H, *m*-C₅H₅N, $^3J_{\text{HH}} = 6.4$ Hz), 6.969 (s, 4 H, *m*-Trip), 7.228 (t, 1 H, *p*-C₅H₅N, $^3J_{\text{HH}} = 7.6$ Hz), 7.249 (t, 2 H, *p*-C₅H₅N, $^3J_{\text{HH}} = 7.6$ Hz), 7.452 (*m*-C₆H₃, $^3J_{\text{HH}} = 7.2$ Hz), 7.818 ppm (d, 4 H, *o*-C₅H₅N, $^3J_{\text{HH}} = 6.6$ Hz); $^{13}\text{C}\{^1\text{H}\}$ NMR (C_6D_6 , 300 K): $\delta = 14.319$ (CH₃–B), 24.198 (*o*-CH(CH₃)₂), 24.424 (*o*-CH(CH₃)₂), 26.381 (*p*-CH(CH₃)₂), 30.819 (*o*-CH(CH₃)₂), 34.862 (*p*-CH(CH₃)₂), 121.118 (*m*-Trip), 124.887 (*m*-C₅H₅N), 125.693 (*p*-C₆H₃), 131.958 ($[\text{H}_3\text{CB}(\text{C}_6\text{F}_5)_3]^-$, ipso-C), 135.448 (*o*-C₅H₅N), 136.770 (ipso-Trip), 137.35 ($[\text{H}_3\text{CB}(\text{C}_6\text{F}_5)_3]^-$, *p*-C), 137.56 ($[\text{H}_3\text{CB}(\text{C}_6\text{F}_5)_3]^-$, *m*-C), 141.235 (*m*-C₆H₃), 145.605 (*o*-Trip), 146.823 (*p*-Trip), 147.475 ($[\text{H}_3\text{CB}(\text{C}_6\text{F}_5)_3]^-$, *o*-C), 147.839 (*o*-C₆H₃), 149.253 ppm (*p*-C₅H₅N); ipso-C₆H₃ not observed; $^{207}\text{Pb}\{^1\text{H}\}$ NMR (C_6D_6 , 300 K): $\delta = 4764$ ppm; $^{11}\text{B}\{^1\text{H}\}$ NMR (C_6D_6 , 300 K): $\delta = -13.62$ ppm; $^{19}\text{F}\{^1\text{H}\}$ NMR (C_6D_6 , 300 K): $\delta = -105.65$ (t, 6 F, $^3J_{\text{FF}} = 24.4$ Hz), -103.34 (s br, 3 F), -70.21 ppm (d, 6 F, $^3J_{\text{FF}} = 20.8$ Hz).

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Keywords: cations · Group 14 elements · lead · structural elucidation · terphenyl ligands

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- $$[\text{R-Pb}] + [\text{toluene}] \xrightarrow{\Delta E} [\text{R-Pb-toluene}]^+ \quad (3)$$
- energy (–9.86 kcal mol⁻¹) is relatively high compared to what we expected for an interaction of this kind. We then performed an additional set of calculations on the hypothetical phenyl–lead complexes with benzene and toluene (Figure 2). A possible explanation for these rather high energy values might be related to the poor quality of the description of the relativistic effects in this particular system in terms of the ECP approximation. Further DFT calculations using basis sets optimized for the use in the zeroth-order regular approximated (ZORA) relativistic equation, such as implemented in the Amsterdam Density Function (ADF) program, are currently in progress. a) W. Kuechle, K. Dolg, H. Stoll, H. Preuss, *Mol. Phys.* **1991**, *74*, 1245. b) Gaussian 03 (Revision B.04), M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, J. A. Pople, Gaussian, Inc., Pittsburgh, PA, **2003**. c) MOLEKEL 4.3, P. Flükiger, H. P. Lüthi, S. Portmann, J. Weber, Swiss Center for Scientific Computing, Manno (Switzerland), **2000–2002**.
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Natural Product Synthesis

Total Synthesis of (–)-Galanthamine by Remote Asymmetric Induction

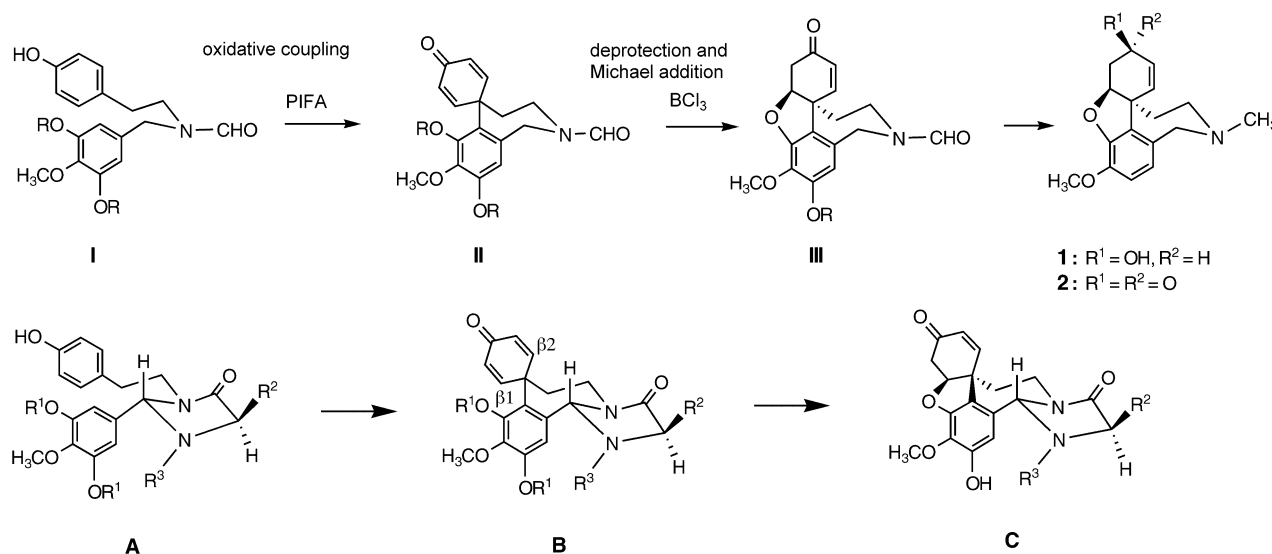
Sumiaki Kodama, Yoshio Hamashima,
Kiyoharu Nishide, and Manabu Node*

(–)-Galanthamine (**1**),^[1] an alkaloid isolated from the *Amaryllidaceae* family, has attracted the attention of synthetic chemists because of its use as a selective acetylcholinesterase inhibitor in the clinical treatment of Alzheimer's disease.^[2] Its limited supply and the high costs associated with isolating this compound from natural sources^[3] are two compelling reasons for the need for an efficient synthesis. (–)-Galanthamine (**1**) has usually been prepared industrially through the crystallization-induced asymmetric transformation^[3,4] of the intermediate (±)-narwedine (**2**).^[5] As the latter compound is highly allergenic, chemists must be extremely careful when working with it, which underscores the necessity for safer and more efficient methods for the synthesis of (–)-**1**. In addition to the classical and biomimetic phenolic oxidative coupling^[4a,b,6] in the presence of metal oxidants, an asymmetric allylic alkylation and intramolecular Heck reaction sequence has also been used in an excellent synthetic strategy toward (–)-**1**.^[7] We recently improved the phenolic oxidative coupling of norbelladine-type derivatives **I**, which contain a pyrogallol moiety, and applied it in a synthesis of (±)-**2** and (±)-**1**.^[8] Herein we report a new asymmetric synthesis of (–)-galanth-

amine (**1**), through remote asymmetric induction with a chiral imidazolidinone auxiliary derived from phenylalanine.

Our strategy for the asymmetric synthesis of (–)-**1** involved the modification of our previous racemic synthesis^[8] by using phenyliodine(III) bis(trifluoroacetate) (PIFA) as the “clean” oxidant, as shown in Scheme 1. The key step in this asymmetric version of the synthesis is the intramolecular Michael addition of the coupling product **II** to give the cyclic ether **III**. This step requires the preferential attack by the phenolic oxygen atom on one olefin of the symmetrical dienone moiety. Therefore, we designed conformational restriction into the seven-membered ring in **II** by introducing an α -amino acid at the benzylic position, α to the N atom in the ring. More precisely, the conformation of the seven-membered ring in the coupling product **B** of the chiral imidazolidinone **A** would be restricted by a fused five-membered ring. It could be reasoned that the intramolecular Michael addition of **B** would then proceed diastereoselectively to afford the cyclic ether **C** by this new type of remote asymmetric induction. Through semiempirical PM3 calculations based on the Monte Carlo techniques for conformer analysis,^[9] we calculated the most stable conformer of the intermediate **B** ($R^1 = H$, $R^2 = Bn$, $R^3 = COCF_3$). The distance between the phenolic O atom and C β 1 (2.61 Å) is 0.55 Å shorter than that between the O atom and C β 2 (3.15 Å). The intramolecular Michael addition should be highly regio- and diastereoselective, thus leading to the desired enantiomer corresponding to (–)-galanthamine.

For the substrate in the coupling reaction, we chose D-phenylalanine as the chiral auxiliary, as its bulky substituent

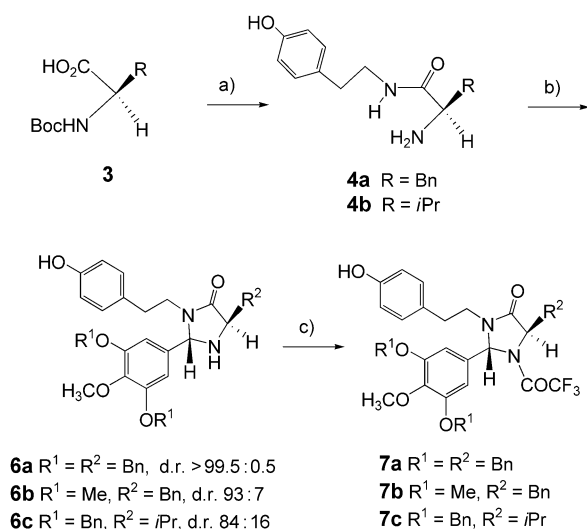


Scheme 1. Strategy for the synthesis of (–)-galanthamine.

[*] S. Kodama, Dr. Y. Hamashima, Prof. Dr. K. Nishide, Prof. Dr. M. Node
Department of Pharmaceutical Manufacturing Chemistry
Kyoto Pharmaceutical University
Misasagi, Yamashina, Kyoto 607-8414 (Japan)
Fax: (+81) 75-595-4775
E-mail: node@mb.kyoto-phu.ac.jp

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under <http://www.angewandte.org> or from the author.

($R^2 = \text{benzyl}$) was expected to be effective in the diastereoselective formation of the chiral imidazolidinone corresponding to **A**. (*R*)-*N*-Boc-D-phenylalanine (**3**, $R = Bn$) was condensed with tyramine in the presence of the dehydrating agent *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl). Removal of the *N*-Boc group then gave the amine **4a** in 92% yield (over two steps; Scheme 2).



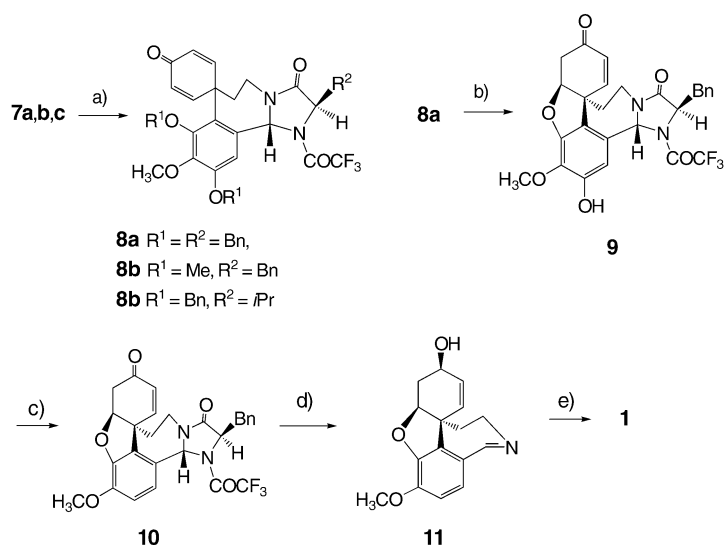
Scheme 2. Synthesis of the oxidative-coupling precursors **7**. Reagents and conditions: a) 1. tyramine (1.2 equiv), EDC·HCl (1.2 equiv), HOBT (0.1 equiv), THF, room temperature, 2 h, 95%; 2. MsOH (2 equiv), MeOH, 40°C, 3 h, 97%; b) for **6a** and **6c**: 1. **5a**, dioxane, room temperature, 1 day; 2. HCl (4 M)/dioxane, room temperature, 2 days, 80% from **4a** and 66% from **4b**, respectively; for **6b**: 1. **5b**, dioxane, room temperature, 12 h; 2. HCl (4 M)/dioxane, room temperature, 12 h, 43% from **4a**; c) (CF₃CO)₂O (2.4 equiv), pyridine, 0°C, 15 min, 94% (**7a**). Boc = *tert*-butoxycarbonyl, HOBT = 1-hydroxy-1*H*-benzotriazole, Ms = methanesulfonyl.

Upon treatment with the pyrogallol-type aldehyde 3,5-dibenzyloxy-4-methoxybenzaldehyde (**5a**), the amine **4a** was transformed into the desired imine intermediate, which underwent cyclization in the presence of 4 M hydrochloric acid to the imidazolidinone **6a** in 80% yield. Exclusive formation of **6a** (R¹ = Bn) was observed in this cyclization, whereas the diastereoselectivity observed in the reaction of **4a** with 3,4,5-trimethoxybenzaldehyde (**5b**) to form **6b** (R¹ = Me) was just 86%. The relative configuration of the two substituents in the imidazolidinone ring was confirmed to be *trans* by NOE experiments.^[10] In the case of the cyclization with **4b**, in which D-valine is incorporated as the chiral auxiliary instead of D-phenylalanine, the desired product **6c** was obtained as a diastereomeric mixture (5.4:1). Phenylalanine was selected as a chiral auxiliary based on the results of these three cyclizations. The secondary amino group in the imidazolidinone ring of **6a** was protected with a trifluoroacetyl group to give the precursor **7a** to the oxidative coupling in high yield. For comparison of the coupling reactions, the imidazolidinone derivatives **7b** and **7c** were also prepared.

The phenolic oxidative coupling of **7a–c** with PIFA in trifluoroethanol resulted in moderate yields (41–61%) of **8a–b** (Scheme 3). In these coupling reactions the yields were lower than we had anticipated based on our earlier studies.^[8] However, **8a** was produced from **7a** in a higher yield (61%) than any reported previously for such a reaction of a norbelladine-type compound.^[4,6] Debenzylation of **8a** with boron trichloride afforded the cyclic ether **9** in 95% yield.

During the debenzylation, an intramolecular Michael addition proceeded smoothly to give the single diastereomer **9**. The stereochemistry of the product **9** was consistent with that predicted by calculation, as confirmed below.

The remaining phenolic hydroxy group in **9** was converted into the triflate and then subjected to palladium(0)-catalyzed reduction with formic acid^[11] to give **10** in 83% yield, as shown in Scheme 3. The stereoselective reduction of the enone **10** with L-selectride, followed by alkaline hydrolysis of the chiral imidazolidinone auxiliary, afforded the imine **11**. Reduction of the imine with sodium borohydride, and *N*-methylation of the product via the corresponding *N*-formamide, completed the asymmetric total synthesis of (–)-galanthamine (**1**). The specific rotation of the synthetic (–)-**1** ([α]_D²⁴ = –121.7 (*c* = 0.30, EtOH)) was consistent with the reported value.^[12] The optical purity of the synthetic (–)-**1** (100% *ee*) was confirmed by HPLC analysis on a chiral phase.^[13]

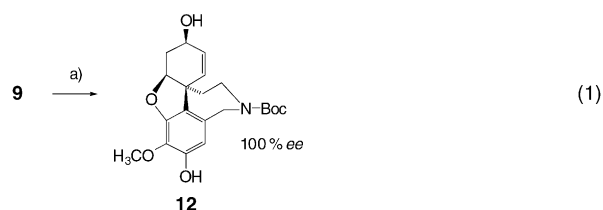


Scheme 3. Synthesis of (–)-galanthamine. Reagents and conditions: a) PIFA (1.1 equiv), CF₃CH₂OH, (for **8a**: –40°C, 10 min, 61%; for **8b**: –40°C, 1.5 h, 41%; for **8c**: –40°C, 15 min, 45%); b) BCl₃ (3.0 equiv), CH₂Cl₂, –78°C, 2 days, 95%; c) 1. Tf₂O (2.4 equiv), pyridine, 0°C, 2.5 h, 83%; 2. Pd(OAc)₂ (0.2 equiv), PPh₃ (0.4 equiv), Et₃N (15 equiv), HCO₂H (10 equiv), DMF, 60°C, 3 days, 100%; d) 1. L-selectride (2.4 equiv), THF, –78°C, 3 h, 78%; 2. KOH (10%), Bu₄NBr (0.5 equiv), EtOH, 80°C, 1 day, 96%; e) 1. NaBH₄ (1.2 equiv), MeOH, 0°C, 5 h; 2. HCO₂Et, 60°C, 2 days, 100% (2 steps); 3. LiAlH₄, THF, 7 h, 94%. DMF = *N,N*-dimethylformamide, Tf = trifluoromethanesulfonyl.

In conclusion, we have described a new and efficient total synthesis of (–)-galanthamine (**1**), which was carried out in 14 steps and 23% overall yield from readily available (*R*)-*N*-Boc-D-phenylalanine. A chiral phenylalanine auxiliary regulated the formation of **6a** as a single diastereomer, then controlled the regio- and stereochemistry of the intramolecular Michael addition of the phenol moiety to the spirodienone in **8a** (R¹ = H) in a novel remote asymmetric induction. This work has provided a straight-forward synthetic route to (–)-galanthamine (**1**) that does not involve the highly allergenic intermediate narwedine (**2**).

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Keywords: alkaloids · asymmetric synthesis · Michael addition · natural products · total synthesis



Reagents and conditions: a) 1. L-selectride (2.0 equiv), THF, -78°C , 1 day, 92%; 2. NaOH (10%), room temperature, 7.5 days, 83%; 3. NaBH_4 (4.0 equiv), MeOH, room temperature, 4 h; 4. $(\text{Boc})_2\text{O}$ (3.0 equiv), THF, room temperature, 2 h, 58% (2 steps).

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- [13] The optical purity of **12**, derived from **9** [Eq (1)], was measured by HPLC on a chiral stationary phase (Daicel CHIRALCEL OD, iPrOH/hexane (9:1), 1.0 mL min⁻¹); (–)-**12**: $R_{\text{t}} = 50.2$ min, (+)-**12**: $R_{\text{t}} = 74.0$ min.

Molecular Devices

A Lockable Light-Driven Molecular Shuttle with a Fluorescent Signal**

Qiao-Chun Wang, Da-Hui Qu, Jun Ren,
Kongchang Chen, and He Tian*

Rotaxanes have received considerable interest in recent years because of their potential applications in nanostructured functional materials and molecular devices.^[1–5] Deeper insight into biological molecular motors have stimulated a surge of activity towards the construction of artificial molecular machines, especially molecular shuttles, which offer possibility of long-range translational motion of a threaded macrocyclic “shuttle” along a “rail track”. Such a supramolecular system could be also used to store information if the shuttling motion (output) is controlled by proper external stimulus (input).^[6] The use of photons as input (light-driven shuttling) is now under prominent consideration because excitation with lasers can lead to a fast response and can function in a small space without producing any by-products.^[7] Temperature change (entropy-driven shuttling) is another clean input because no chemical reaction and no covalent structural changes are involved.^[8] However, recording of the changes by ¹H NMR, absorption, and circular dichroism spectroscopies are the usual methods used to monitor the coconformational

[*] Dr. Q.-C. Wang, D.-H. Qu, J. Ren, Prof. K. Chen, Prof. Dr. H. Tian
Lab for Advanced Materials and
Institute of Fine Chemicals
East China University of Science & Technology
Shanghai 200237 (P. R. China)
Fax: (+ 86) 21-6425-2288
E-mail: tianhe@ecust.edu.cn

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change of rotaxanes, and this might not be convenient in future applications because the difficulty in transforming these spectral signal changes into the easily detected output signals. In contrast, the use of changes in fluorescence as an output signal is preferable because the signal enables easy remote reading and is inexpensive. However, reports based on rotaxanes that can be induced to switch between different fluorescent states (output) in response to clean inputs are rare.

Herein, we report an example of [2]rotaxane α -CD-NPSI (Figure 1; CD is cyclodextrin), in which the hydrogen bonding

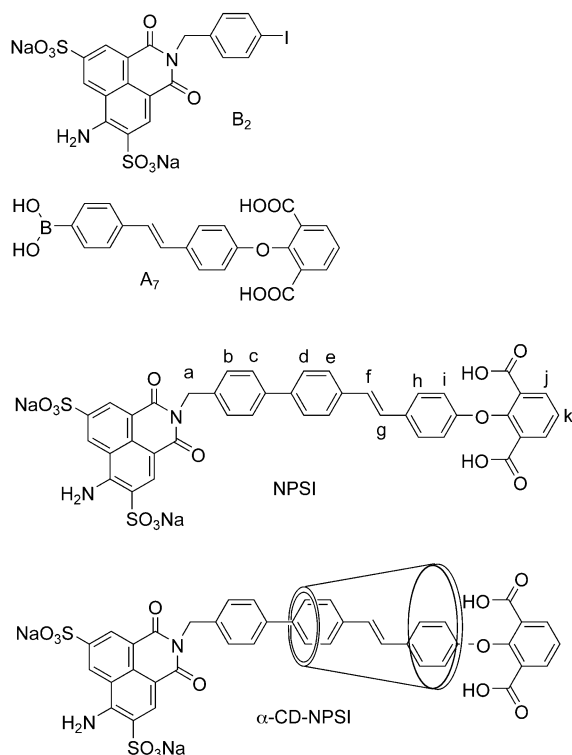


Figure 1. The chemical structures of B₂, A₇, NPSI and [2]rotaxane α -CD-NPSI.

between the α -CD macrocycle and the isophthalic acid unit stops the shuttling motion of the α -CD macrocycle between two binding sites. The disruption of the hydrogen bonding by adding a base enables shuttling, which is exclusively controlled by light energy without the formation of by-products, fully reversible, and accompanied by an obvious change in the intensity of fluorescence. This molecular machine can be a switch, and features the convenient use of optical input and easy reading of the optical output.

α -CD-NPSI consists of an α -CD macrocycle locked into a thread, which has two binding sites—a biphenyl (P) site and a stilbene (S) site—linked covalently, and trapped mechanically by two stoppers: 4-amino-3,6-disulfonic-1,8-naphthalimide disodium salt (N) unit and a isophthalic acid (I) unit as shown in Figure 1. It was reported that [2]rotaxanes can be prepared by the Pd-catalyzed Suzuki coupling in the presence of CD.^[4a,b] We treated A₇ with B₂ in an alkaline aqueous α -CD solution, with a Pd(OAc)₂ catalyst, in the mole ratio A₇:B₂: α -

CD: Pd of 1:1.2:1.5:0.08, and we isolated the rotaxane α -CD-NPSI in 7.6 % yield by chromatography (silica gel, 1:2:5 acetic acid/*n*-butanol/water as the eluent). The “dumbbell” NPSI was also synthesized by using the same conditions as those for the synthesis of α -CD-NPSI but in the absence of α -CD (yield 37 %). The chemical structure of the rotaxane was confirmed by ¹H NMR and ¹H ROESY NMR spectroscopies, and TOF-MS. The NOEs spectra shown in Figure 2 and Figure 3

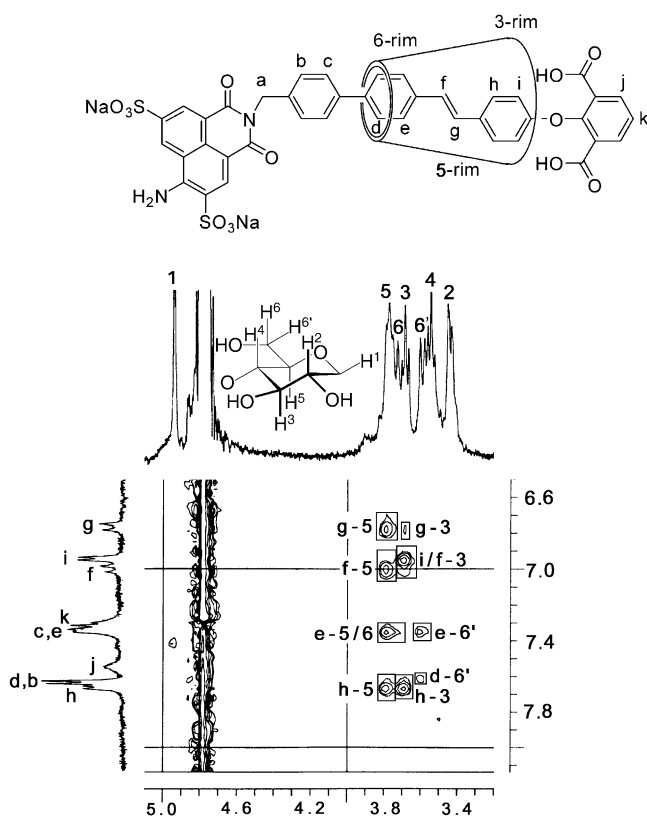


Figure 2. ¹H ROESY NMR (500 MHz) spectrum of α -CD-NPSI in D₂O recorded at 298 K.

observed from the aromatic protons H_e, H_f, H_g, and H_h (no H_i) of the dumbbell to the internal proton 5-H of the α -CD, from H_b, H_g, H_h, and H_i (no H_e) to 3-H, and from the H_e, H_g to 6-H, indicate that the 2, 3-OH rim of the α -CD is near to the isophthalic acid stopper, and that the α -CD ring sits over the stilbene unit. It is to our surprised that only one isomer was obtained, in which the orientation of α -CD was confirmed as shown in Figure 1.

The *E/Z* photoisomerism of the [2]rotaxane α -CD-NPSI in aqueous solution was investigated by monitoring the changes in ¹H NMR spectra as shown in Figure 4 and Figure 5. Efficient photoisomerization was found in a stilbene-based rotaxane set up by Anderson and co-workers, in which the *E/Z* isomerization was accompanied by the shuttling motion of the α -CD ring from the stilbene unit to the biphenyl unit.^[9] But to our surprise, after the irradiation of α -CD-NPSI (5.0 mM in D₂O) at 335 nm for 65 min, no change in ¹H NMR signals was found,^[10] which indicates that no *E* isomer was converted to *Z* isomer. This fact is probably due to

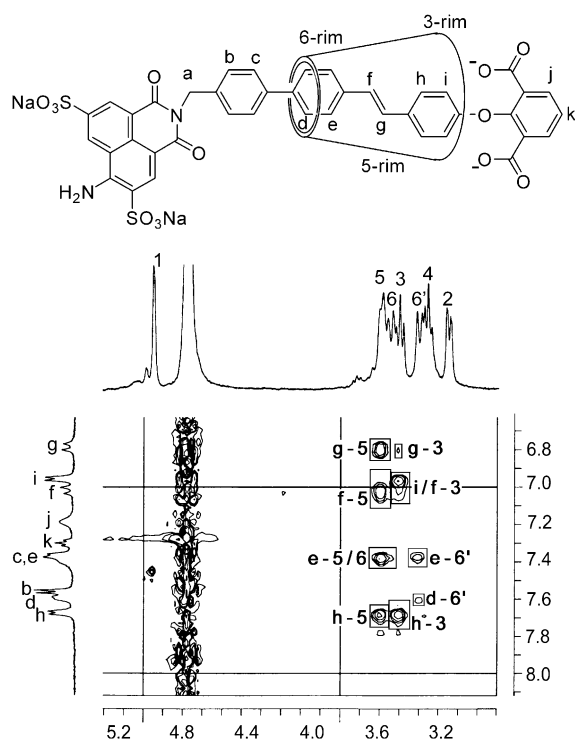


Figure 3. ^1H ROESY NMR (500 MHz) spectrum of $[\alpha\text{-CD-NPSI}]^{2-}$ in D_2O recorded at 298 K.

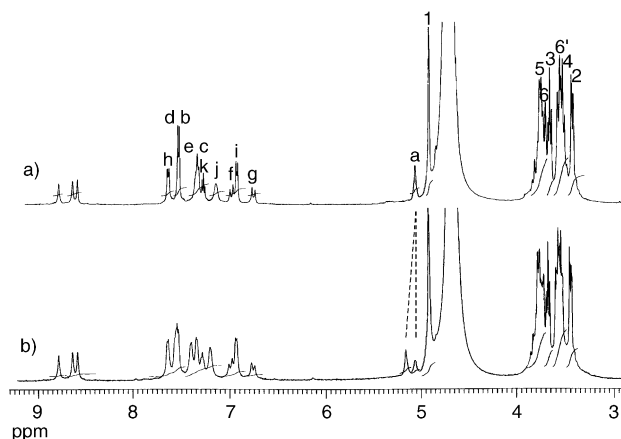


Figure 4. ^1H NMR spectra (500 MHz in D_2O at 298 K) of $[\alpha\text{-CD-NPSI}]^{2-}$ a) before irradiation and b) after irradiation at 335 nm for 65 min (b).

the strong hydrogen bonding between the OH groups in $\alpha\text{-CD}$ and the two carboxyl groups in the isophthalic acid unit, which consequently prevent the $\alpha\text{-CD}$ ring from shuttling away to the biphenyl unit, as illustrated in Figure 6. Conversion of the carboxyl groups into the disodium salt by adding 10 moles of solid Na_2CO_3 to the solution (pH 10.5), which destroys the strong hydrogen bonds, enables the photoisomerization of $[\alpha\text{-CD-NPSI}]^{2-}$. Before irradiation, the same NOEs between the aromatic protons and 3-H, 5-H and 6-H protons on the interior of the $\alpha\text{-CD}$ annulus were found, as shown in Figure 3, which indicates that the ring is

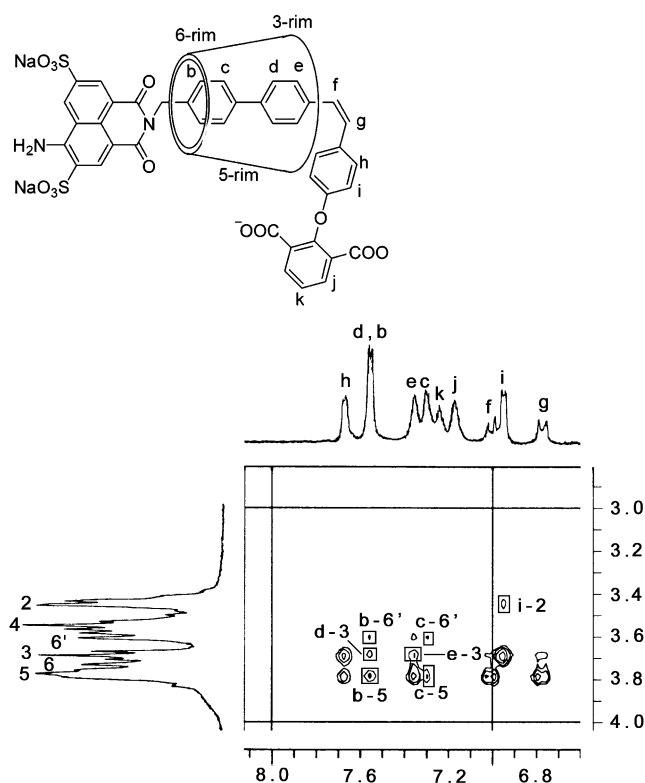


Figure 5. ^1H ROESY NMR (500 MHz) spectrum of $[\alpha\text{-CD-NPSI}]^{2-}$ in D_2O recorded at 298 K after irradiation at 335 nm for 65 min.

still found over the stilbene unit. Irradiation of the product at 335 nm for 65 min leads to new sets of NOEs from H_b , H_c to 5-H and 6-H, from H_d , H_e to 3-H, and from H_i to 2-H, on the external surface of the $\alpha\text{-CD}$, as shown in Figure 5. These results indicate that $[\alpha\text{-CD-NPSI}]^{2-}$ undergoes photoisomerization and the $\alpha\text{-CD}$ ring shifts from the stilbene unit to the biphenyl unit, as shown in Figure 6. Significant signal broadening and a new signal at $\delta = 5.19$ ppm (H_a of the *Z* form) and a decrease in $\delta = 5.07$ ppm (H_a of the *E* form) was found in ^1H NMR spectra after irradiation, as illustrated in Figure 4. Integrals of the two signals at $\delta = 5.07$ ppm and $\delta = 5.19$ ppm appear with a ratio of 3:5, which suggests that at the photostationary about 63% of $[\alpha\text{-CD-NPSI}]^{2-}$ was transformed from the *E* form to the *Z* form.

The absorption changes for the *E/Z* photoisomerism of systems in aqueous solution are shown in Figure 7. After irradiation at 335 nm of $\alpha\text{-CD-NPSI}$ (7.8×10^{-6} M) for 48 h, little change in absorption was found,^[10] which is in accordance with the lack of photoisomerization found, as mentioned above. The conversion of $\alpha\text{-CD-NPSI}$ into its disodium salt $[\alpha\text{-CD-NPSI}]^{2-}$ by adding 10 moles of Na_2CO_3 (pH 9.6) resulted in photoisomerization to give a *E/Z* mixture after irradiation at 335 nm for 48 min, characterized by an increase in the absorption at around 280 nm ($\Delta A = 0.040$), a decrease in the absorption at 335 nm ($\Delta A = 0.026$), and by the presence of two isobestic points at 317 nm and 370 nm as shown in Figure 7. Similarly, the irradiation on the disodium salt of dumbbell NPSI ($\text{Na}^+_2[\text{NPSI}]^{2-}$) for 15 min caused an increase in the absorption at around 280 nm ($\Delta A = 0.049$) and a

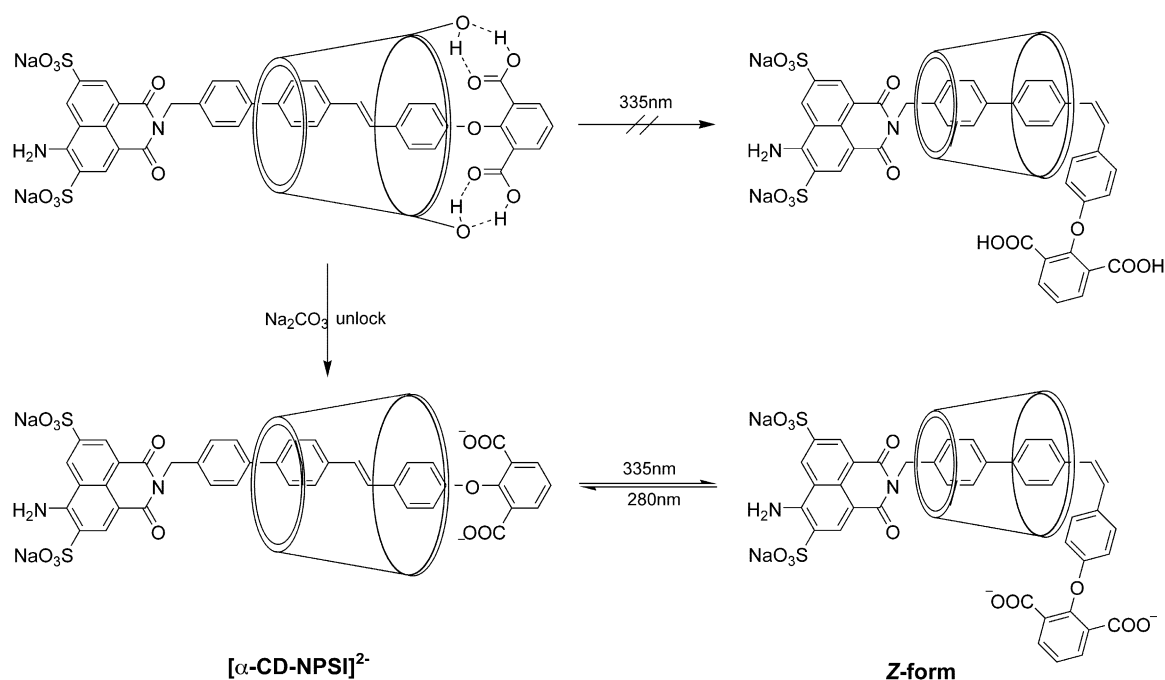


Figure 6. The strong hydrogen bonding between the OH groups in α -CD and the two carboxylic acid groups in the isophthalic acid unit locks the photo-driven motion of α -CD. The addition of Na_2CO_3 destroys the strong hydrogen bonds, thereby unlocking the shuttling motion.

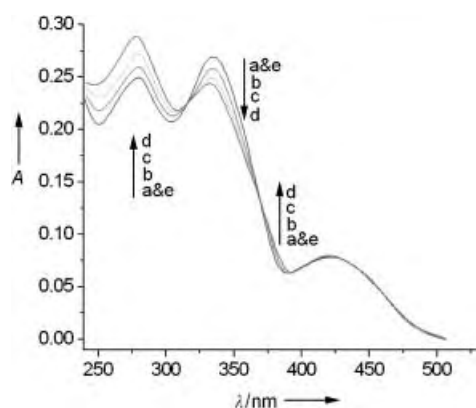


Figure 7. The absorption spectra of an aqueous solution of [α -CD-NPSI]²⁻ a) before and b) after irradiation at 335 nm for 4 min, c) 16 min, d) 48 min. e) These changes in the absorption spectra can be reversed by irradiating the solution at 280 nm (25 min). The absorption changes of an aqueous solution of [NPSI]²⁻ (7.8×10^{-6} M) at 25 °C after irradiation (335 nm) for 15 min were very similar.

decrease at 335 nm ($\Delta A = 0.032$). The fact that the spectral changes for [NPSI]²⁻ are greater than for [α -CD-NPSI]²⁻ indicates that [NPSI]²⁻ undergoes $E \rightarrow Z$ photoisomerization more easily.^[11] This is not surprising as the $E \rightarrow Z$ isomerization becomes more difficult in the presence of the α -CD ring.^[9] The maximum absorption of 4-amino-3,6-disulfonic-1,8-naphthalimide disodium salt at around 422 nm changes little, because this unit is separated from the residues by the methylene group.

The absorption changes in [α -CD-NPSI]²⁻ are accompanied by a parallel increase by about 46% in the intensity of the fluorescence band at $\lambda_{\text{max}} = 530$ nm, as illustrated in

Figure 8, while there is only a small increase by about 4% in the intensity of the same fluorescence band in [NPSI]²⁻.^[10] This behavior is the result of the rigidity of the α -CD ring shifting away from the stilbene unit to the biphenyl unit, which hinders the vibration and the rotation of the bonds in the methylene and biphenyl units. This is confirmed by the finding of the weaker fluorescent intensity of dumbbell [NPSI]²⁻,^[10] in which the vibration and rotation modes more facile without the macrocycle.

When the photoisomerization of [α -CD-NPSI]²⁻ from the E form to the Z form and the consequent α -CD shuttling have

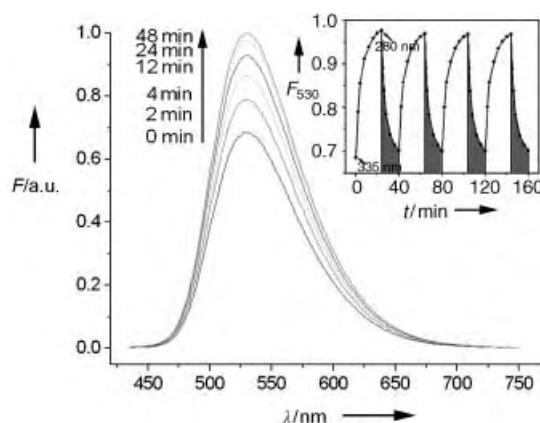


Figure 8. The increasing intensity of fluorescence (emission at 422 nm) of an aqueous solution of [α -CD-NPSI]²⁻ (7.8×10^{-6} M) after irradiation at 335 nm (0 to 48 min). These changes in the absorption spectra can be reversed by irradiating the solution at 280 nm (25 min). Insert: Changes in fluorescence intensity at 530 nm (excited at 422 nm) for aqueous solution of [α -CD-NPSI]²⁻ on alternating irradiation at 335 nm and 280 nm.

been performed, the equilibrium can be shifted back to the *E* form by irradiation at 280 nm. This process is accompanied by a decrease in the absorption at around 280 nm and a rise at 335 nm (Figure 7), as well as a decrease in the intensity of fluorescence at $\lambda_{\text{max}} = 530$ nm (Figure 8). Because of the full reversibility of the photoisomerization process, the photoinduced shuttling motion of the α -CD can be repeated (Figure 6) with reversible fluorescent output signals (Figure 8 insert). Thus, the photoinduced shuttling motion in α -CD-NPSI, which is a lockable molecular switch, can be easily detected by means of a fluorescent output signal at 530 nm.

In summary, a new [2]rotaxane, [α -CD-NPSI]²⁻, is a light-driven molecular shuttle, in which the α -CD macrocycle can shuttle back and forth between the stilbene unit and biphenyl unit by alternating the irradiation frequency. This shuttling is accompanied by obvious changes in the intensity of fluorescence at 530 nm. The shuttle can be locked by simple acidification. It also should be noted that the inputs (switching on at 335 nm and off at 280 nm) are photochemical.

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Cycloadditions

A New Dirhodium(II) Carboxamidate Complex as a Chiral Lewis Acid Catalyst for Enantioselective Hetero-Diels–Alder Reactions**

Masahiro Anada, Takuya Washio, Naoyuki Shimada, Shinji Kitagaki, Makoto Nakajima, Motoo Shiro, and Shunichi Hashimoto*

Over the last decade, the exceptional power of chiral dirhodium(II) carboxylate and carboxamidate catalysts has been demonstrated in a diverse array of enantioselective metal carbene transformations of diazocarbonyl compounds.^[1] Aside from the superiority in diazo decomposition, a dirhodium(II) complex with vacant coordination sites at the

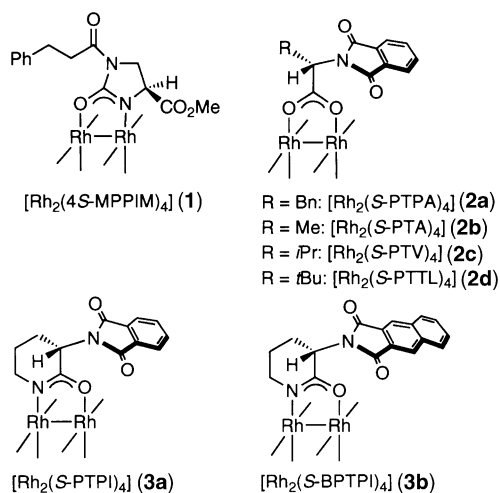
[*] Dr. M. Anada, T. Washio, N. Shimada, Dr. S. Kitagaki, Dr. M. Nakajima, Prof. Dr. S. Hashimoto
Graduate School of Pharmaceutical Sciences
Hokkaido University, Sapporo 060–0812 (Japan)
Fax: (+81) 117-063-236
E-mail: hsmt@pharm.hokudai.ac.jp
Dr. M. Shiro
Rigaku Corporation
3-9-12 Matsubara, Akishima, Tokyo 196-8666 (Japan)

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axial position of each octahedral rhodium center is Lewis acidic as the molecule has a high affinity for axial ligands.^[2] Although this important feature provides a great incentive to develop dirhodium(II) complexes as a new class of chiral Lewis acid catalysts, this goal has remained elusive until a recent breakthrough by Doyle et al.^[3] Doyle and co-workers reported that use of 1 mol % of dirhodium(II) tetrakis[4*S*-methoxycarbonyl-1-(3-phenylpropanoyl)-2-oxoimidazolidinate], $[\text{Rh}_2(4\text{S-MPPIM})_4]$ (**1**, Scheme 1), promoted hetero-



Scheme 1. Chiral dirhodium(II) complexes. MPPIM = 4-methoxycarbonyl-1-(3-phenylpropanoyl)-2-oxoimidazolidinate, PTPA = *N*-phthaloyl-phenylalaninate, PTA = *N*-phthaloylalaninate, PTV = *N*-phthaloylvalinate, PTTL = *N*-phthaloyl-*tert*-leucinate, PTPI = 3-phthalimido-2-piperidinone, BPTPI = 3-(benzene-fused-phthalimido)-2-piperidinone.

Diels–Alder (HDA) reactions^[4–6] between 1-methoxy-3-(tri-methylsilyloxy)-1,3-butadiene (the Danishefsky diene) and nitro-substituted aromatic aldehydes to give, after treatment with trifluoroacetic acid (TFA), dihydropyranones with a maximum of 95% *ee*. Although an exceptionally high turn-over number of 6200 was possible with *p*-nitrobenzaldehyde (10 days, 62% yield, 80% *ee*), a major challenge in terms of reaction rates, enantioselectivity, and the scope with regard to dienes and aldehydes still remained. Herein we report that $[\text{Rh}_2(\text{S-BPTPI})_4]$ (**3b**), a new dirhodium(II) carboxamidate complex that incorporates (*S*)-3-(benzene-fused-phthalimido)-2-piperidinone as chiral bridging ligands, is a more general and highly efficient catalyst for *endo*- and enantioselective HDA reactions.

At the outset of this work, the HDA reaction between 1-methoxy-3-(triethylsilyloxy)-1,3-butadiene (**4a**) and *p*-nitrobenzaldehyde (**5a**) in dichloromethane was explored at room temperature in the presence of 1 mol % of our dirhodium(II) catalysts **2a–d**^[7] and **3a**^[8] (Table 1, entries 1–5). Whereas dirhodium(II) carboxylate catalysts **2a–d** displayed poor enantioselectivities similar to those found by Doyle et al., catalysis with dirhodium(II) tetrakis[3(*S*)-phthalimido-2-piperidinone], $[\text{Rh}_2(\text{S-PTPI})_4]$ (**3a**), provided dihydropyranone (*S*)-**7aa**^[9] in 91% yield with 94% *ee* after desilylation (Table 1, entry 5). Interestingly, the activity of **3a** is similar to that of **2a–d**, although dirhodium(II) carboxamidates are

Table 1. Rh^{II} -catalyzed enantioselective HDA reactions.^[a]

Entry	Rh ^{II}	5	R	T [°C]	t [h]	7	Yield [%] ^[b]	<i>ee</i> [%] ^[c]
1	2a	5a	4-NO ₂ C ₆ H ₄	23	2	7aa	95	6
2	2b	5a	4-NO ₂ C ₆ H ₄	23	2	7aa	97	22
3	2c	5a	4-NO ₂ C ₆ H ₄	23	2	7aa	92	29
4	2d	5a	4-NO ₂ C ₆ H ₄	23	2	7aa	90	45
5	3a	5a	4-NO ₂ C ₆ H ₄	23	2	7aa	91	94
6	3a	5b	C ₆ H ₅	23	32	7ab	92	95
7	3a	5c	4-MeOC ₆ H ₄	23	48	7ac	83	96
8	3a	5d	2-furyl	23	18	7ad	94	93 ^[d]
9	3a	5e	PhC≡C	23	0.5	7ae	97	69
10	3a	5e	PhC≡C	–20	6	7ae	96	87
11	3b	5e	PhC≡C	23	0.5	7ae	95	90
12	3b	5e	PhC≡C	–20	6	7ae	91	92

[a] All reactions were performed on a 0.3-mmol scale (0.5 M) with 1.5 equivalents of diene. [b] Yields of isolated products. [c] Determined by HPLC on a Daicel Chiralcel OD-H (see Supporting Information for details). [d] The absolute stereochemistry was not determined.

commonly regarded as weaker Lewis acids than dirhodium(II) carboxylates. It is also noteworthy that **3a** is even more active than **1**, which is manifested by much shorter reaction times (2 h vs. 24 h).^[3] With respect to the mechanism of the Lewis acid catalyzed HDA reaction, two mechanistic pathways have been proposed: either a pericyclic or a Mukaiyama aldol–Michael pathway.^[5] In the present reaction, the ¹H NMR spectrum of the crude reaction mixture obtained without the use of TFA revealed the exclusive formation of the 2,6-*cis*-dihydropyran **6aa**. Furthermore, no detectable cyclization of the Mukaiyama aldol adduct prepared independently was observed under the present reaction conditions. These results demonstrated that the reaction proceeds through a concerted [4+2] mechanism in an *endo* mode.^[6i,m,10]

The applicability of this catalytic system to a range of aldehydes was then investigated. The use of aromatic aldehydes, including benzaldehyde, *p*-anisaldehyde, and furfural, afforded the corresponding dihydropyranones in similar high yields and asymmetric induction as those found with electron-poor *p*-nitrobenzaldehyde, although these reactions required significantly longer times to reach completion (Table 1, entries 6–8). In contrast, switching from aromatic aldehydes to phenylpropargylaldehyde dramatically accelerated the reaction, but caused a sharp drop in enantioselectivity (69% *ee*; Table 1, entry 9). This result suggests that the steric interaction between the acetylenic moiety and the phthalimido group protruding toward the rhodium–aldehyde adduct might be less severe than that with an aromatic ring (see below). Although the reaction at –20°C gave an improved enantioselectivity (87% *ee*; Table 1, entry 10), we were particularly attracted to the possibility of enhancing the enantioselectivity by extending the phthalimido group with an additional benzene ring, as was with the case with dirhodium(II) carboxylate catalysts in enantioselective carbonyl ylide cycloadditions.^[11] Gratifyingly, the HDA reaction at room temperature under the influence of the newly developed

$[\text{Rh}_2(\text{S-BPTPI})_4]$ (**3b**)^[12] produced **7ae** in 95% yield with 90% *ee* (Table 1, entry 11), in which a slight enhancement (up to 92% *ee*) was observed at -20°C without lowering the yield (Table 1, entry 12).

The present $[\text{Rh}_2(\text{S-BPTPI})_4]$ catalytic system allowed considerable variations in the substitution pattern of the activated diene and the nature of the aldehyde component (Table 2). The applicable aldehydes include aromatic, α,β -acetylenic, α,β -olefinic, aliphatic, and α -alkoxy derivatives (Table 2, entries 1–9). The HDA reaction with 4-methyl-substituted Danishefsky-type dienes **4c** and **4d** produced 2,3-*cis*-dihydropyranones **7ca** and **7da** with essentially complete diastereoselectivities and enantioselectivities in excess of 96% (Table 2, entries 10 and 11), which confirmed the preference for the *endo*-mode transition state. $[\text{Rh}_2(\text{S-BPTPI})_4]$ -catalyzed reactions of monooxygenated dienes **4e** and **4f** with phenylpropargylaldehyde proceeded smoothly at -20°C to yield *all-cis* tetrahydropyranones **8ee** and **8fe** in 99% and 97% *ee*, respectively (Table 2, entries 12 and 13).^[13] As expected from the robustness and high activity of **3b**, selected reactions with highly reactive aldehydes proceeded smoothly with very low catalyst loadings

(0.0075–0.002 mol %) without compromising the yield or enantioselectivity (Table 2, entries 14–16). The turnover numbers (as high as 48 000) are probably among the highest ever reported for Lewis acid catalyzed asymmetric reactions.^[14,15]

The stereochemical outcome of the present HDA reaction can be rationalized based on the crystal structure of the bis(acetonitrile) adduct of $[\text{Rh}_2(\text{S-PTPI})_4]$ (**3a**; Figure 1)^[16]

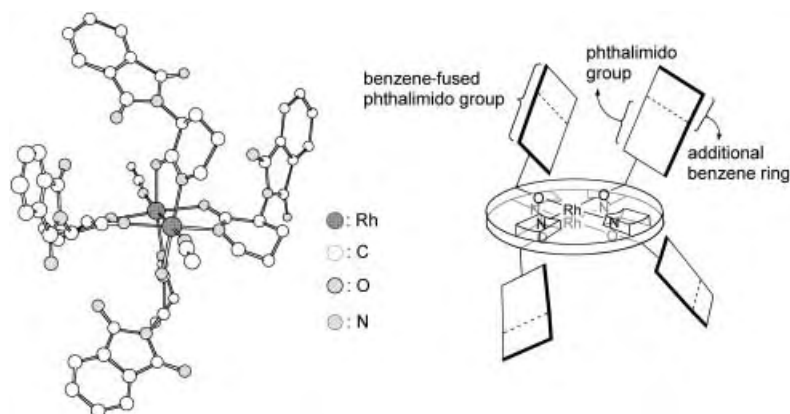


Figure 1. Ball-and-stick (left) and schematic (right) representation of the crystal structure of **3a**·(MeCN)₂.

Table 2: Enantioselective HDA reactions catalyzed by **3b**.

Entry	4	5	R ⁴	T [°C]	t [h]	Prod.	Yield [%] ^[a]	<i>ee</i> [%] ^[b]
1	4a	5f	4-MeC ₆ H ₄	23	24	7af	97	96 ^[c]
2	4a	5g	4-ClC ₆ H ₄	23	6	7ag	95	95 ^[c]
3	4a	5h	4-CNC ₆ H ₄	23	3	7ah	93	95 ^[c]
4	4a	5i	4-CF ₃ C ₆ H ₄	23	1	7ai	93	95 ^[c]
5	4a	5j	<i>n</i> -C ₅ H ₁₁ C≡C	-20	2	7aj	71	93 ^[c]
6	4a	5k	(<i>E</i>)-PhCH=CH	23	36	7ak	86	96
7	4a	5l	PhCH ₂ CH ₂	23	9	7al	89	94
8	4a	5m	BnOCH ₂	-20	18	7am	83	91
9	4b	5n	MOMOCH ₂	-20	24	7bn	86	93 ^[c,d]
10	4c	5a	4-NO ₂ C ₆ H ₄	23	18	7ca	97	96 ^[c]
11	4d	5a	4-NO ₂ C ₆ H ₄	23	10	7da	92	97 ^[c]
12	4e	5e	PhC≡C	-20	20	8ee	87	99 ^[c]
13	4f	5e	PhC≡C	-20	12	8fe	81	97 ^[c]
14 ^[e]	4a	5a	4-NO ₂ C ₆ H ₄	0	48	7aa	96 ^[f]	94
15 ^[g]	4a	5h	4-CNC ₆ H ₄	0	72	7ah	88 ^[h]	96 ^[c]
16 ^[i]	4a	5e	PhC≡C	0	64	7ae	96 ^[j]	91

[a] Yields of isolated products. [b] Determined by HPLC on a Daicel Chiralcel OD-H unless otherwise stated. [c] The absolute stereochemistry was not determined. [d] Determined by HPLC on a Daicel Chiralpak AD. [e] Performed on a 10-mmol scale with 0.0075 mol % of **3b**. [f] Turnover number (TON)=12 800. [g] Performed on a 10-mmol scale with 0.005 mol % of **3b**. [h] TON=17 600. [i] Performed on a 10-mmol scale with 0.002 mol % of **3b**. [j] TON=48 000. MOM= methoxymethyl.

coupled with the formyl C–H...O hydrogen-bond concept proposed by Corey and co-workers.^[17] As in the case of **3a**, **3b** is considered to adopt a conformation with a C₂-like symmetry in which the benzene-fused-phthalimido groups are aligned in a “down-down-up-up” arrangement. Consequently, two stereochemical models of the rhodium catalyst–RCHO complexes, **A** and **B**, can be presented in which a favorable hydrogen bond between the formyl hydrogen atom and the carboxamidate oxygen atom is allowed (Figure 2). The approach of dienes (e.g. **4**) to **A** in an *endo* mode to avoid intrusion into the rhodium framework is preferred over the pathway via **B** owing to the serious repulsion between the incoming diene and the benzene-fused phthalimido wall in **B**, which leads to the observed cycloadduct. In this context, it should be noted that the benzene-fused-phthalimido group,

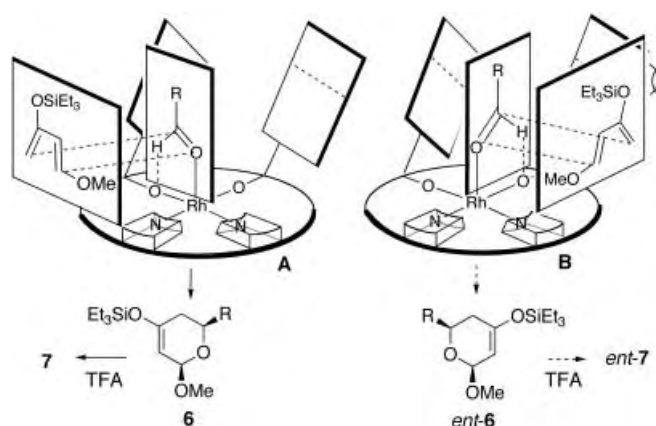


Figure 2. Plausible stereochemical pathway.

which interacts more severely with the aldehyde substituent R than the parent phthalimido group, also greatly favors the formation of a complex **A** as demonstrated by the reaction with the sterically less demanding phenylpropargylaldehyde. This model also explains the preference of dirhodium(II) carboxamidate catalysts **3a,b** over the carboxylate counterparts **2a–d** with similar C₂-symmetry-like conformations^[18] in these reactions, in which four sets of formyl C–H···O hydrogen-bonding interactions are possible.

In conclusion, we have demonstrated that **3b** is an exceptionally effective Lewis acid catalyst for *endo* and enantioselective HDA reactions of a diverse range of aldehydes with Danishefsky-type dienes as well as with monooxygenated dienes, in which up to 99% *ee* and turnover numbers as high as 48000 have been achieved. The catalyst is readily synthesized, air-stable, and easily handled. The absolute stereochemical model proposed herein will provide a useful guide for the development of other classes of Lewis acid catalyzed enantioselective reactions.

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Keywords: aldehydes · asymmetric catalysis · cycloaddition · heterocycles · rhodium

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Cyclopropane Reagents

Formal [3+2] Addition of Acceptor-Substituted Cyclopropylmethylsilanes with Aryl Acetylenes**

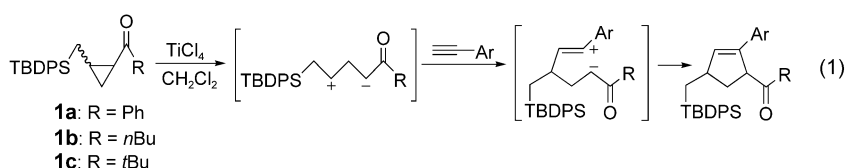
Veejendra K. Yadav* and
Vardhineedi Sriramurthy

Dedicated to Dr. Sukh Dev
on the occasion of his 80th birthday

Cyclopentene-based skeletons are frequently encountered in biologically important natural products. For example, carbosvir, a cyclopentenoid carbocyclic nucleoside,^[1] is a promising drug candidate because of its significant anti-HIV activity.^[2] Consequently, many methods have been developed for the assembly of cyclopentene skeletons.^[3] During our investigations into the synthetic potential of cyclopropylmethylsilanes,^[4] we have discovered that cyclopropyl phenyl ketones with a vicinal *tert*-butyldiphenylsilylmethyl substituent serve as excellent synthetic equivalents of 1,3-dipolar compounds under Lewis acid mediated conditions. We report herein the application of these donor-acceptor-substituted cyclopropane reagents in formal [3+2] addition reactions with aryl acetylenes in the presence of Lewis acids to generate substituted cyclopentenones in excellent yields in a single step. All previously reported addition reactions of acetylenic dipolarophiles have been carried out with hetero-1,3-dipolar compounds to construct heterocycles.^[5]

The special feature of a donor-acceptor-substituted cyclopropane of type **1** is its dual reactivity: Either it can react first with a nucleophile at the silicon-stabilized positive end of the dipole, or the enolate equivalent, that is, the negative end of the dipole, can first be captured by an electrophile.^[6] The reaction outlined in Equation (1) (TBDPS = *tert*-butyldiphenylsilyl) entails initial attack of a terminal acetylenic

carbon center at the positive end of the cyclopropane dipole. The enolate intermediate then intercepts the resulting vinyl cation, and a cyclopentene ring is formed. Our past experience led us to use TiCl₄ as the Lewis acid with substrates **1a–c**.^[4]


Table 1: Formal [3+2] addition of **1a–c** with aryl acetylenes.

Entry	Silane	Acetylene	Product	Yield [%]	<i>cis/trans</i>
1 2 ^[a]	1a (<i>cis</i>) 1a (<i>trans</i>)	$\equiv\text{C}_6\text{H}_5$		75	85:15
3	1a (<i>cis/trans</i>)	$\equiv\text{C}_6\text{H}_4\text{-}p\text{-Cl}$		55	85:15
4 ^[b]	1a (<i>cis/trans</i>)	$\equiv\text{PMP}$		85	
5 ^[c]	1a (<i>cis/trans</i>)	$\equiv\text{PMP}$		60	75:25
6	1b (<i>cis/trans</i>)	$\equiv\text{C}_6\text{H}_5$		70	75:25
7	1b (<i>cis/trans</i>)	$\equiv\text{PMP}$		7: 55 8: 20	57:43
8 9 ^[d]	1c (<i>cis</i>) 1c (<i>trans</i>)	$\equiv\text{C}_6\text{H}_5$		80	95:5

[a] Yield and product ratio were the same as in entry 1. [b] PMP = *p*-methoxyphenyl. [c] Reaction was conducted in the presence of suspended anhydrous K₂CO₃. [d] Yield and product ratio were the same as in entry 8.

[*] Prof. Dr. V. K. Yadav, V. Sriramurthy
Department of Chemistry
Indian Institute of Technology
Kanpur-208016 (India)
Fax: (+91) 512-259-7436
E-mail: vijendra@iitk.ac.in

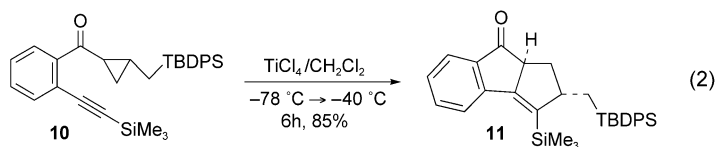
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Supporting information for this article is available on the WWW under <http://www.angewandte.org> or from the author.

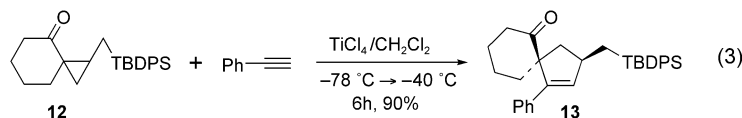
All substrates **1a–c** underwent smooth ring opening at -78°C in CH₂Cl₂ and subsequent reaction with aryl acetylenes (Table 1). Aryl acetylenes with electron-donating substituents reacted better than those substituted with electron-withdrawing groups (see Table 1, entries 3 and 4). A *p*-methoxy substituent caused extensive migration of the double bond under the reaction conditions (Table 1, entries 4 and 7). This double-bond migration appeared to be catalyzed by acid, as it could be completely prevented by conducting the

reaction in the presence of suspended K_2CO_3 (Table 1, entry 5). Simple alkyl acetylenes, such as 1-decyne and benzyl propargyl ether, did not react. The *cis* or *trans* orientation of the *tert*-butyldiphenylsilylmethyl and carbonyl substituents in the adducts was ascertained by NOE measurements.

The intramolecular variant of the above [3+2] addition also proceeded very well. For example, the reaction of *trans*-**10** furnished **11** in 85 % yield [Eq. (2)]. The hydrogen at the ring junction was determined to be *cis* to the silylmethyl substituent based on NOE measurements.



Spiro ring systems, which are present in a large number of natural products, often constitute challenging synthetic targets.^[7,8] The [5,6] spiro skeleton could be constructed readily in high yield by our method. Both *trans*-**12** and *cis*-**12** (formed as a 3:1 mixture) reacted smoothly under the standard conditions to generate **13** as the sole product in 90 % yield [Eq. (3)]. The stereochemical assignment of **13** was possible through comparison of its ^1H NMR spectroscopic data with those of similar species, in combination with NOE measurements.



The high regio- and stereoselectivities of the above addition reactions are remarkable and deserve comment. The predominant *cis* selectivity can be understood by considering the possible transition-state structures for the addition. Based on our previous studies on the ring-opening of similar cyclopropyl ketones, the Ti enolate exists predominantly in the *Z* configuration, and of the ketones studied, the concentration of the titanium enolate was highest for the *tert*-butyl ketone and lowest for the *n*-butyl ketone.^[9]

Four possible transition-state structures, **TS-I** to **TS-IV** (Figure 1), can be constructed for the species generated by attack of the aryl alkyne at the positive end of the cyclopropane dipole and formation of a new C–C bond. For the *Z* enolates of the *tert*-butyl and phenyl ketones, the possible transition structures are **TS-I** and **TS-II**. Likewise, the possible transition structures for the *E* enolate of the *n*-butyl ketone are **TS-III** and **TS-IV**. **TS-I** and **TS-III**, which lead to the *trans* product, are of higher energy than **TS-II** and **TS-IV**, respectively, because of the pseudo-diaxial interactions indicated. Therefore, the *cis* product will be generated predominantly, via **TS-II** and **TS-IV**. However, the pseudo-diaxial interactions will not be as significant in **TS-III** (from the reaction with the *n*-butyl ketone) as in **TS-I** (from the reaction with the phenyl or *tert*-butyl ketones) because of the

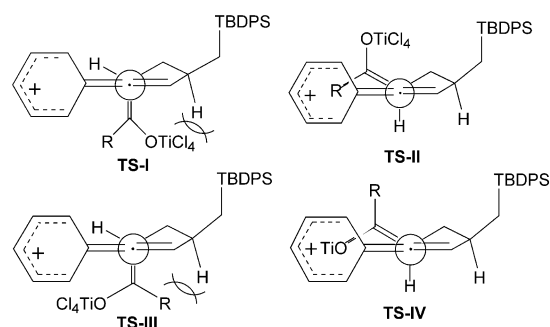


Figure 1. Possible transition states for ring closure.

small size of the *n*-butyl group. For this reason **TS-III** can also participate appreciably, thus leading to a decrease in the observed *cis* selectivity. The exclusive formation of the *cis* isomer **13** from both *trans*-**12** and *cis*-**12**, in which cases only the *Z* enolate is formed, supports the above transition-state analysis.^[10]

In summary, *tert*-butyldiphenylsilylmethyl-substituted cyclopropyl phenyl/alkyl ketones reacted efficiently with aryl acetylenes in the presence of TiCl_4 to form cyclopentene derivatives with high regio- and stereoselectivity. The scope of this methodology for ring construction, including its application to the synthesis of carbocyclic nucleosides and spirocyclic natural products, is being explored further.

Experimental Section

Typical procedure: A solution of TiCl_4 (61 mg, 0.326 mmol) in anhydrous CH_2Cl_2 (0.5 mL) was added slowly under a nitrogen atmosphere to a stirred solution of **1a** (100 mg, 0.251 mmol) and phenylacetylene (33 mg, 0.326 mmol) in anhydrous CH_2Cl_2 (0.8 mL) at -78°C . The resulting deep red mixture was stirred for 3 h at -78°C , then warmed slowly over 1 h to -40°C and stirred for a further 2 h at -40°C . The reaction mixture was then taken up in Et_2O (20 mL) and washed with saturated aqueous NH_4Cl (2×7 mL), then with water (1×7 mL). The combined aqueous washings were extracted with Et_2O (2×7 mL), and the combined organic extracts were washed with brine, dried, and concentrated. Purification of the crude residue by column chromatography on silica gel (EtOAc /hexanes) gave **2** (*cis/trans* 85:15) in 75 % yield as a viscous liquid. The *cis* and *trans* isomers were separated by radial chromatography over silica gel.

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Keywords: alkynes · carbocycles · cyclopropanes · dipolar addition · Lewis acids

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cycle regulation, metabolism, and signal transduction. Protein kinases are thought to comprise the largest enzyme group in the human genome and are important targets in drug discovery. Recent estimates suggest that there are approximately 500 genes encoding protein kinases.^[1] However, only a small fraction of these enzymes have thus far been characterized and little is understood concerning the physiological functions, substrate specificity, and downstream targets of the vast majority of these enzymes. A key step in the biological characterization of kinases and in their use as drug targets is the identification of their cellular interaction partners and the identification of suitable substrates for kinase-inhibitor development.

For a minority of protein kinases, for which cellular substrates have been identified, a knowledge-based approach can be employed to extract information concerning the localization of phosphorylation sites and to deduce peptidic substrates. Alternatively, combinatorial peptide libraries have been used for kinase profiling.^[2] A macroarray-based approach used collections of peptides arranged in a spatially addressable format and allowed the direct identification of peptidic kinase substrates.^[3] The use of peptide macroarrays produced by direct synthesis on cellulose membranes (SPOT technology)^[4] is, however, limited by the number of peptide substrates on the macroarray, the high amount of enzyme needed, and the potential unspecific binding of the kinase and adenosine triphosphate (ATP) to the porous cellulose membrane. These limitations can be overcome by testing peptide microarrays produced either by direct synthesis or by immobilization of peptides on nonporous surfaces, preferentially modified glass or silicon wafers.^[5,6] Recently, proof-of-concept experiments for kinase profiling by using peptide microarrays have been reported.^[5,7] Here, we describe the generation of different types of high-density peptide microarrays, which display up to 6912 covalently attached peptides on one microscope slide, as a robust tool for the characterization of kinase specificity and biology.

Peptides for display on the microarray were synthesized by SPOT technology, released from the cellulose membrane,^[8] and immobilized on glass surfaces. We used the aminooxyacetyl moiety as a reactivity tag coupled to the N terminus of the peptides to allow chemoselective attachment on aldehyde-modified glass surfaces. The hydrophilic linker 1-amino-4,7,10-trioxo-13-tridecanamine succinimide acid^[9] was inserted between the reactivity tag and the peptide sequence to make the peptides more accessible for the enzymes. We used a noncontact printing device for the deposition of the peptides onto the functionalized microarray surfaces (for details, see the Supporting Information).

We extracted 710 human phosphorylation sites as 13-meric peptides from public databases (Swiss-Prot rel. 4.0 and Phosphobase version 2.0)^[10,11] and prepared an appropriate microarray (for the entire list of peptide sequences, see the Supporting Information). Figure 1 summarizes the results of an experiment with cyclic adenosine monophosphate (cAMP) dependent protein kinase (PKA). The sequences of the top 20 substrates that were detected enabled us to deduce a consensus sequence matching the known RRXS consensus for PKA^[2,11] (X = any amino acid). Other kinases have been

Automated Synthesis

High-Content Peptide Microarrays for Deciphering Kinase Specificity and Biology

Mike Schutkowski,* Ulf Reimer, Sören Panse, Liying Dong, Jose M. Lizcano, Dario R. Alessi, and Jens Schneider-Mergener*

Enzymatic protein phosphorylation is one of the key regulatory events in biologically important processes such as cell-

[*] Dr. M. Schutkowski, Dr. U. Reimer, S. Panse, Dr. L. Dong, Prof. Dr. J. Schneider-Mergener
Jerini AG
Invalidenstrasse 130, 10115 Berlin (Germany)
Fax: (+49) 30-97893-105
E-mail: schutkowski@jerini.com
schneider-mergener@jerini.com

Dr. J. M. Lizcano, Prof. Dr. D. R. Alessi
Medical Research Council Protein Phosphorylation Unit
MSI/WTB Complex, University of Dundee
Dow Street, Dundee DD1 5EH (UK)
Prof. Dr. J. Schneider-Mergener
Institut für Medizinische Immunologie
Charité Universitätsmedizin Berlin
10098 Berlin (Germany)



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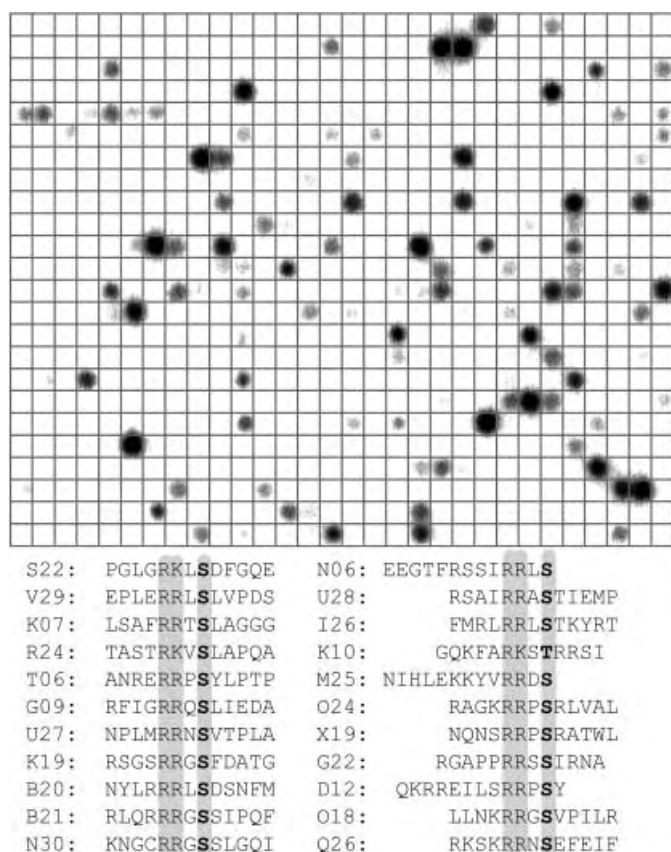


Figure 1. Kinase substrate peptide microarray displaying 710 peptides derived from annotated human phosphorylation sites after incubation with PKA and ^{32}P -ATP. The lower panel shows the coordinates and peptide sequences corresponding to the most intensive signals in decreasing order. The substrate consensus is highlighted in gray.

successfully profiled on the same type of peptide microarray (data not shown and ref. [12]). The proteins related to the substrate peptides identified on the array represent potential downstream targets of the profiled kinase. Another important aspect for kinase regulation is their autophosphorylation. We used overlapping peptide sequences of kinases displayed on microarrays to study autophosphorylation events (see the TIE2 kinase example in the Supporting Information).

Kinase substrate specificities may also depend on prior sequential posttranslational modifications steps, such as phosphorylation by another kinase, an event designated as priming phosphorylation.^[13] This cellularly important regulatory mechanism could be simulated and elucidated with casein kinase 2 (CK2) and the β isoform of glycogen synthase kinase 3 (GSK3 β) on a microarray. Separate incubation of the microarray with either CK2 or GSK3 β and ^{32}P -ATP resulted in a distinct phosphorylation pattern for each kinase (Figure 2). We “preincubated” the microarray with CK2 and nonradioactive ATP to demonstrate that “priming” the peptide substrate with CK2 leads to novel specificities for GSK3. This first experimental step led to nonvisible phosphorylation of the microarray by CK2 kinase. The right panel in Figure 2 displays subsequent phosphorylation of the “prephosphorylated” microarray with GSK3 β , now in the presence of radioactive ^{32}P -ATP. Comparison of the experiments shown in Figure 2 reveals peptide D15 as being sensitive to priming phosphorylation. Peptide D15 (QASS⁶⁵³PQSS⁶⁵⁷DVEDE) is derived from human liver glycogen synthase, a known protein substrate for GSK3 β . According to the literature, serine residue 653 is a GSK3 β substrate only after phosphorylation of serine 657.^[13]

Additionally, we designed a phosphopeptide microarray for analyzing priming phosphorylation in a more general approach. We immobilized 694 annotated human phosphorylation-site peptides together with all possible corresponding monophosphorylated peptides (resulting in 2234 phosphopeptides) on a microarray, thereby simulating all pre-monophosphorylation events in one experiment. After incubation with CK2 (data not shown) we identified two peptides (Figure 3) with improved substrate properties if the tyrosine residue in the +3 position is phosphorylated. The kinetic constants for CK2 were determined in a solution-phase assay and showed a clear improvement in the value of the Michaelis constant K_m for FDNNEEESSYS-pY-E over the unprimed peptide (maximum rate $V_{\max} = 15.7 \mu\text{mol min}^{-1} \text{mg}^{-1}$ and $K_m = 0.3 \text{ mM}$ for primed peptide versus $V_{\max} = 10.2 \mu\text{mol min}^{-1} \text{mg}^{-1}$ and $K_m = 1.4 \text{ mM}$ for nonphosphorylated peptide). In contrast, no substrate properties are detectable for RPRGQRDSSYYWE. However, priming phosphorylation generates a substrate (RPRGQRDSSY-pY-WE: $V_{\max} = 4.4 \mu\text{mol min}^{-1} \text{mg}^{-1}$ and $K_m = 0.4 \text{ mM}$).

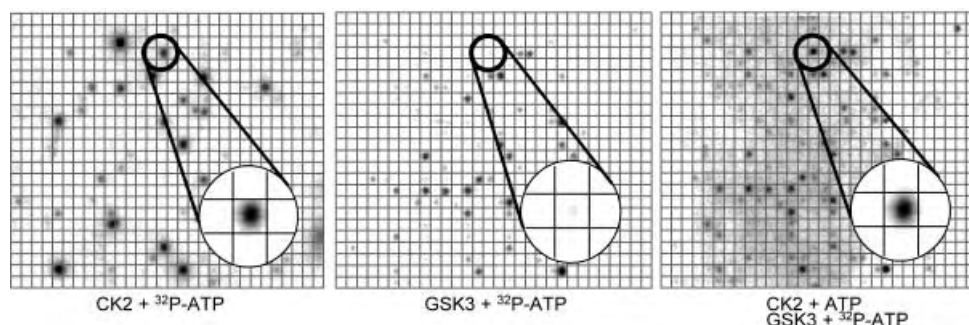


Figure 2. Priming phosphorylation of kinase substrates. Image of a microarray identical to that in Figure 1 after incubation with ^{32}P -ATP and CK2 (left panel) or GSK3 (middle panel), respectively. In the right panel, the array was “preincubated” with CK2 and nonradioactive ATP. This was followed by an incubation with GSK3 in the presence of ^{32}P -ATP. The signal for peptide QASSPQSSDVEDE derived from glycogen synthase is enlarged in each panel.

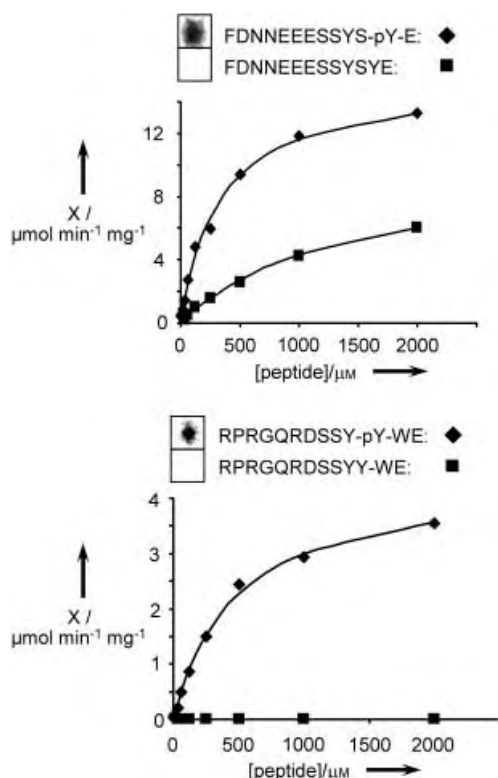
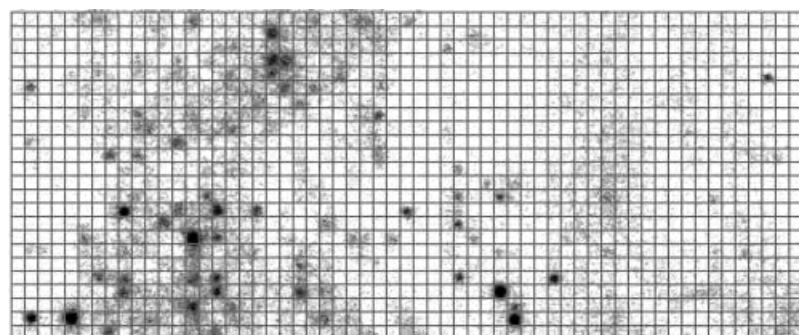


Figure 3. Conversion (X) of the CK2-mediated phosphorylation of peptides FDNNEESSYSY and FDNNEESSYS-pY-E (top) or RPRGQRDSSYYWE and RPRGQRDSSY-pY-WE (bottom, pY = phosphotyrosine). The signals from the corresponding microarray experiments are shown on top of the diagrams and labeled with the appropriate sequence.

The activity of kinases is often regulated by phosphorylation events, mediated by other kinases, in the activation-loop region. To analyze this type of important regulatory event in kinase biology, we prepared a peptide microarray containing 1394 peptides derived from activation loops of human kinases to search for novel downstream targets of 3-phosphoinositide-dependent protein kinase (PDK1; Figure 4). PDK1 is responsible for activation-loop phosphorylation and, thus, activation of certain members of the AGC subfamily of protein kinases that includes protein kinase B (PKB),^[14] p70 S6 kinase,^[15] serum- and glucocorticoid-induced kinase,^[16] protein kinase C isoforms,^[17] p21-activated kinase,^[18] and PKA.^[19] PDK1 itself needs an autophosphorylated activation loop together with an occupied exosite (PIF binding pocket) for full activity.^[20] This activation of PDK1 can be simulated by the addition of an oligopeptide (PIFtide) to the assay solution.^[21] No incorporated phosphate could be detected after incubation of the activation-loop microarray with only PDK1 and ³²P-ATP. However, we found several substrates after the addition of PIFtide to the PDK1 assay solution (Figure 4). Interestingly, many of the detected sequences are not related to the known *in vivo* substrates. The identified NEK2- and SLK-derived peptides represent substrates for PDK1 with improved kinetic parameters compared to the known substrate derived from the activation loop of PKB (T308tide; Figure 5). Moreover, in contrast to T308tide, our novel peptidic substrates are not equipped with



1. FAKTFVGTPYYMS	NEK2	6. LAKSFGSPNRAYT	CDK7
2. LARAFGVPLRTYT	cdc2-related	7. LARAFGVPLRTYT	CDK3
3. DGLLKMLSLVLMH	FRAP	8. RRNTFIGTPYWMA	HPK/GCK like
4. QNMFRNFSFMNPR	PKC-theta	9. MRTLCGTPTYLAP	Chk2
5. RRDSFIGTPYWMA	SLK	10. NSFVGTRSYMSPE	MKK 1

Figure 4. Detection of potential downstream targets for PDK1. A peptide microarray, containing 1394 peptides derived from activation loops^[23] of human kinases, after incubation with PDK1 in the presence of ³²P-ATP and 25 mM PIFtide peptide.^[20] The ten best substrate peptides of PDK1 and the corresponding kinases are listed in the lower panel according to their signal intensity.

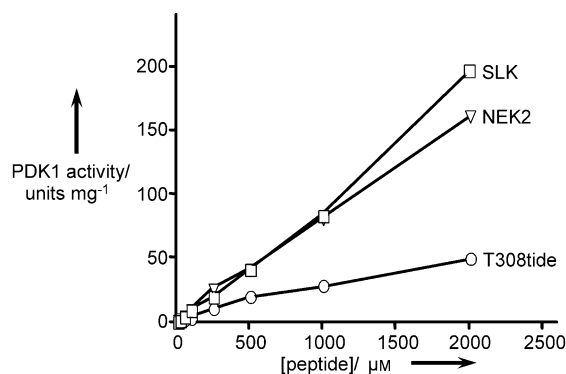


Figure 5. Kinetic analysis of the phosphorylation of two microarray-derived substrates (see Figure 4, sequences 1 and 5) and the known peptidic PDK1 substrate T308tide (KTFCGTPEYLAPEVRR) in solution.

the APE motif on the C-terminal side of the phosphorylation site. This APE motif was thought to be an essential recognition motif for efficient binding to PDK1.^[22] Incorporation of such a moiety into the NEK2- or SLK-derived sequences did not improve the kinetic parameters, a result indicating that, at least for these substrates, the APE motif is not a key determinant for recognition by PDK1 (data not shown). Activation-loop-derived peptides of MKK1 and MKK6 as well as the full-length proteins were also found to be phosphorylated by PDK1 *in vitro*. The phosphorylation is stimulated by PIFtide (Figure 6). There is no physiological evidence that NEK2, SLK, MKK1, or MKK6 are *in vivo* substrates for PDK1 but the results presented in Figures 4–6 demonstrate that peptide microarrays are useful mining tools that enable the rapid identification of *in vitro* substrates for protein kinases.

In summary, we have introduced high-density peptide microarrays as a highly efficient and robust tool in kinase research. The preparation of the arrays was enabled by the ultra-high-throughput automated synthesis of peptides by the

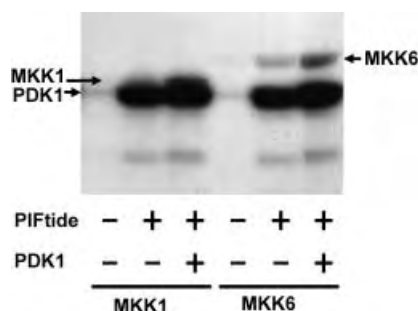


Figure 6. Autoradiography of a polyacrylamide gel electrophoresis (PAGE) gel after phosphorylation of proteins MKK1 and MKK6 with PDK1 in the presence and absence of PIFtide.

SPOT technology, which allows the synthesis of thousands of peptides in parallel on a microscale level. It was shown that the flat glass surface is ideally suited for profiling kinases. This approach potentially allows the highly parallel and efficient study of all human, viral, bacterial, and fungal kinases as potential drug targets.

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Keywords: enzymes · high-throughput screening · microarrays · peptides · substrate specificity

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Natural Product Synthesis

Sceptrin as a Potential Biosynthetic Precursor to Complex Pyrrole–Imidazole Alkaloids: The Total Synthesis of Ageliferin**

Phil S. Baran, Daniel P. O'Malley, and Alexandros L. Zografos*

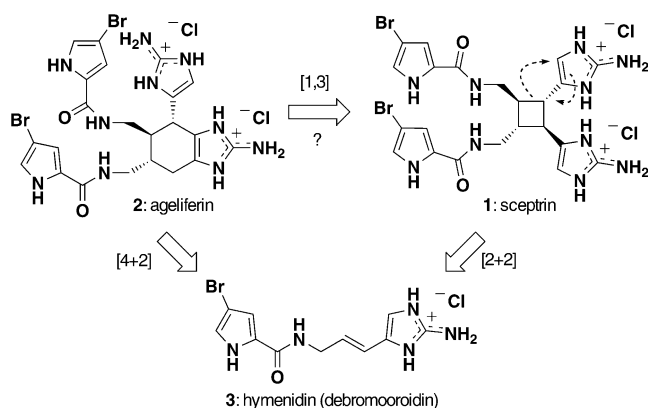
The isolation and characterization in 1981 of the first dimeric pyrrole-imidazole alkaloid, sceptrin (**1**, Scheme 1) by Faulkner, Clardy, and co-workers^[1] was a milestone event in marine

[*] Prof. Dr. P. S. Baran, D. P. O'Malley, Dr. A. L. Zografos
Department of Chemistry
The Scripps Research Institute
10550 North Torrey Pines Road, La Jolla, CA 92037 (USA)
Fax: (+1) 858-784-7375
E-mail: pbaran@scripps.edu

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Scheme 1. Structures of ageliferin (**2**), sceptrin (**1**), and debromooroidin (**3**) as well as retrosynthetic analysis of **1** and **2**.

natural product research as these compounds have extraordinary biological activity and stunning molecular architectures.^[2] Indeed, they have inspired a flurry of research in chemistry.^[2] Recently, the long-standing synthetic challenge posed by sceptrin (**1**) was solved with a concise sequence that proceeds in approximately 24% overall yield, can be conducted on a preparative scale, and does not necessitate chromatography.^[3]

Ageliferin (**2**),^[4] isolated in 1986 from *Agelas conifera* by Rinehart, is an antiviral agent^[4b] and may be a useful chemical tool for mechanistic studies of actin–myosin contractile systems.^[4c] It has been the subject of numerous synthetic efforts,^[5] all of which are based upon a widely accepted biosynthetic hypothesis^[6] wherein **2** is derived from two molecules of hymenidin (debromooroidin, **3**) by an enzymatic “Diels–Alderase” (Scheme 1). We were compelled to question this proposal upon noticing that in every instance in which **2** was isolated, **1** was by far the major constituent (see Figure 1 for an example).^[7] We reasoned that if **1** and **2** were derived from **3** by a divergent pathway (as is proposed^[6]), then the observed ratio of **1** and **2** after isolation should be reversed, solely on thermodynamic grounds. Thus, to explain this apparent discrepancy, we

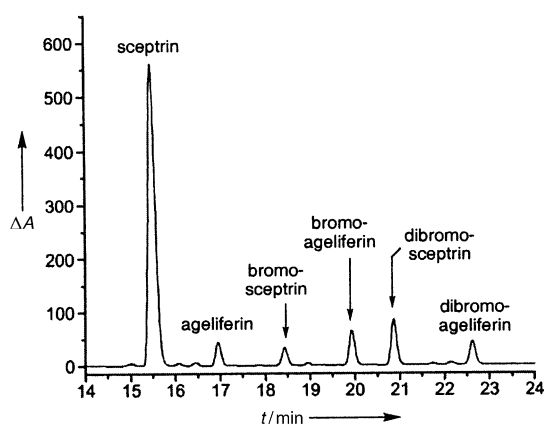
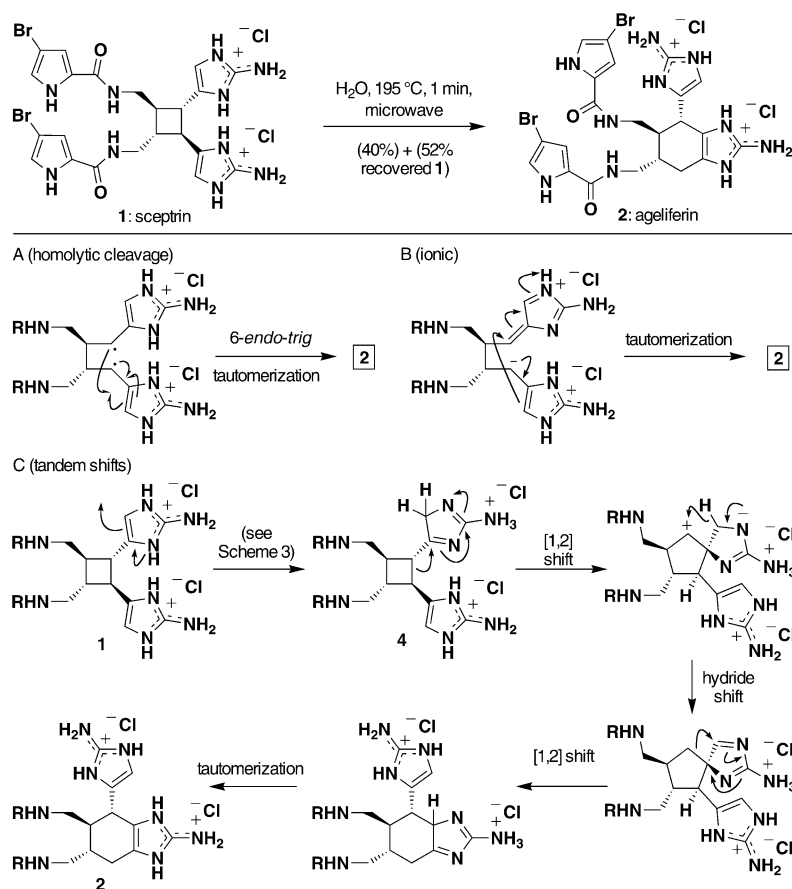


Figure 1. HPLC chromatogram from the extracts of *Agelas conifera*. Reproduced from Ref. [7].

envisioned an alternative scenario wherein **1** rearranges to form **2**. Although such a rearrangement should not proceed thermally in a concerted fashion (see below), it would constitute an “allowed” event if the reaction proceeded in a stepwise fashion (radical or ionic processes) or through photochemical means.^[8,9]

Herein, we report the remarkable thermal conversion of sceptrin (**1**) into ageliferin (**2**). We also present an alternative biogenetic hypothesis commencing from **1** rather than **3** for other complex dimeric pyrrole-imidazole alkaloids, including the axinellamines^[10] and palau’amines.^[11]

Our explorations began with several unsuccessful attempts to effect photochemical [1,3] sigmatropic rearrangement of **1**. Thus, exposure of **1** to a 450-W Hanovia lamp (quartz or pyrex filter) for several days led only to gradual decomposition. Similarly, decomposition was observed when sceptrin (**1**) was heated in methanol at temperatures as high as 150 °C in a microwave. We also found the free base of **1** to be extremely unstable and to decompose into a variety of products which have not been fully characterized. However, when **1** was dissolved in water and heated to 195 °C for 1 minute by using microwave irradiation (Scheme 2), we obtained ageliferin ((±)-**2**) in 40% yield and identical in all respects to a natural sample, along with recovered (±)-**1** (52%). An NMR spectroscopic comparison of synthetic **1**, synthetic **2**, natural **2**, and the products of this reaction is



Scheme 2. Remarkable conversion of sceptrin (**1**) into ageliferin (**2**) and mechanistic analysis.

shown in Figure 2. Given the thermal instability of sceptrin^[3] and ageliferin, it is quite surprising that this reaction proceeds cleanly and reproducibly. In fact, decomposition begins after only 90 seconds at 195°C. If the reaction is performed at 195°C without a microwave, only sceptrin and decomposition products are observed.

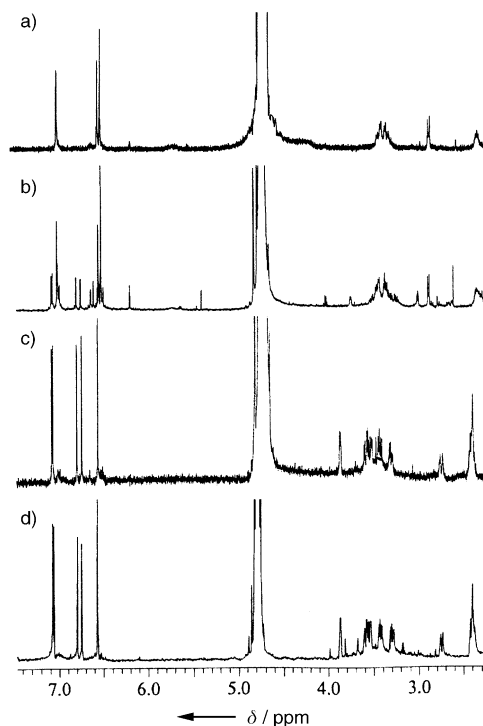


Figure 2. Comparison of the ¹H NMR spectra (600 MHz, D₂O) of a) synthetic sceptrin, b) sceptrin after 1 minute at 195 °C, c) synthetic ageliferin (trifluoroacetic acid salt), and d) natural ageliferin (HCl salt).

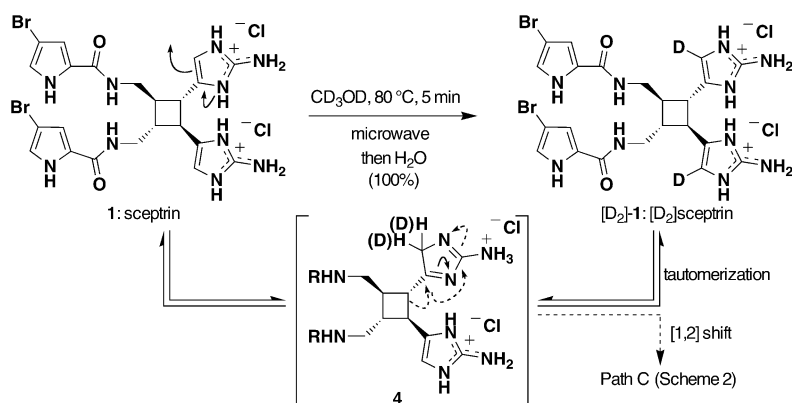
This conversion constitutes, to the best of our knowledge, the first vinyl cyclobutane rearrangement of a natural product. However, perhaps more importantly, it also raises the intriguing question as to whether nature employs an enzyme to catalyze a similar process. It is equally possible that we have unearthed a completely abiotic route to **2**; thermolysis of **1** may just lead to an intermediate that can either return to **1** or irreversibly transform into **2**.

Three selected mechanistic portraits are depicted in Scheme 2. In the first scenario (path A), the cyclobutane is ruptured in a homolytic fashion and this is followed by a 6-*endo-trig* closure. An ionic process, reminiscent of the currently proposed biosynthesis of **2** from **3**,^[6] may also be invoked, as shown in path B. A series of concerted bond shifts, as depicted in path C, can also account for this structural reorganization. Thus, tautomerization to intermediate **4** followed by a thermal [1,2] shift (4→5-membered ring), a hydride shift, another [1,2] shift (5→6-

membered ring), and a final tautomerization could lead to **2**. All three pathways could conceivably lead to the stereochemistry of **2** since the bromopyrrole-bearing carbon centers are incapable of epimerization and could therefore direct ring closure in paths A and B. It follows then that the conversion **1**→**2** should be the same whether racemic or optically pure **1** is employed. Notably, the “allowed” thermal concerted [1,3] sigmatropic pathway would require inversion (suprafacial) of configuration at the migrating carbon atom, while we observe retention.^[8]

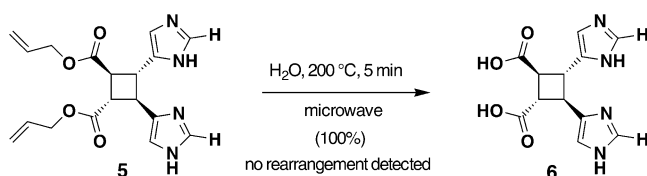
Although theoretical inquiries into the sigmatropic rearrangement of simple monocyclic vinyl cyclobutanes to cyclohexenes point to a radical mechanism,^[9] we believe an ionic process (perhaps similar to paths B or C) is operative here. The ambivalent reactivity of the 2-aminoimidazole C4–C5 double bond contrasts sharply with that of previously studied olefins in this rearrangement.^[12] For example, simply heating **1** at 80 °C in CD₃OD for 5 minutes with microwave irradiation led exclusively to [D₂]sceptrin ([D₂]-**1**) in quantitative yield (Scheme 3). Incidentally, this appears to be the first report of deuterium exchange at a carbon center on a 2-aminoimidazole under neutral conditions. It is postulated that the tautomeric form of sceptrin (**1**), structure **4**, is involved in this proton-addition/proton-loss sequence. In principle, a [1,2] rearrangement could then occur (see dotted arrows, Scheme 3), thus leading to the events shown in path C (Scheme 2) with eventual arrival at ageliferin (**2**). There is ample precedent for this type of ring expansion of cyclobutanes.^[13] Compelling evidence for the requirement of the 2-aminoimidazole subunit in this rearrangement was also garnered by submitting the known cyclobutane **5**^[14] to extended microwave irradiation (200 °C, 5 min) only to obtain the diacid **6** in quantitative yield (Scheme 4).

Based on these findings, it would not be surprising if other complex dimeric pyrrole-imidazole alkaloids such as the



Scheme 3. Facile synthesis of [D₂]sceptrin ([D₂]-**1**) points to the potential viability of path C (see Scheme 2).

palau'amines and axinellamines arise from sceptrin-type intermediates. Indeed, palau'amine (**9**), like ageliferin (**2**), was isolated along with sceptrin (**1**).^[11] For the purpose of inspiring new synthetic approaches to these alkaloids, we propose an alternative biogenic hypothesis for the generation



Scheme 4. Even after 5 minutes at 200 °C, only hydrolysis (no rearrangement) is observed with the known cyclobutane **5**.

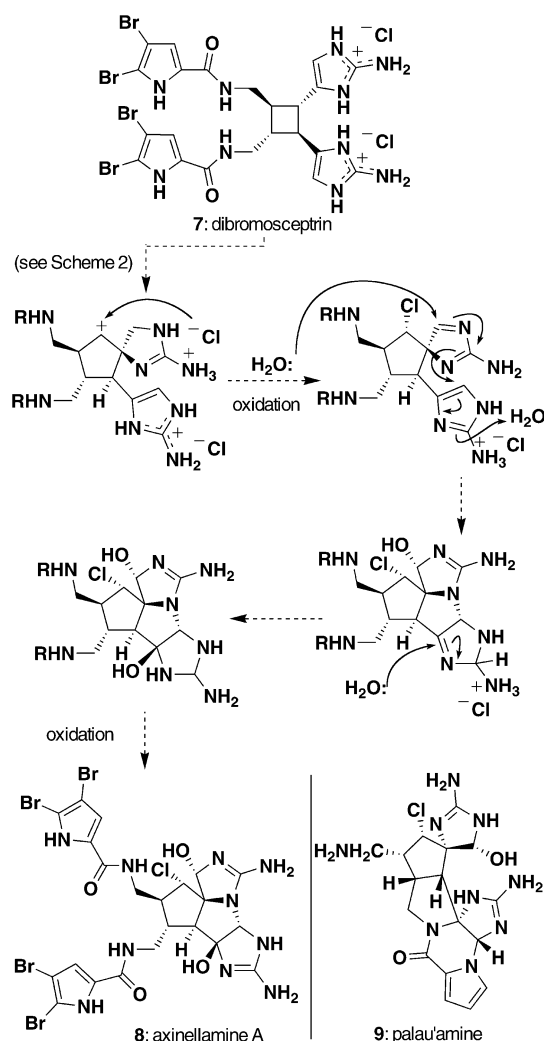
of these compounds: We have chosen to depict the proposed transformation of the known natural product dibromosceptrin (**7**) into axinellamine A (**8**) as an example (Scheme 5); a ring-expansion pathway from a sceptrin-type intermediate could also be drawn for palau'amine (**9**).^[15] A ring-contraction pathway from an ageliferin-type structure could also lead to these alkaloids.^[16]

In summary, we have completed the first total synthesis of ageliferin (**2**, Scheme 1), identified the first vinyl cyclobutane rearrangement of a natural product, and advanced an alternative biosynthetic hypothesis for the formation of

complex pyrrole-imidazole alkaloids. The synthesis adds merely one additional step to the concise, practical, and analogue-friendly sceptrin synthesis developed in our laboratory,^[3] and it can thus easily be scaled up to allow for a full evaluation of the biological potential and structure–activity relationships of these intriguing alkaloids. Efforts are underway to harness the innate symmetry and reactivity of **1** and related structures to effect conversion into other naturally occurring pyrrole-imidazole alkaloids.

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Keywords: alkaloids · biosynthesis · microwave reactions · natural products · total synthesis



Scheme 5. Proposed conversion of dibromosceptrin (**7**) into axinellamine A (**8**) in nature and the related structure of palau'amine (**9**).

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Au₃₂: A 24-Carat Golden Fullerene**

Mikael P. Johansson,* Dage Sundholm, and Juha Vaara

For more than a decade, gold nanostructures have attracted the attention of experimentalists and theoreticians alike. Recent years have witnessed increased interest in gold-containing structures, as several fields of application have found the metal to be not only aesthetical, but also of practical use.^[1] In contrast to carbon where the familiar buckminsterfullerene^[2] came first, later to be followed by carbon nanotubes, gold research started in reverse; gold nanotubes are already a synthetic reality.^[3] Au₃₂ has to date been considered a moderately uninteresting molecule, just one among the many gold clusters. The most stable structure has been suggested to be space-filling,^[4,5] like the majority of all metal clusters studied to date. Using relativistic quantum chemical calculations, we show the existence of another stable isomer: the icosahedral “golden” fullerene Au₃₂, the first all-gold fullerene species. It is spherical and hollow (with a diameter of ≈ 0.9 nm) and structurally very similar to C₆₀. Au₃₂ has a record value of magnetic shielding at its center, and appears to be aromatic.

Considering its place in the periodic table, gold is an unusually relativistic element.^[6] Among other things, this is expressed in its bonding properties. The element manifests aurophilicity, which further enhances the strong gold–gold interactions.^[7] Relativistic effects make many interesting pure gold species such as clusters and nanotubes possible. In addition, heterogenic species are also studied with great interest. The bimetallic icosahedron WAu₁₂, first predicted by Pyykkö and Runeberg^[8] and later synthesized by Li et al.,^[9] is representative of these. Recently, images of multiwalled gold nanowires were published.^[3] No reports of pure “golden” fullerenes exist, however. The closest match is WAu₁₂, where the Au₁₂ shell engulfs a tungsten atom. The icosahedral form of Au₁₂ is itself, however, unstable.^[10]

Few studies of Au₃₂ exist. Work with empirical potentials suggest that the global energy minimum for the molecule is a low-symmetry, lumplike structure of either C₂^[4] or D₂ symmetry.^[5] The scalar relativistic density functional theory (DFT) calculations presented here show that Au₃₂ has another minimum: the icosahedral fullerene form. To determine the stability of the Au₃₂ fullerene, we first optimized its structure. Two different functionals, the popular generalized gradient approximation (GGA) functional BP86^[11] and the nonempirical hybrid GGA functional PBE0,^[12] were used throughout this work. Au₃₂ is composed of triangles in icosahedral symmetry, making a near perfect rhombic triacontahedron. Each atom binds to either five or six neighboring gold atoms. Thus, the symmetry is the same as for the truncated icosahedron C₆₀, with the vertices and planes interchanged. Figure 1 shows the structure of Au₃₂, compared with C₆₀.

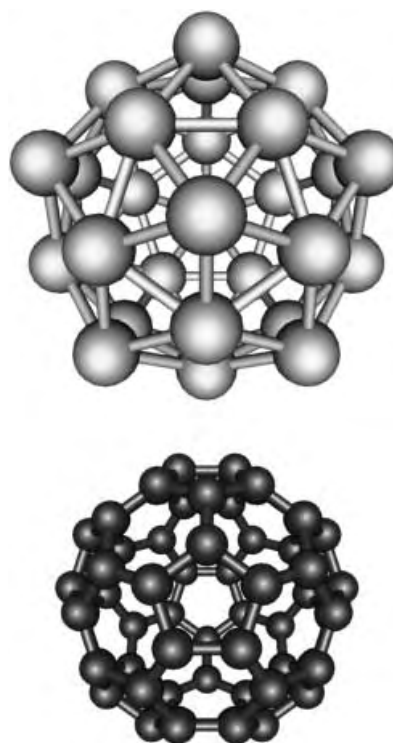


Figure 1. The molecular structures of the Au₃₂ (above) and C₆₀ (below) fullerenes. The calculated Au–Au bond lengths vary between 276 and 287 pm. The figure was prepared using the gOpenMol package.^[32]

[*] M. P. Johansson, Dr. D. Sundholm
Laboratory for Instruction in Swedish
Department of Chemistry, University of Helsinki
P.O. Box 55, 00014 Helsinki (Finland)
Fax: (+358) 9-191-50169
E-mail: mikael.johansson@helsinki.fi

Dr. J. Vaara
Laboratory of Physical Chemistry
Department of Chemistry, University of Helsinki
P.O. Box 55, 00014 Helsinki (Finland)

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Au₃₂ is a closed-shell molecule with an appreciable energy gap between the frontier orbitals, factors important for stability. The gap between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) is 1.7 and 2.5 eV as calculated with the BP86 and PBE0 functionals, respectively. The high symmetry of the molecule increases the density of states in the frontier orbital region; both the HOMO and LUMO are fourfold degenerate.

The harmonic vibrational frequencies of the optimized molecular structure show the icosahedral form to be a minimum; no imaginary frequencies were obtained. A stationary point on the potential energy surface (PES) does not

ensure that other conformations lower in energy do not exist, however. We compared the energy of the Au_{32} fullerene to the structures suggested previously. The energy difference is surprisingly large in favor of the fullerene form. With the BP86 functional, the difference is 150 kJ mol^{-1} to the C_2 structure and 182 kJ mol^{-1} to the D_2 structure. The hybrid PBE0 functional yields a slightly smaller energy difference: 79 and 123 kJ mol^{-1} against C_2 and D_2 , respectively. The cohesive energy of Au_{32} , based on the computed energies of Au_2 and Au_{32} , and the experimental dissociation energy of Au_2 ^[13] and the cohesive energy of bulk gold,^[14] is 60% of the bulk value. As noted by Adamo and Barone, PBE0 is arguably the best available functional for dispersion forces,^[12b] without parameters fitted to experimental data. These energetics should thus be the most reliable that DFT can provide. However, none of the existing functionals are optimal for evaluating the largely dispersion-based aurophilic contribution. An underestimation of gold–gold interactions could artificially raise the energy of bulk gold compared to the fullerene. Also, it has been shown that DFT slightly overestimates the stability of planar gold structures.^[15a] Therefore, it cannot be concluded with absolute certainty that the fullerene form is the true global minimum of Au_{32} , only that it is quite stable.

The stability of Au_{32} seems strongly rooted in relativistic effects. The valence isoelectronic “silvery” fullerene, Ag_{32} , is already higher in energy than space-filling isomers, by at least 100 kJ mol^{-1} (PBE0). As shown by Furché, Häkkinen, and co-workers,^[15] relativity makes the preference for planar structures over 3D structures much more pronounced for gold than for silver. We also compared the stability of Au_{32} to the intriguing Au_{20} tetrahedral cluster, recently reported by Wang and co-workers.^[16] As expected, the stability of Au_{32} is higher than that of Au_{20} , by 10 and $7 \text{ kJ mol}^{-1} \text{ atom}^{-1}$ at the BP86 and PBE0 levels, respectively. In Table 1, we compare other calculated properties of the two highly symmetric gold clusters. C_{60} is included for comparison. Many similarities among the molecules can be noted.

Another factor stabilizing Au_{32} is aromaticity. Strong nuclear magnetic shielding effects in areas lacking electron density, like the center of fullerenes, is an indication of induced ring currents in a molecule.^[17] This in turn correlates

strongly with aromaticity. The negative of the magnetic shielding at the center of the cage equals the nucleus-independent chemical shift (NICS) value,^[18] a popular method for assessing the degree of aromaticity; in general, the more negative the NICS, the stronger the aromaticity. Our magnetic shielding calculations show that the induced currents of Au_{32} are stronger than those of C_{60} and its ions. Up to now, the centers of the C_{60}^{10+} and C_{80}^{8+} ions have held the record for the largest predicted diamagnetic shielding ($\approx 80 \text{ ppm}$).^[19] The shielding at the center of the neutral Au_{32} cluster is even stronger ($\approx 100 \text{ ppm}$). At least two factors can explain this huge shielding. First, Au_{32} has seven times the number of electrons as C_{60} . And second, when the aromatic character is assumed to be accrued by the 32 6s electrons of gold, the $2(N+1)^2$ rule for spherical aromaticity^[19] is fulfilled, with $N=3$. The neutral C_{60} does not fulfill this rule, and indeed shows only a moderate shielding effect. C_{60}^{10+} , on the other hand, is spherically aromatic. Figure 2 shows the magnetic shielding

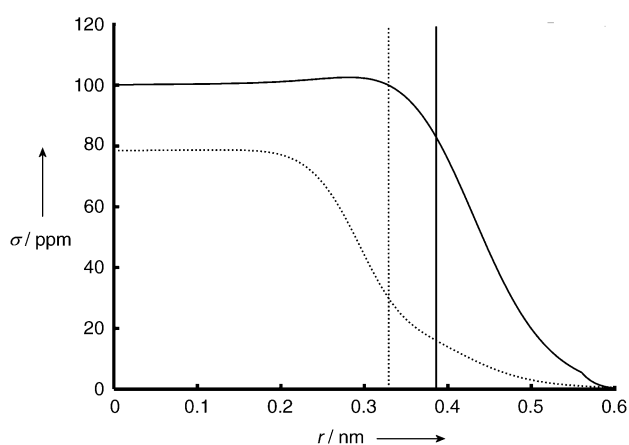


Figure 2. The magnetic shielding σ (in ppm) along a line from the centers of Au_{32} (—) and C_{60}^{10+} (----). The Au_{32} line goes through the midpoint of a triangle. The C_{60}^{10+} line goes through the midpoint of a hexagon; the line through a pentagon (not shown) is almost identical. The vertical lines indicate the distance of the corresponding midpoints from the centers, which equate approximately to the radii of the molecules.

along a line from the centers of Au_{32} and C_{60}^{10+} . Both are seen to behave like miniature magnetic Faraday cages; the shielding inside the spheres is nearly constant. Using the magnetic criterion, Au_{20} is also aromatic. We note that the aromaticity of planar gold compounds has been suggested before.^[20]

The stable gold triangles seem to be ideal building blocks for a variety of molecular-sized geodesic domes, other all-gold fullerenes. Au_{32} is structurally similar to C_{60} in that it can be constructed using the carbon fullerene as a template. By replacing the midpoints of the pentagons and hexagons by 32 gold atoms and then discarding the carbon atoms, one is left with a good model of Au_{32} . Following the same procedure for C_{20} leads to the spherical Au_{12} (which is unstable by itself). Further, by using C_{80} as a template, the icosahedral Au_{42} is obtained, and so on. By not just deleting the carbon atoms of C_{60} after inserting the 32 gold atoms at the centers of the rings,

Table 1: Calculated properties of Au_{32} , Au_{20} , and C_{60} .

Property	Au_{32}	Au_{20}	C_{60}
Diameter [nm] ^[a]	0.9	0.7	0.7
Symmetry point group	I_h	T_d	I_h
HOMO–LUMO gap [eV]			
BP86/PBE0	1.7/2.5	1.9/3.1	1.6/3.0
Frontier orbital config.	$(t_{2u})^6(g_u)^8(g_g)^0$	$(t_2)^6(e)^4(t_2)^0$	$(g_g)^8(h_u)^{10}(t_{1u})^0$
Degeneracy of HOMO/LUMO	4/4	2/3	5/3
Vibrational freq. [cm^{-1}]			
lowest, BP86/PBE0	30/37	28/18	257/273
highest	145/147	172/184	1560/1674
NICS at center of cage, BP86	−100	−36	−2 ^[b]

[a] Approximate values. [b] For C_{60}^{10+} , the corresponding NICS value is −78.

but instead replacing them by gold atoms (and stretching the bonds appropriately), one arrives at Au₉₂. Its icosahedral fullerene form, with a diameter of ≈ 1.5 nm, would need an overall -6 charge to fulfill the spherical aromaticity rule and have a filled HOMO. Gold is highly electronegative, so this charge, especially if donated by electropositive species, is plausible. DFT cannot, however, routinely treat highly charged anions. Preliminary calculations on the closest cation with a filled HOMO, Au₉₂²⁺, show that it is metastable. At the BP86 level, it is a stationary point on the PES, lower in energy than the corresponding planar gold sheet, but higher in energy than space-filling clusters. Au₉₂ could readily be filled with another species. In analogy with nested nanotubes, an obvious thing to insert into Au₉₂ would be Au₃₂. They seem to be a perfect match; the distance between the shells would be about 280 pm. The diameter of the fullerene Au₃₂, about 0.9 nm, matches the most common diameter of the stable nanotubes reported by Kondo and Takayanagi.^[3] This suggests that Au₃₂ and other “golden” fullerenes might be synthesizable by cutting a nanotube of appropriate size, with accompanying closure of the ends.

A wide range of future uses for the “golden” fullerenes can be imagined. Being hollow, they could be used as nanoshells more versatile than the normal, fixed-core variety.^[21] The hollow cavity of the fullerenes offers more flexibility than what can be achieved with space-filling particles. Of course, as with other nanoparticles, the outside of the fullerenes could also be capped with auxiliating ligands. Gold fullerenes could find use as transporters for small molecules, perhaps distributing pharmaceuticals and other chemicals in the human body; gold nanoparticles are for example attachable to certain viruses,^[22] and can even enter cell nuclei.^[23] Decomposition in the body would be harmless, and would enable many other biomedical applications.^[24] A less ambitious but related use would be to use “golden” fullerenes for labeling purposes.^[25] Various fillings and dopants could tune detectable properties of the clusters. In particular, the huge magnetic shielding at the center of Au₃₂ should prove useful.

We have shown that Au₃₂ in its fullerene form is a chemically robust molecule. We hope that our work will inspire the realization of the “golden” fullerenes, not only through computational studies like those reported here, but also in a traditional test tube.

Methods

The molecular structures of the species studied were calculated at the density functional theory (DFT) level using the gradient-corrected functional BP86^[11] and the hybrid, nonempirical functional PBE0.^[12] Where possible, the efficient resolution of the identity (RI) approximation^[26] was employed. Most properties were calculated with the TURBOMOLE^[27] program suite, version 5.6, with the polarized quadruple-zeta basis set VQZPP which includes three *f*-functions. The standard grid size denoted m4 was used. Frequencies were calculated numerically using NUMFORCE. The PBE0 energy comparisons between Au₃₂ isomers were calculated on structures optimized with the TZVPP(2f) triple-zeta quality basis set. Calculations on the silver and carbon fullerenes used the standard TZVPP basis sets. The magnetic shielding calculations were made using the

individual gauges for localized orbitals (IGLO) method,^[28] with a modified version of the DEMON package,^[29] using a (6s5p3d) valence basis set, on the BP86/TZVPP(2f)-optimized structures. For Au₃₂, a three-group orbital localization was necessary. The shielding calculations on C₆₀ used the ECP-MWB-DZP basis set and the ECP-MWB-4VE ECP.^[30] All shielding calculations were made at the BP86 level. The Stuttgart 60- and 28-electron effective core potential (ECP)^[31] modeled the scalar relativistic effects of Au and Ag, respectively. The basis set definitions are detailed in ref. [10].

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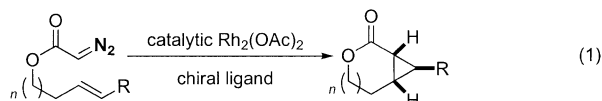
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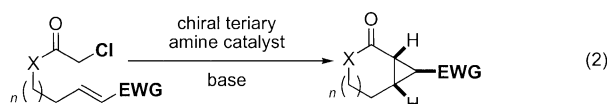
molecular cyclopropanation reaction that forms synthetically versatile $[n.1.0]$ bicycloalkanes by using a nucleophilic tertiary amine catalyst.

The synthesis of these bicycloalkanes is most commonly carried out by inter- or intramolecular metal-catalyzed carbene transfer of diazo compounds to electron-rich alkenes [Eq. (1)].^[5] Other than these methods there are few general

Metal Catalysis: metallocarbenoid-mediated cyclopropanation



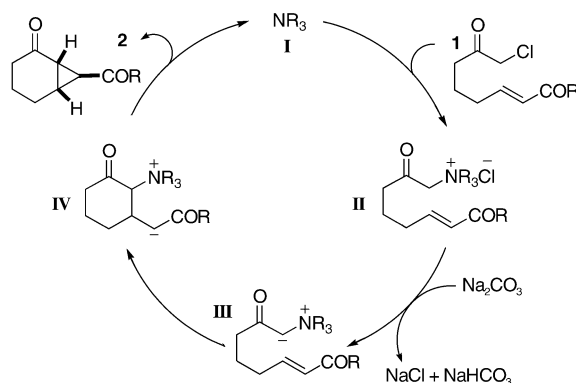
Organocatalysis: ammonium-ylide-mediated cyclopropanation



alternatives to form $[n.1.0]$ bicycloalkanes.^[5c,6] $[n.1.0]$ Bicycloalkanes offer many exciting applications in complex molecule synthesis due to the high levels of stereochemistry and latent reactivity inherent within their structure.^[7] Therefore, new complementary methods for their catalytic enantioselective synthesis are very important. We have identified an interesting organocatalytic intramolecular cyclopropanation process that is stereoselective and produces highly functionalized bicycloalkanes that contain three stereocentres, two rings, and three levels of orthogonal functionality in a single step from linear building blocks [Eq. (2)].

In this approach an α -chloroketone with a tethered electron deficient alkene reacts through a catalytically generated ammonium ylide to form the bicyclic structure [Eq. (2)]. This organocatalytic strategy precludes the use of highly sensitive diazo compounds. It should also offer a wider scope of substrates owing to compatibility with the metal-free catalyst, and produce bicycloalkanes with higher levels of functionality. Furthermore, there are many readily available chiral tertiary amines from which an enantioselective process can be developed.

A proposed catalytic cycle is shown in Scheme 1. The amine catalyst **I** displaces the chloride in **1** to give the



Scheme 1. Proposed catalytic cycle.

Intramolecular Cyclization

An Intramolecular Organocatalytic Cyclopropanation Reaction**

Nadine Bremeyer, Stephen C. Smith, Steven V. Ley, and Matthew J. Gaunt*

Catalytic processes that form functionalized cyclic molecules represent a key transformation for synthetic organic chemistry.^[1,2] Recently, a number of organocatalytic processes have emerged that often provide excellent levels of both enantio- and diastereocontrol for the synthesis of cyclic molecules.^[3,4]

Our recent studies have identified the utility of ammonium ylides for carbon–carbon bond formation. Herein we describe the development of a new organocatalytic intra-

[*] N. Bremeyer, Prof. Dr. S. V. Ley, Dr. M. J. Gaunt
Department of Chemistry, University of Cambridge, Lensfield Road,
Cambridge, CB2 1EW (UK)
Fax: (+44) 122-333-6442
E-mail: mjb32@cam.ac.uk

Dr. S. C. Smith
Syngenta, Jealott's Hill International Research Centre, Bracknell,
Berkshire, RG42 6EY (UK)

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quaternary ammonium salt **II**. Deprotonation forms the ammonium ylide **III** and intramolecular conjugate addition forms **IV** and finally the bicycloalkane **2** is generated through displacement of the ammonium group, concurrently regenerating catalyst **I**.

To assess the viability of this intramolecular process, a range of reaction conditions were investigated by using alkenyl chloroketone **1a**, the results of which are summarized in Table 1. At room temperature little or no reaction was observed in dichloromethane when 20 mol % of 1,4-diazabi-

Table 1: Effect of reaction conditions for the conversion of **1a** into **2a**.

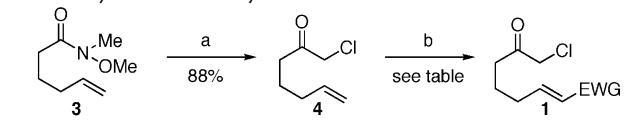
Entry	Solvent	T [°C]	t [h]	Yield	d.r.
1	CH ₂ Cl ₂	22	48	< 10	> 95:5
2	CH ₂ Cl ₂	45	48	45	> 95:5
3	DCE	60	18	91	> 95:5
4	MeCN	80	5	84	> 95:5

cyclo[2.2.2]octane (DABCO) as catalyst and 1.3 equivalents of sodium carbonate as base were used (entry 1). At elevated temperature (45 °C) the yield rose to 45 %, but the reaction was slow (entry 2). With 1,2-dichloroethane (DCE) as solvent and reaction at 60 °C a 91 % yield of **2a** was isolated after 18 h (entry 3). A similar yield was obtained for reaction in acetonitrile (MeCN) at 80 °C with a reduced reaction time of 5 h. Catalytic quantities of quinuclidine could also be used, thus producing similar results to DABCO, although few other tertiary amines gave acceptable reaction. The choice of base was important due to a potential background reaction. After screening a range of organic and inorganic bases, sodium carbonate was found to be the optimal choice. Importantly, a single diastereoisomer was formed and no background reaction was observed in the absence of the catalyst. Therefore, in a single transformation this catalytic process effects the stereocontrolled formation of three stereocenters, two carbon–carbon bonds, and two rings from an acyclic molecule.

Once an intramolecular cyclopropanation process had been identified, a reliable and operationally simple two-step synthesis of the precursors **1** was developed. Alkenyl α -chloroketone **4** was formed by using a modification of the procedure reported by Barluenga et al.,^[9] in which lithiochloromethane (generated in situ through the addition of methyl lithium to a solution of chloriodomethane) reacts with a Weinreb amide **3** to form the desired chloroketone **4** in excellent yield. Alkene cross-metathesis between the alkenyl α -chloroketone **4** and an electron-deficient alkene by using 2.5 mol % of the

Grubbs second-generation catalyst, formed the desired substrate **1** in good yield.^[10] Furthermore, the functional-group sensitivity of alkenyl α -chloroketones clearly demonstrates the mild nature of the alkene cross-metathesis process (Table 2).

Table 2: Synthesis of alkenyl chloroketones **1**.



Alkene-cross metathesis			
Entry	Alkene	t [h]	Yield
1	COMe	12	82
2	CO(CH ₂) ₂ Ph	12	83
3	CHO	9	80
4	CO ₂ ^t Bu	18	85

[a] 2 equiv ClCH₂I, 1.5 equiv MeLi, Et₂O, –78 °C, 0.5 h; [b] the Grubbs second-generation catalyst (2.5 mol %), 1.5 equiv Alkene, CH₂Cl₂, 45 °C. EWG = electron withdrawing group.

The scope of the intramolecular cyclopropanation reaction was subsequently investigated under the optimized conditions.^[11] The results in Table 4 (next page) demonstrate that the reaction is applicable with many types of functionality and all examples produced a single diastereoisomer.

The most reactive substrates were enones **1a–c**, which form the cyclopropanes **2a–c** in excellent yields. The reaction with enoate **1e** also progressed well in MeCN at 80 °C to afford **2e**. Unsaturated sulfone **1f** delivered the desired product **2f** in moderate yield, however, most pleasing was the formation of aldehyde **2d** isolated in 82 % yield from **1d**. This demonstrates that even sensitive functional groups are compatible with this process. Moreover, aldehyde **2d** is a versatile scaffold for subsequent elaboration. The reaction also worked with diene **1g** to form the vinyl cyclopropane **2g** in 72 % yield. [3.1.0]Bicyclohexanes that contained a five-membered ring system could also be formed as single diastereoisomers. Heteroatoms were compatible with the reaction and amide derivative **1i** gave the heterocycle **2i** in 81 % yield. In all examples no background cyclopropanation was observed in the absence of catalyst.

Table 3: Enantioselective organocatalytic cyclopropanation.^[a]

Entry	Catalyst loading	t [h]	Additive	Yield [%]	ee [%]
1	20 mol % 5	96	-	67	64 (+)
2	20 mol % 5	24	40 mol % NaBr	61	94 (+)
3	20 mol % 5	24	40 mol % NaI	64	95 (+)
4	20 mol % 6	24	40 mol % NaBr	48	94 (–)

[a] Reagents and conditions: catalyst, 1.3 equiv Na₂CO₃, MeCN, 80 °C, 24 h.^[12]

Table 4: Scope of the intramolecular cyclopropanation reaction under optimal conditions.

Entry	Substrate	Time	Product	Yield %
1 ^[a]		18		91
2 ^[a]		2		95
3 ^[b]		7		90
4 ^[b]		2		82
5 ^[b]		9		65
6 ^[b]		18		42
7 ^[b]		11		72
8 ^[b]		9		50
9a ^[b]		9		48
9b ^[b]		9		65 ^[c]
10 ^[b]		38		81

[a] DCE at 60°C. [b] MeCN at 80°C. [c] 1 equiv of DABCO.

DABCO was selected as the catalyst because of structural similarity to the cinchona alkaloids, thus making it a racemic model for an enantioselective reaction. On replacement of DABCO with 20 mol % of **5**, cyclopropane **2a** was produced in 67% yield with an enantiomeric excess (*ee*) of 64%, after a 5 day reaction (Table 4).^[11] However, we proposed that the addition of NaBr would accelerate the reaction by facilitating formation of the quaternary ammonium salt. Therefore, the addition of 0.4 equivalents of NaBr led to a 61% yield of **2a** with an *ee* value of 94%. To the best of our knowledge this

excellent result represents the first enantioselective organocatalytic intramolecular cyclopropanation reaction. The use of NaI as an additive gave a similar yield and enantiomeric excess. Moreover, with amine **6** as catalyst, the opposite cyclopropane enantiomer was obtained with an excellent *ee* value (94 %), thus demonstrating the potential efficacy of this organocatalytic process (see Table 3).

In summary, we have developed an organocatalytic intramolecular cyclopropanation reaction for the formation of synthetically versatile $[n.1.0]$ bicycloalkanes as single diastereoisomers. This powerful catalytic process effects the controlled formation of three stereocenters, two carbon–carbon bonds, and two rings in a single transformation. The reaction is enantioselective with a catalytic amount of chiral amine and can form either enantiomer. We are currently exploring the scope of the catalytic enantioselective process and applications towards the synthesis of complex molecules.

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- [11] For full experimental data see the Supporting Information.
- [12] The ee values were determined by using chiral HPLC.

Crystal Engineering

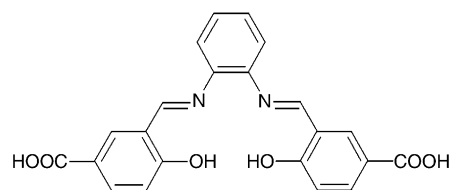
Immobilization of a Metallo Schiff Base into a Microporous Coordination Polymer**

Ryo Kitaura, Goro Onoyama, Hirotoshi Sakamoto,
Ryotaro Matsuda, Sin-ichiro Noro, and
Susumu Kitagawa*

Immobilization of coordinatively unsaturated metal centers (UMCs) into porous frameworks is a very attractive area of research because the porous framework can induce regioselectivity or shape/size selectivity by creating an appropriate environment around the metal center in the restricted

available space. Furthermore, porous frameworks can stabilize the catalytic center by efficiently isolating the sites in a manner similar to the peptide architecture of enzymes in biological systems. Immobilization of UMCs into porous hosts has been attempted with zeolites, polymeric matrices, and clays through ion exchange, impregnation, and isomorphous substitution.^[1,2] However, in these cases, the isolation and uniformity of the UMCs is not sufficient and the environment around the UMCs is not clear. Completely isolated and uniform catalytic centers can be realized if the UMCs are directly incorporated into channel walls of crystalline microporous coordination polymers constructed from transition-metal ions and organic bridging ligands.^[3,4] Such a situation would lead to novel highly selective catalysts and sensors.

In spite of the importance, the incorporation of UMCs into porous frameworks is still rare because of difficulties associated with the formation of UMCs in channel walls by a self-assembly process.^[5–7] Therefore, we focused on the establishment of a rational synthetic method to immobilize various UMCs in the pore walls of microporous coordination polymers. Ligands based on metallo Schiff bases are known to be useful for the generation of supramolecular systems for homogeneous catalyses and so might be suitable for incorporation into infinite porous frameworks for applications in heterogeneous catalyses and sensors.^[8,9] Here, we report a novel three-dimensional (3D) microporous coordination framework obtained from Schiff base type ligands prepared from the reaction of *N,N'*-phenylenebis(salicylideneimine)dicarboxylic acid ($H_4\text{salphdc}$, Scheme 1) with Cu^{II} , Ni^{II} , and Co^{II}



Scheme 1. Schematic representation of $H_4\text{salphdc}$.

ions. These ligands play a key role as linkers with UMCs while Zn^{II} ions act as connectors of the framework. It should be noted that we can rationally immobilize different metal cations into the pore walls of a coordination framework to form complexes of the general form $[\text{Zn}_3(\text{OH})_2(\text{L}^{\text{M}})_2]$ (L^{M} = Schiff base type metalloligand $[\text{M}(\text{salphdc})]^{2-}$, $\text{M} = \text{Cu}^{\text{II}}$, Ni^{II} , and Co^{II}) by using this synthetic method.

In a typical synthesis, the reaction of $\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O}$ and $H_4\text{salphdc}$ in a DMF/MeOH/ H_2O solution at room temperature results in brown needle crystals of $[\text{Cu}(\text{H}_2\text{salphdc})] \cdot \text{H}_2\text{O}$. Figure 1 shows the coordination environment around the Cu^{II} ion of $[\text{Cu}(\text{H}_2\text{salphdc})] \cdot \text{H}_2\text{O}$, together with the numbering scheme. The Cu^{II} ion is coordinated in a square-planar geometry with two nitrogen atoms and two oxygen atoms from the chelating salphdc ligand. The carboxylate groups of the salphdc ligands are not involved in the coordination to the Cu^{II} ion and, therefore, can be utilized for coordination to other metal centers. $[\text{Cu}(\text{H}_2\text{salphdc})] \cdot \text{H}_2\text{O}$ is

[*] Dr. R. Kitaura,⁺ G. Onoyama, H. Sakamoto, R. Matsuda, Dr. S.-i. Noro,⁺⁺ Prof. Dr. S. Kitagawa
Department of Synthetic Chemistry and Biological Chemistry
Graduate School of Engineering
Kyoto University
Katsura, Nisikyo-ku, Kyoto 615-8510 (Japan)
E-mail: kitagawa@sbchem.kyoto-u.ac.jp

[⁺] Current Address: Toyota Central R&D Laboratories, Inc.
Nagakute, Aichi, 480-1192 (Japan)

[⁺⁺] Current Address: Supramolecular Science Laboratory
RIKEN (The Institute of Physical and Chemical Research)
2–1 Hirosawa, Wako-shi, Saitama, 351-0198 (Japan)

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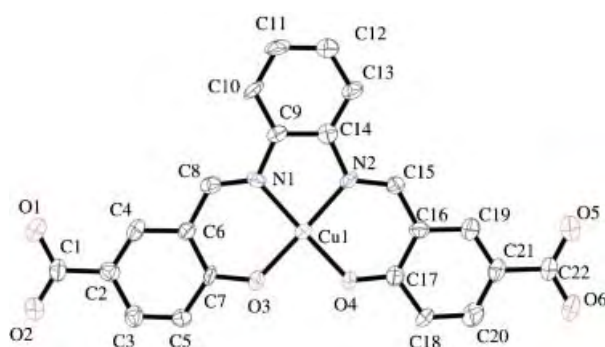


Figure 1. ORTEP drawing of $[\text{Cu}(\text{H}_2\text{salphdc})]\cdot\text{H}_2\text{O}$. Water molecules and hydrogen atoms are omitted for clarity.

soluble in DMF, DMSO, and pyridine and slightly soluble in MeOH.

The metalloligand $[\text{Cu}(\text{H}_2\text{salphdc})]\cdot\text{H}_2\text{O}$ reacts with $\text{Zn}(\text{NO}_3)_2\cdot 6\text{H}_2\text{O}$ to afford a 3D coordination framework **1**·2DMF ($[\text{Zn}_3(\text{OH})_2(\text{L}^{\text{Cu}})_2]\cdot 2\text{DMF}$). Figure 2a shows the coordination environments around the Cu^{II} and Zn^{II} ions of **1**·2DMF. This crystal has two crystallographically independent Zn^{II} ions. One ($\text{Zn}(1)$) is tetrahedrally coordinated to two hydroxide anions and two carboxylate oxygen atoms of L^{Cu} and the other ($\text{Zn}(2)$) is octahedrally coordinated to two hydroxide anions and four carboxylate oxygen atoms of L^{Cu} to form an infinite chain structure. These chains are further

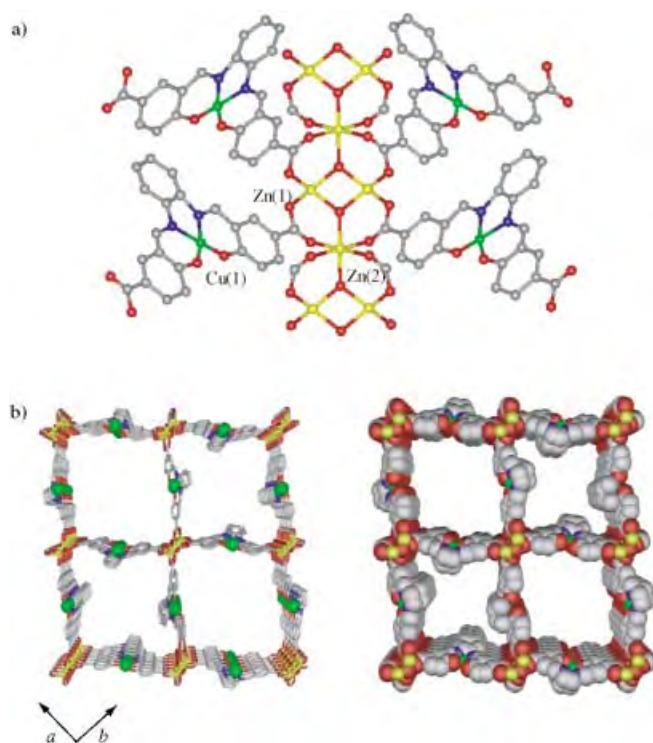


Figure 2. a) The building unit in the 3D porous coordination polymer, **1**·2DMF. The hydrogen atoms and DMF molecule are omitted for clarity. b) 3D porous structure of **1**·2DMF viewed down the c -axis (Zn, yellow; Cu, green; O, red; C, gray; N, blue). One of the doubly disordered phenylenediamine groups and the Cu atoms are omitted for clarity.

linked by L^{Cu} to form a 3D porous structure, which possesses one-dimensional (1D) large channels with a cross-section of approximately $14 \times 14 \text{ \AA}^2$ (Figure 2b) and a large free void space of about 53% if the van der Waals radii of the constituting atoms are considered. Only one DMF molecule per Cu^{II} ion was determined by crystallography to be in the channel, and this is tightly anchored to the framework by a hydrogen bond with the carboxylate oxygen atom. The absence of other guest molecules in the channel of **1**·2DMF was attributed to the disorder of included guest molecules as a result of the large channel size of **1**·2DMF.

Interestingly, coordinatively unsaturated Cu^{II} ions line up along the c -axis with an intermetallic distance of $6.1 \text{ \AA}$. To our knowledge, this is the first example of metallo Schiff base moieties being embedded regularly in the pore wall of a 3D porous framework. Although similar frameworks were reported with 4,4'-biphenyldicarboxylate and 2,6-naphthalenedicarboxylate,^[10] the present porous structure with UMCs has added a new dimension to metal–organic framework chemistry. Bulk samples of this material are stable in air and are insoluble in common organic solvents. The IR spectrum of the material shows no absorptions bands characteristic of any protonated salphdc ligand, thus confirming complete deprotonation of $[\text{Cu}(\text{H}_2\text{salphdc})]$. X-ray powder diffraction (XRPD) measurements with heating under reduced pressure were performed to examine the stability of the framework (Figure 3). The XRPD pattern of as-prepared **1** measured at

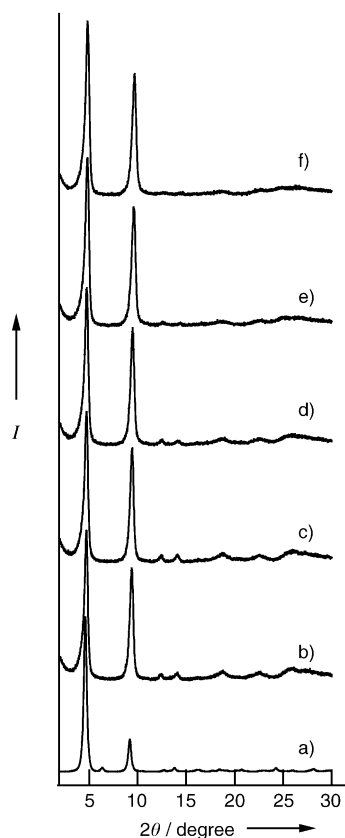


Figure 3. XRPD patterns of as-synthesized **1** with heating from 298 to 523 K. a) Simulated pattern from single-crystal data and the b)–f) XRPD patterns at 298, 373, 423, 473, and 523 K, respectively.

298 K (Figure 3b) is in agreement with that of a simulated pattern obtained from the single-crystal structure (Figure 3a). The sharp peaks obtained over all the temperature regions investigated indicate that the crystallinity was retained. The patterns were almost the same: there was a slight shift in the peak positions from the original ones and a weakening of the peak intensities at higher angles. For example, two strong reflections, (110) and (220), were shifted to the higher angle region by 0.3 and 0.5°, respectively. Consequently, the porous structure was maintained with only slight structure distortions up to 573 K. A detailed study of these structural torsions is underway. We also investigated the use of Co^{II} and Ni^{II} ions instead of Cu^{II} ions to obtain other UMCs. The Co^{II} - and Ni^{II} -incorporated porous coordination polymers $[\text{Zn}_3(\text{OH})_2(\text{L}^{\text{Co}})_2]_n$ (**2**) and $[\text{Zn}_3(\text{OH})_2(\text{L}^{\text{Ni}})_2]_n$ (**3**) were also synthesized by a similar synthetic procedure to **1**. Figure 4 shows the

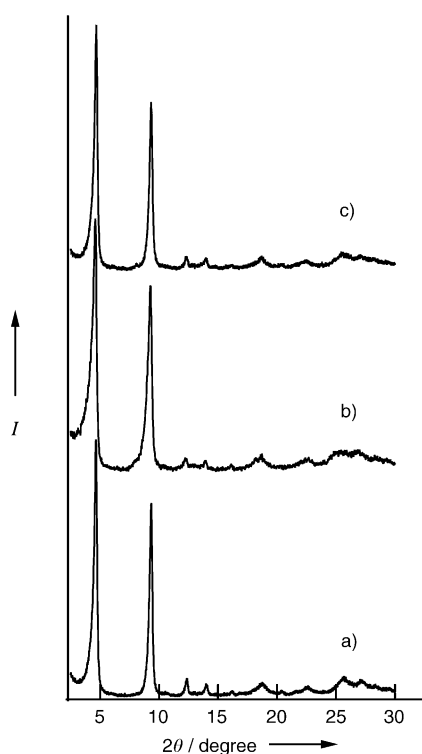


Figure 4. XRPD patterns of a) as-synthesized **1**, b) **2** and c) **3**.

XRPD patterns of **2** and **3**.^[11] The diffraction patterns are in good agreement with that of **1**, thus indicating that the 3D framework is isostructural and, hence indicates that coordinatively unsaturated Co^{II} and Ni^{II} ions had been successfully achieved.

This study demonstrates that completely uniform UMCs (Cu^{II} , Ni^{II} , and Co^{II}) can be embedded in the pore wall of a microporous coordination polymer by using the Schiff base type metalloligand $[\text{M}(\text{salphdc})]^{2-}$ (L^{M}). This synthetic strategy could provide a new type of porous compound that could have applications in highly selective molecular recognition and heterogeneous catalysis.

Experimental Section

H₄salphdc: 3-Formyl-4-hydroxybenzoic acid (**H₂fhba**) was synthesized according to the literature procedure.^[12] A solution of **H₂fhba** (1.08 g, 6.50 mmol) in EtOH (100 mL) was added dropwise to a solution of *o*-phenylenediamine (0.352 g, 3.25 mmol) in EtOH (100 mL) at 333 K. The orange powder precipitate of **H₄salphdc** was collected by filtration, washed with EtOH and THF, and dried under reduced pressure (1.11 g, 77%). ¹H NMR ($[\text{D}_6]\text{DMF}$): δ = 9.06 (s, 2H), 8.33 (d, 2H), 7.95 (dd, 2H), 7.51 (m, 2H), 7.43 (m, 2H), 7.03 ppm (d, 2H).

[Cu(H₂salphdc)]·H₂O: A solution of **H₄salphdc** (5 mM) in DMF/H₂O (4:1, 2.5 mL) was carefully layered on the top of a solution of **Cu(OAc)·H₂O** (5 mM) in DMF/MeOH (1:1, 2.5 mL). Brown needle-shaped crystals began to form in a few weeks. One of these crystals was used for X-ray crystallographic analysis. The bulk product was obtained by the following procedure. Slow addition of a solution of **H₄salphdc** (0.20 g, 1.2 mmol) in DMF (50 mL) to a solution of **Cu(OAc)·H₂O** (0.099 g, 1.2 mmol) in DMF (50 mL) provided a dark brown solution. Addition of MeOH to this dark brown solution resulted in precipitation of a brown powder of **[Cu(H₂salphdc)]·H₂O**. The resulting brown powder was collected by filtration, washed with MeOH, and dried under vacuum for 2 h. Elemental analysis calcd for $\text{C}_{22}\text{H}_{16}\text{CuN}_2\text{O}_7$ (%): C 54.60, H 3.33, N 5.79; found: C 53.11, H 3.31, N 5.78; IR (KBr pellet): $\tilde{\nu}$ = 1668s, 1605s, 1580s, 1529w, 1471w, 1414w, 1383s, 1300m, 1246s, 1191w, 1173w, 1134w, 953w, 843w, 776w, 753w, 696w, 649w, 533w cm^{-1} .

1·2DMF: A solution of **[Cu(H₂salphdc)]·H₂O** (2.3 mg) in DMF (2.5 mL) was carefully layered on the top of a solution of **Zn(NO₃)₂·6H₂O** (2.2 mg, 2.5 mL) in DMF/ CHCl_3 (2 mL/0.5 mL). Brown needle-shaped crystals began to form in a few weeks. One of these crystals was used for X-ray crystallography. The bulk product was obtained by addition of a solution of **H₄salphdc** (0.098 g, 2.0 mmol) in DMF (100 mL) to a solution of **Zn(NO₃)₂·6H₂O** (0.089 g, 3.0 mmol) in DMF/H₂O (50 mL/15 mL) at 333 K. The brown powder obtained was collected by filtration, washed with DMF, and dried under vacuum for 5 h. There is an inherent difficulty in determining the number of guest molecules in such large open structures (**1**) because of disorder of guest molecules, their volatility, and the possibility of exchange. IR (KBr pellet): $\tilde{\nu}$ = 1612s, 1580s, 1472m, 1422w, 1389s, 1357m, 1319m, 1242w, 1191m, 1129m, 958w, 846w, 786m, 752m, 706w, 657w, 557w cm^{-1} .

2: This complex was prepared by a similar procedure as **1**, but using **Co(OAc)₂·4H₂O**. IR (KBr pellet): $\tilde{\nu}$ = 1613s, 1562s, 1510w, 1467w, 1428m, 1392s, 1355m, 1323m, 1248w, 1197m, 1132m, 842w, 786s, 744m, 710w, 661w cm^{-1} .

3: This complex was prepared by a similar procedure as **1** but using **Ni(OAc)₂·4H₂O**. IR (KBr pellet): $\tilde{\nu}$ = 1615s, 1564s, 1506w, 1471w, 1431m, 1401s, 1359s, 1332m, 1251w, 1204w, 1135m, 842w, 814w, 786m, 744m, 712w, 664w cm^{-1} .

Physical measurements: XRPD data were collected on a Rigaku RINT 2000Ultima diffractometer by using $\text{CuK}\alpha$ radiation. Elemental analyses were measured on a Yanaco C,H,N Corder MT-5. IR spectra were recorded on a Hitachi I-5040FT-IR spectrometer with samples prepared as KBr pellets.

X-ray structure determination: X-ray structure determinations were made on a Rigaku Mercury charge-coupled device (CCD) system with graphite monochromated $\text{MoK}\alpha$ radiation. All calculations were performed using the teXsan crystallographic software package of the Molecular Structure Corporation. **[Cu(H₂salphdc)]·H₂O:** Crystal data: $\text{C}_{22}\text{H}_{12}\text{CuN}_2\text{O}_7$, M_r = 479.89, monoclinic, space group $P2_1/c$ (no. 14), a = 16.05(2), b = 7.286(7), c = 18.86(2) Å, β = 93.39(1)°, V = 2201(3) Å³, T = 223.2 K, Z = 4, ρ_{calcd} = 1.448 g cm⁻³, $\lambda(\text{MoK}\alpha)$ = 0.71069 Å, $2\theta_{\text{max}}$ = 55.00°, 4898 reflections measured, 3235 observed ($I > 3.00 \sigma(I)$), 284 parameters; R = 0.0799, R_w = 0.1268. **1·2DMF:** Crystal data: $\text{C}_{50}\text{H}_{40}\text{Cu}_2\text{N}_6\text{O}_{16}\text{Zn}_3$, M_r = 1304.1, monoclinic, space group $C2/m$ (no. 12), a = 27.96(2), b = 26.62(2), c = 6.101(4) Å, β = 94.96(1)°, V = 4523(5) Å³, T = 293.1 K, Z = 2, ρ_{calcd} = 0.962 g cm⁻³,

$2\theta_{\max} = 54.98^\circ$, 5119 reflections measured, 1188 observed ($I > 3.00 \sigma(I)$), 141 parameters; $R = 0.0996$, $R_w = 0.1255$. This framework possesses the disordered phenylenediamine group and the Cu atom (C1–C6, H1–H5, N1, N2, and Cu1). Therefore, the structure refinement was performed with occupancies of these atoms being half. CCDC-216654 and CCDC-216655 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

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Consecutive Cyclic Pentapeptide Modules Form Short α -Helices that are Very Stable to Water and Denaturants**

Nicholas E. Shepherd, Giovanni Abbenante, and
David P. Fairlie*

The α -helix accounts for approximately 30% of protein structures. Often only a few α -helical turns of exposed protein surfaces are recognized by other proteins, DNA, or RNA.^[1] Such helical segments in isolation could be valuable biological probes and drug leads, however, the corresponding short peptides (≤ 15 residues) do not form thermodynamically stable α -helices in water.^[2] Helicity can be stabilized to some extent in longer peptides by using helix-nucleating templates,^[3] metal-ion clips,^[4] unnatural amino acids,^[5] or noncovalent^[6] and covalent^[7–10] side chain constraints (disulfide,^[7] hydrazone,^[8] lactam,^[9] aliphatic^[10]). Small molecules that stabilize or mimic an α -turn have proven elusive, although α -helix side chains have been mounted on non-peptidic scaffolds.^[11] Here we describe a promising modular strategy for mimicking short α -helices by using consecutive sequences of cyclic pentapeptide modules to form short α -helices that are remarkably stable in water, resistant to protein denaturants, likely tolerant of amino acid substitution, easy to synthesize, and with promising utility for biological applications.

Lactam bridges ($i \rightarrow i+3$, $i \rightarrow i+4$, $i \rightarrow i+7$) have previously been reported to increase α -helicity in longer peptides to some extent.^[9] However, consecutive lactam bridges have not previously been reported in short peptides. In principle, cyclic pentapeptides with an $i \rightarrow i+4$ lactam bridge (for example, cyclo(1 $\rightarrow$ 5)-[KARAD], **1**) are α -turn modules that could be directly linked together through amide bonds. Thus, a dimer would have positions i , $i+4$, $i+5$, and $i+9$ occupied by lactam bridges, while exposed positions $i+1$, 2, 3, 6, 7, 8 could in principle be occupied by any peptide side chain (Figure 1). This modular approach to mimicking α -helices is exemplified for the first time below for cyclo(1 $\rightarrow$ 5,6 $\rightarrow$ 10)-Ac-[KARADKARAD]-NH₂ (**2**) and cyclo(1 $\rightarrow$ 5,6 $\rightarrow$ 10,11 $\rightarrow$ 15)-Ac-[KARADKARADKARAD]-NH₂ (**3**). These compounds are shown to be remarkably stable α -helices in water and maintain their extremely high helicity even under strong denaturing conditions.

[*] N. E. Shepherd, Dr. G. Abbenante, Prof. D. P. Fairlie
Centre for Drug Design and Development
Institute for Molecular Bioscience
University of Queensland
Brisbane, Qld 4072 (Australia)
Fax: (+61) 7-3346-2101
E-mail: d.fairlie@imb.uq.edu.au

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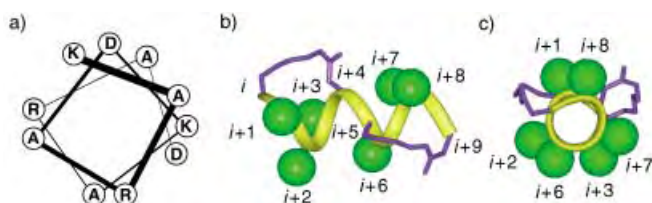


Figure 1. a) Helical wheel structure for dimer **2** (cyclo(1→5,6→10)-Ac-[KARADKARAD]-NH₂) showing the distribution of the side chains; b) side view of **2** showing its helical backbone (yellow), bridging lactam restraints (purple), and exposed side chains (green spheres); and c) **2** viewed end on.

Conventional solid-phase synthesis of **2** and **3** was first attempted using allyl/alloc (alloc = allyloxycarbonyl) orthogonal protection of Asp/Lys residues followed by deprotection and cyclization. However, successive additions of amino acids to the resin-bound cycle **1** were accompanied by extensive aspartimide formation. Consequently, dimer **2** and trimer **3** were instead prepared by solution-phase coupling of cyclo(1→5)-Ac-[KARAD]-OH and cyclo(1→5)-H-[KARAD]-NH₂ (Figure 2). These macrocycles were respec-

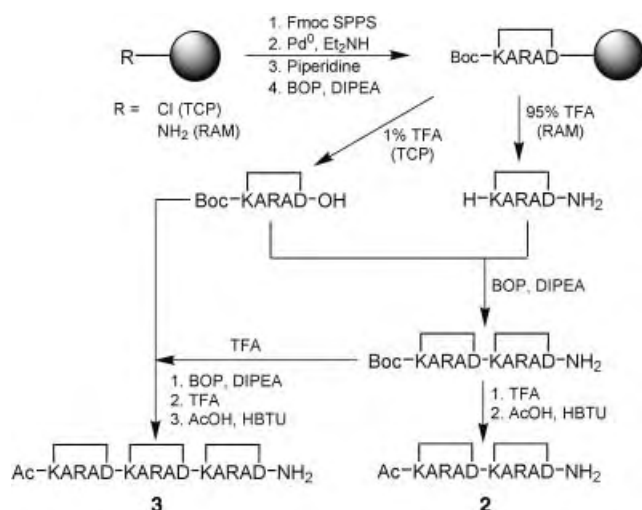


Figure 2. Synthetic strategy for the synthesis of bicyclic **2** and tricyclic **3**. Boc = *tert*-butoxycarbonyl, BOP = 1-benzotriazolyl-*oxy*tris(dimethylamino)phosphonium hexafluorophosphate, DIPEA = *N,N*-diisopropylethylamine, Fmoc = 9-fluorenylmethoxycarbonyl, HBTU = *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate, SPPS = solid-phase peptide synthesis, TFA = trifluoroacetic acid.

tively obtained by standard Fmoc protocols on chlorotrityl (TCP) and rink amide MBHA (RAM) resins using orthogonally protected Fmoc-Asp(OAll)-OH and Boc-Lys(Fmoc)-OH, followed by deprotection, cyclization, and cleavage from the resin.

The circular dichroism spectra recorded in water (Figure 3) reveal the strongly α -helical nature of **2** and **3** (99% and 88%)^[12] compared with their acyclic analogues **4** Ac-(KARAD)₂-NH₂ (3%) and **5** Ac-(KARAD)₃-NH₂ (7%), respectively. The addition of the helix-inducing diluent trifluoroethanol (TFE) failed to increase the helicity in **2**

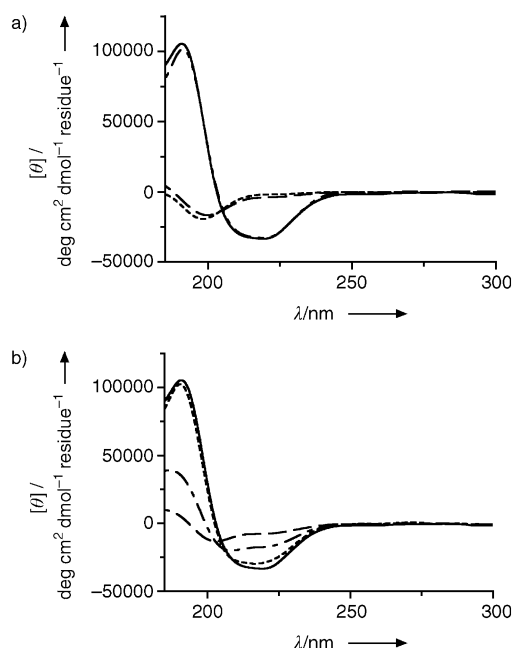


Figure 3. CD spectra obtained in 10 mm phosphate buffer, pH 7.4, 25 °C for 32–44 μ M solutions of a) **2** (—), **3** (---), and acyclic analogues **4** (---) and **5** (---); b) **2** (—) versus **3** (---), **4** (---), and **5** (---) in 50% TFE.

and **3** (85 and 83%, respectively, Figure 3b), which is consistent with high inherent helicity. Compounds **2** and **3** had almost identical molar ellipticity θ_{222} , θ_{208} , $\theta_{192} = -32$, -24.5 , $102 \text{ mdeg cm}^2 \text{ dmol}^{-1} \text{ residue}^{-1}$, respectively (Figure 3a), and a high $\theta_{222}/\theta_{208}$ ratio of approximately 1.3:1.^[13] Calculations of the percentage helicity are derived from equations formulated for longer peptides, and can underestimate the α -helicity of short peptides, especially those containing Ala,^[14a] charged residues,^[14b] or in the presence of significant amounts of TFE.^[14c]

To confirm this compelling CD evidence of high α -helical structure, NMR spectra were obtained for **2** in H₂O:D₂O (9:1). Features (Figure 4a) characteristic of α -helicity included coupling constants $^3J_{\text{NHCH}\alpha} \leq 6 \text{ Hz}$ ^[15] for all the amide NH protons (2.2–5.2 Hz) except D₁₀, a small temperature dependence of the chemical shifts ($\Delta\delta/T < 4 \text{ ppb per deg}$) for seven of the amide NH protons,^[16] which is consistent with all the expected helix-defining H-bonds except for K₁→D₅, and nonsequential medium-range NOE interactions $\delta_{\text{aN}}(i, i+3)$, $\delta_{\text{aN}}(i, i+4)$, and $\delta_{\text{a}\beta}(i, i+3)$ in the NOESY spectra.^[17] In particular, prominent $\delta_{\text{aN}}(i, i+4)$ versus weak $\delta_{\text{aN}}(i, i+2)$ NOE interactions establish α - rather than 3_{10} -helicity and indicate there is only a small number of β - or γ -turns in the conformational mix.^[18]

Three-dimensional structures were calculated for **2** in water, initially using torsional angle dynamic simulated annealing using the program DYANA,^[19] followed by dynamic simulated annealing and energy minimization in Xplor (3.851)^[20] from 122 NOE (32 sequential, 25 medium range, 65 intra-residue) distance restraints, 9 ϕ angle restraints ($^3J_{\text{NHCH}\alpha}$, $\phi = -65 \pm 30^\circ$), and 2 χ_1 angle restraints ($^3J_{\text{NHCH}\alpha}$, $\chi_1 = -60 \pm 30^\circ$). No explicit H-bond restraints were

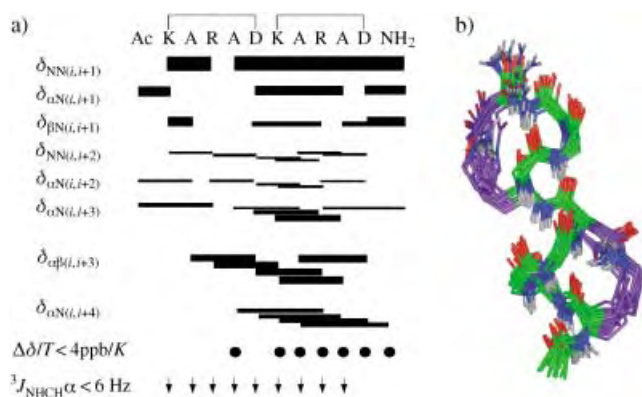


Figure 4. a) Sequential and medium range NOE interactions, temperature coefficients, and coupling constants for **2** in D₂O:H₂O (1:9). The intensities (strong <2.7 Å, medium <3.5 Å, weak <5.0 Å, very weak <6.0 Å) are proportional to the bar width. b) The 20 lowest energy structures for **2** superimposed (backbone, green; lactam bridges, purple; carbonyl O, red; amide N, blue; average backbone pairwise rmsd: 0.577 Å). The C-terminus is at the top and side chains are omitted for clarity.

included in the calculations. The final 20 lowest energy structures contained no dihedral angle ($>2^\circ$) or distance ($>0.17\text{ Å}$) violations. The final structures (Figure 4b) indicate three well-defined α -helical turns for **2** in water, with lactam bridges in locations anticipated from Figure 1c.

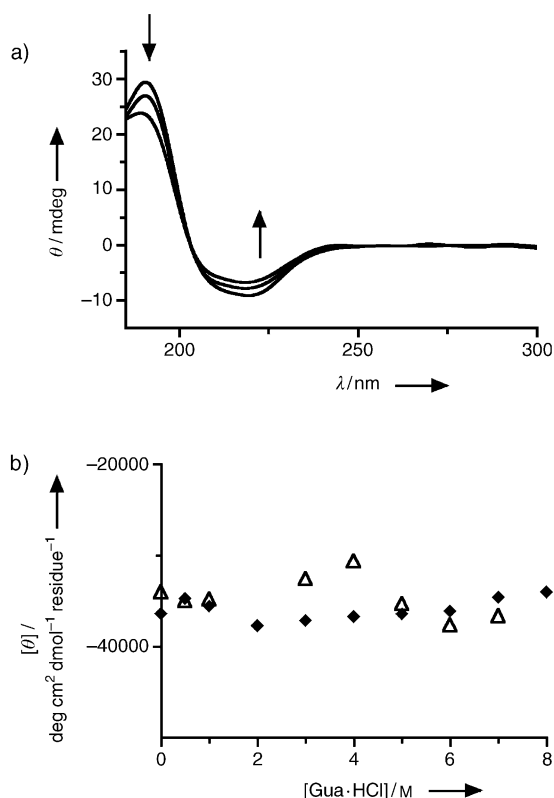


Figure 5. a) CD spectra for **2** in 10 mM phosphate buffer (pH 7.4) at variable temperatures (5, 35, 65 °C). b) Molar ellipticity at 222 nm for **2** (♦) and **3** (△) with varying [guanidine-HCl] at 25 °C.

The helical macrocycles were conformationally very stable even under protein-denaturing conditions, as illustrated by the small dependence of their CD spectra on temperature between 5 and 65 °C (Figure 5a) and on the concentration of guanidine-HCl (Figure 5b). Compound **2** was also found to be highly resistant to proteolytic cleavage by trypsin (ca. 97% intact after 2 h), whereas the linear peptide Ac-(KARAD)₂-NH₂ was degraded in 30 s.

In summary, we have described a promising new generic approach to mimicking α -helices by using sequences of consecutive macrocyclic pentapeptides (for example, cyclo(1→5)-Ac-[KARAD]-NH₂, **1**) to form 3-turn (for example, cyclo(1→5,6→10)-Ac-[KARAD]₂-NH₂, **2**) and 4-turn (for example, cyclo(1→5,6→10,11→15)-Ac-[KARAD]₃-NH₂, **3**) α -helices. These 10- and 15-residue peptides are easy to synthesize and form contiguous α -helical turns through two and three macrocycles that maintain high conformational stability in water, and are resistant to protein-denaturing conditions (65 °C, 8M Gua-HCl, trypsin digestion). CD and 2D-NMR spectra provide compelling evidence of α -helicity, which was not increased by adding TFE. These multicyclic structures permit variation of up to 60% of their component amino acids and thus appear suitable for general mimicry of short α -helical protein segments that bind receptors/ligands on one helical face, and thus confer the major advantages of conformational and proteolytic stability over linear peptides.

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Water-Soluble Supramolecular Fullerene Assembly Mediated by Metallobridged β -Cyclodextrins**

Yu Liu,* Hao Wang, Peng Liang, and Heng-Yi Zhang

Fullerene (C_{60}), which has a spherical π -electron system, shows interesting magnetic,^[1] superconductivity,^[2] electrical,^[3] and biochemical properties.^[4] It is not surprising then that fullerenes and their derivatives have attracted a lot of attention in recent years and have been successfully applied to materials science and biological technology. However, the application of fullerenes and their derivatives has been limited, owing to their low solubility in water and other frequently used solvents. Several approaches have been explored for preparing water-soluble fullerenes,^[5] such as carboxylic acid fullerene derivatives,^[6] amino acid fullerene derivatives,^[7] fullereneol,^[8] peptide and oligonucleotide functionalized fullerene derivatives,^[9] protein–fullerene conjugates,^[10] pendant fullerene polymers,^[11] main-chain fullerene polymers,^[12] and dendrimeric fullerenes.^[13] In addition, cyclodextrins and bridged bis-cyclodextrins as multifunctional molecular receptors can selectively bind a wide variety of organic,^[14] inorganic,^[15] and dye^[16] guest molecules through hydrophobic interactions, forming host–guest inclusion complexes^[17] or nanometer supramolecular assemblies.^[18,19] Therefore, investigations on the molecular recognition and assembly or self-assembly of cyclodextrins and their derivatives are of current interest in chemical and biological systems. Besides, noncovalent supramolecular complexes based on cyclodextrins and fullerenes have been well documented,^[20] and the obtained results indicate that water-soluble cyclodextrin–fullerene complexes show unique photophysical and biological properties. Recently, our studies indicated that the resultant complexes of bis-cyclodextrins and pharmacy molecules significantly enhance both water solubility and biological activity of fullerene derivatives.^[21] Herein, we report a simple way to prepare a water-soluble fullerene assembly with a coordinated metal center through end-to-end intermolecular inclusion complexation of a dimeric cyclodextrin with a fullerene. Furthermore, the assembly behavior of this complex has been comprehensively

[*] Prof. Dr. Y. Liu, Dr. H. Wang, Dr. P. Liang, Dr. H.-Y. Zhang
Department of Chemistry
State Key Laboratory of Elemento-Organic Chemistry
Nankai University
Tianjin, 300071 (P. R. China)
Fax: (+ 86) 22-2350-3625 or 2350-4853
E-mail: yuliu@public.tpt.tj.cn

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investigated by UV/Vis absorption, FTIR, ^1H NMR, and ^{13}C NMR spectroscopies, elemental analysis, thermogravimetric analysis (TGA), scanning tunneling microscopy (STM), transmission electron microscopy (TEM), and scanning electron microscopy (SEM). The biological activity of the assembly was assayed under visible light irradiation and another in dark as a control, which indicates that the assembly may serve as an effective photodriven DNA cleaver.

The supramolecular fullerene polymer, which has coordinated metal centers, was prepared according to the procedures shown in Scheme 1. The 6,6'-*O*-phenylenediselenobridged bis(β -CD) (**1**; CD = cyclodextrin) and its platinum(IV) complex (**2**) were synthesized according to the reported procedures.^[22] The assembly **3** was synthesized with a yield of 4% by treating **2** with fullerene (C_{60}) in a toluene/DMF (v/v = 4:6) solvent mixture. The solubility of **3** in water is approximately 7 mg mL^{-1} (calculated as C_{60} residue), which is larger than that of the 2:1 inclusion complex of natural β -CD and fullerene (4 mg mL^{-1}).^[20c] One reasonable explanation for the increase of solubility is that the intrinsic solubility of bis-CD is larger than that of parent β -CD. The absorption at 343 nm and the peak broadening beyond 350 nm in the UV/Vis spectrum of **3** as well as the typical band at 527 cm^{-1} in the FTIR spectrum were assigned to C_{60} .

Theoretically, bis(β -CD) can form a 1:1 inclusion complex, a 1:1 intermolecular polymer, and a 1:2 inclusion complex with a fullerene guest, as illustrated in Figure 1. Energy-minimized calculations by use of MM2 force-field techniques and of a CPK model indicated that **2** cannot form stable 1:1 inclusion complex with C_{60} owing to steric hindrance. The resonance signal of H-3 protons of the CDs in **3** shift upfield (ca 0.04 ppm) relative to those of free **2** in ^1H NMR spectra (Figure 2), and no significant change in the chemical shift of the H-5 protons of the CD moieties was observed, thus indicating that fullerene was included only shallowly into the cavity from the secondary/wider side of the CDs to form end-to-end CD- C_{60} inclusion complexes (Figure 1 b or 1 c). Furthermore, the results of elemental analysis of **3** showed that the stoichiometry of metallobridged bis(β -CD) and C_{60} is 1:1. These results demonstrate that the bis(β -

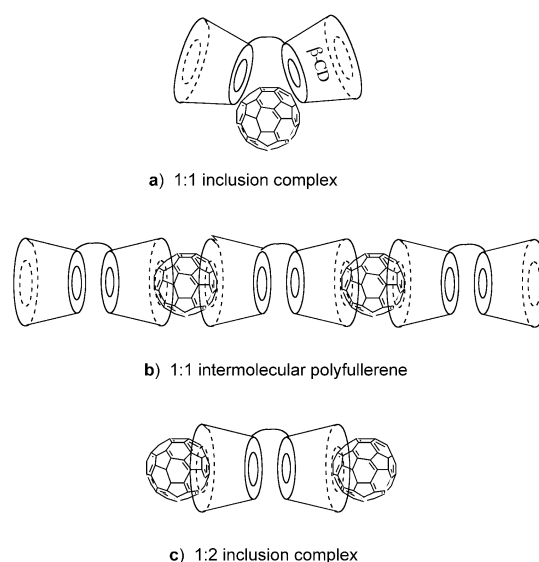


Figure 1. Three possible inclusion modes between metallobridged bis(β -CD) and fullerene.

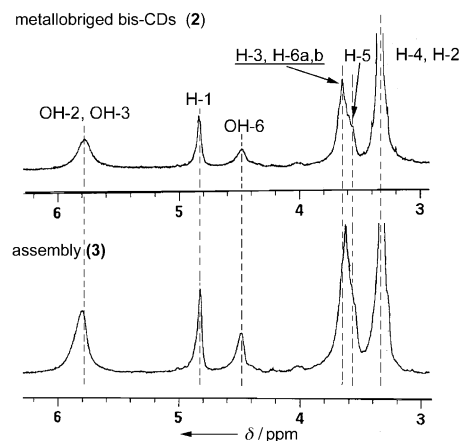
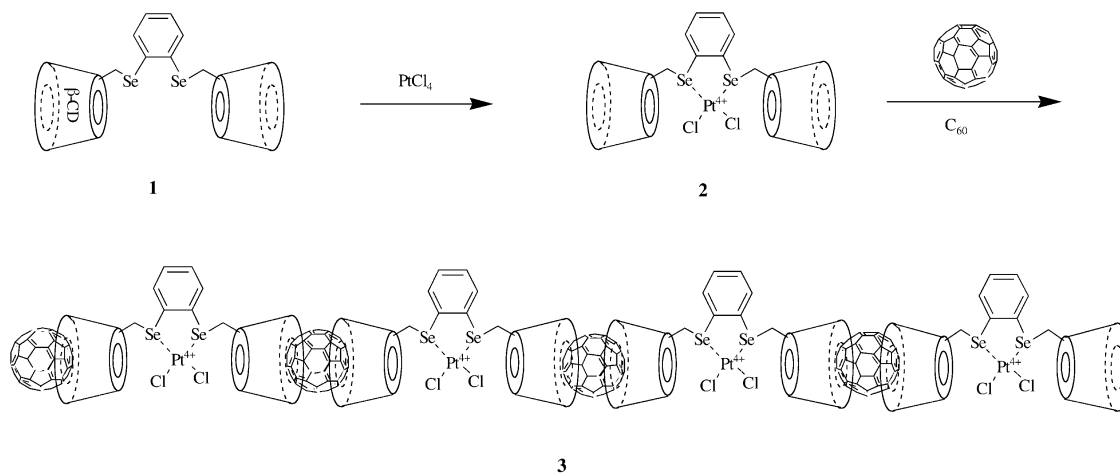


Figure 2. ^1H NMR spectra of **2** (top) and **3** (bottom) taken in $[\text{D}_6]\text{DMSO}$.



Scheme 1. Synthesis of **3**.

CD)–fullerene supramolecular assembly is formed by the 1:1 intermolecular inclusion mode, as shown in Figure 1 b.

The most direct evidence for the formation of bis(β -CD)–fullerene assembly is given by STM. The samples for the STM experiment were prepared by dripping highly dilute aqueous solutions of the substrate onto freshly prepared highly ordered pyrolytic graphite (HOPG) surfaces, followed by evaporation of the aqueous solvents for at least 2 h in a vacuum. The STM image of **2** was recorded for comparison (Figure 3) and shows lots of analogical elliptical bright dots. The space between adjacent elliptical dots is about 1.6 nm. The length and width of an elliptical bright dot are about 2.1 nm and about 1.7 nm, respectively, thus indicating that one elliptical bright dot corresponds to one bis(β -CD) unit. Since the short and rigid bridge chain, which is coordinated to a metal ion, brings the cavities of the CDs close to each other, it is not possible to further distinguish the two CD units (Figure 3c). The height of the dimer is about 1.8 nm (Figure 3b), which is consistent with the actual external diameter for β -CD (1.54 nm). We conclude, from the previous reports by Kunitake and co-workers,^[18j] Miyake et al.,^[18j] and the from this study that the apolar outside wall of **2** interacts with the hydrophobic surface of the HOPG and self-assembles to form regular linear arrays. This intermolecular hydrogen-bonding interaction arises from the presence of the hydroxy groups of the secondary side of the CD (Figure 3d).

A typical STM image of **3** (Figure 4a)^[23] shows a regular linear arrangement observed on HOPG. By combining the size and shape of the patterns in Figure 4a with those of **2** in Figure 3, one can affirm that each bright dot (height ca. 1.9 nm, width ca. 1.8 nm) corresponds to a unit of **2**. The average distance between two adjacent bis(β -CD) units is about 3.0 nm (Figure 4b), which is almost identical to the length of **2** (ca. 2.1 nm) and the diameter of C_{60} (ca. 1.0 nm) added together.^[21c] We note that the height of the wave troughs in Figure 4b is about 1.2 nm, which further confirms that there is a C_{60} molecule between two units of **2** forming the supramolecular assembly, as illustrated in Figure 4c.

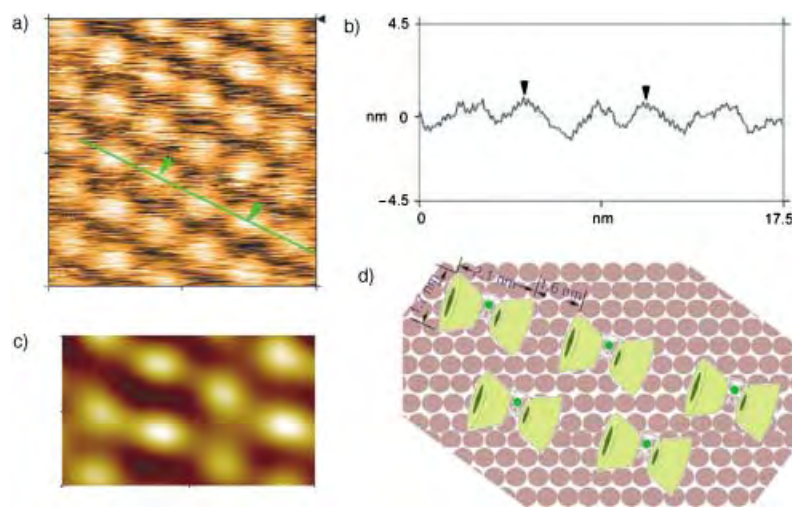


Figure 3. a) STM image of **2** on a HOPG surface (tunneling current 2.0 nA).^[23] b) Line profile of the image shown in (a). c) Sectional image, and d) schematic structure of **2**.

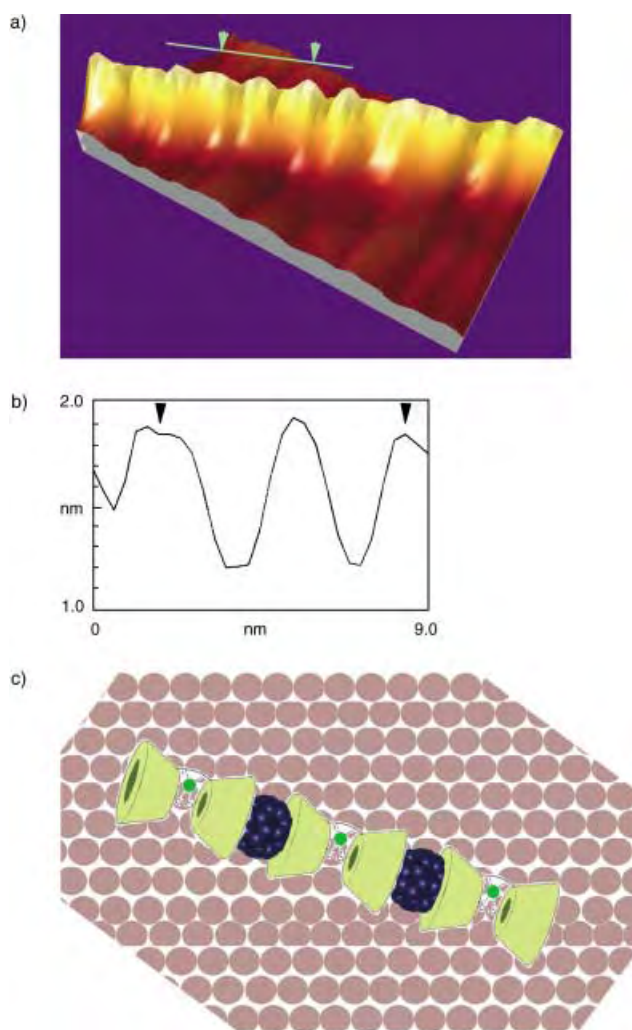


Figure 4. a) STM images of assembly **3** on HOPG a surface (tunneling current 1.0 nA).^[23] b) Line profile of image shown in (a). c) Schematic structure of **3**.

TEM was also performed to provide further insight into the size and shape of the aggregates. For visualization by TEM, a sample was prepared by placing a drop of the solution of assembly **3** onto a carbon-coated copper grid, which was then was shaded with palladium–iridium alloy to thicken and make the images clearer. Thus, the TEM micrographs only were used to measure the length of the supramolecular assembly. From Figure 5, we may note that there exists some linear structures with lengths in the range of 150–200 nm, which are joined together through approximately 60–80 units of the inclusion complex of **2** with a fullerene molecule. In turn, we infer that the average molecular weight of **3** is 250 kg mol^{−1}. On the other hand, SEM images showed the macrostructure of **3** was cylindrical, which is different from the small and irregular morphologies found for **2** with fullerene.

We chose pIRES2-EGFP plasmid as a substrate to investigate the DNA-cutting ability of the assembly. In the dark, DNA was not cleaved in the presence of **1**, **2**,

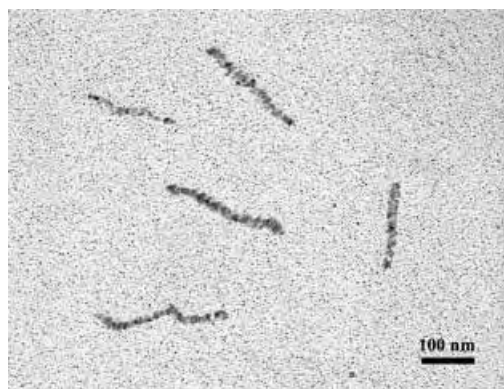


Figure 5. TEM images of **3**.

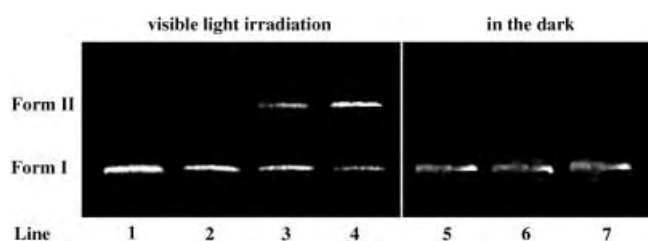


Figure 6. Agarose gel electrophoretic patterns of DNA and nicked DNA. The reaction samples contained $65 \mu\text{mol dm}^{-3}$ of pRES2-EGFP plasmid. Line 1: no reagent in 50 mM Tris-HCl buffer (pH 7.4). Lines 2 and 5: $20 \mu\text{mol dm}^{-3}$ of **1**. Lines 3 and 6: $20 \mu\text{mol dm}^{-3}$ of **2**. Lines 4 and 7: 73 mg dm^{-3} of **3** (corresponding to $20 \mu\text{mol dm}^{-3}$ of **2** and $20 \mu\text{mol dm}^{-3}$ of fullerene). Lines 1–4: incubated under visible light irradiation at 20°C for 6 h. Lines 5–7: incubated in the dark for 6 h. Electrophoresis was performed by using 1% agarose gel containing ethidium bromide ($0.5 \mu\text{g dm}^{-3}$).

or **3** (Figure 6, lines 5–7). Under visible-light irradiation, **2** showed some DNA-cleavage ability (line 3), which supports the argument that the “cationic” metallo bis(β -CD) unit is bound to the “anionic” DNA.^[24] Compound **1** showed no DNA-cleavage ability (line 2). Under the same conditions, **3** showed DNA-cleavage activity (line 4). The closed supercoiled (Form I) DNA was converted into the nicked circular form of DNA (Form II) by incubating under visible-light irradiation, which is attributed to photoinduced electron transfer from the guanine moieties to the fullerenes.^[4,24,25]

In conclusion, a novel supramolecular assembly has been synthesized by the intermolecular inclusion complexation of metallobridged bis(β -CD) and C_{60} . The supramolecular fullerene assembly mediated by the metallobridged bis(β -CD) shows both moderate water-solubility and effective DNA-cleavage ability under light irradiation, which has potential application in biological and medical chemistry.

Experimental Section

A mixture of **2** (0.1 mmol) and fullerene (C_{60} ; 0.1 mmol) were stirred together for three weeks in a mixture of toluene and DMF (v/v = 4:6; 200 mL), during which the deep purple homogenous solution turned deep brown. After the solvent had been removed under vacuum, the crude solid residue was dissolved in water and purified by column chromatography (Sephadex G-25, distilled deionized water as

eluent). Then membrane filtration over a polymer membrane with a molar mass cutoff of 3 kg mol^{-1} was used to obtain high molecular-weight aggregate **3** with a yield of 4%. FTIR (KBr): $\tilde{\nu}$ 3339, 2928, 1723, 1660, 1593, 1430, 1030, 945, 527 cm^{-1} ; $^1\text{H NMR}$ (300 MHz, $[\text{D}_6]\text{DMSO}$, TMS): δ = 3.5–3.8 (m, 12H), 4.8–5.0 (m, 14H), 5.7–6.0 (m, 28H), 7.68–7.71 (m, 2H), 7.94–7.96 ppm (m, 2H); $^{13}\text{C NMR}$ (75 MHz, $[\text{D}_6]\text{DMSO}$): δ = 60.2, 67.6, 72.2, 72.6, 73.3, 81.7, 102.1, 109.5, 125.7, 128.8, 131.8, 133.7, 142–146 ppm (weak); elemental analysis (%) calcd for $\text{C}_{150}\text{H}_{142}\text{O}_{68}\text{Se}_2\text{PtCl}_4 \cdot 6\text{H}_2\text{O}$: C 49.56, H 4.27; found: C 49.60, H 4.30.

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Direct Evidence for a Furtive State in the Degradation of Carbasalatum Calcium**

Philippe Ochsenbein, Michel Bonin, Olivier Masson, Denis Loyaux, Gervais Chapuis, and Kurt J. Schenk*

The soothing effects of the bark, leaves, and fruits of species of the *Spireæ* genus were known in a mystical, intuitive manner to our most distant ancestors.^[1] The knowledge was then formalized, compiled, and propagated throughout the world by the great physicians and pharmacologists, such as Hippocrates, Theophrastus, Dioscorides, and Galen. After this period, this blossoming area of therapeutics lay dormant for almost two thousand years and its renaissance only began in the early eighteenth century when the English pastor and naturalist Edward Stone published his case-study of the effect of willow bark on patients suffering from agues. Novel insights then followed in rapid succession: in 1838 the Italian chemist Rafelle Piria extracted the active component, salicylic acid (SA), in 1874 Hermann Kolbe published its total synthesis, and by 1899 three derivatives devoid of the unpleasant side effects of the pure acid had been discovered. The most useful of these, acetylsalicylic acid (ASA), turned out to be a wonder drug against a broad spectrum of aches: from fever and heart attacks through to inflammations and gout and nowadays is even used to prevent thromboses and embolisms. Recently, salicylates have also been reported to play a role in the reversal of obesity-induced insulin resistance.^[2]

However, since ASA is scarcely soluble in water (1 g in 300 ml), it deploys its beneficial effects only after having been attacked by the alkaline media in the duodenal region of the intestine.^[3] During these reactions it can cause gastric disturbances in sensitive patients. This can be remedied by elaborating derivatives that are more soluble in water and therefore more easily assimilated in the digestive tract.^[3,4] Indeed, improved water solubility and incorporation of biologically active transition metals such as Cu^[5] and Zn^[6] have often been jointly striven for. To date, about two dozen

[*] Dr. P. Ochsenbein
Sanofi-SynthéLabo
371, rue du Professeur Blayac
34184 Montpellier (France)
E-mail: philippe.ochsenbein@sanofi-synthelabo.com
Dr. M. Bonin, Prof. G. Chapuis, Dr. K. J. Schenk
LCr1-IPMC-EPFL
BSP Dorigny, 1015 Lausanne (Switzerland)
Dr. O. Masson
ESRF-BM16
6, rue Jules Horowitz, 38000 Grenoble (France)
Dr. D. Loyaux
Sanofi-SynthéLabo
371, voie No. 1-B.P. 137, 31676 Labège cedex (France)

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ASA complexes are known. An early commercialized example is carbasalatum calcicum (CC, $[\text{Ca}(\text{ASA})_2(\text{urea})]$) which is claimed to be amazingly soluble in water (25%,^[4] 231 mg mL⁻¹ of solution at 37°C^[7]), unlike other ASA complexes, for example, Cu_2ASA_4 .^[5] Even though CC has become renowned through the Pharmacopoeia Europea,^[8] the details of the solubility and other subtle points regarding its chemistry and physiology are still awaiting elucidation. However, few investigations have been devoted to this elucidation and only a few common analytical results are available. The elemental analysis is consistent with the empirical formula $\text{C}_{19}\text{H}_{18}\text{CaN}_2\text{O}_9$. The lines in the IR (KBr) trace ($\tilde{\nu}_{\text{h}} = 1760$ and 1730 (C=O); $\tilde{\nu}_{\text{as}} = 1400$ and 1590 (COO); $\tilde{\nu}_{\text{s}} = 1230$ and 1190 (COO) cm⁻¹) compare favorably with those of $\text{VO}^{2+}/\text{ASA}$ ^[9] and $[\text{Zn}(\text{ASA})_2] \cdot \text{H}_2\text{O}$ ^[6] and with those given in refs. [10,11]. The $[M+H]^+$ signal of CC cannot be found in FAB mass spectra, probably because Ca forms weak complexes. NMR spectra recorded in D₂O confirm the presence of ASA, but the indications for urea are marginal. All these results do not provide any definite corroboration of the nature of the complex. A solid-state ¹⁷O NMR investigation, possibly involving the use of coupling tensors, might offer such an answer but would require huge single crystals and appears to be a formidable task. Since not even tiny crystals are available (the Merck Index, 13th ed., 2001, talks about an amorphous powder as recently as 2001), we undertook a synchrotron powder diffractometry^[12] study, which furnished the proof-of-existence of CC through its crystal structure. Furthermore, we present here novel details concerning the degradation of CC in pure water,^[3,4,7] namely the isolation and structure of the furtive intermediate species acetylsalicylatum calcicum (AC). The high solubility of CC is also reassessed.

The well-established synthetic procedure^[8] yields CC as a white powder containing well-developed square platelets too tiny for single-crystal diffraction studies. Recrystallization of CC showed that a) slow diffusion of various poor solvents into aqueous solutions was rather unsuccessful, except that acetone gave rise to an oddity, namely colorless cuboctahedra that turned out to be ASA,^[13] and b) slow evaporation of a supersaturated (> 25%^[4,7]) aqueous solution did not afford crystals if the process, which takes a few hours, was allowed to go to completion. Spectroscopic analysis of the residues reveals that the deacetylation^[3,4] of ASA is almost complete at this stage.

In view of this chemical evolution of CC in water we decided to scrutinize its behavior in this solvent by optical microscopy. CC was added to water in a Petri dish until the crystals no longer dissolved but floated on the surface of the mother liquor. These undissolved grains of CC could easily be seen on the surface by means of a binocular microscope. Surprisingly, the grains then began to disappear and, successively, two new habitats nucleated in the heart of the mother liquor: thickish joists first and sturdy laths about half a minute later. After about 90 seconds of coexistence, the joists began to disappear, but, with swift action, crystals of this furtive form could be isolated and, as they were stable enough in air, analyzed by X-ray diffraction. They were found to be *catena*-bis(μ_2, η^2 -acetylsalicylato)-diaquo-calcium dihydrate (acetyl-

salicylatum calcicum, AC).^[14] Their structure (Figure 1) contains columns (rod group $P(12/c)1$) of edge-sharing CaO_8 polyhedra (approximate point group C_{2v}) embedded in a matrix of acetylsalicylato groups bound by van der Waals

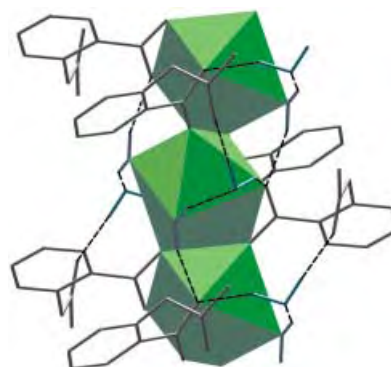


Figure 1. A [001] column of edge-sharing CaO_8 polyhedra (green) in acetylsalicylatum calcicum. Hydrogen bonds (black, dashed) between the bridging acetylsalicylato groups and coordinated (dark blue)/crystalline (turquoise) water molecules are also shown.

forces (shortest H...H contacts: 2.92–3.60 Å). These columns, separated by about 12.5 Å, are stabilized by extensive hydrogen-bonding interactions (normalized bond lengths^[15] of 1.77–2.00 Å and angles of 160–166°) between the coordinated water molecules, the crystalline water molecules, and the carbonyl oxygen atoms of the ester groups. A small decay in intensity occurred during the three to four days of data collection,^[14] which might arise from the adsorption of some moisture (the crystal surface turned white). This phenomenon is reminiscent of the problem mentioned by Lawrence^[3] in his patent. Indeed, he claimed a maximal number of water molecules of 3.5 for a synthesis leading to storable AC. In view of the presence of two molecules of water in our structure, we conject that 1.5 additional water molecules might be at the crystal surface. The laths, once dried, are also stable in air and have been shown to be ASA^[13] by single-crystal X-ray diffraction studies.

Since CC is instable in water it was studied by synchrotron powder diffractometry (Figure 2) on beamline BM16 at the European Synchrotron Radiation Facility. The successful solution of this structure (with 31 non-hydrogen atoms) shows that CC is indeed a unique phase,^[16] namely *catena*-bis(μ_2, η^2 -acetylsalicylato)- μ_2 -urea-calcium. CC^[16] (Figure 3) contains chains (rod group $P(1)2_1(1)$) of corner-sharing CaO_6 polyhedra (very approximate D_2 symmetry). These columns (separated by $c/2$) are cross-linked along the c axis by way of N–H...O=C bonds (N–O distances of 2.97–3.29 Å) to form semirigid (100) sheets held together by van der Waals forces only along the a axis. The ester groups in both acetylsalicylates are roughly at right angles (84 and 89°) with respect to their benzene rings, whereas the carboxylate groups form angles of 32 and 35° with the latter. For the major part, the freely refined distances in CC fall well within the bracket of distances (2.293–2.415 Å) found in the CCDC

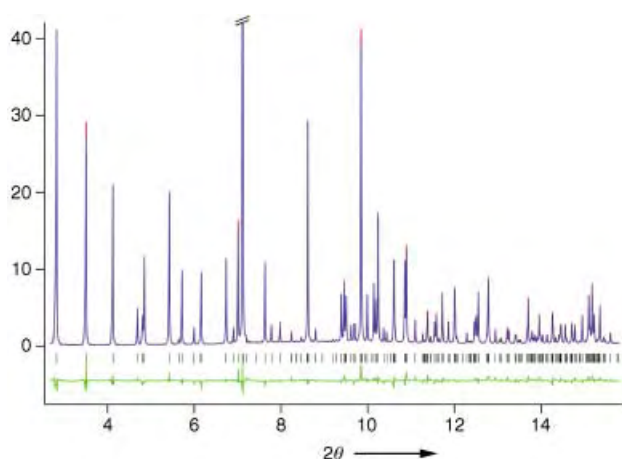


Figure 2. Excerpt from the observed (red), calculated (blue), and difference (green) profiles resulting from the Rietveld refinement for carbasalatum calcium. The black marks indicate reflection positions.

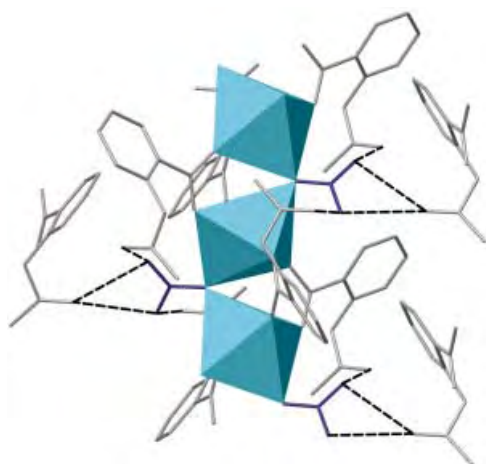


Figure 3. A [010] column of corner-sharing CaO_6 octahedra (turquoise) in carbasalatum calcium. Hydrogen bonds (black, dashed) between the bridging acetylsalicylate groups and coordinated urea molecules (dark blue) are also shown.

database.^[20] Our Ca-O(urea) distances, however, are the longest ones reported (2.503(6) and 2.519(6) Å). The extreme values in CC might arise from the fact that the urea molecule reconciles several roles, namely bridging the two CaO_6 octahedra, participating in hydrogen-bonding interactions with the acetyl ester moiety, and optimally embedding itself in the general packing of the structural components. Thus, the Ca-O(urea) bond acquires an almost van der Waals like character which we believe to play an essential role in the solvation behavior of CC. We should like to emphasize that this uncommon crystallization sequence could not have been established without the help of the human eye, much as in the recent report^[21] about concomitant polymorphs. Indeed, an attempt to corroborate the degradation of CC in a heat-flow calorimeter coupled with a focused-beam reflectance-meas-

urement probe failed;^[22,23] no optical or thermal signal of the intermediate AC could be detected.

Finally, we endeavor to explain the various phenomena observed while crystallizing CC. It appears that in a super-saturated aqueous solution, coordinated urea in CC rapidly becomes unstable and is replaced by two water molecules. This instability might be indicated by the particularly long Ca-O(urea) distances. This hypothesis is further endorsed by the complexation constants of the exchange $\text{Ca}^{2+} + 2\text{urea} \rightarrow \text{Ca(urea)}_2$ for which $\log \beta_2$ is -0.6 in 4M NaClO_4 .^[24] The exchange is reinforced by the large number of potential hydrogen bonds with the water molecules. However, the thus-formed AC does not survive for a long time either and the acetylsalicylate groups are quickly replaced by water molecules. The high solubility of CC in water is definitely relegated to the realm of myth and CC therefore joins the ranks of other ASA complexes. Indeed, with the Ca^{2+} ion being a hard acid and H_2O being a hard base, the dissolved species is most likely $[\text{Ca}(\text{H}_2\text{O})_6]^{2+} \cdot 2\text{ASA}$.

We have added two pharmaceutically important structures to the class of complexes containing ASA (until now only the structure of $[\text{Cu}^{\text{II}}(\text{ASA})_2]$ ^[25] had been reported). Besides the mere knowledge of the molecular arrangement in CC and AC, the careful observation of the solvation behavior of CC has provided us with a better understanding of certain crucial features of this complicated quaternary system. Finally, this study has confirmed the value of a simple, regrettably neglected, tool, namely careful observation of crystals with a microscope, which has led to the detection, isolation, and characterization of a furtive species.

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Keywords: bridging ligands · calcium · hydrogen bonds · optical microscopy · structure elucidation

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Dissolution at the Nanoscale: Self-Preservation of Biomaterials**

Ruikang Tang, Lijun Wang, Christine A. Orme,
Tammy Bonstein, Peter J. Bush, and
George H. Nancollas*

Despite the complicated hierarchical structures of natural materials such as shells, bones, and teeth, their basic building blocks are generally in the nanometer size range^[1–6] to ensure optimum physical and biological functions.^[5–7] Herein we reveal that this nanostructural optimization also confers on the biomaterials remarkable characteristics of dynamic preservation. Generally, dissolution of minerals is regarded as a spontaneous reaction in which all the solid phase can be dissolved in undersaturated solutions. However, it has been found that demineralization reactions actually involve particle-size-dependent critical conditions of energetic control at the molecular level.^[8–10] It suggests that the dissolution of crystallites may be inhibited or even suppressed when their sizes fall into the same range as that of a certain critical value—always at a nanoscale level. Therefore, these nanostructured biomaterials can be resistant to dissolution on account of their sizes and can remain relatively stable in the biological milieu even though the latter may be undersaturated. This new lesson from nature enriches our understanding of nanostructured materials and biological demineralization.

Enamel layers at tooth surfaces are among the hardest biological tissues.^[4,11,12] In this Communication, enamel is selected as an example in the context of nanodemineralization since it is highly mineralized and exhibits features that are close to pure synthetic apatites with about 97 % mineral phase and about 3 % water and organic matrix.^[11–13] Scanning electron micrographs (SEM) of enamel surfaces prior to significant dissolution (Figure 1a) show the well-organized

[*] Dr. R. Tang, Dr. L. Wang, Prof. G. H. Nancollas

Department of Chemistry
University at Buffalo

The State University of New York
Buffalo, NY 14260 (USA)

Fax: (+1) 716-645-6947

E-mail: ghn@buffalo.edu

Dr. C. A. Orme

Department of Chemistry and Materials Sciences

Lawrence Livermore National Laboratory
Livermore, California 94550 (USA)

Dr. T. Bonstein, P. J. Bush

Instrumentation Center, School of Dental Medicine
University at Buffalo

The State University of New York

Buffalo, New York 14260 (USA)

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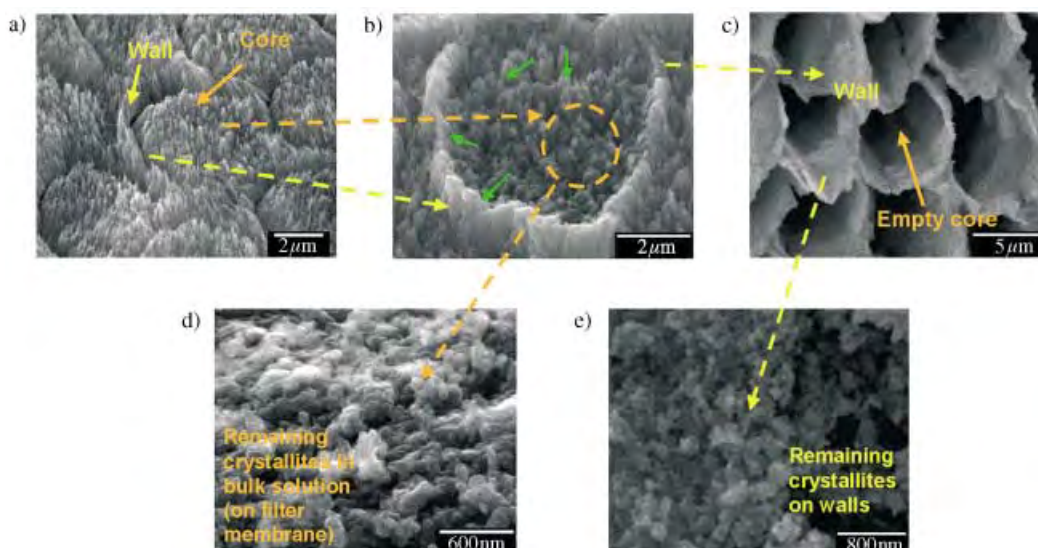


Figure 1. Demineralization of dental enamel: yellow and orange labels mark the dissolution of the walls and cores, respectively, thus showing that they undergo similar dissolution processes. a) Well-organized rod structures on mature human enamel surfaces: both the walls and cores are composed of numerous needlelike apatites that have the same chemical and physical properties. However, the crystallites in the cores are oriented perpendicular to the enamel surface while those on the walls are inclined by 10–40°. b) During dissolution, crystallites become smaller and nanosized apatite particles (shown by green arrows; see also Figure 2) are formed on both walls and cores. c) After 7 days of dissolution, the cores are emptied but the walls remain. d) Nanosized apatite particles collected from the bulk solution by filtration (Nucleopore N003 filter membrane) at the end of dissolution experiment. These particles have escaped from cores and walls (Figure 1 b) but are resistant to further dissolution even though the solution is undersaturated. e) SEM of the wall at higher magnification; nanosized apatite residues, retained on the wall surfaces, are kinetically protected against further dissolution.

rodlike structures. Previous^[4,12] studies and our current results of in situ electron dispersive spectroscopy (EDS), X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS) and infrared (IR) indicate identical chemical and crystallographic properties of the minerals in cores and on walls, both being composed of numerous needlelike inorganic apatites, but the crystallites in the cores are oriented perpendicular to the enamel surface, while those on the walls are inclined by 10–40°. During enamel maturation, it has been suggested that the proteins (mainly amelogenins) that induce/control the crystallization of apatite are almost completely degraded or removed.^[4,14,15]

Under physiological conditions of temperature (37°C, $T = 310$ K) and ionic strength (0.15 M), it is found that demineralization of enamel surfaces will only take place at relatively high undersaturations ($S < 0.4$, S is undersaturation, see Experimental Section) and at lower pH (<5.5); otherwise, no dissolution can be experimentally detected. In this study, the demineralization of enamel has been investigated over an extended experimental period of 1–10 day(s) at $S = 0.10$ and pH 4.5 by using a nanomolar-sensitive constant-composition (CC) technique^[16] combined with in situ atomic force microscopy (AFM). It is found that apatite crystallites both in the cores and on the walls undergo similar demineralization processes (Figure 1). Figure 1 b shows walls and cores at an intermediate stage during the dissolution reaction, in which the nanosized apatite particles (≈ 100 nm) that result from dissolution can be seen on both surfaces (Figure 2). However, further dissolution of these nanosized particles was suppressed: 1) Those from cores were released directly into the solution by fluid diffusion flux, thus resulting in empty cores

(Figure 1 c); these particles were stable against dissolution in the bulk solution (Figure 1 d; it should be noted that they are of similar size to those in Figure 1 b, ≈ 80 –100 nm). 2) The small particles from the walls also did not dissolve but they adhered to the wall framework. It has been shown that almost all of the organic components in enamel are found in the

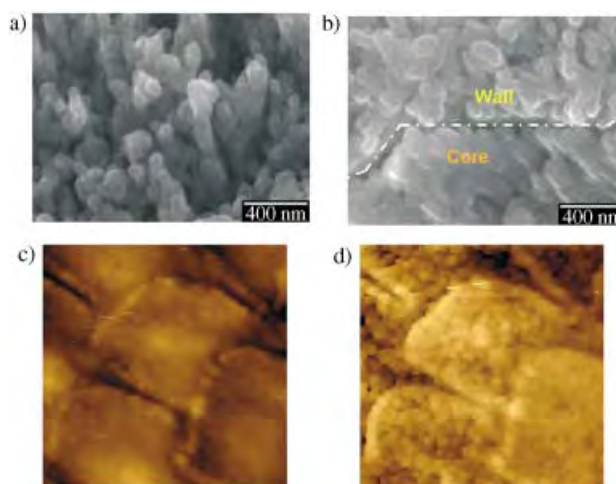


Figure 2. Enlargement of Figure 1 b, both the core (a) and wall surfaces (b) are covered by numerous apatite particles of similar nanosize following demineralization. This phenomenon is also observed and confirmed by in situ AFM studies, c) and d) show the enamel surfaces in solution near the beginning of the dissolution experiment and after 4 h of reaction, respectively. The formation of the nano particles (2 d, 100–200 nm) is observed in this real time experiment. The AFM image scales are $8 \times 8 \mu\text{m}$.

walls,^[17] which remain intact at the end of the dissolution experiments (Figure 1 c). High magnification SEM confirms that the remaining wall surfaces are actually covered by numerous small crystallites 100 ± 20 nm in size (Figure 1 e). This demineralization of enamel surfaces was also confirmed by in situ AFM studies (Figure 2 c and d); the formation and release of the nanoparticles during the dissolution reaction were observed in real time. All the results of in situ EDS, XRD, XPS, and IR studies indicate that these residual crystallites are apatitic phases similar to those prior to demineralization. The apatitic residues of the demineralization reactions, both from walls and cores, have the same size distribution range (Figures 1 d,e and 2) and are stabilized against further dissolution in the undersaturated aqueous solution.

To exclude the influence of complicating biological factors such as the possible presence of organic matrix components, CC dissolution studies have also been made by using synthetic hydroxyapatite ($\text{Ca}_5(\text{PO}_4)_3\text{OH}$) samples of high purity ($>99.4\%$) with needlelike morphology (400–600 nm length, 60–100 nm width). Similar reproducible self-inhibited dissolution was observed with these nanosized crystallites. At a high undersaturation ($S=0.02$), they undergo rapid dissolution ($4.0 \pm 0.3 \times 10^{-6} \text{ mol m}^{-2} \text{ min}^{-1}$) and almost all of solid phase is dissolved in less than 3 h. However, at lower undersaturation, the rates decrease during the reaction; at $S=0.580$, the initial rate, $3.2 \pm 0.2 \times 10^{-7}$, decreases to $1.6 \pm 0.1 \times 10^{-7}$ and $0.8 \pm 0.1 \times 10^{-7} \text{ mol m}^{-2} \text{ min}^{-1}$ at 50 min and 90 min, respectively. The CC dissolution curves reach plateaux prior to complete dissolution (Figure 3 a), thus indicating the creation of metastable states in which the reaction is effectively terminated even though the apatite crystallites remain in contact with the undersaturated solutions. This result is similar to the observations made during enamel demineralization. The residual crystallites are confirmed to be of the same chemical compositions as the initial seed crystals. At $S=0.315$, 90% (by mass) of the seed crystals are dissolved and this decreases markedly with decreasing undersaturation; at an undersaturation of 0.580, only a relatively small fraction (38%) of the apatite crystallites undergo dissolution before the reaction is effectively stopped or reduced to a very low rate. No immediate CC dissolution of the seed crystallites could be detected at lower undersaturations (e.g. $S=0.828$, Figure 3).

Clearly, this dissolution termination is a kinetic phenomenon and cannot be attributed to reaction retardation as a result of surface modification by additives. However, it can be explained in terms of a dissolution model that incorporates particle size considerations. It has been suggested, and confirmed by experiment, that demineralization of sparingly soluble salts such as apatite is generally initiated and accompanied by the formation and development of pits on the crystal surfaces and that the dissolution rates are also determined by the pit densities and spreading velocities.^[8–10,18,19] Analogous to the formation of two dimensional nuclei/hillocks for crystal growth, in dissolution the rate of

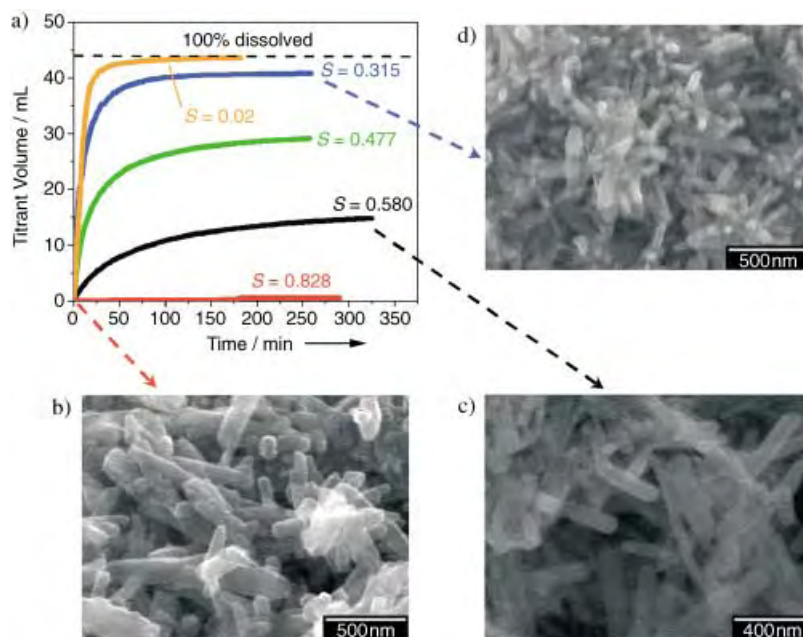


Figure 3. In vitro CC dissolution of synthetic hydroxyapatite. a) CC plots of titrant volume against time at different undersaturations. The red lines indicate the titrant volumes for full dissolution of the added seeds. Only at very high undersaturation ($S=0.02$), does the dissolution go to completion. The dissolution rates decrease with time and eventually, only a fraction of the added seeds undergo dissolution before the rates approach zero. Near equilibrium ($S=0.828$) no dissolution can be detected in the undersaturated solutions. For the smaller hydroxyapatite seeds (length, 200–300 nm and width, 50–80 nm), no CC dissolution can be detected at an even higher undersaturation of $S \geq 0.720$. This value for enamel is $S \geq 0.4$, showing the much less extensive dissolution. b) SEM of seed crystals and c) crystallites remaining at the end of dissolution experiments at $S=0.580$ and d) $S=0.315$. The results are similar to those for enamel dissolution.

step movement from a pit of radius r can be obtained from treatments similar to the model of Burton, Cabrera, and Frank,^[8–10,20,21]

In Equation (1), r^* , the critical radius for the formation of a two-dimensional pit/dissolution step, is given by Equation (2)^[20,21]

$$R(r) = R_{\infty} \left[1 - \frac{e^{(1-S)r^*/r-1}}{e^{1-S}-1} \right] \approx R_{\infty} \left(1 - \frac{r^*}{r} \right) \quad (1)$$

$$r^* = \frac{\gamma_{SL} \Omega}{|\Delta g|} \quad \text{and} \quad \Delta G = k T \ln S \quad (2)$$

in which, γ_{SL} is interfacial tension, k , the Boltzmann constant, Ω , the area occupied by each dissolution unit and ΔG , the change of the Gibbs free energy for dissolution. It has been shown that only pits larger than r^* , which provide the active dissolution sites, contribute to the reaction. In Equation (1), R_{∞} is the velocity of dissolution steps at $r \rightarrow \infty$. It follows that when r is closer to r^* , its dissolution rate decreases and approaches zero when $r \rightarrow r^*$. When the dimensions of the crystallites (l) are of the same order as r^* (l is less than $20r^*$), it can be assumed that the formation of active pits becomes difficult on such a small crystal faces (e.g. formation of new pits with radius r^* on surfaces $2-5r^*$ in size) and their enlargement is strongly inhibited by the limitation of pit/crystal size (the pit sizes, r , are confined by crystallite size, l).

With decreasing active pit densities (which always decrease during dissolution,^[8,19] especially when $l \rightarrow r^*$) and pit sizes ($r \propto l$), dissolution is retarded or even undetectable by CC if etch pits cannot grow to values larger than r^* . This is consistent with the CC dissolution results, which show that for crystallite sizes approaching the critical values, the rates (slopes of the CC curves in Figure 3a) decrease markedly with time despite the sustained driving force. By introducing the apatite interfacial tension, $\gamma_{SL} \approx 105 \text{ mJ m}^{-2}$,^[22,23] and lattice structure ($a = b = 0.942 \text{ nm}$, $c = 0.685 \text{ nm}$), one can be estimated from Equation (1) that the value of r^* is about 20–50 nm for enamel apatites under these dissolution conditions. It is significant that the observed dissolution apatite residues in Figure 1d have very similar size distributions.

Lasaga and Lutge as well as our group have suggested that if the undersaturation (driving force for demineralization) is low (e.g. $S = 0.828$ for pure hydroxyapatite in the present work), pits will not open up and defect sites will not lead to stepwaves that form far from the localized etch pits due to the low driving force.^[8,10,18] Alternately, in terms of crystallite sizes, due to the high value of r^* , active dissolution pits (250–500 nm at $S = 0.828$) are close to the crystallite size (400–600 nm). Here, we show that, although high undersaturations can induce the initial dissolution, the decreasing crystallite size also leads to the possible inhibition and even suppression of dissolution when it approaches the critical value for the active pit, r^* . The phenomena of dissolution termination and their “critical” sizes are not arbitrary; rather, these self-tuned and dissolution-insensitive effects for tiny crystallites and biomaterials such as tooth enamel occur specifically at the nanoscale.

The critical size of pits, r^* , is a function of undersaturation [Eq. (2)] and the residual apatite crystallites have sizes similar to their critical dissolution values, assuming the mean crystal size of the dissolution residues, l , is in some manner proportional to r^* . The direct relationship between the sizes of crystallites remaining after dissolution and the undersaturations can be studied by precisely controlled CC experiments and SEM (Figure 3). These confirm that the size decreases with increasing undersaturation. In terms of a 3D macroscale dissolution model for pure hydroxyapatite,^[24,25] the extent of dissolution and the particle critical sizes can be estimated from the CC kinetics results.^[8] From Equation (2), the ratios of the critical sizes for S of 0.580, 0.477, and 0.315 are 2.11:1.55:1.00, respectively. The results from CC dissolution kinetic experiments, 1.97:1.60:1.00, are in excellent agreement. The data are also supported by scanning electron micrographs (Figure 3) which show that although the initial seed crystals are not uniform in size, the small nanosized dissolution residues (Figure 3d) have similar size distributions (length $120 \pm 20 \text{ nm}$, width $40 \pm 10 \text{ nm}$). It is interesting to speculate that this marked crystallite size effect in the dissolution reactions could provide the simplest route for the formation of nanoparticles of sparingly soluble salts. Their sizes can be readily adjusted by changing the dissolution conditions.

Dissolution experiments by using smaller synthetic apatite crystallites (length, 150–250 nm, width, 20–50 nm) give similar results but the unusual dissolution phenomenon is

magnified as the sizes approach the critical conditions; no dissolution can be detected even at higher undersaturation ($S = 0.720$). In contrast to conventional models (e.g., Ostwald–Freundlich) which assume smaller particles have higher solubilities and therefore dissolve faster,^[26] at $S = 0.580$, the CC dissolution rates of synthetic apatite are $2.2 \pm 0.2 \times 10^{-7} \text{ mol min}^{-1} \text{ m}^{-2}$ for smaller crystallites (length, 150–250 nm and width, 20–50 nm), whereas, larger particles (length 500–600 nm and width 60–100 nm) exhibit greater dissolution rates, $3.2 \pm 0.2 \times 10^{-7} \text{ mol min}^{-1} \text{ m}^{-2}$. Our dissolution model would suggest that since the sizes of the smaller finely divided apatite crystallites are closer to the critical values, the dissolution rate is more readily self-inhibited as predicted by Equation (1) and stronger dissolution conditions must be used to induce demineralization. Similar results of size-dependent dissolution kinetics have also been shown in the case of another calcium phosphate, brushite ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$), which further suggest that traditional dissolution models may be applied for larger crystallites ($> 100r^*$).^[9] Our model also explains the generally observed existence of nanoparticles in aqueous solutions: they are dynamically stable despite being thermodynamically metastable.

In a large number of biological systems, the mineral phases are composed of tiny sparingly soluble crystallites tens to thousands of nanometers in size; it appears that this is the most natural selection. It is well-known that there is a close relationship between solubility and γ_{SL} . During dissolution, some neighboring ions on the surface are replaced by water molecules to form units that escape into the bulk solution. Higher values of γ_{SL} indicate a greater difficulty in forming such an interface between the solid and aqueous phases. Thus, sparingly soluble salts in aqueous solution always have much higher interfacial free-energy values than soluble salts, thus resulting in greater values of r^* (10–100 nm). Biological minerals have sizes in this critical range and may be protected from dissolution since the reaction in the metastable region is significantly inhibited [Eq. (1)]. This accounts for the observation that the dissolution of biomineral powders is always slower than that of synthesized relatively large crystallites after normalization for specific surface area. In addition to these interesting conclusions from CC dissolution studies, it is also widely accepted that the growth of tiny apatite seeds is rarely observed at low supersaturations ($S < 4$)^[27] due to extremely slow growth rates, which can again be attributed to a kinetic size effect.

For many systems in nature, nanosized mineral particles constitute the basic building blocks; typically tens to hundreds of them can be tuned to give the self-assembled mineral a remarkable “biological function and activity”. However, the present results show that the biomaterials become insensitive to dissolution or even crystal growth at the nanoscale level. An excellent example is seen in studies of caries lesion formation. Enamel surfaces remain undissolved in water (pH 5.5–5.8) in spite of the undersaturation. Caries (dissolution) is only induced at localized sites where bacteria have produced acidic conditions. The mineral crystallites can be stabilized in the fluctuating physiological fluids, thus exercising a degree of self-preservation as the result of this kinetic size-effect at the nanoscale level. Furthermore, this nanodissolution behavior

of nanoparticles is also of great significance for the solvent stability and reactivity in working nanoparticle-based structures and sensors.

Experimental Section

Constant Composition (CC)

Dissolution experiments were made in magnetically stirred double-walled pyrex vessels. The undersaturated reaction solutions (200 mL) were prepared by mixing calcium chloride and potassium dihydrogen phosphate with sodium chloride to maintain the ionic strength, I , at 0.15 mol L^{-1} . The pH was adjusted to the desired value, 4.50 ± 0.01 . Nitrogen, saturated with water vapor at 37°C , was purged through the reaction solutions to exclude carbon dioxide. The dissolution reactions were initiated by the introduction of either enamel blocks ($\approx 4 \times 4 \times 0.2 \text{ mm}$) or apatite seed crystallites (10.0 mg). Titrant addition was potentiometrically controlled by glass and Ag/AgCl reference electrodes. During dissolution, the electrode potential was constantly compared with a preset value and the difference, or error signal, activated a motor-driven titrant buret. Thus a constant thermodynamic dissolution driving force was maintained. Concentrations of the titrant solutions are given by Equations (3) and (4),

$$T_{\text{NaCl}} = W_{\text{NaCl}} + W_{\text{KOH}} + 6 C_{\text{eff}} \quad (3)$$

$$T_{\text{HCl}} = 14 C_{\text{eff}} - W_{\text{KOH}} \quad (4)$$

in which W and T are the total concentrations in the reaction solutions and titrants, respectively, and C_{eff} is the effective titrant concentration with respect to hydroxyapatite. During the reactions, slurry samples were periodically withdrawn, filtered and the solutions analyzed for calcium and phosphate. The total concentrations of calcium and phosphate remained constant to within $\pm 1.5\%$ during the experiments.

The dissolution flux rate, J , can be calculated by using Equation (5),

$$J = \frac{C_{\text{eff}}}{A_T} \frac{dV}{dt} \quad (5)$$

in which dV/dt is the gradient of the CC titrant curves and A_T is the surface area. The initial value of A_T was calculated from the specific surface area of the seeds, which was determined by BET nitrogen adsorption (30:70 N_2/He , Quantasorb II, Quantachrome); subsequent values during dissolution were estimated from a well-established 3-dimensional dissolution model for HAP.^[28]

In situ atomic force microscopy: AFM images of enamel surfaces were collected in contact mode by using a Digital Instruments Nanoscope III microscope. All images were acquired in height and deflection modes by using the lowest tip force possible to reduce the tip-surface interaction. The enamel was anchored inside the fluid cell and undersaturated solution was passed through while the images were taken.

Scanning electron microscopy: Samples, under vacuum, were sputter-coated with a thin carbon deposit to provide conductivity, and then examined by using a field-emission SEM (Hitachi S-4000), typically at 20 or 30 KeV.

Supersaturation and solution speciation: The undersaturation, S , is given by Equation (6),

$$S = \left[\frac{IP}{K_s} \right]^{1/9} \quad (6)$$

in which, IP is ionic activity product and the solubility activity product of apatite, K_s , is 2.35×10^{-59} . The solution is undersaturated when $S < 1$; higher values reflect lower undersaturations. Solution speciation calculations were made by using the extended Debye-Hückel equation proposed by Davies from mass-balance expressions for total calcium and total phosphate with appropriate equilibrium

constants by successive approximation for the ionic strength. Values of the dissociation constants of phosphoric acid were $K_1 = 6.22 \times 10^{-3}$, $K_2 = 6.59 \times 10^{-8}$, and $K_3 = 6.6 \times 10^{-13} \text{ mol L}^{-1}$, and the water ionic product was $2.40 \times 10^{-14} \text{ mol}^2 \text{ L}^{-2}$. The formation constants for the ion pairs $\text{CaH}_2\text{PO}_4^+$, CaHPO_4 , CaPO_4^- , and CaOH^+ were taken as 28.1, 589, 1.40×10^6 , and 25 L mol^{-1} , respectively.

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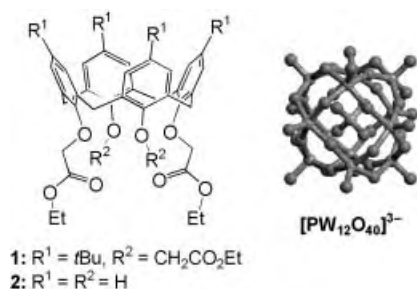
Keywords: apatites · bioinorganic · biomineralization · enamel · nanostructures

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Porous Organic–Inorganic Assemblies Constructed from Keggin Polyoxometalate Anions and Calix[4]arene–Na⁺ Complexes: Structures and Guest-Sorption Profiles**

Yusuke Ishii, Yasumasa Takenaka, and Katsuaki Konishi*

The rational design of crystalline solids by self-organization of multiple molecular components is one of the interesting subjects in supramolecular chemistry, as it allows a defined spatial arrangement of the components and the formation of a diverse range of three-dimensional structures with unique functions.^[1] In particular, the combination of organic compounds and inorganic metal clusters is interesting owing to their inherently different natures and possible synergetic effects in the crystal lattice. Polyoxometalates, anionic early transition metal oxide clusters, are attractive inorganic building blocks because of their discrete structures and notable acid/base, redox, and photochemical properties, which lead to a vast range of applications.^[2,3] Herein we report two porous hybrids assembled from a Keggin polyoxotungstate sodium salt (Na₃PW₁₂O₄₀) and calix[4]arene derivatives (**1**, **2**) by host–guest interaction between the



organic hosts and the sodium ion.^[4] We also demonstrate the guest-sorption capabilities of these porous materials.

Solutions of **1** in CHCl₃ and Na₃PW₁₂O₄₀ in MeOH were mixed and allowed to stand at room temperature to give

single crystals of the 3:1 assembly [1-Na]₃[PW₁₂O₄₀] (**3**). X-ray crystallographic studies revealed the formation of an infinite ionic crystal with a cubic crystal system, which contained a head-to-tail columnar array of the 1-Na⁺ complexes as a fundamental component. As shown in Figure 1 a, the columns

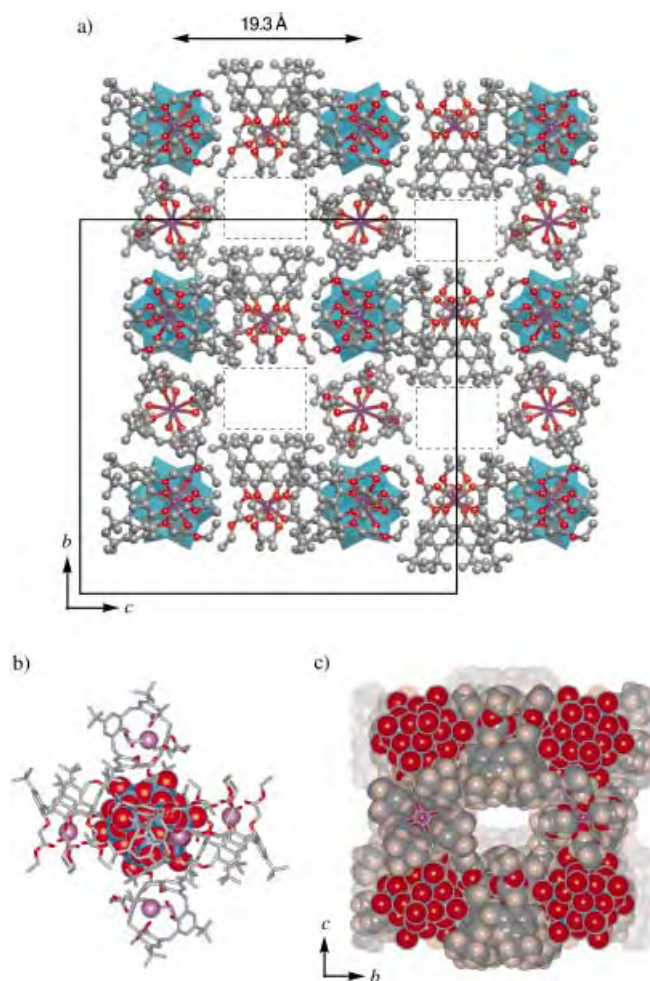


Figure 1. Crystal structure of **3** with solvent molecules omitted. a) A view of the packing structure along the *a* axis illustrating orthogonal stacking of the 1-Na⁺ columns running along the *a*, *b*, and *c* axes. b) Octahedral orientation of 1-Na⁺ units around a cluster anion. c) A cross section of the microchannel viewed along the *a* axis. The black lines in (a) indicate the unit cell, and the dotted lines the microchannels.

propagate in three directions along the *a*, *b*, and *c* axes and are stacked alternately in orthogonal configurations to generate a three-dimensional gridlike framework, which serves as a scaffold for the α-[PW₁₂O₄₀]³⁻ anions. One anion is surrounded by six 1-Na⁺ units, whereby no bonding interactions between sodium and cluster oxygen atoms are observed (Figure 1 b).^[5] The side faces of each calix[4]arene unit are involved in weak intermolecular interactions with the anions (e.g., van der Waals forces).^[6] Packing of the cationic and anionic components in this manner leaves micropores as the residual space. As shown in Figure 1 a and c, cavities with sizes of about 6 × 9 Å are created between four calix[4]arene units, which extend along the *a* axis to form channels. Channels are

[*] Y. Ishii, Dr. Y. Takenaka, Prof. Dr. K. Konishi
PRESTO, Japan Science and Technology Agency (JST), and
Division of Material Science
Graduate School of Environmental Earth Science
Hokkaido University
North 10 West 5, Sapporo 060–0810 (Japan)
Fax: (+81) 11-706-4538
E-mail: konishi@ees.hokudai.ac.jp

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also formed in the *b* and *c* directions, and these tridirectional channels intersect each other to form a three-dimensional microporous network. The micropores are occupied by diffuse solvent molecules, and the solvent-accessible volume of the unit cell was estimated, (PLATON program^[7]) to be 19624 Å³, which is approximately 34% of the unit-cell volume (57066 Å³).

A porous structure was also observed for the assembly with calix[4]arene **2** ([2-Na]₃[PW₁₂O₄₀], **4**), but the fundamental packing profile and the pore characteristics are rather different. As shown in Figure 2a, the crystal structure is constructed by alternating stacking of **2**-Na⁺ and α-[PW₁₂O₄₀]³⁻ layers on the *bc* plane, whereby one-dimensional channels are created along the [101] direction. The size

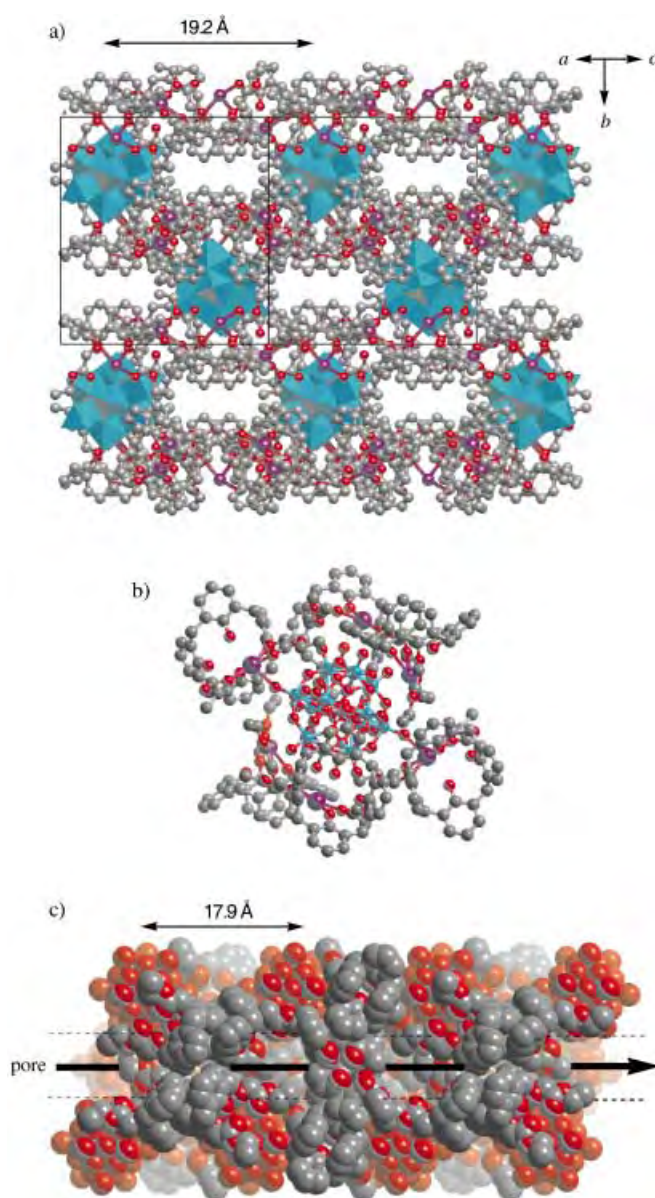


Figure 2. Crystal structure of **4** with hydrogen atoms and solvent molecules omitted. a) View of the packing structure along the 101 direction. b) Orientation of **2**-Na⁺ units around a cluster anion. c) Top view of the microchannel with the anion units above the pore omitted for clarity. The lines in c) represent the pore rims.

and volume percentage of the channels were estimated to be about 4 × 8 Å and 15%, respectively. The orientation of **2**-Na⁺ around the anion is also different. Two of the six calix[4]arene units surrounding one cluster anion are linked to the anion by Na–O coordinative bonds (Na–O 2.434 Å) to form a 1:2 molecular fragment, while the other four undergo only weak intermolecular interactions with the anion (Figure 2b).^[8] The anion surfaces are not completely covered by the calix[4]arene units and are partially exposed to the micropore. Accordingly, as shown in the top view of a microchannel (Figure 2c), the pore wall is constructed from both calix[4]arene and anion units. This contrasts with **3**, in which the anions are completely shielded by the calix[4]arene units.

These porous architectures containing polyoxometalates prompted us to investigate their guest-sorption capabilities, since such polyoxometalate-based materials have potential for selective reactions and recognition.^[9,10] After evacuation at 100°C for 2 h, the crystal of **4** showed a powder XRD profile identical to that simulated from the single-crystal data (Figure 3b, I and II), that is, the porous structure shown in Figure 2 was maintained after thermal pretreatment.^[11] This pretreated solid efficiently sorbed small alcohols in the one-

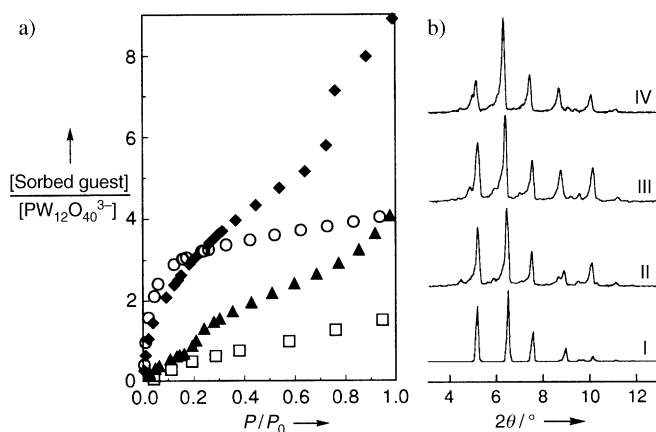


Figure 3. a) Sorption isotherms of **4** for ethanol (○, 298 K), methanol (◆, 288 K), water (▲, 298 K), and 1-butanol (□, 303 K). b) XRD profiles of **4** simulated from the single-crystal data (I), before (II) and after (III) ethanol sorption, and after two ethanol sorption/desorption cycles (IV).

dimensional micropores. For example, as shown in Figure 3a, the sorption isotherm of ethanol showed a sharp rise in the low-pressure region, characteristic of sorption in micropores.^[12] The sorption/desorption cycle occurred in a completely reversible fashion, and the powder XRD profiles remained unchanged throughout the cycle (Figure 3b).^[13] Methanol also showed a reversible sorption/desorption isotherm with retention of the porous structure of the sorbent, but also a second increase in the high-pressure region in addition to the initial sharp increase in the low-pressure region. Interestingly, these alcohols showed higher binding activities than water, which has an inherently strong affinity towards the parent salt Na₃PW₁₂O₄₀. This unique selectivity may arise from the amphiphilic character of the pore wall of **4**, which is constructed from both hydrophobic calix[4]arene and

hydrophilic $[\text{PW}_{12}\text{O}_{40}]^{3-}$ units. The steric bulk and/or the hydrophobic/hydrophilic balance of guests also appear to be important factors: Higher alcohols such as 1-butanol showed small binding activities.

Assembly **3** also sorbed alcohols, but the broad powder XRD pattern after guest sorption indicated an amorphous character. The broad diffraction pattern did not change on evacuation of the amorphous sample. The strong binding of the guests to the anionic moiety may disturb the orientation of the calix[4]arene units in the original crystalline sorbent (Figure 1) and thus cause irreversible destruction of the crystal lattice.

In conclusion, we have presented two porous organic–inorganic assemblies composed of calix[4]arene– Na^+ complexes and polyoxotungstate anions. To the best of our knowledge, assembly **4** is the first porous calixarene-based ionic material to display reversible guest sorption with retention of the original framework.^[14] Exploitation of the spatially restricted channels for catalysis is worthy of further investigation.

Experimental Section

3: $\text{Na}_3\text{PW}_{12}\text{O}_{40}$ (198 mg, 0.067 mmol) in EtOH (10 mL) was mixed with **1** (200 mg, 0.20 mmol) in CHCl_3 (10 mL), and the resulting clear solution was allowed to stand at room temperature for 7 days to give colorless transparent cubic crystals, which were collected by filtration (325 mg, 82 %). Elemental analysis (%): calcd for $[\text{1-Na}]_3[\text{PW}_{12}\text{O}_{40}] \cdot 6\text{H}_2\text{O} \cdot 2\text{CHCl}_3$: C 34.85, H 4.08, Cl 3.39; found: C 34.97, H 3.86, Cl 3.87.

4: $\text{Na}_3\text{PW}_{12}\text{O}_{40}$ (246 mg, 0.083 mmol) in CH_3OH (20 mL) was mixed with **2** (150 mg, 0.25 mmol) in CHCl_3 (20 mL), and the resulting clear solution was allowed to stand at room temperature for 2 days to give orange rectangular crystals, which were collected by filtration (278 mg, 70 %). Elemental analysis (%): calcd for $[\text{2-Na}]_3[\text{PW}_{12}\text{O}_{40}] \cdot 2\text{H}_2\text{O} \cdot \text{CHCl}_3$: C 26.77, H 2.33, Cl 2.17; found: C 26.41, H 2.39, Cl 2.95.

Crystal data for **3**: $\text{C}_{182}\text{H}_{320}\text{Cl}_6\text{Na}_3\text{O}_{131}\text{PW}_{12}$, cubic, space group $Ia-3$ (no. 206), $a = b = c = 38.5884(9)$ Å, $\alpha = \beta = \gamma = 90^\circ$, $V = 57460.6(23)$ Å³, $Z = 8$, $\rho_{\text{calcd}} = 1.647$ g cm⁻³, $T = 153.1$ K, $2\theta_{\text{max}} = 55^\circ$, $\mu(\text{MoK}\alpha) = 4.935$ mm⁻¹, 19255 reflections measured, of which 11877 were unique ($R_{\text{int}} = 0.046$). The carbon atoms of the *t*Bu group (C16–C18, C32–C34) were disordered over two positions and hence were refined isotropically at fixed positions with occupation factors of 0.5. The oxygen atoms of water molecules (O15–O40), which were disordered over multiple positions, were refined isotropically at fixed positions with appropriate occupation factors without hydrogen atoms. Final R and R_w values were 0.046 and 0.058, respectively, for 6774 observed reflections ($I > 5.0\sigma(I)$) and 467 variable parameters.

Crystal data for **4**: $\text{C}_{108}\text{H}_{109}\text{Cl}_3\text{Na}_3\text{O}_{66}\text{PW}_{12}$, monoclinic, space group $C2/c$ (no. 15), $a = 36.395(8)$, $b = 20.451(4)$, $c = 19.229(4)$ Å, $\beta = 107.079(9)^\circ$, $V = 13680.6(51)$ Å³, $Z = 4$, $\rho_{\text{calcd}} = 2.401$ g cm⁻³, $T = 153.1$ K, $2\theta_{\text{max}} = 55^\circ$, $\mu(\text{MoK}\alpha) = 4.935$ mm⁻¹, 30586 reflections measured, of which 15293 were unique ($R_{\text{int}} = 0.034$). One of the sodium atoms (Na2) was disordered over two positions, and so was refined isotropically with occupation factors of 0.5. The non-hydrogen atoms of the solvents (chloroform and water), one of the oxygen atoms attached to the phosphorous atom, and the carbon atoms of one of the ethyl groups (C28, C29) were refined isotropically. Final R and R_w values were 0.046 and 0.068, respectively, for 9001 observed reflections ($I > 5.0\sigma(I)$) and 926 variable parameters.

Crystal data were collected on a Mercury CCD area detector coupled with a Rigaku AFC-8S diffractometer with graphite-monochromated $\text{MoK}\alpha$ radiation ($\lambda = 0.7107$ Å) using CrystalClear (Rigaku Co.). Data were corrected for Lorentzian and polarization effects. An

empirical absorption correction was applied by using REQABA. The structures were solved with CrystalStructure (Molecular Structure Co.). The initial positions of tungsten atoms were determined by direct methods, and other non-hydrogen atoms were located by Fourier and difference Fourier techniques and refined anisotropically unless otherwise noted. Full-matrix least-squares refinement was employed against F . Hydrogen atoms were located at calculated positions and refined isotropically. CCDC-197385 (**3**) and -227498 (**4**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

Powder XRD measurements: All spectra were measured with a Rigaku Mini Flex diffractometer using $\text{CuK}\alpha$ radiation ($\lambda = 1.54056$ Å, 30 kV, 15 mA). Scans were performed in the range of $2\theta = 1.5$ – 20° at a rate of 0.02°s^{-1} .

Sorption measurements: The sorption isotherms were measured with a Japan Bel Belsorp 18 sorption instrument.

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Keywords: calixarenes · crystal engineering · microporous materials · organic–inorganic hybrid composites · polyoxometalates

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Electron Diffraction

Ultrafast Electron Crystallography of Surface Structural Dynamics with Atomic-Scale Resolution**

Franco Vigliotti, Songye Chen, Chong-Yu Ruan, Vladimir A. Lobastov, and Ahmed H. Zewail*

Progress over the past two decades has made it possible to study atomic-scale femtosecond molecular dynamics in all phases of matter. The atomic-scale spatial resolution has been achieved with both X-ray and electron diffraction, and only recently has the combination of spatial and temporal resolution^[1] been advanced successfully for studies of isolated

chemical reactions by ultrafast electron diffraction^[2,3] and bulk crystal phonons and melting^[4–12] by X-ray diffraction. For surface structures and molecules on surfaces, ultrafast electron crystallography (UEC) is unique, and herein we demonstrate its potential for the direct determination of surface structural dynamics of crystalline solids (GaAs), following impulsive fs laser excitation. From the change of Bragg diffraction (shift, width, and intensity), we show, by direct inversion of the diffraction data, that *compression* and *expansion* of the atoms occur on the -0.01 Å to $+0.02$ Å scale, respectively, and that the transient temperature reaches its maximum value (1565 K) in 7 ps. The onset of structural change lags behind the rise in the temperature, demonstrating the evolution of non-equilibrium structures. These structural dynamics results are compared with those of nonthermal fs optical probing, and the agreement for the temperature response from the fluence dependence of the dielectric function is impressive.^[13,14] The success in the direct observation of surface (monolayers) structural dynamics with combined ultrafast temporal and atomic-scale spatial resolutions promises many new applications of UEC.

GaAs is an ideal system to demonstrate this potential of UEC for surface studies, especially as its crystalline and semiconducting properties are well-quantified.^[15] This has allowed a wide range of ultrafast optical experiments that vary from the probing of carrier properties^[16] to electronic disordering or change in symmetry.^[17] In addition to these optical studies, recent ultrafast X-ray experiments on GaAs revealed *bulk* lattice dynamics following fs laser excitation.^[8] However, these ultrafast X-ray experiments could not probe the surface owing to the large penetration depth into the crystal by X-rays, typically up to several μm . On the other hand, optical techniques that probed the surface on the scale of a few nanometers could not directly determine the structure with atomic-scale resolution, but gave valuable information on the response of the dielectric function and lattice disordering.^[13,14,17] The large scattering cross-section of electrons combined with ultrafast time resolution allows the bridging of this gap in addressing the dynamics of surface structures in real time.

The experiments were performed in the newly developed UEC apparatus,^[18] briefly as follows (Figure 1). Under ultra-high vacuum (10^{-10} Torr), and following surface characterization by low-energy electron diffraction and Auger spectroscopy, the sample was brought to the scattering position where beams of the laser-pulse excitation and electron-pulse probe intersected in space, with an adjustable time delay Δt (the zero of time was determined in situ). We followed a published procedure^[19] to terminate semiinsulating GaAs (111) crystals by a monolayer of chlorine with a Cl atom atop each Ga atom, saturating the otherwise dangling bond of Ga. The surface retained its integrity throughout the experiments, as evidenced by the unchanged quality of the diffraction patterns (symmetry, spot profiles, and intensities)—see below. The crystal, placed on a goniometer with three degrees of freedom in translation and two axes of rotation, was positioned in space with a precision of 0.01° in rotation, and of $10\ \mu\text{m}$ in translation. All experiments were carried out at room temperature.

[*] Dr. F. Vigliotti, S. Chen, Dr. C.-Y. Ruan, Dr. V. A. Lobastov, Prof. Dr. A. H. Zewail
Arthur Amos Noyes Laboratory of Chemical Physics
California Institute of Technology
Pasadena, CA 91125 (USA)
Fax: (+1) 626-792-8456
E-mail: zewail@caltech.edu

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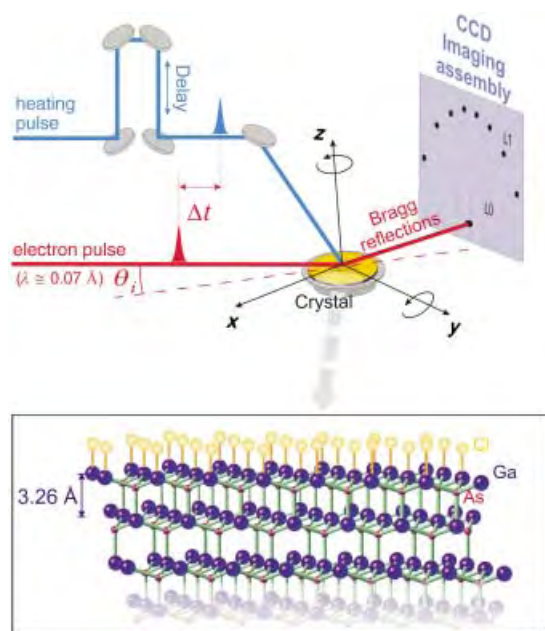


Figure 1. Concept of UEC and structure of the chlorine-terminated GaAs (111) crystal. The output of a Ti:Sa fs amplifier (120 fs, 800 nm, 2 mJ, 1 kHz) was frequency tripled to yield a beam at 266 nm (400 μJ, <300 fs, 1 kHz). This UV beam was brought to the scattering chamber to provide the initial heating pulse at $t=0$. A very small fraction of this beam was directed synchronously onto a back-illuminated Ag photocathode to generate the electron pulses. These electron pulses, after acceleration and focusing, were guided to the crystal, where they overlapped in space and time (delay Δt) with the heating pulse. Although electron pulses were made as short as 500 fs (see text), in this grazing incidence, the spatial extent decreases this resolution to tens of ps. However, because of the sensitivity achieved (20 s per frame), we were able to reduce the experimental temporal cross-correlation to 7 ps; with convolution and at the level of signal reported herein, we readily obtained a 1–2 ps response. This was made possible by reducing the spatial extent of the substrate to 400 μm by masking techniques (which will be described elsewhere in detail), resulting in a transit time of 4 ps. The sample, placed on a high-precision five-axis goniometer, was positioned to allow the electron beam to impinge at the chosen incidence angle ($\theta_i < 5^\circ$), azimuthally along the $\langle 112 \rangle$ direction. L0 and L1 refer to the zero-order and first-order Laue zones. The resulting diffraction patterns were recorded in the far field by an imaging CCD camera system. In the inset, the structure of the crystal is shown, with the bilayer spacing of 3.26 Å.

Femtosecond laser heating at $t=0$ initiated the dynamics, which were subsequently probed by an ultrashort packet of electrons of 30 keV ($\lambda_{\text{deBroglie}} \approx 0.07 \text{ Å}$). The electrons impinged the surface at a small incidence angle ($\theta_i \approx 1^\circ$) and at this grazing incidence, reflection high-energy electron-diffraction methods^[20] are uniquely suited and have been used for studies of superheating with a temporal resolution of $\approx 100 \text{ ps}$.^[21,22] The electron pulses were generated by a modified Williamson–Mourou^[23] streak camera arrangement. In our UEC machine, we have obtained pulses of duration as short as $\approx 500 \text{ fs}$,^[24–26] but for the grazing geometry of the experiment, the temporal cross-correlation was 7 ps. By controlling the arrival of the electron pulse (Δt in Figure 1), we can probe the structure prior to or following the heating pulse excitation. Structural changes were followed in real

time by monitoring the Bragg reflections and rocking curves, as recorded by a CCD (charge-coupled device) imaging assembly capable of single electron detection.^[2,3] Three features of the diffraction were followed as a function of time: the Bragg peak shift, width, and intensity.

Figure 2 presents a typical static diffraction image, which displays the very strong (0,0) and other Bragg reflections. Figure 2a shows a diffraction pattern, for which the incidence

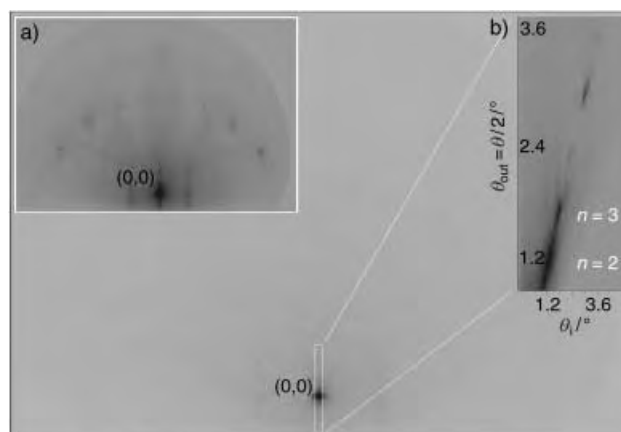


Figure 2. Static diffraction images of the crystal (111) surface obtained by the ultrashort electron pulses without time resolution. a) Diffraction image showing the intense (0,0) in-phase reflection, together with the streaks and spots in the Laue zones. b) Experimental rocking curve for the (0,0) reflection–(111) lattice planes. The periodicity allows the unequivocal identification of the Bragg reflections and gives the interlayer spacing (see text).

angle was tuned to reveal higher-order diffraction peaks, as well as diffraction streaks in the zero-order Laue zone. These and similar data allow the precise determination of the camera distance from the scattering position ($170 \pm 1 \text{ mm}$), by direct inversion of the angular separation between the streaks at low angles, or from the Bragg spots in the higher Laue zones. This inversion is direct because the lattice rods are separated in reciprocal space by an in-plane inverse distance of 3.14 Å^{-1} along the intersection with the Ewald sphere of radius $2\pi\lambda^{-1} = 90 \text{ Å}^{-1}$. By gating the detection on the (0,0) Bragg spot and following the diffraction position as a function of the incidence angle, we also obtained the experimental rocking curve, which gives the GaAs lattice periodicity along the (111) direction ($n=1, 2, \dots$). This is shown in Figure 2b, where the incidence angle was varied over several degrees. The experimental periodicity in θ_i of $0.60^\circ \pm 0.02^\circ$ is in quantitative agreement with the expected value of 0.61° obtained for the lattice bilayer spacing of 3.26 Å.

In the time-resolved experiment, depicted schematically in Figure 1, the excitation pulse at $t=0$ defines the initial temperature and structural change. In Figure 3, we follow the center position and intensity of the $n=2$ (0,0) Bragg spot as a function of time. We also monitor the width, as shown in Figure 3b. Figure 3a presents the change in the peak center position, which maps out the change in lattice spacing in the (111) direction. Results are shown for fluences of 9% and 45% of the experimentally determined 4.5 mJ cm^{-2} damage

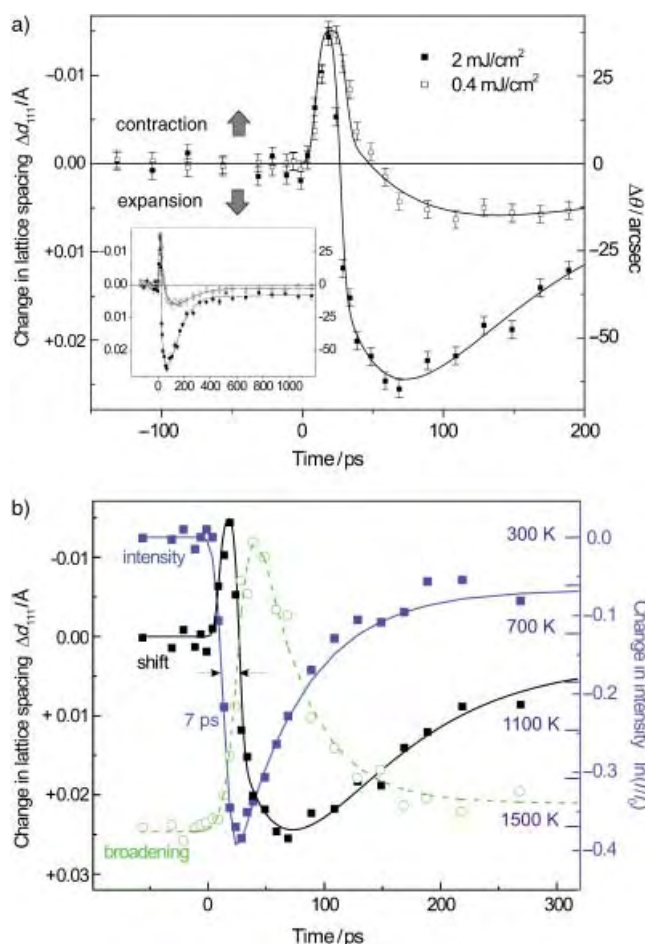


Figure 3. Time dependence of the Bragg reflection center position, intensity, and width, following the laser heating excitation at $t=0$. a) Center position of the Bragg spot as a function of time and fluence. The vertical axis on the right gives the angular deviation and the left axis shows the corresponding change in lattice spacing, perpendicular to the (111) surface plane. A contraction takes place at early times ($\Delta d < 0$), followed by lattice expansion ($\Delta d > 0$). The inset shows the evolution at long time. b) Comparison of the integrated intensity of the Bragg spot (temperature) with the change in lattice spacing for the data set obtained at 2 mJ cm^{-2} . The right axis gives the ratio of the time-dependent integrated intensity of the Bragg spot (I) to its counterpart at negative delay (I_0) on a logarithmic scale, from which the temperature scale is obtained. The lattice expansion is also shown with its scale on the left axis, together with the broadening of the Bragg spot, depicted by the dashed line. The apparent delay between the temperature rise and the lattice expansion is noted by the two arrows (see text).

threshold at 266 nm. The angular deviation ($\Delta\theta$) of the Bragg spot directly gives the change in lattice spacing (Δd_{111}), from the relation $\Delta d_{111} = -\Delta\theta \cdot d_{111} \cdot \{2 \sin(\theta/2)\}^{-1}$ (θ is the total scattering angle). A deviation to larger or smaller angles ($\Delta\theta > 0$ or $\Delta\theta < 0$) is therefore the signature of lattice contraction or expansion.

From the results shown in Figure 3 a, it is evident that the top surface layers of the crystal immediately contract following excitation at $t=0$. The amplitude of this initial contraction is given for two fluences, but the complete fluence dependence is presented in Figure 4. After the initial contraction

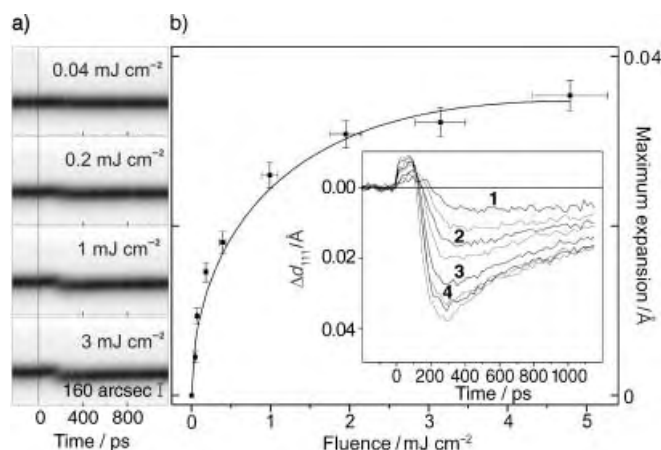


Figure 4. Fluence dependence of the structural dynamics. a) Experimental traces for a set of data at the indicated fluences. b) The amplitude at maximum change is shown as a function of excitation fluence. The time-dependence is displayed in the inset (1: 0.04 , 2: 0.2 , 3: 1 , 4: 3 mJ cm^{-2}). Notably, the temporal resolution is lower than that in Figure 3, because these fluence-dependent measurements were made without masking in order to explore the lowest possible range of fluences.

($-0.015 \text{ \AA}$), the system expands to a maximum amplitude ($+0.025 \text{ \AA}$), which strongly depends on the fluence: the larger the fluence the more ample the expansion. The data also show that both the onset time and the velocity of the expansion ($\approx \text{ms}^{-1}$) strongly depend on the fluence: expansion occurs earlier and faster at higher fluences. After reaching its maximum expansion, the system contracts again toward the original lattice spacing on a much longer time scale, beyond 50 ps, but a finite smaller expansion persists for at least several ns. Observations were also made for 800-nm fs excitation at various laser fluences and the behavior is similar, that is, an initial contraction, followed by an expansion and subsequent return toward the initial lattice spacing. This similarity in form, compared to that in the 266-nm experiment, indicates that the observed structural dynamics is not dominated by a charging of the surface by photoemission, as excitation at 800 nm and/or at low fluences results in a similar behavior.

The transient temperature is evident in the change in the diffraction-integrated intensity with time. This is presented in Figure 3b, for excitation at 45% of damage threshold (2 mJ cm^{-2}), by plotting the evolution of the integrated intensity of the Bragg spot as a function of time. Using the tabulated Debye–Waller factors for bulk GaAs,^[27] and taking into account the two-dimensionality of the surface,^[28] we obtained an initial temperature jump to $1565 \pm 83 \text{ K}$. The system cools down on the timescale of a few hundred ps to reach $\approx 510 \text{ K}$ after 1 ns. The initial temperature jump has a rise time of 7 ps (10 ps before deconvolution), in perfect agreement with results from fs optical studies of the dielectric function.^[13,14] Moreover, the maximum temperature reported above is close to the value (1300–1500 K) extrapolated from these optical studies at corresponding fluence. For the lower

fluence regime (0.4 mJ cm^{-2}) presented in Figure 3a, we find a temperature jump to $420 \pm 18 \text{ K}$, with a decay leveling off at $320 \pm 5 \text{ K}$ after 1 ns. In this case too, the rise time and the maximum temperature are consistent with the optical study.

The evolution of the lattice expansion and that of the temperature are juxtaposed in Figure 3b, together with the width. Strikingly, the temperature evolution precedes the lattice expansion, and we measured a delay of 15 ps between the rise in temperature and the structural expansion. This lag in structural expansion provides direct evidence for the proposed delayed lattice changes following an impulsive initial temperature.^[13,14] We note that the temperature jump to $1565 \pm 83 \text{ K}$ is similar to (or even exceeds) the 1513 K melting point,^[15] whereas the excitation fluence is only half of the damage threshold. However, as evident from Figure 3b, the peak temperature does not persist for a long time and the system does not lose its crystalline structure.

The lagged structural change reaches its maximum expansion of $+0.025 \text{ \AA}$ at a temperature of $\approx 1000 \text{ K}$ (Figure 3b). This lattice expansion in the nm-scale structure may now be compared with the expansion in bulk GaAs. From the linear expansion coefficient of bulk crystals,^[15] a temperature of 1000 K would correspond to a linear lattice expansion of $0.013 \text{ \AA}$, and this value differs by a factor of 2 from our experimental value of $0.025 \pm 0.001 \text{ \AA}$. At much longer times ($\approx 1 \text{ ns}$), when the change in expansion levels off, a temperature of 510 K would correspond to a linear expansion of $0.0038 \text{ \AA}$, and our experimental value is $0.0032 \pm 0.0005 \text{ \AA}$ (Figure 3b). This temporal decrease in the disparity in spacing is indicative of the change in surface to bulk-type behavior. From modeling of strain propagation in X-ray studies, a $0.0082\text{-\AA}$ surface strain amplitude was inferred for GaAs.^[8] Our measurements indicate a larger deformation (by a factor of 3).

Because of their small incidence angle, the probing electrons have a very small penetration depth (a few $\text{\AA}$ for 30-keV electrons at $\theta_i \approx 1^\circ$) and thus probe only the very top surface layers of the crystal; in the geometry of our experiment, the excitation pulse (30° incidence angle) has a vertical penetration depth also of nm scale (3.5 nm at 266 nm). These small and comparable penetration depths for the photons and electrons provide a unique condition for monitoring the local structural dynamics of these surface layers. In X-ray experiments, a heating pulse typically has an absorption length of $0.3 \text{ }\mu\text{m}$, and the probing X-ray pulse has a μm -scale penetration depth. Such scales require consideration of strain propagation in the bulk and over the $\mu\text{m(s)}$ length.^[8] Clearly, direct probing of the surface motions of atoms is critical to the understanding of the surface initial dynamics and to the connection to bulk propagation at different temperatures (fluences). The effect of fluence on structural changes is mapped out in Figure 4a, and representative examples of experimental traces that follow the time-dependent Bragg reflection are shown in Figure 4b.

Additional experiments were carried out on silicon to isolate the effect of chlorination and to test the generality of the approach and the scope of application. Both chlorinated and non-chlorinated silicon (111) surfaces were subjected to the same experimental conditions (excitation wavelength and

fluence). Similar behavior to that of GaAs was found—whereas hydrogen-terminated silicon did not present noticeable surface contraction preceding the expansion, the chlorinated surface showed a prompt contraction, which precedes the expansion. The overall phase of the signal in Figure 3 depends on the overlap at the mask (Figure 1, caption) and it is possible that optical phonon generation at $t=0$ contributes to the observed scattering. It should be noted that the phase corresponding to the expansion is unequivocally established and confirmed by the temporal evolutions of the temperature and structural dynamics at different fluences. Several mechanisms were considered for the striking nature of the contraction, as will be described in detail elsewhere. However, the observations with chlorine are consistent with a potential-driven change at the earliest times, prior to coherent acoustic phonon generation.

A general structural-dynamics picture now emerges from the observations of the structure changes and the timescales of the motion (Figure 3b), and from the observations on silicon surfaces. In the nonthermal regime,^[13] the initial fs transient excitation, which creates the electron-hole pairs, distorts the potential, and structural changes occur on the ultrashort timescale by this deformation prior to significant motion in the lattice (phonons), as experimentally verified above. This highly non-equilibrium state of the solid is followed by energy dissipation and redistribution, which ultimately lead to expansion of the lattice and restructuring at longer times. With this in mind, only an expansion of the surface atoms would be expected, contrary to the *contraction* and *expansion* observed in the studies reported herein. However, for the chlorine-terminated surface, the large electronegativity shifts the electronic charge distribution towards the chlorine (ionic potential). The ensuing Coulombic interaction with the underlying layers contracts the interatomic layers, as observed in the early-time ultrafast rise of the contraction (Figure 3a), and on this timescale, the dynamics is driven by the potential change. Along with the observations made in the case of silicon and supporting this proposed mechanism for contraction, we note that atomic chlorine chemisorbed on GaAs was found to be an electron acceptor.^[29]

Following the contraction, expansion proceeds on a similar timescale. Through Auger processes (at a density of $\approx 10^{21} \text{ cm}^{-3}$), which take place in a few ps, the carrier density decreases, but the total electronic energy remains unchanged.^[13,30,31] The drop in the Coulombic potential along with electron-phonon coupling now drives the system in a reversed motion toward expansion (Figure 3a). The expansion of the lattice requires 7 ps to define surface-layer temperature and this is evident in the rise of the intensity profile (Figure 3b); only after this rise can we define the temperature acquired through electron-phonon coupling. The structural change (expansion) follows the temperature rise, but after the apparent delay of 15 ps, reaching its maximum of $+0.025 \text{ \AA}$ expansion at yet longer time. This thermal expansion in the (111) direction must be due to anharmonicity of lattice vibrations. Remarkably, the width of the Bragg spot reaches its maximum before the peak of structural lattice expansion. Lattice dynamics is first driven by

coherent collective phonons followed by the isotropic expansion that ensues when anharmonicity becomes effective. This nascent lattice expansion must first overcome the persisting contraction (Figure 3a). From our data, we obtained an onset for the expansion of ≈ 5 ps after the temperature has risen to half of its maximum and an additional delay of ≈ 10 ps to overcome the initial contraction. It should be noted that this picture of structural dynamics is robust at lower fluences (Figure 4). However, in the lower fluence regime, the initial temperature is decreased, electron–phonon coupling dominates, and diffusive processes become pronounced at longer times.

The return to the original structure is observed in the decrease in Δd_{111} , from $+0.025$ to $+0.003$ Å, but this restructuring takes place on a much longer timescale (Figure 3). We note that diffusive processes must begin beyond 50 ps, as up to this time Δd_{111} continues to increase—cooling down the surface by diffusion leads to a decrease in Δd_{111} . Thus, the structure at the expanded value of $\Delta d_{111} = 0.025$ Å is vibrationally in a non-equilibrium state of collective modes, which cools down by energy redistribution and diffusion at longer times. Theoretical calculations of the heat diffusion using the known thermal properties of GaAs (heat capacity and thermal conductivity^[15]) gave a good match to the temperature behavior from the point of leveling off shown in Figure 3b.

The above results show the new dimensions of ultrafast electron crystallography at time, length, and sensitivity scales ideally suited for atomic-scale structural dynamics of surfaces and interfaces. This is achieved because of the ability to directly determine—from the diffraction periodicity and changes with time—the evolution of atomic spacings (Bragg peak positions), transient temperatures (Bragg integrated intensity), and the involvement of coherent lattice vibrations (Bragg peak width). The timescales for the non-equilibrium surface structures, and restructuring at longer times, are critical for understanding the surface phenomena and for propagation in bulk materials. We believe that UEC is now poised for many applications in this general area of surface science and nanometer-scale materials, and macromolecular structures.^[32]

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Please note: Minor changes have been made to this manuscript since its publication in *Angewandte* Early View. The Editor.

Synthesis and Application of Fluorescence-Labeled Ras-Proteins for Live-Cell Imaging**

Reinhard Reents, Melanie Wagner, Jürgen Kuhlmann,* and Herbert Waldmann*

Live-cell imaging employing fluorescent-labeled probes is rapidly developing into one of the most important techniques of biological research.^[1] A significant bottleneck in the application of this technology is the efficient and site-selective labeling of proteins.^[2] Molecular biological introduction of green fluorescent protein (GFP) and congeners like cyan fluorescent protein (CFP) has emerged as a powerful tool for this purpose.^[3] However, since the biological fluorophore GFP has a mass of 27 kDa, its influence on the in-vivo readout cannot be excluded, in particular if protein–protein interactions are involved. For instance, restrictions in the use of the GFP marker due to steric constraints and undesired interactions were reported in studies of tubule formation^[4] and of the secretory pathway in yeast.^[5] An additional limitation is given by the overlapping emission and excitation spectra of the accessible fluorescent proteins that restrict utilization in FRET (fluorescence resonance energy transfer) assays or dual-wavelength confocal microscopy. Also, GFP shows “flickering” where phases of active and inactive fluorescence emission alternate.^[6] These drawbacks can be overcome by the use of semisynthetic proteins carrying small fluorophores, and this general approach has been widely explored.^[7]

Such protein constructs could be invaluable tools for studying the chemical biology of the signal-transducing Ras-proteins and their relatives, in particular, the processes regulating their cellular localization and transport. These S-palmitoylated and S-farnesylated small GTPases regulate various important biological processes such as control of growth and differentiation, and impaired Ras is found in approximately 30% of all human cancers.^[8] Only when they are fully lipid-modified are the Ras-proteins biologically

active and localized at the plasma membrane. Live-cell imaging experiments with GFP- and CFP-labeled Ras-proteins have been reported,^[9] but the synthesis and biological application of fully functional Ras-proteins site-specifically equipped with a small fluorophore and suitable for live-cell imaging has not been achieved.

Herein we report on the synthesis and preliminary biological characterization of Ras-proteins that carry a small fluorophore at the C-terminus, are substrates for the cellular palmitoylation machinery, and whose localization within the cell can be monitored successfully by means of confocal laser fluorescence microscopy.

In initial experiments semisynthetic Ras-proteins were synthesized following a methodology previously described,^[10] in which a Mant, NBD, or Bodipy fluorophore was attached to the farnesyl residue, and which contain a second cysteine. If such proteins are microinjected into PC12 cells, the cysteine is recognized by the cellular palmitoylation machinery, and the doubly lipidated proteins formed in vivo may induce a quantifiable outgrowth of neurites, indicating localization to the plasma membrane.^[10]

However, these lipoproteins could not be used successfully owing to practical limitations of cell-imaging microscopy optics (Mant), or the Ras chimera could be visualized by confocal microscopy but they lost differentiating efficiency in the PC12 cell assay (NBD, Bodipy). Although not successful, these experiments revealed that the Bodipy fluorophores display the most advantageous optical properties and indicated that the fluorophore should not be incorporated into a lipid group but rather attached to the peptide chain.

Based on the notion that the C-terminal methyl ester present in all native Ras-proteins most likely serves only to enhance the lipophilicity of the proteins' C-terminus,^[11,12] we decided to replace the C-terminal methyl ester by functional groups that incorporate a Bodipy-FL or a Bodipy-TR fluorophore. To minimize possible steric interactions between the fluorophore and the protein an ethylenediamine spacer was introduced.

The synthesis of the fluorescent-labeled Ras-peptides and their coupling to an N-Ras mutant are shown in Scheme 1. Peptide **5** was assembled on a polystyrene resin **1** equipped with a 4-methyltrityl linker. After attachment of Fmoc-protected ethylenediamine **2** and removal of the Fmoc group, the peptide was synthesized employing Fmoc-protected amino acid building blocks **3** including S-farnesylated Fmoc-cysteine.^[13] After N-terminal deprotection of the final glycine, maleimidocaproic acid **4** was attached to the N-terminus. Peptide **5**, which embodies the seven C-terminal amino acids found in native N-Ras, was released from the solid support by treatment with dilute trifluoroacetic acid. Under these conditions, the acid-labile farnesyl group and the S-*t*Bu-disulfide protecting function of the second cysteine are unharmed. The Bodipy-FL and -TR labels were then attached to peptide **5** employing EDC/HOBt as the condensing reagents to give fluorescent-labeled peptides **6a** and **6b** in high yields.

Peptides **6a** and **6b** were then coupled to N-Ras protein by a method described previously.^[10] Briefly, an oncogenic RasG12 V-mutant terminating at position 181 in a cysteine was generated by introduction of a stop codon in positions 182

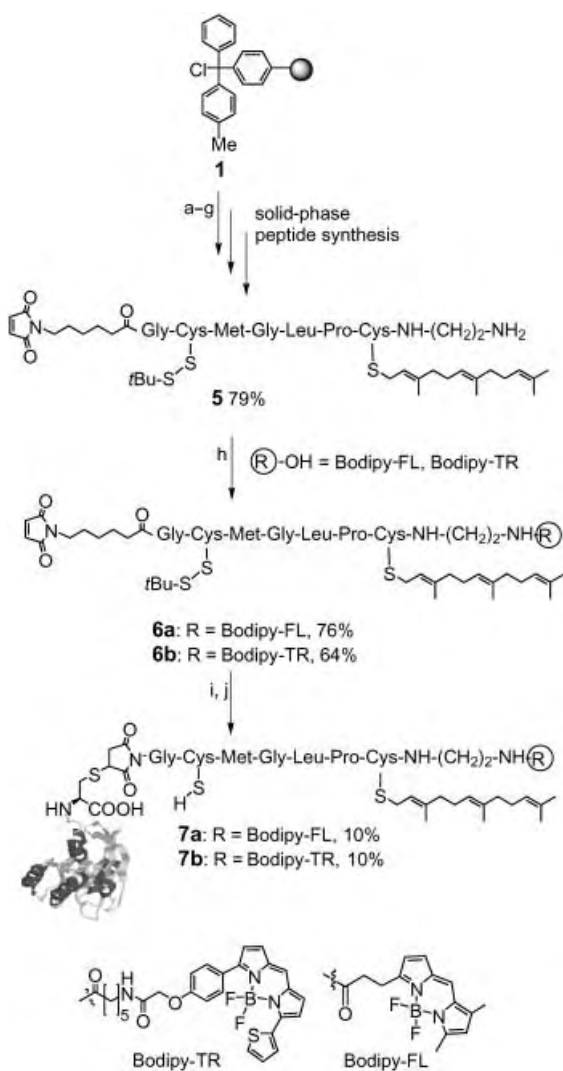
[*] Dr. M. Wagner, Dr. J. Kuhlmann
Max-Planck-Institut für Molekulare Physiologie
Abteilung Strukturelle Biologie
Otto-Hahn-Strasse 11, 44227 Dortmund (Germany)
Fax: (+49) 231-133-1435
E-mail: juergen.kuhlmann@mpi-dortmund.mpg.de

Dr. R. Reents, Prof. Dr. H. Waldmann
Max-Planck-Institut für molekulare Physiologie,
Abteilung Chemische Biologie
Otto-Hahn-Strasse 11, 44227 Dortmund (Germany) and
Universität Dortmund, Fachbereich 3, Organische Chemie
Fax: (+49) 231-133-2499
E-mail: herbert.waldmann@mpi-dortmund.mpg.de

[†] These authors contributed equally to this work.

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Scheme 1. Synthesis of fluorescent-labeled Ras-proteins **7**: a) Fmoc-NH-(CH₂)₂-NH₂ (**2**), *i*Pr₂NEt; b) 20% piperidine in DMF; c) 4 equiv Fmoc-AA-OH (**3**), HBTU, HOBT, *i*Pr₂NEt, DMF, 90 min; repeat for each amino acid building block; d) Ac₂O (capping); e) 20% piperidine in DMF; f) maleimidocaproic acid (**4**), HBTU, HOBT, *i*Pr₂NEt, DMF, 90 min; g) 1% TFA in CH₂Cl₂: **5** (79%); h) EDC, HOBT, Bodipy-TR or Bodipy-FL, triethylamine, CH₂Cl₂: **6a** (76%), **6b** (64%); i) N-Ras, buffer pH 8.5, Triton X114, 4°C, j) DTE, buffer pH 8.5, 37°C: **7a** (10%), **7b** (10%). DMF = dimethylformamide, DTE = dithioerythritol, EDC = 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide, Fmoc = 9-fluorenylmethoxycarbonyl, HBTU = 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, HOBT = 1-hydroxy-1*H*-benzotriazole, TFA = trifluoroacetic acid.

and 183 of N-Ras cDNA, and the resulting PCR product was cloned into the *E. coli* ptac expression vector. Expression was performed in *E. coli* strain CK600K. The C-terminal cysteine of the obtained Ras mutant is the only surface-accessible cysteine, and when exposed to the maleimido-modified peptides in Triton X114 buffer it adds chemoselectively to the double bond. Protein isolation is facilitated by separation of the coupling buffer into a detergent-rich and an aqueous phase upon warming to 37°C. The lipidated proteins accumulate in the Triton phase, nonlipidated protein remains in the aqueous phase. The proteins were purified by ion-

exchange chromatography and characterized by SDS-PAGE (Figure 1). For biophysical experiments an additional size-exclusion chromatography was performed to ensure >95% purity of the lipoprotein product. In addition, a MALDI mass

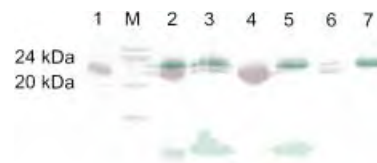


Figure 1. Results of the synthesis and purification of the semisynthetic Ras-protein **7a**. The coupled and uncoupled proteins were separated by extraction with Triton X114. An excess of uncoupled protein in the Triton phase was removed by washing with aqueous buffer. Samples were analyzed by SDS-PAGE. Overlay of coomassie staining (gray) and fluorescence image (green). 1) Truncated N-Ras (1–181), M: molecular weight marker, 2) reaction mixture after 16 h, 3) Triton phase after extraction, 4) aqueous phase after extraction with Triton, 5) Triton phase after washing with aqueous buffer, 6) aqueous phase after washing of Triton phase, 7) coupling product after DEAE column.

spectrometric investigation of the coupling products proved that the lipidated peptide was attached only once to the Ras-protein. Ras-protein and peptides were employed in equimolar amounts to give access to the desired fluorescent-labeled Ras chimera in multimilligram amounts with yields of 10%. Finally the *S*-*t*Bu disulfide still attached to the coupling products obtained from peptides **6a** and **6b** was cleaved by treatment with dithioerythritol (DTE) at 37°C.

The Ras-protein **7a** obtained was then subjected to biological evaluation. When microinjected into PC12 cells Bodipy-FL-modified Ras-protein **7a** was accepted by the cellular palmitoylation machinery and induced a differentiated phenotype (Figure 2). It reached a maximum differ-

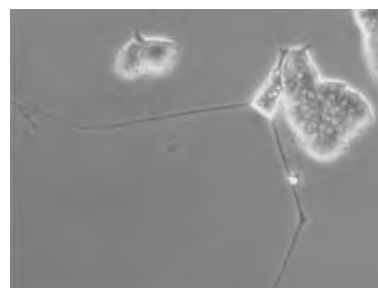


Figure 2. Transformation of PC12 cells after microinjection with the Bodipy-FL-labeled Ras-protein **7a**. The picture proves the biological activity of this protein conjugate.

entiation efficiency of 76%, which is in the range of the efficiency observed for the analogous semisynthetic Ras-protein bearing a methyl ester at the C-terminus (88%) and wild-type Ras itself (84%).

Encouraged by the finding that protein **7a** indeed is biologically active, we microinjected it into MDCK and COS7 cells and inspected them by confocal fluorescence microscopy (Figure 3). Due to the excellent properties of the Bodipy-FL



Figure 3. Localization of the Bodipy-FL-labeled Ras-protein **7a** after microinjection of 50 μM protein solution into COS7 cells and b) after microinjection of 50 μM protein solution into MDCK cells. The cells were incubated for 4 h after injection and Bodipy-FL was excited with the laser line $\lambda = 488 \text{ nm}$. In both cells selective plasma membrane and Golgi staining is observed. Golgi localization was confirmed by staining with the Golgi marker ceramide-BodipyTR; c) after coinjection of 50 μM protein with 2 μM cerulenin analogue **8**. Cells were inspected after 7 h incubation, and Bodipy-FL was excited as described above. Staining of the plasma membrane is almost abolished compared to the controls without **8**.

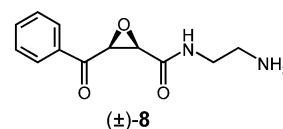
fluorophore, protein concentrations of only 10 μM in the injection needle were sufficient to generate satisfactory images.

Inspection of the cells one hour after injection revealed that the Ras construct with a Bodipy-FL as substitute for the carboxymethyl function inserted into endomembranes and was completely excluded from the nucleus. It induced a distinct staining of the plasma membrane of MDCK and COS7 cells after further incubation at 37°C for three to four hours with a clear prevalence of fluorescence signal in the endomembranes. These results demonstrate that the Bodipy-FL-bearing Ras chimera are biologically active and can be visualized in conventional fluorescence microscopes at relatively low concentrations. The Bodipy-FL does not flicker and gives a continuous readout. These characteristics make the Ras-proteins with non-protein fluorophores particularly suitable for biochemical and biophysical analysis, for example, for reconstitution and interaction studies in artificial membranes and for cellular assays in medicinal chemistry research. The application of such protein probes gives control over the final protein concentration in the experiments usually not attainable by transfection methods. Furthermore the availability of purified proteins in sufficient amounts allows correlation of the results of cellular assays with observations in vitro, for example gleaned from biophysical experiments. In particular, the control over the structure of the lipid modifications and the fluorophore gained by means of chemical synthesis provides the opportunity to introduce nonnatural lipids. Thereby membrane anchors can be generated that are not accessible from the cellular machinery.^[10]

In order to demonstrate the applicability of the Ras probes we attempted to visualize Ras localization in cells depending on the presence of a compound that exerts a known biological effect. Cerulenin inhibits fatty acid biosynthesis by blocking the fatty acid synthase complex.^[14] Smith et al. have shown that treatment of T24 human bladder carcinoma cells with the natural product or analogues thereof results in the formation of non-palmitoylated H- and N-Ras proteins.^[15] The cellular target(s) of these electrophilic compounds were not identified. Non-palmitoylated H- and N-Ras

should not be localized to the plasma membrane, and this should be demonstrable with the live-cell imaging Ras probes described above.

As a representative compound cerulenin analogue **8** (for the synthesis of **8** see the Supporting Information) was



analyzed in the PC12 cell assay mentioned above and described earlier.^[10] When an oncogenic S-palmitoylatable semisynthetic Ras-protein was injected alone or coinjected with compound **8** into PC12 cells, it was observed that in the presence of the cerulenin analogue the differentiation rate of the cells was significantly less than that observed after injection of the Ras-protein alone (data not shown); this indicates interference with the biological activity of the protein. In order to determine whether the application of cerulenin analogue **8** indeed influences localization of Ras-proteins to the plasma membrane, semisynthetic, fluorescent Ras-protein **7a** was coinjected with the compound into MDCK cells. Inspection of the cells by means of confocal fluorescence microscopy revealed that the protein accumulated in the cytosol. Staining of the plasma membrane was strongly reduced (see Figure 3c).

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The Chemistry of Ca^{II} and Yb^{II} : Astoundingly Similar But Not Equal!**

Sjoerd Harder*

The structures and chemical behavior of heavier alkaline-earth metal complexes (Ca , Sr , and Ba)^[1] have often been compared to that of early d- and f-block metal complexes.^[2–11] In particular, parallels with “hot” lanthanide(II) complexes^[12] have been observed. Lanthanide complexes are d^0 species with a limited radial extension of their f orbitals. Therefore, orbital factors do not affect their chemistry as much as in transition-metal chemistry. As for complexes of the heavier alkaline-earth metals, their bonding and structures are mainly determined by electrostatic and steric factors.

There is ample evidence for the striking similarity in the chemistry of Ca^{II} and Yb^{II} : 1) crystal structures of Ca and Yb analogues are always isomorphous and have exceptionally similar cell constants,^[7,8,10] 2) the observation of nearly identical IR spectra of Ca and Yb analogues suggests a striking similarity in bonding,^[2] 3) analogous Ca and Yb complexes display similar gas-phase behavior: electron-diffraction studies of $[\text{Cp}^*\text{Ca}]$ (Cp^* is pentamethyl cyclopentadiene) and $[\text{Cp}^*\text{Yb}]$ reveal similar $\text{Cp}^*_{\text{centroid}}\text{-M-Cp}^*_{\text{centroid}}$ angles of $154(3)^\circ$ and $158(4)^\circ$, respectively,^[13] and 4) analogous reactivities of Ca^{II} and Yb^{II} complexes have been reported.^[8,9]

These parallels in the chemistry of Ca and Yb complexes prompted us to repeat the calcium chemistry recently reported by us, but with ytterbium. The substitution of Ca for Yb in benzylcalcium-based initiators for stereoselective styrene polymerization^[15,20] and an investigation of its polymerization chemistry is here of special importance.

The syntheses and crystallization of the Yb^{II} complexes were greatly facilitated by the strong similarity with their calcium analogues: in all cases exactly the same procedures as for their Ca analogues were followed. The Yb^{II} complexes **1(Yb)**–**5(Yb)** were prepared and the structures of **1(Yb)**–

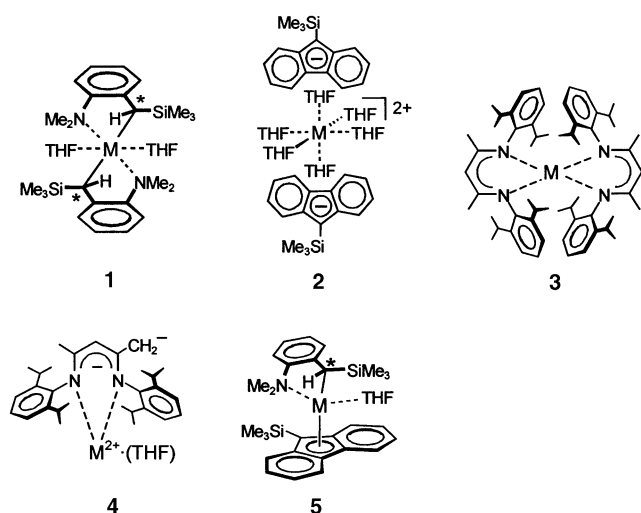
[*] Priv.-Doz. Dr. S. Harder⁺
Fachbereich Chemie
Universität Konstanz
Postfach 5560, M738, 78457 Konstanz (Germany)
Fax: (+49) 7531-883-137
E-mail: harder@chemie.uni-konstanz.de

[⁺] New Address:
Institut für Anorganische Chemie
Universität Duisburg-Essen
45117 Essen (Germany)

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4(Yb) were determined by X-ray diffraction. Not only a striking similarity in cell parameters (Table 1) but also in molecular geometry is observed. Close similarity in their molecular geometries can be explained by their nearly identical ionic radii (Ca^{2+} 1.00 Å; Yb^{2+} 1.02 Å).^[14]

The molecular similarity of **1(Yb)** and **1(Ca)** is demonstrated in Figure 1 a. Both structures are nearly identical and show very similar bond lengths and angles. Even the puckering in the THF rings, which usually shows strong disorder, is identical. In solution **1(Yb)** and **1(Ca)** both exist as a pair of diastereomers and have close similarity in their ^1H NMR and IR spectra (see Supporting Information).^[15]

Crystals of the solvent-separated ion-pair **2(Yb)** were obtained by a procedure similar to that used for the Ca analogue:^[16] the addition of THF to a solution of bis(9-Me₃Si-fluorenyl)ytterbium·(THF)₂ in benzene results in crystallization of **2(Yb)**. The structure of **2(Yb)** is identical to its Ca analogue and can also be described with the “metal-in-a-box” model: the $[\text{Yb}^{2+}(\text{THF})_6]$ ion is encapsulated in a box of fluorenyl ligands and cocrystallized benzene molecules.^[16]

Both (dipp–nacnac) complexes, **3(Yb)** and **3(Ca)**, crystallize as C_2 -symmetric molecules, which are stabilized by C–

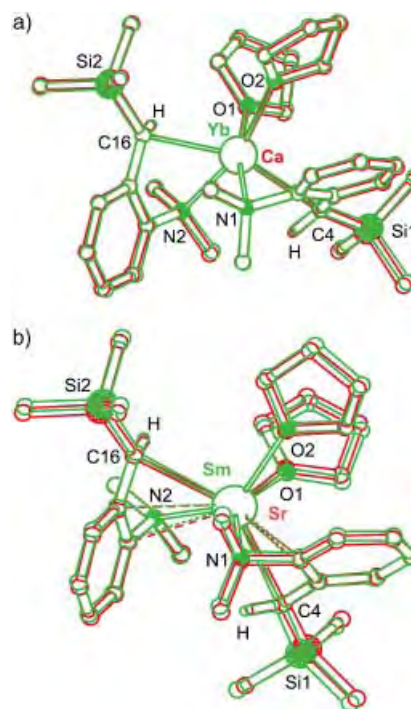


Figure 1. a) Fit of the crystal structures of **1(Yb)** (green) and **1(Ca)** (red). Selected bond lengths [Å] and angles [°]; the values for the Ca complex are given in square brackets: M–C4 2.661(3) [2.638(3)]; M–C16 2.640(3) [2.618(3)]; M–O1 2.491(2) [2.441(2)]; M–O2 2.417(3) [2.373(2)]; M–N1 2.577(3) [2.567(2)]; M–N2 2.652(3) [2.632(2)]; C4–M–C16 157.3(1) [157.5(1)]; O1–M–O2 79.9(1) [80.8(6)]; N1–M–N2 102.7(1) [102.2(1)]. b) Fit of the crystal structures of **1(Sm)** (green) and **1(Sr)** (red). Selected bond lengths [Å] and angles [°]; the values for the Sr complex are given in square brackets: M–C4 2.768(3) [2.770(2)]; M–C16 2.789(3) [2.798(2)]; M–O1 2.578(2) [2.532(2)]; M–O2 2.550(2) [2.560(2)]; M–N1 2.756(2) [2.759(2)]; M–N2 2.771(2) [2.783(2)]; C4–M–C16 142.48(9) [143.46(6)]; O1–M–O2 75.68(8) [76.08(6)]; N1–M–N2 127.76(6) [127.07(5)].

H··· π interactions between the dipp–nacnac ligands.^[17] Not only their molecular geometries^[18] but also their dynamic behavior in solution is strikingly similar: the activation energy for inversion of puckering in the metal–N–C–C–C–N ring, which was shown to be metal-dependent,^[17] is 17.6(1) kcal/mol for both the Ca as well as the Yb complex.

Ligand exchange between the homoleptic complexes **1(Ca)** and **3(Ca)** resulted in a heteroleptic complex, which through C–H activation reacted to give **4(Ca)**.^[11] The Yb analogues react under exactly the same conditions and **4(Yb)** could be isolated as “black” (extremely dark red) crystals in a similar yield. Again, the molecular structures of **4(Ca)** and **4(Yb)** show a striking similarity.^[19]

The half-sandwich Ca complex **5(Ca)** was recently introduced by

Table 1: Comparison of the crystal data for the Yb/Ca pairs **1–4** and the Sm/Sr pair **1**.

	1(Yb) [1(Ca)]	2(Yb) [2(Ca)]	3(Yb) [3(Ca)]	4(Yb) [4(Ca)]	1(Sm) [1(Sr)]
<i>a</i> [Å]	16.3290(8) [16.302(2)]	12.593(1) [12.569(2)]	19.475(1) [19.483(2)]	13.667(2) [13.617(1)]	9.9406(8) [10.0146(5)]
<i>b</i> [Å]	12.7720(7) [12.761(2)]	16.552(2) [16.550(2)]	12.451(1) [12.491(1)]	21.245(2) [21.267(2)]	10.3050(8) [10.3125(5)]
<i>c</i> [Å]	17.780(1) [17.772(2)]	18.095(2) [18.121(2)]	22.091(2) [22.082(2)]	21.376(3) [21.456(2)]	20.189(2) [20.208(1)]
α	90 [90]	90 [90]	90 [90]	90 [90]	85.935(7) [85.290(5)]
β	107.62(4) [107.52(5)]	128.570(8) [128.555(7)]	100.42(1) [100.302(8)]	90 [90]	89.237(7) [89.994(5)]
γ	90 [90]	90 [90]	90 [90]	90 [90]	75.335(6) [74.878(4)]
<i>V</i> [Å ³]	3534.1(8) [3525.7(8)]	2948.9(6) [2947.8(7)]	5270.3(7) [5287.2(9)]	6206.6(14) [6213.5(8)]	1995.7(3) [2007.4(2)]
color ^[33]	orange [light-yellow]	red [orange-red]	black/dark red [light-yellow]	black/dark red [light-yellow]	black/dark red [yellow]

us as an initiator for the living and syndiotactic polymerization of styrene.^[20] Unfortunately, the quality of the crystals of its Yb analogue was poor and only a unit cell and rough structure of **5(Yb)** could be determined.^[21] The ¹H NMR spectra of **5(Yb)** and **5(Ca)** show nearly equal chemical shifts (Figure 2). This is all the more remarkable, since it is known

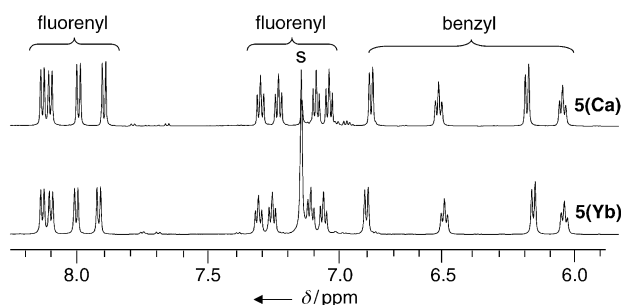


Figure 2. Aromatic region in the ¹H NMR (600 MHz, [C₆D₆]) spectra of **5(Yb)** and **5(Ca)**; s = residual solvent signal.

that the metal in benzyl complexes markedly influences the extent of charge delocalization and thus the chemical shift of the *ortho* and *para* protons.^[22] Almost identical charge delocalization is confirmed by the hybridization at the benzylic carbon CH(SiMe₃): **5(Yb)** and **5(Ca)** show an identical ¹J(¹³C,¹H) of 118.7 Hz, which corresponds to a pyramidal sp³ geometry. Chirality at the benzylic carbon gives two enantiotopic sides in the fluorenyl ligand: all hydrogen atoms are unique. Both complexes, **5(Yb)** and **5(Ca)**, show at higher temperature fast inversion about the benzylic carbon atom and coalescence of the fluorenyl signals. Interestingly, the concentration-independent coalescence temperatures and activation energies are significantly different: *T*_{coal} = 55(1) °C and Δ*G*[‡] = 17.0(1) kcal mol^{−1} for **5(Yb)**; *T*_{coal} = 90(1) °C and Δ*G*[‡] = 18.8(1) kcal mol^{−1} for **5(Ca)**.

The benzylcalcium complexes **1(Ca)** and **5(Ca)** have been tested as initiators in styrene polymerization.^[15,20] The homoleptic complex **1(Ca)** yields atactic polystyrene (Figure 3a) but a polymer with a slightly higher syndiotactic character is observed for polymerizations in pure styrene (Figure 3b). Polymerization in pure styrene at 20 °C with the heteroleptic initiator **5(Ca)** yields a polymer of considerable syndioregularity (Figure 3c; *r* = 91.2 %, *rr* = 83.1 %).^[23] The syndiotacticity can be increased by lowering the polymerization temperature from 20 to −20 °C (*r* = 92.6 %, *rr* = 85.7 %).

Hitherto well-defined Yb^{II} alkyls have not been tested in styrene polymerization^[24] (the first heteroleptic Yb^{II} half-sandwich complex was published only very recently).^[25] Styrene polymerization with the initiator **5(Yb)** gives a polymer with a significantly lower syndiotacticity than found for its Ca analogue (Figure 3d; *r* ≈ 82, *rr* ≈ 67 %). One reason for the observed difference could be faster inversion of its chiral chain end, which generates a stereo-error (Scheme 1).^[20] The lower activation energy for the inversion process in **5(Yb)** (see above) is in line with this assumption.

We were startled, however, to find that styrene polymerization with the homoleptic complex **1(Yb)** in pure styrene at

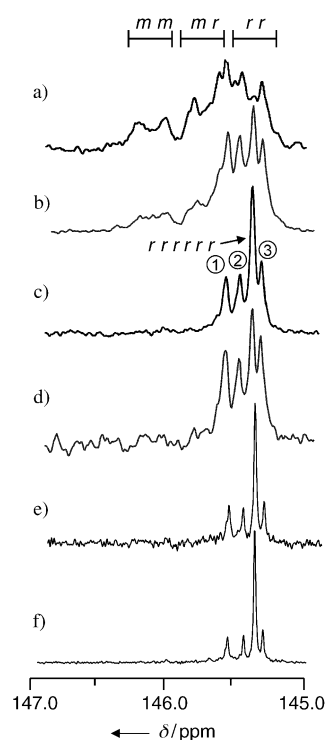
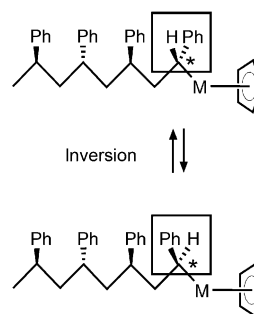


Figure 3. The ¹³C-signal of the phenyl C₁₀ in polystyrene (600 MHz spectrometer, 100 °C, [C₂D₂Cl₄]): a) initiator **1(Ca)**, 10% styrene solution in cyclohexane, 50 °C, *M*_n = 1.09·10⁵, *D* = 1.37; b) initiator **1(Ca)**, 100% styrene, 20 °C, *M*_n = 3.77·10⁴, *D* = 2.47; c) initiator **5(Ca)**, 100% styrene, 20 °C, *M*_n = 9.60·10⁴, *D* = 2.35; d) initiator **5(Yb)**, 100% styrene, 20 °C, *M*_n = 2.01·10⁵, *D* = 2.30; e) initiator **1(Yb)**, 100% styrene, 20 °C, *M*_n = 1.68·10⁵, *D* = 1.78; f) initiator **1(Yb)**, 100% styrene, −20 °C, *M*_n = 5.1·10⁴, *D* = 2.04. Signals are resolved at the heptade level: main signal = rrrrrr, 1 = rrrrrr, 2 = rrrrrr, 3 = mrrrrr.^[23]

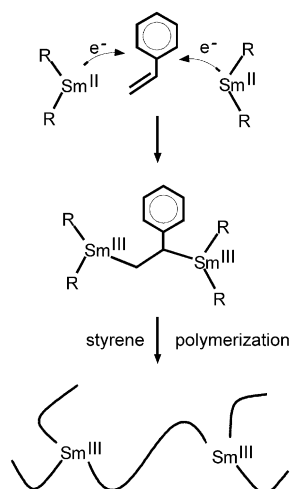


Scheme 1. Inversion of the chiral carbanionic chain end results in a stereo-error.

20 °C gives a polymer of high syndiotacticity (Figure 3e; *r* = 93.1 %, *rr* = 86.6 %). A decrease in temperature to −20 °C even lowers the number of stereo-errors further (Figure 3f; *r* = 94.9 %, *rr* = 90.0 %). Molecular-weight analysis of the polymer produced in solution suggests that there are two growing chains per metal center.^[26] This observation undermines the idea that stereoselective polymerization has to proceed by a single-site catalyst of general formula: [L_nM-alkyl] (L = spectator ligand). It is likely that the stereoselectivity is not determined by stereoselective mono-

mer coordination (the presence of two chiral centers leads to four possible diastereomeric coordination complexes), but is determined in the transition state for insertion. One explanation for the unexpected high stereoselectivity of the styrene polymerization with **1(Yb)** could be the much higher insertion rate observed for the ytterbium complex.^[27] Although the rate of inversion of the chiral polystyryl chain ends is currently unknown, it is the competition between stereoselective insertion and chain-end inversion that determines the syndiotacticity in the polymer obtained.^[20]

The remarkably high syndiospecificity in styrene polymerization with **1(Yb)** prompted us also to explore the chemistry of the analogue **1(Sm)**. The crystal structure of this samarium(II) complex shows strong similarity with that of the strontium analogue^[28] (Table 1; Figure 1b). This Sr/Sm similarity has been observed before^[8] and is due to nearly identical ionic radii: Sr^{2+} 1.21 Å; Sm^{2+} 1.22 Å.^[13] Both structures are similar to those of the Ca/Yb pair, except for the more extended coordination of the benzylic ligand to the somewhat larger metal ion. Styrene polymerization (10% solution in cyclohexane, 50 °C) with **1(Sm)** gave a polymer with a bimodal distribution of the molecular weights. The mean of the smaller fraction (ca. 20%) is approximately double that of the main fraction (ca. 80%).^[29] It is therefore likely that **1(Sm)** is oxidized by styrene to a catalytically active Sm^{III} species (Scheme 2; a similar intermediate with a



Scheme 2. Twofold electron transfer gives a bridged dimetallic Sm^{III} initiator.

bridging styryl group has been characterized by X-ray diffraction).^[30] This situation would result in a polymer with a 80:20 bimodal distribution as observed (the bridging chain grows twice as fast as the terminal chains). A similar mechanism for **1(Yb)** is excluded on the basis of: 1) the formation of a polymer with a unimodal distribution, 2) the lower standard oxidation potential E^0 ($\text{M}^{2+}/\text{M}^{3+}$) for ytterbium (+1.15 V) compared to samarium (+1.55 V),^[31] and 3) the styrene polymerization can be followed by NMR spectroscopy (paramagnetic Yb^{III} species would result in disturbance of field homogeneity).

The large differences observed in styrene polymerization with benzylcalcium and benzylytterbium initiators contrast

sharply with the surprising similarities of their molecular structures and NMR spectra. At this stage it is unclear which factors are responsible for the different behavior of analogous Ca/Yb compounds in polymerization reactions. It is unlikely that back-bonding of the f electrons plays a role in the intermediate Yb–styrene complex.^[32] Ligand–Ca and ligand–Yb bonds might be of similar length (the ligand–Yb bonds are generally slightly longer than the corresponding ligand–Ca bonds) but are not necessarily of similar strength and might have a different potential-energy profile, thus resulting in a different reactivity. The ligand–Yb bond can be weakened by electron–electron repulsion in the completely filled f shell ($4f^{14}$), as has been observed in $[\text{Cp}_2^*\text{Yb}(\text{CO})]$ complexes for which an unusual Yb–OC bond is proposed.^[32] A weaker ligand–Yb bond is in line with the low activation energy for inversion of the benzylic carbon in **5(Yb)**. It is possible that slight differences in ligand–metal bonding might have great impact on the stereoselectivity of a polymerization reaction.

We are currently extending our investigations on similarities and differences in the chemistry of the alkaline-earth and lanthanide metals.

Experimental Section

All synthetic methods and characterization of the Yb compounds (including the X-ray diffraction information) are reported in the Supporting Information. The standard polymerization procedure has been described earlier.^[28]

CCDC-217554–217558 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/contents/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

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Keywords: alkaline-earth metals · lanthanides · polystyrene · stereoselective polymerization

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Electron Density Studies

A Simple Approach to Nonspherical Electron Densities by Using Invariants***Birger Dittrich,* Tibor Koritsánszky, and Peter Luger*

The Hohenberg–Kohn theorem^[1] highlights the importance of the electron density $\rho(\mathbf{r})$ as a fundamental physical property that uniquely characterizes an electronic system. It states that the ground-state energy of a nondegenerate quantum chemical system is a function of the electron density. The electron density of a crystalline state can, in principle, be measured by high-resolution X-ray diffraction. In spite of the significantly improved experimental conditions (availability of area detectors, synchrotron radiation, and low-temperature devices), the collection of high-resolution data ($d \leq 0.5 \text{ \AA}$ or $\sin \theta \lambda^{-1} \geq 1.0 \text{ \AA}^{-1}$)—prerequisite for X-ray charge density

[*] Dr. B. Dittrich, Prof. Dr. P. Luger
Institut für Chemie/Kristallographie
Freie Universität Berlin
Takustrasse 6, 14195 Berlin (Germany)
Fax: (+49) 308-385-3464
E-mail: birger@chemie.fu-berlin.de

Prof. Dr. T. Koritsánszky
Department of Chemistry, Middle Tennessee State University, P.O.
Box 68, Murfreesboro, TN 37132 (USA)

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studies—remains technically difficult, especially for biopolymers, such as proteins, that usually form soft crystals of low scattering power. Owing to their size, the applicability of ab initio calculations to this important class of compounds is also limited.

The conventional interpretation of atomic-resolution X-ray data is based on the promolecule model (a superposition of spherically symmetric, isolated atomic densities). At this level, the exactness of the reconstruction of the diffraction image is limited to the main characteristic of the topology of the crystalline electron-density distribution, that is, the occurrence of maxima at the nuclear positions.

The popular pseudoatom formalism^[2] provides an atom-centered representation of electron-density deformations arising from chemical bonding. It was demonstrated recently, that pseudoatoms exhibit chemical transferability provided the deformation radial functions are localized.^[3] In crystallographic content, the above argument implies that the hidden/missing experimental information in the diffraction data can be revealed/supplemented by electron-density modeling that goes beyond the isolated-atom approximation.

Herein we present a method for modeling electron density, which is based on the locality of pseudoatoms and rationalized within the nearest-neighbor approximation (NNA),^[3] which can be used to model any molecular systems, including biopolymers. The NNA has been used successfully as a working hypothesis for modeling of larger molecular systems.^[4–6]

We introduce the concept of invariums. In this approach invariums (invariant atoms) are assigned to each chemically unique atom in a structure. For an individual atom the corresponding invarium is defined in that the nearest neighbors are the same as those of the chosen atom in terms of element and bond order. In line with this definition, more than one, but a finite number of, invariums can be assigned to one element, depending upon the atom's chemical environment. The atomic electron density of an invarium is generated from a geometry-optimized small model compound containing the invarium by using a quantum chemical approximation (in this work: B3LYP6-311++G(3df,3pd)^[7]). In such model species the next-nearest-neighbor atoms are then replaced by hydrogen atoms. For this optimized geometry theoretical structure factors^[8] are calculated and used as simulated data in a least-squares refinement,^[9] to determine the multipole parameters of an invarium. Figure 1 illustrates

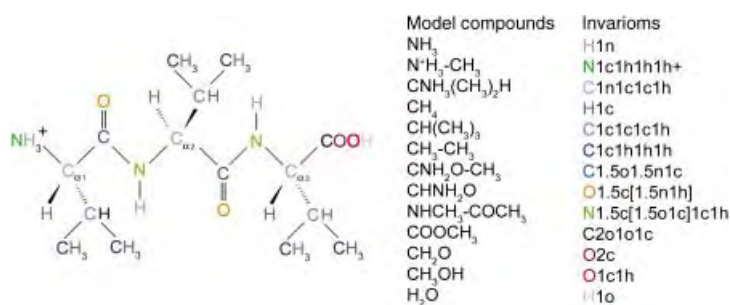


Figure 1. Schematic representation of the positively charged tri(L-valine) molecule with colored invariums, their nomenclature, and the corresponding model compounds.

the selection of invariums and their associated model molecules for the tri(L-valine) structure. The scheme also suggests a simple invarium nomenclature.^[10] The total electron density of a molecule is obtained as a superposition of its constituting invariums. In some cases of mesomerism and delocalized systems the next-nearest-neighbor atoms are considered for the corresponding invarium model compounds.

The method described here differs in important aspects from the experimental determination of multipole parameters suggested by Pichon-Pesme et al.^[11,12] for protein crystallography. Since our approach does not rely on experimental data, measurement errors and biases arising from inadequate modeling of the thermally smeared density, are excluded. Furthermore, we provide a consistent definition for transferable sites in terms of invariums that can be generated, in principle, for all chemically relevant bonding situations at any level of theory.

Results for the invarium analysis of two molecules are presented below and demonstrate the feasibility of the method. The first one is the tri(L-valine) structure which has recently been determined in our laboratory using a low-resolution ($\sin \theta/\lambda = 0.72 \text{ \AA}^{-1}$ or $d = 0.69 \text{ \AA}$) data set. The tripeptide crystallizes with two molecules in the asymmetric unit (one zwitterionic and the other one positively charged) together with a disorder propanol solvent and a trifluoroacetic acid counterion (Figure 2).

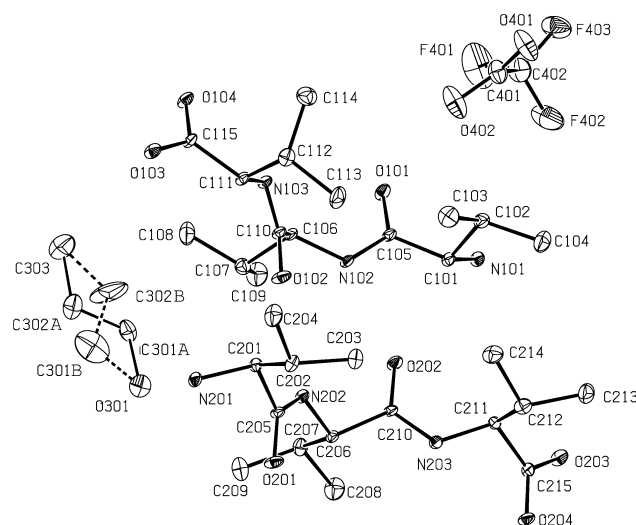


Figure 2. ORTEP^[20] representation of the asymmetric unit of the tripeptide investigated (thermal ellipsoids set at 50% probability); hydrogen atoms are omitted for clarity.

After the structure solution^[13] the molecule was spherically refined^[14] (parameters x , y , z , U_{ij}). The conventional structure refinement resolved the propanol disordered sites with $1/8$ and $7/8$ occupations. The spherical refinement was then repeated with the program XDLSM^[9] using a weighting scheme of $1/\sigma^2$. After transfer of the invarium-model multipole parameters the neutrality of the unit cell (a total invarium charge of 0.07 electrons) was reestablished

by rescaling the monopole populations. The scale factor, positional and thermal parameters were then refined to convergence.^[15]

The topological analysis of the invariom-model density (Table 1) led to bond descriptors that are statistically equal

Table 1: Topological analysis of covalent bonds at the bond critical points in tri(L-valine).^[a]

Bond	$\rho(r)_{\text{bcp}}$	$\nabla^2\rho(r)_{\text{bcp}}$	d	ε
$\text{C}_{\text{pept}}-\text{N}_{\text{pept}}$	2.32 2.39(8)	-28 -23(2)	0.8146	0.08
$\text{C}_{\alpha}-\text{N}_{\text{pept}}$	2.07 1.84(6)	-13 -13(2)	0.7598	0.13
$\text{C}_{\alpha}-\text{C}_{\text{pept}}$	1.75 1.77(4)	-13 -14(1)	0.7567	0.17
$\text{C}_{\text{pept}}-\text{O}_{\text{pept}}$	2.92 2.94(10)	-31 -36(6)	0.7358	0.13
$\text{C}_{\alpha}-\text{C}_{\beta}$	1.79 1.67(5)	-15 -11(2)	0.7416	0.09
$\text{C}_{\alpha}-\text{N}_{\text{amino}}$	1.91 1.80(6)	-8 -13(2)	0.7560	0.10
$\text{C}_{\alpha}-\text{C}'$	1.65 1.73(8)	-12 -13(2)	0.7679	0.17
$\text{C}'-\text{O}_{\text{meso}}$	2.71 2.72(9)	-29 -32(5)	0.7519	0.12

[a] Average values from both tri(L-valine) molecules. $\rho(r)_{\text{bcp}}$ and $\nabla^2\rho(r)_{\text{bcp}}$ are the electron density and the Laplace function at the bond critical point (bcp), d is the distance from the bcp to the first named atom of the bond, ε is the ellipticity. Units are e and Å. The second lines contain average bcp values from six oligopeptide studies.^[16]

(three standard deviations) to average values obtained for oligopeptides from high-resolution charge density studies.^[16]

Through the transfer of the invariom multipole parameters it was possible to determine a detailed nonspherical electron density despite the comparatively low resolution (Figure 3 a). The comparison of Figure 3 b and c clearly shows that the residual electron density is strongly reduced after the invariom refinement.

Molecular electronic properties, such as the dipole moment and the electrostatic potential (Figure 4, top for

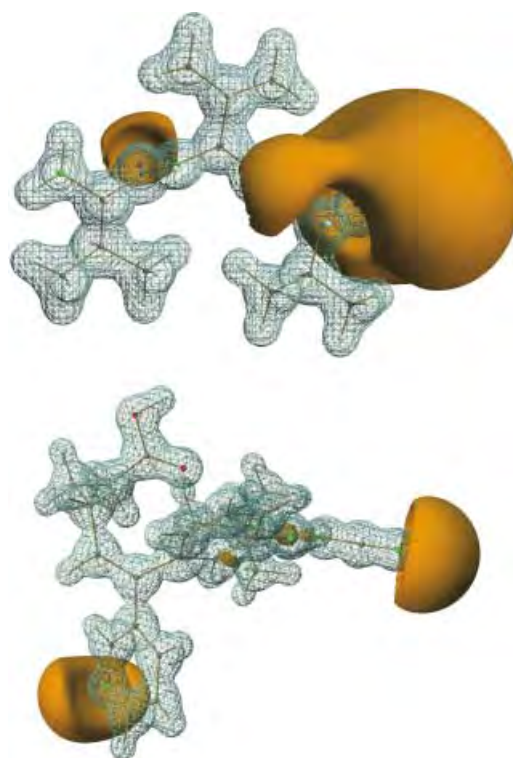


Figure 4. Electrostatic potentials: Top: the zwitterionic tri(L-valine) molecule from the structure of (VVV)₂·TFE·PrOH; Bottom: terbogrel, turquoise isosurface at +1 (terbogrel) and 0.5 (VVV), orange isosurface with -0.1 eÅ⁻¹.

zwitterionic tri(L-valine)) were also evaluated using the nonspherical invariom electron density. As expected, the dipole moment for the positively charged molecule 1 (6.5 D) is smaller than that for the zwitterionic molecule 2 (17.9 D).

Our second example is terbogrel, an anti-thrombotic drug molecule of more than 50 atoms. The experimental density of this molecule was extracted earlier from a 100 K data set of almost 220000 reflections, and compared with a theoretical electron density derived by ab initio calculations requiring 85 days of computing time.^[17] The invariom refinement of a

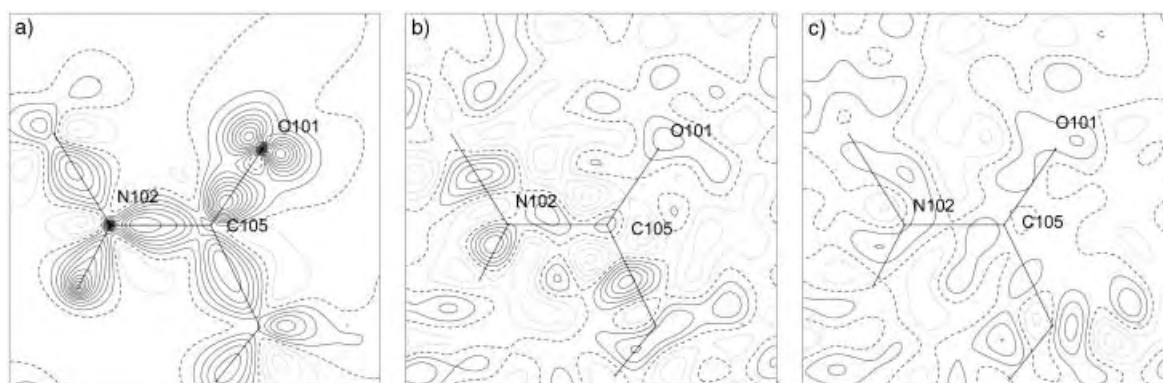


Figure 3. a) Deformation density in the plane of a peptide bond after the transfer of invarioms. Contours at 0.1 eÅ⁻³, b) residual electron density in this plane for the promolecule and c) for the invariom model. Contour lines at 0.05 eÅ⁻³. A significant reduction of residual electron density is clearly visible.

room-temperature low-resolution ($d = 1.1 \text{ \AA}$) data set of less than 3000 reflections took only a few minutes and led to an electron density and electrostatic potential (Figure 4, bottom) that resemble those derived in the more extended study.

We presented a novel scheme for interpretation of conventional X-ray diffraction data in terms of ab initio derived, transferable nonspherical atoms. Invariums can be acquired rapidly for any atom type in any bonding situation from appropriately designed small molecules or fragments and stored in a database. It has the advantage that no experimental errors are included in the invarium parameters. An invarium-based structure refinement leads, from knowledge of x , y , z , and U_{ij} to precise nonspherical electron density and related properties in principle for all structures including biomolecules. Future applications will include the calculation of Hirshfeld surfaces^[18,19] associated with invariums. Since conventional "low-order" data collection is becoming a routine task, the automated interpretation of these data in terms of the invarium formalism is a potential tool for high-throughput screening investigations commonly used in drug design.

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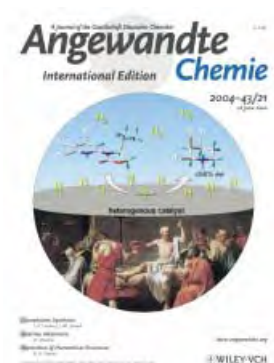
Keywords: electron density · structure elucidation · theoretical chemistry · X-ray structure analysis

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- [15] Crystal size $0.6 \times 0.21 \times 0.15 \text{ mm}^3$, monoclinic, $P2_1$, $Z = 2$, $a = 9.705(1)$, $b = 19.270(1)$, $c = 11.820(1) \text{ \AA}$, $\beta = 100.26^\circ$, $\rho = 1.229 \text{ g cm}^{-3}$, $V = 2175(1) \text{ \AA}^3$, $T = 120 \text{ K}$, $F(000) = 868e$, $\lambda = 0.7107 \text{ \AA}$ ($\text{Mo K}\alpha$), $\sin \theta/\lambda_{\text{max}} = 0.72 \text{ \AA}^{-1}$, routine measurement of less than < 12 h duration with Bruker Smart 1 K diffractometer. 35573 measured reflections, of which 6828 were symmetry independent and 6048 (with $F_o > 2\sigma(F_o)$) used in the refinement against F , hydrogen atoms from a difference Fourier synthesis except for the disordered propanol molecule, 798 parameters, after spherical refinement: $R_1 = 0.040$, $R_w = 0.030$, after transfer of the invarium multipole populations (not refined): $R_1 = 0.035$, $R_w = 0.025$. Structure was not ideally suited for a high-resolution measurement due to disorder and solvent molecules. CCDC 227052 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).
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Cover Picture

Frank Glorius,* Nick Spielkamp, Sigrid Holle, Richard Goddard, and Christian W. Lehmann

The stereoselective hydrogenation of oxazolidinone-substituted pyridines shown in the cover picture gives piperidines containing up to four new stereocenters. One of the biologically active compounds readily accessible by this method is (*S*)-coniine, the poisonous hemlock alkaloid used in ancient Greece for executions. The famous painting by David shows Socrates shortly before drinking from the poison chalice. F. Glorius et al. describe this synthetic method on pages 2850 ff.



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J. Vollet, J. R. Hartig, H. Schnöckel*:

Al₅₀C₁₂₀H₁₈₀: A Pseudofullerene Shell of 60 Carbon Atoms and 60 Methyl Groups Protecting a Cluster Core of 50 Aluminum Atoms
DOI: 10.1002/anie.200453754
Published online: May 6, 2004

T. Kato, C. A. Reed*:

Putting *tert*-Butyl Cation in a Bottle
DOI: 10.1002/anie.200453931
Published online: May 6, 2004

Y. Chen, M. Wang, C. Mao*:

An Autonomous DNA Nanomotor Powered by a DNA Enzyme
DOI: 10.1002/anie.200453779
Published online: May 6, 2004

R. E. Looper, R. M. Williams*:

A Concise Asymmetric Synthesis of the Marine Hepatotoxin 7-Epicylindrospermopsin
DOI: 10.1002/anie.200454208
Published online: May 6, 2004

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Author(s), *Angew. Chem. Int. Ed.*, online publication date, DOI.

Obituary

Hugh Christopher Longuet-Higgins (1923–2004) and John A. Pople (1925–2004) **2740** W. Kutzelnigg

Books

Organosilicon Chemistry V ——— **2744** Norbert Auner, Johann Weis *reviewed by G. Linti*

The Role of the Solvent in Chemical Reactions ——— **2744** Erwin Buncel, Robert Stairs, Harold Wilson *reviewed by J. B. F. N. Engberts*

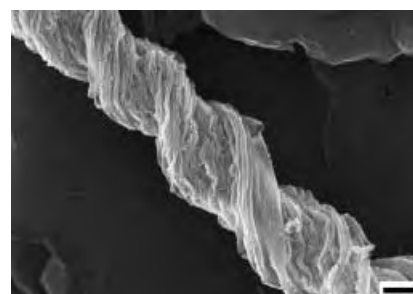
Highlights

Replication Processes

R. A. Caruso* ——— **2746–2748**

Micrometer-to-Nanometer Replication of Hierarchical Structures by Using a Surface Sol–Gel Process

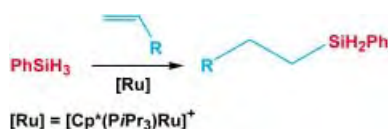
The sol–gel process has been shown to replicate materials with high precision on both the micro- and nanometer scales. Paper, cotton, and cloth templates combine with a surface sol–gel process in order to maintain details of hierarchical structural properties by producing a self-supporting material consisting of interwoven and branched tubes that result from fibers less than 100 nm in diameter (see SEM image; scale bar = 2 μm).



Selective Alkylations

H. Brunner* ——— **2749–2750**

A New Hydrosilylation Mechanism—New Preparative Opportunities



One up in limiting reactivity: The mono-substitution of multifunctional molecules is a general problem in synthetic chemis-

try. The catalyst [Cp*(P*i*Pr₃)(H)₂Ru=Si(H)Ph·Et₂O][B(C₆F₅)₄] (Cp* = η⁵-C₅Me₅) can be used for the alkylation of phenylsilane PhSiH₃ with simple olefins. The reaction stops at the monoalkylation level to give PhSi(Alk)H₂ (see scheme; R = H, C₆H₅, C₄H₉).

Reviews

Hydrocyanations

J.-M. Brunel, I. P. Holmes* – 2752–2778

Chemically Catalyzed Asymmetric Cyanohydrin Syntheses



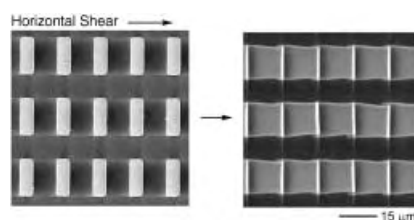
Versatile synthetic intermediates: Chiral cyanohydrins (see formula) can be readily converted into a number of key functional groups with control of absolute stereochemistry. Advances in chemically catalyzed asymmetric cyanation reactions of aldehydes and ketones to form cyanohydrins are described and compared.

Communications

Nanotechnology

B. D. Gates, Q. Xu, V. R. Thalladi, T. Cao, T. Knickerbocker, G. M. Whitesides* – 2780–2783

Shear Patterning of Microdominos: A New Class of Procedures for Making Micro- and Nanostructures

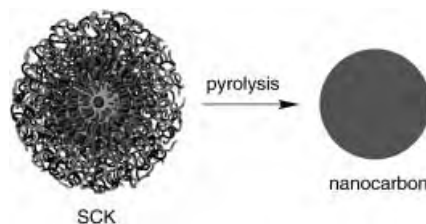


A simple technique for fabricating complex, repetitive 3D microstructures, and the application of this procedure to the generation of arrays of nanoscale (in-plane) features is described. This technique, which involves the use of shearing forces, is capable of fabricating regular, 3D arrays of microstructures that are difficult or impossible to obtain by other methods (see picture).

Nanostructures

C. Tang, K. Qi, K. L. Wooley, K. Matyjaszewski, T. Kowalewski* – 2783–2787

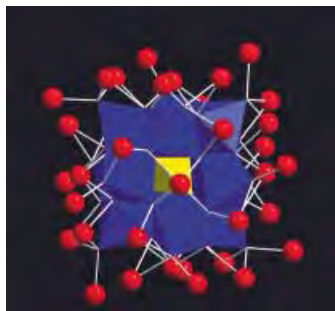
Well-Defined Carbon Nanoparticles Prepared from Water-Soluble Shell Cross-linked Micelles that Contain Polyacrylonitrile Cores



An alternative approach to nanostructured carbon materials relies on the use of covalently stabilized micellar precursors that are soluble in aqueous systems (see picture). These precursors belong to a class of shell cross-linked nanoparticles (SCKs) that are formed by self-assembly and stabilization of amphiphilic block copolymers.

Keggin Ions

M. Nyman,* F. Bonhomme, T. M. Alam, J. B. Parise, G. M. B. Vaughan – 2787–2792

 $[\text{SiNb}_{12}\text{O}_{40}]^{16-}$ and $[\text{GeNb}_{12}\text{O}_{40}]^{16-}$: Highly Charged Keggin Ions with Sticky Surfaces


The α -Keggin ions $[\text{TNb}_{12}\text{O}_{40}]^{16-}$ (T = Si, Ge) have been synthesized hydrothermally as water-soluble sodium salts. These clusters are very highly charged, and are bound to their charge-balancing sodium atoms in the solid state (see picture). NMR spectroscopy experiments show that the sodium atoms remain strongly associated with the clusters in solution.

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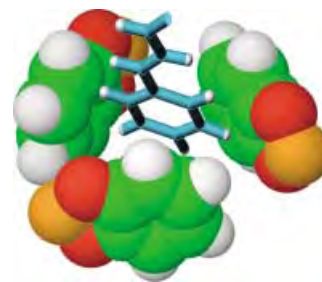
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Supramolecular Chemistry

P. Sozzani,* A. Comotti, S. Bracco,
R. Simonutti — 2792 – 2797

A Family of Supramolecular Frameworks of Polyconjugated Molecules Hosted in Aromatic Nanochannels

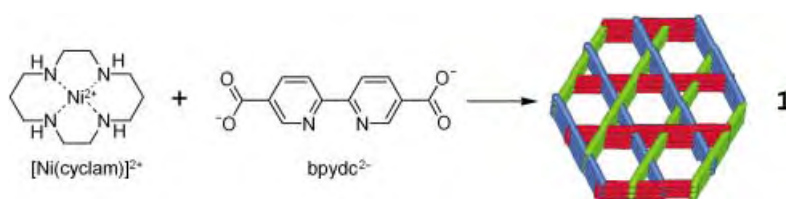
Aromatic nanochannels were assembled about linear π -conjugated molecules. The novel supramolecular architectures are cemented by weak interactions and form crystals that melt at much higher temperatures than the standard molecular arrangement. The perfect fitting of the chains in the unusual environment is shown by the close contacts of 2.5 Å that occur between guest hydrogen atoms and the surrounding aromatic groups (see picture).



Metal–Organic Framework

E. Y. Lee, M. P. Suh* — 2798 – 2801

A Robust Porous Material Constructed of Linear Coordination Polymer Chains: Reversible Single-Crystal to Single-Crystal Transformations upon Dehydration and Rehydration



Hole in one: A metal–organic framework with permanent microporosity has been constructed from linear coordination polymer chains consisting of $[\text{Ni}(\text{cyclam})]^{2+}$ and bpydc^{2-} . In framework **1** the chains run in three directions (red, green, blue in

the picture) and are stacked to form 1D channels that are filled with water molecules, which can be removed completely and reabsorbed with retention of the single crystallinity.

Single-Molecule Magnets

H. Miyasaka,* R. Clérac,*
W. Wernsdorfer,* L. Lecren, C. Bonhomme,
K.-i. Sugiura, M. Yamashita — 2801 – 2805

A Dimeric Manganese(III) Tetradentate Schiff Base Complex as a Single-Molecule Magnet

Slow relaxation of the magnetization reveals the single-molecule magnetic behavior of $[\text{Mn}_2(\text{saltmen})_2(\text{ReO}_4)_2]$ ($\text{saltmen}^{2-} = N,N'-(1,1,2,2\text{-tetramethylethylen-})\text{bis}(\text{salicylideneimine})$), a simple, out-of-plane Mn^{III} dimeric complex containing a $[\text{Mn}^{\text{III}}(\mu\text{-O})_2\text{Mn}^{\text{III}}]$ core (see structure shown, Mn brown, O red, N blue, Re pink). The two anisotropic Mn^{III} ions are ferromagnetically coupled, which induces an $S_T = 4$ ground state.



Controlled NO Transfer

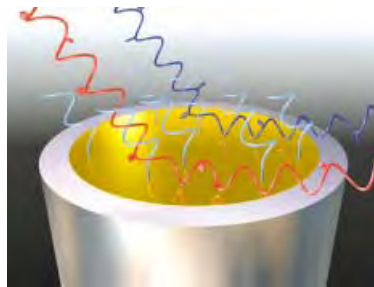
J. T. Mitchell-Koch, T. M. Reed,
A. S. Borovik* — 2806 – 2809

Light-Activated Transfer of Nitric Oxide from a Porous Material

A **light-dark-light-dark sequence** was used to demonstrate that only NO is released from a new mesoporous material that contains immobilized $\text{Ru}(\text{NO})$ sites. No release is observed during the dark phases (shown schematically). Since this release is regulated by light, the controlled transfer of NO to cobalt(II) tetraphenylporphyrin as well as equine skeletal muscle myoglobin is possible.



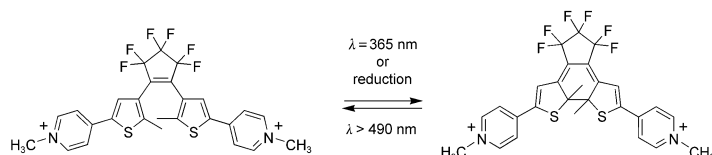
Ferrocene and anthraquinone were used to label RNA (red, dark blue), which could then be detected at an electrode surface bearing complementary DNA oligonucleotide probes (light blue). This approach is an electrochemical analog of popular fluorescence methods for gene expression analysis.



RNA Detection

D. A. Di Giusto, W. A. Wlassoff,
S. Giesebrecht, J. J. Gooding,
G. C. King* ————— 2809–2812

Enzymatic Synthesis of Redox-Labeled
RNA and Dual-Potential Detection at
DNA-Modified Electrodes



Getting the blues: Electrochemical ring-closing is observed for the photochromic bis(pyridinium) dication of the dithienylethene backbone in which the pale yellow ring-open isomer can be converted to its

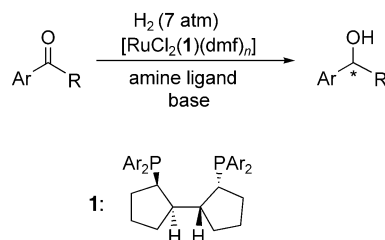
blue ring-closed isomer by either irradiation with UV light or by electrochemical reduction (see picture). The electrochemical ring-closing reaction also produces a photochemically unobtainable product.

Photochromism

B. Gorodetsky, H. D. Samachetty,
R. L. Donkers, M. S. Workentin,
N. R. Branda* ————— 2812–2815

Reductive Electrochemical Cyclization of a
Photochromic 1,2-Dithienylcyclopentene
Dication

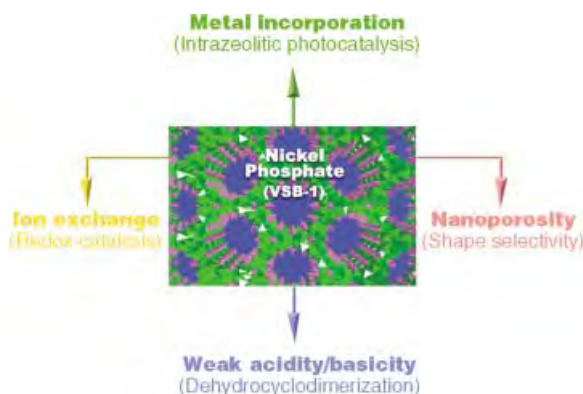
Variations on a theme: A Ru^{II}–bicp complex used in combination with an achiral 2-(alkylthio)amine ligand (or achiral 1,2-diamine ligand) is an efficient catalyst for the highly enantioselective hydrogenation of a variety of ketones (see scheme). In some cases simply changing the amine ligand and/or the solvent leads to opposite enantioselectivity.



Stereoselective Reduction

D. G. Genov,* D. J. Ager — 2816–2819

Asymmetric Hydrogenation of Ketones
Catalyzed by Ru^{II}–bicp Complexes



A substitute for zeolites: The nanoporous nickel phosphate VSB-1 exhibits zeolitic properties and very weak acidity and basicity (see picture). When VSB-1 is modified by metal-ion exchange or

through metal incorporation into the framework, it offers promising catalytic properties, such as shape selectivity, and activity in both redox catalysis and photocatalysis.

Porous Materials

J.-S. Chang,* J.-S. Hwang, S. H. Jhung,
S.-E. Park,* G. Férey,
A. K. Cheetham* ————— 2819–2822

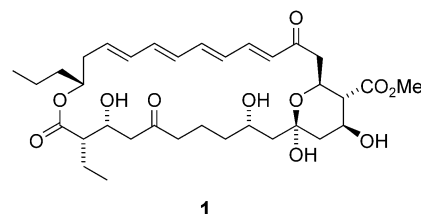
Nanoporous Metal-Containing Nickel
Phosphates: A Class of Shape-Selective
Catalyst

Natural Products Synthesis

G. K. Packard, Y. Hu, A. Vescovi,
S. D. Rychnovsky* — 2822–2826

Synthesis of Rimocidinolide Methyl Ester,
the Aglycone of (+)-Rimocidin

An efficient, convergent route based on cyanohydrin acetonide couplings was used for the synthesis of the aglycone of rimocidin, rimocidinolide methyl ester (**1**). Cyanohydrin acetonides were used as β -hydroxyketone synthetic equivalents in the assembly of the polyol chain.

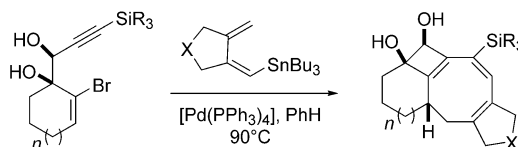


Domino Reactions

B. Salem, J. Suffert* — 2826–2830

A 4-*exo*-dig Cyclocarbopalladation/
 8π Electrocyclization Cascade:
Expeditious Access to the Tricyclic Core
Structures of the Ophiobolins and
Aleurodiscal

New perspectives for an unpopular reaction? An 8π electrocyclization has been used to construct the eight-membered ring of 5,8,5- and 6,4,8,5-polycyclic systems in a one-pot 4-*exo*-dig cyclocarbo-



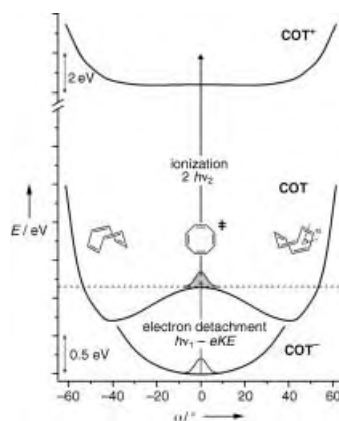
palladation/Stille coupling/electrocyclization sequence (see scheme). The polycycles obtained have the framework of biologically important compounds such as the ophiobolins. R = Et, Me; $n = 0, 1$.



Femtochemistry

D. H. Paik, D.-S. Yang, I.-R. Lee,
A. H. Zewail* — 2830–2834

The Transition State of Thermal Organic
Reactions: Direct Observation in Real
Time

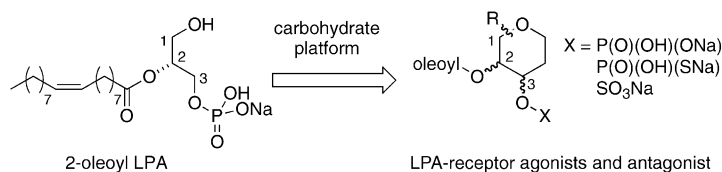


A classic in physical organic chemistry, the inversion of cyclooctatetraene (COT), was studied with femtosecond resolution by negative-ion-beam techniques. The transition state for the thermal inversion was accessed directly by detachment of the electron from the planar COT⁻ ion.

Molecular Recognition

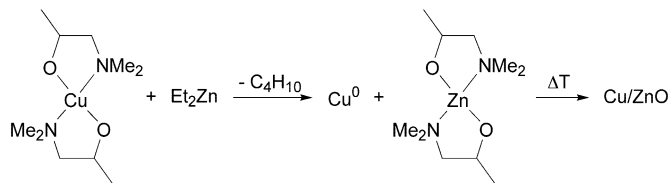
Y. Tamaruya, M. Suzuki, G. Kamura,
M. Kanai,* K. Hama, K. Shimizu, J. Aoki,
H. Arai, M. Shibasaki* — 2834–2837

Identifying Specific Conformations by
Using a Carbohydrate Scaffold: Discovery
of Subtype-Selective LPA-Receptor
Agonists and an Antagonist



Stable and potent subtype-selective lyso-phosphatidic acid (LPA) analogues (agonists and an antagonist) were developed by using carbohydrates as a core structure (see scheme). An array of molecules with the recognition motifs of LPA (a phos-

phate anion, an oleoyl group, and a hydrogen-bond acceptor) attached to carbohydrate isomers in different three-dimensional arrangements were tested for LPA-receptor activation or inhibition. R = alkyl.



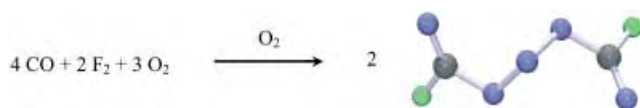
Consecutive vaporization cycles for the preparation of heterogeneous catalysts: Active Cu- and ZnO-containing methanol-synthesis catalysts were obtained by vaporizing suitable organometallic precursors into mesoporous silicates, fol-

lowed by thermal treatment (see scheme). The catalytically active components in the final material have nanoscopic dimensions. The methanol production by the catalysts is similar to that of systems synthesized by the classical route.

Heterogeneous Catalysis

R. Becker, H. Parala, F. Hipler, O. P. Tkachenko, K. V. Klementiev, W. Grünert, H. Wilmer, O. Hinrichsen, M. Muhler, A. Birkner, C. Wöll, S. Schäfer, R. A. Fischer* ————— **2839–2842**

MOCVD-Loading of Mesoporous Siliceous Matrices with Cu/ZnO: Supported Catalysts for Methanol Synthesis



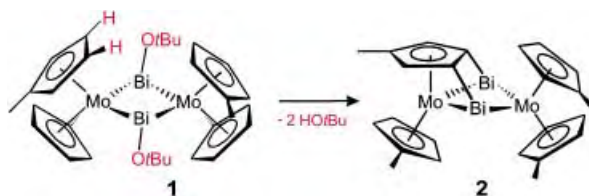
Three of a kind: The open-chain trioxide bis(fluoroformyl)trioxide, FC(O)OOOC(O)F, is prepared on preparative scale by treating CO with F₂ in an oxygen atmosphere (see scheme; F green, O blue, C gray). The unstable FC(O)OOOC(O)F is

characterized fully including X-ray diffraction. In the crystal structure the most stable rotamer (*trans-syn-syn*) is present as only one of two possible chiral helical molecules.

Chirality

H. Pernice, M. Berkei, G. Henkel, H. Willner,* G. A. Argüello, M. L. McKee, T. R. Webb ————— **2843–2846**

Bis(fluoroformyl)trioxide, FC(O)OOOC(O)F



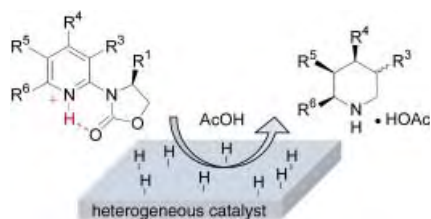
“Banana bonds” between Bi and C are a feature of **2**, which forms in the course of intramolecular alcohol eliminations from

1. C–H activations through complex-induced proximity effects are responsible for these unexpected eliminations.

Bismuth Complexes

S. Roggan, C. Limberg,* B. Ziemer, M. Brandt ————— **2846–2849**

Intramolecular C–H Activation in Complexes with Mo–Bi Metal Bonds



Up to four stereocenters can be created efficiently in a single step by the asymmetric hydrogenation of oxazolidinone-substituted pyridines (see scheme). Furthermore, selective chirality transfer and nondestructive cleavage of the chiral auxiliary occur under the same reaction conditions, making an additional cleavage step unnecessary.

Asymmetric Synthesis

F. Glorius,* N. Spielkamp, S. Holle, R. Goddard, C. W. Lehmann ————— **2850–2852**

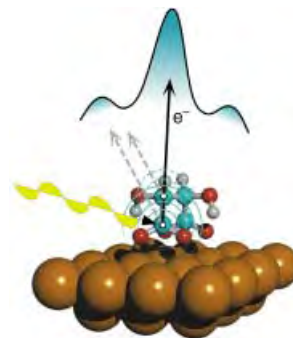
Efficient Asymmetric Hydrogenation of Pyridines

Chirality

R. Fasel,* J. Wider, C. Quitmann,
K.-H. Ernst,* T. Greber — 2853–2856

Determination of the Absolute Chirality of
Adsorbed Molecules

Intramolecular “electron lamp” throws bright shadows: Angle-scanned X-ray photoelectron diffraction (XPD) allows the absolute configuration of adsorbed molecules to be determined in a straightforward way. Owing to intramolecular scattering, the C 1s XPD pattern of tartaric acid adsorbed on Cu(110) (see picture; Cu brown, C blue, O red) has pronounced forward-focusing maxima that directly yield the absolute molecular conformation without the need for complex calculations.



Communications labeled with this symbol have been judged by two referees as being “very important papers”.

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Corrigendum

In the Communication by A. Berndt and co-workers published in *Angew. Chem. Int. Ed.* **2003**, 42, 1717–1719, Figure 1 (the crystal structures of compounds **4a**·Li₂(DME)₂ and **5c**) was inadvertently not printed. Figure 1 is shown below.

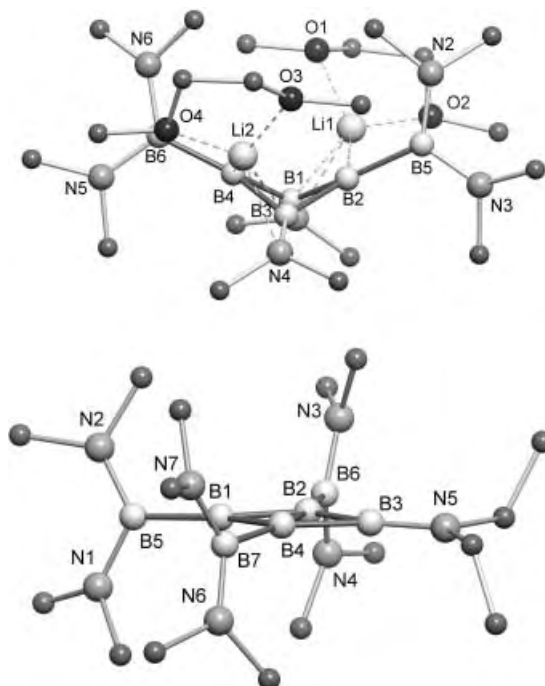


Figure 1. Structures of **4a**·Li₂(DME)₂ (top) and **5c** (bottom). Selected bond lengths [pm] and angles [°]: **4a**·Li₂(DME)₂: B1–B2 162.7(3), B2–B3 163.4(3), B3–B4 162.0(4), B4–B1 163.9(3), B1–B3 190.6(4), B1–N1 150.2(3), B2–B5 168.4(4), B3–N4 150.0(3), B4–B6 168.6(4), B5–N2 144.9(3), Li1–O1 198.7, Li1–B1 215.7, Li1–B2 235.4, Li1–N1 216.7(4); B1–B2–B3 71.5(2), B2–B1–B4 89.4(2), B2–B1–B3–B4 120.6(2). **5c**: B1–B2 160.5(3), B2–B3 162.2(3), B3–B4 161.3(3), B4–B1 160.5(3), B2–B4 160.5(3), B1–B5 169.4(3), B2–B6 167.9(3), B4–B7 170.8(3), B3–N5 138.7(3), B6–N3 142.9(3); B1–B2–B6 113.6(2), B3–B4–B7 138.8(2), B2–B3–N5 147.0(2), B1–B4–B7 100.3(1), B1–B2–B4–B3 177.7(2).

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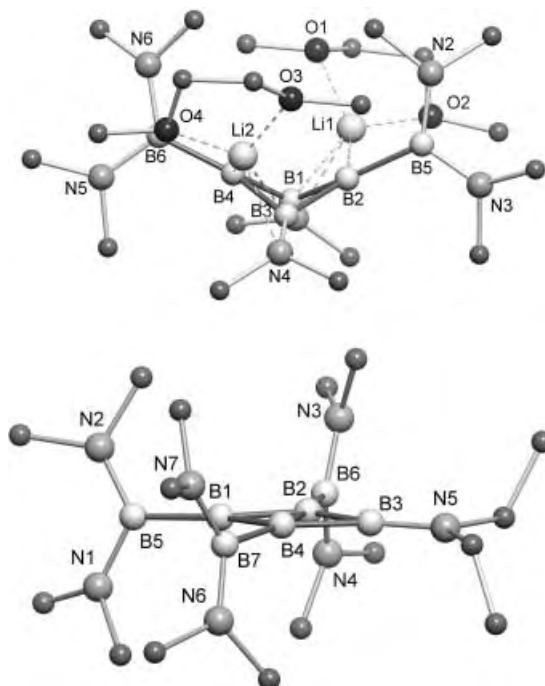


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H. C. Longuet-Higgins and J. A. Pople: Theoretical Chemistry**

Werner Kutzelnigg*

Within just a few weeks two remarkable theoretical chemists have left us forever. Although they had some common background, they could hardly have been more different. Each in his own way made a noticeable impact on science. Both were English, from the famous schools of Lennard-Jones and Coulson. Longuet-Higgins and Pople collaborated on a few publications together, for example, on the Renner-Teller effect.^[1] Both initiated their careers with studies on the Hückel molecular orbital theory (HMO) of π -electron systems, but their later roles were very different. Whereas Longuet-Higgins favored the kind of theory that could be formulated on the back of an envelope, Pople became a major protagonist of computational chemistry. The spectacular success of the latter field resulted not only from the intelligence and ingenuity of quantum chemists, but also from the extraordinarily rapid development of computer technology. Pople bet on the winning horse.

Hugh Christopher Longuet-Higgins (1923–2004)

Hugh Christopher Longuet-Higgins studied with C. A. Coulson in Oxford. Their very nice work on HMO theory made this theory esthetically appealing and mathematically elegant,^[2–4] for example, through the formulation of HMO-based perturbation theory.^[2] I particularly like one article, in which, for a special class of compounds, the MO coefficients are constructed without

any calculations.^[3,4] Longuet-Higgins was only 31 when he took up a position in Cambridge in 1954 as the successor of Lennard-Jones, who had left Cambridge for the recently founded reform university in Keele.

Longuet-Higgins's studies on electron-deficient boron compounds were very important for chemistry. Although one usually associates the theory of this class of compounds—certainly with good reason—with W. N. Lipscomb, the decisive publication on the electronic structure of B_2H_6 was written by Longuet-Higgins while he was still a graduate student.^[5] Mainly by using group-theoretical arguments, he both explained the stability of CaB_6 and predicted the existence of $B_{12}H_{12}^{2-}$ before its experimental preparation.^[4,6]

There is another context in which Longuet-Higgins was given less credit for his contributions than he was due. He was the first to apply the concept of correlation diagrams to explain electrocyclic reactions in terms of what are now known as the Woodward-Hoffmann rules, whereby he went far beyond the somewhat vague concept of the conservation of orbital symmetry.^[7]

Two further discoveries of Longuet-Higgins will certainly guarantee his lasting reputation. One is the first example of what is now known as a Berry phase, namely, the change of sign of the wave function on a closed loop around a conical intersection.^[8] Another seminal contribution was his theory of the symmetry groups of nonrigid molecules, into which feasible permutations of equivalent atoms enter as symmetry elements.^[9] It is impossible to understand the rotation-vibration spectra of fluxional molecules, especially in the millimeter region, without this theory.^[10]



H. C. Longuet-Higgins

Longuet-Higgins had a sharp mind, but was also somewhat sarcastic and tended to make jokes at the expense of others. Löwdin was apparently quite angry about Longuet-Higgins's parody of the “green technical reports” from Uppsala. S. F. Boys in particular suffered from the way his superior, Longuet-Higgins, treated him. Longuet-Higgins did not care for the

numerical work of Boys. Perhaps Longuet-Higgins was aware that Boys's proposal to perform quantum-chemical computations by using a Gaussian basis^[11] would have at least as much impact as his own most important contributions. Perhaps he sensed that Boys's work would turn out to be the beginning of a new and powerful branch of theoretical chemistry in which he, Longuet-Higgins, would neither have, nor wish to have, a share. This attitude may help explain Longuet-Higgins's decision to abandon his successful career as a theoretical chemist in 1967, to leave his chair in Cambridge after only 13 years, and to devote himself in Edinburgh to new challenges in the realm of neuroinformatics and theoretical psychology, for

example, the perception of music.^[12] I was unable to find out whether the standing Longuet-Higgins achieved in the neurosciences was comparable to the prestige he had formerly gained in chemistry. Six years ago I had

the chance to ask him (at an event in commemoration of S. F. Boys) whether he regretted having left theoretical chemistry. His only answer was that he never regretted anything. Longuet-Higgins's legacy as a theoretical chemist might be characterized by his Faraday Discussion lecture in Brighton,^[13] during which he proposed to divide chemists into three classes: experimentalists, computationalists, and theorists. This sharp distinction between theory and computational chemistry did not gain many adherents.

Theory on the back of an envelope

[*] Prof. Dr. W. Kutzelnigg
Fakultät für Chemie der
Ruhr-Universität Bochum
(Germany)

[**] The author thanks Paul von Ragué Schleyer for his help in preparing the English version of this manuscript.

John A. Pople (1925–2004)

John A. Pople studied with Lennard-Jones in Cambridge. Pople was a mathematician who felt (accurately, as it



J. A. Pople

turned out) that he could make more of a mark in chemistry. Although he started with studies in statistical mechanics, Pople's main achievements were in three fields: the theory of the magnetic

properties of molecules, semiempirical quantum chemistry, and *ab initio* quantum chemistry. His theoretical contributions to NMR spectroscopy, based mainly on the HMO method, were summarized in his monograph with Schneider and Bernstein.^[14] For over a decade, this book had considerable impact on the developing field of high-resolution NMR spectroscopy.

It is reported that Pople, then head of the basic-physics division of the National Physical Laboratory at Teddington, was appointed to a royal commission charged with finding out why so many British scientists were moving to the USA. Apparently he did find out, because shortly afterwards, in 1964, he accepted a position in Pittsburgh, PA, where he pursued most of his scientific career at Carnegie-Mellon University. He spent his last years at Northwestern University, near Chicago.

Until the late 1950s, molecules of chemical interest with more than a few atoms could only be treated by drastically simplified methods. These methods could, in principle, be derived from quantum mechanics.^[4] However, such crude approximations were required that useful results were only possible if certain quantities were treated as empirically adjustable parameters. Originally the HMO method was the most important semiempirical scheme, but it was limited to the description of π -electron

systems. It is a “one-electron” theory, as the electron–electron interactions are not treated explicitly. Pople invented a straightforward improvement of HMO theory, which, akin to an SCF theory, treats electron interaction (SCF: self-consistent field).^[15] At about the same time, Pariser and Parr^[16] proposed an HMO theory in which a semiempirical correction of the electron interaction was an important part. The two treatments merged into the famous PPP (Pariser–Parr–Pople) approximation, which dominated the theory of UV/Vis spectroscopy of conjugated π -electron systems for more than two decades.

For a remarkably long time only the so-called “mobile” π electrons in conjugated systems could be described semiempirically.^[4] One had to wait for semiempirical schemes for all electrons (including the largely localized σ electrons) until R. Hoffmann^[17] proposed the extended Hückel theory (EHT; a one-electron theory, though with inclusion of the overlap between the atomic orbitals) and Pople suggested

CNDO^[18,19] as a generalization of the PPP method (that is, with electron interaction, but without overlap).

The notation CNDO (complete neglect of differential overlap)

has created some confusion, because the neglect of overlap is hardly legitimate, especially for σ electrons. However, it can be justified indirectly if one regards orthogonalized atomic orbitals (AOs), rather than the original AOs, to be the basis functions.^[4] The EHT method has probably been more popular in inorganic chemistry, whereas CNDO has found most applications in organic chemistry. The literature is rich in modifications of CNDO, mainly from the school of M. J. S. Dewar. The most sophisticated of these, by W. Thiel,^[20] is still used successfully, in particular for very large conjugated systems. Methods such as PPP and CNDO were usually regarded—often with a polemic undertone—as extremely crude simplifications of the exact theory. However, one can regard them in a more positive light as refinements of the Hubbard model, which is still extremely popular in solid-state theory.

The essential aspect of this model and its refinements is the competition between (overlap-dependent) short-range forces and long-range electrostatic (Coulomb-like) interactions in a molecule or a solid.

When Pople decided to abandon semiempirical theory^[21] (for which Dewar, his companion in its development, apparently never forgave him) and to join the *ab initio* branch, the latter field had already undergone significant development and was quite successful for smaller molecules. Therefore, Pople's role was not to initiate an entirely new methodology. Although he was first regarded as an outsider by the inner circle of *ab initio* theorists, or even as someone who wished to reinvent the wheel, to organic chemists interested in theory he soon became the uncontested authority on *ab initio* theory. This popularity was due not so much to Pople's praiseworthy improvements to methods and programs (e.g. in the form of an accelerated integral program), as to his concept of computational chemistry, which he had developed during his experiences with semiempirical theory. A “traditional” *ab initio* theorist would study one system at a time (such as the potential hypersurface of a chemical reaction) and try to apply the best method available for a particular problem. The same theorist's investigation of the next system would likely involve a different theoretical level, again chosen as the best available for the new purpose. Hence, it was difficult for a non-expert to compare different publications, even by the same author, to say nothing of the results of different authors. Pople, on the other hand, published computations based on a well-defined method, which was used uniformly for 100 or more molecules, rather than just a single species. He also insisted on making careful and comprehensive comparisons with the corresponding experimental data. His notion of a “model chemistry”,^[22] defined by a chosen approximation (such as SCF) and a particular basis set (such as STO-3G or 4-31G*), was central to this concept. Each model chemistry was thoroughly documented, especially with regard to its reliability and the limits of its validity, based on comparisons with experiment. This strategy was like the

From semiempirical methods ...

application of standardized empirical methods, such as CNDO and INDO, which were parameterized to reproduce experimental data. The concept of a model ab initio chemistry also implied standardization and, consequently, a loss of flexibility, but it had the advantage of portability. Authors around the world could not only perform computations fully consistent with one another on different machines, but they could also build upon the published computations of others. Major support for this concept came from the decision by Pople and his co-workers to develop a series of user-friendly programs (key-word GAUSSIAN) and to make them generally available (even the source code) at affordable prices. The authors of other programs for quantum chemistry were much more reticent in these respects. Pople's goal was to develop and distribute a tool for general use by chemists. Consequently, GAUSSIAN became the market leader, and gained for Pople authority on the ab initio nomenclature.

When Pople decided to perform computations including electron correlation (that is, the effects ignored in the SCF approximation),^[23] he might have had recourse to existing methods that had already been developed and were being applied successfully in Germany, such as IEPA (independent electron pair approximation)^[24] or CEPA (coupled electron pair approximation).^[25,26] However, he preferred to establish his own (not very different) hierarchy of approximations for electron correlation, which then largely eclipsed IEPA and CEPA. Much later, in private conversations, he admitted that it might not have been fair to completely ignore the debt to these earlier approaches.

Like most ab initio theorists, Pople initially regarded density functional theory with skepticism, especially approaches of the LDA (local density approximation) type,^[27] for the simple reason that these were too inaccurate

for chemical purposes. The situation changed dramatically when A. Becke^[28] and J. Perdew^[29] proposed so-called second-generation density functionals, which included "gradient corrections" and made chemical accuracy possible. Thereafter Pople, ever a pragmatic scientist, did not hesitate to implement density functionals, such as B3LYP, in his program packages.^[30]

Without Pople's contributions, the unexpected triumph of numerical ab initio quantum chemistry, which has made it an irreplaceable tool for most chemists, would very likely not have occurred. It was extremely helpful that Pople found competent partners, who helped to bridge the gap to "real chemistry". P. von R. Schleyer was an important convert.^[31] For example, he used theory to reveal entire domains of chemistry that were unknown from experiment.

It was entirely deserved, and came as no surprise, that John Pople was awarded the Nobel Prize in Chemistry in 1998 for his life's work.^[32] The prize was shared by Walter Kohn in recognition of his original proposals of density functionals,^[27] which had been developed rapidly^[28,29] and implemented into quantum-chemical program packages.^[30] However, its "marriage" with density functional theory was not necessary for ab initio quantum chemistry to achieve its big breakthrough. Density functional formalism is just one stone (albeit a very bright one) in the rich mosaic of ab initio quantum chemistry. Other stones include the evaluation of energy gradients and of relativistic corrections.

Together with many others—who, willingly or not, remained in the background—Pople contributed greatly to the fulfillment of the goal, which has now even been surpassed: that theoretical chemistry should attain uncontested importance in chemistry. Although I am sincerely pleased about this achievement, I wonder whether my own dream^[33] (shared by other theoretical

chemists) was really only the reduction of theory to a black box, which can be used routinely like a scientific instrument, even by someone who has no knowledge of or interest in what is happening inside. Pople himself shared these misgivings. Chemistry is much more complex than most of its practitioners appreciate.

Sir John, as he was entitled to call himself in the last year of his life, was a pleasant person and always enjoyable to talk to. His arguments were clear and lucid. His wit was pithy. Unlike many less famous colleagues, he never took himself to be very important. I liked him.

... to ab initio theory and GAUSSIAN ...

... to the Nobel Prize in Chemistry

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Micrometer-to-Nanometer Replication of Hierarchical Structures by Using a Surface Sol–Gel Process**

Rachel A. Caruso*

Keywords:

oxides · replication · sol–gel processes · template synthesis · titanium dioxide

Paper, cloth, and cotton are not new materials, however, Huang and Kunitake have recently shown these cellulose materials to have yet another use: they are excellent supports upon which to build layers of titanium dioxide and hence produce hierarchical titania structures with intricate replication from the macroscopic to the nanometer scale.^[1]

Titania (TiO_2) is known for its wealth of useful characteristics: It is an inexpensive semiconductor material that exhibits low toxicity. It is also a photoactive compound, and is stable over a range of chemical and thermal conditions. Hence the technological importance of TiO_2 is great, with applications prevalent in keeping the environment clean and playing a role in the conversion of solar energy to electricity. For example, particles and films of TiO_2 are used for the photodecomposition of organic pollutants in both air and wastewaters.^[2] Thin titania films applied to glass or ceramic surfaces result in self-cleaning windows^[3] and tiles.^[4] The dye-sensitized titania solar cell, a cell mimicking photosynthesis for the conversion of sunlight into energy, contains a porous TiO_2 film.^[5] Studies on the ability to control the morphology of titania are abundant, since structural properties and total accessible surface area both

play a significant role in the efficiency of titania in many of its applications.

One method of building controlled or tailored structures is replication. The literature abounds with studies displaying inverse replication (giving a negative copy of the original object), hollow replication (hollow, positive copy), and true replication. Biological structures are a great inspiration in this field of research as the imitation of biological structures could result in the enhancement of material properties.^[6] Quoting from Cook et al.,^[7] “the synthetic replication of evidently useful biological structures by a simple casting process is expected to introduce some of the superb properties of biological structures into man-made materials.” Materials from nature can be morphologically complex with sophisticated structure and ordering. The rich variety of forms in biological species provides many possible structures suitable for imitation. Imitating nature on the small scale brings about bio-inspired nanoscience.

Template-directed synthesis has proved an ideal approach to replication, by the design and fabrication of materials with predetermined structural properties. There is quite a list of biological species that have been used as templates for the formation of porous inorganic structures, which includes bacterial superstructures,^[8] cotton/cloth textile,^[9] diatoms,^[10] dog and human hair,^[11] insect wings,^[7] living cells,^[12] mushroom gills,^[11] plant leaves,^[7] paper,^[13] pollen grains,^[14] shell membrane,^[15] silk fiber,^[11] skeletal plates,^[16] spider silk,^[7,11] wood,^[17] and wool.^[9] Scanning electron microscopy (SEM) images of a titania replication of cloth, a silica replication of a butterfly wing, and a silica replica-

tion of pine wood are shown as examples in Figure 1. These biological tem-

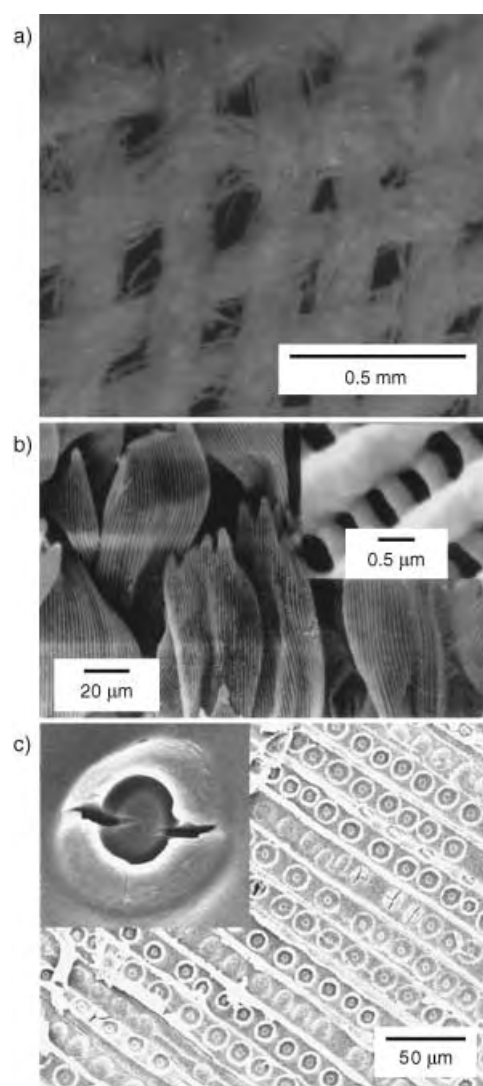


Figure 1. SEM images of inorganic replications of biological species: a) A titania replication of cloth;^[9] b) a silica replication of a peacock butterfly wing (obtained by chemical vapor deposition);^[7] c) a silica replication of pine wood plus a surfactant.^[17b]

[*] Dr. R. A. Caruso
School of Chemistry
The University of Melbourne
Melbourne 3010 (Australia)
Fax: (+61) 393-475-180
E-mail: rcaruso@unimelb.edu.au

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plates generally consist of hierarchical structuring. However, the unique morphological features of these templates over multiple length scales, especially down to the nanometer level, were not maintained in the final materials.

The coating of a template results in hollow replication as opposed to a negative copy or true replication.^[18] Sol-gel chemistry applied to three-dimensional (3D) materials allows homogeneous coating of complex structures.^[19] This approach applied to membrane filters or paper has shown micrometer-scale replication for the preparation of porous metal-oxide structures,^[20] and on a smaller scale, viroid particles^[21] and carbon nanotubes^[22] have shown nanoscale replication. So, hollow replication exists individually on the macro-scale as well as the nanoscale. However, a constant challenge has been to maintain a significantly thin coating that can give intricate details on the nanometer level, yet on the other hand be sufficiently stable to maintain the overall structure on the micrometer scale, hence allowing the faithful reproduction (as a hollow replication) of complex hierarchical structures. Sol-gel coatings applied

to fibers with a large diameter (on the micrometer scale) and secondary structure on the 100-nm scale gave both structural stability of the large-diameter tube and fine structural details on the order of 100 nm as nodules within the tubes.^[23] Employing a repetitive sol-gel layering technique, Huang and Kunitake^[1] have shown replication of multiscale materials with outstanding precision (tens of nanometers) while maintaining stability on the macroscale using templates that are abundant in nature, low cost, and environmentally benign, such as simple paper, cotton, and cloth. The procedure used was a repeated surface sol-gel process^[24] undertaken by filtration/deposition cycles: the chemisorption of metal alkoxides from solution onto hydroxylated template surfaces followed by hydrolysis and condensation reactions. This resulted in a subnanometer-thick layer coating the morphologically complex template surface. The metal alkoxide was once again exposed to the now hybrid material surface and the procedure repeated a number of times to build up the intricate layers. The “surface sol-gel process”^[24] is considered most appropriate for this

purpose because it traces the sizes and shapes of the template molecules.^[25] The template was removed by heating the sample to 500°C for 6 h. This surface-adsorption technique has been shown to proceed with molecular precision^[26] giving a titania gel layer that was 0.4 ± 0.1 nm thick per deposition cycle.^[27] Zirconia structures could also be obtained by using the same technique.^[1]

SEM and transmission electron microscopy (TEM) images of the titania materials portrayed a “faithful replication” of the templates (Figure 2a) with a slight shrinkage that is commonly observed after calcination of a sol-gel material. A self-supporting sheet containing a porous structure characteristic of the original template was obtained. Nanotubular structures with outer diameters between 30 and 100 nm were found to be interwoven and branched (Figure 2b) mimicking the original cellulose fibers, and to maintain the spiral twist of cotton lint (Figure 2c). The wall thickness of the titania could be controlled by the number of layers and was shown to be 10 nm after 20 layer cycles (Figure 2d).

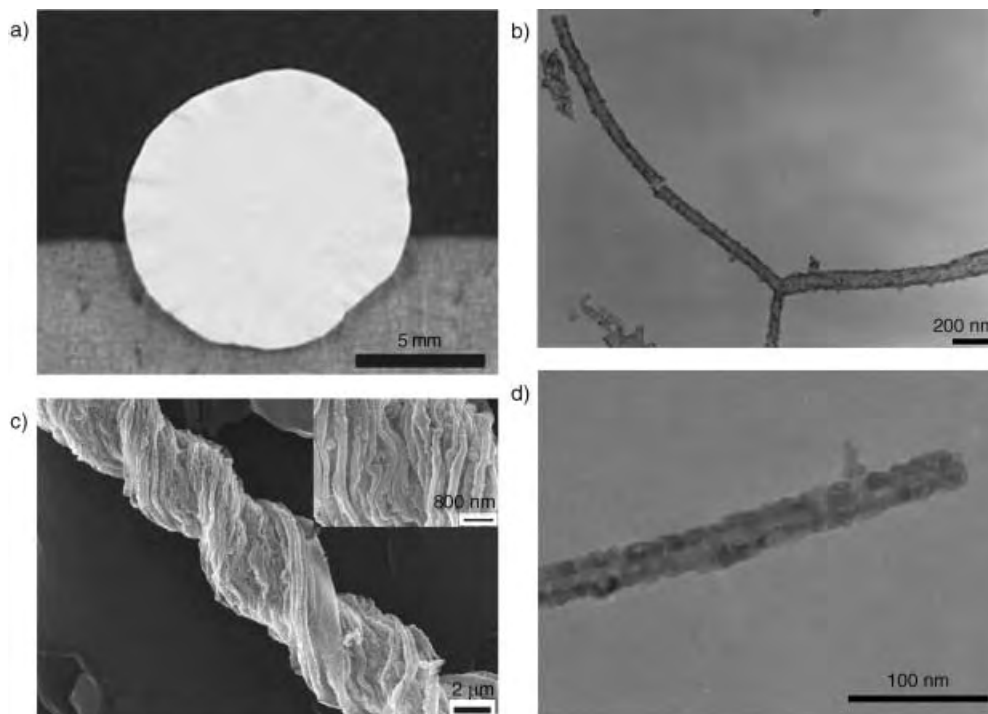


Figure 2. a) Photograph of the final titania structure obtained after applying the surface sol-gel process to paper (cellulose filter); b) TEM image of a branched titania nanotube; c) SEM image of the titania fiber obtained after applying the surface sol-gel process to cotton (inset: the same fiber at a higher magnification); d) TEM image of the tubular structure indicating the wall thickness.^[1]

As is commonly the case where intricate detail and precision are of foremost importance, the time required for fabrication can become an issue. The challenge will be the ability to scale-up the procedure to a convenient large-scale production that is not too time consuming.

Huang and Kunitake see this synthetic procedure as a practical and environmentally friendly approach to produce nanotubular metal oxides that is both low-cost and efficient, as well as a nanocopying tool to probe structures of biosystems on nanometer scales. Thus, this new methodology extends the range of replication techniques that already exist, by allowing hollow replication on both the micron and the nanometer scale simultaneously.

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Selective Alkylations

A New Hydrosilylation Mechanism—New Preparative Opportunities

Henri Brunner*

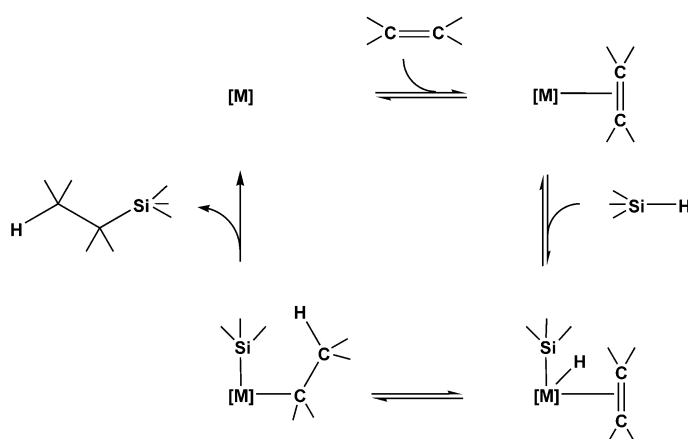
Keywords:

homogeneous catalysis · hydrosilylation · reaction mechanisms · silylene ligands

The Si–H bond is polar, long, and weak, and therefore, very reactive. Its addition across a double bond (hydrosilylation) is an important reaction not only in organosilicon chemistry but also in organic synthesis and dendrimer and polymer chemistry. It is a catalytic reaction for which a large variety of catalysts is available. They control the regio- and stereoselectivity including the enantioselectivity. Most of these catalysts operate according to the well-established Chalk–Harrod mechanism^[1] or one of its variants^[2] resulting in the *cis* addition of a Si–H bond to an alkene.

In the classical Chalk–Harrod mechanism of olefin hydrosilylation a Si–H bond adds oxidatively to the metal atom of an olefin complex (Scheme 1). Migration of the hydride ligand onto the coordinated olefin generates the silyl alkyl intermediate, which undergoes reductive elimination to form the Si–C bond of the product. Normally, reactions following Chalk–Harrod-type mechanisms are accompanied by isomerization and scrambling of deuterium labels.

Transition-metal silylene complexes, the analogues of the carbene complexes, have been known for quite a while.^[3,4] However, no currently accepted hydrosilylation mechanism includes a silylene intermediate, although such proposals have appeared.^[5–7] In a recent publication Tilley et al. describe a catalytically active silylene complex, the Si–H bond



Scheme 1. Classical Chalk–Harrod mechanism for the hydrosilylation of olefins.

of which adds to olefins.^[8] Interestingly, this catalysis opens up the way to a new selective reaction, the monoalkylation of monosubstituted silanes RSiH_3 .

The catalyst for this special type of hydrosilylation is the base-stabilized silylene complex $[\text{Cp}^*(\text{P}i\text{Pr}_3)(\text{H})_2\text{Ru}=\text{Si}(\text{H})\text{Ph-Et}_2\text{O}][\text{B}(\text{C}_6\text{F}_5)_4]$ ($\text{Cp}^* = \eta^5\text{-C}_5\text{Me}_5$), in which the ether is only loosely bound. It is prepared starting from the 16-electron complex $[\text{Cp}^*(\text{P}i\text{Pr}_3)\text{RuCl}]$, which readily adds PhSiH_3 to afford $[\text{Cp}^*(\text{P}i\text{Pr}_3)\text{Ru}(\text{Cl})(\text{H})\text{SiH}_2\text{Ph}]$.^[9] In this oxidative addition reaction the first hydrogen atom of the silane PhSiH_3 is removed from the silicon atom and transferred to the metal atom. Treatment with $\text{Li}[\text{B}(\text{C}_6\text{F}_5)_4] \cdot 3\text{Et}_2\text{O}$ abstracts the halogen and in an α -elimination with respect to the silyl substituent SiH_2Ph the actual catalyst $[\text{Cp}^*(\text{P}i\text{Pr}_3)(\text{H})_2\text{Ru}=\text{Si}(\text{H})\text{Ph}][\text{B}(\text{C}_6\text{F}_5)_4]$ is formed. In this α -elimination the second hydrogen atom is shifted from Si to Ru such that only a single hydrogen atom remains on the silicon atom—an

important prerequisite for the selectivity of the catalytic monoalkylation of silanes RSiH_3 . Recently, a comparison on the basis of DFT calculations was carried out for silyl complexes $[(\text{P-P})\text{Pt-SiHR}_2]$ and the isomeric hydridosilylene complexes $[(\text{P-P})\text{HPt}=\text{SiR}_2]$.^[10]

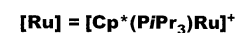
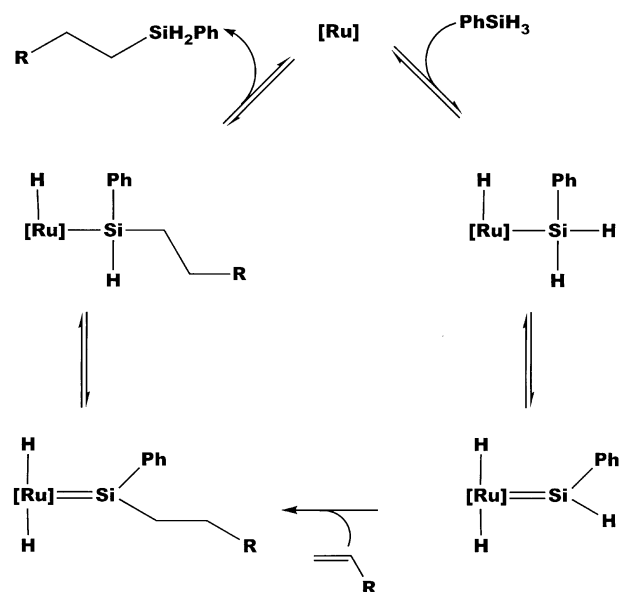
For catalysis a solution of the catalyst in CH_2Cl_2 was treated with a 50-fold excess of both 1-hexene and PhSiH_3 . In a clean reaction $\text{Ph}(\text{Hex})\text{SiH}_2$ (Hex = *n*-hexyl) was formed as the only silicon-containing product. There was no “over-reaction” to $\text{Ph}(\text{Hex})_2\text{SiH}$ and $\text{Ph}(\text{Hex})_3\text{Si}$. Other olefins tested with PhSiH_3 include ethene, cyclohexene, styrene, and 1-methylcyclohexene. The latter, a trisubstituted olefin, gave ($\pm$)-*trans*-1- Ph_2HSi -2-methylcyclohexene.

The product of this reaction reveals that Si–H adds *cis* to the olefin. The combination $\text{HexSiH}_3/1$ -hexene afforded Hex_2SiH_2 . Characteristically, all the reactions stopped at the monoalkylation level. In addition, the products obtained from the substituted olefins show that

[*] Prof. Dr. H. Brunner
Institut für Anorganische Chemie
Universität Regensburg
93040 Regensburg (Germany)
Fax: (+49) 941-943-4439
E-mail: henri.brunner@chemie.uni-regensburg.de

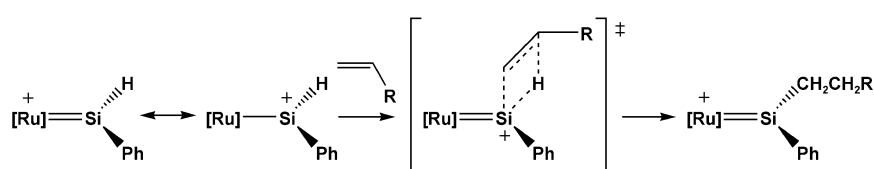
the addition occurred exclusively in an anti-Markovnikov fashion—unusual for hydrosilylation reactions.

The catalytic cycle for this new reaction type is shown in Scheme 2. The first step is the oxidative addition of PhSiH_3 to the unsaturated fragment $[\text{Ru}]$ ($=[\text{Cp}^*(\text{P}i\text{Pr}_3)\text{Ru}]^+$) of the catalyst. Then, an α -elimination generates the silylene intermediate. The next step is the insertion of the olefin into the Si–H bond of the silylene ligand. In this irreversible step the new alkyl substituent is formed. The irreversibility of this step was supported by the fact that



Scheme 2. Catalytic cycle for the hydrosilylation of olefins according to Tilley and Glaser.^[8]

reaction of PhSiH_3 with an excess of $[\text{D}_4]\text{ethene}$ afforded $\text{PhH}_2\text{SiCD}_2\text{CD}_2\text{H}$ as the only observed isotopomer. Reversibility of this insertion step—normal for Chalk–Harrod-type hydrosilylations—would lead to a scrambling of the deuterium label. This is not observed here. In the final steps the reversal of the α -elimination regenerates the silyl substituent $\text{SiH}(\text{CH}_2\text{CH}_2\text{R})\text{Ph}$, and a re-



Scheme 3. Transition state in the hydrosilylation of olefins ($[\text{Ru}] = [\text{Cp}^*(\text{P}i\text{Pr}_3)\text{Ru}]^+$).

ductive elimination removes the monoalkylated silane $\text{SiH}_2(\text{CH}_2\text{CH}_2\text{R})\text{Ph}$ from the complex.

The selective monoalkylation and the anti-Markovnikov regiochemistry are consequences of the reaction mechanism, which involves a four-centered transition state including the Si–H bond of the $\text{Ru}=\text{Si}(\text{H})\text{Ph}$ moiety and the olefinic substrate (Scheme 3). Such a transition state is analogous to that of the B–C bond-forming step in the hydroboration of alkenes^[11,12] and, in fact, unlike the four-coordinate silicon atom in a silyl metal compound the three-coordinate cationic silicon center in a silylene complex resembles a monomeric borane. Anti-Markovnikov regiochemistry results because the olefin turns its large substituents away from the metal fragment (Scheme 3).

The monoalkylation selectivity has its origin in the fact that in the silylene catalyst only one Si–H bond is left because two of the hydrogen atoms of the starting material PhSiH_3 are “parked” at the metal center. They return to the silicon atom only after the olefin insertion to eliminate the product $\text{Ph}(\text{Hex})\text{SiH}_2$ for the olefin 1-hexene or in general $\text{R}(\text{R}')\text{SiH}_2$. Compounds of the type $\text{R}(\text{R}')\text{SiH}_2$ do not qualify as substrates for another alkylation since both hydrogen atoms are lost in the oxidative addition and the α -elimination, the initial steps of the catalytic cycle, and no Si–H bond is available in

the silylene intermediate for olefin insertion, the key feature of the selectivity of this monoalkylation.

Monosubstitution of trifunctional molecules such as RSiH_3 generally is a problem. To some extent the formation of by-products due to di- and trisubstitution can be controlled by stoichiometry and by substituent effects. In the monoalkylation of monosubstituted silanes discussed here selectivity is elegantly achieved by the mechanism, although the new catalytic system is far from perfect. The protocols use only low substrate/catalyst ratios, and the yields are not quantitative. This is probably due to the build-up of silylene species no longer containing Si–H bonds, which tie up the catalyst and reduce the concentration of the actual catalytic species.

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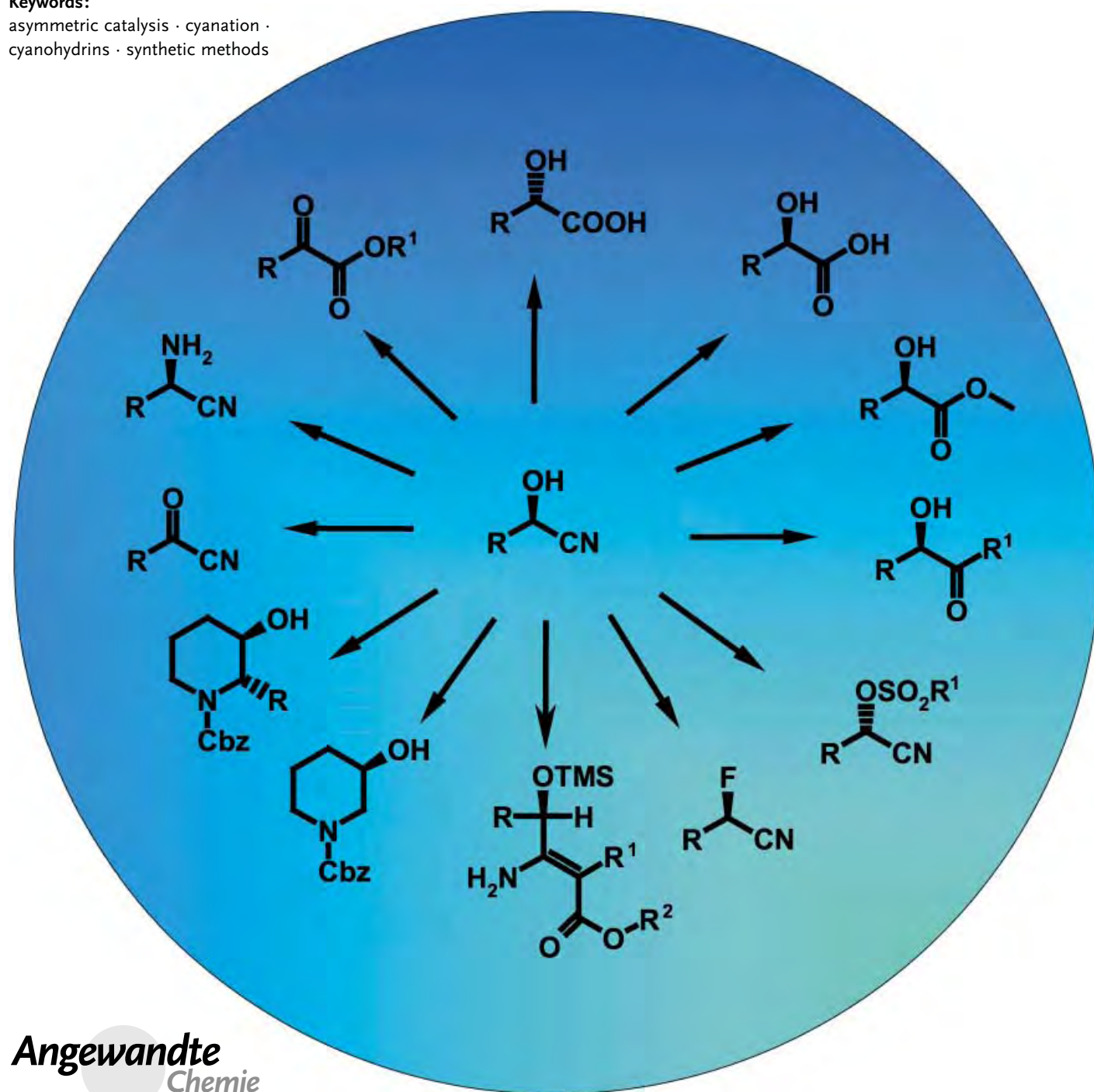
Hydrocyanations

Chemically Catalyzed Asymmetric Cyanohydrin Syntheses

Jean-Michel Brunel and Ian P. Holmes*

Keywords:

asymmetric catalysis · cyanation ·
cyanohydrins · synthetic methods



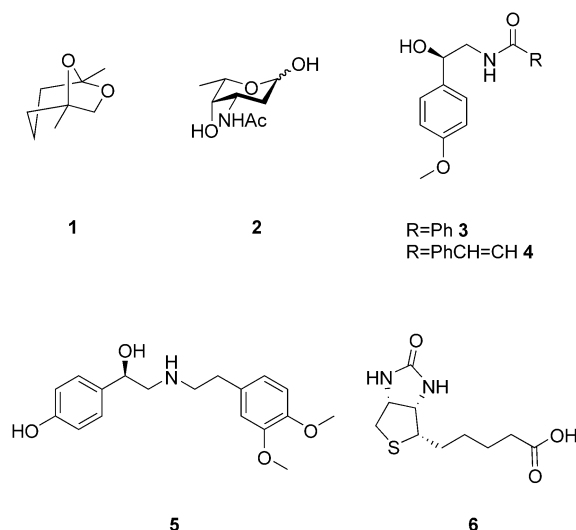


Figure 1. Examples of compounds synthesized via enantioenriched cyanohydrins.

Chemically catalyzed cyanation methods now offer viable alternatives and are starting to be used in the total synthesis of complex molecules. For example, epothilones A (**7**) and B (**8**),^[25] and an advanced intermediate of (20*S*)-camptothecin (**9**)^[26] were prepared by chemically catalyzed asymmetric cyanation. Chemically catalyzed methodology has also been applied in the synthesis of purely synthetic compounds, for example, the muscarinic receptor antagonist (*S*)-oxybutynin (**10**; Figure 2).^[27]

1.3. Overview

Over the past two decades, significant advances have been made towards developing catalytic asymmetric cyanohydrin syntheses.^[1–3] The resulting reactions can be subdivided into three classes: chemically, enzyme-, and peptide-mediated procedures. In this Review we focus exclusively on chemically catalyzed methodologies; the areas of enzyme^[1–3] and peptide catalysis^[1,3] have been covered comprehensively in recent reviews.

When comparing preparative reactions, a number of factors need to be taken into account. Herein, we have

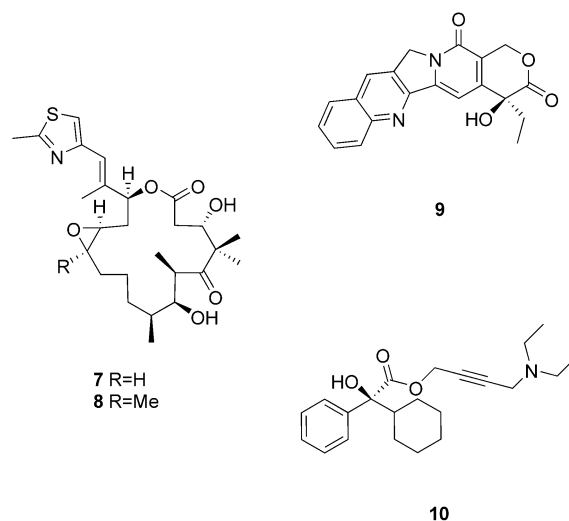


Figure 2. Examples of compounds synthesized through chemically catalyzed asymmetric cyanation.

focused on the following key parameters: yield, enantioselectivity, substrate tolerance, reagents used, temperature, and time. Synthetic practicality—how easy the procedure is to perform—is also considered. When selecting a reaction protocol from the many available possibilities, these factors need to be assessed and balanced with reference to the desired application. We hope that the data presented and our commentary will offer a rough guide to allow a comparison of available procedures.

It should be noted that a number of the methods detailed herein are not catalytic, as stoichiometric quantities of reagents are used. However, through their inclusion, we have tried to give an historical perspective of the development of true catalytic systems and to provide a more complete overview of the area from its inception to the present day.

1.4. Cyanide Sources

In all the reactions reviewed herein, the cyanohydrins have been prepared by the addition of a cyanide source to an aldehyde or ketone. Nearly all the procedures described use trimethylsilylcyanide (TMSCN) as their cyanide source. The



Jean Michel Brunel was born in 1968 in Marseille, France. He graduated from the Ecole Supérieure de Chimie de Marseille (1991) and obtained his PhD in 1994 from the Université Aix Marseille III. In 1994, he joined the group of Prof. H. B. Kagan (Université Paris Sud) as a postdoctoral fellow, where he worked on the enantioselective catalytic oxidation of sulfides. In 1997, he joined the CNRS as Chargé de Recherche and now works in the Institut Méditerranéen de Recherche en Nutrition (IMRN INRA 1111). His research interests include asymmetric catalysis and the synthesis of novel antifungal agents.



Ian Holmes was born in 1971 in Beverley, England. He completed both undergraduate studies (1993) and postgraduate studies (1996, with Dr. M. Mascal) at the University of Nottingham. After working for Zeneca Pharmaceuticals for two years as a senior synthetic chemist he returned to academia and worked with Prof. H. B. Kagan (Université Paris Sud) as a postdoctoral researcher. He now works as a medicinal chemist within Discovery Research at GlaxoSmithKline in the area of 7-transmembrane receptor chemistry.

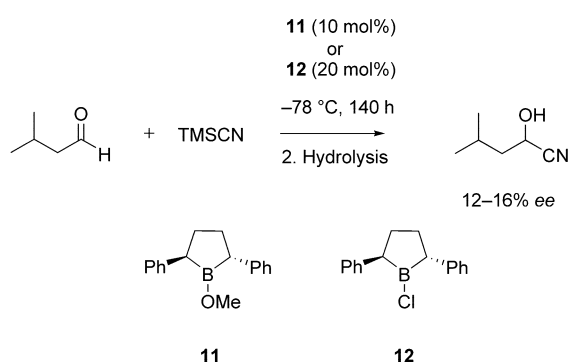
use of TMSCN allows the desired cyanohydrin to be prepared directly as the corresponding trimethylsilyl (TMS) ether. The stability of the TMS adduct, conferred by an energetically favorable Si–O bond, prevents the reverse cyanation reaction from occurring and hence removes a potential pathway for racemization. Unfortunately, TMSCN is relatively expensive and will contribute considerably to the financial cost, particularly if used on a large scale. It is also highly toxic and volatile which complicates its use on anything other than small scales. The major alternative investigated has been hydrogen cyanide (HCN), which is not without its own associated problems. Whilst comparatively inexpensive, HCN is a highly toxic gas and requires careful containment and handling.

Only recently have asymmetric cyanohydrin syntheses been developed that use alternative cyanide sources. Reagents such as potassium cyanide (Sections 2.2.5 and 2.3), acetone cyanohydrin (Section 2.10), and cyanoformates (Sections 2.6, 2.7, and 3) have been studied, and some excellent results have been reported. Alternate cyanide sources and synthetic strategies are areas that will undoubtedly continue to be the focus of much research.

2. Catalysts for the Cyanation of Aldehydes

2.1. Boron-Containing Catalysts

In 1986, Reetz et al. reported the first enantioselective addition of TMSCN to an aldehyde catalyzed by an optically active Lewis acid. TMSCN was added to isobutanal in the presence of **11** (10 mol%) or **12** (20 mol%) to give, after hydrolysis, the corresponding cyanohydrin in yields of 45–55% (Scheme 2). Enantiomeric excesses of 12–16% were



Scheme 2. Asymmetric trimethylsilylcyanation mediated by boron-based Lewis acid catalysts.

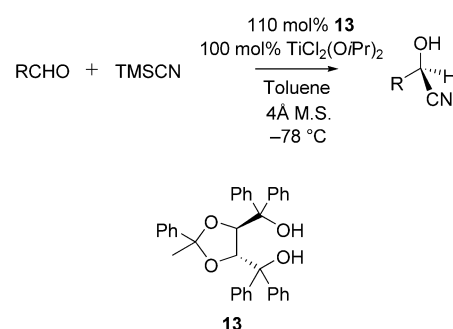
obtained, although the absolute configurations of the products were not determined.^[28] Despite the poor enantioselectivity and the low temperature for extended periods of time, this isolated report demonstrated the potential of Lewis acid catalyst systems in asymmetric cyanohydrin synthesis.

2.2. Titanium-Based Catalysts

Following the disclosure by Reetz et al., numerous Lewis acid systems with applicability to asymmetric cyanohydrin synthesis have been described and investigated. The area that has received the most attention is without doubt that of titanium(IV) catalysis.

2.2.1. Taddol Ligand Systems

The first detailed example of a titanium(IV)-based reagent for the asymmetric formation of cyanohydrins was reported by Narasaka et al. in the late 1980s.^[29] They described the reaction of a range of aldehydes with excess TMSCN in the presence of a titanium(IV) complex of the 1,4-diol **13** and 4-Å molecular sieves (Scheme 3, Table 1). The use of a stoichio-



Scheme 3. Cyanation of aldehydes catalyzed by the taddol system of Narasaka et al.^[29] M.S. = molecular sieves.

Table 1: Cyanation of aldehydes catalyzed by the taddol system of Narasaka et al. (Scheme 3).^[29]

Entry	R	T [°C] ^[a]	t [h]	Yield [%]	ee [%]
1	Ph	22	122	68	73 (R)
2	Ph	–78	12	79	96 (R)
3	PhCH ₂ CH ₂	22	20	88	91 (R)
4	PhCH ₂ CH ₂	–78	12	89	74 (R)
5	<i>c</i> -C ₆ H ₁₁	–78	48	77	68 (R)
6	<i>n</i> -C ₈ H ₁₇	22	23	85	93 (R)
7	<i>n</i> -C ₈ H ₁₇	–78	24	66	76 (R)

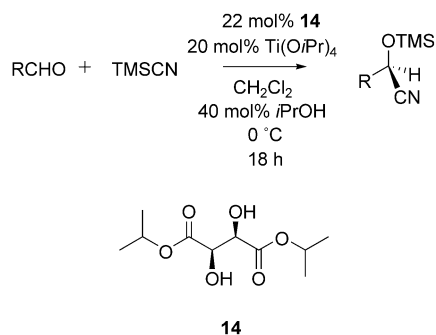
[a] Temperature of catalyst generation.

metric quantity of the reagent was necessary; attempted substoichiometric reactions gave yields that correlated to the amount of titanium species present. It was also observed that the temperature at which the titanium(IV) reagent was formed influenced the selectivity. Aromatic aldehydes gave the best enantioselectivities for reactions in which the reagent was formed at low temperatures. However, when the reagent was formed at room temperature, aliphatic systems were found to give superior results. Notably, in all the reactions carried out, the cyanohydrin product was isolated directly, not the corresponding TMS adduct.^[30] Further studies also revealed that the reactions gave only kinetic products; treatment of a racemic sample of cyanohydrin under enantioselective conditions gave no change in enantioselectivity.

Although this procedure is not catalytic, it demonstrated that high enantioselectivity could be obtained by using a synthetic Lewis acid based reagent. It was only a short time before a true catalytic system was reported.

2.2.2. Tartrate Ester Ligand Systems

The first example of a substoichiometric titanium(IV) system for the generation of enantioenriched cyanohydrins was developed from the Sharpless catalyst for asymmetric epoxidation. Oguni and co-workers investigated the reaction of aldehydes with TMSCN catalyzed by equimolar (and later substoichiometric) quantities of titanium(IV) complexes of L-(+)-diisopropyl tartrate (**14**).^[31] It was found that additives were essential to obtain good enantioselectivity. Two equivalents of propan-2-ol with respect to titanium complex gave optimal conditions.^[32] In the presence of propan-2-ol, high yields and *ee* values of up to 91 % were obtained for reactions employing 20 mol % of catalyst (Scheme 4, Table 2).



Scheme 4. Trimethylsilylcyanation of aldehydes catalyzed by the diisopropyl tartrate system reported by Oguni and co-workers.^[31]

Table 2: Trimethylsilylcyanation of aldehydes catalyzed by the diisopropyl tartrate system of Oguni and co-workers (Scheme 4).^[31]

Entry	Aldehyde	Equimolar Reaction		20 mol % Catalyst	
		Yield [%]	<i>ee</i> [%]	Yield [%]	<i>ee</i> [%]
1	benzaldehyde	63	88	84	91 (<i>R</i>)
2	<i>p</i> -methylbenzaldehyde	89	77	79	65 (<i>R</i>)
3	<i>p</i> -anisaldehyde	90	81	88	77 (<i>R</i>)
4	2-naphthaldehyde	89	73	80	60 (<i>R</i>)
5	thiophene-2-carbaldehyde	92	81	84	83 (<i>R</i>)

Interestingly, the reaction gave the cyanohydrins as their trimethylsilyl adducts, in contrast to the results obtained when using the Narasaka taddol-derived reagent described above. The authors proposed a catalytic cycle for the reaction in which hydrogen cyanide, generated in situ by hydrolysis of TMSCN with propan-2-ol, initiates the reaction. The resulting titanium complex then reacts with TMSCN or HCN to give a chiral titanium species, the proposed asymmetric silylcyanation species. Mechanistic investigations were carried out which supported this proposal.

This protocol clearly demonstrated that high yields and *ee* values could be obtained by using a substoichiometric chiral Lewis acid system. Furthermore, these results were obtained

at 0 °C, offering a major advantage in terms of synthetic utility over previous preparations. Unfortunately, the reaction conditions proved not to be very substrate-tolerant. Minor modifications in the structure of the aldehyde resulted in significantly lower enantioselectivities. Also, the catalyst loading required was still considerable (20 mol %).

2.2.3. A Titanium–Triol Complex

With the potential of titanium(IV) complexes as substoichiometric catalysts for cyanohydrin formation demonstrated, other ligand systems were investigated. De Vries and co-workers investigated hydrocyanations catalyzed by a titanium–chiral alcohol complex with a view to developing a system with industrial applicability. For economic reasons, HCN is preferable to TMSCN for cyanation when working on large scales. As all titanium–diol complexes disclosed at the time were incompatible for use with HCN, the authors prepared and studied the asymmetric titanium–triol complex **15** (Figure 3). It was hoped **15** would be less prone to alkoxide displacement by cyanide than the previously studied diols. Unfortunately, no reaction was observed between HCN and benzaldehyde, even in the presence of a stoichiometric quantity of **15**. However, **15** was shown to be an active catalyst for the trimethylsilylcyanation of benzaldehyde.^[33] After refinement of the reaction conditions, it was found that the use of a stoichiometric quantity of **15**, in the presence of molecular sieves at –20 °C, led to the conversion of benzaldehyde into (*S*)-mandelonitrile in 92 % yield with 76 % *ee* after 2 h (Scheme 5). This reaction

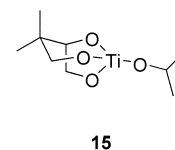


Figure 3. Titanium complex **15**.



Scheme 5. The reaction of benzaldehyde with TMSCN mediated by a stoichiometric quantity of **15**.

was also shown to proceed when using only 10 mol % of catalyst **15**; however, a longer reaction time (12 h) was required, and the yield and enantioselectivity decreased (70 %, 10 % *ee*). A higher catalyst loading (200 mol %) also gave inferior results.

The scope of the reaction was not fully investigated. Only one other aldehyde, 3-hydroxybenzaldehyde, was studied. It was found that it was necessary to protect the phenol group prior to reaction. With this established, the trimethylsilyl adduct was shown to react to give the corresponding cyanohydrin with 30 % *ee*. The addition of TMSCN to a ketone was also attempted in the presence of **15**, but without success.

These results do not compare favorably with those obtained with the diol complexes described above. Higher *ee* values may be obtained for both the stoichiometric and

substoichiometric reactions. However, this catalyst system was subsequently used with good effect in the historically problematic area of ketone cyanation, albeit under very forcing conditions (Section 3).

2.2.4. *C*₁-Symmetric Schiff Base and Related Ligands

The hydrocyanation and trimethylsilylcyanation of aldehydes mediated by titanium complexes derived from Schiff bases have proved to be a rich area of research. Initial reports in this field came from the groups of Inoue and Oguni, who prepared titanium complexes of *C*₁-symmetric ligands.

Inoue and co-workers entered this field whilst studying biomimetic transformations catalyzed by synthetic peptides. After showing that certain dipeptides catalyze the asymmetric addition of HCN to aldehydes,^[1,3] they investigated the use of titanium complexes of amino acid derived Schiff bases.^[34,35] A range of titanium complexes were prepared and screened as catalysts for the reaction of benzaldehyde with HCN. It was found that dipeptides were superior to mono-peptides, provided that both residues were of the same absolute configuration. The nature of the N-terminal residue was found to influence the absolute configuration of the product, and the C-terminal residue affected the degree of enantioselectivity. The presence of an amide bond was found to be essential to obtain an efficient catalyst. It was also found that decreased steric congestion at the C-terminal residue (e.g., by the replacement of the amino acid with either aminocyclohexane or piperidine) and increased steric bulk around the Schiff base results in the inversion of the absolute configuration of the product. Consequently, either enantiomer of the product can be obtained by using only naturally occurring amino acids.^[36]

A number of highly active titanium catalysts were prepared from a range of peptide ligands (Figure 4). These included Nap-S-Val-S-Trp-OMe (**16**), the titanium(IV) complex of which gave *ee* values of up to 90 % for the catalytic additions of HCN to aromatic aldehydes; Dbs-S-Val-Pip (**17**), which afforded the product with the opposite absolute configuration with up to 97 % *ee*; and the dipeptide Nap-S-Val-S-Phe-OMe (**18**), which gave comparatively good yields for α,β -alkenyl and aliphatic aldehydes (Scheme 6, Table 3).^[37]

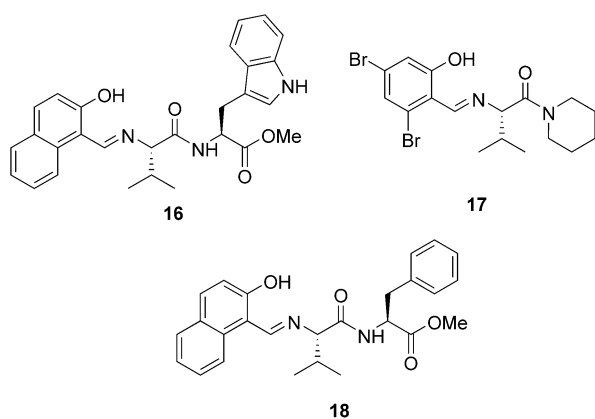
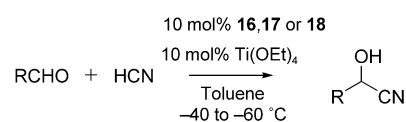


Figure 4. Peptide-derived ligands synthesized by Inoue and co-workers.^[37]



Scheme 6. Hydrocyanation of aldehydes catalyzed by the *C*₁-symmetric Schiff base system of Inoue and co-workers.^[37]

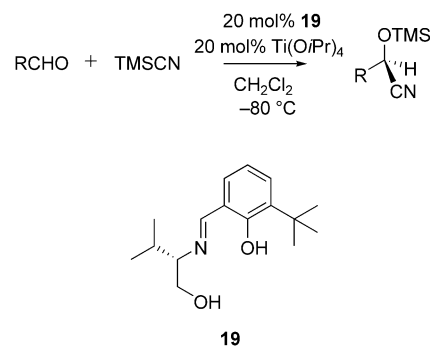
Table 3: Hydrocyanation of aldehydes catalyzed by the *C*₁-symmetric Schiff base systems of Inoue and co-workers (Scheme 6).^[37]

Entry	Aldehyde	Ligand	<i>t</i> [h]	Yield [%]	<i>ee</i> [%]
1	2-naphthaldehyde	16	7.5	55	90 (R)
2	2-naphthaldehyde	17	84	63	72 (S)
3	<i>m</i> -anisaldehyde	16	4	71	85 (R)
4	<i>m</i> -anisaldehyde	17	41	79	97 (S)
5	heptanal	18	13	96	76 (R)
6	cyclohexanecarbaldehyde	18	1.5	85	54 (R)

The activity of these titanium complexes with TMSCN was then studied. It was found that whilst reactions with HCN worked well, reactions with TMSCN gave poor selectivity. However, replacement of titanium with aluminum resulted in an active catalyst that gave high levels of enantioselectivity with TMSCN (Section 2.6).^[38]

Oguni and co-workers also developed systems for the asymmetric cyanation of aldehydes catalyzed by titanium(IV) complexes based on *C*₁-symmetric Schiff bases. Catalysts prepared from β -amino alcohols led to enantioselectivities of up to 91 % for the addition of TMSCN to aldehydes. The authors first reported the screening of a number of chiral Schiff bases derived from β -amino alcohols (e.g., L-valinol and L-tert-leucinol) as chiral ligands. Significant enhancements in reaction rates were observed when using these ligands, for example, the reaction of benzaldehyde with TMSCN in the presence of the titanium complex of **19** (20 mol %) was sixfold faster than the reaction with Ti(OiPr)₄ (20 mol %) alone.^[39] Trimethylsilylcyanation of a range of aldehydes was investigated with the catalyst derived from **19** to demonstrate its general applicability (Scheme 7, Table 4).

Mechanistic investigations—studies of solvent, concentration, stoichiometry, temperature, and catalyst loading—were also carried out to optimize the results. An interesting discovery was that catalytic reactions (20 mol %) were more



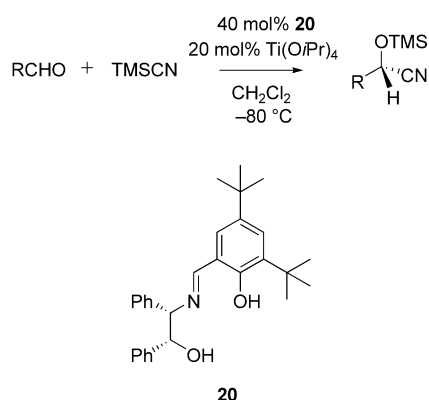
Scheme 7. Trimethylsilylcyanation of aldehydes catalyzed by the *C*₁-symmetric Schiff base system of Oguni and co-workers.^[39]

Table 4: Trimethylsilylcyanation of aldehydes catalyzed by the C₁-symmetric Schiff base system of Oguni and co-workers (Scheme 7).^[39]

Entry	Aldehyde	<i>t</i> [h]	Yield [%]	<i>ee</i> [%]
1	benzaldehyde	36	67	85 (<i>R</i>)
2	<i>p</i> -methylbenzaldehyde	36	68	71 (<i>R</i>)
3	<i>p</i> -anisaldehyde	36	62	91 (<i>R</i>)
4	2-naphthaldehyde	36	76	73 (<i>R</i>)
5	cinnamaldehyde	36	81	72 (<i>R</i>)
6	cyclohexanecarbaldehyde	12	72	65 (<i>R</i>)
7	pivalaldehyde	36	58	70 (<i>R</i>)

enantioselective than reactions run in the presence of a stoichiometric quantity of the catalyst. The nature of the active catalyst was studied with a variety of techniques, and a structure was proposed for the active catalyst. This, in turn, led to a proposed mechanism in which the stereochemical outcome of the reaction is dictated by the shielding of one face of the activated aldehyde by the substituents on the amino alcohol.^[40,41]

Schiff bases derived from β-amino alcohols were also investigated by Yaozhong et al. They used enantiopure ligands based on 2-amino-1,2-diphenylethanol to generate active titanium catalysts, for example, that based on ligand **20**, which gave *ee* values of up to 92 % for the addition of TMSCN to aldehydes (Scheme 8, Table 5).^[42]

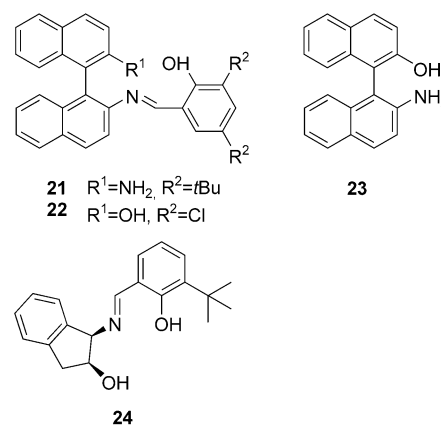
**Scheme 8.** Trimethylsilylcyanation of aldehydes catalyzed by the C₁-symmetric Schiff base system of Yaozhong et al.^[42]**Table 5:** Trimethylsilylcyanation of aldehydes catalyzed by the C₁-symmetric Schiff base system of Yaozhong and co-workers (Scheme 8).^[42]

Entry	Aldehyde	<i>t</i> [h]	Yield [%]	<i>ee</i> [%]
1	benzaldehyde	60	72	92 (<i>S</i>)
2	3-phenoxybenzaldehyde	60	58	49 (<i>S</i>)
3	<i>o</i> -chlorobenzaldehyde	60	76	91 (<i>S</i>)
4	(<i>E</i>)-cinnamaldehyde	60	60	54 (<i>S</i>)
5	<i>p</i> -anisaldehyde	60	31	48 (<i>S</i>)

A number of variables were found to be pivotal in obtaining enantioselective conditions. The substituents on the salicylaldehyde portion of the catalyst influenced the *ee* obtained, the di-*tert*-butyl derivative being optimal. Also, the ligand/titanium stoichiometry had a marked effect on the

enantioselectivity. In contrast to the system of Oguni and co-workers, the reactions catalyzed by stoichiometric quantities of catalyst were found to give better *ee* values than those run with substoichiometric loading.^[43]

Other amino alcohol systems have also been investigated. Recently, Che and co-workers studied catalysts **21** and **22** based on nobin (**23**).^[44] Somanathan, Walsh, and co-workers described a series of active catalysts, including **24**, derived from *cis*-1-amino-2-indanol (Figure 5).^[45] Tang and co-workers also described a series of titanium-based catalysts employing chiral Schiff base ligands derived from camphor. These catalysts resulted in *ee* values of up to 66 %.^[46]

**Figure 5.** C₁-symmetric Schiff base systems.

A related strategy was employed by Choi and co-workers, who investigated ligands (for titanium) based on sulfonamides of chiral 1,2-amino alcohols. The titanium complex generated in situ from titanium isopropoxide and ligand **25** was shown to be a good catalyst for the trimethylsilylcyanation of aldehydes (Scheme 9, Table 6). At -65°C , a range of aldehydes were converted into the corresponding cyanohydrins with up to 96 % *ee* in the presence of 10 mol % of catalyst. Notably, both aliphatic and aromatic aldehydes gave excellent *ee* values.^[47]

The majority of the above-mentioned C₁-symmetric systems deliver cyanohydrins with high enantioselectivity.

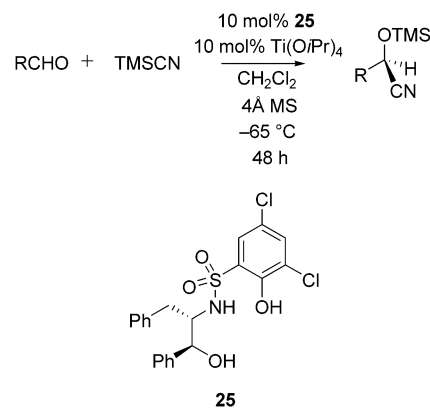
**Scheme 9.** Trimethylsilylcyanation of aldehydes catalyzed by the sulfonamide system of Choi and co-workers.^[47]

Table 6: Trimethylsilylcyanation of aldehydes catalyzed by the sulfonamide system of Choi and co-workers (Scheme 9).^[47]

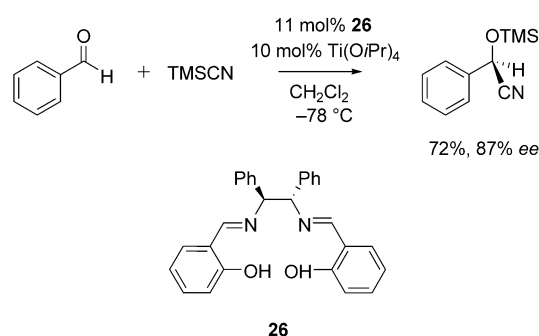
Entry	Aldehyde	Yield [%]	ee [%]
1	benzaldehyde ^[a]	90	94 (R)
2	<i>p</i> -chlorobenzaldehyde	93	90 (R)
3	<i>p</i> -anisaldehyde	100	94 (R)
4	<i>o</i> -anisaldehyde	100	86 (R)
5	2-naphthaldehyde	95	96 (R)
6	(<i>E</i>)-cinnamaldehyde	100	93 (R)
7	isobutyraldehyde	100	95 (R)

[a] 5 mol% catalyst.

As one would expect, the general applicability and average *ee* values delivered by the systems has improved over time, and the excellent results of Choi and co-workers detailed above are probably the best achieved in the area to date. Each system described has its own merits, be it the ability to invert stereochemistry by subtle modifications of the catalyst structure, the ability to use HCN as cyanide source, or generic applicability. However, all these protocols suffer from a number of fundamental problems—the low temperatures required, extended reaction times, and the use (in all but one case) of TMSCN as the cyanide source. Although these conditions are acceptable for the synthesis of individual compounds on small scales, they are not appropriate for use with modern array-synthesis technologies or for scale up in the preparation of target molecules or synthetic monomers. Therefore, although such procedures are of utility in an academic or classical medicinal-chemistry environment, they do not lend themselves to modern drug-discovery processes or ultimately to the commercial synthesis of molecules.

2.2.5. *C*₂-Symmetric Schiff Bases and Related Ligands

In 1996, Jiang and co-workers reported examples of catalysts derived from *C*₂-symmetric Schiff bases for the trimethylsilylcyanation of aldehydes. Reasoning that related ligands had been used with great effect in other catalytic asymmetric processes; they prepared a number of ligands derived from enantiopure 1,2-diphenylethylenediamine and a series of salicylaldehyde derivatives, for example, **26**. The titanium complex of **26** was shown to be an active enantioselective catalyst, which led to *ee* values of up to 87% for the conversion of benzaldehyde into (*R*)-mandelonitrile (Scheme 10). It was found that a number of experimental parameters were important to obtain good conversions and *ee* values. In contrast to the selectivity trend displayed for *C*₁-symmetric systems, less-hindered salicylaldehyde-derived catalysts were found to be more enantioselective. The titanium/ligand ratio was also shown to have a marked effect on the outcome of the reaction, as did catalyst loading. High *ee* values were observed when stoichiometric amounts of catalyst were used; however, the enantioselectivity decreased with lower loading (50 mol%). Subsequent studies revealed that higher *ee* values were obtained at lower loading still (optimum: 10 mol%). This was postulated to be due to aggregation effects, which have been observed in a number of titanium-based catalyst systems. A number of cyanohydrins



Scheme 10. Trimethylsilylcyanation of aldehydes catalyzed by the *C*₂-symmetric Schiff base system of Jiang and co-workers.^[48, 49]

were prepared by using this optimized methodology: Aromatic aldehydes gave higher selectivity than aliphatic aldehydes (Table 7).^[48, 49]

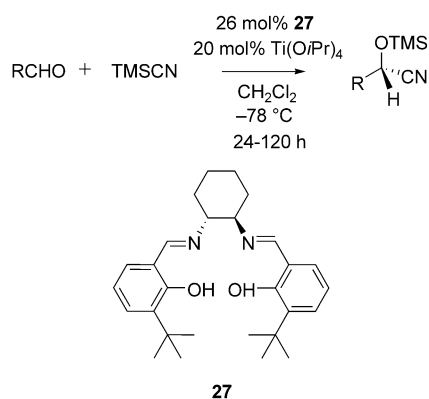
Table 7: Trimethylsilylcyanation of aldehydes catalyzed by the *C*₂-symmetric Schiff base system of Jiang and co-workers (Scheme 10).^[48, 49]

Entry	Aldehyde	<i>t</i> [h]	Yield [%]	ee [%]
1	benzaldehyde	24	72	87 (R)
2	<i>p</i> -chlorobenzaldehyde	36	76	84 (R)
3	<i>p</i> -methylbenzaldehyde	36	60	82 (R)
4	<i>p</i> -anisaldehyde	36	68	74 (R)
5	(<i>E</i>)-cinnamaldehyde	36	70	72 (R)
6	pivalaldehyde	36	85	73 (R)
7	<i>n</i> -nonanal	36	58	22 (R)

Working independently, Belokon, North, and co-workers also reported catalysts derived from *C*₂-symmetric Schiff bases in 1996. Choosing to investigate ligands derived from enantiopure 1,2-diaminocyclohexane and substituted salicylaldehydes, they succeeded in preparing active enantioselective titanium-based catalysts. In contrast to the work of Jiang and co-workers, it was found that substituents on the salicylaldehyde ring improved the activity of the system; of the ligands tested, 3-*tert*-butyl analogue **27** gave the best results. In the presence of the titanium catalyst derived from **27** (20 mol%) a range of aldehydes was converted into cyanohydrins (Scheme 11, Table 8). The catalyst system was tolerant to changes in reaction temperature to an unprecedented extent.^[50]

Building on this work, Belokon and co-workers investigated a number of systems prepared from a chiral paracyclophane. It was found that enantiopure 4-hydroxy-5-formyl-[2.2]paracyclophane (FHPC, **28**) could be used to generate *C*₂-symmetric Schiff bases in combination with a number of diamines, and some of the resulting ligands were used to generate highly selective titanium complexes (Figure 6). The optimal system identified, **29**, catalyzed the addition of TMSCN to benzaldehyde to give mandelonitrile in 90% yield with 84% *ee* in the presence of 10 mol% of catalyst at −78 °C. However, the reaction was very slow, requiring 120 h to go to completion.

Owing to the high tolerance of system **27** to reaction temperature, studies were made to determine if **29** behaved



Scheme 11. Trimethylsilylcyanation of aldehydes catalyzed by the titanium complex of ligand **27**.

Table 8: Trimethylsilylcyanation of aldehydes catalyzed by the titanium complex of ligand **27** (Scheme 11).

Entry	Aldehyde	Yield [%]	<i>ee</i> [%]
1	benzaldehyde	68	75 (S)
2	<i>p</i> -anisaldehyde	70	62 (S)
3	(<i>E</i>)-cinnamaldehyde	60	77 (S)
4	pivalaldehyde	55	77 (S)

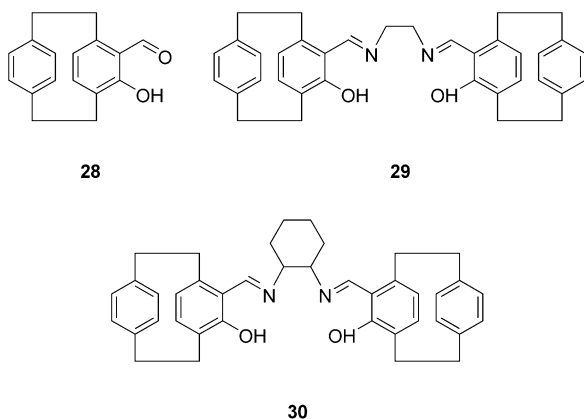
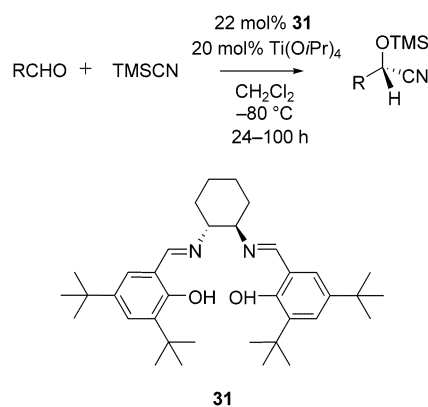


Figure 6. Schiff base ligands derived from paracyclophanes.

similarly. Unfortunately, although good yields were obtained when using the most selective catalyst, a marked drop in enantioselectivity was observed for reactions carried out at ambient temperature. Interestingly, the less-selective catalyst **30** was considerably more tolerant of reaction temperature, with only a 5 % decrease in the *ee* value observed when the reaction was carried out at room temperature instead of at -78°C . Also of note was the observation that the *ee* value obtained with catalyst **30** increased as the reaction proceeded. A reaction carried out at -78°C gave 90 % yield with 35 % *ee* after 24 h. However, when the reaction was left for 120 h, the yield remained 90 %, but the *ee* value increased to 48 %.^[51]

Building on their preliminary studies, attempts were made to optimize the structure of the chiral [(salen)Ti] complex further. A number of catalysts derived from Schiff bases of

1,2-diaminocyclohexane were screened for the addition of TMSCN to aldehydes. Tetra-*tert*-butylsalen **31** was found to give optimal results. In the presence of 20 mol % of catalyst at -80°C , a range of aldehydes was converted into the corresponding TMS cyanohydrins with *ee* values of up to 92 % (Scheme 12, Table 9).^[52,53]



Scheme 12. Trimethylsilylcyanation of aldehydes catalyzed by the titanium complex of ligand **31**.

Table 9: Trimethylsilylcyanation of aldehydes catalyzed by the titanium complex of ligand **31** (Scheme 12).

Entry	Aldehyde	Yield [%]	<i>ee</i> [%]
1	benzaldehyde	89	72 (S)
2	<i>p</i> -methylbenzaldehyde	40	88
3	<i>p</i> -anisaldehyde	40	84
4	<i>p</i> -nitrobenzaldehyde	60	10
5	<i>o</i> -methylbenzaldehyde	90	80
6	cinnamaldehyde	100	54
7	pivalaldehyde	100	36

In light of the excellent initial results obtained with **31**, further investigations were carried out in an attempt to obtain enhanced selectivity with a lower catalyst loading. It was found that the use of titanium tetrachloride to generate the active species led to the isolation of complex **32** (Figure 7). This result was in marked contrast to earlier work with alkoxides, in which case the active catalyst was only generated in solution, and evidence suggests at least three catalytic species. Species **32** was shown to be an excellent catalyst for the trimethylsilylcyanation of benzaldehyde at loadings as low as 0.01 mol %. Furthermore, the reaction was shown to have a low temperature coefficient. The cyanation of benzaldehyde

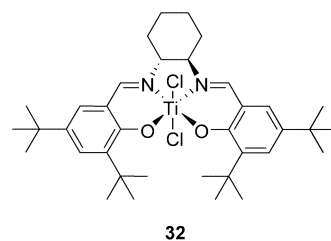
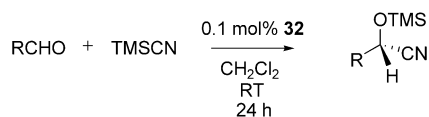


Figure 7. Titanium-based catalyst **32**.

catalyzed by **32** at room temperature led to the product with 86% *ee*, in comparison with 90% *ee* at -80°C . A number of aldehydes were converted into cyanohydrins to test the general applicability of **32** (Scheme 13, Table 10).



Scheme 13. Trimethylsilylcyanation of aldehydes catalyzed by **32**.

Table 10: Trimethylsilylcyanation of aldehydes catalyzed by **32** (Scheme 13).

Entry	Aldehyde	<i>ee</i> [%]
1	benzaldehyde	86 (S)
2	<i>p</i> -methylbenzaldehyde	72
3	<i>p</i> -anisaldehyde	84
4	<i>p</i> -nitrobenzaldehyde	30
5	<i>o</i> -anisaldehyde	72
6	pivalaldehyde	46
7	propanal	44

The observation was then made that the presence of additives, especially traces of water, were crucial for reproducible high-yielding reactions. Under exacting anhydrous reaction conditions, it was shown that neither the titanium complex of **31** nor that of **32** were efficient catalysts. Reasoning that there was possibly a common active catalytic intermediate in both reactions, the authors found that treatment of **32** with 1 equivalent of water gave complex **33** (Figure 8), which was isolated and characterized by X-ray

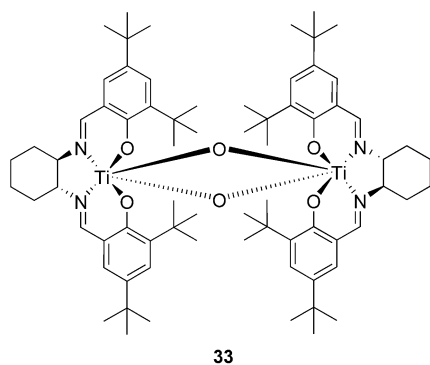
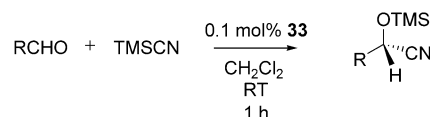


Figure 8. Titanium-based catalyst **33**.

crystallographic analysis. Experimental evidence suggests that **33** is most probably generated as the active catalyst in reactions with complex **31**.

Complex **33** can be used to generate cyanohydrins at a loading of 0.1 mol% at room temperature. Reactions are rapid, typically complete in less than 1 h (less than 5 min in the case of benzaldehyde). These results suggest that the low reaction temperatures that have been employed with most previous titanium-based catalysts were necessary only to suppress the catalytic activity of competing poorly selective

catalysts. It was noted that the reaction is relatively solvent-independent, and no nonlinear effects were observed. A number of aldehydes were converted into their corresponding cyanohydrins in the presence of **33** (Scheme 14, Table 11).^[54]



Scheme 14. Trimethylsilylcyanation of aldehydes catalyzed by **33**.

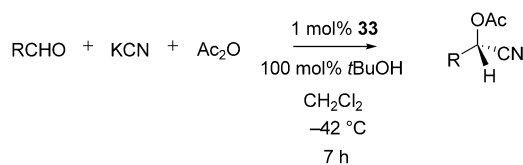
Table 11: Trimethylsilylcyanation of aldehydes catalyzed by **33** (Scheme 14).

Entry	Aldehyde	<i>ee</i> [%]
1	benzaldehyde	86 (S)
2	<i>p</i> -methylbenzaldehyde	87 (S)
3	<i>p</i> -anisaldehyde	84 (S)
4	<i>p</i> -nitrobenzaldehyde	50 (S)
5	<i>o</i> -anisaldehyde	88 (S)
6	pivalaldehyde	66 (S)
7	propanal	52 (S)

Extensive mechanistic studies were carried out to investigate the exact mode of action of **33** and to determine if further improvements were possible to the already excellent enantioselectivities observed. Techniques applied include X-ray crystallographic, NMR and IR spectroscopic, and CD analysis of an active titanium–ligand complex, and a comprehensive kinetic investigation of a number of active, differently substituted, catalysts was carried out. Based on the results obtained from these investigations, a catalytic cycle was proposed in which both the aldehyde and cyanide components of the reaction are simultaneously activated.^[55]

To improve the industrial applicability of ligand **33**, alternate sources of cyanide have also been studied. As TMSCN is relatively expensive and hydrogen cyanide is highly toxic and difficult to handle, Belokon, North, and co-workers investigated nonvolatile cyanide sources. In the presence of acetic anhydride—to trap the formed cyanohydrin as an acetate ester—potassium cyanide was identified as the cyanide source of choice. After careful optimization of reaction conditions, including studies of stirring rates, solvents, concentration, and additives *ee* values of up to 93% were observed for the addition of KCN to a diverse range of aldehydes (Scheme 15, Table 12).^[56,57]

Che and co-workers have also been active in the area of cyanation reactions in the presence of titanium catalysts with C_2 -symmetric Schiff bases. They prepared a range of ligands,

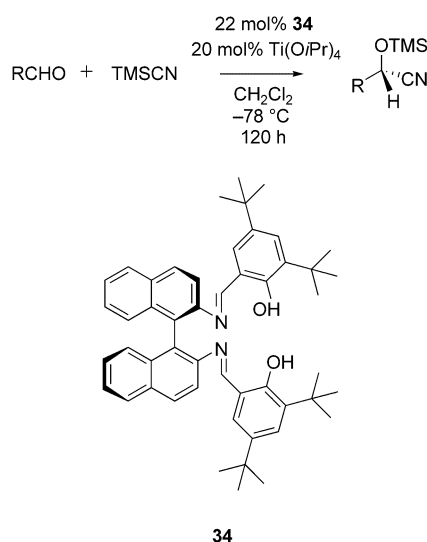


Scheme 15. Cyanation of aldehydes catalyzed by **33**.

Table 12: Cyanation of aldehydes with KCN catalyzed by **33** (Scheme 15).

Entry	Aldehyde	Yield [%]	ee [%]
1	benzaldehyde	93	90 (S)
2	<i>p</i> -fluorobenzaldehyde	98	92 (S)
3	<i>o</i> -fluorobenzaldehyde	87	85 (S)
4	<i>m</i> -phenoxybenzaldehyde	99	90 (S)
5	3-phenylpropionaldehyde	80	84 (S)
6	pivalaldehyde	40	62 (S)
7	isobutanal	64	69 (S)

including **34**, which have been used with both titanium and ruthenium systems. Excellent selectivity was observed (up to 96% *ee*) with a catalyst formed from **34** and titanium isopropoxide (Scheme 16, Table 13).^[44]



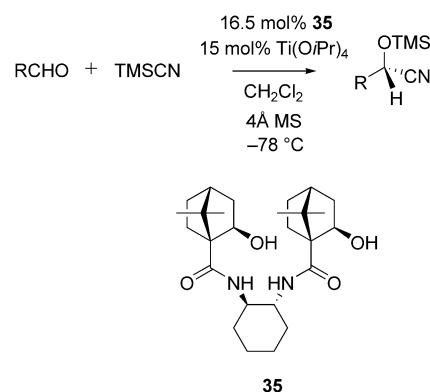
Scheme 16. Trimethylsilylcyanation of aldehydes catalyzed by the C₂-symmetric Schiff base system of Che and co-workers.^[44]

Table 13: Trimethylsilylcyanation of aldehydes catalyzed by the C₂-symmetric Schiff base system of Che and co-workers (Scheme 16).^[44]

Entry	Aldehyde	Yield [%]	ee [%]
1	benzaldehyde	92	93 (S)
2	<i>p</i> -methylbenzaldehyde	82	88 (S)
3	<i>m</i> -methylbenzaldehyde	75	96 (S)
4	<i>o</i> -methylbenzaldehyde	87	95 (S)
5	<i>o</i> -chlorobenzaldehyde	82	51 (S)
6	phenylacetaldehyde	63	71 (S)
7	2-methylpropenal	75	42 (S)

Uang and co-workers investigated C_2 -symmetric systems based on amides. Using ligand **35**—derived from enantiopure 1,2-diaminocyclohexane and ketopinic acid chloride—and titanium isopropoxide, they generated an extremely active catalyst for the trimethylsilylcyanation of aldehydes. Optimum results were obtained at -78°C when using 15 mol % of catalyst in the presence of molecular sieves. A number of aldehydes were screened and the corresponding cyanohydrins were formed with up to 97% *ee*. Notably, high *ee* values are

obtained with both aromatic and aliphatic aldehydes (Scheme 17, Table 14).^[58] Building on these excellent initial results, Uang and co-workers demonstrated that enhanced



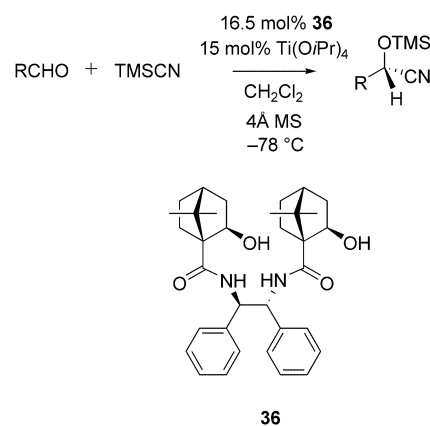
Scheme 17. Trimethylsilylcyanation of aldehydes catalyzed by the diamide system of Uang and co-workers.^[58]

Table 14: Trimethylsilylcyanation of aldehydes catalyzed by the diamide system of Uang and co-workers (Scheme 17).^[58]

Entry	Aldehyde	<i>t</i> [h]	Yield [%]	<i>ee</i> [%]
1	benzaldehyde	48	79	94 (S)
2	<i>p</i> -anisaldehyde	120	53	97 (S)
3	<i>o</i> -methylbenzaldehyde	120	68	97 (S)
4	2-naphthaldehyde	120	76	96 (S)
5	3-phenoxybenzaldehyde	120	57	97 (S)
6	(<i>E</i>)-cinnamaldehyde	120	51	94 (S)
7	valeraldehyde	36	96	89 (S)

enantioselectivities may be obtained for aliphatic aldehydes with a closely related catalyst **36** (Scheme 18, Table 15).^[59]

As is the case for the C_1 -symmetric systems described above, most procedures detailed for C_2 -symmetric systems require experimental protocols at low temperatures for extended time periods combined with the use of TMSCN as the cyanide source. The notable exception to this is the later work of Belokon, North, and co-workers. By refinement and



Scheme 18. Trimethylsilylcyanation of aldehydes catalyzed by the modified diamide system of Uang and co-workers.^[59]

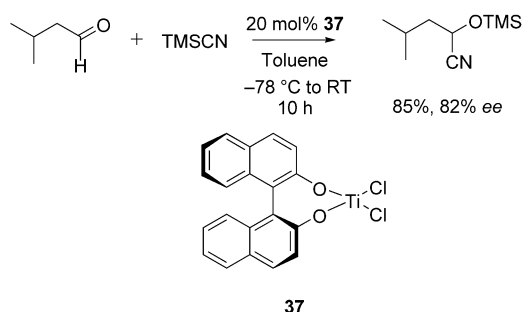
Table 15: Trimethylsilylcyanation of aldehydes catalyzed by the modified diamide system of Uang and co-workers (Scheme 18).^[59]

Entry	Aldehyde	Yield [%]	ee [%]
1	benzaldehyde	87	93 (S)
2	<i>p</i> -anisaldehyde	72	99 (S)
3	<i>o</i> -methylbenzaldehyde	80	94 (S)
4	2-naphthaldehyde	78	99 (S)
5	3-phenoxybenzaldehyde	78	95 (S)
6	(<i>E</i>)-cinnamaldehyde	74	97 (S)
7	valeraldehyde	92	97 (S)

careful study of their catalyst structure, they developed a system which led to high *ee* values at ambient temperature with a 1-h reaction time for the cyanation of a range of aldehydes with low catalyst loading. Furthermore, they addressed the use of TMSCN as the cyanide source and reported conditions for the use of KCN as an alternative, thus making this a procedure of choice for large-scale work—though relatively low temperatures have to be employed for the 7-h reaction. Their catalyst system **33** is also applicable to unhindered aromatic ketones. Unfortunately, electron-poor aromatic aldehydes and aliphatic systems do not perform particularly well with catalyst **33**. Currently, this catalyst or the related vanadium system **43** (Section 2.3) are probably the cyanation reagents of choice for simple aromatic aldehyde systems when balancing synthetic utility with enantioselectivity.

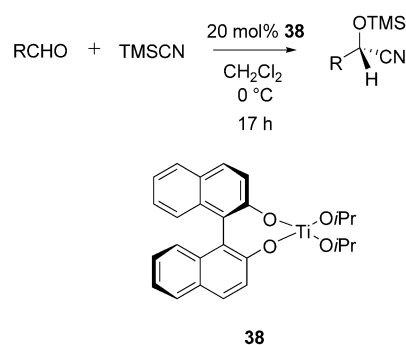
2.2.6. Binol-Derived Complexes

Binol-derived complexes have also been investigated as trimethylsilylcyanation catalysts. The initial report in this area, and the first of titanium-mediated catalytic trimethylsilylcyanation, came from Reetz et al. In an isolated example, they demonstrated that in the presence of **37** (20 mol %), isobutanal could be converted into the corresponding cyanohydrin silyl ether in 85% yield with 82% *ee* (Scheme 19).^[60] The absolute configuration of the product was not determined.



Scheme 19. The asymmetric addition of TMSCN to isobutanal with the titanium system of Reetz et al.^[60]

Nakai and co-workers reported the trimethylsilylcyanation of a range of aldehydes catalyzed by the related complex **38** (Scheme 20, Table 16). When **38** was used, the reactions



Scheme 20. Trimethylsilylcyanation of aldehydes catalyzed by the binol system of Nakai and co-workers.^[61]

Table 16: Trimethylsilylcyanation of aldehydes catalyzed by the binol system of Nakai and co-workers (Scheme 20).^[61]

Entry	Aldehyde	Yield [%]	ee [%]
1	benzaldehyde	> 90	< 10
2	<i>p</i> -anisaldehyde	> 90	< 10
3	<i>p</i> -chlorobenzaldehyde	> 90	< 10
4	ethanal	> 90	< 10
5	<i>n</i> -nonanal	> 90	72 (S)
6	cyclohexanecarbaldehyde	> 90	33
7	pivalaldehyde	> 90	75

could only be run catalytically at temperatures above -30°C . Below -30°C the reaction proceeded only when using stoichiometric quantities of **38**. A mechanistic explanation backed by NMR spectroscopic studies was proposed. In the presence of **38** (20 mol %), a series of aldehydes were converted into the corresponding cyanohydrins at 0°C and with up to 75% *ee*. Interestingly, aliphatic aldehydes gave much higher *ee* values than aromatic aldehydes, in marked contrast to nearly all the other catalytic systems reported herein.^[61]

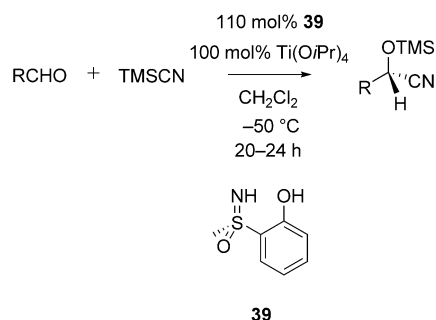
Recently, Seebach and co-workers studied a polymer-bound binol analogue of the Nakai system. It was shown that the use of 20 mol % of the bead-based catalyst led to an *ee* value of 72% in the trimethylsilylcyanation of pivalaldehyde, comparable with the homogeneous reaction (see Table 16, entry 7). The catalyst was readily separated and reused. Interestingly, the enantioselectivity increased on recycling, to a maximum of 83% *ee* after five consecutive uses of the catalyst. Subsequent experiments showed that the titanium leached slowly from the polymer after five runs, but that reactivation was possible by treatment with titanium isopropoxide.^[62]

Without question, binol systems suffer from issues of general applicability. They are of interest, however, as they result in relatively high *ee* values for a number of aliphatic systems in reactions carried out at easily attainable temperatures.

2.2.7. Sulfoximine Ligands

Bolm and co-workers studied the use of chiral sulfoximines as ligands for titanium-derived catalysts for trimethyl-

silylcyanation. Ligand **39** and titanium isopropoxide were used to generate a complex that gave cyanohydrins with up to 91 % *ee* in reactions involving a stoichiometric quantity of the catalyst. Lower catalyst loadings were studied, but were found to give inferior results (Scheme 21, Table 17).^[63, 64] The methodology of Bolm and co-workers is a stoichiometric procedure and is included here only for completeness.



Scheme 21. Trimethylsilylcyanation of aldehydes catalyzed by the stoichiometric sulfoximine system of Bolm and co-workers.^[63, 64]

Table 17: Trimethylsilylcyanation of aldehydes catalyzed by the stoichiometric sulfoximine system of Bolm and co-workers (Scheme 21).^[63, 64]

Entry	Aldehyde	Yield [%]	<i>ee</i> [%]
1	benzaldehyde	72	91 (S)
2	<i>p</i> -anisaldehyde	60	87 (S)
3	1-naphthaldehyde	92	76 (S)
4	<i>n</i> -hexanal	64	89 (S)
5	(<i>E</i>)-cinnamaldehyde	63	79 (S)
6	cyclohexanecarbaldehyde	70	89 (S)
7	pivalaldehyde	70	81 (S)

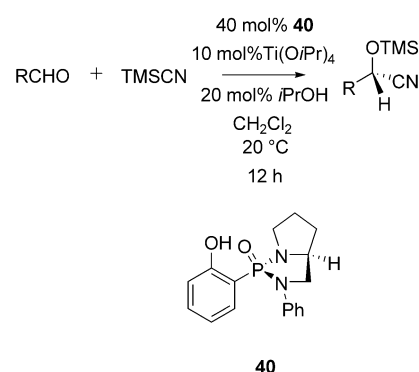
2.2.8. Bifunctional Lewis Acid/Lewis Base Catalysts

An important development in catalytic trimethylsilylcyanation, with wider implications across the field of asymmetric catalysis, was the discovery of bifunctional catalyst ligands. Buono and co-workers and Shibasaki and co-workers (see Sections 2.6 and 3) reported examples of this class of ligand with applicability to silylcyanation in early 1999.

Buono and co-workers found that cyanohydrins could be prepared with excellent enantioselectivities by using 10 mol % of a titanium catalyst derived from the *o*-hydroxyaryl phosphonodiamide ligand **40** at room temperature. The ratio of the components used to generate the active complex was pivotal in obtaining good enantioselectivities. A range of aldehydes was investigated and the products were obtained with up to 98 % *ee* (Scheme 22, Table 18).^[65]

Tang and co-workers investigated bifunctional *o*-hydroxyaryl phosphonodiamide ligands. They used 40 mol % of the readily prepared ligand **41** to prepare cyanohydrins with up to 90 % *ee* from aromatic aldehydes. The reactions were typically carried out at 0 °C and gave high yields (Scheme 23, Table 19).^[66]

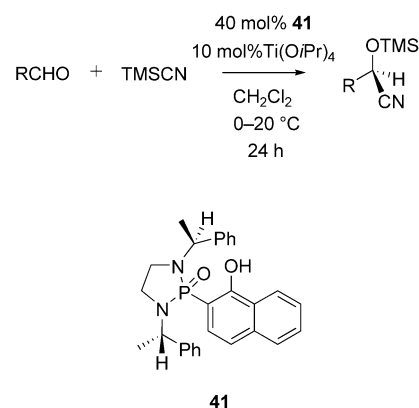
Sulfoxide-containing ligands for bifunctional asymmetric titanium catalysts were also investigated. Rowlands prepared



Scheme 22. Trimethylsilylcyanation of aldehydes catalyzed by the system of Buono and co-workers.^[65]

Table 18: Trimethylsilylcyanation of aldehydes catalyzed by the system of Buono and co-workers (Scheme 22).^[65]

Entry	Aldehyde	Yield [%]	<i>ee</i> [%]
1	benzaldehyde	95	94 (S)
2	<i>p</i> -anisaldehyde	86	98 (S)
3	1-naphthaldehyde	92	90 (S)
4	2-phenoxybenzaldehyde	75	42 (S)
5	3-trifluoromethylbenzaldehyde	70	15 (S)
6	furan-2-carbaldehyde	79	3 (S)



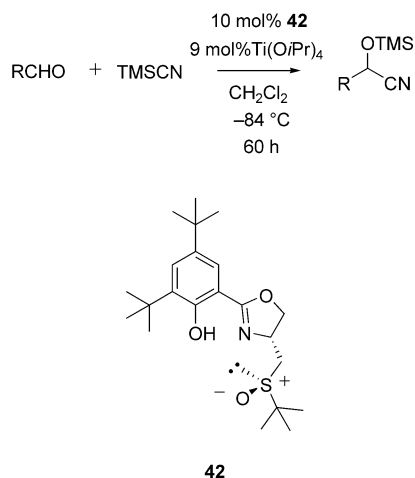
Scheme 23. Trimethylsilylcyanation of aldehydes catalyzed by the system of Tang and co-workers.^[66]

Table 19: Trimethylsilylcyanation of aldehydes catalyzed by the system of Tang and co-workers (Scheme 23).

Entry	Aldehyde	Yield [%]	<i>ee</i> [%]
1	benzaldehyde	98	43 (R)
2	<i>o</i> -anisaldehyde	92	90 (R)
3	<i>p</i> -anisaldehyde	92	50 (R)
4	<i>o</i> -methylbenzaldehyde	80	72 (R)
5	<i>p</i> -methylbenzaldehyde	95	41 (R)
6	<i>o</i> -nitrobenzaldehyde	96	8 (R)
7	2-naphthaldehyde	90	76 (R)

and studied a series of ligands based on a phenolic oxazoline scaffold. Cyanohydrins were prepared with up to 61 % *ee* by using 10 mol % of ligand **42** and 9 mol % titanium isoprop-

oxide (Scheme 24, Table 20). The presence of the weakly Lewis basic sulfoxide was shown to be the key to the activity of the catalysts.^[67]



Scheme 24. Trimethylsilylcyanation of aldehydes catalyzed by the sulfonamide system of Rowlands and co-workers.^[67]

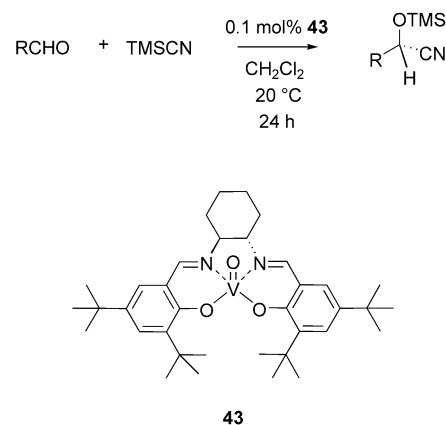
Table 20: Trimethylsilylcyanation of aldehydes catalyzed by the system of Rowlands and co-workers (Scheme 24).^[67]

Entry	Aldehyde	Yield [%]	ee [%]
1	benzaldehyde	85	54 (<i>R</i>)
2	<i>p</i> -anisaldehyde	80	57 (<i>R</i>)
3	<i>o</i> -nitrobenzaldehyde	48	10 (<i>R</i>)
4	2-naphthaldehyde	80	40 (<i>R</i>)
5	cinnamaldehyde	78	50 (<i>R</i>)
6	pivalaldehyde	26	40 (<i>R</i>)
7	heptanal	62	37 (<i>S</i>)

The synthesis of aldehydes by using bifunctional catalysis based on titanium systems currently suffers from issues of general applicability. Furthermore, the more selective system described requires high loadings of asymmetric ligands. All the systems described use TMSCN as the cyanide source; however, the work from the groups of Buono and Tang was carried out at easily attainable reaction temperatures. This fact together with and related work with aluminum-based bifunctional catalysts (see Sections 2.6 and 3) suggest that there is much room for enhancement in the area.

2.3. Vanadium Catalysts

Building on their mechanistic understanding of asymmetric cyanohydrin syntheses catalyzed by chiral titanium–salen complexes, Belokon, North, and co-workers developed a catalyst system based on vanadium(IV). The chiral (salen)V=O catalyst **43** is more enantioselective than the corresponding titanium-based system, and 0.1 mol% of **43** is sufficient to convert aromatic and aliphatic aldehydes into the corresponding cyanohydrins with up to 95% ee at room temperature (Scheme 25, Table 21).^[68]



Scheme 25. Trimethylsilylcyanation of aldehydes catalyzed by vanadium catalyst **43**.

Table 21: Trimethylsilylcyanation of aldehydes catalyzed by vanadium catalyst **43** (Scheme 25).

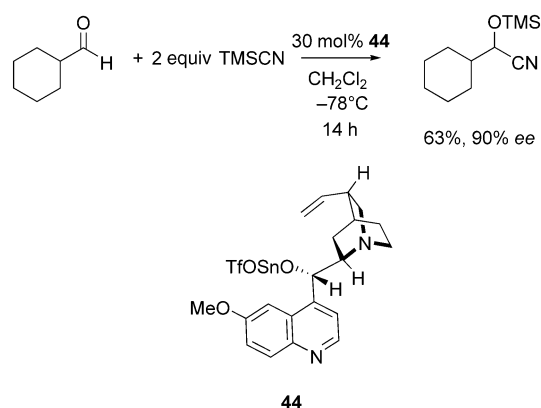
Entry	Aldehyde	Yield [%]	ee [%]
1	benzaldehyde	> 95	94 (<i>S</i>)
2	<i>p</i> -anisaldehyde	> 95	90
3	<i>o</i> -methylbenzaldehyde	> 95	90
4	<i>p</i> -nitrobenzaldehyde	> 95	73
5	propanal	> 95	77
6	pivalaldehyde	> 95	68

It has been demonstrated that catalyst **43** may be used with KCN as the cyanide source in a manner similar to titanium catalyst **33**.^[57] Studies have also been carried out towards developing solid-supported variants of **43**^[69] and around the use of ionic liquids as “green” solvents for its use.^[70]

Although higher ee values were obtained for both aromatic (notably electron-poor systems) and aliphatic aldehydes, reactions with **43** proceed substantially slower than those with the corresponding titanium complex. A reaction complete in < 5 min with **33** took 18 h with **43**. Catalyst **43** was inactive in attempted cyanation reactions of ketones. In terms of synthetic utility, this system offers a reasonable enhancement in ee values over **33** when time is not a key factor in the choice of method.

2.4. Tin Catalysts

In 1991, Kobayashi et al. described the use of a chiral tin(II) Lewis acid **44** (which was prepared from 1,1'-dimethylstannocene, trifluoromethanesulfonic acid, and (+)-cinchonine) in the enantioselective addition of TMSCN to cyclohexanecarbaldehyde. In the presence of a substoichiometric amount of complex **44** (30 mol%), the corresponding cyanohydrin was obtained in 63% yield and with 90% ee as its trimethylsilyl adduct (Scheme 26).^[71] A range of other aldehydes were studied and ee values of up to 96% were found for aliphatic systems—though the absolute configura-



Scheme 26. The addition of TMSCN to cyclohexanecarbaldehyde catalyzed by **44**.

tion of the products were not determined (Table 22). Notably, no reaction was observed with benzaldehyde.

Table 22: Trimethylsilylcyanation of aliphatic aldehydes catalyzed by the tin system of Kobayashi et al. (Scheme 26).^[71]

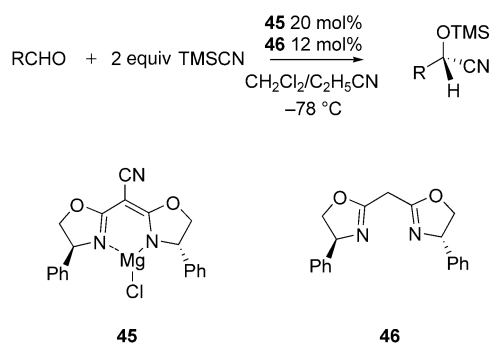
Entry	Aldehyde	Yield [%]	ee [%]
1	nonanal	89	72
2	heptanal	79	96
3	isobutanal	67	95
4	pivalaldehyde	49	83
5	benzaldehyde	0	–

Despite TMSCN as the cyanide source, the high catalyst loadings, and the low temperatures required, the Kobayashi system is of interest owing to its unusual reactivity and selectivity profile. It unfortunately requires 2 equivalents of TMSCN with respect to the aldehyde, and hence care is required when working up the reaction.

2.5. Magnesium Catalysts

In 1993 Corey and Wang reported an enantioselective method for the synthesis of chiral cyanohydrins in the presence of the magnesium-based catalyst **45** (20 mol%) and ligand **46** (12 mol%). This protocol was used in the conversion of a number of aldehydes into their corresponding cyanohydrin TMS ethers with enantioselectivities varying from 52 to 95% *ee*. Better results were obtained for aliphatic aldehydes (Scheme 27, Table 23).^[72] The Corey system shows more activity than the Kobayashi tin catalyst for aromatic systems and gives excellent *ee* values for a number of aliphatic systems. As is the case with the Kobayashi tin catalyst, 2 equivalents of TMSCN are used with respect to aldehyde.

It is currently still unclear if the selectivities observed for the Corey magnesium system and the Kobayashi tin catalyst are a function of ligand structure or the choice of metal used. Possible future work to explain the nature of this aliphatic selectivity would clearly benefit from a mix-and-match approach in which these metals are tested with some of the



Scheme 27. Trimethylsilylcyanation of aldehydes catalyzed by the magnesium system of Corey and Wang.^[72]

Table 23: Trimethylsilylcyanation of aldehydes catalyzed by the magnesium system of Corey and Wang (Scheme 27).^[72]

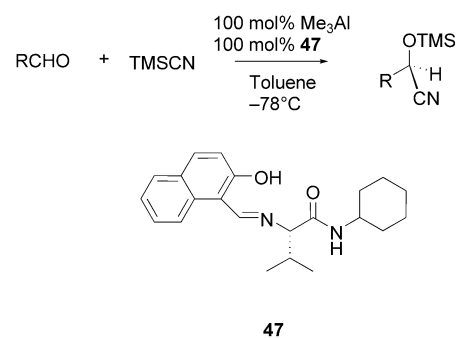
Entry	Aldehyde	<i>t</i> [h]	Yield [%]	ee [%]
1	benzaldehyde	26	88	52 (<i>S</i>)
2	sorbaldehyde	24	24	84 (<i>S</i>)
3	2-ethylbutyraldehyde	6	86	91 (<i>S</i>)
4	geranial	45	31	63 (<i>S</i>)
5	cyclohexanecarbaldehyde	< 5	94	94 (<i>S</i>)
6	pivalaldehyde	30	57	90 (<i>S</i>)

more established ligand systems (typically assessed with benzaldehyde as a reference compound—which clearly may not be appropriate in all cases).

2.6. Aluminum Catalysts

As described above in Section 2.2.4, Inoue and co-workers developed aluminum complexes of Schiff base capped peptides (e.g. **47**), which promote the enantioselective addition of trimethylsilylcyanide to aldehydes. Such systems were used in the conversion of a range of aldehydes with up to 71% *ee* (Scheme 28, Table 24).^[38]

Iovel et al. also studied aluminum-based complexes as catalysts for the asymmetric synthesis of cyanohydrins. Three asymmetric ligand systems were investigated, of which (*S,S*)-2,6-bis(4-isopropylloxazolin-2-yl)pyridine (pybox, **48**) gave

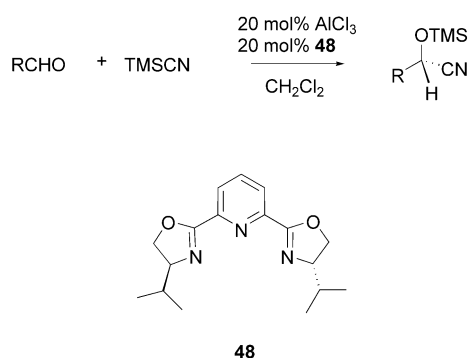


Scheme 28. Trimethylsilylcyanation of aldehydes catalyzed by the aluminum system of Inoue and co-workers.^[38]

Table 24: Trimethylsilylcyanation of aldehydes with the aluminum system of Inoue and co-workers (Scheme 28).^[38]

Entry	Aldehyde	<i>t</i> [h]	Yield [%]	<i>ee</i> [%]
1	benzaldehyde	5	66	71 (<i>R</i>)
2	<i>o</i> -methylbenzaldehyde	3	75	58 (<i>R</i>)
3	<i>m</i> -anisaldehyde	4	92	56 (<i>R</i>)
4	cyclohexanecarbaldehyde	0.5	86	56 (<i>R</i>)
5	<i>n</i> -heptanal	24	84	37 (<i>R</i>)

the best results. A catalyst generated in situ from pybox with AlCl₃ was used to convert a number of aromatic aldehydes into the corresponding cyanohydrins with TMSCN (Scheme 29, Table 25).^[73] Both NMR spectroscopic and modeling studies were used to gather information on the structure of the catalyst.



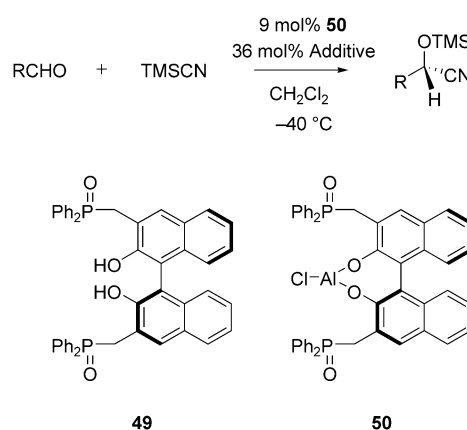
Scheme 29. Trimethylsilylcyanation of aldehydes catalyzed by the aluminum system of Iovel et al.^[73]

Table 25: Trimethylsilylcyanation of aldehydes catalyzed by the aluminum system of Iovel et al. (Scheme 29).^[73]

Entry	Aldehyde	Yield [%]	<i>ee</i> [%]
1 ^[a]	benzaldehyde	90	44 (<i>S</i>)
2 ^[b]	furan-2-carbaldehyde	96	n.d. ^[c]
3 ^[b]	thiophene-2-carbaldehyde	87	n.d. ^[c]
4 ^[b]	benzaldehyde	92	90 (<i>S</i>)

[a] Reaction carried out at room temperature, 4 h. [b] Reaction carried out at 0–10 °C, 16 h. [c] Not determined.

An exciting new area in aluminum-based catalysis was opened up by Shibasaki and co-workers, who described the synthesis and use of a binol-derived bifunctional ligand **49** with both Lewis acid and Lewis base functionality. Its aluminum complex **50**, in the presence of additives, was shown to give outstanding results for the trimethylsilylcyanation of a range of aldehydes. Under optimal conditions *ee* values of up to 98% were obtained for both aromatic and aliphatic systems (Scheme 30, Table 26).^[74] Based on these excellent results, the mechanism of the reaction was investigated and a catalytic cycle proposed. The reaction was also applied, with excellent enantioselectivity, to the synthesis of a complex fragment of the natural product epothilone.^[75]

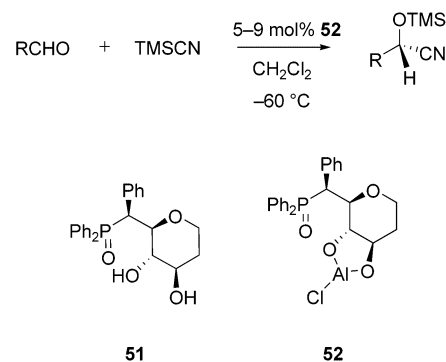


Scheme 30. Trimethylsilylcyanation of aldehydes catalyzed by the binol–aluminum system of Shibasaki and co-workers.^[74]

Table 26: Trimethylsilylcyanation of aldehydes catalyzed by the binol–aluminum system of Shibasaki and co-workers (Scheme 30).^[74]

Entry	Aldehyde	Additive	<i>t</i> [h]	Yield [%]	<i>ee</i> [%]
1	benzaldehyde	CH ₃ P(O)Ph ₂	96	98	96 (<i>S</i>)
2	<i>p</i> -methylbenzaldehyde	CH ₃ P(O)Ph ₂	79	87	90 (<i>S</i>)
3	cinnamaldehyde	Bu ₃ P(O)	40	99	98 (<i>S</i>)
4	furan-2-carbaldehyde	CH ₃ P(O)Ph ₂	70	86	95 (<i>S</i>)
5	3-phenylpropionaldehyde	Bu ₃ P(O)	37	97	97 (<i>S</i>)
6	pivalaldehyde	Bu ₃ P(O)	45	96	90 (<i>S</i>)
7	<i>n</i> -heptanal	Bu ₃ P(O)	58	100	98 (<i>S</i>)

Shibasaki and co-workers also investigated other scaffolds applicable to bifunctional catalyst ligands. They used a carbohydrate-derived system **51** to prepare aluminum complex **52**, which gave up to 80% *ee* for the trimethylsilylcyanation of aldehydes (Scheme 31, Table 27). Although the *ee* values obtained with **52** were lower than those with **50**, additives were not necessary, slow addition of TMSCN could be avoided, and a lower catalyst loading was tolerated. Furthermore, catalyst **52** was shown to be effective for the trimethylsilylcyanation of acetophenone (20% *ee*).^[76] This observation was investigated further and led to the development of a catalyst with outstanding activity for the trimethyl-



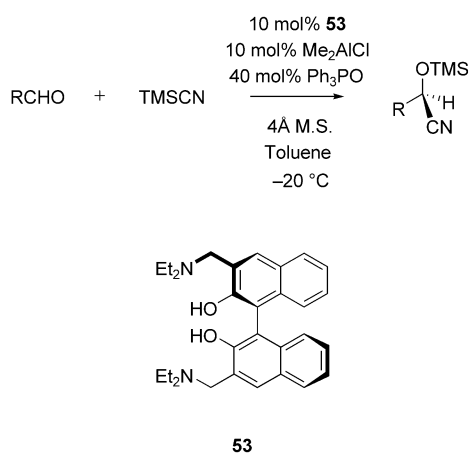
Scheme 31. Trimethylsilylcyanation of aldehydes catalyzed by the carbohydrate-derived aluminum system of Shibasaki and co-workers.^[75]

Table 27: Trimethylsilylcyanation of aldehydes catalyzed by the carbohydrate-derived aluminum system of Shibasaki and co-workers (Scheme 31).^[75]

Entry	Aldehyde	<i>t</i> [h]	Yield [%]	<i>ee</i> [%]
1	benzaldehyde	50	96	80 (<i>S</i>)
2	(<i>E</i>)-cinnamaldehyde	76	82	76 (<i>S</i>)
3	3-phenylpropionaldehyde	50	96	70 (<i>S</i>)
4	heptanal	38	98	80 (<i>S</i>)

silylcyanation of ketones (Section 3). The development and application of these systems were recently reviewed.^[77]

Building on the work of Shibasaki and co-workers, Nájera, Saá, and co-workers designed binolam (**53**) with the aim of preparing a highly enantioselective catalyst that could be readily recycled. By using 10 mol % of the catalyst generated from **53** and dimethylaluminum chloride in the presence of molecular sieves and 40 mol % triphenylphosphane oxide, they converted a range of aldehydes into the corresponding cyanohydrins with > 98 % *ee* (Scheme 32, Table 28). Further-

**Scheme 32.** Trimethylsilylcyanation of aldehydes catalyzed by the aluminum complex of **53**.**Table 28:** Trimethylsilylcyanation of aldehydes catalyzed by the aluminum complex of binolam (**53**) (Scheme 32).

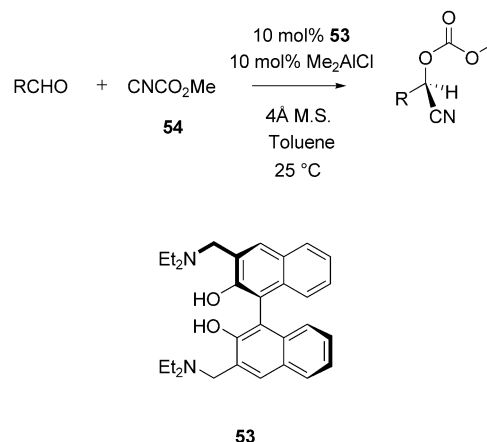
Entry	Aldehyde	<i>t</i> [h]	Yield [%]	<i>ee</i>
1	benzaldehyde	6	99	> 98 (<i>R</i>)
2	<i>p</i> -anisaldehyde	20	87	> 98 (<i>R</i>)
3	cinnamaldehyde	6	99	82 (<i>R</i>)
4	furan-2-carbaldehyde	5	99	76 (<i>R</i>)
5 ^[a]	furan-2-carbaldehyde	12	99	92 (<i>R</i>)
6	<i>p</i> -chlorobenzaldehyde	21	99	> 98 (<i>R</i>)
7	4-phenoxybenzaldehyde	48	70	70 (<i>R</i>)
8 ^[a]	heptanal	3.5	99	76

[a] Reaction temperature: -40 °C.

more, the catalyst was readily recovered through a simple acid–base workup. The catalyst could then be reused without loss of efficiency.^[78]

The use of alternative cyanide sources to TMSCN has also been studied for catalyst **53**. Methyl cyanofornate (**54**) was

used as the cyanide source to convert a range of aldehydes into their cyanofornate analogues with up to 82 % *ee*. Notably, these reactions were carried out at room temperature (Scheme 33, Table 29).^[79] When using methyl cyanofornate

**Scheme 33.** Cyanofornylation of aldehydes catalyzed by the aluminum complex of **53**.**Table 29:** Cyanofornylation of aldehydes catalyzed by the aluminum complex of binolam (**53**) (Scheme 33).

Entry	Aldehyde	Equiv 54	<i>t</i> [h]	Yield [%]	<i>ee</i>
1	benzaldehyde	3	28	> 98	78 (<i>R</i>)
2	<i>p</i> -anisaldehyde	4	20	> 98	78
3	cinnamaldehyde	4	24	95	66
4	<i>p</i> -chlorobenzaldehyde	4	24	> 98	80
5	2-methylbut-2-enal	4	12	> 98	82
6	heptanal	3	20	> 98	68

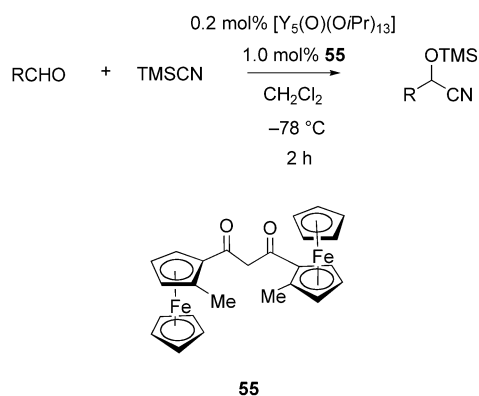
mate instead of TMSCN, the use of a phosphane oxide additive was unnecessary. However, multiple equivalents of methyl cyanofornate were required and lower *ee* values were obtained.

Aluminum-based reagents are rapidly become the second most studied Lewis acid system for asymmetric cyanohydrin synthesis. Although TMSCN and exacting experimental conditions are required for the Shibasaki binol-based system, it does offer high conversions and enantioselectivities for a broad range of aromatic and aliphatic aldehydes. The related system of Nájera, Saá, and co-workers offers comparable *ee* values for a number of aromatic aldehydes combined with synthetically easier methodology (a single addition of TMSCN), faster reaction times at generally higher temperatures, and enhanced ease of catalyst recovery. However, this is offset against the *ee* values obtained for aliphatic systems. Should high optical cyanohydrin purity be the only factor considered in the choice of reaction, then both systems are worthy of consideration along with the titanium systems of Choi and co-workers (Section 2.2.4) and Uang and co-workers (Section 2.2.5) as well as the yttrium system of Shibasaki and co-workers (Section 2.7). The initial communication of Iovel et al. also offers the intriguing possibility of

an aluminum-based catalyst system that operates at room temperature, or at least comparatively high temperatures.

2.7. Yttrium Catalysts

Abiko and Wang found that an yttrium complex of the chiral acetoacetate analogue 1,3-bis(2-methylferrocenyl)propane-1,3-dione (**55**) is an effective catalyst (active at less than 0.2 mol %) for trimethylsilylcyanation of aldehydes. Aromatic aldehydes, except those with electron-withdrawing groups, gave excellent enantioselectivity (up to 99 % *ee*), although the experimental procedure required multiple additions of reagents. Moderate selectivity was possible with aliphatic aldehydes. The absolute configuration of the products were inverted with respect to those of the aromatic systems (Scheme 34, Table 30).^[80] Although the exact structure of the active catalyst has not yet been identified, a mechanism for the reaction was proposed on the basis of number of experimental observations.

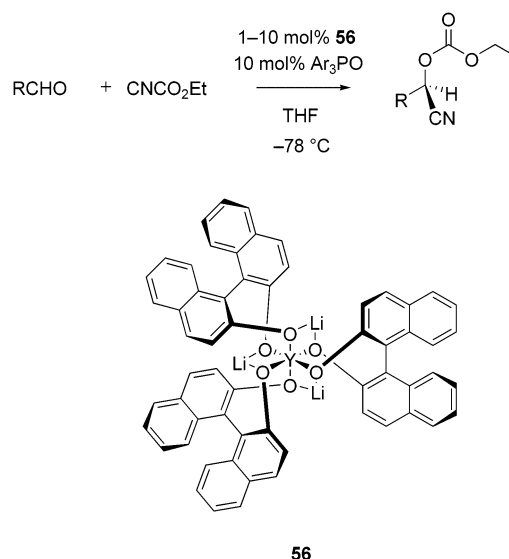


Scheme 34. Trimethylsilylcyanation of aldehydes catalyzed by the yttrium complex of **55**.

Table 30: Trimethylsilylcyanation of aldehydes catalyzed by the yttrium complex of **55** (Scheme 34).

Entry	Aldehyde	Yield [%]	<i>ee</i> [%]
1	benzaldehyde	98	90 (<i>S</i>)
2	<i>p</i> -methylbenzaldehyde	> 95	91 (<i>S</i>)
3	<i>p</i> -fluorobenzaldehyde	> 95	81 (<i>S</i>)
4	<i>p</i> -cyanobenzaldehyde	> 95	30 (<i>S</i>)
5	cyclohexanecarbaldehyde	> 95	49 (<i>R</i>)
6	pivalaldehyde	> 95	49 (<i>R</i>)

Shibasaki and co-workers studied the heterobimetallic complex [YLi₃[tris(binaphthoxide)]] (**56**; 1–10 mol %) as a catalyst in the cyanoformylation of a range of aldehydes in the presence of additives (Scheme 35, Table 31). Notably, only 1.2 equivalents of ethyl cyanofornate were used—substantially less than in the cyanoformylation protocol of Nájera, Saá, and co-workers (Section 2.6). The optimal additive identified was 2,6-dimethoxy-phenylphosphane oxide. This catalyst system has one of the broadest substrate generalities



Scheme 35. Cyanoformylation of aldehydes catalyzed by the yttrium-based catalyst **56**.

Table 31: Cyanoformylation of aldehydes catalyzed by the yttrium-based catalyst **56** (Scheme 35).

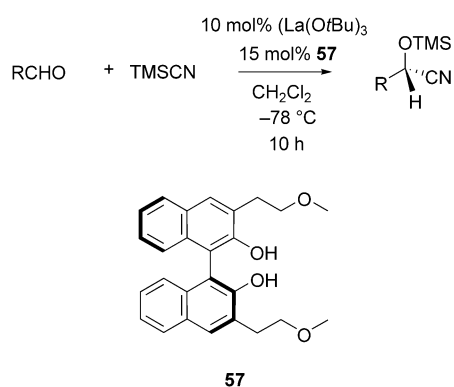
Entry	Aldehyde	56 [mol %]	<i>t</i> [h]	Yield [%]	<i>ee</i> [%]
1	benzaldehyde	10	2	96	94 (<i>R</i>)
2	1-naphthaldehyde	10	2	97	90
3	cinnamaldehyde	10	3	100	91
4	3-methylbutanal	10	2	88	98
5	3-methylbutanal	1	9	96	90
6	hexanal	10	3	93	94
7	cyclohexanecarbaldehyde	10	2	97	96
8	pivalaldehyde	10	3	93	87

reported; furthermore, the reaction times required are relatively short.^[81]

2.8. Lanthanide Catalysts

Qian et al. found that substituted aromatic and aliphatic aldehydes are converted into cyanohydrins with up to 71 % *ee* in the presence of 10 mol % of the chiral lanthanum catalyst generated from La(*Or*Bu)₃ and (*S*)-3,3'-bis(methoxyethyl)binol (**57**; Scheme 36, Table 32).^[82] Greeves et al. also developed trimethylsilylcyanation reactions of aldehydes catalyzed by complexes based on lanthanum (and a number of other lanthanides, in particular ytterbium). Catalysts formed in situ from lanthanide trichlorides and pybox ligands **58** gave *ee* values of up to 89 % for a range of aliphatic and aromatic aldehydes (Scheme 37, Table 33). Catalyst loadings of 10 mol % were routinely used, though initial studies indicated that loadings as low as 1 mol % were tolerated without significant losses in enantioselectivity.^[83]

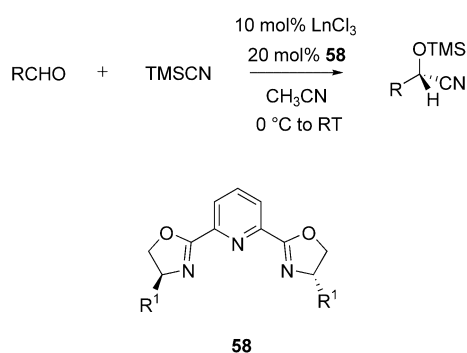
These results combined with those of the aluminum system of Iovel et al. (Section 2.6) suggest that the pybox ligand system is a good candidate for further investigation. High yields and enantioselectivities and short reaction times



Scheme 36. Trimethylsilylcyanation of aldehydes catalyzed by the lanthanum catalyst of Qian et al.^[82]

Table 32: Trimethylsilylcyanation of aldehydes catalyzed by the lanthanum catalyst of Qian et al. (Scheme 36).^[82]

Entry	Substrate	Yield [%]	ee [%]
1	benzaldehyde	77	71 (S)
2	<i>p</i> -methylbenzaldehyde	80	73 (S)
3	<i>p</i> -chlorobenzaldehyde	82	48 (S)
4	<i>p</i> -anisaldehyde	56	63 (S)
5	cyclohexanecarbaldehyde	76	54 (S)
6	3-phenylpropionaldehyde	80	66 (S)



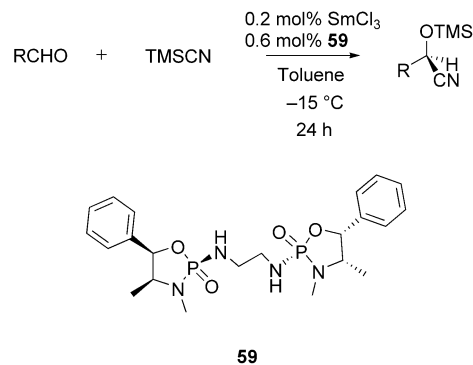
Scheme 37. Trimethylsilylcyanation of aldehydes catalyzed by the ytterbium catalyst system of Greeves and co-workers.^[83]

Table 33: Trimethylsilylcyanation of aldehydes catalyzed by the ytterbium catalyst of Greeves and co-workers (Scheme 37).^[83]

Entry	Aldehyde	R ¹	t [h]	Yield [%]	ee [%]
1	benzaldehyde	<i>i</i> Pr	1	94	75 (S)
2	benzaldehyde	Ph	1	61	89 (S)
3	<i>p</i> -methylbenzaldehyde	<i>i</i> Pr	3	93	70 (S)
4	<i>p</i> -chlorobenzaldehyde	<i>i</i> Pr	16	60	62 (S)
5	furan-2-carbaldehyde	<i>i</i> Pr	2	86	67 (S)
6	cyclohexanecarbaldehyde	<i>i</i> Pr	2	86	60 (S)
7	pivalaldehyde	<i>i</i> Pr	2	83	49 (S)

have been obtained for reactions carried out at relatively high temperatures. There is potential for considerable optimization of these systems in terms of ligand structure, metal, additives, etc.

Fang and Yang described the use of a catalyst generated from samarium(III) chloride and a chiral phosphorus(V) ligand **59** in the asymmetric addition of TMSCN to aromatic aldehydes. Notably, this system gives good *ee* values at comparatively high temperatures in a number of instances. Products with up to 90 % *ee* have been obtained with catalyst loadings of less than 0.2 mol % (Scheme 38, Table 34).^[84]



Scheme 38. Trimethylsilylcyanation of aldehydes catalyzed by the samarium catalyst of Fang and Yang.^[84]

Table 34: Trimethylsilylcyanation of aldehydes catalyzed by the samarium catalyst of Fang and Yang (Scheme 38).^[84]

Entry	Aldehyde	Yield [%]	ee [%]
1	benzaldehyde	> 95	84 (R)
2	<i>p</i> -methylbenzaldehyde	> 95	66 (R)
3	<i>p</i> -fluorobenzaldehyde	> 95	77 (R)
4	<i>p</i> -anisaldehyde	> 95	90 (R)
5	<i>p</i> -cyanobenzaldehyde	> 95	35 (R)
6	<i>p</i> -nitrobenzaldehyde	> 95	29 (R)

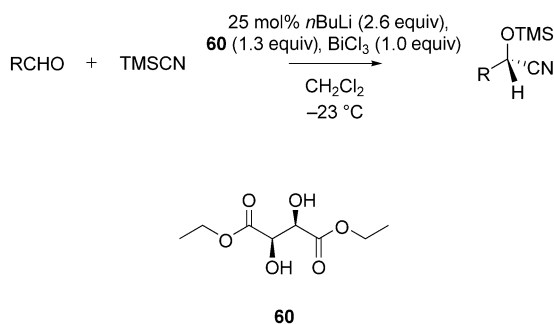
Lanthanide-derived systems are proving to be a very fruitful area of research. As mentioned above, the pybox ligand system seems to offer a potential approach to a high-temperature asymmetric cyanation system for aldehydes.

2.9. Bismuth Catalysts

The asymmetric trimethylsilylcyanation of a variety of aromatic and aliphatic aldehydes in the presence of a catalyst prepared from bismuth(III) chloride and L-diethyl tartrate (**60**) was described by Wada, Smith, and co-workers. Enantiomeric excesses of 20–73 % were obtained by using 25 mol % of the catalyst (Scheme 39, Table 35).^[85] Poorer results are obtained for aromatic aldehydes with this bismuth–tartrate catalyst than with titanium–tartrate system of Oguni and co-workers (Section 2.2.2). The use of highly toxic bismuth salts is a further disadvantage this catalyst system.

2.10. Zirconium Catalysts

Developing on methodology initially disclosed for alkynyl transfer, Maruoka and co-workers used a zirconium catalyst



Scheme 39. Trimethylsilylcyanation of aldehydes catalyzed by the bismuth catalyst of Wada, Smith, and co-workers.^[85]

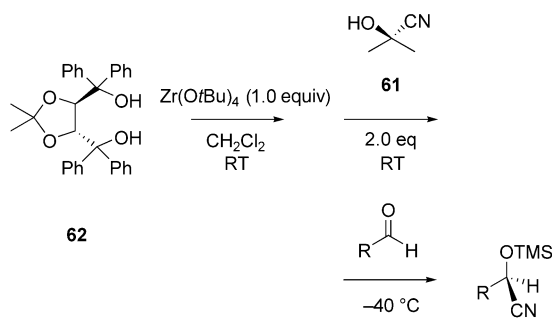
Table 35: Trimethylsilylcyanation of aldehydes catalyzed by the bismuth catalyst of Wada, Smith, and co-workers (Scheme 39).^[85]

Entry	Aldehyde	<i>t</i> [h]	Yield [%]	<i>ee</i> [%]
1	benzaldehyde	0.5	100	73 (S)
2	3-phenoxybenzaldehyde	3	100	58
3	(<i>E</i>)-cinnamaldehyde	20	100	20
4	3-phenylpropionaldehyde	0.5	100	53
5	cyclohexanecarbaldehyde	3.5	87	58
6	<i>n</i> -nonanal	0.5	100	53

to facilitate cyanation with acetone cyanohydrin (**61**) as a source of hydrogen cyanide. This achievement is of special note because of the unusual cyanide source. Acetone cyanohydrin is frequently used in enzyme-catalyzed processes,^[1,2,3] but has seen little use in chemical catalysis.

Screening of a number of systems revealed that taddol **62** was the ligand of choice for use with zirconium(IV) butoxide for cyanation reactions under stoichiometric conditions. Yields of up to 80% were obtained for the reaction of acetone cyanohydrin with 3-phenylpropanal; the enantioselectivity was found to be temperature-dependant. As the temperature of the reaction was lowered from -40 to -78°C , the *ee* value increased from 85 to 91%; however, a substantial decrease in the rate was also observed. A number of aldehydes were tested under the optimized stoichiometric conditions, with the unusual result that aliphatic systems gave both higher *ee* values and yields (Scheme 40, Table 36).^[86]

With an effective stoichiometric cyanation system in hand, a catalytic variant was then sought. Provisional results indicated that this is the case: The reaction of 3-phenyl-

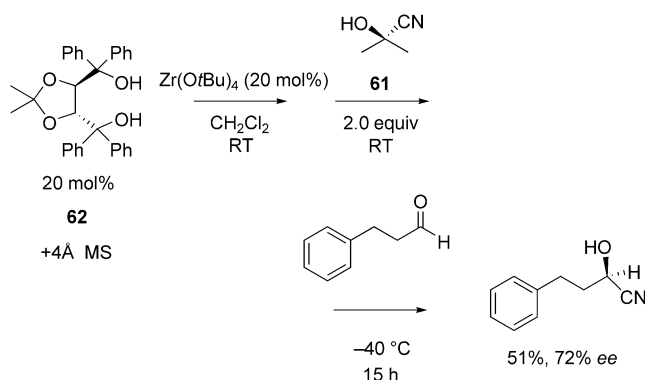


Scheme 40. Hydrocyanation of aldehydes mediated by the stoichiometric zirconium system of Maruoka and co-workers.^[86]

Table 36: Hydrocyanation of aldehydes mediated by the stoichiometric zirconium system of Maruoka and co-workers (Scheme 40).^[86]

Entry	Aldehyde	<i>t</i> [h]	Yield [%]	<i>ee</i> [%]
1	benzaldehyde	18	45	63 (R)
2	furan-2-carbaldehyde	18	30	61 (R)
3	3-phenylpropionaldehyde	7.5	63	85 (R)
4	cyclohexanecarbaldehyde	5	55	79 (R)
5	<i>n</i> -nonanal	5	63	84 (R)
6	pivalaldehyde	5	36	72 (R)

propanal with acetone cyanohydrin (2 equiv) in the presence of 20 mol% of the catalyst derived from **62** gave the product in 51% yield with 72% *ee* (Scheme 41). Notably, the inclu-

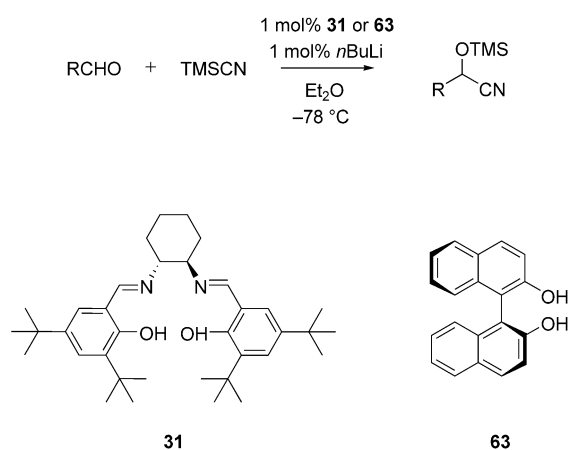


Scheme 41. Hydrocyanation of 3-phenylpropionaldehyde catalyzed by the zirconium system of Maruoka and co-workers.^[87]

sion of molecular sieves was imperative to obtain good yields and enantioselectivity under catalytic conditions.^[87] These provisional results are highly encouraging as they indicate the potential for the use of cyanide sources other than the classically used TMSCN or HCN. Although the *ee* values obtained to date are relatively low, it has been demonstrated that acetone cyanohydrin can be used in conjunction with asymmetric Lewis acid mediated procedures.

2.11. Chiral Base Catalysis

Kagan and Holmes investigated the addition of TMSCN to aldehydes catalyzed by lithium salts of chiral phenols. Templated asymmetric additions were performed by taking advantage of the propensity of silicon to form penta- and hexavalent complexes that are strong Lewis acids. Notably, this process does away with the need for a transition metal owing to the presence of the metalloid silicon. Both (*S*)-(-)-binol (**63**)^[88] and (*R,R*)-(-)-salen (**31**)^[89] were identified as precursors to active enantioselective catalysts, which after optimization of the reaction conditions were used to convert a range of aldehydes into their corresponding cyanohydrins with up to 97% *ee* (Scheme 42, Table 37). Whilst this procedure is interesting from a mechanistic point of view, the highly exothermic nature of the reaction and the poor substrate tolerance render it currently only of academic



Scheme 42. Chiral-base-catalyzed trimethylsilylcyanation reactions.^[88, 89]

Table 37: Trimethylsilylcyanation with chiral base catalysis (Scheme 42).^[88, 89]

Entry	Aldehyde	Ligand	Yield [%]	ee [%]
1	benzaldehyde	63	96	56 (<i>S</i>)
2	benzaldehyde	31	98	86 (<i>R</i>)
3	benzaldehyde	31	88	97 (<i>R</i>)
4	<i>p</i> -anisaldehyde	63	95	54 (<i>S</i>)
5	<i>p</i> -anisaldehyde	31	93	2 (<i>R</i>)
6	<i>p</i> -trifluoromethylbenzaldehyde	31	88	0
7	cyclohexanecarbaldehyde	63	94	30 (<i>S</i>)
8	pivalaldehyde	63	62	26 (<i>S</i>)

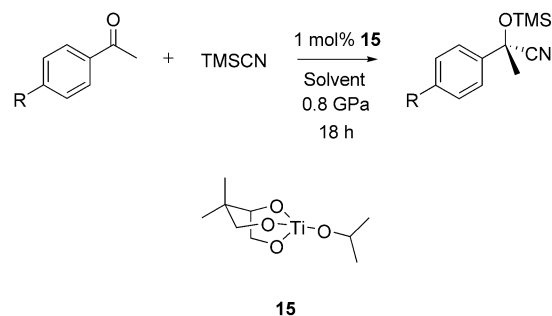
interest. Also, it is unclear whether hypervalent silicon intermediates are responsible for the observed reactions or whether lithium acts as a Lewis acid.

3. Catalysts for the Cyanation of Ketones

The asymmetric cyanation of ketones has been historically considered as problematic. There is more steric hindrance around the carbonyl group of ketones than of aldehydes, significantly decreasing the propensity of the former to undergo cyanation. Despite this inherent problem, the area has received considerable attention as a consequence of the synthetic utility of homochiral cyanohydrins of ketones.

The first chemically catalyzed system for the asymmetric cyanation of ketones was described in 1997 by Choi and co-workers, who built on the work of de Vries,^[33] by investigating **15** as a catalyst for the addition of TMSCN to simple aromatic ketones. Reasoning that high pressure (in the order of GPa) might be used to drive cyanohydrin synthesis, the reaction of acetophenone and TMSCN was investigated at 18 °C with 1 mol% of **15** at 0.8 GPa in a range of solvents.^[90] In dichloromethane, (*S*)-acetophenone cyanohydrin was obtained after 18 h in 93% yield with 60% *ee*. It was found that **15** could be isolated from the crude reaction mixture and reused to give products with similar *ee* values (59%) but in lower yields (69%). In a control reaction carried out at atmospheric pressure, an *ee* value of only 7% was obtained. This suggests that high pressures suppress racemization of the

product, which normally occurs at ambient pressure—presumably by forcing the equilibrium of the reaction toward the product and preventing the reverse reaction. High-pressure reaction conditions were shown to be applicable to a range of simple substituted aromatic methyl ketones with moderate enantioselectivities (Scheme 43, Table 38). This catalyst system demonstrated for the first time that enantioselectivity was attainable for chemically catalyzed cyanations of ketones. However, the forcing conditions employed make its use unattractive for standard laboratory use.



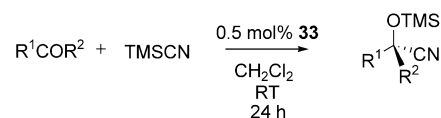
Scheme 43. Reaction of acetophenone with TMSCN catalyzed by **15**.

Table 38: Trimethylsilylcyanation of ketones catalyzed by **15** (Scheme 43).

Entry	Ketone	Solvent	Pressure	Yield [%]	ee [%]
1	acetophenone	THF	0.8 GPa	5	32
2	acetophenone	CH ₂ Cl ₂	0.8 GPa	93	60
3 ^[a]	acetophenone	CH ₂ Cl ₂	0.8 GPa	69	59
4	acetophenone	CH ₂ Cl ₂	Ambient	23	7
5	1-(4-chlorophenyl)-ethanone	CH ₂ Cl ₂	0.8 GPa	39	32
6	1-(4-methoxyphenyl)-ethanone	CH ₂ Cl ₂	0.8 GPa	24	36
7	1- <i>p</i> -tolylethanone	CH ₂ Cl ₂	0.8 GPa	27	45

[a] Recycled catalyst.

The development of the first ambient-pressure system for chemically catalyzed asymmetric ketone cyanation came as a result of advancements made in the asymmetric cyanation of aldehydes. In light of the superb results obtained with their titanium catalysts (Section 2.2.5), Belokon, North, and co-workers investigated the applicability of **33** as a catalyst for the addition of TMSCN to aromatic ketones. It was found that the use of 0.5 mol% of **33** resulted in *ee* values of up to 70% in the cyanation of methyl and ethyl aromatic ketones (Scheme 44, Table 39).^[68, 91] Notably, substantially longer reaction times were required for ketones than those reported



Scheme 44. Trimethylsilylcyanation of ketones catalyzed by **33** (see Figure 8).

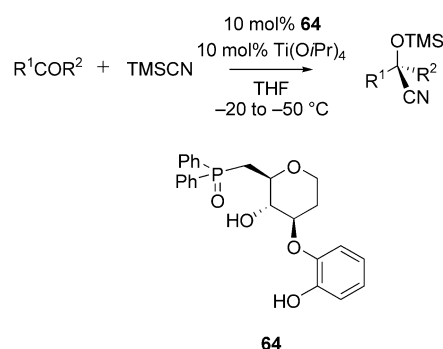
Table 39: Trimethylsilylcyanation of ketones catalyzed by **33** (Scheme 44).

Entry	R ¹	R ²	Yield [%]	ee [%]
1	Ph	Me	93	66 (S)
2	Ph	Et	64 ^[a]	32
3	Ph	<i>i</i> Pr	No Reaction	
4	Ph	<i>t</i> Bu	No Reaction	
5	4-Me-C ₆ H ₄	Me	100	66
6	4-OMe-C ₆ H ₄	Me	100	60
7	4-CF ₃ -C ₆ H ₄	Me	100	56
8	2-OMe-C ₆ H ₄	Me	100 ^[b]	72

[a] Reaction time: 96 h. [b] Reaction time: 48 h.

for aldehydes. Also, only relatively unhindered simple ketones were tolerated as reaction substrates.

The first truly broad-spectrum ketone cyanation catalyst also came about as a result of advances in the asymmetric cyanation of aldehydes. Shibasaki and co-workers described a bifunctional ligand–titanium catalyst system discovered whilst studying an aluminum complex for the cyanation of aldehydes (Section 2.6). It was found that 10 mol % of the titanium complex of **64** could be used to promote reactions at between –50 and –20 °C in THF with outstanding results. A range of ketones were studied, including aliphatic and cyclic compounds, and enantioselectivities of up to 92 % *ee* were obtained (Scheme 45, Table 40).^[92] Further studies revealed that the enantioselectivity could be improved by ligand tuning (up to 97 % *ee* with acetophenone). Lower catalyst loadings were also tolerated.^[93]

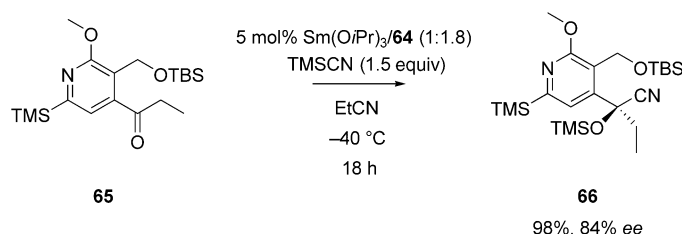

Scheme 45. Trimethylsilylcyanation of ketones catalyzed by the system of Shibasaki and co-workers.^[92]

The work of Shibasaki and co-workers is an outstanding contribution to this area. Their system is applicable to a range of substrates, many of which had been thought of as extremely difficult to address, for example, hindered and cyclic ketones. Although the system requires subzero temperatures, these are not too extreme and are attainable with modern array-synthesis platforms. The issues of TMSCN as a cyanide source and reaction time still remain to be addressed.

Table 40: Trimethylsilylcyanation of ketones catalyzed by the system of Shibasaki and co-workers (Scheme 45).^[92]

Entry	Ketone	<i>t</i> [h]	Yield [%]	ee [%]
1	acetophenone	36	85	92 (R)
2	1- <i>p</i> -tolylethanone	84	80	90
3	methyl 2-naphthyl ketone	80	82	95
4	indan-1-one	96	72	69
5	1-phenylpropan-1-one	64	89	91
6	4-phenylbut-3-en-2-one	88	72	91
7	1-cyclohexylethanone	36	86	90
8	4-phenylbutan-2-one	36	92	85
9	heptan-2-one	36	88	76

Shibasaki, Curran, and co-workers also investigated lanthanide-based bifunctional catalysts. Working towards the synthesis of a member of the camptothecin family, they developed a samarium catalyst based on ligand **64** that can generate **66** from precursor **65** in 98 % yield with 84 % *ee* (Scheme 46).^[26] Building on this excellent result, they went on


Scheme 46. Asymmetric addition of TMSCN to **65**.

to study the use of other lanthanides with ligand **64** for the silylcyanation of ketones. Gadolinium gave impressive results for both aryl and alkyl ketones (Scheme 47, Table 41). Notably, the gadolinium system gave the opposite absolute stereochemistry to that obtained with the titanium system. On the basis of mechanistic studies, a working model for the

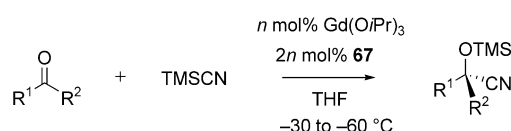

Scheme 47. Trimethylsilylcyanation of ketones catalyzed by the gadolinium catalyst of Shibasaki, Curran, and co-workers.

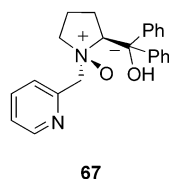
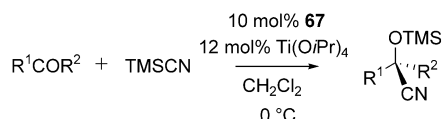
Table 41: Trimethylsilylcyanation of ketones catalyzed by the gadolinium catalyst of Shibasaki, Curran, and co-workers (Scheme 47).

Entry	Ketone	<i>n</i> [mol %]	<i>T</i> [°C]	<i>t</i> [h]	Yield [%]	ee [%]
1	acetophenone	5	–40	2	92	92 (S)
2 ^[a]	acetophenone	10	–30	36	85	92 (R)
3	1-(4-chlorophenyl)-ethanone	5	–60	55	89	89 (S)
4	methyl 2-naphthyl ketone	5	–60	24	95	87 (S)
5	1-phenylpropan-1-one	5	–60	14	93	97 (S)
6	4-phenylbut-3-en-2-one	10	–60	14	97	86 (S)
7	4-phenylbutan-2-one	5	–60	1	90	62 (S)

[a] With titanium.

catalyst structure and the reaction mechanism was proposed.^[26]

Feng, Jiang, and co-workers also investigated bifunctional catalysis as an approach to the cyanation of ketone. They prepared and screened a series of ligands that bear a Lewis basic *N*-oxide and their titanium complexes. Ligand **67** gave the best results of those studied: the cyanation of a range of ketones with 10 mol % of **67** proceeded with up to 69 % *ee* (Scheme 48, Table 42).^[94]



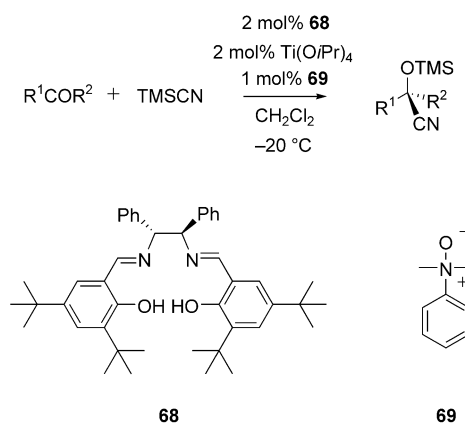
Scheme 48. Trimethylsilylcyanation of ketones catalyzed by the bifunctional catalyst derived from ligand **67**.^[94]

Table 42: Trimethylsilylcyanation of ketones catalyzed by the bifunctional catalyst derived from ligand **67** (Scheme 48).^[94]

Entry	Ketone	<i>t</i> [h]	Yield [%]	<i>ee</i> [%]
1	acetophenone	96	78	54 (<i>R</i>)
2	1- <i>p</i> -tolylethanone	96	63	41
3	methyl 2-naphthyl ketone	96	73	69
4	α-tetralone	120	61	37
6	4-phenylbut-3-en-2-one	96	77	25
7	benzylacetone	120	87	68

Building on these results and with an eye to using more readily available ligands, Feng and co-workers also investigated the cyanation of ketones in the presence of Lewis acid complexes of commercially available ligands in combination with achiral Lewis base additives. By combining the titanium complex of ligand **68** (2 mol %) and achiral *N*-oxide **69** (1 mol %), they identified a system that gives up to 84 % *ee* in the cyanation of a range of ketones (Scheme 49, Table 43).^[95] This practical approach uses a readily available chiral ligand and gives substantially higher *ee* values than the related bifunctional catalysts for a range of ketones. The reaction times are, however, slightly extended.

Research has not been limited to Lewis acid systems for the cyanation of ketones; Lewis base catalysis has also been investigated. Deng and Tian investigated chiral tertiary amine base systems that give outstanding results. Modified cinchona alkaloids were used as catalysts (Figure 9) and led to *ee* values of up to 97 % for the addition of ethyl cyanoformate to a structurally diverse range of ketones. Remarkably, this reaction is applicable to alkyl, cyclic, and hindered ketones, which are converted into the corresponding cyanohydrins



Scheme 49. Trimethylsilylcyanation of ketones catalyzed by the catalyst system derived from ligand **68**.^[95]

Table 43: Trimethylsilylcyanation of ketones catalyzed by the catalyst system derived from ligand **68** (Scheme 49).^[95]

Entry	Ketone	<i>t</i> [h]	Yield [%]	<i>ee</i> [%]
1	acetophenone	120	75	84 (<i>S</i>)
2	1- <i>p</i> -tolylethanone	120	57	73
3	methyl 2-naphthyl ketone	120	50	84
4	α-tetralone	120	37	81
6	4-phenylbut-3-en-2-one	120	79	64
7	benzylacetone	120	85	84

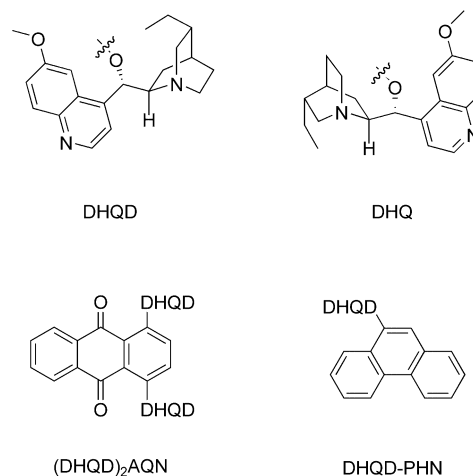
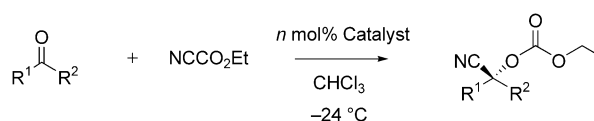


Figure 9. Modified cinchona alkaloid catalysts.

with high *ee* values (Scheme 50, Table 44).^[96] The scope of this reaction was recently extended to acetal ketones.^[97]

The choice of cyanide source—ethyl cyanoformate—is of particular interest. Interestingly, moderate enantioselectivity



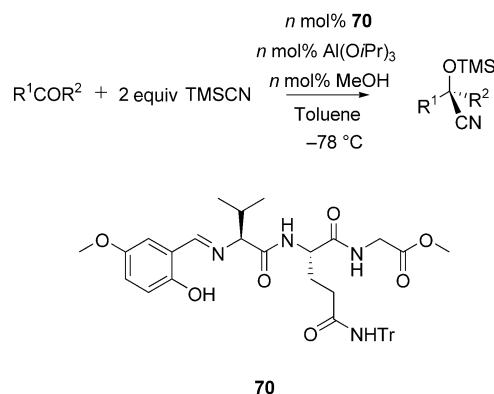
Scheme 50. Cyanoformylation of ketones catalyzed by the Lewis base systems of Deng and Tian.^[96]

Table 44: The cyanoformylation of ketones catalyzed by the Lewis base systems of Deng and Tian (Figure 9, Scheme 50).^[96]

Entry	Ketone	Catalyst (mol %)	t [h]	Yield [%]	ee [%]
1	2,2-dimethylcyclopentanone	(DHQD) ₂ AQN (15)	48	66	97
2	2,2-dimethylcyclohexanone	(DHQD) ₂ AQN (20)	96	62	91
3	1-cyclohexylethanone	(DHQ) ₂ AQN (20)	48	52	87
4	3,3-dimethylbutan-2-one	(DHQD) ₂ AQN (30)	120	55	88
5	2,2-diethoxycyclopentanone	DHOD-PHN (20)	72	96	93
6	3,3-diethoxybutan-2-one	DHOD-PHN (35)	96	65	90
7	heptan-2-one	(DHQD) ₂ AQN (20)	12	54	59

is observed when using this reagent with base catalysis, even at room temperature. This is in marked contrast to the often highly exothermic, poorly selective reactions normally observed with TMSCN in the presence of bases with sufficient nucleophilicity to catalyze cyanohydrin formation under similar conditions. Choi, Matsumoto, and co-workers investigated simple cinchona alkaloid systems as catalysts for the reaction of TMSCN with acetophenone. After the variation of a number of reaction parameters, including solvent and pressure, a maximum of 10 % ee was obtained.^[98]

Finally, Snapper, Hoveyda, and co-workers described a peptide-ligated aluminum-based Lewis acid system that gives excellent generic applicability. Furthermore, the ligand is readily recycled and is easily modified. The addition of TMSCN to ketones with 10 mol % of **70** proceeded with up to 91 % ee (Scheme 51, Table 45).^[99] This system gives excellent



Scheme 51. Trimethylsilylcyanation of ketones catalyzed by the system of Snapper, Hoveyda, and co-workers, derived from ligand **70**.^[99]

Table 45: Trimethylsilylcyanation of ketones catalyzed by the system derived from ligand **70** reported by Snapper, Hoveyda, and co-workers (Scheme 51).^[99]

Entry	Ketone	X mol %	t [h]	Yield [%]	ee [%]
1	acetophenone	10	72	84	91 (R)
2 ^[a]	acetophenone	20	48	93	88
3	1-(2-chlorophenyl)-ethanone	20	48	87	85
4	methyl 2-naphthyl ketone	20	48	83	94
5	1-phenylpropan-1-one	10	48	97	82
6	4-phenylbut-3-en-2-one	20	48	67	95
7	indan-1-one	20	48	87	88
8	nonan-1-one	20	48	93	86

results for aromatic and aliphatic ketones (saturated and unsaturated).

In the space of only 6 years, the field of chemically catalyzed asymmetric cyanation has progressed from its first reported example—under forcing conditions—to systems that give excellent enantioselectivities for a diverse range of substrates. The recent synthesis of the antitumor agent fostriecin by

Shibasaki and co-workers elegantly demonstrates the application of this methodology.^[100]

4. Summary and Outlook

The past two decades have seen significant advances in the field of chemically mediated asymmetric cyanohydrin syntheses. Methods currently available rival, and in many cases surpass, enzymatic procedures in terms of synthetic utility, applicability, and enantioselectivity. Procedures that historically required stoichiometric quantities of reagents and lengthy reaction times at low temperatures for specific substrates are being revolutionized by a new generation of catalysts.

4.1. Synthesis of Cyanohydrins from Aldehydes.

For the synthesis of cyanohydrins from aldehydes, procedures have been developed that work at room temperature, with reaction times measured in minutes rather than days, and give ee values of > 90 % for a range of aromatic substrates at catalyst loadings of less than 1 mol %. The best understood of these are the titanium and vanadium systems of Belokon, North, and co-workers (Sections 2.2.5 and 2.3, respectively), which offer high yields and comparatively high enantioselectivities in practically simple reactions carried out at room temperature. These catalysts evolved from poorly understood aggregates formed in situ (in reality, probably mixtures of several active species), to discrete, fully characterized pre-formed species or precursors. There are also indications that the outstanding performance of these systems at room temperature may not be unique and that other high temperature systems may be possible (see Sections 2.6 and 2.8).

Catalyst systems have been developed that deliver the cyanation products of a wide range of aldehyde substrates with extremely high ee values. In cases in which the enantiomeric excess of the product is the primary consideration, the procedures of Choi and co-workers (Section 2.2.4), Uang and co-workers (Section 2.2.5), Shibasaki and co-workers (Sections 2.6 and 2.7), and Nájera, Saá, and co-workers (Section 2.6) are the pinnacle of what has been achieved to date. These procedures tend to require extended reaction times at low temperatures, and in some cases multiple additions of reagents are required.

New sources of cyanide are now being investigated. As discussed above, both the classical cyanide sources, TMSCN and HCN, have considerable problems associated with their use. Notably, the first Lewis acid catalyzed asymmetric cyanohydrin synthesis used TMSCN, and it is only in the last 3 years that viable alternatives have been reported. The use of KCN (Sections 2.2.5 and 2.3), cyanoformates (Sections 2.6, 2.7, and 3.0), and acetone cyanohydrin (Section 2.10) clearly demonstrate that alternate sources are possible.

Now that it has been demonstrated that excellent yields and enantioselectivities can be obtained for catalytic asymmetric cyanation reactions, attention must be paid to the development of user-friendlier experimental protocols suitable for use outside academic and classical medicinal chemistry laboratories. Reaction temperature, times, potential for modifications in scale, and ease of experimental protocol are all areas that undoubtedly will see considerable research effort.

4.2. Synthesis of Cyanohydrins from Ketones

The historically challenging area of ketone cyanation is being actively addressed. Procedures for the cyanation of even problematic dialkyl ketones have been reported, and recently reported methods provide conditions for the preparation of cyanohydrins from cyclic, aliphatic, and hindered ketones with excellent enantioselectivity.

From initial conditions requiring high pressures, systems have been developed that give excellent enantioselectivities at ambient pressure. The titanium system of Shibasaki and co-workers, the lanthanide systems of Shibasaki, Curran, and co-workers, the nucleophilic catalysts of Deng and co-workers, and the aluminum system of Snapper, Hoveyda, and co-workers are all methods of choice. The ambient-temperature system of Belokon, North, and co-workers gives *ee* values of up to 72% is an alternative only if enantioenrichment of the product is acceptable.

As is the case for the addition of cyanides to aldehydes, alternate cyanide sources are now being investigated for use with ketones. The work of Deng and co-workers with ethyl cyanoformate demonstrates that such sources may be realistically identified.

Despite the numerous advances made in chemically catalyzed asymmetric cyanohydrin syntheses, a number of key challenges still remain. These include the identification of ambient-temperature systems that give high *ee* values for electron-deficient and aliphatic aldehydes, the time-economic preparation of ketone cyanohydrins with high *ee* values at room temperature, and the identification of user-friendlier reaction conditions and reagents. The recent applications of nonclassical cyanide sources, the discovery of ambient-temperature catalyst systems, and advances in both bifunctional and nucleophilic catalysis seem to offer possible ways forward towards these goals.

Alternate approaches to cyanohydrins are also being investigated. Asymmetric cyanation may not be the only viable method for the preparation of chiral cyanohydrins.

Indeed, results for the α -hydroxylation of nitriles with a variety of oxidizing agents suggests an alternate route that entirely avoids the problematic use of TMSCN or HCN.

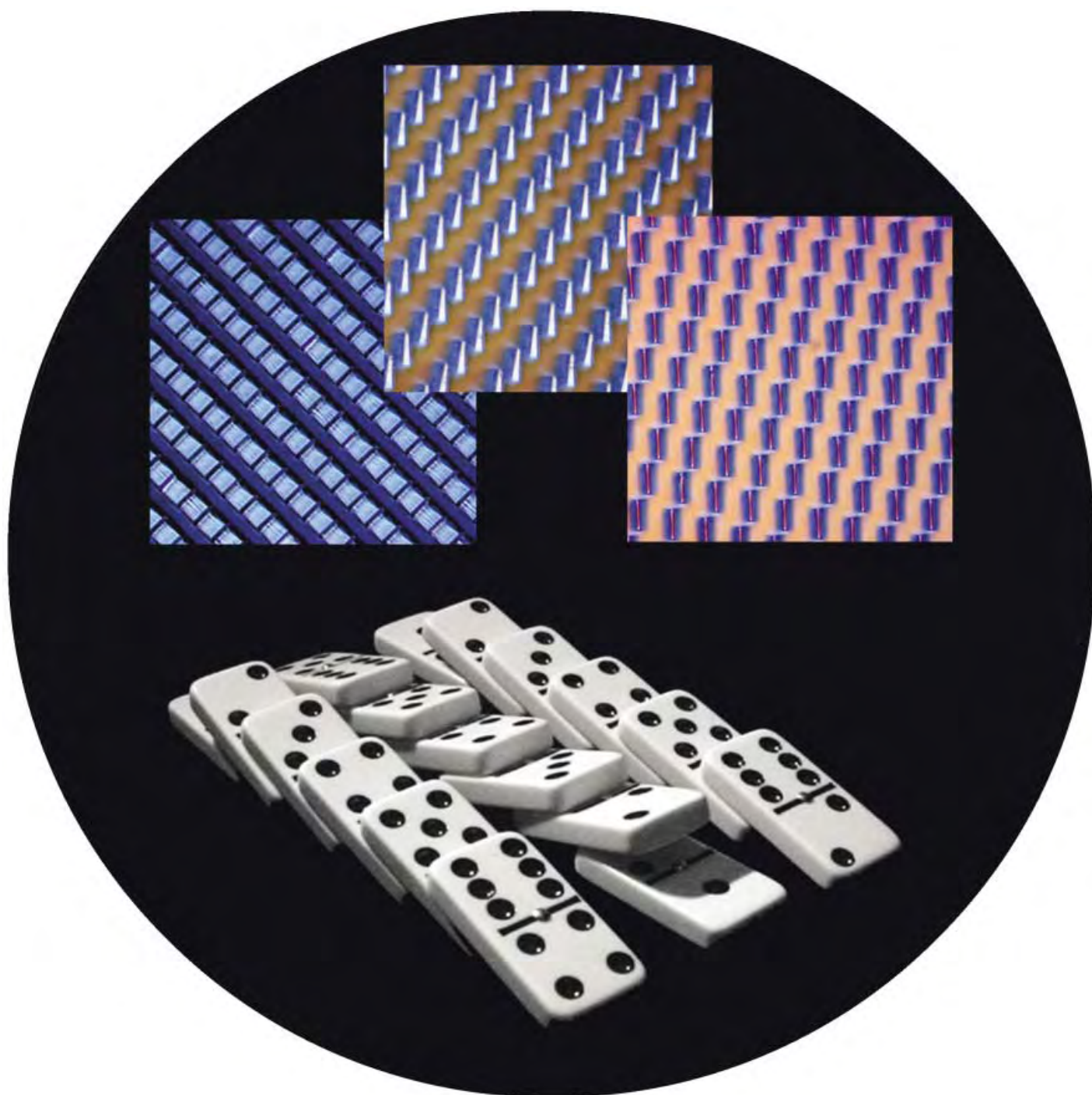
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Communications



Tipping large arrays of micrometer-scale dominos onto their side generates complex 3D topographies and arrays of nanoscale electrodes. These microdominos collapse in parallel when a shearing force is applied uniformly to an array of free-standing pillars. For more information, see the Communication by G. M. Whitesides and co-workers on the following pages.

Shear Patterning of Microdominos: A New Class of Procedures for Making Micro- and Nanostructures**

Byron D. Gates, Qiaobing Xu, Venkat R. Thalladi, Tingbing Cao, Tanya Knickerbocker, and George M. Whitesides*

This paper describes the preparation of fields of micrometer-scale pillars (“microdominos”), and the tipping of these pillars onto their sides by shearing. Previously, the repositioning of micrometer-scale features has focused on the use of surface tension forces to bring features out-of-plane (e.g., mirrors in microelectromechanical systems, or MEMS),^[1] or to align in-plane features.^[2] Figure 1 illustrates our approach schematically, and gives a representative result showing the uniformity of the process. This process provides a route to repetitive 3D microstructures that would be difficult or impossible to prepare by conventional micro/nanolithography. Since the top surfaces of these pillars can be coated with nanometer-thick films by evaporation, tipping also provides a method of generating regular arrays of features with nanometer-scale lateral dimensions in-plane. Although the use of thin-film deposition techniques to generate features with functional, nanoscale edges is well established,^[3] this method provides a simple route to arrays of nanoscale edges that can be addressed electrically.

We fabricated arrays of microdominos on a silicon support by photolithography by using SU-8 photoresist.^[4] This silicon substrate was positioned on a 2 mm thick glass substrate by using a poly(dimethyl siloxane) (PDMS) slab as an adhesion layer. We mounted these substrates on a horizontal translation stage equipped with a stepper that had 1 μm resolution. A second PDMS slab was placed on another glass substrate positioned parallel to, and above, the silicon support. Each PDMS block was ≈ 3 mm thick. We moved the top PDMS slab into contact with the microdominos by using a vertical translation stage that also had 1 μm resolution. The transparency of the top substrates allowed us to use optical microscopy to observe the contact between the PDMS slab and the microdominos. Horizontal translation of the bottom substrate created a shear force between the array of microdominos and the upper elastomeric block. The applied shear induced cohesive failure of the SU-8 at the base of each

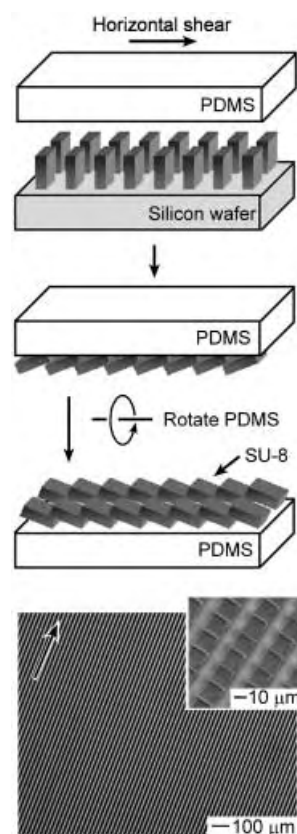


Figure 1. Schematic of the procedure used to reorient microdominos. The sheared polymer structures transferred to the PDMS surface when the PDMS block was separated from the silicon wafer. The optical microscopy image shows overlapping 20 μm tall rectangles of SU-8 supported on PDMS. We fabricated this topography by shearing an array of rectangles (with a 5 $\mu\text{m} \times 15 \mu\text{m}$ cross section) in the direction indicated by the arrow—perpendicular to the 15 μm edge. The inset shows a scanning electron microscopy (SEM) image of these overlapping microdominos.

polymer structure, and each microdomino in the area was tipped onto one of its sides. When we separated the two substrates, the fractured microdominos transferred from the silicon wafer to the top PDMS slab. The pattern of the collapsed structures was determined by the geometry of the microdominos and the direction of the horizontal component of the shear.

To be able to tip the dominos reproducibly, they must fracture from the substrate under shear. Slight modifications were made to the manufacturer's recommended conditions for photolithography by using SU-8 when fabricating the microdominos: we used a shorter (3 min) pre-exposure bake at 95°C than that recommended, followed by a lower integrated intensity (150 mJcm^{-2}) UV exposure.^[5] These altered parameters decreased the cross-linking density in the microdominos; when sheared, the resulting polymer posts fractured at the interface with the silicon substrate.

By shearing the microdominos with a PDMS slab, we achieved uniform pattern generation and transfer. In our hands, other materials (e.g., poly(methyl methacrylate) (PMMA) and polyvinyl chloride) when coated onto a silicon support or a PDMS slab, generated irregular patterns of

[*] Dr. B. D. Gates, Q. Xu, Dr. V. R. Thalladi, Dr. T. Cao, T. Knickerbocker, Prof. G. M. Whitesides
Department of Chemistry and Chemical Biology
Harvard University
Cambridge, MA 02138 (USA)
Fax: (+1) 617-495-9857
E-mail: gwhitesides@gmwgroup.harvard.edu

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collapsed microdominos on shearing; many of these structures remained on the silicon wafer. PDMS has two important roles in obtaining uniform patterns of sheared microdominos: 1) this polymer adheres to the SU-8 microstructures non-covalently; and 2) the elastomeric properties of PDMS permit it to deform upon contact with the polymer posts, thus absorbing some of the force upon contact.

This procedure will generate uniform patterns of tipped microdominos covering up to about 1 cm².^[6] A uniform applied force is important to obtain homogeneous patterns over this large area. To address this concern, we cured the PDMS substrates on a flat surface and used a level to align the substrates prior to applying the shear. Uniform collapse of the microdominos can be prevented by dust particles on either substrate; conveniently, the elastomeric slab conforms to surface contaminants and minimizes the effect of these abnormal topographies.^[6] To limit surface contamination from airborne particles, we cured the PDMS against a flat substrate without exposing this PDMS surface until it was used for shearing the microdominos.

Figure 2 shows some of the topographies that can be formed by shearing rectangular, triangular, and circular posts. Figures 2 A and B show a ribbed structure that was created by shearing 20 μm tall rectangles in a direction perpendicular to their longest lateral edge. The height and spacing between the microdominos determines the amount of overlap between the neighboring structures. We patterned different topographies by applying a shear to the rectangles at various angles (Figures 2 C–E). The rectangles in Figures 2 C and D were tipped onto the narrowest edge (5 μm wide). In Figures 2 E and F the structures were also tipped onto the narrowest edge; this arrangement was, however, unstable and these microdominos subsequently collapsed onto their widest (15 μm) face.^[6] Collapse in this second direction, perpendicular to the applied horizontal shear, is difficult to control as the intermediate stage (microdominos tipped onto the 5 μm edge) is unstable towards the slightest horizontal translation. Figure 2 G–J show microdominos of different shapes tipped onto one side. The sheared triangles in Figures 2 G and H and the cylindrical rods in Figures 2 I and J each provide a topography that is impossible to obtain by conventional photolithography.^[7]

Electrets are dielectric materials with the ability to store an electric field—either charge or polarization induced.^[8] We have previously patterned charge in electrets by using electrodes with dimensions down to about 150 nm, patterned by conventional lithography.^[9] Here we used an array of narrow electrodes obtained by shearing microdominos to print, in parallel, a pattern of charge into a film of PMMA (Figure 3). A 15 nm metal film, deposited by electron-beam deposition at a 20° angle relative to the plane of the silicon substrate, coated the top and part of one of the 15 μm faces of the original microdominos (Figure 3 B). We deposited Pd onto the microdominos because of the small grain size (15–20 nm) of this metal when deposited by electron-beam evaporation and its resistance to oxidation.^[10] The microdominos were sheared by using a substrate of conducting polyaniline (PANI) grafted onto a PDMS slab, which also served as an electrode.^[6,11] The array of 15 μm tall rectangles,

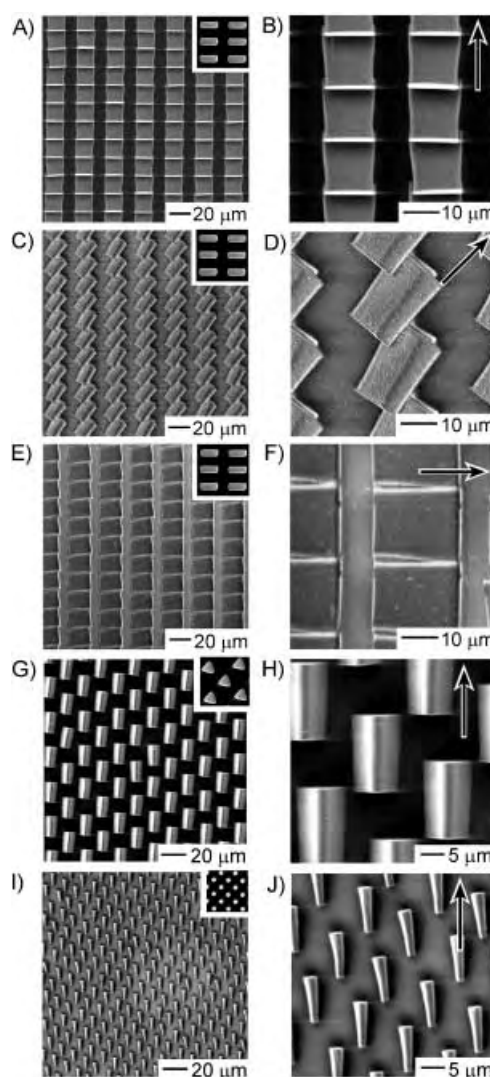


Figure 2. SEM images of rectangles, triangles, and cylinders of SU-8 that were transferred to a PDMS block after applying a shear. The insets show a top view of the original, uncollapsed pillars and the arrows indicate the direction of the shear. (A, B) Stacked microdominos (LxWxH: 15 μm $\times$ 5 μm $\times$ 20 μm) oriented by a horizontal force applied in a direction perpendicular to the 15 μm edge of the rectangles. The structure of the stacked rectangles can be changed by controlling the direction of shear relative to the 15 μm edge: (A, B) 90°; (C, D) 45°; (E, F) 0°. Distinct patterns were obtained from a square lattice of equilateral triangles (5 μm on a side) sheared at about 45° relative to the orientation of the lattice (G, H). Shearing 10 μm tall cylindrical posts reorients the vertical array into a planar array of parallel, approximately cylindrical rods (I, J).

capped with Pd, were tipped onto this conducting polymer surface (Figures 3 C and D) with one of the metal-coated surfaces in contact with the conducting polymer electrode. We imaged the exposed Pd features by SEM after etching the SU-8 with reactive ion etching, thus confirming an edge thickness of about 15 nm.^[6] Figures 3 D and E show the configuration of the metal-capped microdominos, after tipping, for printing charge into PMMA.

Printing charge from the (now electrically connected) array of edges is straightforward. The dimensions and shape

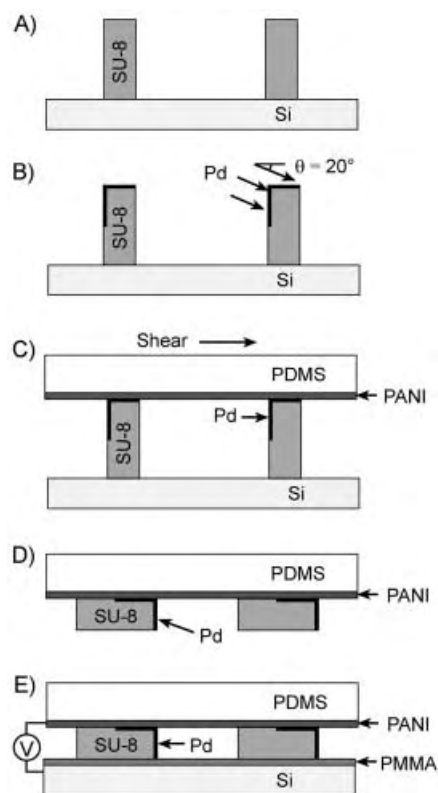


Figure 3. Schematic for preparing arrays electrodes with narrow lateral dimensions by using microdominos as templates. The microdominos of SU-8 (A) defined by photolithography were coated with a Pd film (B) at an angle of 20° relative to the silicon substrate. C) A slab of PDMS with PANI grafted onto the surface was used to apply a horizontal shear to the microdominos. D) The collapsed microdominos transferred to the PANI/PDMS substrate, and were brought into contact with a PMMA film supported on a silicon wafer. We used the Pd films as electrodes with narrow lateral dimensions (E) by applying a potential between the PANI and the silicon substrate.

of the microdominos determined the pattern. Figures 4 A and B show SEM images of an array of Pd capped $15\ \mu\text{m}$ tall rectangles transferred onto the polyaniline surface. After bringing a thin film of PMMA into contact with this array of electrodes, we applied a voltage of $+50\ \text{V}$ for 30 s (current density of ca. $20\ \text{nA}\ \text{nm}^{-2}$, or ca. $2 \cdot 10^6$ electrons per Pd atom) between the conducting polymer and the silicon wafer (Figure 3 E). We separated the patterned electrode from the PMMA, and imaged the surface potential of the PMMA by Kelvin probe force microscopy (KFM). Figures 4 C and D show the topography of the PMMA surface (ca. $0.5\ \text{nm}$ rms roughness), and an array of $600\ \text{nm}$ wide regions of charge. These lines of charge show a peak surface potential of about $400\ \text{mV}$.

In summary, we have demonstrated that a vertical structure defined with photolithography can be easily repositioned onto one side face by shear. The procedure requires tuning the conditions of conventional photolithography to acquire an array of polymer posts that can easily collapse upon applying the shear. This patterning technique has been applied to, but is not limited to, arrays of rectangular, triangular, and cylindrical posts. The reorientation of micro-

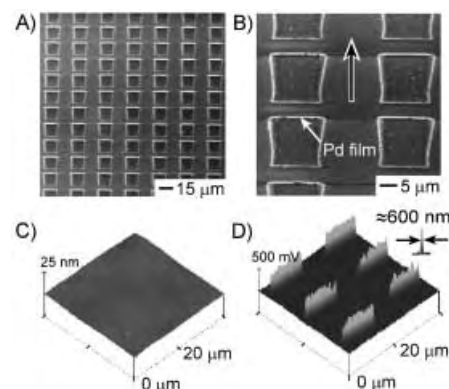


Figure 4. Printing charged regions in electrets from arrays of nanometer scale conducting features: (A, B) SEM images of $15\ \mu\text{m}$ tall rectangles capped with a $15\ \text{nm}$ Pd film and sheared onto a polyaniline coated PDMS substrate. The arrow indicates the direction of the applied shear. (C, D) Topography ($25\ \text{nm}$ vertical scale) and surface potential ($500\ \text{mV}$ vertical scale) of the $100\ \text{nm}$ thick PMMA surface after patterning regions of charge (ca. $600\ \text{nm}$ wide, ca. $400\ \text{mV}$ peak surface potential). Each 3D plot is shown at a 45° angle for clarity. The inset in (D) shows the side profile for one region of charge.

dominos offers routes to 3D micrometer-scale structures that are now difficult or impossible to prepare. This tunable fabrication approach to 3D topographies could find several applications in microfabrication and materials science. For example, simple arrays of Pd structures with narrow lateral dimensions were prepared by tipping over Pd capped micrometer-scale structures, which could be electrically addressed to pattern charge in electrets.

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Nanostructures

Well-Defined Carbon Nanoparticles Prepared from Water-Soluble Shell Cross-linked Micelles that Contain Polyacrylonitrile Cores**Chuanbing Tang, Kai Qi, Karen L. Wooley,
Krzysztof Matyjaszewski, and Tomasz Kowalewski*

Carbon-based materials have traditionally played an important role in modern technologies, ranging from the manufacture of everyday-use products such as automobile tires (carbon fillers), through reinforcements in composites (carbon fibers), electrode materials for a variety of processes, to activated carbons widely used in separation techniques, to name just a few. In addition to those commodity materials, in recent decades there has been a dynamic growth in the area of advanced engineering carbon materials. Seminal events in this area include the development of synthetic diamond and highly oriented pyrolytic graphite in 1960.^[1,2] In the last two decades, this field has been particularly reenergized by the discovery of fullerenes in 1985^[3] and carbon nanotubes in 1991.^[4] In addition to generating tremendous fundamental interest, these nanostructured forms of carbon have found, or are expected to find, numerous applications such as advanced fillers,^[5,6] materials for energy and gas storage,^[7,8] templates,^[9] nanoprobe and sensors,^[10] and elements for molecular electronics devices.^[10,11] Two main groups of strategies for

the preparation of engineering carbon materials include:^[12,13] 1) pyrolysis of organic precursors (mostly polymeric) under inert atmosphere to yield large-scale engineering carbon materials and 2) physical/chemical vapor deposition techniques to produce well-defined nanostructured forms of carbon. Whereas techniques from the first group are applicable to large-scale production, they offer very limited control of the carbon (nano)structure. Techniques from the second group, while allowing for atomic-scale precision in nanostructure control, are relatively expensive, have limited yield, and require complex equipment.

Recently, we developed a novel, low-cost route to well-defined nanostructured carbon materials based on the pyrolysis of block copolymer precursors that contain polyacrylonitrile (PAN) and a sacrificial block (e.g., poly(*n*-butyl acrylate)).^[14,15] By this method, the carbon nanostructure is templated after the nanostructure of the PAN domains, which are formed through self-assembly and driven by phase separation between PAN and the sacrificial block. Upon pyrolysis, PAN domains are converted into increasingly graphitic carbon, whereas the sacrificial phase is volatilized. The prerequisite for this approach is the ability of the PAN domains to retain their nanostructure upon thermal treatment. The necessary stabilization is achieved through thermal treatment (heating in the presence of air to 230 °C), a process well-known in the field of carbon fibers,^[16] which causes the PAN precursor to form cyclic, ladder, and eventually cross-linked species. Nanostructured carbon materials derived through this novel route hold considerable promise in many areas such as: field emitters, supercapacitors, and photovoltaic cells. One appealing advantage of this approach is the possibility of combining it with currently used device-fabrication techniques that rely on thin-film processing and lithography. One of the potential issues here, however, is the processability of the precursors: PAN and its block copolymers are soluble in a narrow range of solvents such as: *N,N*-dimethylformamide (DMF), ethylene carbonate (EC), dimethylsulfoxide and *N*-methylpyrrolidone (NMP).

As one of the possible ways of addressing this challenge, herein we present an alternative approach, which now relies on the use of covalently stabilized micellar precursors that are soluble in aqueous systems. These precursors belong to a class of shell cross-linked nanoparticles (shell cross-linked knedels, SCKs), formed by self-assembly and covalent stabilization of amphiphilic block copolymers.^[17–22] PAN block constitutes the core of the SCKs and the sacrificial block (e.g., poly(acrylic acid), PAA) forms a water-soluble shell, and some of the carboxylic acid groups from the PAA block within the shell layer are covalently cross-linked following the micellization process, which occurs in a mixture of DMF and water.

The use of nanostructured amphiphilic block copolymers as a template to form inorganic nanostructures has numerous precedents.^[23–26] The use of SCKs rather than simple micelles in this study is motivated by their colloidal stability and their ability to retain their nanostructure upon processing into thin and ultrathin films,^[18,23] and, as demonstrated below, upon subsequent thermal treatment. Processability into thin films is of particular importance when nanostructures are to be patterned or organized into ordered arrays.

[*] C. Tang, Prof. K. Matyjaszewski, Prof. T. Kowalewski
Department of Chemistry
Carnegie Mellon University
4400 Fifth Avenue, Pittsburgh, PA 15213 (USA)
Fax: (+1) 412-268-6897
E-mail: tomek@andrew.cmu.edu

K. Qi, Prof. K. L. Wooley
Department of Chemistry
Washington University
Missouri 63130 (USA)

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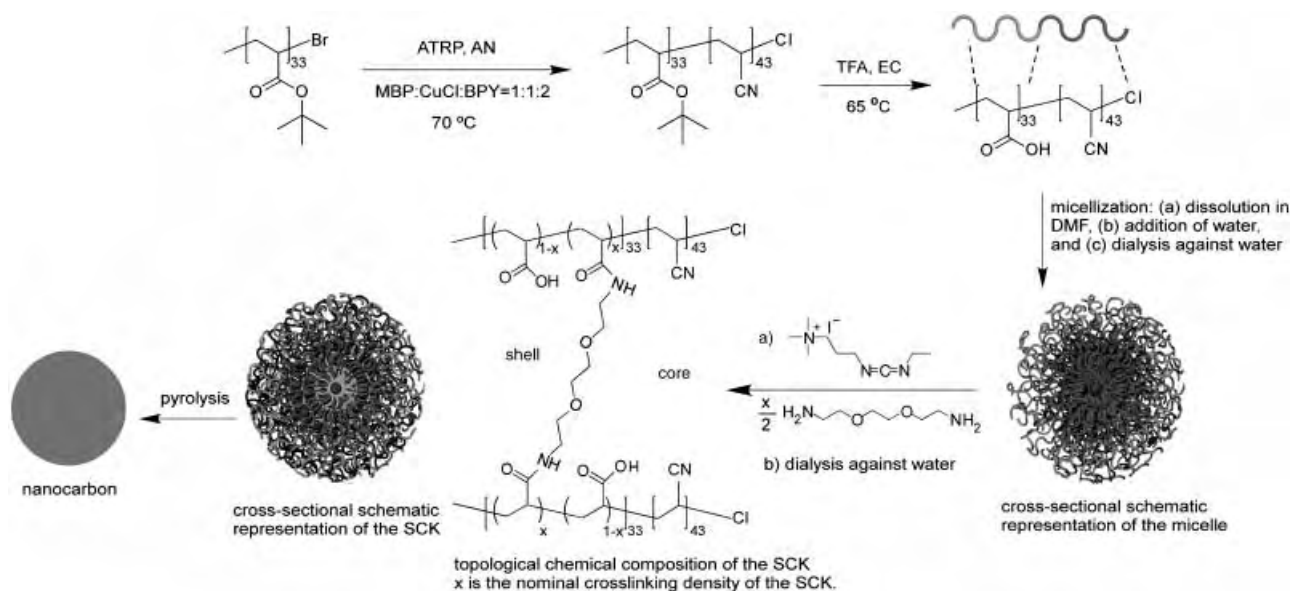
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The spherical SCKs used throughout this study were formed from poly(acrylic acid)-*b*-polyacrylonitrile (PAA-*b*-PAN) copolymers, which in turn, were obtained by hydrolysis of poly(*tert*-butyl acrylate)-*b*-polyacrylonitrile (PtBA-*b*-PAN) (Scheme 1). Well-defined block copolymers with polydispersity indexes (PDI) < 1.2 were prepared by atom-transfer radical polymerization (ATRP),^[27,28] which is one of the most robust controlled/living radical polymerizations, thus providing polymers with narrow molecular-weight distributions, controlled molar masses, complex architectures and functionalities.^[28–33] A bromine-terminated poly(*tert*-butyl acrylate) (PtBA-Br) macroinitiator was synthesized and then chain extended with acrylonitrile by halogen exchange^[34,35] to increase the relative rate of initiation versus propagation (for experimental conditions see Supporting Information). With the same starting PtBA-Br, different block lengths of PAN or the relative ratios of PAN and PtBA were achieved by controlling the conversion of acrylonitrile (AN) during the reactions. In this study, we focused on one well-defined block copolymer: P(*t*BA)₃₃-*b*-P(AN)₄₃. The hydrolysis of PtBA-*b*-PAN was performed at 65 °C in ethylene carbonate by reactions with anhydrous trifluoroacetic acid (TFA), which was confirmed by ¹H NMR spectroscopy to give high conversion of *t*BA into acrylic acid. Micellization of PAA-*b*-PAN was carried out by dissolving the block copolymer in DMF and then adding water at a controlled rate (15 mL h⁻¹) to induce micelle formation. Low final concentrations, about 0.25 mg mL⁻¹, were used, to prevent intermicellar crosslinking at the later step. Following the removal of DMF by dialysis (cellulose membrane dialysis tubing, MWCO 3500 Da) against water and activation with a water-soluble carbodiimide (1-[3'-(dimethylamino)propyl]-3-ethylcarbodiimide methiodide), the acrylic acid groups present within the shell layer of the micelles were then cross-linked with a diamine,

2,2'-(ethylenedioxy)-bis(ethylamine), to yield covalently stabilized SCK nanoparticles. Results of our previous work with SCKs that contain PAA shells indicate that partial shell crosslinking renders charged nanoparticles with ionizable acrylic acid groups still present in the shell.^[19] Electrostatic repulsion between such SCKs has been shown to favor formation of sparse ordered arrays, it may also aid in preventing the aggregation of colloidal suspensions. Thus, this study has focused on SCKs with 50 % shell-crosslinking density, based on the stoichiometry of the amine functional groups of the diamino crosslinker to the carboxylic acids from PAA-*b*-PAN block copolymers present in the shell.

Thin and ultrathin films of the SCK carbon precursors were prepared by drop casting aqueous SCK solutions onto freshly cleaved mica then the samples were allowed to dry in air. The dried samples were then subjected to 2 h annealing at 230 °C in contact with air to stabilize the PAN domains, and subsequently pyrolyzed by heating at 20 °C min⁻¹ from room temperature to 600 °C under flowing nitrogen gas. Because of the limited thermal stability of mica, samples to be subjected to pyrolysis at 900 °C were deposited on clean silicon wafers.

The morphology and size distributions of the SCK precursors and carbon nanoparticles obtained after pyrolysis were characterized with the aid of tapping-mode AFM (see Supporting Information). The use of solutions with concentrations ranging from 0.1 to 0.025 mg mL⁻¹ led to the deposition of isolated SCKs on the surface (Figure 1a–d), thus facilitating size analysis of individual objects. Height distributions of the particles obtained with the aid of custom-developed AFM image analysis software are shown in Figure 1e–h. The widths of the distributions were characterized through the ratio of weight-average and number-average heights $\bar{H}_w/\bar{H}_n$. As deposited, the SCKs samples exhibited a very narrow distribution of heights ($\bar{H}_w/\bar{H}_n=1.01$) with a



Scheme 1. Illustration of the five-step synthesis of carbon nanoparticles: 1) formation of a diblock copolymer PtBA-*b*-PAN by ATRP; 2) formation of an amphiphilic block copolymer PAA-*b*-PAN; 3) formation of polymer micelles through self-assembly; 4) formation of SCKs after cross-linking of the shell layer of polymer micelles; 5) formation of nanostructured carbon particles through pyrolysis. MBP = methyl 2-bromopropionate, BPY = 2, 2'-bipyridine, TFA = trifluoroacetic acid, AN = acrylonitrile.

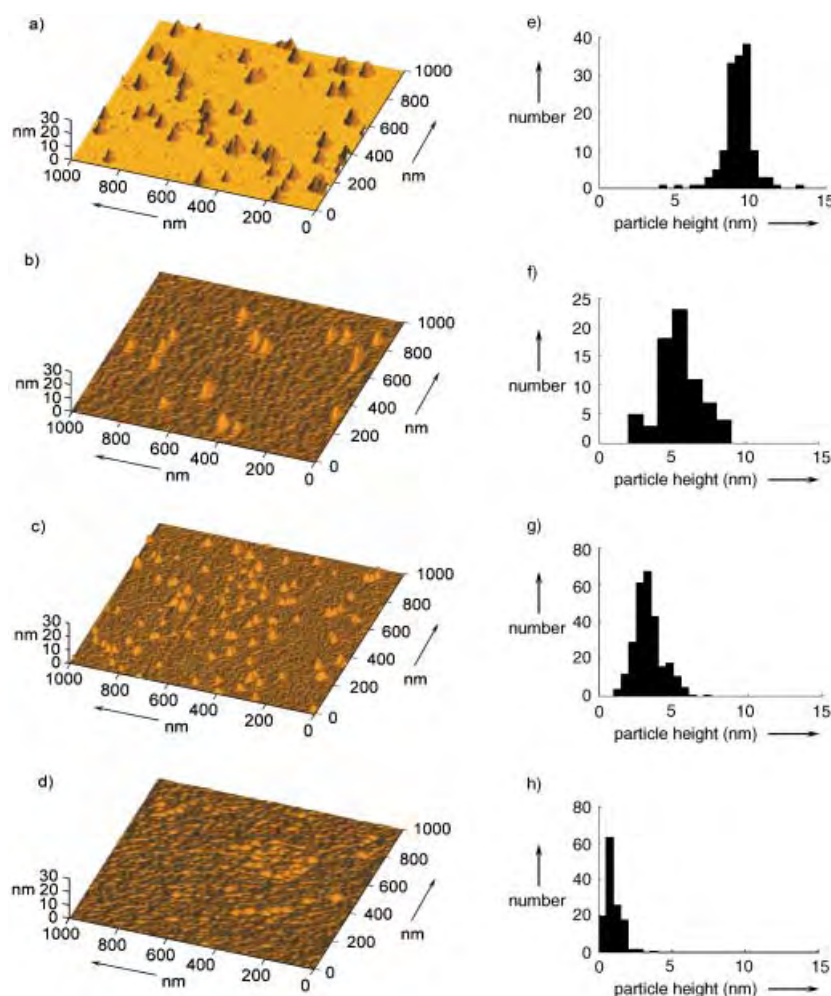


Figure 1. a)–d) Tapping mode AFM images of nanoparticles: a) SCKs on mica; b) nanoparticles stabilized on mica in air at 230 °C for 2 h and c) after heating under nitrogen at 20 deg min⁻¹ from room temperature to 600 °C; d) after pyrolysis of thermally stabilized SCKs on a silicon wafer, obtained by heating under nitrogen at 20 °C min⁻¹ from 230 °C to 900 °C. e)–h) Height distributions of SCKs and carbon nanoparticles determined from AFM images: e) SCKs; f) carbon nanoparticles stabilized at 230 °C; g) carbon nanoparticles pyrolyzed at 600 °C and h) carbon nanoparticles pyrolyzed at 900 °C

number-average height $\bar{H}_n = 9.0 \pm 0.5$ nm. In comparison, the diameter of these SCKs in solution determined by dynamic light scattering (DLS) was equal to 34 ± 2 nm. This difference indicated that upon deposition on mica, the SCK nanoparticles became flatter, as described earlier for partially cross-linked SCKs.^[36,37] Upon thermal stabilization and pyrolysis, the average heights of the nanoparticles decreased with an increase of processing temperature, as expected for mass loss due to volatilization of the sacrificial shell block and carbonization of PAN.

The progress of PAN carbonization was monitored with the aid of FTIR spectroscopy (Figure 2) and Raman scattering spectroscopy (Figure 3). To facilitate sufficient IR-absorption and Raman scattering intensity, multilayer SCK films deposited on silicon wafers, rather than sub(monolayers), were used in this part of the study (for surface morphology of such films after pyrolysis at 900 °C see Supporting Information). The FTIR spectra taken prior to

thermal treatment showed a characteristic strong absorption band at approximately 2240 cm⁻¹, which corresponds to CN groups of PAN. The spectra of samples pyrolyzed at 600 °C and 900 °C show broad and strong absorption bands between 1600 and 1300 cm⁻¹, indicating the formation of C=C and C=N bonds derived from the cyclization and cross-linking reactions involving CN groups. At the same time, the intensity of the absorption bands due to CN groups diminished with the increase in pyrolysis temperature and finally disappeared after treatment at 900 °C. These observations are consistent with reported data on PAN pyrolysis, according to which, heating in air at 230 °C (thermal stabilization stage) leads predominantly to cyclization, whereas subsequent pyrolysis under an inert atmosphere is dominated by progressive dehydrogenation and denitrogenation of PAN.^[16] The observed red shift of C=C and C=N absorptions upon increase of pyrolysis temperature is consistent with an increase in conjugation.

Graphitization of carbon upon pyrolysis of organic precursors can be conveniently studied with the aid of Raman scattering, by monitoring the characteristic set of bands between 1200 and 1700 cm⁻¹. The peak around 1600 cm⁻¹ indicates the presence of graphitic species that corresponds to the Raman-allowed E_{2g} mode, called the graphite mode (G-band).^[38,39] The presence of disordered species is indicated by the presence of a defect-induced Raman band called the defect A_{1g} mode (D-band), centered on 1350 cm⁻¹.^[40] Typically, the progress of graphitization upon pyrolysis is followed by comparing the growth and sharpening of the G-band and diminution of the D-band. A similar trend has been observed in this study, although the G-band was not

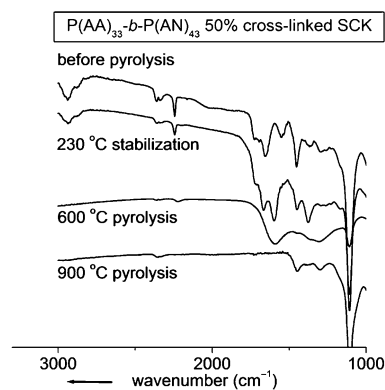


Figure 2. FTIR spectra of multilayer films of nanoparticles on a silicon wafer (transmittance in arbitrary units).

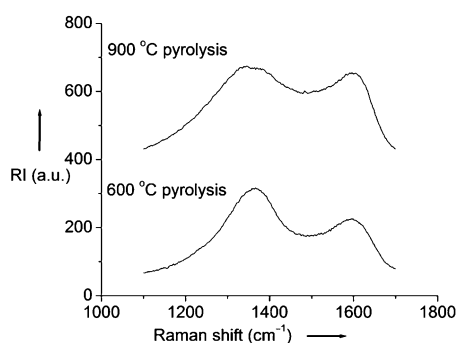


Figure 3. Raman spectra of carbon nanoparticles pyrolyzed at 600 °C and 900 °C on a silicon wafer (RI = Raman Intensity).

nearly as sharp and distinct as those typically reported for PAN-derived carbon fibers^[41,42] or even as in nanostructured carbons derived from “bulk” block copolymers that contain PAN, as described in our earlier work.^[14] The less-ordered nature of the graphitic species formed upon pyrolysis of SCKs can be explained by very high surface-to-volume ratio of this system, which leads inevitably to a more frequent occurrence of structural defects.

In conclusion, well-defined carbon nanoparticles were developed through pyrolysis of shell cross-linked PAA-*b*-PAN nanoparticles prepared by micellization and cross-linking of amphiphilic block copolymers in aqueous systems. These low-dimensional nanostructures were characterized by AFM and DLS. FTIR and Raman spectroscopy confirmed their partially graphitic nature. Nanostructured carbon materials prepared according to this route are expected to find many applications, such as antistatic coatings, materials for field emitters, specialized electrodes, and sensors. One of the particularly important advantages of this route is the use of covalently stabilized water-soluble precursors, thus facilitating environmentally friendly processing conditions. In addition, manipulation of solvent systems and copolymer composition may open the way to extend this approach to other low-dimensional nanostructures similar to those observed in other block copolymer systems.^[43,44] Some of these extensions envisioned include: the preparation of carbon nanorods from shell-crosslinked cylindrical micelles; hollow carbon nanospheres made from shell cross-linked polyacrylonitrile-*b*-poly(acrylic acid) grafted from silica nanoparticles; hollow carbon nanotubules derived from shell-crosslinked densely-grafted single polymer brushes with polyacrylonitrile inner layer and poly(acrylic acid) outer layer.

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Keggin Ions

$[\text{SiNb}_{12}\text{O}_{40}]^{16-}$ and $[\text{GeNb}_{12}\text{O}_{40}]^{16-}$: Highly Charged Keggin Ions with Sticky Surfaces**

May Nyman,* François Bonhomme, Todd M. Alam,
John B. Parise, and Gavin M. B. Vaughan

Heteropolyanions are negatively charged clusters of corner-sharing and edge-sharing early transition-metal MO_6 octahedra and heteroatom XO_4 tetrahedra, in which the tetrahedra are usually located in the interior of the cluster.^[1] The geometry, composition, and charge of these clusters are varied through synthesis parameters, and cluster properties are highly tunable as a function of these characteristics. Heteropolyanions have been employed in a range of applications that include virus-binding inorganic drugs,^[2] homogeneous

and heterogeneous catalysts,^[3,4] electro-optic and electrochromic materials,^[5,6] metal and protein binding,^[7] and as building blocks for nanostructuring of materials.^[8] The α -Keggin geometry, which was first structurally characterized in 1933 by J.F. Keggin^[9] for the phosphotungstic acid ($\text{H}_3\text{PW}_{12}\text{O}_{40}$) is one of the most widely recognized and thoroughly studied heteropolyanion geometries.^[10] Following Keggin's initial discovery, solid-state and solution chemistries of the heteropolytungstates and heteropolymolybdates (i.e., $\text{H}_4\text{SiMo}_{12}\text{O}_{40}$) with the Keggin geometry have been well developed. These Keggin ions have been synthesized with a variety of atoms in the XO_4 tetrahedral site, numerous addenda atom substitutions in the MO_6 octahedral sites, as acids, and as salt compounds with a range of counter cations.^[11,12] Given the apparent ubiquity of the Keggin geometry, it is not surprising that the first-observed heteropolyniobate (reported Nyman et al. in 2002^[13]) was a Keggin ion. However, we first identified^[13] the $[\text{SiNb}_{12}\text{O}_{40}]^{16-}$ α -Keggin ion in the solid state as a 1D inorganic polymer that features alternating Nb-Keggin ions and $[\text{Ti}_2\text{O}_2]^{4+}$ bridges, rather than isolated clusters. Presented here is the first synthesis and structural characterization of the dodecaniobate Keggin ion in the form of a water-soluble salt that contains isolated clusters. Solution and solid-state multinuclear NMR spectroscopies were used to better characterize the relationship between the solid-state structure and solution behavior of these highly charged species. To our knowledge, the $[\text{TNb}_{12}\text{O}_{40}]^{16-}$ ($\text{T} = \text{Si}, \text{Ge}$) Keggin ions reported herein have the highest negative charge observed for clusters that have the plenary Keggin geometry; and also higher charge than the typical mono-, bi- and trivacant lacunary Keggin ions. The unprecedented high charge should render these clusters unique with regard to metal binding and other applications that involve anion-cation electrostatic interactions in solution or at interfaces.

These $[\text{TNb}_{12}\text{O}_{40}]^{16-}$ clusters ($\text{T} = \text{Si}, \text{Ge}$): $\text{Na}_{16}[\text{SiNb}_{12}\text{O}_{40}] \cdot 4\text{H}_2\text{O}$ **1**; $\text{Na}_{16}[\text{GeNb}_{12}\text{O}_{40}] \cdot 4\text{H}_2\text{O}$ **2** are chemically quite different from the related heteropolymolybdate and heteropolytungstate Keggin ions in their synthetic approach, their pH stability, and their charge. The Nb Keggin ions are synthesized and stable in basic solutions (pH 7–12.5) and decompose in acidic solutions, whereas the Mo and W Keggin ions are synthesized and stable in acidic solutions (pH $\approx$ 1–3) and decompose in more basic solutions. The Nb Keggin ions are synthesized hydrothermally at 190 °C from amorphous, hydrous niobium oxide and alkoxides of germanium or silicon, whereas the Mo and W Keggin ions are generally precipitated at room temperature from tungstic or molybdic acid or alkali precursors. Use of sodium silicate or sodium germanate as precursors rather than the alkoxides results in mixtures that contain the heteropolyniobate only as a minor phase, with poorly crystalline sodium niobates^[14] as the major phase. The general differences between synthesis of heteropolyniobates and synthesis of heteropolymolybdates and heteropolytungstates have been discussed in detail previously.^[13] The hydrothermal formation of $\text{Na}_{16}[\text{TNb}_{12}\text{O}_{40}] \cdot 4\text{H}_2\text{O}$ ($\text{T} = \text{Si}, \text{Ge}$) is quite sensitive to processing parameters. Time-dependent investigations of the synthetic pathway of the Nb Keggin ion revealed that after 1 hour of

[*] Dr. M. Nyman, Dr. F. Bonhomme
Sandia National Laboratories
Department of Geochemistry
Albuquerque, NM 87185 (USA)
Fax: (+1) 505-844-7354
E-mail: mdnyman@sandia.gov

Dr. T. M. Alam
Sandia National Laboratories
Department of Organic Materials
Albuquerque, NM 87185 (USA)
Prof. Dr. J. B. Parise
Center for Environmental Molecular Sciences
Department of Geosciences
Chemistry Department
State University of New York
Stony Brook, NY 11794–2100 (USA)

Dr. G. M. B. Vaughan
Materials Science, European Synchrotron Radiation Facility, BP 220,
38043 Grenoble CEDEX (France)

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hydrothermal treatment at 180 °C, the Lindqvist ion, $\text{Na}_7(\text{H}_3\text{O})^+[\text{Nb}_6\text{O}_{19}]\cdot 14\text{H}_2\text{O}$,^[15] is precipitated. After 2 h, a clear solution is observed with all solids dissolved, and 3 h results in the Nb Keggin ion salt as the only crystalline phase. Yet, if the Lindqvist ion is used as the niobium precursor (with a 1:1 molar replacement of Nb), 1D Nb Keggin chain materials (unpublished results) are the major products of hydrothermal processing. However, if either NaOH or NaCl is added to this Lindqvist ion reaction, the product is again the isolated Nb Keggin clusters, which suggests the common-ion effect plays a role in these syntheses. In the case of the synthesis of the silicon analogue, a slight decrease in NaOH concentration in the precursor solution results in the formation of a different cluster geometry with the composition $\text{Na}_{14}[\text{H}_2\text{Si}_4\text{Nb}_{16}\text{O}_{56}]\cdot 45.5\text{H}_2\text{O}$.^[13]

From the microcrystalline powder of **1**, an approximately cube-shaped crystal with a diameter of 15 μm was selected for single-crystal X-ray diffraction analysis at the European Synchrotron Radiation Facility (ESRF).^[16] The structure of **1** was solved in the cubic space group, $P4_3n$. Rietveld refinement (program Fullprof2K^[17]) of the diffraction patterns of **1** and **2**, based on the results from a single crystal, confirmed that the single crystal is representative of the bulk microcrystalline powders, that no crystalline impurities are present, and that **1** and **2** are isostructural. The X-ray powder-diffraction spectra of **1** and **2**, along with the difference pattern between the observed X-ray powder data for **1** and the diffraction pattern calculated from the single-crystal structure of **1** are shown in Figure 1. The lattice constants of

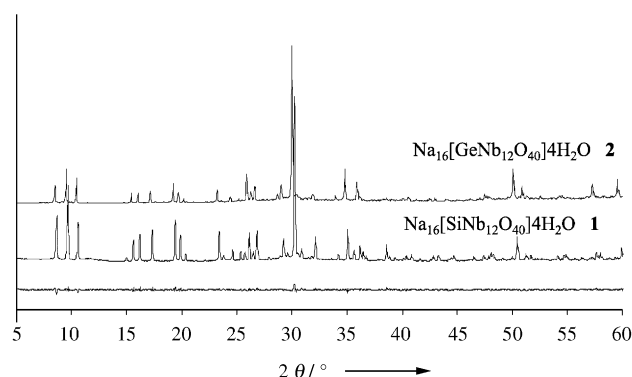


Figure 1. Observed powder X-ray diffraction patterns of **1** and **2**, and difference between the diffraction pattern calculated from single-crystal data and observed X-ray powder data of **1**.

1 and **2** were refined from powder-diffraction data by using the Le Bail method,^[18] with silicon SRM640 from the National Institute of Standards and Technology (NIST) as internal standard: **1** $a = 20.4875(2)$ Å, **2** $a = 20.5349(4)$ Å. The single-crystal data of **1** reveals two crystallographically distinct $[\text{SiNb}_{12}\text{O}_{40}]^{16-}$ α -Keggin ions in the unit cell. Keggin 1 resides at the origin and in the center of the cubic cell and pairs of Keggin 2 are located on the faces of the unit cell, giving a 1:3 ratio of Keggin 1 to Keggin 2. Figure 2 shows the two Keggin ions in a ball-and-stick model. The Nb_1O_6 octahedra of Keggin 1 and the Nb_2O_6 , Nb_3O_6 , and Nb_4O_6

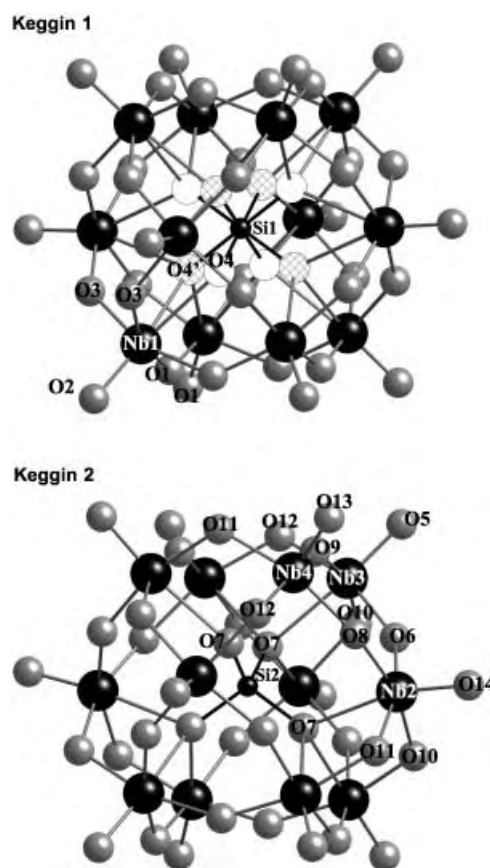


Figure 2. Ball-and-stick representation of the two crystallographically unique Keggin ions of **1**. Bond lengths [Å] of Keggin 1 (top), O4 and O4' are half occupied: Si1–O4 1.62(2), Si1–O4' 1.60(2), Nb1–O2 1.735(9), Nb1–O3a 1.92(1), Nb1–O3 1.94(1), Nb1–O1 1.97(1), Nb1–O4 2.46(1), Nb1–O4' 2.49(1). Bond lengths [Å] of Keggin 2 (bottom): Si2–O7 1.659(7), Nb2–O14 1.778(8), Nb2–O6 1.937(8), Nb2–O8 1.953(7), Nb2–O10 1.995(8), Nb2–O11 2.026(8), Nb2–O7 2.395(7), Nb3–O5 1.765(9), Nb3–O10 1.976(8), Nb3–O9 1.981(8), Nb3–O6 1.991(8), Nb3–O12 2.03(1), Nb3–O7 2.408(7), Nb4–O13 1.765(8), Nb4–O9 1.975(8), Nb4–O12 1.987(9), Nb4–O8 2.003(7), Nb4–O11 1.998(8).

octahedra of Keggin 2 are distorted in the regular fashion of polyoxometalate d^0 metal centers with a long axial Nb–O_c bond to the center of the cluster (2.395(7)–2.49(1) Å), a short axial Nb–O_t terminal bond (ranging from 1.735(9) to 1.778(8) Å) to the outside of the cluster, and four intermediate equatorial Nb–O_e bonds (between 1.92(1) and 2.03(1) Å). Sixteen sodium atoms per cluster were located from the single-crystal data of **1**, which is the necessary amount to balance the charges of the cluster. Bond-valence sum calculations (BVS)^[19] for the oxygen atoms of the clusters ruled out the presence of protons bonded to the Keggin oxygen atoms.

The ratio of Keggin 1 to Keggin 2 in **1** was confirmed by solid-state ^{29}Si MAS NMR spectroscopy. The ^{29}Si MAS NMR spectrum (Figure 3) shows a signal at -74.1 ppm (77 %) with a shoulder at -72.6 ppm (23 %) which is in agreement with a 3:1 ratio of the two Keggin ions. The SiO_4 tetrahedron of Keggin 1 is disordered with Si1 surrounded by eight half-occupied oxygen atoms (O4 and O4' sites). The Si1–O4 bond length is 1.62(2) Å and the Si1–O4' bond length is 1.60(2) Å.

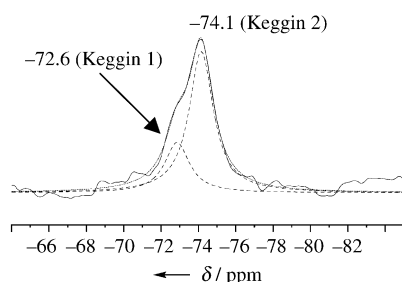


Figure 3. Solid-state ^{29}Si MAS NMR spectrum of $\text{Na}_{16}[\text{SiNb}_{12}\text{O}_{40}]\cdot 4\text{H}_2\text{O}$ **1**. The spectral deconvolution of the overlapping resonances is shown. The two resolvable peaks are assigned to SiO_4 and Si_2O_4 of Keggin 1 and Keggin 2, respectively.

In Keggin 2, Si_2 is tetrahedrally bound to four O_7 atoms, with a bond length of 1.659(7) Å. Therefore these two sites are distinguishable by ^{29}Si NMR based on the slight difference in geometry of the SiO_4 tetrahedra. In solid-state structures, ^{29}Si signals shift downfield with both increasing Si–O bond length^[20] and increasing Si–O–X bond angle.^[21] Both Si–O–Nb bond angle and Si–O bond length are slightly larger for Keggin 2, which confirms the ^{29}Si NMR assignment based on the ratio of the resonances.

The ^{17}O solid-state NMR spectrum of the germanium analogue **2** (Figure 4) shows two distinct environments for oxygen atoms (O_c) bonded to the central tetrahedral germanium, but the solid-state ^{17}O spectrum of the Si-analogue **1** clearly shows a single O_c site. The ^{17}O MAS NMR spectroscopic experiments were run at different magnetic-field strengths to prove the two O_c peaks of **2** are not a result of a second-order quadrupolar-broadening effect. These data suggest that the O_c sites of Keggin 1 (O_4 , O_4') and Keggin 2 (O_7) are more similar in the Si analogue than in the Ge analogue. The two solid-state ^{17}O peaks for the O_c positions of **2** become a single peak in solution, and the O_c peak for **1** remains a single peak in solution (see Figure 4 and Table 1). The O_c signals of **1** and **2** have approximately the same chemical shift in solution. The O_c signal of **1** does not shift significantly upon dissolution, whereas the two O_c peaks of **2** undergo a downfield shift of around 15 and 30 ppm, respectively. Since we do not have a single-crystal structure of **2**, the differences in O_c geometry between **1** and **2** cannot be quantitatively described. However, it is likely to be related to the difference between Ge–O and Si–O bond lengths (Ge–O bond lengths typically exceed Si–O bond lengths by about 0.1 Å), and how these T–O bonds are affected by packing distortions in the solid state. The packing distortions are eliminated in solution since the two O_c resonances of the Ge analogue becomes a single signal. An explanation for this would be that the cluster geometry becomes more flexible upon dissolution, or that a fluxional molecular rearrangement occurs. The atoms within the clusters are allowed to “relax” to optimum coordination geometries, which may not necessarily be the same constrained geometries in the solid-state lattice.

The ^{17}O and ^{23}Na NMR spectroscopic analyses provide evidence that **1** and **2** display an unusual behavior of strongly associating with their charge-balancing cations upon dissolution, and these results are described in the following

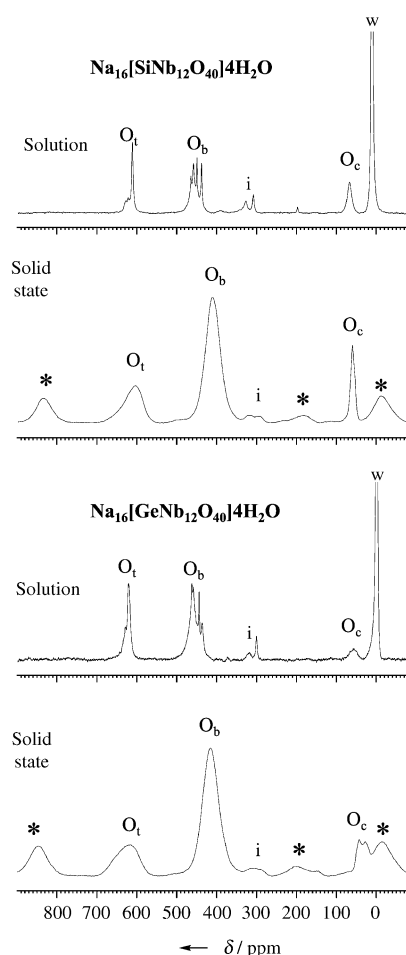


Figure 4. Solid-state and solution ^{17}O NMR spectra of isotopically enriched $\text{Na}_{16}[\text{SiNb}_{12}\text{O}_{40}]\cdot 4\text{H}_2\text{O}$ (left) and $\text{Na}_{16}[\text{GeNb}_{12}\text{O}_{40}]\cdot 4\text{H}_2\text{O}$ (right). O_t , O_b , and O_c are terminal, bridging, and central oxygen atoms. Spinning sidebands are marked with *; signals identified as impurities are marked with i (sodium carbonate and niobium oxide) w is water oxygen. The assignments of the solid-state and solution ^{17}O O_t , O_b , and O_c signals were made based on the previous NMR spectroscopic studies on the molybdate and tungstate Keggin ions.^[25–28]

Table 1: ^{17}O NMR spectroscopic chemical shifts for **1** and **2** in the solid state and solution

Sample	δ [ppm]		
	$\text{O}_t^{[a]}$	$\text{O}_b^{[a]}$	$\text{O}_c^{[a]}$
$\text{Na}_{16}[\text{GeNb}_{12}\text{O}_{40}]\cdot 4\text{H}_2\text{O}$ (2)	609	415	43
solid-state	643		26
$\text{Na}_{16}[\text{GeNb}_{12}\text{O}_{40}]\cdot 4\text{H}_2\text{O}$ (2)	621.3	437.3	57.3
solution	629.4	444.7	
		459.4	
		463.1	
$\text{Na}_{16}[\text{SiNb}_{12}\text{O}_{40}]\cdot 4\text{H}_2\text{O}$ (1)	601	406	50
solid-state	636		
$\text{Na}_{16}[\text{SiNb}_{12}\text{O}_{40}]\cdot 4\text{H}_2\text{O}$ (1)	608.6	433.0	56.9
solution	625.1	444.4	
	619.3	452.8	
		459.9	

[a] Compared to H_2O , 0 ppm.

discussions. In the solid state, the sodium atoms (16 per cluster) reside between the Keggin clusters, and each sodium atom bridges at least two separate Keggin ions. This arrangement lends a structural resemblance to a 3D network of linked Keggin ions. Keggin 1 has 30 Na atoms bonded to its terminal and bridging oxygen atoms with a total of 60 Na–O bonds; and Keggin 2 is bonded to 38 Na atoms through 74 Na–O bonds (all Na–O bonds are between 2.3–2.7 Å). Figure 5 emphasizes this bonding between the sodium and

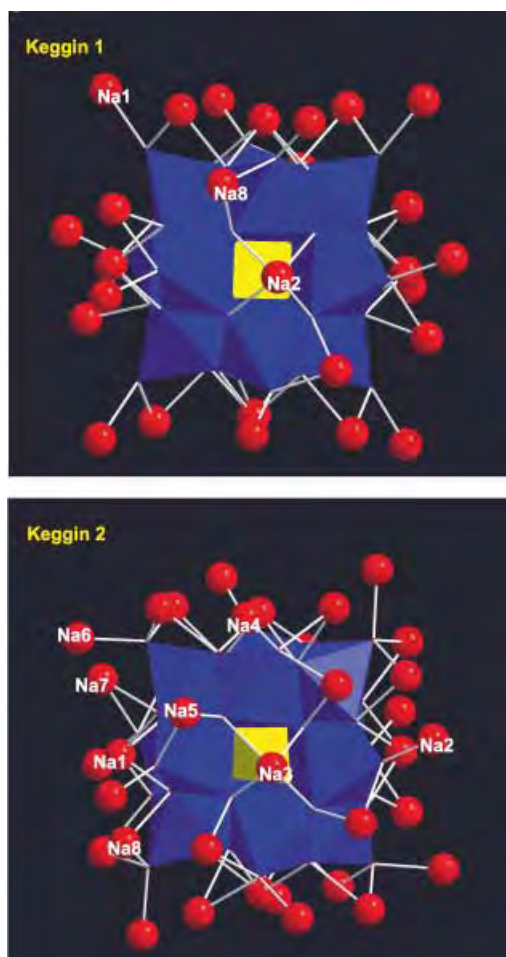


Figure 5. Polyhedral representation of Keggin 1 (top) and Keggin 2 (bottom), showing the sodium ion bound to the surface in the solid state (red spheres).

Keggin ions in the solid state. In conjunction with the extensive binding between the Na cations and the anionic cluster in the solid state, this crystal lattice contains remarkably few water molecules: only four water molecules per cluster, or 0.25 per Na cation. Normally, lattices of the Nb-based polyoxometalate salts,^[13,15,22–24] as well as those of the polyoxotungstates and polyoxomolybdates,^[1] carry 15–50+ water molecules per cluster that both solvate the charge-balancing cations and hydrogen bond to the oxygen atoms of the cluster. This unusual feature of strong binding between the sodium atoms and the anionic Keggin clusters observed here is likely to be related to stabilization of the high surface

charge of the clusters. Furthermore, the arrangement of the Keggin ion oxygen atoms apparently provides good coordination sites for metal cations. For instance, every “four-ring window” of Keggin 1 and Keggin 2 hosts a sodium cation in a cryptate-like fashion (see Figure 5, Na1, Na2, and Na3).

The ^{17}O solution and solid-state spectra of **1** and **2** are compared in Figure 4. As observed for the related molybdates and tungstates, the signals of the solid-state ^{17}O spectra are sufficiently broadened so that only one signal position is identified for the O_t , O_b , and O_c sites (with the exception of the O_c site of **2**, discussed above). The surprising result of the ^{17}O solution experiments is the number of resolvable peaks in the spectral region of terminal and bridging oxygen atoms for **1** and **2** (see Table 1). Based on similar work on the related molybdates and tungstates, we expected to see only two peaks for the bridging oxygen atoms,^[25–28] one for the $\mu_2\text{-O}$ bridging two edge-sharing NbO_6 octahedra and one for the $\mu_2\text{-O}$ bridging two corner-sharing NbO_6 octahedra. Yet we observe four signals. Furthermore, we anticipated only one signal for the terminal oxygen, rather than two or three that are observed. The splitting of the O_t and O_b ^{17}O solution NMR spectroscopic signals is attributed to symmetry-reducing distortions of the clusters. The biggest contribution to distortion of the terminal and bridging oxygen sites of Keggin 1 and Keggin 2 of **1** and **2** in the solid state is likely to be binding of the clusters to sodium in the crystal lattice, thus resulting in differences in bond angles and lengths about the oxygen atoms. However, we expect these differences to be eliminated upon dissolution if the clusters were to shed their bound sodium cations. This is clearly not the case, given the number of resolvable oxygen sites observed by solution ^{17}O NMR. Therefore, this peak splitting can be attributed to the retention of cluster-bound sodium atoms in solution, and perhaps also dissolution of **1** and **2** as small oligomeric units, linked together by the bound sodium atoms.

Solution ^{23}Na NMR spectroscopy provided further evidence for this solution behavior of the Nb Keggin ions. The ^{23}Na NMR spectra of three different solution concentrations of **1**, and the same three solution concentrations of $\text{Na}_7\text{H}_3\text{O}^+[\text{Nb}_6\text{O}_{19}]\cdot 14\text{H}_2\text{O}$ ^[15] normalized to sodium concentration were analyzed (see Table 2). The hexaniobate Lindqvist ion was used for comparison because it has a similar Na:Nb ratio as **1**, an equal negative charge per NbO_6 octahedron (−1.33), and similar solution pH to concentration correlation.

Table 2: Solution ^{23}Na NMR spectroscopy of $\text{Na}_{16}[\text{SiNb}_{12}\text{O}_{40}]\cdot 4\text{H}_2\text{O}$ and $\text{Na}_7\text{H}_3\text{O}^+[\text{Nb}_6\text{O}_{19}]\cdot 14\text{H}_2\text{O}$

Concentration Na [ppm]	Solution pH	^{23}Na signal position [ppm] ^[a]	Signal width [Hz, FWHM] ^[a]
$\text{Na}_{16}[\text{SiNb}_{12}\text{O}_{40}]\cdot 4\text{H}_2\text{O}$			
5	8.1	−0.27	23
50	9.4	−0.37	87
500	10.2	−0.69	242
$\text{Na}_7\text{H}_3\text{O}^+[\text{Nb}_6\text{O}_{19}]\cdot 14\text{H}_2\text{O}$			
5	7.6	−0.17	16
50	9.5	−0.23	10
500	9.8	−0.01	16

[a] Compared to 1 M NaCl, 0 ppm, FWHM = 18.5 Hz.

The charge-balancing sodium atoms of the dissolved Lindqvist ion are clearly hydrated, in that the ^{23}Na resonance is narrow and close to 0 ppm, as expected for a water-soluble sodium salt. On the other hand, the resonance for the charge-balancing Na atoms of the Keggin ion shifts slightly and broadens significantly with increasing concentration of the dissolved cluster. The peak broadening is likely attributed to a dynamic equilibrium of the sodium ions between binding to the clusters and completely solvating in solution. Additionally the clusters may oligomerize in solution by linking through the sodium ions, as is observed in the solid-state structure. Both cluster linking and the degree of association of the sodium ions with the clusters is expected to increase with increasing solution concentration, which correlates with the increased peak broadening. Consistent with this observation, the ^{23}Na NMR resonances broadened upon cooling and sharpened with heating of the solution of **1**. However, resolution of different Na sites was not observed before reaching the freezing temperature of the solution.

With further study, the high negative charge of these new heteropolyniobates may be exploited for greater selectivity or strength of binding in applications that utilize the electrostatic interaction between negatively charged polyoxometalate clusters and positively charged species or species that feature positively charged regions (i.e., amino acids, proteins, metals, viruses). Additionally, a direct synthetic approach to $[\text{TNb}_{12}\text{O}_{40}]^{16-}$ makes substitutional variants between the niobate Keggin ion and heteropolyacids, such as the tungstate Keggin ion, potentially more attainable. The development of clusters with properties intermediate to the heteropolyacids and heteropolybases would allow access to highly tunable pH stability, charge, and composition. Therefore the introduction of these new basic and highly charged members to the Keggin family expands the potential of these greatly studied and applied materials.

Experimental Section

Synthesis of 1: Sodium hydroxide (0.26 g, 6.5 mmol) was dissolved in water (8 mL) in the teflon liner of an autoclave. Amorphous $\text{Nb}_2\text{O}_5 \cdot x\text{H}_2\text{O}$ (0.35 g, 2.0 mmol Nb–Nb precursor, 30 wt % water; Reference Metals Co. Inc., Bridgeville, PA) and tetraethylorthosilicate (0.27 g, 1.3 mmol) were added and the mixture was stirred for approximately 30 min. The pH of the precursor mixture was about 12.7. The teflon liner was loaded and sealed in a 23 mL stainless-steel autoclave, and heated at 190 °C for 12–24 h. The white, microcrystalline product was collected by filtration. Yield: 0.27 g $\text{Na}_{16}[\text{SiNb}_{12}\text{O}_{40}] \cdot 4\text{H}_2\text{O}$, 0.12 mmol; 73 % based on Nb (Si is in excess).

Synthesis of 2: Sodium hydroxide (0.16 g, 4.0 mmol) was dissolved in water (8 mL) in the teflon liner of an autoclave. Amorphous, hydrous $\text{Nb}_2\text{O}_5 \cdot x\text{H}_2\text{O}$ (0.35 g, 2.0 mmol Nb; Reference Metals Co. Inc., Bridgeville, PA) and tetraethoxygermane (0.33 g, 1.3 mmol, Gelest, Inc.) were added and the mixture was stirred for approximately 30 min. The pH of the precursor mixture was about 11.5. The teflon liner was loaded and sealed in a 23 mL stainless-steel autoclave, and heated at 190 °C for 12–24 h. The white, microcrystalline product was collected by filtration. Yield = 0.20 g $\text{Na}_{16}[\text{GeNb}_{12}\text{O}_{40}] \cdot 4\text{H}_2\text{O}$, 0.09 mmol; 53 % based on Nb (Ge is in excess).

X-ray powder diffraction of **1** and **2** was performed on a Bruker D8 Advance diffractometer in Bragg–Brentano geometry with Ni filtered $\text{CuK}\alpha$ radiation. Samples were prepared on a zero-background

quartz plate and data were recorded from 5–90° with a step time of 25 s.

Elemental analysis: Elemental analysis was carried out on **1** by using an Perkin Elmer Elan 6100 ICP-MS. A solution of **1** was prepared by dissolving in DI water, diluting to an approximate concentration of 100 ppm of **1**, and acidifying with a small amount of nitric acid. Further dilutions to approximately 10 ppm and 1 ppm of **1** were made. Standards of Na, Si, and Nb were prepared by diluting 1000 ppm standards that had also been acidified with nitric acid. Results calcd (%): Nb 50.15, Na 16.15, Si 1.26; found: Nb 53.6, Na 15.7, Si 1.45.

The ^{17}O labeled samples of **1** and **2** were both produced by the identical reactions described above, except that they were scaled down by four and the solvent was 40.2 atom % ^{17}O water (Isotec, Inc., Miamisburg, Ohio).

The solid-state ^{17}O NMR MAS spectra were obtained on a Bruker Avance 600 instrument by using an observe frequency of 81.40 MHz, and a Bruker Avance 400 instrument with an observe frequency of 54.28 MHz. For the 81.40 MHz data the ^{17}O MAS NMR spectra were obtained by using a 2.5 mm broadband probe with spinning speeds between 30 and 35 kHz, with 2 μs $\pi/6$ pulses ($\pi/2$ pulse length 6 μs), 1 k scan averages and 5 s recycle delay. For the 54.28 MHz ^{17}O MAS NMR data and the ^1H – ^{17}O cross-polarization (CP) MAS NMR data, a 4 mm broadband MAS probe spinning between 12.5 and 15 kHz was used. For all ^{17}O MAS NMR spectra high-power ^1H decoupling was employed by using the two-pulse phase modulation (TPPM) decoupling sequence.^[29]

The solution ^{17}O NMR spectroscopic data were obtained on a Bruker Avance 600 instrument at 81.40 MHz by using a 5 mm broadband probe. The ^{17}O direct Bloch decay spectra were obtained by using a 0.5 s recycle delay, 8 μs $\pi/2$ pulses, 512 scan averages and ^1H decoupling. The ^{17}O NMR spectra were all referenced to external neat H_2^{17}O ($\delta = 0.0$ ppm) at 25 °C.

The solid-state ^{29}Si MAS NMR spectra were obtained on a Bruker Avance 600 instrument by using an observe frequency of 119.2 MHz, a spinning speed of 5 kHz, and a single-pulse Bloch decay sequence with 1640 scan averages, 3 μs $\pi/4$ pulses, 240 s delay, with the spectra being referenced to a secondary external Q_8M_8 ($\delta = 12.4$ ppm) standard.

The solution ^{23}Na NMR spectra were obtained on a Bruker Avance 400 instrument using 100 scan averages, 6.75 μs $\pi/2$ pulses with a 10 ms recycle delay. The ^{23}Na chemical shifts were referenced to a secondary external 1M NaCl standard at RT ($\delta = 0.0$ ppm).

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A Family of Supramolecular Frameworks of Polyconjugated Molecules Hosted in Aromatic Nanochannels**

Piero Sozzani,* Angiolina Comotti, Silvia Bracco, and Roberto Simonutti

Organic molecules that contain extended conjugated π systems are an important class of active molecules, which show a number of attractive photochemical and electro-optical properties: they have applications as optical and electronic materials for photonics and data storage, and as semiconductors and probes in biological media.^[1] Their inclusion in solid matrices extends the range of thermal stability, provides protection from chemical damage and improves their processability. Organized materials used as hosts are of particular interest, since they can transfer the benefits of their peculiar topological properties and modulate the functions of active molecules.^[2] The application of the principles of self-assembly and crystal engineering^[3] permits the manipulation of conjugated molecules through new interactions and the shaping of specific nanoscale environments in a way that mimics the control exerted by proteins on biological light receptors.^[4] Noncovalent soft interactions are enough to bring about the spontaneous aggregation of host and guest molecules, even if they are very different in mass and their chemical nature. The tunability of the constraints and the variety of groups that can be involved in the weak interactions indicates a strategy that, besides modulating guest dynamics and conformations, allows us to explore a wide variety of intermolecular interactions.

The tris(*o*-phenylenedioxy)cyclotriphosphazene (TPP) molecule exhibits high D_{3h} symmetry, that disfavors close packing and promotes the formation of host–guest adducts.^[5] The versatility of TPP is such that it has intriguing properties; it can self-assemble, and can form a nanoporous material with permanent nanochannels of 0.5 nm in diameter that can absorb molecules from the gas phase.^[6] Herein we show that a variety of linear π -conjugated molecules can be encapsulated in infinite nanochannels lined with aromatic groups, which offer tailored surroundings of weak interactions. Self-organization with TPP resulted in nanostructured adducts of exceptional stability in which polyconjugated molecules sit aligned in the parallel nanochannels of the crystalline framework (Figure 1). The novel host–guest architectures with

[*] Prof. P. Sozzani, Dr. A. Comotti, Dr. S. Bracco, Dr. R. Simonutti
Department of Materials Science
University of Milano-Bicocca
Via R. Cozzi 53, 20125 Milan (Italy)
Fax: (+39) 02-6448-5400
E-mail: piero.sozzani@unimib.it

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

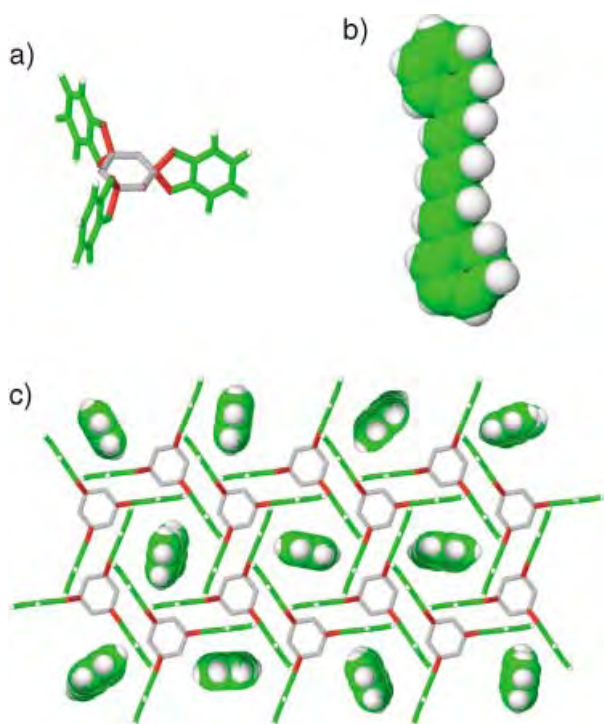


Figure 1. An example of adducts of tris(*o*-phenylenedioxy)spirocyclotriphosphazene (TPP) with polyconjugated molecules (green: carbon; white: hydrogen; violet: phosphorous; gray: phosphazenic ring). a) Side view of TPP (ball-and-stick mode) as arranged in the hexagonal crystal cell of the compounds **1–10**. b) Space-filling mode of *trans,trans,trans*-1,6-diphenyl-1,3,5-hexatriene molecule. c) Projection of the hexagonal crystalline adduct of TPP with guest molecules viewed along the channel axis (*c* axis). The guest molecules are aligned uniaxially along the nanochannels.

conjugated molecules are sustained by a diffuse network of $\text{CH}\cdots\pi$ and $\pi\cdots\pi$ interactions that, through cooperation, improve the robustness of the supramolecular adducts. The stabilizing interactions were recognized by using advanced high-resolution 2D hydrogen–carbon solid-state NMR techniques, now available for elucidating the structure and dynamics of large supramolecular assemblies.^[7] Linear conjugated molecules that contain various chains of building blocks (i.e., double bonds, phenyl and thiophene rings) could be included to form the adducts **1–10** (Table 1).

The self-assembly process is evidenced by the spontaneous formation of the supramolecular adducts, shown in the differential scanning calorimetry (DSC) traces by exotherms that occur simultaneously with, or subsequently to, the melting of the guests (Supporting Information). The congruent melting of the adducts are at temperatures higher than that of both the matrix (250°C) and the guests. Thus, the range of thermal stability of the nanostructured materials **1–10** extends up to 310°C ($\Delta H_m \approx 90 \text{ J g}^{-1}$), 100–200°C higher than the melting points of the guests.^[8] Their crystal structures show a virtually identical hexagonal architecture dictated by the host (space group $P6_3/m$), as determined by the resolution of single-crystal structures of selected samples and the simulation of the powder-diffraction profiles^[9] (the crystallographic data and refinements are given in the Supporting

Information). Concerning the guest atoms, Fourier-difference synthesis could determine a residual electron-density distribution diffused along the nanochannels without any specificity, thus indicating the occurrence of static or dynamic disorder of the guests in the incommensurate structures. Adducts **1–10** and the bulk guests were characterized by ^1H , ^{13}C , and ^{31}P MAS NMR spectroscopy at a spinning speed of 15 kHz (chemical-shift assignments are given in the Experimental Section and Supporting Information). The low multiplicity of the matrix signals indicates the high symmetry of the TPP molecule in the hexagonal honeycomb structure, which is consistent with results from X-ray diffraction (Figure 1).

Advanced 2D NMR spectroscopy experiments based on fast magic-angle spinning and Lee–Goldburg decoupling (Supporting Information) can reduce hydrogen–hydrogen homonuclear coupling and result in high resolution in the hydrogen region of the spectrum. The resolution and sensitivity in 2D reduces the overlapping of signals so that isotope labeling is not required to detect intermolecular interactions.^[7] Analysis of the compound with diphenylhexatriene (**8**) at 1 ms cross-polarization time results in a 2D ^1H , ^{13}C HETCOR Lee–Goldburg NMR spectrum with well separated host and guest signals (Figure 2a); cross signals of the guest molecules are indicated by the blue area. The remarkable resolution shows the hydrogen signals assigned to the

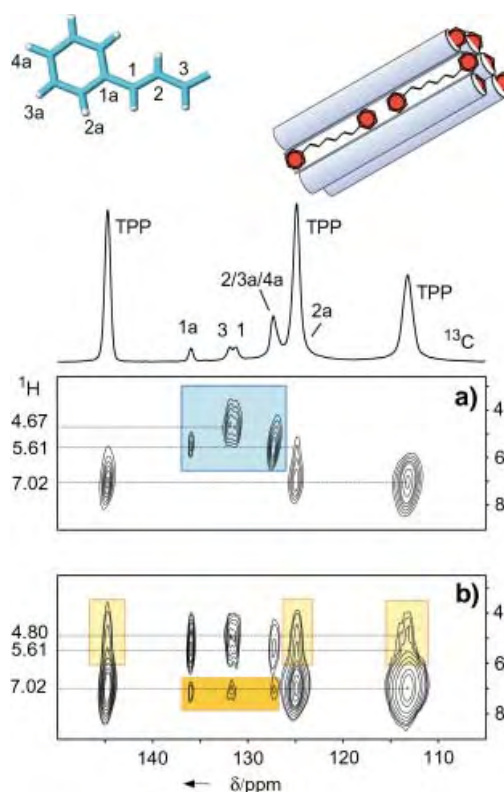
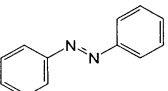
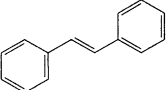
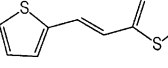
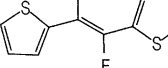
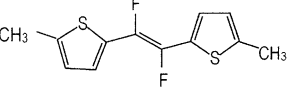
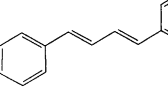
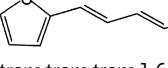
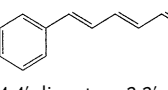
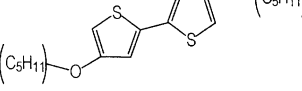
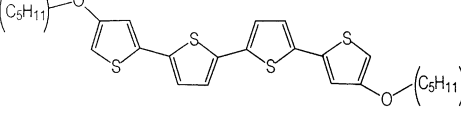


Figure 2. Contour plots of 2D phase-modulated Lee–Goldburg PMLG decoupled heteronuclear (^1H , ^{13}C) dipolar correlation spectra of compound **8**. The spectra were performed in a magnetic field of 7.04 T by using a spinning speed of 15 kHz. Ramped cross-polarization times of a) 1 ms (blue area: guest–guest cross signals) and b) 5 ms (yellow area and orange area: guest–TPP and TPP–guest cross signals, respectively) were applied.

Table 1: Guest molecules and their adducts with TPP 1–10 along with their melting properties.

	Name and structural formula of the guest molecules ^[a]	<i>T_m</i> pure [°C] ^[b]	<i>T_m</i> of the adduct [°C] ^[b]
1	<i>trans</i> -azobenzene 	68	266
2	<i>trans</i> -stilbene 	125 ≈ 97 %	275
3	<i>trans</i> -1,2-di(2-thienyl)-ethylene 	134 > 98 %	283
4	<i>trans</i> -1,2-difluoro-1,2-di(2-thienyl)-ethylene 	94	284
5	<i>trans</i> -1,2-difluoro-1,2-di(5-methyl-2-thienyl)-ethylene 	86 > 98 %	290 ^[c]
6	<i>trans,trans</i> -1,4-diphenyl-1,3-butadiene 	153 ≥ 99 %	289 ^c
7	1,4-di(2-thienyl)-1,3-butadiene 	173	301
8	<i>trans,trans,trans</i> -1,6-diphenyl-1,3,5-hexatriene 	203 ≥ 99 %	309 ^c
9	4,4'-dipentoxo-2,2'-dithiophene 	52	296 ^c
10	4,4'''-dipentoxo-2,2':5',2'':5'',2''':5'''-tetrathiophene 	119	313 ^c

[a] The guest molecules are listed according to molecular masses. [b] The accuracy of the melting point is $\pm 1^\circ\text{C}$; the calorimetric traces of the adducts are reported in the Supporting Information. [c] The adduct was also prepared by crystallization from *o*-xylene solution.

polyene chains ($\delta_{\text{H}} = 4.7$ ppm) and the aromatic heads ($\delta_{\text{H}} = 5.6$ ppm) of the guest.

The nanochannels of TPP develop aromatic walls with the phenylenedioxy groups facing the guests, which generates a notable upfield shift of about 2 ppm ($\Delta = \delta_{\text{adduct}} - \delta_{\text{liquid}} = -2$ ppm) due to the diamagnetic susceptibility produced by the host paddles wrapping around the confined guests. Longer cross-polarization times (5 ms) result in new correlation signals (Figure 2b) that pertain to through-space intermolecular interactions between TPP and diphenylhexatriene nuclei,

thus indicating an intimate relationship among the assembly components.

In the 2D HETCOR experiment, proton magnetization evolves during t_1 under its chemical-shift Hamiltonian and then is transferred to the nearby carbon atoms by cross polarization: the intensity of the correlation signal is a measurement of the distance d according to the scaling law of $1/d^6$. The effectiveness of the transfer highlights the short hydrogen-to-carbon distances. Compounds **2** and **6**, as well as the diazobenzene adduct **1**, show similar host-guest interactions and diamagnetic upfield shifts. The adduct **9** formed with a dithiophene derivative that bears the pentoxo side chains is an example of one of the thiophene-containing compounds (**3**, **4**, **5**, **7**, **9**, and **10**). Selected 2D ^1H , ^{13}C HETCOR spectra are shown in Figure 3.

The exceptional resolution in the hydrogen domain and the large magnetic susceptibility was exploited to understand the precise topology of the hydrogen atoms in the adduct. In a control experiment on the pure bulk material, only intramolecular correlations of protons to vicinal carbon atoms were active (Figure 3a): an exception being methyl and γ -methylene protons that show intermolecular correlations with thiophene ring carbon (C3 and C5), since the *trans* aliphatic chains point towards the thiophene ring of a vicinal molecule in the herringbone arrangement^[10] (Figure 3c). In contrast, the 2D spectrum of adduct **9** (Figure 3b) shows a multiplicity of host-guest interactions and a strong upfield shift of the guest signals in the carbon and hydrogen dimension (compare projections of Figure 3b to Figure 3a). The dramatic shifts of the hydrogen-atom signal of $-1.8/-2.2$ ppm have such an influence in the ^1H MAS NMR spectrum (Figure 3e) that methyl protons resonate upfield to TMS at $\delta_{\text{H}} = -1.1$ ppm, methyleneoxy protons move from $\delta_{\text{H}} = 3.6$ to 1.8 ppm and thiophene protons at $\delta_{\text{H}} = 4.10$ ppm are prevented from overlapping with the TPP signal.^[11]

The marked upfield shifts are observed here for the first time in the spectrum of the polyconjugated molecules embedded in aromatic nanochannels, and the 2D cross signals are a clear demonstration of the intimate relationship of the guest with the host aromatic groups. The strong magnetic susceptibility, derived from the TPP aromatic paddles, permits the exact determination of the distance of a probe atom from the phenylenedioxy ring (Figure 4). Nucleus-independent chemical-shift maps give the calculated chemical shifts in the region around the aromatic ring, as derived from

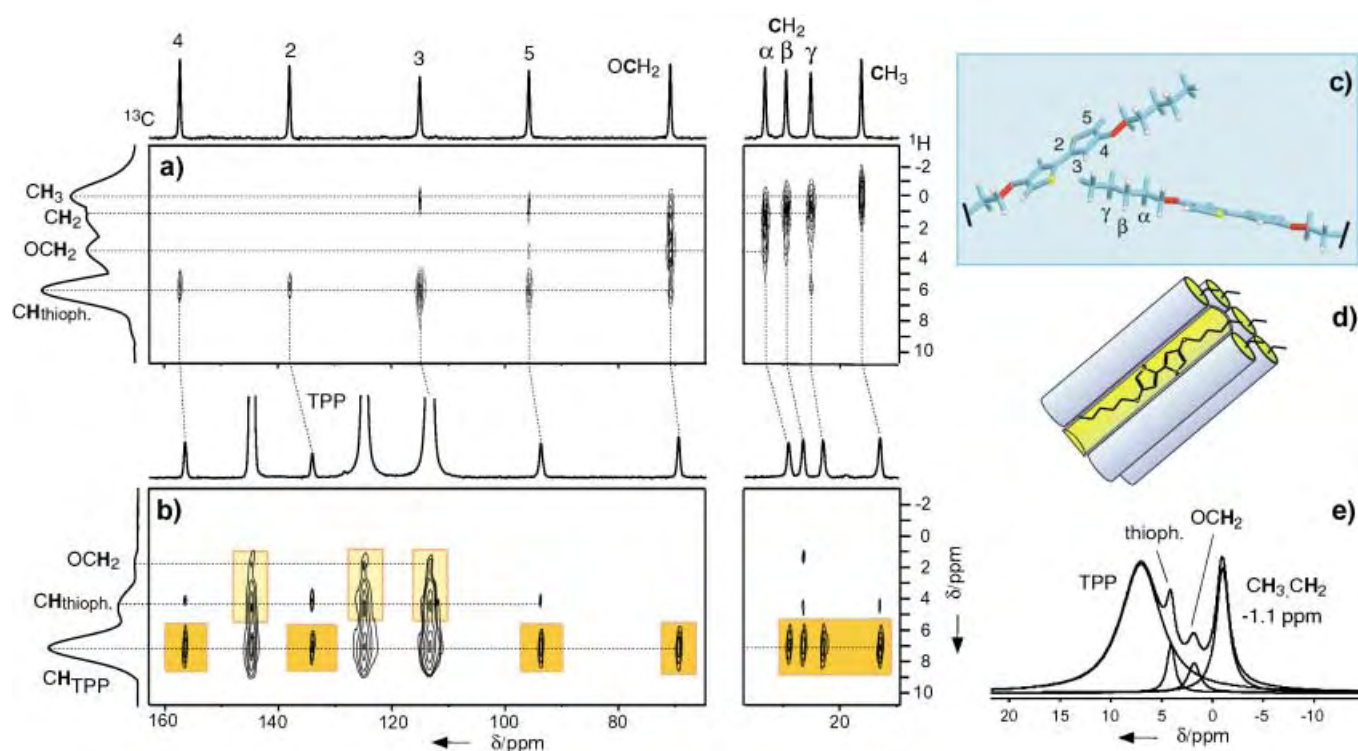


Figure 3. Contour plots of 2D PMLG decoupled heteronuclear (^1H , ^{13}C) dipolar correlation spectra of a) the 4,4'-dipentoxo-2,2'-dithiophene and b) compound **9**. The spectra were performed in a magnetic field of 7.04 T by using a spinning speed of 15 kHz. Ramped cross-polarization times of a) 1 ms and b) 5 ms were applied (yellow and orange areas: guest-TPP and TPP-guest cross signals). c) A view of two molecules in the monoclinic unit cell, space group $P2_1/c$ $a = 9.823(1)$, $b = 11.942(2)$, $c = 7.885(1)$ Å, $\beta = 90.13^\circ(2)$ (blue: carbon; yellow: sulfur; white: hydrogen). d) Schematic picture of the 4,4'-dipentoxo-2,2'-dithiophene confined to the aromatic nanochannels. e) ^1H MAS NMR spectrum of **9** obtained with a spinning speed of 15 kHz.

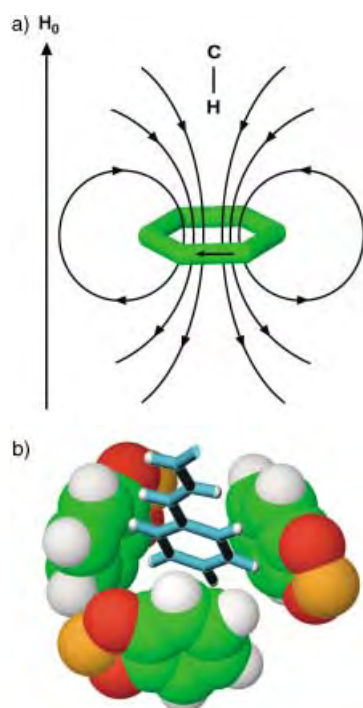


Figure 4. a) Topology of guest hydrogen atoms located at 2.5 Å above the plane of the benzene rings: the ring currents generate an upfield shift of 2 ppm. b) An example of polyconjugated guest molecule encapsulated in the nanochannel and surrounded at a close contact by three aromatic paddles of the TPP host.

the electronic current density generated by the main magnetic field.^[12] Proton resonances shifted 2 ppm upfield indicate the topology of the guest hydrogen atoms above the plane of benzene rings at a distance of 2.5 Å (Figure 4a).^[13] Short intermolecular distances, such as those determined, imply close contact between the π -electron clouds and hydrogen atoms, and favorable Van der Waals interactions. Both theoretical studies and the survey of a number of crystal structure determinations have established that energy minima occur at distances of hydrogen atoms from the center of aromatic rings of between 2.5 and 2.8 Å.

The $\text{CH}\cdots\pi$ arrangements account for about 2 kcal mol $^{-1}$ of energy.^[14] Recently, energy minima of 2.5 kcal mol $^{-1}$ were accurately determined for benzene dimers in the favorable T-shaped and slipped-parallel arrangements. At the minima, the intermolecular distance of CH hydrogen atoms from the aromatic center is 2.5 Å, as in the present case.^[15] The XRD structural resolution, although unable to detect the exact location of the guests, describes the crystal buildings in which the guests are encapsulated. The existence of $\text{CH}\cdots\pi$ interactions is compatible with the size of the 5 Å nanochannels that are recognizable in the crystal structure. Three contiguous phenylenedioxy rings are present at each cross section of TPP structure (Figure 4b) and another three, related by a 6 $_3$ screw axis, are found on the next TPP layer along the channel.

Thus, both the aromatic and aliphatic CH groups of the guest find, with high probability, any of the π -receptors lining

the walls and move to vicinal receptors through low activation energies. Multiple CH $\cdots\pi$ interactions simultaneously sustain the architecture, and make a notable contribution to the exothermic self-assembly. Melting enthalpies evaluated by DSC and referenced to one mole of TPP, give values of 6 kcal mol $^{-1}$ for bulk TPP in the nanoporous modification (host–host interactions) and about 12 kcal mol $^{-1}$ for the adducts (host–host and host–guest interactions).^[16] Since the host arrangement is the same in the bulk nanoporous modification as in the adducts, the difference in enthalpy is mostly due to the host–guest interactions, accounting for 2 kcal mol $^{-1}$ per each TPP paddle involved in CH $\cdots\pi$ interactions. Also, the conformational arrangement of the guests differs from that of the bulk by as much as it does from that of the solutions. Conformations contribute to additional upfield shifts, even reaching on some carbon atoms a shift value of –4 ppm with respect to the bulk. The large shift must be ascribed to the γ -gauche shielding effect^[17] added with the same sign to the magnetic susceptibility. In the nanostructured environment, although the molecules are squeezed to elongated shapes, departure from *trans* conformations is largely tolerated, since the guests are softly embraced within an organized environment. In fact, the interactions are loose enough to permit a diffusional spinning motion of the guests within the nanochannels, which is consistent with carbon spin-lattice relaxation times of 10–30 s in the extreme narrowing regime of motions, with correlation times of $\tau_c < 10^{-8}$ s for all the guests.^[18] Interestingly, entropy decreases moderately while weak interactions lead to the formation of host–guest complexes: if the host–guest interactions were strong and tight enough to impede librations and torsions of the guests, the entropy loss would be also greater, thus leading the enthalpy/entropy balance to compensate, without gaining much advantage through thermal stability.^[19] Thus, guest dynamics concur with the favorable host–guest interactions resulting in a surprising overall stabilization, which allows adducts to exist at high temperatures.

In summary, linear π -conjugated molecules have been engineered in unique supramolecular structures in such a way as to be completely surrounded by aromatic groups. The molecules are confined by self-assembly within a crystalline honeycomb network and have uniform alignment along the crystallographic *c* axis, with the stimulating perspective of orienting anisotropically the active molecules in thermally stable single crystals. Unconventional NMR spectroscopic techniques were able to localize, with rare accuracy, the hydrogen atoms involved in weak hydrogen-atom– π interactions and found the central motif common to the novel nanostructures, that is, the organization of π receptors wrapping about the guests and sustaining a diffuse network of weak $\pi\cdots\pi$ and aliphatic CH $\cdots\pi$ interactions. Weak host–guest interactions form collectively a robust, exceptionally stable, architecture that nevertheless provides a soft environment for the guest: an ideal locus at which the encapsulated molecules are balanced between freedom and constriction. Interestingly, the infinite fully aromatic nanochannels, shaped by the host, resemble carbon nanotubes^[20] with the aromatic rings parallel to the channel axis. The novel series of supramolecular adducts contribute to the evolution of new generations of 1D

ordered assemblies of important families of functional molecules with the strategy of exploiting weak interactions to modulate the molecular arrangements and functions.

Experimental Section

TPP was synthesized according to a reported procedure.^[6c] Compounds **1–10** were prepared either by crystallization from *o*-xylene solutions of TPP (0.01 mole L $^{-1}$) and guest molecules or by heating intimately mixed guest and TPP powders above the melting point of the guests (host–guest weight ratio of about 6:1); *o*-xylene was previously purified by *n*-nonane impurities by using molecular sieves and TPP itself as entrapment agent of linear alkanes. No inclusion compound with 3,3'-dipentoxo-2,2'-dithiophene is formed due to the steric hindrance of the molecule.

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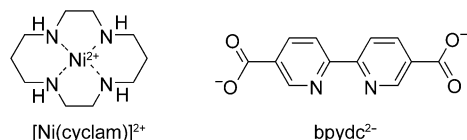
Metal–Organic Framework

A Robust Porous Material Constructed of Linear Coordination Polymer Chains: Reversible Single-Crystal to Single-Crystal Transformations upon Dehydration and Rehydration**

Eun Young Lee and Myunghyun Paik Suh*

Metal–organic open-framework solids have been the focus of research interest because of their potential applications in molecular adsorption and separation processes,^[1–4] ion exchange,^[5,6] catalysis,^[7,8] sensor technology,^[9–11] and optoelectronics.^[12] They can be designed and assembled to generate cavities or channels of various sizes and shapes by appropriate choice of the building blocks.^[13–18] However, despite extensive studies, information on how to design and synthesize metal–organic frameworks (MOFs) with permanent porosity is still limited.^[1,16,17] Moreover, applications of MOFs are scarce compared to zeolites. This is because 1) they often collapse when the guest molecules occupying the voids are removed, 2) they are in general thermally unstable compared with inorganic zeolites and are destroyed at high temperatures (>200 °C) or even at low temperatures under vacuum, and 3) they are often soluble in solvents and dissociate into their building blocks.^[3,4,13–15] Porous materials that retain their crystallinity during the reversible desorption/adsorption of guest molecules could be relevant as sensing devices.

Here we report a robust metal–organic open framework, namely, [Ni(cyclam)(bpydc)]·5H₂O (**1**), which is constructed of linear coordination polymer chains made of the nickel–macrocyclic complex [Ni(cyclam)](ClO₄)₂ (cyclam = 1,4,8,11-tetraazacyclotetradecane) and 2,2′-bipyridyl-5,5′-dicarboxylate (bpydc^{2−}). Open framework **1** shows a reversible type I^[19]



N₂ gas sorption isotherm that indicates permanent microporosity (Langmuir surface area of 817 m² g^{−1} and pore volume of 0.37 cm³ cm^{−3}). It adsorbs H₂ gas up to 1.1 wt% at 77 K and 1 atm. The framework is thermally stable up to 300 °C. It differentiates phenol, pyridine, and benzene in isooctane. When solid **1** was dehydrated, its color changed from yellow to pink, and it returned to yellow on exposure to

moisture. Interestingly, the single-crystal nature of **1** was retained during these reversible dehydration and rehydration processes. Although various linear coordination polymers have been prepared,^[15,20] those exhibiting permanent porosity, gas adsorption, or selective guest binding are rare.^[21]

The X-ray crystal structure of **1** is shown in Figure 1; crystallographic parameters are listed in Table 1. Each

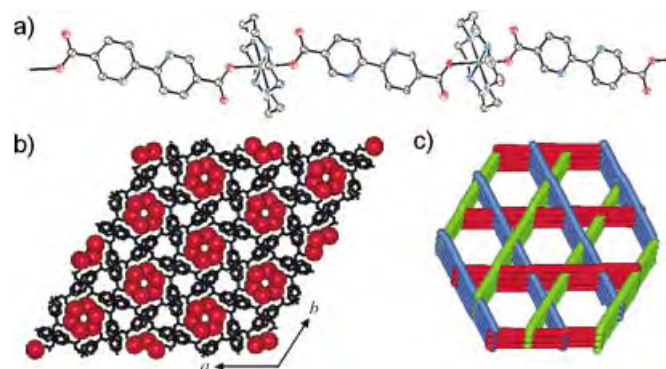


Figure 1. X-ray structure of **1**. a) Structure of the linear coordination polymer. b) View on the *ab* plane showing the linear coordination polymer chains extending in three directions; water guest molecules occupying the channels are depicted in CPK style (red). c) View showing the stacking of the linear chains, which generates 1D channels.

Table 1: Crystallographic parameters for **1**, **1′**, and **1″**.

Compound	1	1′	1″
space group	<i>R</i> $\bar{3}$	<i>R</i> $\bar{3}$	<i>R</i> $\bar{3}$
<i>a</i> [Å]	26.143(1)	26.019(1)	26.136(2)
<i>c</i> [Å]	11.3245(3)	11.3009(4)	11.3231(4)
<i>V</i> [Å ³]	6702.7(4)	6625.6(4)	6698.4(8)
Ni–O [Å]	2.144(2)	2.119(2)	2.143(3)
av Ni–N [Å]	2.068(2)	2.067(2)	2.067(2)
Pyr–CO ₂ [−] [°] ^[a]	5.1(4)	2.4(3)	5.3(4)
Ni...Ni [Å] ^[b]	8.438	8.403	8.436
C–H...π [Å] ^[b]	3.687	3.670	3.685

[a] Dihedral angle between pyridine and carboxylate planes in the bpydc^{2−} unit. [b] Shortest distance between nearest chains.

bpydc^{2−} ligand binds two Ni^{II} ions in a bis-monodentate mode, and each Ni^{II} macrocyclic complex with a square-planar coordination geometry is coordinated to two carboxylate oxygen atoms of bpydc^{2−} at *trans* positions, which results in a linear coordination polymer chain. The uncoordinated carbonyl oxygen atom of bpydc^{2−} interacts with the secondary amine group of the macrocycle to form a hydrogen-bonded six-membered ring (O2...N1 3.010(6) Å, O2...H1–N1 137.7°), which reinforces the linear chain. The intrachain Ni...Ni separation is 15.56 Å. The bpydc^{2−} ligand is planar and almost perpendicular to the coordination plane of the macrocycle (dihedral angle 85.1(1)°).

The structure contains three series of linear polymer chains extending in the [2,1,1], [1,2,−1], and [−1,1,1] directions (Figure 1 b and c). They are linked to one another by C–H...π interactions^[22] between C2 of the macrocycle and the pyridyl ring of bpydc^{2−} (distance between C2(*x*−*y*−1/3, *x*−2/3, −*z*+1/3) and the centroid of the pyridine ring: 3.687 Å) and by hydrogen bonding with the guest water

[*] E. Y. Lee, Prof. Dr. M. P. Suh
School of Chemistry and Molecular Engineering
Seoul National University
Seoul 151-747 (Republic of Korea)
Fax: (+82) 2-886-8516
E-mail: mpsuh@snu.ac.kr

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

molecule (OW1...O2 2.801(8) Å). The shortest interchain Ni...Ni distance is 8.438 Å. The packing of 1D coordination polymer chains generates 1D channels with honeycomb-like openings of 10.0 Å diameter (effective size 5.8 Å). The channels are filled with guest water molecules. The packing of the rods have been mathematically analyzed.^[23] Compound **1** exhibits a rod-packing mode corresponding to the double network of threefold braids, that is, a threefold braid of slanting rods is packed with inversion of the braid on translating in the *c* direction. In the present compound, however, the central vertical rod of the mathematical threefold braid is missing, and this creates the channel in **1**.

Compound **1** is insoluble in water and in common organic solvents. Thermogravimetric analysis on a crystalline sample of **1** showed loss of all guest water molecules at 25–110 °C. No chemical decomposition was observed up to 300 °. The N₂ gas-sorption study revealed a reversible type I isotherm characteristic of a microporous material (Figure 2). The isotherm

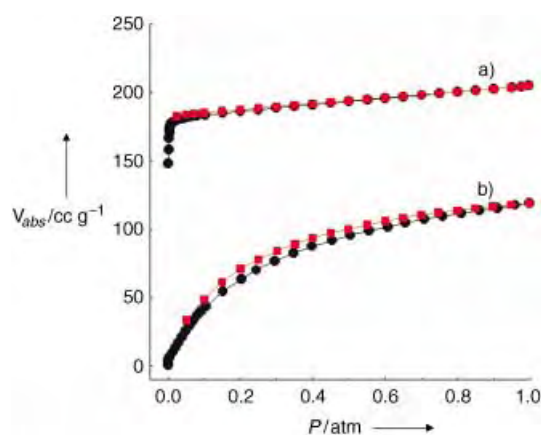


Figure 2. Gas-sorption isotherms for **1** (■ in red indicates desorption). a) N₂, b) H₂. *P*_o(N₂) = 760 torr at 77 K.

shows no hysteresis on desorption of gas from the pores. The Langmuir surface area and pore volume, estimated by applying the Langmuir and Dubinin–Radushkevich equations, are 817 m² g^{−1} and 0.37 cm³ cm^{−3}, respectively. These values compare favorably to zeolites, whose pore volumes range from 0.18 to 0.47 cm³ cm^{−3}.^[24] In addition, the compound adsorbs H₂ gas up to 1.1 wt % (2.7 H₂ molecules per formula unit of host) at 77 K and 1 atm (Figure 2), which is superior to the best zeolite ZSM-5 (0.7 wt %).^[25] Compared with MOF-5 (Langmuir surface area 2900 m² g^{−1}, pore volume 0.61 cm³ cm^{−3}, H₂ gas uptake 4.5 wt %),^[1a,b] **1** is inferior. However, **1** performs better than other recently published metal–organic open frameworks with respect to porosity and H₂ uptake capacity; for example, the {Mn(HCO₂)₂} framework does not adsorb N₂ gas but adsorbs H₂ gas (0.9 wt %).^[26]

Interestingly, **1** retains its single-crystal nature even after removal of the guest water molecules by heating the crystal at 150 °C under 10^{−5} torr for 2–40 h, which is accompanied by a color change from yellow to pink (Figure 3). The X-ray structure of the dehydrated crystal (**1'**) indicates that the porous framework structure constructed by packing of the linear chains is intact, and that only bond lengths and angles involving the Ni^{II} center and the carboxylate ligand are



Figure 3. Photographs of a) original crystal **1** in the mother liquor, b) after evacuation of **1** at 150 °C and 10^{−5} torr for 2 h (**1'**), and c) after exposure of **1'** to water vapor for 5 min (**1''**).

slightly changed (Table 1 and Supporting Information). This is contrary to our previous observation on a pillared bilayer open framework, which exhibits a spongelike behavior on removal of the guests.^[4] The C–H...π interaction^[22] between the linear chains in **1'** (C2(*x*−*y*−1/3, *x*−2/3, −*z*+1/3)–centroid distance 3.670 Å) apparently sustains the porous framework structure. In general, Ni^{II} ions in an octahedral coordination geometry show a pink color, and those in square-planar environment result in a yellow color. The color change on dehydration may be partly attributable to shortening (by 0.025 Å) of the Ni–O(carboxylate) bond, which makes the Ni^{II} coordination environment in **1'** closer to octahedral compared to the hydrated solid (Table 1). In hydrated solid **1**, water guest molecules are involved in hydrogen bonding to the host framework through carbonyl oxygen atoms and C–H groups of the pyridyl rings of the bpydc ligand. However, these interactions cannot explain the yellow color in **1**, since the Ni^{II} ions in **1** still maintain the octahedral coordination geometry.

When a dehydrated crystal of **1'** was exposed to air or water vapor the pink color of the crystal returned to yellow over a few minutes with retention of the single-crystallinity (Figure 3). Powder samples of **1'** instantly change color on exposure to air. The X-ray crystal structure of the rehydrated crystal **1''** was same as that of **1** (Table 1 and Supporting Information). In this regard, compound **1** is a new member of a relatively small group of molecular materials that reversibly desorbs guest molecules with retention of the original structure and single-crystallinity.^[1a,16,27]

In iso-octane the dehydrated host solid binds ethanol, phenol, pyridine, and benzene, for which Langmuir isotherms are observed (Figure 4), while it does not bind toluene. The amount of organic molecules bound to the solid at various guest concentrations was measured by GC, and the formation constant *K_f* and the number of binding sites (BS) for guest molecules in moles per gram of host [BS]₀/ω were estimated (Table 2). The *K_f* values indicate that the host binds guests in the order of ethanol ≈ phenol > pyridine > benzene. It is evident that the host favors guests that can form hydrogen bonds with its carbonyl groups, which are exposed to the channels.^[4,28]

In conclusion, we have shown that a metal–organic framework having permanent porosity can be constructed by packing of linear coordination polymer chains. Framework **1** exhibits multiple functions: high porosity, hydrogen-storage capacity, and selective guest-binding ability. The framework exhibits reversible single-crystal to single-crystal transformation on dehydration and rehydration, which is accompanied by a fast color change of the crystal.

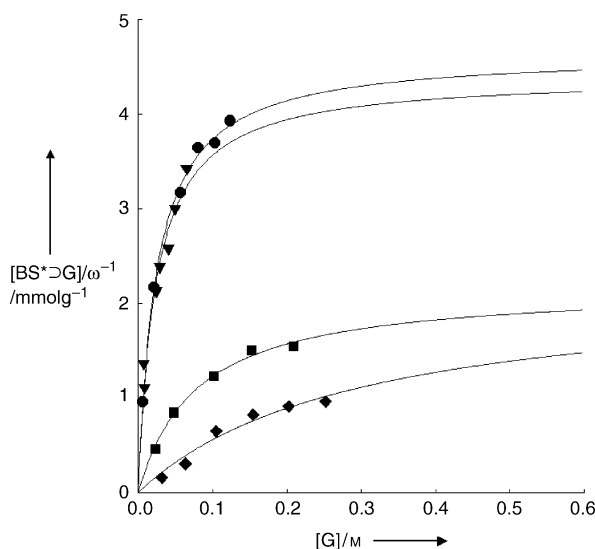


Figure 4. Binding of host solid **1** with the organic guests EtOH (●), PhOH (▼), pyridine (■), and benzene (◆). K_f , $[G]$, and $[BS\supset G]/\omega$ are defined in the Experimental Section.

Table 2: Guest binding data for **1'**.^[a]

	K_f [M ⁻¹]	$[BS]_0/\omega$ [mmol g ⁻¹]	GIC ^[b] [mol]
EtOH	41.1 ± 3.4	4.65 ± 0.11	2.33
PhOH	42.6 ± 10.7	4.41 ± 0.44	2.21
pyridine	12.9 ± 1.8	2.18 ± 0.12	1.09
benzene	3.37 ± 1.73	2.31 ± 0.71	1.11

[a] K_f and $[BS]_0/\omega$ indicate the binding constant and the binding capacity of host solid (per g) for guest molecules, respectively. [b] Guest inclusion capacity per formula unit of **1'**, [Ni(cyclam)](bpydc)].

Experimental Section

1: [Ni(cyclam)](ClO₄)₂ (0.314 g, 0.69 mmol) was dissolved in hot water (7 mL), and a hot aqueous solution (2 mL) of Na₂bpydc (0.199 g, 0.69 mmol) was added dropwise. The yellow solution was allowed to stand at room temperature until pale yellow crystals formed, which were filtered off, washed with water, and dried in air. Yield: 0.306 g, 75%. FTIR (Nujol mull): ν = 3370 (m, br), 3195 (m), 1596 (s), 1537 (m), 1099 (s), 1022 (m), 837 (s), 783 (s) cm⁻¹; UV/Vis (diffuse reflectance): $\lambda_{\max}$ = 510 nm, 386 (sh) nm. Elemental analysis (%) calcd for NiC₂₂H₄₀N₆O₉: C 44.68, H 6.82, N 14.21; found: C 44.83, H 6.81, N 14.24.

1': The single crystal **1** was isolated after the X-ray intensity data were collected at 293 K in the mother liquor. The crystal was introduced into a 0.5 mm glass capillary having an open end, which was inserted into the 9 mm cell of a gas sorption apparatus. It was heated at 150 °C under 10⁻⁵ torr for 2 h, and then cooled to room temperature under the same vacuum. The removal of all solvent guest molecules was checked by monitoring the out-gases. The cell was filled with He gas (1.0 atm). As soon as the capillary was removed, it was sealed for X-ray structure determination.

Gas-sorption study: Gas sorption was measured on a Quantachrome Autosorb-1 instrument. An exact amount of **1** was introduced into the gas sorption apparatus, and then the compound was dehydrated at 150 °C and 10⁻⁵ torr until all the guest molecules were removed from the pores. The N₂ gas-sorption isotherm was monitored at 77 K at each equilibrium pressure by the static volumetric method. The H₂ gas-sorption isotherm was measured by a similar procedure at 77 K.

Guest-binding study: The formation constant K_f for the complexes formed between a binding site (BS) of the insoluble host and a guest molecule (G) was defined as k_{ad}/k_{de} [Eqs. (1) and (2)] by analogy



$$K_f = \frac{k_{ad}}{k_{de}} = \frac{[BS \supset G]}{[BS][G]} \quad (2)$$

with the Langmuir isotherm for adsorption of gas molecules on solid surfaces.^[4,28,29] The concentration of G bound to BS ($[BS\supset G]$) was plotted against $[G]$, and K_f and $[BS]_0/\omega$ were estimated by analysis of the data according to Equations (3) and (4). In the experiment, the total concentration of guest ($[G]_0$) was varied to keep the θ values in the range of 20–80%.

$$\theta = \frac{[BS \supset G]}{[BS]_0} = \frac{[G]}{[G] + \frac{1}{K_f}} \quad (3)$$

$$\frac{[BS \supset G]}{\omega} = \frac{\left(\frac{[BS]_0}{\omega}\right)[G]}{\left([G] + \frac{1}{K_f}\right)} \quad (4)$$

If θ is defined as the fractional coverage then where ω is the amount of host solid per unit volume of the solution (mg mL⁻¹).

Pale pink crystals of **1'** were dried in a Schlenk tube at 80–100 °C under vacuum for 2–3 h. The solid (20.0–36.0 mg) was weighed exactly and immersed in a measured volume of isooctane (1–2 mL) containing ethanol, phenol, pyridine, or benzene for 16–20 h at 20 °C. The initial concentrations of ethanol, phenol, pyridine, and benzene were varied in the ranges of (0.206–2.57) × 10⁻¹ M, (0.222–1.27) × 10⁻¹ M, (0.198–2.37) × 10⁻¹ M, and (0.337–2.81) × 10⁻¹ M, respectively, to keep the saturation values θ in the range of 20–80%. The concentration change of the organic guest was measured by GC, with dodecane as internal standard. The GC system was fitted with a 30 m × 0.32 mm × 0.25 μm cross-linked polydimethylsiloxane capillary column and interfaced with a GC ChemStation. The column temperature was programmed from 80 °C (3 min) to 140 °C (2 min) at a rate of 30 °C min⁻¹. A flame-ionization detector was used.

X-ray crystallography: Diffraction data for **1**, the dehydrated crystal **1'**, and the rehydrated crystal **1''** were collected with an Enraf Nonius Kappa CCD diffractometer (MoK α , λ = 0.71073 Å, graphite monochromator). Crystal data for **1**: NiC₂₂H₄₀N₆O₉, M_r = 591.31, trigonal, space group $R\bar{3}$, a = 26.143(1), c = 11.3245(3) Å, V = 6702.9(4) Å³, Z = 9, T = 293 K, R_1 = 0.0662 ($I > 2\sigma(I)$), $wR_2(F^2)$ = 0.1981 ($I > 2\sigma(I)$), GOF = 1.109. Although microanalysis and thermogravimetric analysis indicate that the compound contains five water molecules per formula unit, only four water molecules could be located in the structure because of disorder. Full-site occupancy was assigned to OW1 because it forms a hydrogen bond with the carbonyl oxygen atom of the host and adjacent OW1 molecules, and 2/3 and 1/3 site occupancy factors were assigned to OW2 and OW3, respectively. Crystal data for **1'**: NiC₂₂H₃₀N₆O₄, M_r = 501.23, trigonal, space group $R\bar{3}$, a = 26.019(1), c = 11.3009(4) Å, V = 6625.6(4) Å³, Z = 9, T = 293 K, R_1 = 0.0503 ($I > 2\sigma(I)$), $wR_2(F^2)$ = 0.1391 ($I > 2\sigma(I)$), GOF = 1.210. Crystal data for **1''**: NiC₂₂H₄₀N₆O₉, M_r = 591.31, trigonal, space group $R\bar{3}$, a = 26.136(2), c = 11.3231(4) Å, V = 6698.4(8) Å³, Z = 9, T = 293 K, R_1 = 0.0622 ($I > 2\sigma(I)$), $wR_2(F^2)$ = 0.1749 ($I > 2\sigma(I)$), GOF = 1.110. Crystallographic data for **1**, **1'**, and **1''** are summarized in the Supporting Information.

CCDC 224802 (**1**), 224803 (**1'**), and 224804 (**1''**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union

Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

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Single-Molecule Magnets

A Dimeric Manganese(III) Tetradentate Schiff Base Complex as a Single-Molecule Magnet**

Hitoshi Miyasaka, Rodolphe Clérac,*
Wolfgang Wernsdorfer,* Lollita Lecren,
Claire Bonhomme, Ken-ichi Sugiura, and
Masahiro Yamashita*

In the early 1990s the storage of information at the molecular level became potentially feasible owing to the discovery of

[*] Dr. H. Miyasaka, Prof. K.-i. Sugiura, Prof. M. Yamashita[†]
Department of Chemistry, Graduate School of Science
Tokyo Metropolitan University
1-1 Minami-ohsawa, Hachioji, Tokyo 192-0397 (Japan)
and

“Structural Ordering and Physical Properties”, PRESTO
Japan Science and Technology Agency
4-1-8 Honcho Kawaguchi, Saitama 332-0012 (Japan)
Fax: (+81) 426-77-2525
E-mail: miyasaka@comp.metro-u.ac.jp

Dr. R. Clérac, L. Lecren, C. Bonhomme
Centre de Recherche Paul Pascal
CNRS, UPR 8641
115 avenue du Dr. A. Schweitzer, 33600 Pessac (France)
Fax: (+33) 5-5684-5600
E-mail: clerac@crpp-bordeaux.cnrs.fr

Dr. W. Wernsdorfer
Laboratoire Louis Néel
CNRS, BP 166
25 Avenue des Martyrs, 38042 Grenoble Cedex 9 (France)
Fax: (+33) 4-7688-1191
E-mail: wernsdor@grenoble.cnrs.fr

[[†]] Present address:
CREST, Japan Science and Technology Agency
4-1-8 Honcho Kawaguchi, Saitama 332-0012 (Japan)

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multinuclear transition-metal complexes acting as single-molecule magnets (SMMs).^[1,2] To date, several SMM families based on complexes of Mn,^[3] Fe,^[4] Ni,^[5] V,^[6] Co,^[7] or mixed transition metals^[8] have been reported. These systems exhibit slow relaxation of their magnetization induced by the combined effect of a high-spin ground state S_T and uniaxial anisotropy D . These two ingredients create an energy barrier $\Delta = |D|S_T^2$ between spin-up and spin-down states, which leads to a hysteresis phenomena with reversal of the magnetization, similar to that observed in bulk magnets. When a magnetic field is applied to saturate the magnetization of these complexes and then removed, the magnetization decays with a relaxation time τ that follows the Arrhenius law with an activation energy equal to Δ . At lower temperatures, τ may reach saturation as the thermally activated relaxation pathway becomes slower than quantum tunneling through the energy barrier. Hence, SMM complexes appear to be unique systems for studying fundamental phenomena, such as quantum spin tunneling and quantum phase interference, which may be used for future applications in molecular electronics.^[9]

In this field, the minimization of the SMM nuclearity remains an important issue for obtaining simple model systems with a small number of quantum energy levels. To date, although some claims have been made that mononuclear^[10,11] or dinuclear iron systems^[12] could display SMM or SMM-like behavior, the smallest complexes unambiguously exhibiting SMM behavior are tetramers of transition metals.^[3a-d,4b-4d,5c,6,7] Herein we report a simple out-of-plane Mn^{III} dimer possessing an $S_T = 4$ spin ground state and exhibiting slow relaxation of its magnetization at low temperatures with the signature of SMM behavior.

Following the synthetic method used for similar complexes,^[13] $[\text{Mn}_2(\text{saltmen})_2(\text{ReO}_4)_2]$ (**1**, $\text{saltmen}^{2-} = \text{N,N'-(1,1,2,2-tetramethylethylene)bis(salicylideneimine)}$) was prepared in high yield as large crystals (see the Experimental Section). The $[\text{Mn}_2(\text{saltmen})_2]^{2+}$ dimeric unit in **1** is very similar to that observed in $[\text{Mn}_2(\text{saltmen})_2(\text{H}_2\text{O})_2](\text{ClO}_4)_2$ and its derivatives.^[13] As shown in Figure 1a, the $[\text{Mn}_2(\text{saltmen})_2]^{2+}$ motif lies on an inversion center, and hence the asymmetric unit contains only one Mn^{III} site that assumes an axial-elongated square-bipyramidal six-coordination geometry. The Mn^{III} atom is surrounded by N_2O_2 atoms of the saltmen^{2-} ligand in the equatorial plane and two axial oxygen atoms, O(3) and O(1)*, from the $\{\text{ReO}_4\}^-$ ion and the neighboring $\{\text{Mn}(\text{saltmen})(\text{ReO}_4)\}$ moiety, respectively. In the equatorial plane, the average Mn(1)–X bond length is 1.948(4) Å (X = N or O atom of the saltmen^{2-} ligand). As usually observed for octahedral Mn^{III} ions, the Jahn–Teller distortion leads to elongated axial Mn(1)–O(3) and Mn(1)–O(1)* bonds (2.459(4) and 2.184(4) Å).

Two symmetrical arrangements of Mn^{III} dimers are found in the crystal packing. The Jahn–Teller axes of these two units lie roughly in the bc plane and are arranged along the c axis with a tilt angle of about 70° (Figure 1b). The interdimer Mn...Mn separations are particularly large in **1**: 9.29 and 9.54 Å in the a and c axis directions, respectively. These separations are notably longer than those observed in similar dimeric compounds (5.59–8.28 Å).^[13] Moreover, no signifi-

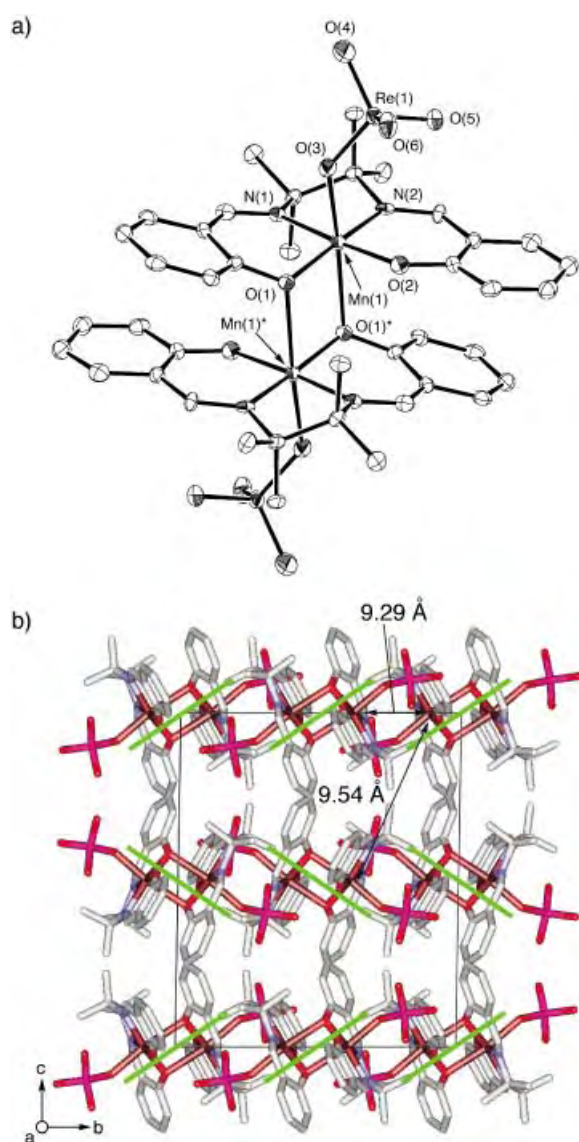


Figure 1. a) An ORTEP drawing of **1** (thermal ellipsoids set at 50% probability). The hydrogen atoms have been omitted for clarity. Selected bond lengths [Å] and angles [°]: Mn(1)···Mn(1)* 3.330(1), Mn(1)–O(1) 1.913(4), Mn(1)–O(1)* 2.459(4), Mn(1)–O(3) 2.184(4); Mn(1)–O(1)–Mn(1)* 98.5(2), O(1)–Mn(1)–O(1)* 81.5(1), O(3)–Mn(1)–O(1)* 172.1(1). The asterisk indicates the symmetry operation $2-x, -1/2+y, 1/2-z$. b) The crystal packing of **1** as projected along the a axis. The green lines indicate the Jahn–Teller distortions of the two symmetry-related dimers. Mn brown, Re pink, O red, N blue, C gray.

cant intermolecular π – π contact has been found between the packing molecules.

The dc magnetic susceptibility measured on a nujol-restrained polycrystalline sample of **1** is depicted in Figure 2. The product χT gradually increases from 6.19 cm³ K mol^{–1} at 300 K to reach a maximum of 9.58 cm³ K mol^{–1} at 8.0 K and then decreases to 8.17 cm³ K mol^{–1} at 1.8 K.^[14] This behavior is typical for $[\text{Mn}_2(\text{saltmen})_2]^{2+}$ units that exhibit a ferromagnetic interaction J between Mn^{III} ions as well as a zero-field splitting effect induced by the uniaxial anisotropy D_{Mn} of each Mn^{III} ion.^[13] Therefore, the Hamiltonian given in Equation 1

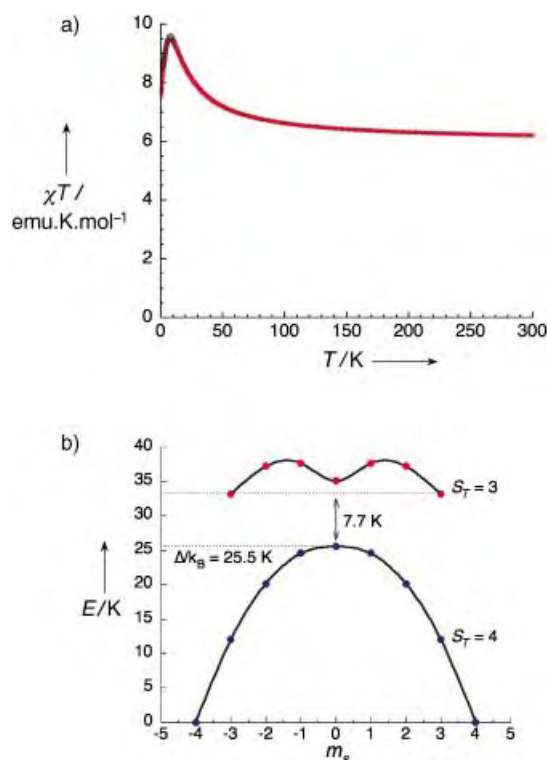


Figure 2. a) The temperature dependence of the product χT for **1** under 1 kOe. The red line represents a best simulation using an $S_{\text{Mn}} = 2$ dimer model described in the text. b) Energy diagram of the two lowest spin states calculated from the fitting of the temperature dependence of the magnetic susceptibility. E = energy.

has been used to model the magnetic susceptibility ($S_1 = S_2 = S_{\text{Mn}}$, S_{iz} is the z component of the S_i operator).

$$\mathcal{H} = -2J S_1 S_2 + D_{\text{Mn}} \sum_{i=1,2} S_{iz}^2 + g\mu_B H \sum_{i=1,2} S_i \quad (1)$$

The susceptibility was simulated using the general procedure developed by Clemente-Juan and co-workers (MAGPACK program).^[15] The best set of parameters obtained is $g = 2.00(1)$, $J/k_B = +2.65(5)$ K, and $D_{\text{Mn}}/k_B = -4.0(2)$ K, where g is the g factor of the Mn ion and k_B is the Boltzmann constant (the solid line in Figure 2). These parameters are very similar to those obtained for $[\text{Mn}_2(\text{saltmen})_2(\text{H}_2\text{O})_2](\text{ClO}_4)_2$ ($J/k_B = +2.57$ K, $D_{\text{Mn}}/k_B = -3.60$ K) and its derivative.^[13] From the estimated values of J and D_{Mn} , the energy diagram can be calculated (Figure 2b). As observed in SMMs, the uniaxial anisotropy D_{Mn} creates an energy barrier Δ/k_B between the $m_s = \pm 4$ levels estimated at 25.5 K (since Δ/k_B is equal to $|D_{\text{Mn}_2}| S_T^2/k_B$, we can deduce that $D_{\text{Mn}_2}/k_B = -1.59$ K, where D_{Mn_2} is the uniaxial anisotropy for the Mn^{III} dimer).

Above the $S_T = 4$ ground state, the first excited state $S_T = 3$ lies at 7.7 K. Therefore, as a first approximation only the ground state $S_T = 4$ was considered to fit the plot of M versus H/T (Figure 3). The experimental data have been accurately simulated with $g = 2.00$ and $D_{\text{Mn}_2}/k_B = -1.63$ K (solid lines in Figure 3). These parameters lead to a theoretical energy barrier Δ/k_B of 26 K, which is in excellent agreement with that

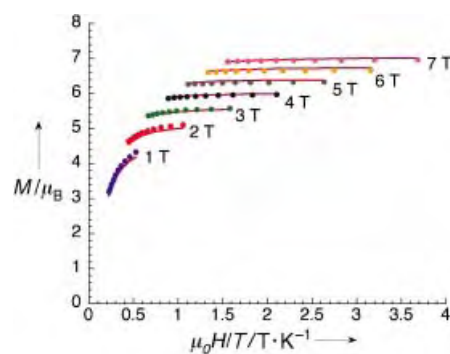


Figure 3. Plots of the magnetization M versus the ratio of the external field H to temperature ($T = 1.9$ – 4.5 K) for **1**. The red lines are the best fit of the data obtained for $S_T = 4$ with $g = 2.00$ and $D_{\text{Mn}_2}/k_B = -1.63$ K.

expected from the simulated temperature dependence of the susceptibility and the deduced energy diagram (see above).

The frequency dependence of the ac susceptibility on an aligned single crystal of **1** was measured at different temperatures under zero applied dc field. As seen in Figure 4, and as

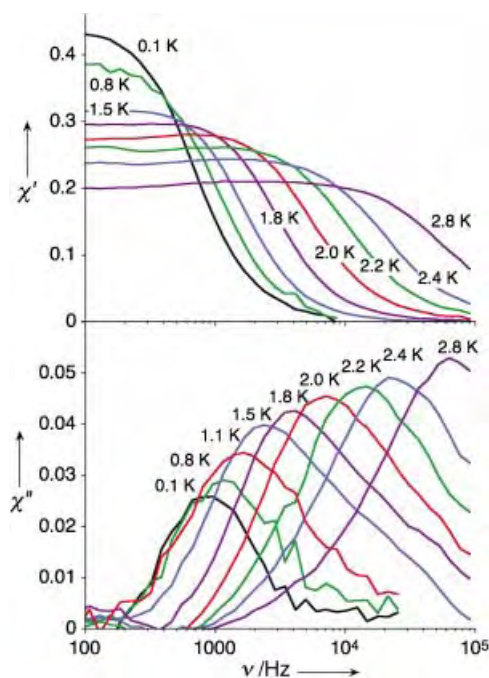


Figure 4. The frequency dependence of the in-phase (χ') and out-of-phase (χ'') ac susceptibility of **1** at temperatures below 2.8 K at zero dc field and with an ac field amplitude of 5 Oe. These measurements were carried out on a single crystal aligned along one of the two easy directions. χ' and χ'' are given in arbitrary units.

expected for SMMs, a single relaxation process is observed for both components of the ac susceptibility. The relaxation time τ has been estimated from the maximum of the χ'' versus ν curves ($\tau = 1/2\pi\nu_{\text{max}}$) and plotted as a function of $1/T$ (Figure 5).

Between 4 and 1.9 K, the relaxation follows the Arrhenius law ($\tau = \tau_0 \exp(E_a/k_B T)$, see the inset of Figure 5) with an activation energy E_a of 16 K and a pre-exponential factor $\tau_0 =$

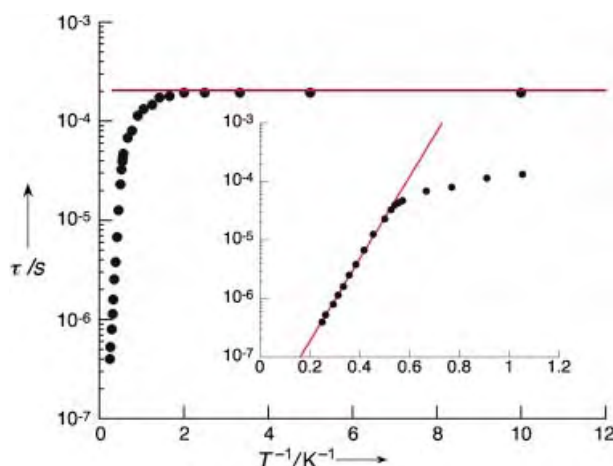


Figure 5. Plot of the relaxation time τ versus $1/T$ for **1**. Inset: Enlarged view of the high-temperature region of the plot to emphasize the Arrhenius behavior.

8×10^{-9} s. Below 1.9 K, τ reaches saturation and becomes temperature independent at 1.9×10^{-4} s below 0.6 K. This feature is characteristic of SMM behavior when quantum tunneling of the magnetization (QTM) is dominant,^[3a,5a,16] in this case between the lowest energy $m_s = \pm 4$ levels of the $S_T = 4$ state. E_a is lower than the expected value calculated from $D_{Mn_2} (\Delta/k_B = |D_{Mn_2}| S_T^2/k_B = 26 \text{ K})$. This result clearly indicates that QTM is operative even at higher temperatures and reduces the energy barrier, as seen in other SMM systems.^[3a] In agreement with this idea, the value of E_a measured at different dc fields increases up to 23 K at 800 Oe.

An important characteristic of the SMM behavior is magnetization hysteresis, which was investigated with a micro-SQUID setup^[17] in the temperature range of 0.04 to 7 K. Figure 6 presents hysteresis loops measured on a single

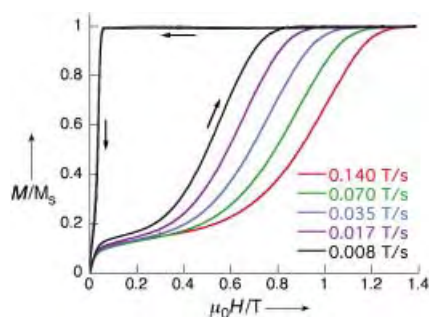


Figure 6. The field dependence of the normalized magnetization at 0.04 K and several field sweep rates.

crystal of **1** with the magnetic field applied along the easy axis of one of the two molecular directions.^[18] The hysteresis loops were measured starting at zero field because of the fast tunneling. Hysteresis appears below 2 K (for field sweep rates of 0.14 T s^{-1}) and becomes temperature independent below 0.6 K but is strongly time dependent (Figure 6). Both features

are expected for SMMs. A close examination of the hysteresis loops at zero field revealed a very small antiferromagnetic intermolecular coupling of about 0.2 K (0.035 T).

In summary, complex **1** is a simple out-of-plane dimer of Mn^{III} ions that unambiguously exhibits single-molecule magnetic behavior. To date, this compound is the smallest magnetic unit which has been reported to behave as a magnet at the molecular level and to show quantum tunneling.

Experimental Section

1: Complex **1** was prepared following a reported method.^[13] A solution of manganese(III) acetate dihydrate (1.34 g, 5 mmol) in methanol (20 mL) was added to H_2 saltmen (1.62 g, 5 mmol) in methanol (50 mL). After the brown solution was heated at 50°C and stirred for 30 min, solid sodium perrhenate (1.37 g, 5 mmol) was added. Hot water (50 mL) was added to the hot solution in methanol, and the solution was collected by filtration. The filtrate was left to stand for one week at room temperature to form dark brown crystals. The crystals were collected by suction filtration, washed with a minimum amount of water, and dried in air (92% yield based on the Mn precursor). Elemental analysis (%) calcd for $C_{40}H_{44}Mn_2N_4O_{12}Re_2$: C 38.28, H 3.53, N 4.46; found: C 38.14, H 3.63, N 4.48; IR (KBr pellet): $\nu = 1605$ (C=N), 922, 910 cm^{-1} (Re–O).

X-ray crystallography: The data were collected on a Rigaku CCD diffractometer (Sutern70) using graphite-monochromated $Mo_{K\alpha}$ radiation ($\lambda = 0.71069 \text{ \AA}$). The structures were solved by direct methods (SIR92)^[19] and expanded using Fourier techniques.^[20] The non-hydrogen atoms were refined anisotropically, while hydrogen atoms were introduced as fixed contributors. Full-matrix least-squares refinements on F^2 based on 4630 unique reflections were employed, where the unweighted and weighted agreement factors of $R = \sum ||F_o| - |F_c|| / \sum |F_o|$, and $wR = [\sum w(F_o^2 - F_c^2)^2 / \sum w(F_o^2)^2]^{1/2}$ were used. All calculations were performed using the CrystalStructure software package.^[21] Crystal and experimental data for **1** ($C_{40}H_{44}Mn_2N_4O_{12}Re_2$): $M_r = 1255.10$, orthorhombic space group $Pbca$ (no. 61), $T = 100 \pm 1 \text{ K}$; $a = 16.765(2)$, $b = 14.416(3)$, $c = 17.179(4) \text{ \AA}$; $V = 4150.6(1) \text{ \AA}^3$, $Z = 4$, $\rho_{\text{calcd}} = 2.008 \text{ g cm}^{-3}$, $F_{000} = 2432.00$, $2\theta_{\text{max}} = 54.6^\circ$. Final $R = 0.036$ ($I > 2.00\sigma(I)$), $R = 0.050$ (all data), $wR = 0.094$ (all data), $GOF = 1.014$ for 293 parameters and a total of 38179 reflections, 4630 unique reflections ($R_{\text{int}} = 0.110$); equivalent reflections were merged. The linear absorption coefficient μ for $Mo_{K\alpha}$ radiation is 64.78 cm^{-1} . An empirical absorption correction was applied. The data were corrected for Lorentzian and polarization effects; max./min. electron density in the ΔF map: $2.72 / -2.06 \text{ e \AA}^{-3}$. CCDC 226818 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

Magnetic measurements: Magnetic susceptibility measurements were carried out on finely ground polycrystalline samples (nujol restricted) of **1** with the use of a Quantum Design SQUID (superconducting quantum interference device) magnetometer MPMS-XL. Experimental data were corrected for the sample holder and for the diamagnetic contribution calculated from Pascal constants.^[22] Hysteresis loops on a single crystal were performed with an array of micro-SQUIDS.^[17] The ac susceptibility measurements on single crystals of the order of 0.1 mm were performed with a home-built Hall probe susceptometer that works at temperatures between 0.030 and 30 K, frequencies between 1 and 10^6 Hz , and applied fields up to 1.4 T.

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Controlled NO Transfer

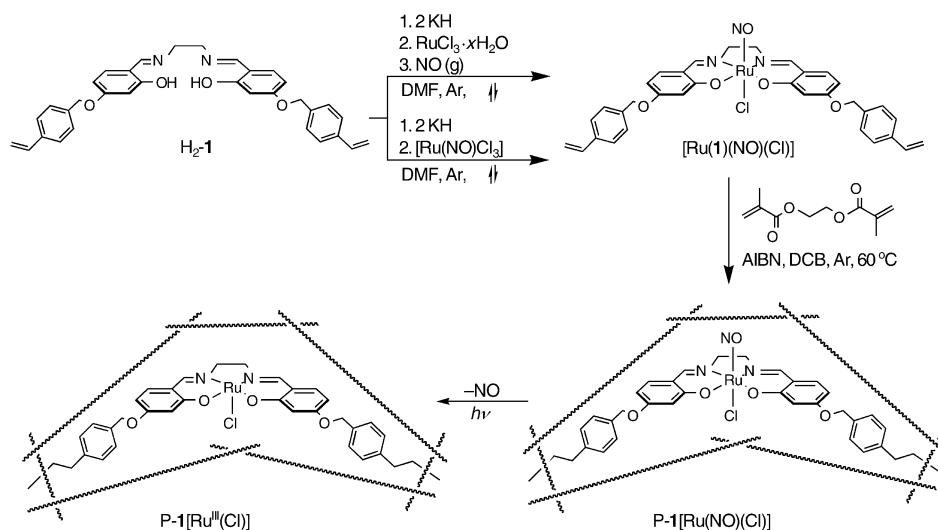
Light-Activated Transfer of Nitric Oxide from a Porous Material**

Jeremy T. Mitchell-Koch, Timothy M. Reed, and A. S. Borovik*

Nitric oxide (NO) is an important biomolecule in mammalian biology, with a range of effects that include regulatory functions in the cardiovascular, respiratory, and nervous systems.^[1] The linkage of abnormal NO levels to numerous diseases^[1,2] and the potential use of NO as an anti-biofouling agent for in vivo medical devices^[3] further underscores the need for new storage–release systems. There is ample evidence that a diverse group of systems will be necessary to meet therapeutic demands.^[4] One continuing challenge is the preparation of materials that controllably release NO without leaching toxic by-products.^[3,5] A promising approach for such materials utilizes covalently immobilized metal nitrosyl (M–NO) complexes within porous polymeric hosts.^[6] These materials combine the properties of the M–NO units, such as reversible NO binding, with those of the porous hosts. We report herein the synthesis and properties of a highly cross-linked, network polymer containing immobilized Ru–NO sites. Light triggers the controlled release of NO, as demonstrated by the transfer of NO in solution to a metalloporphyrin and myoglobin.

We have previously described a material containing covalently immobilized cobalt(II) bis[2-hydroxy-4-(4-vinylbenzyloxy)benzaldehyde]ethylenediimine complexes ([Co(**1**)], see Scheme 1 for H₂-**1**) within porous hosts for the storage and release of NO.^[6] This material, P-**1**[Co(**1**)], bound NO rapidly and selectively with slow release under ambient conditions (about 40 % removal of NO over 14 days). It was prepared by a template copolymerization method, similar to that used to make molecularly imprinted polymers.^[7] Molecular precursors are used in polymerization, since this allows for structural manipulation of the immobilized sites. In our design,^[6,8] substitutionally inert metal complexes containing polymerizable groups are used as templates to form the immobilized sites: Multiple covalent connections of the metal–NO template complex to the polymeric hosts minimizes leaching. Monomeric Ru–NO complexes with ligands related to **1**^{2–} photolytically transfer NO in solution.^[9] We reasoned that the Ru–NO complex with **1**^{2–} could be prepared and utilized as a template to make a new material containing NO. Therefore, the template complex [Ru(**1**)(NO)(Cl)] was synthesized and used to produce the corresponding porous methacrylate polymer P-**1**[Ru(NO)(Cl)], a material with which controllable transfer of NO is achieved with light.

The two methods for preparing [Ru(**1**)(NO)(Cl)] are outlined in Scheme 1. The use of NO and RuCl₃ follows literature methods and gives yields of about 45 %. A second method uses [Ru(NO)Cl₃] and produces [Ru(**1**)(NO)(Cl)] in a slightly higher yield of 54 %. This latter method is advanta-



Scheme 1. Preparative routes for [Ru(**1**)(NO)(Cl)], P-**1**[Ru(NO)(Cl)], and P-**1**[Ru(Cl)]. AIBN = azobisisobutyronitrile, DCB = 1,2-dichlorobenzene.

[*] Dr. J. T. Mitchell-Koch, T. M. Reed, Prof. A. S. Borovik
Department of Chemistry
University of Kansas
2010 Malott Hall, 1251 Wescoe Drive
Lawrence, KS 66045-7582 (USA)
Fax: (+1) 785-864-5396
E-mail: aborovik@ku.edu

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

geous because it does not require the use of NO gas during synthesis. Data from NMR, electronic absorbance, and FT-IR spectroscopy as well as mass spectrometry, are consistent with the formulation of this monomer as [Ru(**1**)(NO)(Cl)] (see the Supporting Information).

[Ru(**1**)(NO)(Cl)] contains polymerizable styryloxy groups for covalent immobilization within a porous network polymer. The copolymerization procedure for preparing P-**1**[Ru(NO)(Cl)] employs [Ru(**1**)(NO)(Cl)], ethylene glycol dimethacrylate (EGDMA) as the cross-linking agent, the

radical initiator AIBN, and 1,2-dichlorobenzene as the porogen (Scheme 1). The monomer $[\text{Ru}(\text{I})(\text{NO})(\text{Cl})]$ is stable towards NO release under the reaction conditions, as long as light is absent during polymerization. A monolithic solid was isolated, which was washed continuously with CH_2Cl_2 , pulverized, and sieved to an appropriate particle size. Particles between 125 and 75 μm have an average pore diameter of 60 $\text{\AA}$, a pore volume of 0.63 mL per gram of polymer, and a surface area of 400 m^2 per gram of polymer.

The spectroscopic analysis of $\text{P-1}[\text{Ru}(\text{NO})(\text{Cl})]$ indicates that the Ru–NO complex remains intact during polymerization. The FT-IR spectrum of $\text{P-1}[\text{Ru}(\text{NO})(\text{Cl})]$ shows a band at 1824 cm^{-1} , which is assigned to the NO stretch,^[10] and the electronic absorption spectrum of the polymer suspended in toluene has an absorbance band at $\lambda_{\text{max}} = 373\text{ nm}$. Furthermore, $\text{P-1}[\text{Ru}(\text{NO})(\text{Cl})]$ is EPR silent at 77 and 4 K, consistent with antiferromagnetic coupling between the unpaired electron of NO and the low-spin $d^5\text{ Ru}^{\text{III}}$ center. The spectroscopic features found for $\text{P-1}[\text{Ru}(\text{NO})(\text{Cl})]$ are nearly identical to those for the monomeric precursor $[\text{Ru}(\text{I})(\text{NO})(\text{Cl})]$ (see the Supporting Information).

Photolytic release of NO is observed for $\text{P-1}[\text{Ru}(\text{NO})(\text{Cl})]$, and broad-band irradiation with a Hg arc lamp converts $\text{P-1}[\text{Ru}(\text{NO})(\text{Cl})]$ into $\text{P-1}[\text{Ru}(\text{Cl})]$. This process was monitored with EPR spectroscopy (Figure 1).

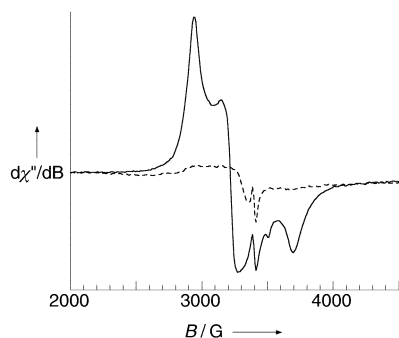


Figure 1. The X-band EPR spectra (4 K) for $\text{P-1}[\text{Ru}(\text{NO})(\text{Cl})]$ (----) and after broad-band irradiation for 2 h to form $\text{P-1}[\text{Ru}^{\text{III}}(\text{Cl})]$ (—).

The X-band EPR spectrum of photolyzed $\text{P-1}[\text{Ru}(\text{NO})(\text{Cl})]$ contains a rhombic signal with g values at 2.3, 2.1, and 1.8, which is indicative of immobilized ruthenium(III) complexes with $S = 1/2$ ground states (i.e., Ru^{III} complexes that have lost NO). This spectrum is identical to that of $\text{P-1}[\text{Ru}(\text{Cl})]$ prepared independently (see the Supporting Information).

Nitric oxide is also photoreleased from suspensions of $\text{P-1}[\text{Ru}(\text{NO})(\text{Cl})]$ in various solvents. For instance, irradiation of $\text{P-1}[\text{Ru}(\text{NO})(\text{Cl})]$ suspended in toluene with 370 nm light results in the formation of $\text{P-1}[\text{Ru}(\text{Cl})]$, which has characteristic absorbance bands at $\lambda_{\text{max}} = 400\text{ nm}$ (shoulder) and 660 nm. The photolysis of NO appears to be dependent on the solvent. Nitric oxide loss from $\text{P-1}[\text{Ru}(\text{NO})(\text{Cl})]$ is slower from suspensions in noncoordinating solvents, including CH_2Cl_2 , toluene, and cyclohexane, than in coordinating solvents, such as acetonitrile and water. A likely explanation for this observation is that coordinating solvents bind to the site on the ruthenium center left vacant after photolysis,

which prevents NO rebinding, thus hastening release. Similar observations were found for solution studies of related monomeric Ru–NO complexes.^[9]

Irradiation of a suspension of $\text{P-1}[\text{Ru}(\text{NO})(\text{Cl})]$ at 370 nm causes the heterogeneous transfer of NO to cobalt(II) tetraphenylporphyrin ($[\text{Co}^{\text{II}}(\text{TPP})]$) dissolved in CH_2Cl_2 . The photolytic transfer was monitored with electronic absorbance spectroscopy (Figure 2) and shows clean conver-

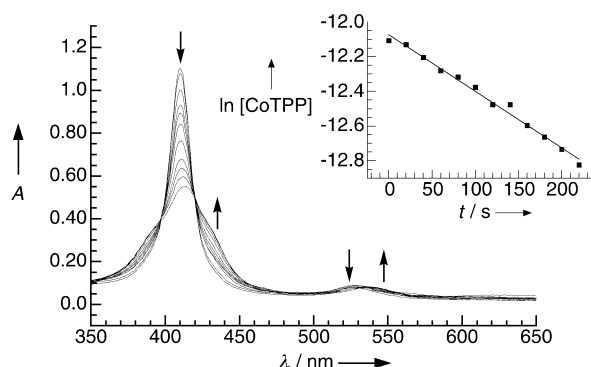


Figure 2. Overlaid electronic absorption spectra demonstrating the photolytic transfer of NO from $\text{P-1}[\text{Ru}(\text{NO})(\text{Cl})]$ to $5.5 \times 10^{-6}\text{ M}$ $[\text{Co}^{\text{II}}(\text{TPP})]$ in CH_2Cl_2 . Each spectrum was collected after 20 s of irradiation at $\lambda = 370\text{ nm}$. Inset: Plot used to determine a first-order rate constant of $k_1 = 0.0030\text{ s}^{-1}$. A = absorbance.

sion of $[\text{Co}^{\text{II}}(\text{TPP})]$ into $[\text{Co}^{\text{II}}(\text{TPP})(\text{NO})]$ with sharp isosbestic points at $\lambda_{\text{max}} = 397, 420,$ and 425 nm . The NO transfer to $[\text{Co}^{\text{II}}(\text{TPP})]$ is completed in less than 20 min and gives an apparent first-order rate constant k_1 of 0.0030 s^{-1} (inset of Figure 2). This rate is slower than that found for homogeneous NO transfer from the monomer $[\text{Ru}(\text{I})(\text{NO})(\text{Cl})]$ ($k_1 \approx 0.01\text{ s}^{-1}$, see the Supporting Information).

Figure 3 shows an NO-release profile for aqueous suspensions of particulate $\text{P-1}[\text{Ru}(\text{NO})(\text{Cl})]$. Continuous irradiation for 50 min at pH 7.2 for particles smaller than 75 μm produces an increase in the amount of NO released to a maximum of $15.5(4)\text{ }\mu\text{mol}$ of NO per gram of polymer (19% yield);^[11] no additional release was observed at longer irradiation times (Figure 3a). $\text{P-1}[\text{Ru}^{\text{III}}(\text{Cl})]$ was also produced from this photolysis and gave the expected EPR signal, whose spin concentration is $14(1)\text{ }\mu\text{mol}$ of Ru^{III} sites per gram of polymer. Figure 3b illustrates that larger particles (0.5 to 1.0 mm) produce a maximum of $2.0(4)\text{ }\mu\text{mol}$ of NO per gram of polymer after 80 min of irradiation. To demonstrate that release is only caused during irradiation, light-dark-light sequences were examined (Figure 3b). The amount of NO released correlated with the time period of irradiation, and no release was observed during the dark phase. The amount of NO released in these studies was monitored indirectly by determining the nitrite concentration with the Griess spectrophotometric method,^[12] and shows that photolysis of $\text{P-1}[\text{Ru}(\text{NO})(\text{Cl})]$ gives controllable NO production in an aqueous medium at physiological pH.

Equine skeletal muscle myoglobin (Mb) was used to establish that $\text{P-1}[\text{Ru}(\text{NO})(\text{Cl})]$ can transfer NO to proteins. Many heme proteins, such as Mb, bind NO.^[13] Moreover, Mb has been implicated as an in vivo NO scavenger^[14] because of

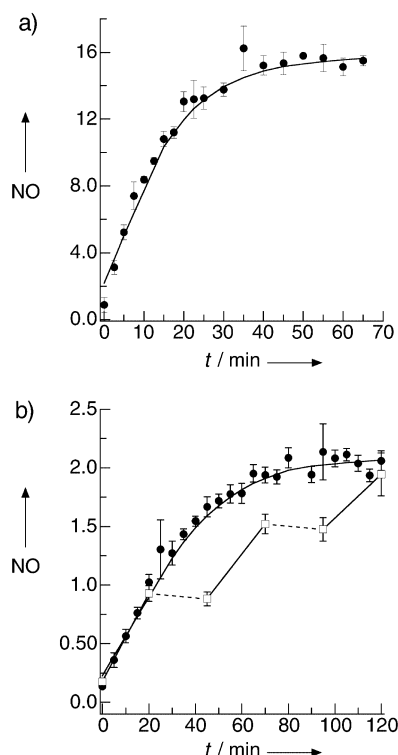


Figure 3. Plots of the release of NO (in $\mu\text{mol NO}$ per gram of polymer) as a function of time for particles a) smaller than $75\ \mu\text{m}$ and b) between 0.5 and $1\ \text{mm}$. (●) Continuous irradiation, (□) light-dark-light sequences, (—) irradiation phases, (----) dark phases.

its high concentrations in cardiac cells and striated muscles, both of which use NO as an intercellular messenger.^[15] Irradiation of P-1[Ru(NO)(Cl)] with $370\ \text{nm}$ light at pH 7.2 converted 81 % of reduced Mb into Mb(NO) within 20 min. The NO transfer is illustrated in Figure 4 by the shift in the Mb Soret band from $\lambda_{\text{max}} = 435$ to $420\ \text{nm}$. Nearly identical shifts were reported for Mb and Mb(NO);^[16] furthermore, control spectra of equine skeletal muscle Mb and Mb(NO) display identical properties. The spectral data corresponded to a first-order rate constant of $k_1 = 5.0 \times 10^{-4}\ \text{s}^{-1}$ (inset of Figure 4).

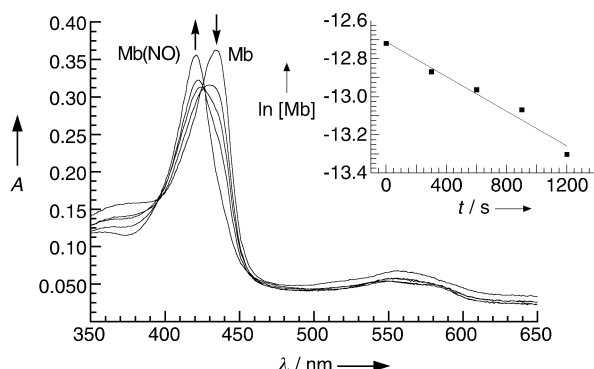


Figure 4. Overlaid electronic absorption spectra demonstrating the photolytic transfer of NO from P-1[Ru(NO)(Cl)] to $3.0 \times 10^{-6}\ \text{M}$ Mb in $50\ \text{mM}$ phosphate buffer, pH 7.2. Each spectrum was collected after 5 min of irradiation at $\lambda = 370\ \text{nm}$. Inset: Plot used to determine a first-order rate constant of $k_1 = 5.0 \times 10^{-4}\ \text{s}^{-1}$. A = absorbance.

Our findings with P-1[Ru(NO)(Cl)] for the release and transfer of NO illustrate that photolytic processes can be incorporated into biomaterials to controllably dispense this biologically important compound. While photolytic release of NO has been reported from compounds^[9,17,18] and a self-assembled monolayer,^[19] our findings represent the first example, to our knowledge, in which light triggers NO release from a material. A photorelease mechanism as shown for P-1[Ru(NO)(Cl)] complements those reported for other materials, in particular materials containing diazeniumdiolates, which control NO release by chemical manipulations. Materials such as P-1[Ru(NO)(Cl)] can potentially be used to accurately distribute NO to specific targets for in vitro and in vivo studies.

Experimental Section

P-1[Ru(NO)(Cl)]: Under an argon atmosphere, [Ru(1)(NO)(Cl)] ($0.0745\ \text{g}$, $0.107\ \text{mmol}$, $5\ \text{mol}\%$), EGDMA ($0.40\ \text{g}$, $2.0\ \text{mmol}$, $94\ \text{mol}\%$), and AIBN ($0.0040\ \text{g}$, $0.021\ \text{mmol}$, $1\ \text{mol}\%$) were combined in a thick-wall polymerization tube (Ace Glass). The porogen DCB ($1.5\ \text{g}$) was added to the mixture, and the tube was sealed, covered with aluminum foil, and heated to 60°C . After 24 h a brown monolithic polymer was obtained and continuously extracted with CH_2Cl_2 overnight. The resulting polymer was pulverized in a mortar and pestle, sieved to a particle size between 0.5 and $1.0\ \text{mm}$, and dried under reduced pressure to yield $0.482\ \text{g}$ of P-1[Ru(NO)(Cl)].

NO release studies: Suspensions of P-1[Ru(NO)(Cl)] particles were prepared by adding $2\ \text{mL}$ aliquots of phosphate buffer solution ($50\ \text{mM}$, pH 7.2) to P-1[Ru(NO)(Cl)] ($20\ \text{mg}$) in Pyrex test tubes, which were sealed and stored in the dark. Samples were shaken and exposed to light at $\lambda = 350\ \text{nm}$ ($\approx 24\ \text{W}$) from a Rayonet photochemical reactor for defined time periods, after which a sample ($50\ \mu\text{L}$) was transferred to a well of a 96-well microtiter plate. Each sample well was treated with components of the Griess reagent system (Promega). The amount of nitrite was determined spectrophotometrically with a SpectraMax 190-plate reader (Molecular Devices) monitoring absorbance at $520\ \text{nm}$.

NO transfer studies: In a typical experiment, a solution of the NO acceptor with known concentration ($\approx 10\ \text{mg}$ for [Co(TPP)], $\approx 100\ \text{mg}$ for Mb) and P-1[Ru(NO)(Cl)] particles (0.5 – $1\ \text{mm}$) were placed in a 1.00-cm Suprasil quartz cuvette and sealed under an inert atmosphere with a rubber septum. The sample was irradiated with a broad emission Hg arc lamp (Oriel) equipped with a $370\ \text{nm}$ wavelength band pass filter (Omega Optical). Spectra were collected in defined intervals and recorded on a Varian Cary 50 spectrophotometer at room temperature. Reduced equine skeletal muscle Mb was prepared from metMb (Sigma) using dithionite (≈ 1.2 equiv).

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Enzymatic Synthesis of Redox-Labeled RNA and Dual-Potential Detection at DNA-Modified Electrodes**

Daniel A. Di Giusto, Wjatschesslaw A. Wlassoff,
Susanne Giesebrecht, J. Justin Gooding, and
Garry C. King*

Electrochemical techniques offer attractive approaches to the detection and identification of nucleic acids, a central activity of modern chemical biology. High sensitivity,^[1,2] low instrumentation costs, a ready capacity for miniaturization,^[3,4] and direct electronic readout offer strong motivations for the development of this field.^[5–7] Various approaches have been explored, including conjugation of DNA oligonucleotides with electroactive reporters,^[8–10] use of soluble electroactive mediators or intercalators,^[11–14] redox enzyme mediation,^[15] and measurement of direct label-free electrochemical processes^[16,17] or field effects.^[18,19] All of these methods rely critically upon hybridization of complementary nucleic acid sequences for specific recognition while some utilize charge transport through the π -stack of duplex DNA.^[20] Of the known methods, explicit electroactive labeling has the combined advantages of a positive detection signal, a low background, and the ability to introduce several electrochemically distinguishable tags.^[9,21]

The utility and fidelity of nucleic-acid-modifying enzymes such as polymerases, ligases, and nucleases is widely exploited in common genotyping approaches^[22] but has been relatively underutilized in electrochemical methods of nucleic acid detection. Moreover, amplification and labeling of RNA by polymerases has been central to the development of gene expression analysis. A number of steps must be taken to integrate electrochemistry and molecular biology more effectively. Stable, enzyme-compatible, electrochemically discrete labels, robust detection interfaces, and sensitive and

[*] D. A. Di Giusto, Dr. W. A. Wlassoff, S. Giesebrecht, Dr. G. C. King
School of Biotechnology and Biomolecular Sciences
The University of New South Wales
Sydney NSW 2052 (Australia)
Fax: (+61) 2-9385-1483
E-mail: garry@kinglab.unsw.edu.au
Dr. J. J. Gooding
School of Chemical Sciences
The University of New South Wales
Dr. W. A. Wlassoff
Institute of Cytology and Genetics
Lavrientev Ave. 10
Novosibirsk 630090 (Russia)
S. Giesebrecht
Botanisches Institut der Universität Bonn
Venusbergweg 22, 53115 Bonn (Germany)

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selective assay methods must all be developed. In pursuit of these goals, electrochemically labeled nucleoside triphosphates for enzymatic production of nucleic acids have recently been introduced. Electroactive deoxy-,^[23–26] dideoxy-,^[27] and acyclic nucleotides^[26,28] have been described. Although detection of electroactive nucleic acids can be achieved with high-performance liquid chromatography or capillary electrophoresis combined with an electrochemical detector (ECD-HPLC, ECD-CE),^[8,9] these transport methods are not necessarily the most suitable for miniaturized parallel analysis. Self-assembled monolayers (SAMs) of DNA oligonucleotide probes attached to a conductive substrate such as gold have been developed as electrode surfaces for nucleic acid hybridization and electrochemical analysis.^[4,26,29,30] Because each electrode monolayer is analogous to a spot on a microarray, this is a good candidate system for the required level of miniaturization and parallelization.^[31]

Although most electrochemical analyses have focussed upon DNA, the ease of linear amplification, high heteroduplex stability, and ready fragmentation offered by RNA are highly advantageous to analysis. RNA has previously been detected electrochemically, but earlier methods were not designed to identify specific transcripts, only bulk RNA extracts.^[8,32] Other schemes have measured specific transcript levels, but only following conversion of RNA into DNA by reverse-transcriptase-PCR.^[4,33] Here we describe two electrochemically distinct, enzyme-compatible ribonucleoside triphosphates (“electrotides”), which are incorporated into RNA transcripts and detected specifically at DNA-functionalized electrodes.

Ferrocene- and anthraquinone-labeled UTP derivatives ((Fc1-UTP (**1**) and Aq1-UTP (**2**), respectively, Figure 1a) were synthesized by coupling 5-(3-aminopropen-1-yl)uridine-5'-triphosphate with the carboxylic acids of the electroactive species using HATU as a condensing reagent. Yields for both triphosphate products were 20–30% after ion-exchange chromatography. Product integrity was confirmed by UV absorption, ¹H NMR spectroscopy, and cyclic voltammetry (CV). Fc1-UTP displayed a half-wave potential ($E_{1/2}$) of +375 mV vs Ag/AgCl with a near-ideal peak separation (ΔE_{peak}) of 75 mV. Aq1-UTP showed a reproducible quasi-reversible electrochemical behavior at a glassy carbon electrode, with $E_{1/2} = -570$ mV and $\Delta E_{\text{peak}} = 342$ mV. Free anthraquinonecarboxylate ($E_{1/2} = -460$ mV and $\Delta E_{\text{peak}} = 297$ mV) displayed a similar quasi-reversible behavior at this surface.

To determine the compatibility of **1** and **2** with the RNA polymerases conventionally employed for in vitro transcription reactions, a 232-basepair DNA template derived from the human PAI-2 coding sequence was generated by PCR. This template, which contained opposing T7 and SP6 promoter sequences for bidirectional transcription, consistently yielded a discrete major RNA transcript approximately 408 nucleotides (nt) in length from either promoter alone (Figure 1b). The predominance of this long transcript indicated efficient self-templated RNA extension of the kind described elsewhere,^[34] yielding an essentially double-stranded looped RNA product (Figure 1c). Substrates **1** and **2** were similarly accommodated by T7 and SP6 RNA polymerases, displaying

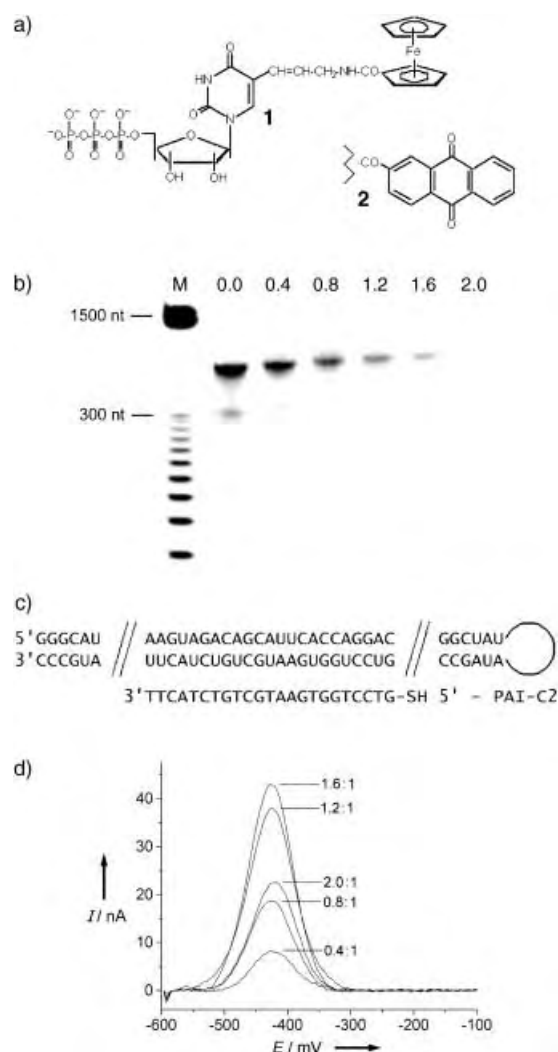


Figure 1. a) Electrotide constructs **1** and **2**. b) In vitro transcription of PAI-2 RNA at Fc1-UTP:UTP ratios between 0.0:1 and 2.0:1. Molecular weight markers (M) are indicated. c) Central region of the 408-nt PAI-2 RNA transcript with the sequence of the PAI-C2 DNA capture oligonucleotide. d) Baseline-corrected OSW voltammograms of PAI-2 RNA transcripts at various Aq1-UTP:UTP ratios.

significant incorporation over a broad concentration range (Figure 1b). Increasing the modified:unmodified UTP ratio in the transcription mixture produced more-heavily labeled transcripts as judged by the decreased electrophoretic mobility of the RNA product, an effect which is directly attributable to the higher molecular weight of the labeled nucleotide. Greater labeling was accompanied by lower RNA yields (Figure 1b), consistent with the known discrimination of phage RNA polymerases against most modified nucleoside triphosphate substrates.

For electrochemical detection of labeled RNA, self-assembled monolayers of the 5'-thiolated PAI-C2 oligonucleotide (Figure 1c) and 6-sulfanylnhexanol were prepared on gold electrodes. Following hybridization of redox-labeled RNA and brief washing in PBS (phosphate-buffered saline), Osteryoung square-wave voltammetry (OSWV) of PAI-C2-derivatized electrodes produced a clear peak corresponding

to the correct electroactive moiety. Hybridization of RNA products from transcription reactions performed at several labeled:unlabeled UTP ratios yielded an optimal signal at an Aq1-UTP:UTP ratio between 1.2:1 and 1.8:1 (Figure 1 d). For Fc1-UTP, the optimal ratio was very similar despite the large difference in the structures of the electroactive groups. The cause of this optimal labeled:unlabeled UTP ratio lies directly in the association of greater labeling density with lower RNA yields (Figure 1 b). Subsequent experiments employed labeled:unlabeled UTP ratios within the optimal range.

To verify the specificity of the detector interface, Fc1-U-labeled RNA was also incubated with electrodes bearing the noncomplementary oligonucleotides 2A and EP. Neither noncomplementary oligonucleotide yielded a discernable signal under conditions where the complementary PAI-C2 oligonucleotide gave a strong response (Figure 2 a). The detector interface is thus selective for complementary RNA sequences carrying either electrochemical moiety. To examine the concentration dependence of the interface, 1.2:1-labeled PAI-2 RNA was incubated at various concentrations with PAI-C2 electrodes. A plot of voltammogram peak area vs. RNA level (Figure 2 b) appears to curve somewhat, which suggests the onset of electrode binding site saturation at high RNA levels. Approximately 200 pM RNA, equivalent to 20 fmol or 3 ng, could be reliably detected, a value that compares favorably with that of an alternative electrochemical RNA detection method.^[32] In relation to the electrode surface area, 200 pM RNA is equivalent to 2.3 pmol RNA per cm². Because the present test system involves a worst-case hybridization where the RNA target is double stranded, we expect substantially better sensitivity with further development. Recent studies of ferrocene-modified oligonucleotides on electrode surfaces indicate that attomole sensitivities should be achievable.^[35] In future studies we will investigate the sensitivity improvements to be obtained by controlling monolayer size, regularity, oligonucleotide coverage, and hybridization conditions.

In gene expression analysis, the common two-color fluorescence implementation compares the signals from treatment and control RNA samples labeled with two different fluorophores. An equivalent electrochemical implementation requires co-detection of two electrochemically distinguishable redox groups. Although different redox potentials can be produced by altering the linker or substituents on a single electroactive species, this often does not yield baseline signal resolution, requiring sophisticated AC voltammetry for signal discrimination.^[9,21] As an alternative approach we have employed the two distinct electrochemical moieties of Fc1- and Aq1-UTP to achieve complete signal separation. To test this system, independent Aq1- and Fc1-RNA pools were separately transcribed from an identical template sequence at a labeled:unlabeled UTP ratio of 1.4:1. The two RNAs were mixed at different ratios and hybridized to electrodes bearing the complementary oligonucleotide probe. Characteristic signals for both the Fc1- and Aq1-labeled RNAs were observed with baseline peak separation (Figure 2 c), even in the presence of large amounts of RNA. Moreover, the respective peak areas permitted to measure the relative abundance of each labeled RNA. Although the two electro-

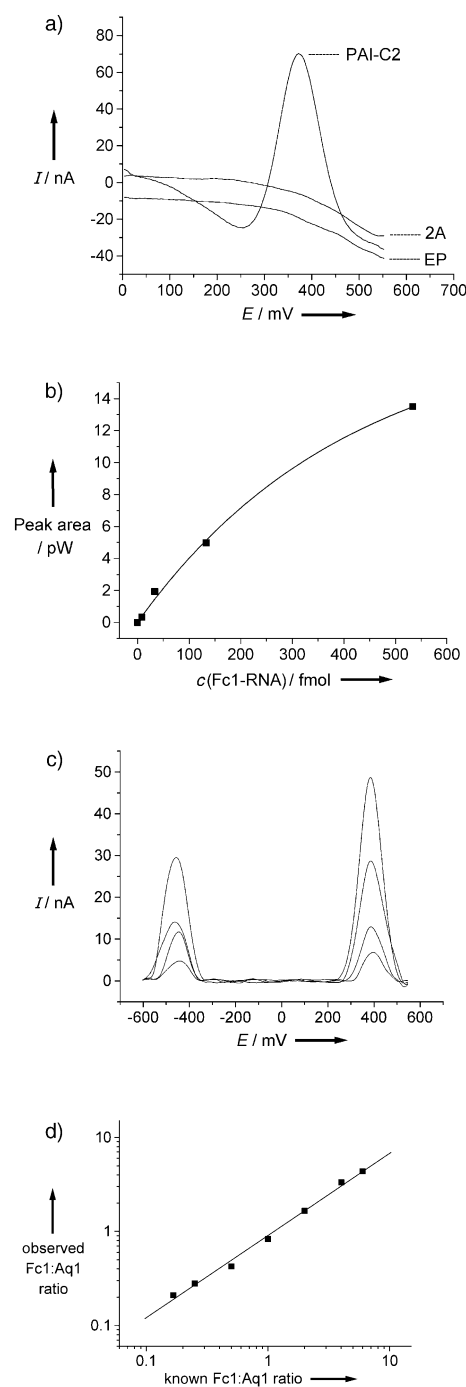


Figure 2. a) Uncorrected OSW voltammogram following incubation of Fc1-U-labeled RNA with electrodes bearing the complementary PAI-C2 oligonucleotide or the noncomplementary 2A or EP oligonucleotides. b) Target dependence of the binding of labeled PAI-2 RNA to PAI-C2-modified electrodes. c) OSW voltammograms of Fc1-U- and Aq1-U-labeled RNAs co-hybridized to electrodes. Traces derived from Fc1-RNA:Aq1-RNA ratios of 6:1, 2:1, 1:2, and 1:6 are shown. d) Comparison of known Fc1-RNA:Aq1-RNA ratios applied to electrodes with observed Fc1:Aq1 OSW voltammogram peak area ratios. The line of best fit is shown.

active groups were detected with slightly differing efficiencies, there was a strong linear correlation between the known Fc1-RNA:Aq1-RNA ratios applied to the electrodes and the

OSW voltammogram peak area ratios (Figure 2d), indicating that these two groups are compatible with RNA quantitation.

We have described the direct, specific and quantitative detection of electroactive RNA in an electrode-based format amenable to miniaturization and parallelization. The use of readily synthesized, polymerase-compatible, electrochemically distinct electrotides broadens the application of electrochemical detection methods to nucleic acids. With further development of nucleotides, interfaces, and detection methods, we expect this approach to be useful not only for the co-detection of same-sequence transcripts, but also to the development of electrochemical RNA biosensors for a variety of applications including detection of single nucleotide polymorphisms (SNPs) and genome identification.

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Photochromism

Reductive Electrochemical Cyclization of a Photochromic 1,2-Dithienylcyclopentene Dication**

*Brian Gorodetsky, Hema D. Samachetty, Robert L. Donkers, Mark S. Workentin, and Neil R. Branda**

The construction of functional molecular devices requires access to molecular components with physical properties that can be reversibly modified by applying one or more external stimuli. Molecular isomerization reactions triggered by a physical stimulus such as heat, light, electricity, or through the

[*] B. Gorodetsky, H. D. Samachetty, Prof. N. R. Branda
Department of Chemistry
Simon Fraser University
8888 University Drive, Burnaby, BC V5A 1S6 (Canada)
Fax: (+1) 604-291-3765
E-mail: nbranda@sfu.ca

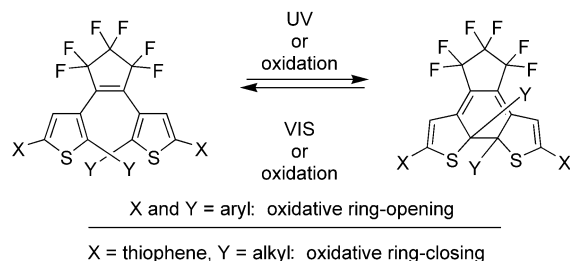
R. L. Donkers, Prof. M. S. Workentin
Department of Chemistry
The University of Western Ontario
London, ON N6A 5B7 (Canada)

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remote complexation of ions or molecules have all been instrumental in the development of molecular systems that display active switching functions.^[1] Photochromic^[2] compounds containing the dithienylethene (DTE) backbone^[3] undergo thermally irreversible cyclization reactions between colorless and colored forms when stimulated with UV and visible light (Scheme 1). Because of the notable differences in



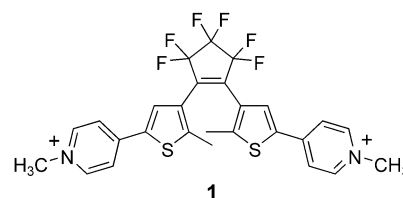
Scheme 1. The photo- and electrochemical (oxidation) ring-closing and ring-opening reactions of 1,2-dithienylethenes.

the nature of π conjugation between the two DTE isomers, these photoresponsive systems offer several choices of changes in properties such as absorbance, luminescence, refractive index, and redox potential, all of which are useful in photo-optical and photoelectronic device applications.

Developing examples of more sophisticated switching components that take advantage of more than one stimulus is an important goal. We have recently demonstrated that when several DTE derivatives are chemically or electrochemically oxidized, they undergo the same ring-closing and ring-opening reactions that are induced by light.^[4] Although the precise structural requirements and mechanism of these important electrochromic reactions are still to be fully defined, the tendency for ring closure or ring opening to dominate appears to be dictated by where aryl groups are located on the photochromic skeleton: the presence of aryl rings on the two carbon atoms involved in forming the new C–C single bond induces oxidative ring opening while their absence results in electrochemical cyclization of easily oxidized bis(dithiophene) derivatives (Scheme 1). This exciting new discovery prompted us to examine the electrochemical properties of other photochromic dithienylethene derivatives.

One such example is dicationic bispyridinium **1**, a versatile compound designed by Lehn and co-workers^[5] to demonstrate how the redox properties of DTE derivatives can be optically modulated due to the enhanced electronic communication between the two easily reduced pyridinium groups through the linearly π -conjugated DTE backbone in the ring-closed form. More recently, the groups of Irie^[6], Tian,^[7] and our group^[8] have used the unalkylated neutral dipyrindine precursor of **1** as a binary Lewis base to construct supramolecular coordination complexes. Herein we show that the electrochemical reduction of the ring-open isomer of dication **1** results in cyclization to generate the same ring-closed isomer as is produced photochemically as well as an additional ring-closed isomer that has never been observed.

Cyclic voltammetry was used to study the reduction of the ring-open isomer of dication **1**.^[9] Representative cyclic



voltammograms (CVs) are shown in Figure 1. The CV exhibits an irreversible cathodic reduction at -0.95 V versus a standard calomel electrode (SCE) at 0.2 V s⁻¹, and a smaller

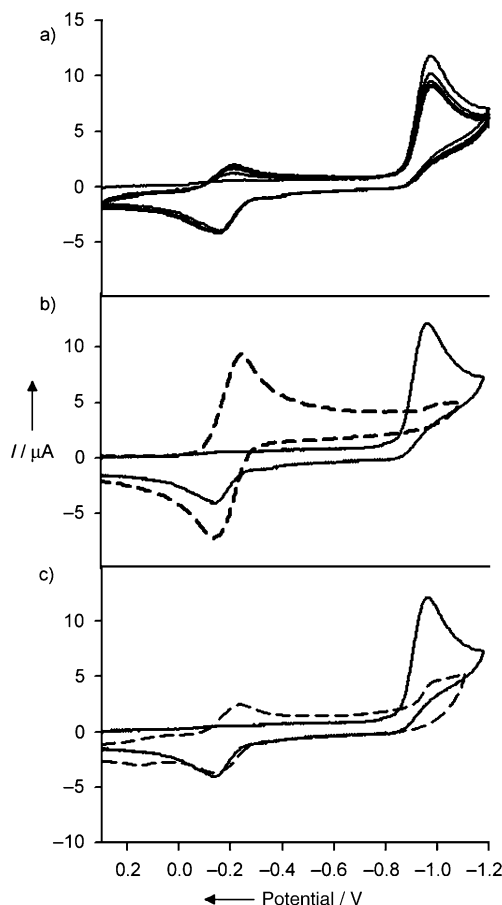


Figure 1. Cyclic voltammograms of DMF solutions (1.6×10^{-3} M) of ring-open isomer **1** containing tetrabutylammonium (0.1 M) perchlorate at 0.2 V s⁻¹ a) showing repetitive cycling through the irreversible reduction, b) before (solid line) and after (dashed line) irradiation with 365 nm light, and c) before (solid line) and after (dashed line) electrolysis at -0.9 V vs SCE.

anodic peak on the return scan. This latter anodic peak is part of a reversible couple formed in the initial reduction and it has a standard potential of -0.18 V versus SCE. This redox couple is illustrated in Figure 1a, which shows the result of repetitively cycling through the potential range. The standard potential of this couple is the same as that measured for the reduction of the ring-closed isomer of **1**. This is illustrated in

Figure 1 b, which shows CVs measured by using solutions of ring-open isomer of dication **1** before (solid line) and after irradiation with 365 nm light (dashed line). The photochemical ring closure of ring-open **1** is essentially quantitative and yields an equivalent of the ring-closed dication **1** (see Figures 2 a and d), thus the CVs before and after photolysis

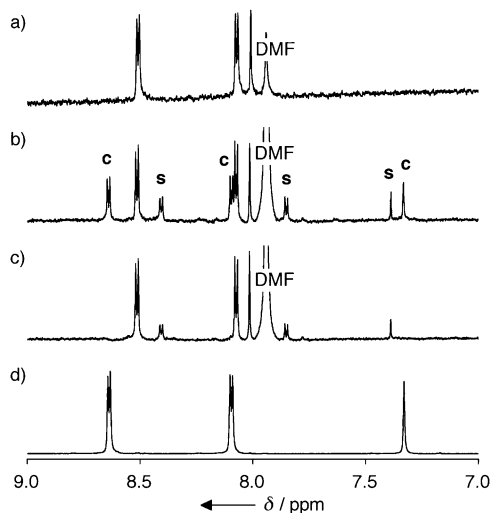


Figure 2. Partial ^1H NMR spectra of CD_3CN solutions of the ring-open isomer **1**, a) before electrolysis, b) after 10 min of electrolysis (-1.2 V) followed by quenching with AgPF_6 , c) after the electrolysis experiment followed by 10 min of irradiation ($> 490\text{ nm}$) and d) the PSS of **1** obtained by irradiating a solution of **1** with 365 nm light until no spectral changes were observed. Signals corresponding to the ring-closed isomer ('c') and the side product ('s') are highlighted in plot (b).

are indicative of equimolar solutions of the ring-open and ring-closed isomers of **1**, respectively.

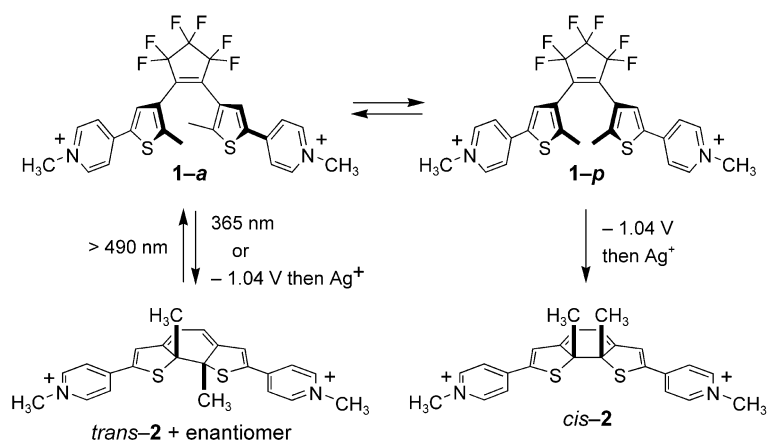
Electrolysis of the solution of ring-open **1** at the position of the irreversible reduction wave (ca. -0.9 V) consumes 1 F mol^{-1} , or one electron equivalent. Cyclic voltammograms recorded before and after 95% electrolysis are shown in Figure 1 c and illustrate the formation of the ring-closed isomer of **1** as the main electroactive (and soluble) product. This result was confirmed by a ^1H NMR spectroscopy study of the electrolysis mixture (discussed below). By comparing the relative peak currents of the ring-closed isomer of **1** formed after electrolysis (Figure 1 c) with that formed photochemically (Figure 1 b), the yield of electrochemically induced ring-closure can be estimated to be about 22%. These electrolysis experiments suggest that the ring-open isomer of dication **1** undergoes an electrochemically induced cyclization to its ring-closed counterpart via its radical cation. Although this process has never been reported in DTE systems, a reductive ring-closing reaction was reported for a fulgide derivative as early as 1984.^[11]

A UV/Vis absorption spectrum measured after electrolysis of **1** exhibits intense absorptions with maxima at 766 and

689 nm. These absorptions, presumably due to the radical cation of the ring-closed form of **1**, disappear on addition of silver ions and generate a deep-blue solution and a UV/Vis spectrum characteristic of the ring-closed form of the dication of **1**. The electrochemically induced cyclization is not quantitative and the majority (75%) of the radical cation of the ring-open form of **1** must be consumed in other reactions, such as radical cation dimerization or coupling reactions with the dication. During the electrolysis, there is a significant amount of a dark precipitate that forms and settles out of the solution, and this precipitate is likely to be made up of products formed by the reaction of the radical cation of the ring-open form of **1**.

The progress of the electrochemical ring closure can also be monitored by using ^1H NMR spectroscopy by treating the electrolysis reaction with an excess (10 equiv) of AgPF_6 to oxidize the paramagnetic products back to their dications.^[10] The results of these experiments are shown in Figure 2. After 10 minutes of electrolysis, signals arising from a substantial amount of the ring-closed isomer of **1** are observed in the ^1H NMR spectrum along with signals corresponding to an additional minor product that have similar chemical shifts (Figure 2 b). Irradiation of the postelectrolysis samples with light greater than 490 nm results in the regeneration of the ring-open form of **1** at the expense of its ring-closed counterpart, however, the side product is unaffected by this irradiation (Figure 2 c).

We are suggesting that this side product is the previously unobserved ring-closed compound *cis*-**2** (Scheme 2) although it has yet to be isolated. This ring-closed diastereomer has its two methyl groups in the *cis*-orientation and is not formed under photochemical conditions, as it can only result from the



Scheme 2. Photoexcitation ring closure of conformers **1-a** and **1-p** only produces the *trans*-stereoismer, while electrochemical ring-closure can produce both *cis* and *trans* products.

disrotatory pericyclic reaction of the parallel conformer, **1-p**, which has the two thiophene rings in mirror symmetry. Despite the fact that this conformer coexists in almost equal proportion to the antiparallel conformer, **1-a**, which has the two thiophene rings in a C_2 orientation, and the interconver-

sion rate is estimated to be much slower than the lifetimes of the photoexcited states, only photoexcited **1-a** can be converted to its ring-closed form *trans-2*.^[12] Irie and co-workers have shown that locking the dithienylethene skeleton into its parallel conformation completely prevents ring closure,^[13] while increasing the population of the antiparallel conformation enhances the extent of ring closure.^[14] Because the electrochemical ring-closing reaction proceeds via radical intermediates, it is not prone to the same limitations as the photochemical reaction and the formation of *cis-2* should be expected. It is, however, generated to a smaller extent than its *trans*-counterpart as it is less stable.^[15]

We offer the following evidence to support our claim. The side product is not a result of the electrolytic reaction pathways typically observed for viologen derivatives, including polymerization, demethylation to generate the pyridines, or α oxidation to form the corresponding pyridinones.^[16] All of these possibilities can be ruled out based on the fact that the ¹H NMR spectrum of the side-product does not resemble those of the mono-*N*-methyl and bis(pyridine) derivatives in either their ring-open and ring-closed forms, and the symmetry of the spectrum does not correspond to a pyridinone. The similarity of the ¹H NMR spectrum of the side product to that of the ring-closed isomer is one support for assigning the side product as *cis-2*. Another support is the fact that only one redox couple is observed in the CV experiment. As *cis-2* and *trans-2* would be expected to have similar (or identical) reduction potentials, they would appear as a single redox couple. Electrochemically formed polymers may be produced after extended periods of electrolysis and could be responsible for the precipitate observed in the electrolysis experiments.

Irie and co-workers^[17] as well as our group^[18] have reported that the ring-closed forms of some DTE derivatives undergo photochemical rearrangements to a non-photochromic isomer. Although the mechanism of this reaction is not fully understood, we rule out this rearrangement as a possibility in the case discussed here based on the thermal instability of both the side-product and the ring-closed isomer *trans-2*. When samples of the electrochemically generated products are heated to 80 °C for 3 h or left at room temperature for one week, the signals in the ¹H NMR spectrum corresponding to both ring-closed products disappear and only those corresponding to the ring-open isomer are observed. This thermal reaction would be highly unlikely for the known rearranged product and was not witnessed in either our or Irie's case. Another observation that supports this claim is that the ¹H NMR spectrum for the electrochemically generated side product does not display similarities with the rearranged photoproducts reported by Irie and co-workers as well as us. Current efforts are focused on isolating this side product and elucidating the precise mechanism for its formation. Both will be reported in due course.

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Keywords: chromophores · electrochemistry · isomers · molecular devices · photochromism

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Stereoselective Reduction

Asymmetric Hydrogenation of Ketones Catalyzed by Ru^{II}-bicp Complexes**

Daniel G. Genov* and David J. Ager

Enantiomerically pure secondary alcohols are among the most valuable key intermediates for the manufacture of pharmaceuticals and advanced materials. The simplest and most powerful way to produce chiral alcohols is the asymmetric hydrogenation of ketones.^[1] The most general and efficient catalyst system for the enantioselective hydrogenation of a variety of simple ketones reported so far is Noyori's homochiral Xylbinap/daipen/Ru^{II} combination with *i*PrOH as the solvent and in the presence of *t*BuOK.^[2] The high degree of enantioselectivity is a result of the synergistic effects of the chiral diphosphane and diamine ligands. The Ru complexes of the parent phosphane ligand of the series, binap (2,2'-bis(diphenylphosphanyl)-1,1'-binaphthyl), afford significantly lower selectivities in this reduction.^[2] Studies have been reported showing that Noyori's homogeneous hydrogenation follows a nonclassical mechanism, whereby a hydride on the Ru center and a proton of the NH₂ ligand are transferred simultaneously to the C=O function through a six-membered pericyclic transition state.^[2,3]

A few years ago Zhang et al. reported the synthesis of a new chiral 1,4-diphosphane, (2*R*,2'*R*)-bis(diphenylphosphanyl)-(1*R*,1'*R*)-dicyclopentane ((*R,R*)-bicp, **1a**) (Figure 1), and its application in asymmetric Rh- and Ru-catalyzed hydrogenations.^[4] High enantioselectivities are observed for the Ru-catalyzed hydrogenation of acetophenones when a chiral diamine is also used as a ligand for the metal.^[4b] One shortcoming for the general use of bicp as a ligand is that its original synthesis involves a hydroboration with Alpineborane, which is available as only one isomer.^[4] We have solved this problem by developing a different synthetic sequence that affords both bicp enantiomers.^[5] Our synthetic

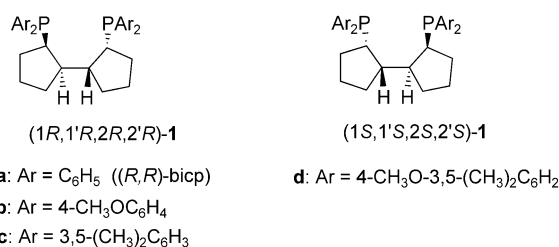


Figure 1. Chiral diphosphane ligands in the bicp family.

procedure also allowed the preparation of new phosphane ligands in the bicp family (**1b**, **1d**, Figure 1). This paper describes a method to access either enantiomer of a secondary alcohol through a bicp/Ru^{II} reduction of the corresponding prochiral ketone and the appropriate solvent.

The initial studies carried out in our laboratory showed that [RuCl₂](*R,R*)-bicp)(dmf)_{*n*} used in combination with an achiral 2-(alkylthio)amine such as 2-ethylthioaniline (**2a**, Figure 2) or an achiral diamine such as 4,5-dimethyl-1,2-

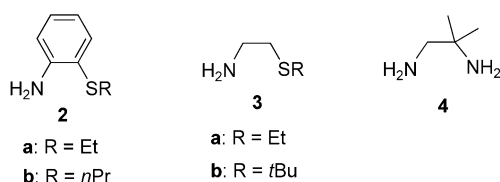
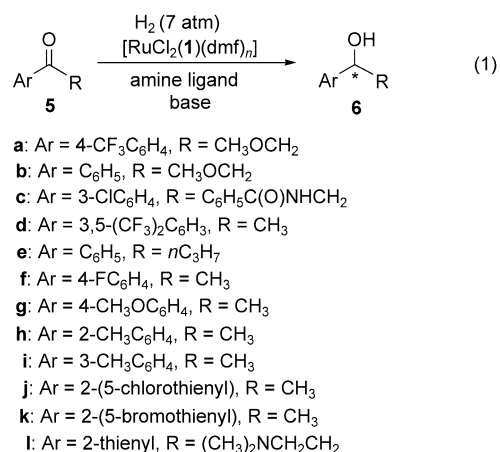


Figure 2. Achiral amine ligands.

phenylenediamine and *i*PrONa in *i*PrOH catalyzed the hydrogenation of various aryl alkyl ketones to give enantioselectivities similar to those achieved with chiral 1,2-diamines.^[6] However, the selectivities obtained, between 70 and 80 % *ee*, were still less than satisfactory. For comparison, very low selectivities were obtained when [RuCl₂](*S*)-binap(**2a**) or [RuCl₂](*S*)-MeObiphep(**2a**) (MeObiphep = 6,6'-dimethoxy-2,2'-bis(diphenylphosphanyl)-1,1'-biphenyl) were used as catalysts.^[6]

After these initial studies, we carried out a comprehensive investigation on the asymmetric hydrogenation of ketone **5a** [Eq. (1)] and observed unexpected solvent effects on the



[*] Dr. D. G. Genov,* Dr. D. J. Ager
DSM Pharma Chemicals
5900 NW Greenville Boulevard
Greenville, NC 27835 (USA)
E-mail: danielgenov@hotmail.com

[†] Present address:
Theravance, Inc.
901 Gateway Boulevard
South San Francisco, CA 94080 (USA)
Fax: (+1) 650-808-6120

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

enantioselectivity. These observations ultimately led us to optimize conditions to achieve excellent selectivities. The results of selected hydrogenation experiments with **5a** are listed in Table 1. When the hydrogenation of **5a** was carried

receptor antagonist (HIV entry inhibitor).^[7] The change of the diphosphane ligand to the more hindered (*S,S*)-**1d** (Figure 1) gave (*R*)-**6a** in a similar selectivity (97% *ee*, Table 1, entry 10).

Table 1: Asymmetric hydrogenation of 2-methoxy-4'-trifluoromethylacetophenone (**5a**) catalyzed by Ru/**1** complexes.^[a]

Entry	Ligand	Amine	S/C	Solv.	T [°C]	Conv. [%] ^[b]	<i>ee</i> [%] ^[c]
1	(<i>R,R</i>)- 1a	3a	200	MeOH	RT	> 99	80 (<i>R</i>)
2	(<i>R,R</i>)- 1a	3a	200	<i>i</i> PrOH	RT	> 99	8 (<i>R</i>)
3	(<i>R,R</i>)- 1a	3a	200	EtOH	RT	> 99	53 (<i>S</i>)
4	(<i>R,R</i>)- 1a	3b	200	MeOH	RT	> 99	53 (<i>S</i>)
5	(<i>R,R</i>)- 1a	3b	200	<i>i</i> PrOH	RT	> 99	61 (<i>S</i>)
6	(<i>R,R</i>)- 1a	3b	200	EtOH	RT	> 99	87 (<i>S</i>)
7	(<i>R,R</i>)- 1a	3b	200	<i>n</i> BuOH	RT	> 99	90 (<i>S</i>)
8 ^[d]	(<i>R,R</i>)- 1a	3b	500	<i>n</i> BuOH	−10	> 99	96 (<i>S</i>)
9	(<i>R,R</i>)- 1a	3b	1000	<i>n</i> BuOH	−10	98	95 (<i>S</i>)
10	(<i>S,S</i>)- 1d	3b	500	<i>n</i> BuOH	−10	> 99	97 (<i>R</i>)
11	(<i>R,R</i>)- 1a	2a	200	MeOH	RT	> 99	83 (<i>R</i>)
12	(<i>R,R</i>)- 1a	2a	200	EtOH	RT	> 99	83 (<i>R</i>)
13	(<i>R,R</i>)- 1a	2a	200	<i>i</i> PrOH	RT	> 99	74 (<i>R</i>)
14	(<i>R,R</i>)- 1a	4	200	MeOH	RT	25	70 (<i>R</i>)
15	(<i>R,R</i>)- 1a	4	200	EtOH	RT	> 99	85 (<i>R</i>)
16	(<i>R,R</i>)- 1a	4	200	<i>i</i> PrOH	RT	> 99	57 (<i>R</i>)

[a] Unless otherwise stated, reactions were carried out with 0.5 mmol **5a** and 7 atm H₂ pressure for 15 h. The catalyst was formed in situ by the addition of [RuCl₂(**1**)(dmf)_{*n*}] solution in the corresponding solvent, amine (molar ratio of substrate to amine = 50) and base (molar ratio of substrate to base = 25) to the reaction mixture. [b] The yields were determined by ¹H NMR and GC analysis. [c] The *ee* values were determined by chiral GC with γ-DEX 225 column. The absolute configurations were determined using authentic samples. [d] The reaction was carried out with 1 mmol **5a**. The yield of the product isolated by column chromatography was 99%.

out in MeOH using [RuCl₂((*R,R*)-**1a**)(dmf)_{*n*}] in combination with 2-ethylthioethylamine (**3a**) (Figure 2) as a catalyst and *t*BuONa as a base, the *R* enantiomer of **6a** was obtained with 80% *ee* (Table 1, entry 1). Changing the solvent from MeOH to EtOH resulted in the opposite enantiomer of **6a** (*S*) in 53% *ee*. In *i*PrOH the selectivity was very low (8% *ee* of (*R*)-**6a**) (Table 1, entry 2). It has been reported previously that the rate of the hydrogenation can be affected by changing the alcohol solvent,^[3b] but to our knowledge this is the first example of a reversal of the configuration of the product by simply changing the solvent from methanol to ethanol. When *tert*-butylthioethylamine (**3b**) (Figure 2) was used as the amine ligand, (*S*)-**6a** was obtained predominantly in all alcohol solvents (MeOH, *i*PrOH, *i*BuOH, EtOH, and *n*BuOH) at room temperature, but the enantioselectivities in EtOH and *n*BuOH were significantly higher (Table 1, entries 4–7). Thus, while (*S*)-**6a** was obtained in 61% *ee* when *i*PrOH was used as the solvent (Table 1, entry 5), the enantioselectivity rose to 90% *ee* when the hydrogenation was carried out in *n*BuOH (Table 1, entry 7). The enantioselectivity achieved in the hydrogenation of **5a** in *n*BuOH was further enhanced when the experiment was conducted at a lower temperature. Thus, when **5a** was hydrogenated in *n*BuOH at −10°C using Ru/(*R,R*)-**1a**/**3b** (molar ratio of substrate to catalyst = 500) as the catalyst and *t*BuONa as the base, (*S*)-**6a** was obtained in 96% *ee* (Table 1, entry 8). (*S*)-**6a** is a precursor for the synthesis of a second generation CCR5

each other, as each of them is efficient for asymmetric reduction of a different structural type of ketone substrate.

The amine **3b** is the best ligand for the asymmetric hydrogenations of α-functionalized ketones (**5a–c**). The selectivities obtained in the asymmetric hydrogenation of **5b** were analogous to those observed for the structurally similar **5a** (Table 2, entries 1 and 2). The asymmetric hydrogenation of ketone **5c** using [RuCl₂((*R,R*)-**1a**)(dmf)_{*n*}] (molar ratio of substrate to catalyst = 1000) in combination with **3b** and *t*BuONa in *n*BuOH at room temperature afforded the corresponding *S* alcohol (**6c**) in a quantitative yield with 96% *ee* (Table 2, entry 3). (*R*)-**6c** is a building block for the preparation of a very potent β3-adrenergic receptor agonist^[8] and can be obtained by using (*S,S*)-**1a**^[5] as the diphosphane ligand. In the hydrogenation of ketone **5c** the parent ligand **1a** leads to better selectivities than the more substituted **1d** (Table 2, entry 4).

The amine **3b** was also a very efficient ligand for the asymmetric hydrogenation of 3,5-bis(trifluoromethyl)acetophenone [**5d** in Eq. (1)]. The use of [RuCl₂((*S,S*)-**1d**)(dmf)_{*n*}] (molar ratio of substrate to catalyst = 1000) in combination with **3b** and *t*BuONa in *n*BuOH at −10°C gave the corresponding *S* alcohol (**6d**) in 93–94% *ee* (Table 2, entry 6). The *R* enantiomer of this alcohol, which can be obtained by using (*R,R*)-**1d**,^[5] is a precursor for the synthesis of potent NK₁ receptor antagonists.^[9] The enantioselectivity obtained with our system is the same as that obtained with

Table 2: Asymmetric hydrogenation of ketones **5b–l** catalyzed by Ru/**1** complexes.^[a]

Entry	Ketone	Ligand	Amine	S/C	Solv.	T [°C]	Conv. [%] ^[b]	ee [%] ^[c]
1	5b	(<i>R,R</i>)- 1a	3b	500	<i>n</i> BuOH	−10	> 99	93 (<i>S</i>)
2	5b	(<i>S,S</i>)- 1d	3b	500	<i>n</i> BuOH	−10	> 99	96 (<i>R</i>)
3 ^[d]	5c	(<i>R,R</i>)- 1a	3b	1000	<i>n</i> BuOH	RT	> 99	96 (<i>S</i>)
4	5c	(<i>S,S</i>)- 1d	3b	500	<i>n</i> BuOH	RT	> 99	93 (<i>R</i>)
5	5d	(<i>R,R</i>)- 1a	3b	1000	<i>n</i> BuOH	−10	> 99	89 (<i>R</i>)
6 ^[e]	5d	(<i>S,S</i>)- 1d	3b	1000	<i>n</i> BuOH	−10	> 99	93 (<i>S</i>)
7	5d	(<i>S,S</i>)- 1d	2b	1000	EtOH	RT	> 99	90 (<i>R</i>)
8	5e	(<i>R,R</i>)- 1a	2b	500	EtOH	RT	> 99	90 (<i>S</i>)
9	5e	(<i>S,S</i>)- 1d	2b	500	EtOH	RT	> 99	91 (<i>R</i>)
10	5f	(<i>R,R</i>)- 1a	2b	1000	EtOH	RT	> 99	87 (<i>S</i>)
11	5f	(<i>S,S</i>)- 1d	2b	1000	EtOH	RT	> 99	90 (<i>R</i>)
12	5g	(<i>R,R</i>)- 1a	2b	500	EtOH	RT	> 99	88 (<i>S</i>)
13	5g	(<i>S,S</i>)- 1d	2b	500	EtOH	RT	> 99	90 (<i>R</i>)
14	5h	(<i>R,R</i>)- 1a	2b	500	EtOH	RT	> 99	76 (<i>S</i>)
15	5h	(<i>R,R</i>)- 1c	2b	500	EtOH	RT	> 99	87 (<i>S</i>)
16	5i	(<i>R,R</i>)- 1a	2b	500	EtOH	RT	> 99	88 (<i>S</i>)
17	5i	(<i>R,R</i>)- 1c	2b	500	EtOH	RT	> 99	93 (<i>S</i>)
18	5j	(<i>R,R</i>)- 1a	4	200	EtOH	RT	> 99	94 (<i>S</i>)
19	5k	(<i>R,R</i>)- 1a	4	200	EtOH	RT	96	94 (<i>S</i>)
20 ^[f]	5l	(<i>R,R</i>)- 1a	4	2000	EtOH	RT	> 99	96 (<i>S</i>)

[a] Unless otherwise stated, reactions were carried out with 0.5 mmol **5** and 7 atm H₂ pressure for 15 h. The catalyst was formed in situ by the addition of [RuCl₂(**1**)(dmf)_n] solution in the corresponding solvent, amine (molar ratio of substrate to amine = 50), and base (molar ratio of substrate to base = 25) to the reaction mixture. [b] The yields were determined by ¹H NMR and GC analysis. [c] The *ee* values were determined by chiral GC with a γ-DEX 225 column. The absolute configurations were determined using authentic samples or by comparison of the sign of optical rotation and the retention times with those in the literature.^[4b, 6, 9a, 11] [d] The yield of the product isolated by column chromatography was 99%. The *ee* was determined by chiral HPLC using a Chiracel OD-H 150×4.6-mm column. [e] The reaction was carried out with 1 mmol **5d**. The product was isolated by column chromatography (99%). [f] The reaction was carried out with 5.5 mmol **5l**, molar ratio of substrate to amine = 100, and molar ratio of substrate to base = 50. The yield of the product isolated after column chromatography was 90%. The *ee* was determined by chiral HPLC using a Chiracel OD 250×4.6-mm column.

Corey's oxazaborolidine-catalyzed borane reduction of **5d**.^[9] When the hydrogenation of **5d** was carried out in EtOH using the same enantiomer of the diphosphane ligand **1d** as above (molar ratio of substrate to catalyst = 1000), but with another nonchiral (alkylthio)amine instead of **3b**, **2b** (Figure 2), the opposite *R* enantiomer of **6d** was obtained again with high *ee* (90% *ee*, Table 2, entry 7).

For ketones **5e–i** (mono *para*-, *ortho*-, and *meta*-substituted acetophenones without functional groups in α -position to the carbonyl function), **2b** (Table 2) was the amine ligand that combined with Ru/**1d**, and *t*BuONa in EtOH gave the highest enantioselectivities (90–93% *ee*). The substituent at the sulfur atom in the 2-(alkylthio)aniline ligand **2** was varied extensively for each ketone, and the highest enantioselectivities were achieved with **2b**. The enantioselectivities obtained in the reductions of ketones **5e–i** in EtOH were only 1–2% higher than those achieved with *i*PrOH as the solvent.

For the heteroaromatic ketones **5j–l** (Table 2, entries 18–20) containing thienyl substituents, the highest enantioselectivities (94–96% *ee*) were obtained in EtOH with Ru/**1a/4** complexes in the presence of *t*BuONa. The more substituted diphosphane ligands **1c,d** gave selectivities lower than those achieved with the parent ligand **1a**. When the 2-thienyl ketone **5l**, which possesses a β -dimethylamino group, was hydrogenated with the Ru/(*R,R*)-**1a/4** complex as a catalyst

(molar ratio of substrate to catalyst = 2000), (*S*)-**6l** was obtained in 96% *ee* and 99% yield (90% yield after column chromatography) (Table 2, entry 20). This amino alcohol is an intermediate for the synthesis of (*S*)-duloxetine, a potent inhibitor of serotonin- and norepinephrine-uptake carriers.^[10] For comparison, (*S*)-**6l** was obtained in 92% *ee* with the Ru/(*R*)-Xylbinap/(*R,R*)-daipen catalyst system with the same molar ratio of substrate to catalyst.^[11] We believe our process is advantageous since it uses the parent bicip ligand and the relatively inexpensive achiral amine ligand **4** rather than Xylbinap and daipen. In addition the molar ratio of substrate to catalyst in our process can be further increased by carrying out the reaction in a 1:1 mixture of EtOH and *i*PrOH. In this way full conversion and selectivities of 93–94% *ee* were obtained with molar ratio of substrate to catalyst = 4000. Furthermore, EtONa can be used instead of *t*BuONa without affecting the yield or the selectivity, thus reducing the number of the components in the reaction mixture.

At this point, the mechanism of the hydrogenation reactions reported here and the solvent effects are not fully understood. To gain some insight into the factors influencing the hydrogenation reactions, we carried out experiments with higher H₂ pressure (10 atm). The enantio-

selectivities observed were the same as those achieved with 7 atm H₂ pressure. Hydrogenations using *t*BuOLi or *t*BuOK as the base instead of *t*BuONa did not reveal any cation effect on the enantioselectivity. Lastly, experiments were performed in which each of the commercially available chiral alcohols **6e** was used in the hydrogenation reaction mixture instead of the corresponding ketone **5e**. No loss of enantiopurity was observed, thus ruling out reversible transfer hydrogenation. Full mechanistic studies are still being carried out and will be reported in due course.

In summary, a new catalyst system comprising Ru/**1** complexes in combination with inexpensive nonchiral 2-(alkylthio)amine or 1,2-diamine and an alkoxide as a base for the highly enantioselective hydrogenation of a variety of aryl ketones has been developed. The hydrogenation tolerates various substituents including CH₃O, NHCOPh, N(CH₃)₂, F, Cl, Br, and CF₃. The new catalyst system is of great practical potential because of the low cost and availability of the achiral auxiliary amine ligand used.^[12]

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Porous Materials

**Nanoporous Metal-Containing Nickel
Phosphates: A Class of Shape-Selective
Catalyst****

Jong-San Chang, Jin-Soo Hwang, Sung Hwa Jung,
Sang-Eon Park,* Gérard Férey, and
Anthony K. Cheetham**

The discovery of a large family of nanoporous aluminum phosphates in the early 1980s has generated widespread interest in non-aluminosilicate-based microporous materials,^[1] and there has been an enormous growth in the chemical diversity of open-framework inorganic materials in new compositional domains during the last decade.^[2] Commercial applications of open-framework materials continue to be dominated by the aluminosilicate zeolites,^[3] but attempts are

[*] Dr. J.-S. Chang, Dr. J.-S. Hwang, Dr. S. H. Jung
Catalysis Center for Molecular Engineering
Korea Research Institute of Chemical Technology (KRICT)
P.O. Box 107, Yusung, Taejeon 305–600 (Korea)
Fax: (+82) 42-860-7676
E-mail: jschang@kRICT.re.kr
Prof. S.-E. Park
Department of Chemistry, Inha University
Incheon 402-751 (Korea)
Fax: (+82) 32-874-7674
E-mail: separk@inha.ac.kr
Prof. A. K. Cheetham
Materials Research Laboratory, University of California
Santa Barbara, CA 93106 (USA)
Fax: (+1) 805-893-8797
E-mail: cheetham@mrl.ucsb.edu
Prof. G. Férey
Institut Lavoisier, UMR 8637
Université de Versailles Saint Quentin
45 avenue des Etats-Unis, 78035 Versailles Cedex (France)

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being made to exploit some of the exciting properties of the newly discovered systems.^[4] Recent synthetic efforts have led to the discovery of many new phases,^[2,5] for example, V-P-O, Fe-P-O, Co-P-O and Zn-P-O. However, as is the case with most nonsilicate open frameworks, the poor thermal stability of these systems leads to the collapse of the pore structures on activation, thus rendering them unsuitable for applications that require porosity. The challenge, therefore, is to design a nanoporous system that is thermally stable with respect to chemical or structural degradation and can be applied in catalytic reactions that are carried out at relatively high temperatures. In this respect, the need for new classes of catalysts has led a quest for creating novel nanoporous materials that might also exhibit catalytic activity.

We recently showed that the open-framework nickel(II) phosphate, VSB-1 (Versailles/Santa Barbara-1), is sufficiently stable to be rendered nanoporous and exhibits typical zeolitic properties, making it the first example of a nanoporous nickel(II) phosphate material.^[6] The VSB-1 structure is based on a 3D network of NiO₆ and PO₄ tetrahedra defining a large, unidimensional channel, with an estimated free diameter of 8.8 Å.^[6] Structural analysis indicates that HPO₄ and extra-framework cations (NH₄⁺ or K⁺) line the channel in VSB-1. Its surface area and chemical composition are 183 m² g⁻¹ (after dehydration) and Ni₁₈(HPO₄)₁₄(OH)₃F₉(H₃O/NH₄)₄·12H₂O, respectively. We also discovered a second nickel phosphate molecular sieve (VSB-5); this has several applications such as hydrogen storage^[7] and selective hydrogenation and base catalysis.^[8] Catalytically active centers in porous oxides can give rise to highly selective new catalysts that are of great importance in the development of clean technology.^[9] Here, we describe a new class of shape-selective catalyst based on nanoporous nickel phosphates.

We first tested the acid–base properties of VSB-1 by examining its reactivity with 2-methyl-3-buten-2-ol (MBOH) as a model reaction for acid–base catalysis, showing that VSB-1 possesses only weak acidity and basicity (see Supporting Information).

To examine the possible accessibility of Pd species in the pores during catalytic reactions, we have explored catalytic activities of Pd^{II}-exchanged VSB-1 (Pd-VSB-1) for the direct formation of H₂O₂ from H₂ and air in aqueous media. Such a process is an attractive alternative^[10] to the current technology that involves the hydrogenation of an alkylanthraquinone to the corresponding hydroquinone, followed by the reaction of the hydroquinone with oxygen to yield H₂O₂. We previously demonstrated that Pd^{II}-exchanged zeolite catalysts (Pd-BEA) exhibit more effective performance relative to those prepared from other types of zeolites.^[11] In this work, Pd-VSB-1 containing 0.72 wt % Pd is fairly active for the reaction, yielding more H₂O₂ than Pd-BEA, as illustrated in Table 1. It is evident that the nanoporous structure of VSB-1 is suitable as a catalyst support of palladium to produce H₂O₂.

Employing the nanoporous VSB-1 material as a support for a noble metal enables the shape-selective properties of the nanoporous structure to be conferred on the hydrogenation function. For instance, the combination of noble metal/nanoporous material is expected to be beneficial for selective hydrogenation of cycloolefins.^[12] As shown in Figure 1, Pd-

Table 1: Direct production of H₂O₂ from H₂ and air over Pd-exchanged VSB-1 and zeolite catalysts.^[a]

Catalyst	Pd loading [wt %] ^[b]	[H ₂ O ₂] [mM]	TOF ^[c]
Pd-VSB-1	0.72	7.1	414
Pd-BEA	0.70	5.6	338
Pd-L	0.95	2.7	119

[a] Reaction conditions: *T* = 20 °C, *P* = 1 atm, 0.01 M HCl (400 mL), H₂:air = 10:40 mL min⁻¹, 0.1 g catalyst, 1 h. [b] Analyzed by ICP-MS. [c] TOF (turnover frequency) = moles of H₂O₂ formed per mole of Pd per hour.

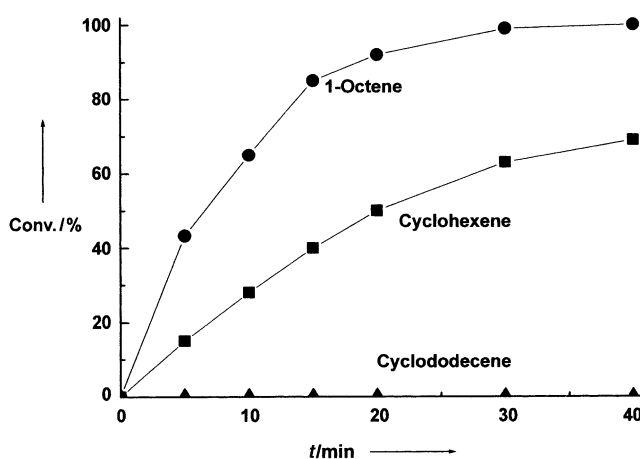


Figure 1. Competitive hydrogenation of an olefin mixture over Pd-VSB-1 containing 0.72 wt % Pd. Reaction conditions: substrate = 1 mol % each of 1-octene, cyclohexene, and cyclododecene in *n*-hexane (90 mL), *T* = 30 °C, H₂ pressure = 2 atm, catalyst weight = 0.2 g.

VSB-1 containing 0.72 wt % Pd exhibits shape selectivity for competitive hydrogenation of 1-octene, cyclohexene, and cyclododecene. Negligible hydrogenation rates are found for larger substrates such as cyclododecene, clearly demonstrating the sieving properties of the catalyst. Furthermore, it proves that the active hydrogenation centers are mainly located within the micropores of Pd-VSB-1, which indicates the potential of the noble metal in the VSB-1 system to act as a new type of shape-selective catalyst.

Catalytic hydroxylation of phenol to dihydroxybenzenes such as catechol and hydroquinone is an important industrial reaction for the production of fine chemicals and has been extensively investigated.^[13] Microporous titanasilicates with large surface areas have proved to be the most important catalysts for phenol hydroxylation with hydrogen peroxide, mainly in organic solvents.^[14] However, in this work, Cu^{II}-exchanged VSB-1 (Cu-VSB-1) exhibits promising catalytic activity for phenol hydroxylation with H₂O₂ in an aqueous solution. As shown in Table 2, it is found that Cu-VSB-1 is very active for phenol hydroxylation. Phenol conversion with Cu-VSB-1 increases up to 40.1 % after 3 h when increasing the copper content to 2.9 wt % (corresponding to 70 % ion exchange). It gives high catechol selectivity (67–70 %) regardless of the copper content, which is higher than that

Table 2: Activity of Cu-VSB-1 for phenol hydroxylation with H₂O₂, in terms of copper content.^[a]

Catalyst ^[b]	Phenol conv. [%] ^[c]	H ₂ O ₂ efficiency [%] ^[d]	Product selectivity [%] ^[e]		
			CAT	HQ	BQ
Cu-VSB-1 (14)	17.2	61.9	68.7	28.3	3.0
Cu-VSB-1 (39)	25.1	65.8	67.6	30.0	2.4
Cu-VSB-1 (50)	28.0	64.8	68.3	29.7	2.0
Cu-VSB-1 (70)	40.1	73.4	69.8	30.2	–

[a] Reaction conditions: 0.2 g catalyst, solvent: water (60 mL), phenol/H₂O₂ molar ratio = 1:1, amount of phenol used = 22 mmol, 3 h, *T* = 60 °C. [b] Numbers in parenthesis denote the degree of ion-exchange, as determined by ICP-MS. [c] Phenol conversion [%] = 100 × [phenol before reaction (moles) – phenol after reaction (moles)] / phenol before reaction (moles). [d] H₂O₂ efficiency [%] = 100 × [products (CAT + HQ + BQ) (moles)] / total H₂O₂ (moles) converted. [e] Product selectivity was calculated for the dihydroxybenzenes and benzoquinone. CAT = catechol, HQ = hydroquinone, BQ = benzoquinone.

of the titanosilicate TS-1 (49%) reported in the literature,^[14] revealing that the large-pore VSB-1 material exhibits reduced pore limitation in the formation of diphenols, compared to TS-1. No catalyst deactivation or leaching of copper species from the substrate was observed when catalytic measurements were twice repeated over a catalyst that had been filtered after being used for a first run. XP spectra of Cu-VSB-1 indicates that the VSB-1 structure produces copper oxide clusters in the pore together with the exchanged copper cations upon ion-exchange of copper (see Supporting Information), which suggests that not only copper cations but also copper oxide species within the pore of VSB-1 are effective for the hydroxylation of phenol with H₂O₂. These two species might provide a beneficial effect to improve the catalytic activity. In the H₂O₂/Cu^{II} oxidation system, cupric ions (Cu²⁺) added to hydrogen peroxide were found to generate hydroxyl radicals (HO[•]) capable of benzoate hydroxylation.^[15] In the present case, copper(II) species dispersed in the pores of VSB-1 might also generate the hydroxyl radicals from H₂O₂ that are active for the phenol hydroxylation.

We prepared Fe-doped VSB-1 (Fe-VSB-1) and explored the framework iron species as a chromophore and a redox site for photoinduced chemistry or catalysis. We believe that the unique and fascinating properties of zeolites involving transition-metal ions within the zeolite frameworks or cavities open up new possibilities for light-induced applications, not only in photocatalysis but also for various photochemical processes.^[16] This is because the micropore of a zeolite confines the organic reactant in a nanoscopic vessel and, moreover, the transition-metal ions in metal-containing porous materials are considered to be highly dispersed at the atomic level and well defined, existing in a specific structure within the zeolite framework. The UV/Vis DRS spectrum of Fe-VSB-1 shows a strong absorption in the 250–350 nm interval, which is typically assigned to an O^{2–} to Fe³⁺ charge-transfer transition ([Fe³⁺ ← O^{2–}]) of isolated Fe³⁺ species.^[17] It is confirmed that the framework Fe^{III} species, possibly octahedral iron species, are sensitive to UV irradiation, which leads to photoreduced Fe^{II} species (see Supporting Information). Thus, UV irradiation for Fe-VSB-1 containing 0.69 wt % Fe gives rise to a decrease in the intensity of the charge transfer ([Fe³⁺ ← O^{2–}]) band centered at 280 nm due to enhancement of charge separation, and leads to the

formation of the corresponding charge-transfer excited state, [Fe²⁺–O[–]]*. Moreover, UV irradiation for methanol-adsorbed Fe-VSB-1 also decreases the intensity of the O^{2–} to Fe³⁺ charge-transfer band due to electron transfer from MeOH to Fe³⁺. These results encouraged us to utilize Fe-VSB-1 as a photocatalyst. As an example, we have investigated UV photochemical oxidation of ammonia in an aqueous solution using Fe-VSB-1, assisted by H₂O₂. It follows the pathway of the photo-Fenton reaction for the degradation of pollu-

ants, in which photoreduced transition-metal species lead to the formation of HO[•] radicals^[18] and thereby convert ammonia to nitrate or nitrite ions. VSB-1 itself shows negligible activity for this reaction, but Fe-VSB-1 exhibits very good activity to oxidize ammonia into nitrate and nitrite ions under UV irradiation (Table 3). The activity is much better than that

Table 3: Activities of TiO₂, FeCl₂, and Fe-VSB-1 in the photo-Fenton oxidation of ammonia in water.^[a]

Catalyst	NH ₃ conversion [%]	TOF ^[b]
TiO ₂ (P-25)	11	1.8
FeCl ₂	18	4.7
Fe-VSB-1 ^[c]	43	7200

[a] Reaction conditions: NH₄OH (758 ppm), 30% H₂O₂ (20 mL), H₂O (660 mL), 0.5 g catalyst, UV wavelength: 200 nm, UV power: 450 W, irradiation time: 10 min. [b] TOF (turnover frequency) is moles of NH₃ decomposed per mole of Ti (or Fe) per hour. [c] Fe content = 0.69 wt %.

of anatase TiO₂ (P25) as a typical photocatalyst and pure FeCl₂ as a photo-Fenton reagent, and its superiority is evident when the activity is displayed in terms of turnover frequency (TOF). This result indicates that highly dispersed Fe^{III} sites in the framework of VSB-1 can behave as active centers for intrazeolite photocatalysis.

In summary, the novel material, VSB-1, is found to have important properties such as nanoporosity, zeolitic properties, and very weak acidity and basicity. When VSB-1 is modified with metal ion-exchange or metal-incorporation into the framework, it can offer promising catalytic properties: e.g., shape selectivity, redox catalysis, and photocatalysis. Indeed, the catalytic results exemplified in this work clearly demonstrate that nanoporous metal-containing nickel phosphates can be classified as a new class of shape-selective catalyst.

Experimental Section

Syntheses of VSB-1 and metal-modified VSB-1 materials: VSB-1 was initially prepared in a hydrothermal reaction at 180 °C for 6 days between nickel(II) chloride hexahydrate and phosphoric acid (85% by weight) in the presence of tris(2-aminoethyl)amine in a pyridine/HF/water solvent system; further details are given elsewhere.^[6] Iron-incorporated VSB-1 was synthesized likewise using an aqueous

solution of $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (Aldrich, 99%). For the Pd^{II} and Cu^{II} ion-exchanged materials, palladium and copper were introduced by ion exchange of the proton form of VSB-1 using a 0.01M aqueous solution of $[\text{Pd}(\text{NH}_3)_4\text{Cl}_2]$ and a 0.02M aqueous solution of $\text{Cu}(\text{NO}_3)_2$, respectively. The resulting metal-loaded materials were dried at 120°C overnight and calcined at 350°C for 4 h in air. For catalytic measurements, Pd-VSB-1 was reduced for 4 h in 5% H_2 at 300°C. Metal loading in the final products was determined with ICP-MS.

Catalytic measurements were conducted with a plug-flow reactor (for MBOH decomposition) or a glass reactor (for H_2O_2 production, olefin hydrogenation, phenol hydroxylation, and ammonia decomposition). Detailed methods for catalytic measurements and analysis are described in the Supporting Information.

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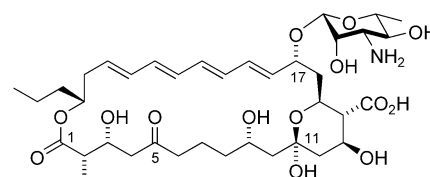
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Synthesis of Rimocidinolide Methyl Ester, the Aglycone of (+)-Rimocidin**

Garrick K. Packard, Yueqing Hu, Andrea Vescovi, and Scott D. Rychnovsky*

Polyene macrolide antibiotics have found widespread use as antifungal agents.^[1] The family of polyene macrolides incorporating a mycosamine sugar represent the most common and widely used members of the class of natural products, and include amphotericin B, nystatin, candidin, pimaricin, and rimocidin.^[2] These stereochemically complex, highly oxygenated structures have attracted the attention of synthetic chemists.^[3] Amphotericin B was synthesized by Nicolaou and co-workers,^[4] and the aglycone of amphotericin B, amphoteronolide B, was prepared by Masamune and co-workers.^[5,6] We have been interested in the synthesis of polyene macrolides, but most of our work has focused on the oxopolyene subclass.^[7] We report herein the first synthesis of the rimocidin aglycone, rimocidinolide methyl ester (**2**).

Rimocidin (**1**) is a 28-membered polyene macrolide which was isolated from *Streptomyces rimosus* in 1951.^[8] Cope et al.



Rimocidin (**1**)

established the carbon skeleton in the mid-1960s,^[9] and its flat structure was determined independently by the groups of Borowski and Rinehart in the 1970s.^[10] The stereochemistry of rimocidin was determined by Borowski and co-workers group in 1995 through 2D NMR analysis.^[11] Rimocidin includes 14 stereogenic centers, a conjugated tetraene, a tetrahydropyran hemiketal, and a macrocyclic lactone and is β -linked to mycosamine. These functionalities make rimocidin sensitive to both strongly acidic and strongly basic reaction conditions. Smith et al. recently reported a synthesis of the C1–C18 segment of rimocidin^[12] through a dithiane

[*] Dr. G. K. Packard, Dr. Y. Hu, Dr. A. Vescovi, Prof. S. D. Rychnovsky
Department of Chemistry
University of California, Irvine
516 Rowland Hall, Irvine, CA 92697–2025 (USA)
Fax: (+1) 949-824-6379
E-mail: srychnov@uci.edu

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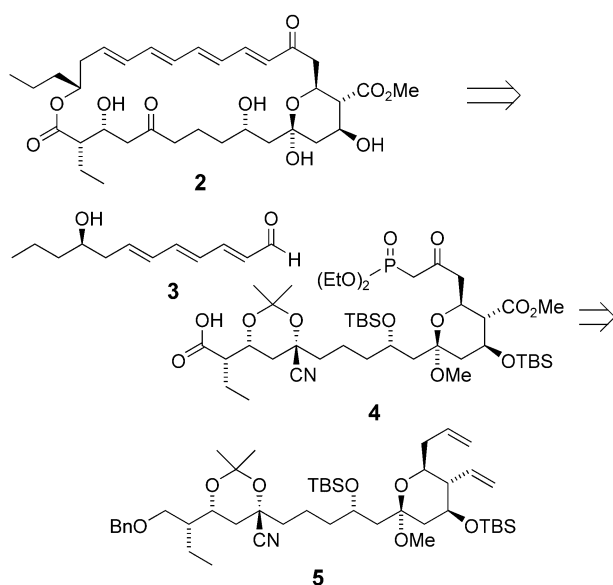


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linchpin strategy.^[13] Our strategy is based on cyanohydrin acetonide couplings to facilitate the convergent assembly of the rimocidin aglycone.

Cyanohydrin acetonides have been used extensively in our laboratories as synthons for *syn*-1,3-diols,^[14] and this strategy has been applied, for example, to syntheses of roxatidin^[15] and dermostatin.^[16] The structure of rimocidin, however, does not contain any *syn*-1,3-diol segments. In our approach to rimocidin, cyanohydrin acetonides would be used as synthons for β -hydroxyketones rather than for *syn*-1,3-diol acetonides. Thus the cyanohydrin would revert to its earlier application as an acyl anion equivalent,^[17] and the strategy is comparable to the widely used dithiane disconnection.^[18]

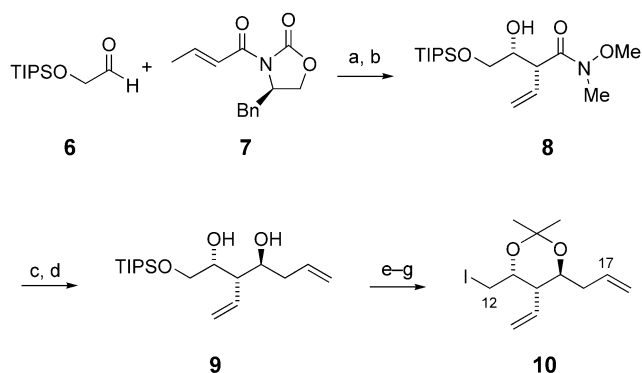
A retrosynthetic analysis of rimocidinolide methyl ester (**2**) is presented in Scheme 1. The macrolide ring would be



Scheme 1. Retrosynthetic analysis of rimocidinolide methyl ester **2**.

prepared from two segments, an unsaturated aldehyde **3** and the complex phosphonate **4**. Two bonds would be formed between **3** and **4**: an ester linkage and an alkene. The ester and alkene could be formed in either order, but in general the Horner–Emmons cyclization route has better precedent and would be investigated first. The phosphonate **4** would be prepared from protected polyol **5**. We planned to assemble the polyol **5** through cyanohydrin acetonide couplings.

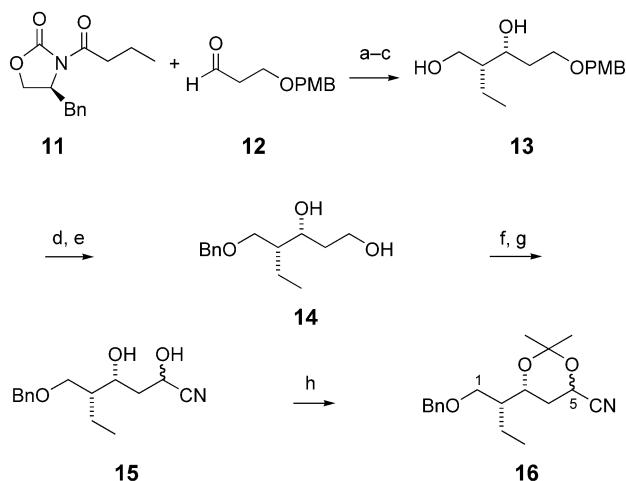
The most complex segment of the polyol chain is the C12–C17 segment **10**, the precursor to the hemiacetal ring. Its synthesis, based on an enantioselective aldol reaction, is outlined in Scheme 2. An Evans aldol reaction^[19] between **7** and aldehyde **6** gave the expected product contaminated with the deconjugated crotonate. The aldol adduct was most conveniently isolated after conversion into its Weinreb amide **8**.^[20] Treatment with allyl magnesium bromide and subsequent *anti*-selective reduction with the Evans triacetoxyborohydride^[21] generated diol **9** as a single diastereomer. Acetonide formation did not proceed to completion, and the recovered starting material was recycled to improve material through-



Scheme 2. Reagents and conditions. a) Bu₂BOTf, Et₃N, CH₂Cl₂, then **6**, –78 °C; b) MeONHMe·HCl, Me₃Al, CH₂Cl₂, 53% over two steps; c) AllylMgBr, THF, –10 °C, 92%; d) Me₄N(AcO)₃BH, CH₃CN/HOAc, –20 °C, 94%; e) 2,2-DMP, acetone, CSA, reflux, repeat; f) TBAF, THF, 99% over two steps; g) I₂, PPh₃, imidazole, benzene/Et₂O, 99%. TIP-S = triisopropylsilyl, Tf = trifluoromethanesulfonyl, 2,2-DMP = 2,2-dimethoxypropane, CSA = $\pm$ -camphorsulfonic acid, TBAF = tetrabutylammonium fluoride.

put. Cleavage of the TIPS group and introduction of the iodide substituent with PPh₃ and I₂ completed the synthesis of **10**^[22] in an overall yield of 44% in seven steps.

Two cyanohydrin acetonides, **16** and **20**, were prepared for the synthesis. The synthesis of **16** is presented in Scheme 3 and also uses an Evans aldol coupling.^[19] Aldol coupling between **11** and aldehyde **12** led to the expected adduct. The optimal procedure for producing diol **13** involved hydrolytic removal of the auxiliary and reduction of the acid with LAH. Direct reduction of the adduct led to complex mixtures. Several approaches were investigated for the selective benzylation of

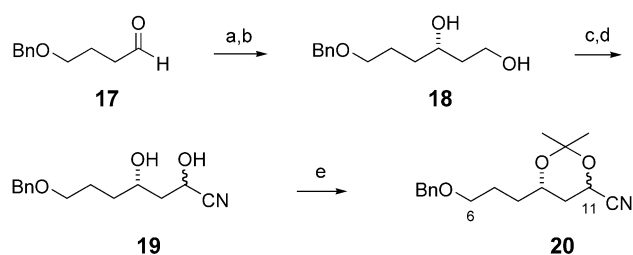


Scheme 3. Reagents and conditions. a) Bu₂BOTf, Et₃N, CH₂Cl₂, then **12**, –78 °C, 92%; b) LiOH, H₂O₂; c) LAH, THF, reflux, 74% over two steps; d) NaH, BnBr, DMF, –50 °C, 70%; e) HCl (1 N), EtOH, reflux, 98%; f) TEMPO, KBr, NaOCl; g) TMSCN, KCN·18[crown]-6, 92% over two steps; h) 2,2-DMP, acetone, PPTS, reflux, 97% (3:2 ratio). PMB = *p*-methoxybenzyl, LAH = lithium aluminum hydride, DMF = *N,N*-dimethylformamide, TEMPO = 2,2,6,6-tetramethylpiperidin-1-yl oxyl, TMS = trimethylsilyl, PPTS = pyridinium toluene-*p*-sulfonate.

the primary alcohol **13**,^[23] but the most effective method used excess NaH and 1 equivalent of BnBr.^[24] The PMB group was cleaved selectively by treatment with TMSCl, SnCl₂, and anisole,^[25] but the deprotection step was more conveniently carried out by treatment with HCl (1N) in refluxing ethanol. Conversion of the 1,3-diol into a cyanohydrin acetonide made use of the selective oxidation of primary alcohols to aldehydes with TEMPO.^[26] Oxidation of **14** catalyzed by TEMPO led to a solution of the β -hydroxyaldehyde in CH₂Cl₂. The solution was treated directly with TMSCN and KCN/[18]crown-6 to generate the cyanohydrins **15**. Experience has shown that β -hydroxyaldehydes rapidly dimerize in the absence of solvent, but solutions can be handled without trouble.^[27] Acetonide formation catalyzed by PPTS gave the cyanohydrin acetonide **16** as an inconsequential 3:2 mixture of isomers at the cyanohydrin center. We consider this strategy for the conversion of 1,3-diol **14** into cyanohydrin acetonide **16** to be the most reliable and convenient method for the preparation of cyanohydrin acetonides.

Synthesis of the second cyanohydrin is presented in Scheme 4. Enantioselective allylation of aldehyde **17**, followed by ozonolysis and reduction with NaBH₄, produced the diol **18** with 89% *ee*. Oxidation with TEMPO, cyanohydrin formation, and acetonide formation completed the five-step synthesis of cyanohydrin acetonide **20**.

The cyanohydrin acetonide segments and iodide **10** were combined as illustrated in Scheme 5. In the past, we alkylated cyanohydrins by forming the anion and then adding the electrophile.^[14] Several years ago we reported that deprotonation was compatible with most alkyl halide electrophiles.^[28] The alkylation of cyanohydrin **20** was most conveniently and reliably conducted through an in situ deprotonation method. The less precious cyanohydrin **20** was used in twofold excess relative to the iodide **10**.^[29] The two segments were combined in THF at -40°C, and a solution of LDA was added. If the

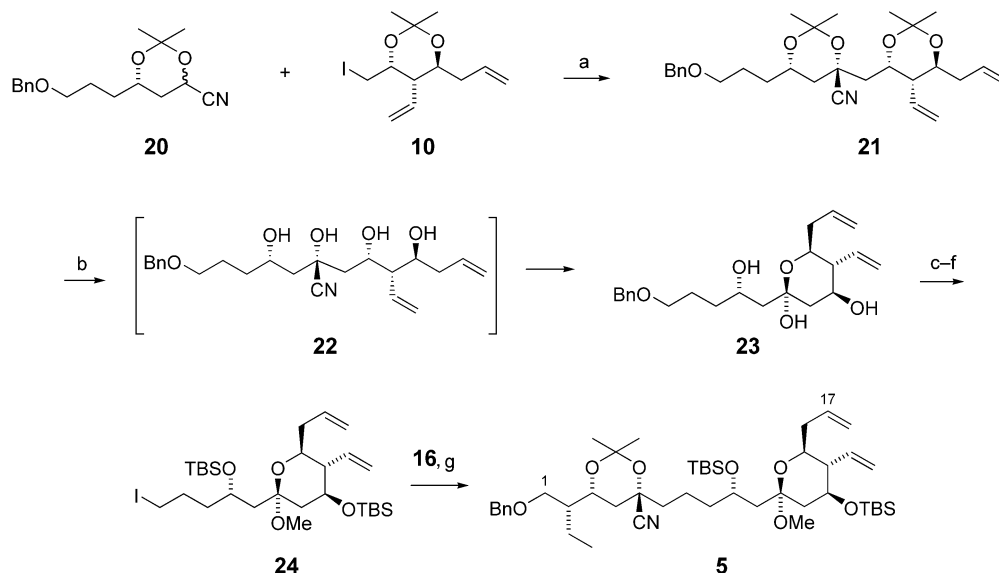


Scheme 4. Reagents and conditions. a) 1. (-)-Ipc₂BAllyl, THF, -100°C; 2. H₂O₂, NaOH (1 N), MeOH; b) 1. O₃, CH₂Cl₂/MeOH, -78°C; 2. NaBH₄, -78°C, 82% over two steps; c) TEMPO, KBr, NaOCl; d) TMSCN, KCN/[18]crown-6, 94% over two steps; e) 2,2-DMP, acetone, PPTS, reflux, 88% (1:1 ratio). lpc = isopinocampheyl.

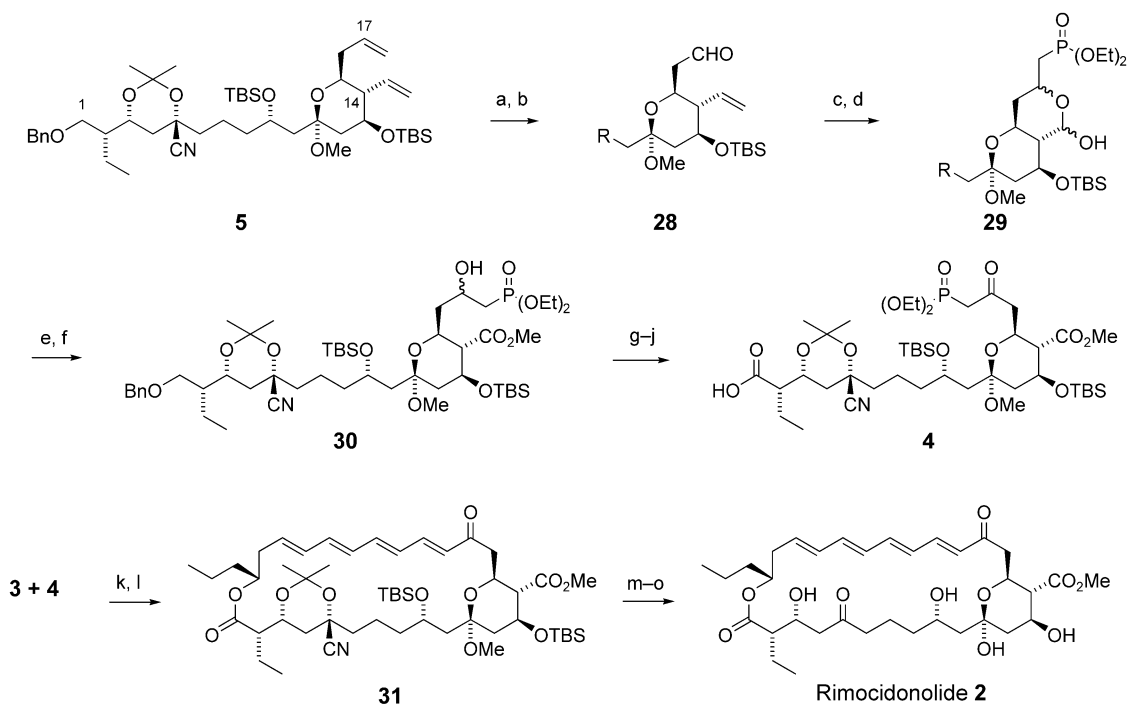
reaction were incomplete by TLC analysis, more LDA solution was added and the process was repeated until no starting iodide remained. This approach is robust and works well on both small (< 100 mg) and large (ca. 10 g) scales.

The hemiacetal was introduced by deprotection of the cyanohydrin acetonide. Treatment of **21** with acidic Dowex resin in wet methanol liberated the alcohols, and exposure of the solution to Et₃N led to spontaneous generation of the hemiacetal **23**. Reprotection of **23** began with methyl acetal formation and protection of the secondary alcohols as TBS ethers. Reductive debenzoylation and iodide formation gave iodide **24**. Cyanohydrin **16** (2 equiv) was combined with iodide **24** by using the in situ deprotonation method to yield the alkylated product **5**. Both cyanohydrins **5** and **21** were formed as single isomers in the alkylation steps, apparently owing to a kinetic anomeric effect.^[14] Thus the C1–C17 protected polyol segment **5** was prepared in seven steps and in 55% overall yield from iodide **10**.

Oxidation of the diene **5** to a dialdehyde was much more difficult than we had anticipated, and we opted for a selective



Scheme 5. Reagents and conditions. a) LDA, THF, DMPU, -40°C, 92%; b) 1. wet MeOH, Dowex 50X2-100; 2. Et₃N, 87%; c) HC(OMe)₃, MeOH, PPTS, 89%; d) TBSCl, imidazole, DMAP, DMF, 97%; e) Li/NH₃, THF, -78°C, 93%; f) I₂, PPh₃, imidazole, benzene, 92%; g) LDA, THF, DMPU, -40°C, 93%. LDA = lithium diisopropylamide, DMPU = *N,N'*-dimethyl-*N,N'*-propyleneurea, TBS = *tert*-butyldimethylsilyl, DMAP = 4-dimethylaminopyridine.



Scheme 6. Reagents and conditions. a) OsO_4 , NMO, 90%; b) $\text{Pb}(\text{OAc})_4$, 91%; c) $(\text{EtO})_2\text{P}(\text{O})\text{CH}_3$, BuLi, THF; d) O_3 , MeOH/ CH_2Cl_2 , -78°C , 95%; e) TPAP, NMO, 87%; f) LiOMe, MeOH, 98%; g) Dess–Martin periodinane, CH_2Cl_2 , 95%; h) $\text{Pd}(\text{OH})_2$, H_2 , EtOAc, 96%; i) Dess–Martin periodinane, CH_2Cl_2 ; j) NaClO_2 , 2-methyl-2-butene, *t*BuOH, 99% over two steps; k) Yamaguchi esterification, 79%; l) K_2CO_3 , [18]crown-6, toluene, 60°C , 5 h, 74%; m) HF/pyridine, pyridine, THF, 23°C , 30 min; n) HCl (6 N), THF/ H_2O , 23°C , 5 h; o) Et_3N , CH_2Cl_2 , 23°C , 10 min, 55% over three steps. NMO = *N*-methylmorpholine-*N*-oxide, TPAP = tetra-*n*-propylammonium perruthenate.

oxidation route. Osmium tetroxide selectively oxidized the C17 alkene in preference to the C14 alkene (Scheme 6). Presumably the C14 alkene is hindered by the flanking equatorial substituents. Cleavage of the diol gave the C17 aldehyde **28**, and the phosphonate was introduced by using lithiated diethyl methylphosphonate. Lactol **29** was produced upon ozonolysis. The lactol was converted into the methyl ester by oxidation with TPAP–NMO^[30] followed by treatment with lithium methoxide in methanol. Synthesis of phosphonate **4** was completed by oxidation of the alcohol, hydrogenation to cleave the benzyl ether, and then a two-step oxidation of the alcohol function at C1 to an acid. The described conversion of polyol **5** into phosphonate **4** is the result of many different attempts using a variety of approaches. Functional-group interactions thwarted most of the routes investigated. The final route is reliable and avoids the common pitfalls.

The synthesis of rimocidinolide **2** was completed as illustrated in Scheme 6. The acid **4** was esterified with alcohol **3**^[31] by using the Yamaguchi protocol.^[32] (Alternatives such as DCC, HATU, and PyBOP produced the ester but in lower yields.^[33]) An attempted cyclization of the resulting ester with LiCl/DBU resulted in elimination of acid **4**, which was reisolated in 75% yield.^[34] On the other hand, Horner–Wadsworth–Emmons macrocyclization with K_2CO_3 and [18]crown-6 in toluene produced the macrolide **31** in 74% yield.^[35] Deprotection of macrolide **31** began with removal of the TBS ethers with HF–pyridine in pyridine/THF. The methyl acetal was partially removed under these conditions.

Cleavage of the acetonide and hydrolysis of the methyl acetal took place upon treatment with HCl (6 N) in THF. Finally, the C7 ketone was unmasked by brief exposure to Et_3N . Thus, rimocidinolide methyl ester (**2**) was synthesized in 29 steps in the longest linear sequence and in 4.3% overall yield from aldehyde **6**. In practice, a number of these steps were combined for convenience. The synthetic rimocidinolide **2** was characterized by NMR and IR spectroscopy as well as HRMS and CD.^[36]

The synthesis of the rimocidinolide and in particular of the polyol segment **5** illustrates the usefulness of cyanohydrin acetonides as β -hydroxyketone synthons for the convergent synthesis of polyol chains.^[14] Segment **5** was assembled in a 14-step linear sequence in nearly 25% overall yield from aldehyde **6**. The cyanohydrin acetonides **16** and **20** were prepared in high yield from terminal 1,3-diols by using a new protocol. Alkylation of these synthons with alkyl iodides **24** and **10** proceeded in 93% and 92% yields, respectively, through an in situ deprotonation technique. Ketones can be liberated from cyanohydrin acetonides by standard deprotection methods. Cyanohydrin acetonide alkylations represent a very efficient strategy for assembling ketone-containing chains, and this approach complements our previous use of these materials and *syn*-1,3-diol synthons. Polyol syntheses based on cyanohydrin acetonide couplings are therefore a competitive alternative to the more common dithiane strategies.^[18]

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Keywords: C–C coupling · cyanohydrins · macrocycles · natural products · total synthesis

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Domino Reactions**A 4-*exo*-dig Cyclocarbopalladation/
8 π Electrocyclization Cascade: Expeditious
Access to the Tricyclic Core Structures of the
Ophiobolins and Aleurodiscal****

*Bahaâ Salem and Jean Suffert**

The design and elaboration of complex molecules from simple starting materials in the minimum number of synthetic steps is one of the most challenging goals in organic synthesis today.^[1] Towards this end, transition-metal-catalyzed processes have become a powerful tool for the construction of sensitive functionalized polycyclic molecules under very mild conditions.^[2] A particularly attractive goal in this field is the development of new methodologies for synthesizing cyclo-

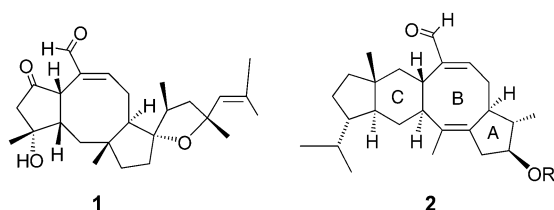
[*] B. Salem, Dr. J. Suffert
Faculté de Pharmacie
Université Louis Pasteur de Strasbourg (UMR 7081 CNRS/ULP)
74 Route du Rhin, 67401 Illkirch Cedex (France)
Fax: (+33) 3-9024-4310
E-mail: jeansu@aspirine.u-strasbg.fr

[**] Prof. Michael Harmata is acknowledged for stimulating discussions and Dr. André De Cian for X-ray crystal-structure analysis.



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

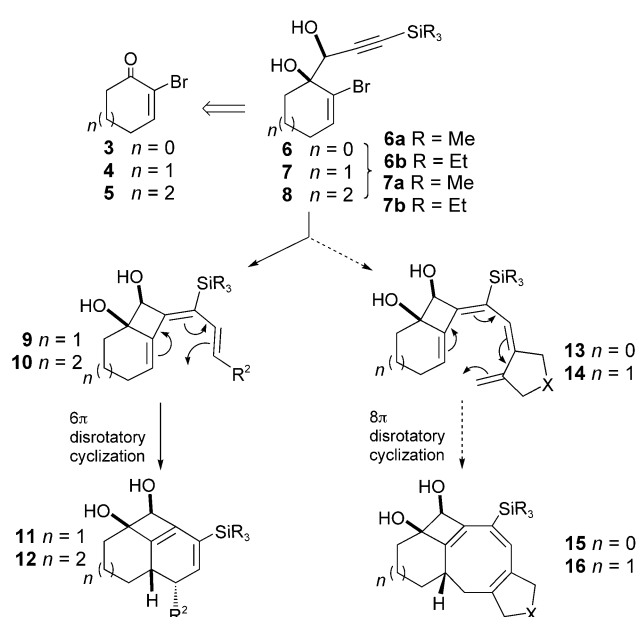
octanoid ring systems.^[3] This substructure is present in over 100 different natural products, many of which exhibit exceptional and broad-ranging biological activity. One of the most notable examples is the diterpene paclitaxel (taxol).^[4] However, because of the high degree of ring strain, transannular interactions, and unfavorable entropic and enthalpic factors, the synthesis of compounds with eight-membered rings remains difficult. Several authors have reported elegant approaches to such systems based on transition-metal catalysis.^[5] The most recent reports^[5h–j] described the formation of bicyclic structures by using a rhodium catalyst in a one-pot operation. However, none of these approaches involved the direct formation of a 5,8,5-tricyclic skeleton. Ophiobolin A (**1**)^[6] and aleurodiscal (**2**)^[7] are natural compounds that



contain a 5,8,5-tricyclic and 5,6,8,5-tetracyclic core, respectively. Ophiobolins have a broad spectrum of biological activity against nematodes, fungi, and bacteria,^[8,9] as well as potent antitumor activity.^[10] Aleurodiscal is an antifungal antibiotic produced by mycelial cultures of *Aleurodiscus mirabilis* collected from the bark of *Cinnamomum camphora*, which is endemic to Japan. Extensive studies toward these polycyclic skeletons have been carried out by several research groups,^[11] and the total synthesis of ophiobolin C was completed by Kishi and co-workers in 1989.^[12]

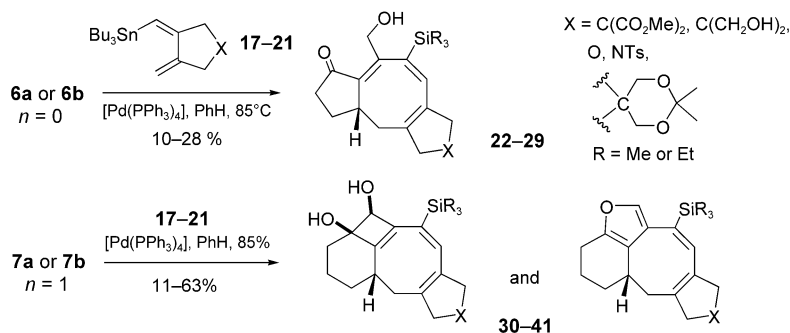
We recently disclosed an efficient reaction that led to the formation of unusual polycyclic compounds through an initial 4-*exo*-dig cyclocarbopalladation of propargylic diols.^[13] In a one-pot operation, the products of this reaction can undergo reaction with a suitable vinyl stannane, followed by a disrotatory 6 π electrocyclization to give unusual tricyclic systems of the type **11** and **12**, which contain a new six-membered ring and a cyclobutene ring whose double bond is located at the junction of the three rings (Scheme 1). These were the first examples of a metal-catalyzed exclusively 4-*exo*-dig cyclization. The process has been applied to a wide variety of propargylic diols and tributylstannane derivatives and can be scaled up to afford multigram quantities of new highly strained compounds. One limitation was the failure of the diols **6**, which contain a five-membered ring, to undergo the desired reaction. Instead only decomposition of the starting material was observed, probably because of the highly strained nature of the [3.2.0] bicyclic system in the hypothetical intermediate.

During our studies towards the extension of this new methodology to the synthesis of other valuable polycyclic structures, such as those present in the biologically active natural products mentioned above, we decided to examine the possibility of including a conrotatory



Scheme 1. 4-*exo*-dig cyclocarbopalladation/6 π electrocyclization cascade extended to 8 π electrocyclization.

8 π electrocyclization in the reaction sequence. It was proposed that the functionalized tetracyclic trienes **15** and **16**, which contain an eight-membered ring, could be formed from the potential tetraene intermediates **13** and **14** (Scheme 1). The two diols **6** and **7**, which contain a five- and a six-membered ring, respectively, were tested as substrates in presence of the stannylated dienes **17–21**. The stannanes were prepared by a straightforward method developed by Lautens et al.^[14] We describe herein a new cascade reaction based on three consecutive transformations starting from these two types of diol: An initial 4-*exo*-dig cyclocarbopalladation followed by a Stille cross-coupling reaction and finally a concerted conrotatory 8 π electrocyclization allows the preparation of highly functionalized tetracyclic compounds. Three main types of product were obtained in this reaction, depending on the ring size of the substrate (Scheme 2). The first product type (**22–29**, Table 1) was generated from the cyclopentene diols **6a** or **6b** in a sequence of four reactions. The other two product types (**30–41**, Table 1) resulted from a cascade of three or five consecutive reactions starting from



Scheme 2. Access to the core skeleton of the ophiobolins and aleurodiscal. Ts = *p*-toluenesulfonyl.

Table 1: Synthesis of various 5,8,5- and 6,4,8,5-polycyclic systems.

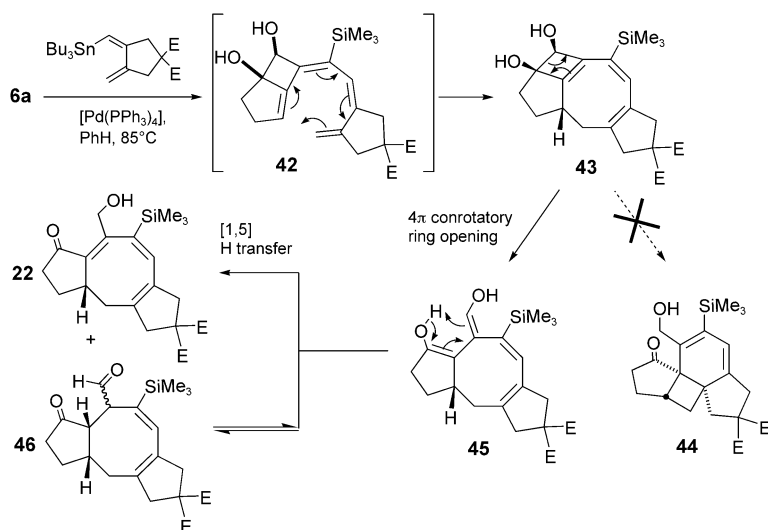
Entry	Starting diol	Products (yield [%])				
		(with 17)	(with 18)	(with 19)	(with 20)	(with 21)
1						
2						
3						
4						
			32:33 = 59:41 (61^[b])		36:38 = 48:52 (56^[b]) 37:39 = 34:66 (41^[b])	

[a] Reaction conducted in toluene as the solvent at 110°C. [b] Combined yield.

the cyclohexene diols **7a** or **7b**. All reaction sequences proceeded in a one-pot operation without isolation of the intermediates.

This reaction cascade facilitates the expeditious preparation of 5,8,5-tricyclic structures of the ophiobolane family and new 6,4,8,5-tetracyclic structures, which include the ABC core of aleurodiscal. The polycyclic products are produced in just two steps from the readily available bromocyclopentenone **3** or bromocyclohexenone **4** (Scheme 1). To our knowledge, this is the shortest reported route to functionalized polycyclic structures of this type and degree of complexity.^[11] The starting *anti* diols **6** and **7** were prepared on a large scale by the addition of a suitably protected metalated propargylic

alcohol to **3** and **4**, respectively, followed by deprotection and chromatographic separation of the *anti* and *syn* diastereomers.^[13a] When the diol **6a** was treated with the tributylstannyldiene **17** ($X = C(CO_2Et)_2$) in the presence of $[Pd(PPh_3)_4]$ (5 mol%) in benzene at 85°C, an exclusively 4-*exo*-dig cyclocarbopalladation gave the bicyclic [4.2.0] product **42**, which could not be isolated, but underwent a rapid 8 π electrocyclic cyclization to afford the tetracyclic derivative **43** in 16% yield after 2 h (Scheme 3). Pericyclic reactions of this type have few precedents in the synthesis of natural products.^[15,16] One of the most well-known examples was described in the total synthesis of endiandric acids A and B by Nicolaou et al. in 1982.^[15]



Scheme 3. Formation of the 5,8,5-tricyclic structure of the ophiobolane family.

The 1,3,5-cyclooctatriene **43** thus obtained did not undergo the usual subsequent 6π electrocyclization^[17] to the corresponding tetracyclic diene **44**, because of the unlikely formation of this very strained spirocyclic diene. The stereospecificity of the conrotatory 8π electrocyclization allowed the synthesis of **43** as a single diastereomer. Each of the 1,3,5-cyclooctatrienes **30–41**, derived from the diols **7a** and **7b**, were also formed as a single diastereomer. Whereas the diols **7a** and **7b** reacted to give a stable 6,4,8,5-tetracyclic system, the initial products of the reactions of **6a** and **6b**, when heated for several hours, underwent opening of the cyclobutene ring to give the ketoallylic alcohol **22** and ketoaldehyde **46**. The formation of **22** and **46** can be explained by a 4π electrocyclic ring opening of **43** to the intermediate bis(enol) **45**, which can either undergo a 1,5-hydrogen shift to give **22** or tautomerization to its γ -ketoaldehyde form **46** (Scheme 3). At 85°C the ketoaldehyde **46** undergoes thermal decomposition, and none of it was isolated after 6 h in the crude reaction mixture. The generality of this new expeditious process was explored by using several stereodefined cyclic stannanes (Table 1).

In two cases the yields of the 6,4,8,5-polycyclic systems were limited by the formation of furanyl derivatives, which probably result from a rearrangement of the corresponding cyclobutenediol. The yields observed (10–53 %) were modest to acceptable if the complexity of the new products formed in just one step from readily prepared diols is considered. The most efficient cascade process started from the diol **7b** (R =

SiEt₃) and the dienyl stannane **19** and afforded the tetracycle **35** in 53 % yield. The exact influence of the silyl group on the reaction has not been yet clarified, as no clear trend was observed in the results (compare entries 1 and 2 with entries 3 and 4 of Table 1). The structures of all compounds were proposed based on ¹³C and 2D ¹H NMR spectroscopy, including NOESY, COSY, HMQC, and HSQC experiments, and mass spectral analysis. Finally, these structures were unambiguously confirmed by X-ray crystal-structure analysis on two compounds,^[18] the tetracycles **30** and **38** (Figure 1).

In conclusion, we have reported the direct synthesis of 5,8,5- and 6,4,8,5-polycyclic systems in one operation from readily prepared substrates. The modest yields observed are compensated by the structural complexity of the final products obtained and the limited number of steps in the reaction sequence. The strategy developed is based on a cascade reaction consisting of a 4-*exo*-dig cyclocarbopalladation, Stille cross-coupling reaction, and conrotatory 8π electrocyclization. To date, only rare examples of this pericyclic reaction for the direct formation of eight-membered rings have been described. Thus, we have shown that its potential as an efficient route to stable cyclooctatrienes should be reconsidered. Further extension of this chemistry to the rapid synthesis of compounds of biological importance is underway.

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Keywords: domino reactions · electrocyclic reactions · medium-ring compounds · polycycles · Stille reaction

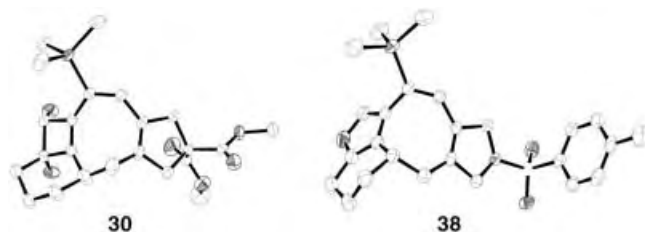


Figure 1. X-ray crystal structures of compounds **30** and **38**.

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- [18] Crystal data for **30**: $C_{23}H_{32}O_6Si$, $M_r = 432.59$, monoclinic, space group $P2_1/c$, $a = 19.1201(5)$, $b = 12.9474(4)$, $c = 21.2609(6)$ Å, $\beta = 116.360(5)^\circ$, $V = 4716.0(2)$ Å³, $Z = 8$, $\rho_{\text{calcd}} = 1.22$ g cm⁻³, $\mu = 0.134$ mm⁻¹, 10841 data (3161 reflections with $I > 3\sigma(I)$), $R = 0.055$, $wR = 0.065$, residual electron density maxima 0.47 e Å⁻³. Crystal data for **38**: $C_{25}H_{31}NO_3SSi$, $M_r = 453.68$, orthorhombic, space group $Pbca$, $a = 8.0812(1)$, $b = 38.0184(2)$, $c = 15.5969(6)$ Å, $V = 4791.9(2)$ Å³, $Z = 8$, $\rho_{\text{calcd}} = 1.26$ g cm⁻³, $m = 0.211$ mm⁻¹, 13493 data (3007 reflections with $I > 3\sigma(I)$), $R = 0.042$, $wR = 0.062$, residual electron density maxima 0.34 e Å⁻³. Measurements were made on a Kappa CCD diffractometer (Mo K_{α} , graphite monochromated, $\lambda = 0.71073$ Å). These structures were solved by using direct methods. After refinement of the non-hydrogen atoms, difference Fourier maps revealed maxima of residual electron density close to positions expected for hydrogen atoms. Hydrogen atoms were introduced as fixed contributors at calculated positions ($C-H = 0.95$ Å, $B(H) = 1.3$ Beqv(C)). All calculations were made by using the Nonius OpenMoleN package.^[19] Neutral atom-scattering-factor coefficients and anomalous dispersion coefficients were taken from a standard source.^[20] CCDC 228625 (**30**) and 228626 (**38**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).
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The Transition State of Thermal Organic Reactions: Direct Observation in Real Time**

D. Hern Paik, Ding-Shyue Yang, I-Ren Lee, and Ahmed H. Zewail*

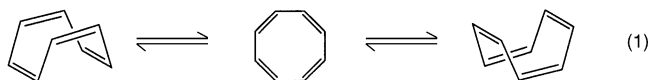
The motion of atoms during chemical reactions occurs on the femtosecond time scale, and only with this resolution can the transition state be frozen in time. Numerous examples of femtochemistry studies have been reported for reactions on excited surfaces and in all phases of matter (see, for example, recent references [1] and [2]). Combining the femtosecond time resolution and negative-ion-beam techniques provides a unique way to study the nuclear motions of *neutral* molecules in the electronic *ground* state, and this has been successful for Ag₃ and Ag₄.^[3–7]

For stable (positive electron affinity) negative ions prepared in the gas phase the electronic states are lower in energy than the neutral ground state. Thus, the ground state of the neutral reaction can be prepared with well-defined nuclear configuration (coherent wave packet) by photodetaching the electron from the anion with a femtosecond laser pulse. Because the geometries of the anion and the neutral molecule are different, the vertical transition results in significant nuclear motion toward the equilibrium geometry of the ground state. This motion can be monitored in real time by using a second femtosecond laser pulse, with variable time delay, through ionization and detection with mass spectrometry.

[*] D. H. Paik, D.-S. Yang, I.-R. Lee, Prof. Dr. A. H. Zewail
Laboratory for Molecular Sciences
Arthur Amos Noyes Laboratory of Chemical Physics
California Institute of Technology
Pasadena, CA 91125 (USA)
Fax: (+1) 626-792-8456
E-mail: zewail@caltech.edu

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In the present work we report the femtosecond (fs) time-resolved dynamics of the transition state of the reaction of 1,3,5,7-cyclooctatetraene (COT) [Eq. (1)]. This reaction of



neutral COT on the ground-state potential is not a photochemical one, thus the transition state is that of a thermal reaction.

For this system a number of experimental and theoretical works have shown that neutral COT has a “tub-shaped” (D_{2d}) structure in the electronic ground state,^[8–16] whereas the COT anion has a planar (D_{4h}) structure that resembles the geometry of neutral COT at the transition state of the ring inversion.^[17–21] From the photoelectron spectrum of COT^- , Wenthold et al. showed that the vertical transition from COT^- can directly access the transition state of the ring inversion in the electronic ground state of neutral COT.^[20]

In our experiment the coherent wave packet of the nuclear motion was launched from the negative ion using a 405-nm femtosecond pulse (Figure 1). The COT^- ion was mass-selected (Figure 2a), and the pump pulse (3.1 eV) photodetaches the electron from the anion to yield the

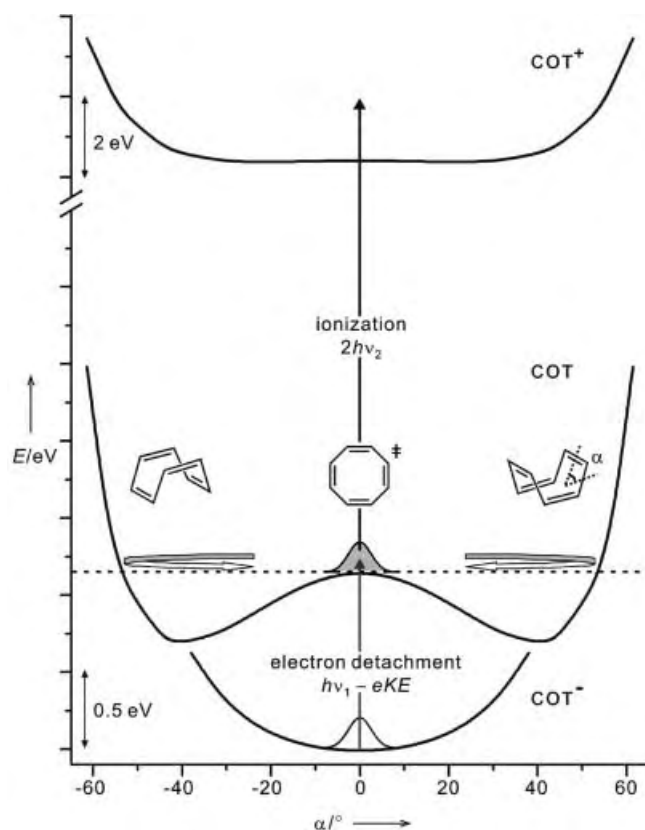


Figure 1. Schematic illustration of the experiment, with calculated potential energies for COT^- , COT, and COT^+ as a function of the bending angle (α). The electron detachment by the pump pulse and ionization by the probe pulse are indicated by upward arrows. The initial wave packet prepared by the pump pulse is indicated by a Gaussian profile at the transition state of the ring inversion.

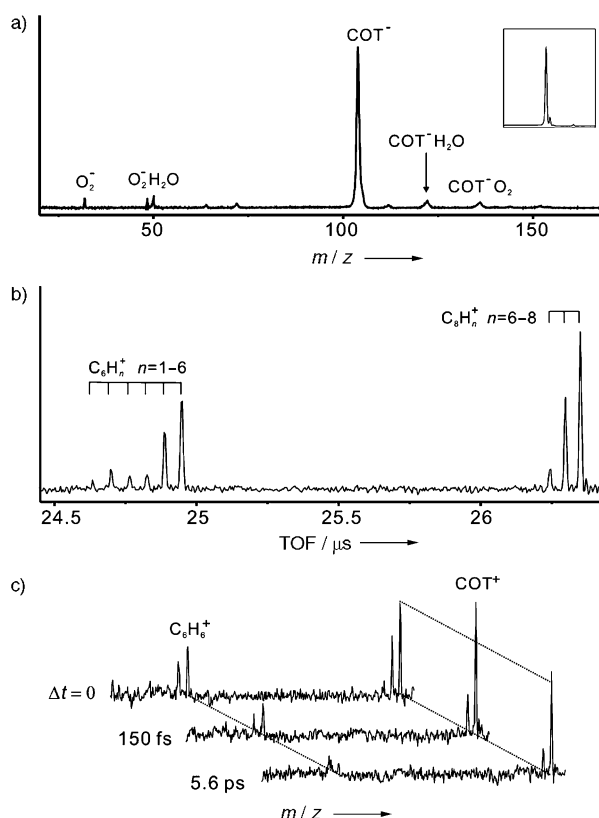


Figure 2. a) Mass spectrum of negative ions produced by the electron-impact source. Inset: High-resolution mass spectrum obtained by the linear reflectron mass spectrometer. b) Positive-ion time-of-flight (TOF) mass spectrum generated by intercepting COT^- with the pump and probe pulses at 160 fs. c) Time-dependent mass spectra of the positive ions obtained at three different delay times (0, 160 fs, and 5.6 ps), which display the increment of the positive-ion signals with respect to the reference at -1 ps [$I(t) - I_{t < 0}$]. With the pump and probe pulses we always observed enhancement of fragment cations as well as the parent COT^+ at all positive delay times. Thus, the enhanced COT^+ signal is due to the generation of neutral COT by the pump pulse at positive delay rather than from the depletion of the background COT^+ at negative delay. For the latter, the fragment-ion signal should appear as a dip in the difference spectrum. Note that the peak for COT^+ first increases and then decreases with time (see Figure 3).

transition state (TS) of the neutral reaction. The released electron carries most of the energy, and the wave packet is launched directly at the transition state (Figure 1). We then ionized the neutral COT with two photons of the probe pulse (270 nm) with variable time delay and monitored the temporal evolution of the mass spectrum of COT^+ (Figure 2c).

The mass spectrum of negative ions is shown in Figure 2a, together with the high-resolution peak obtained by the linear reflectron mass spectrometer. The photoelectron (PE) spectrum (not shown) of COT^- for detachment at 405 nm with a femtosecond pulse is consistent with that reported by the Lineberger group.^[20] Our spectrum shows the main peaks, but the broadening is mainly due to the Doppler effect in the magnetic-bottle detection.^[22] In our ion source some minor intensity (hot bands) was observed at the < 1 -eV threshold. The important point is that the PE spectrum displays the

electron detachment to both the singlet (1.1 eV) and triplet (1.6 eV) states.

Figure 2b shows the positive-ion time-of-flight mass spectrum obtained by using the two femtosecond pulses at a positive delay time. Under our experimental conditions COT⁺ was generated even with the probe pulse alone, but this resulted in a background signal. However, when both the photodetaching pump pulse and the ionizing probe pulse were temporally synchronized, we observed the transient behavior in the mass spectrum of the positive ions shown in Figure 2c for the three delay times 0 fs, 160 fs, and 5.6 ps.

In order to follow the temporal evolution, we recorded many such transients, and the behavior is shown in Figure 3 for gating at the mass of COT⁺. The observed transient exhibits two characteristics: 1) positive signal offset (shown by a thin line) and 2) an apparent damped oscillatory behavior. The positive offset is understood because the detachment of electron from COT⁻ yields both the singlet and triplet states of COT, as evident in the PE spectrum. The offset is flat within 4 ps (Figure 3) and up to 12 ps (not shown), and this is consistent with a contribution from a long-lived triplet state ($\approx 100 \mu\text{s}$).^[23]

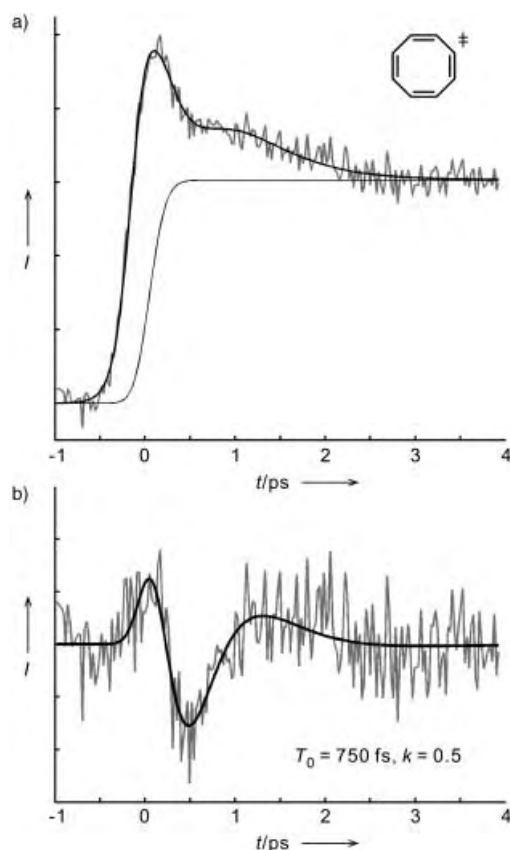


Figure 3. Transition-state dynamics of ground-state COT. Femtosecond transients of COT generated by the pump–probe scheme described in Figure 1 and gated using the COT⁺ ion signal. a) The measured transient is shown together with the theoretical fit of Equation (2). The oscillatory feature ($a=0$) is illustrated in b (see text for details). There is a small contribution at negative time because of the reverse “probe–pump” signal. This is clear in our analysis because the initial peak is a factor of two broader than our cross correlation.

The oscillatory behavior is reminiscent of the transient behavior observed by our research group for the photoisomerization of *cis*-stilbene.^[24] The transient fit shown in Figure 3 was obtained using the molecular-response function with proper convolution given in Equation (2), where γ is the

$$M(t) = \exp(-\gamma t) \cdot \{a + b \cdot \cos[\omega(t)t]\} \quad (2)$$

decay rate and a and b are constants. The time-dependent frequency is $\omega(t) = 2\pi/(T_0 + kt)$, where T_0 is the initial period and k gives its change (dispersion) with time. From the fit we obtained $\gamma^{-1} = 750$ fs, $a/b = 2.6$, $T_0 = 750$ fs, and $k = 0.5$. The residual sinusoidal component ($a=0$) is shown in Figure 3b.

These results indicate that the prepared wave packet of COT nuclear motions (the planar structure of the TS) initially decays (and dephases) on a time scale similar to the oscillatory period between the two tub-shaped structures. Also, as the wave packet moves away from the TS the period increases by 50 % in 750 fs.

In order to elucidate the nature of the oscillatory motion on the ground-state surface, we first carried out a quantum simulation of wave-packet trajectory along the one-dimensional ring inversion (Figure 1). The potential energy curve was calculated using density functional theory, and we obtained all normal modes at different geometries including the ring inversion of imaginary frequency. Regardless of the probe-window position, we obtained a recurrence period of ≈ 350 fs, which is about half of our experimental value. This large discrepancy led us to examine the importance of the multidimensional energy landscape in view of the fact that transverse (to the TS) mode(s) can be very important.

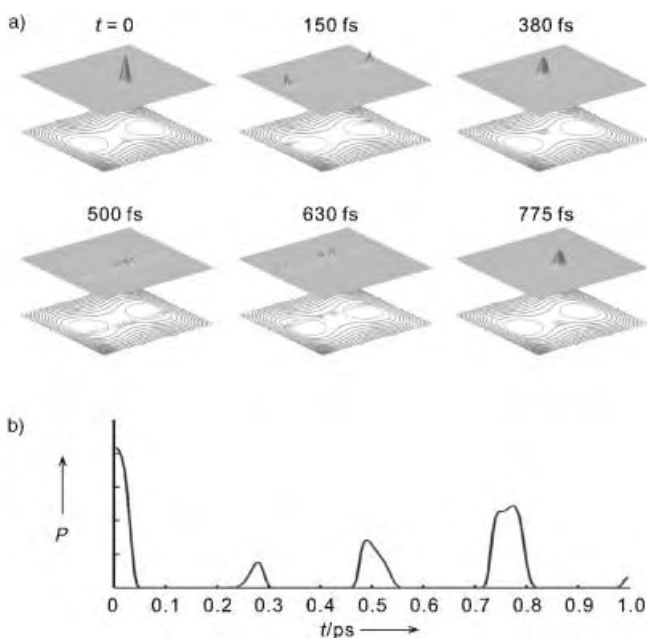


Figure 4. a) Snapshots of wave-packet evolution on the two-dimensional contour (R1, R2). Note that the wave packet returns to the initial position at 775 fs. b) The simulated molecular-response function obtained by opening the probe window at the initial wave-packet position. P = probability.

On a two-dimensional surface, which includes both the reaction coordinate for ring inversion (RI1) and a second coordinate whose frequency is close to that of another ring inversion (RI2, $\approx 200\text{ cm}^{-1}$), we obtained the snapshots shown in Figure 4; the RI1 and RI2 coordinates correspond to the flipping motions along the double and single bonds, respectively (Figure 5). From these quantum calculations we plotted the probability as a function of time, with the detection window located at the TS launching position.^[25] We can see that the prominent wave-packet period is now in agreement with experiment (775 fs). However, we must account for the selectivity imposed on the window of detection along the harmonic transverse mode (RI2). This asymmetry of detection can be rationalized by considering the multiple transition states shown in Figure 5. COT, through

bond shifting (BS, $\approx 1700\text{ cm}^{-1}$; 20 fs), can acquire a D_{8h} TS which is located only 4 kcal mol⁻¹ from the D_{4h} geometry.^[19] As shown in Figure 5, this ultrafast interconversion can induce such asymmetry. The two lowest frequency normal modes (degenerate E_{2u}) at the D_{8h} geometry are the linear combination of the first (reaction coordinate) and the second ring-inversion modes, which suggests that Duschinsky rotation plays an important role, especially at the transition state of the reaction coordinate.^[26]

In conclusion, we have demonstrated real-time probing of the transition-state dynamics of cyclooctatetraene in the electronic ground state. This transition state is unique in its definition for thermal reactions, and here it was directly accessed by detachment of the electron from the planar COT⁻ anion. The motion in the multidimensional nuclear space in

the reaction of neutral COT was monitored by ionization/mass spectrometry. The oscillatory feature observed in the transients reflects trajectories of motion (resonance) along the reaction coordinate, the ring-inversion motion. With the aid of quantum calculations we examined the nature of the modes involved and the energy landscape. It should now be possible to apply this methodology to other ground-state thermal reactions for the real-time probing of their transition states.

Experimental Section

The ion-beam apparatus was described elsewhere.^[27] Briefly, COT⁻ was generated by crossing a $\approx 1\text{-keV}$ electron pulse with the gaseous mixture of COT/O₂/Ar. The COT vapor at 25°C was expanded into a high-vacuum chamber by flowing through premixed Ar/O₂ gas (approximately 95% Ar and 5% O₂) at a total backing pressure of 400 psig. A small amount of O₂ was introduced in the gas line, which produced more COT⁻ and stabilized the ion intensity. The ion beam was collimated by a skimmer (1.0-mm hole) and then directed into the field-free time-of-flight region by applying a -3.0-kV electric pulse to a two-stage accelerator. In the field-free region, ions were separated by their masses, and the anion packet of COT⁻ was intercepted with femtosecond laser pulses by adjusting the switching time of the ion accelerator with respect to the laser arrival time.

The femtosecond laser pulses (110 fs) at 810 nm and 820 nm were generated from a Ti:sapphire oscillator and amplified by the regenerative and the two-stage multipass amplifiers. The IR fundamental was split (50:50), and one of the beams was frequency-doubled. The other beam was converted to the third harmonic by sequential doubling and sum-frequency mixing. The second harmonic (405 nm or 410 nm)

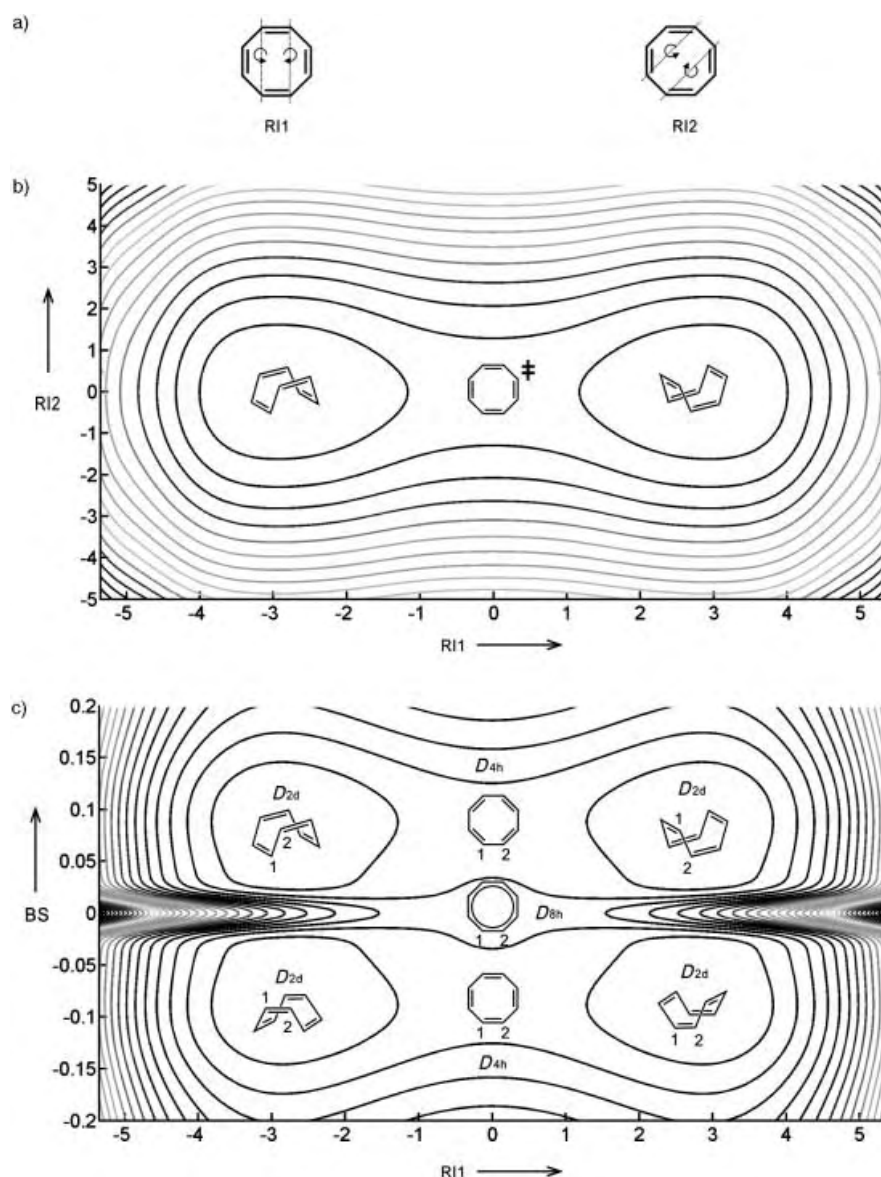


Figure 5. a) Representation of the two coordinates of ring inversion. b) Two-dimensional representation of the potential-energy surface of the electronic ground state along RI1 and RI2. The contour is constructed by adding two one-dimensional curves. c) Potential contours schematic along the ring inversion RI1 and bond-shifting (BS) coordinates.

was used as the pump pulse to photodetach the electron from COT^- , and the third harmonic (270 nm or 273 nm) was used as the probe pulse to ionize the nascent neutral COT by two-photon absorption. Positive ions generated in the laser-interaction region were collected by the reflectron mass spectrometer, and the detached electron was analyzed by the magnetic-bottle PE spectrometer. Time-dependent transient was obtained by integrating the COT^+ ion peak as a function of the delay time between the pump and probe pulses (cross correlation 300 fs).

Ab initio calculations for the potential energy along the reaction coordinate (RI1) were made using the Gaussian 98 program at the B3LYP/cc-pVTZ level of theory.^[28] Wave-packet motions were calculated by numerically solving the Schrödinger equation.

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Keywords: cyclooctatetraene · femtochemistry · photodetachment · transition states · wave-packet dynamics

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Molecular Recognition

Identifying Specific Conformations by Using a Carbohydrate Scaffold: Discovery of Subtype-Selective LPA-Receptor Agonists and an Antagonist**

Yoko Tamaruya, Masato Suzuki, Goshu Kamura, Motomu Kanai, Kotaro Hama, Kumiko Shimizu, Junken Aoki, Hiroyuki Arai, and Masakatsu Shibasaki**

Lysophosphatidic acids (LPA) are a group of important extracellular signaling molecules that elicit a wide variety of fundamental biological responses, such as cell-growth stim-

[*] Y. Tamaruya, M. Suzuki, G. Kamura, Dr. M. Kanai, K. Hama, K. Shimizu, Dr. J. Aoki, Dr. H. Arai, Dr. M. Shibasaki
Graduate School of Pharmaceutical Sciences
The University of Tokyo
Hongo, Bunkyo-Ku, Tokyo 113-0033 (Japan)
Fax: (+81) 3-5684-5206
E-mail: kanai@mol.f.u-tokyo.ac.jp
mshibasa@mol.f.u-tokyo.ac.jp

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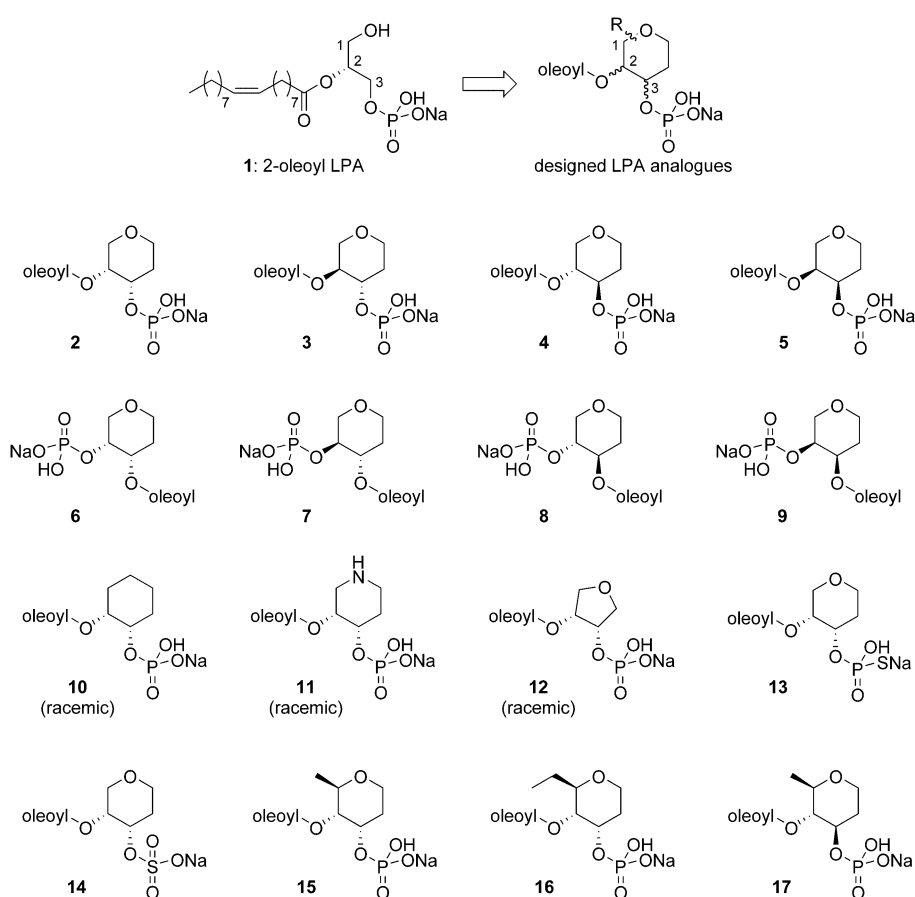
Supporting information for this article is available on the WWW under <http://www.angewandte.org> or from the author.

ulation, calcium mobilization, escape from apoptosis, tumor-cell invasion, and smooth-muscle contraction.^[1] The diverse properties are mediated through interactions of LPA with G-protein-coupled receptors named LPA₁, LPA₂, and LPA₃. Studies to explore the specific role of each subtype are in progress, and are essential for further advances, for example, for designing drugs to modulate a specific LPA-mediated signal-transduction pathway. Studies with subtype-selective agonists and antagonists are very powerful for this purpose. Herein, we describe a new strategy for the discovery of subtype-selective ligands for LPA receptors, based on the identification of specific active conformations of LPA by using carbohydrates as scaffolds.

2-Oleoyl LPA (**1**) is the most potent physiological molecule in the LPA family. Studies on this LPA-receptor ligand have focused mainly on the generation of stable analogues by preventing acyl migration between the 1- and 2-hydroxy groups.^[2–4] These studies led to the proposed LPA pharmacophore,^[5] as well as to several subtype-selective agonists and antagonists.^[6] The activity and selectivity of these analogues, however, are not yet satisfactory for their use as biological tools. This might be partly because of the conformational flexibility of these analogues. The population and/or lifetime of analogues in the active three-dimensional conformation might not be sufficient for selective receptor activation as a result of a thermal disturbance. Therefore, we planned to restrict the conformational flexibility by introducing a ring structure.^[7]

Our molecular design for LPA analogues is summarized in Scheme 1. Based on the hypothesis that different LPA-receptor subtypes distinguish different three-dimensional arrangements of a negative charge (phosphate anion), a long hydrophobic tail (oleoyl group), and a hydrogen-bond acceptor (oxygen atom at the 1-position),^[8] we synthesized an array of molecules that display these recognition motifs at various relative positions defined by core carbohydrate scaffolds.^[9] By employing a variety of readily available enantiomerically pure carbohydrate configurational isomers, the arrangement can be finely tuned. The diversity of the arrangements can be further increased by introducing substituents on the ring to modulate the ring conformation. For each arrangement, a particular recognition motif can be modified independently to examine the effect of the motif. Furthermore, problematic acyl migration is prevented in these analogues, because the oxygen atom corresponding to the 1-hydroxy group of 2-oleoyl LPA is incorporated into the pyran ring. Based on this idea, we synthesized approximately 40 molecules; selected compounds are shown in Scheme 1.^[10]

First, the agonist activity of **2–9** was examined. Upon stimulation with the LPA analogues, the increase in Ca²⁺



Scheme 1. Design of LPA analogues and selected synthesized compounds.

concentration in insect Sf9 cells that express LPA₃ receptors was assessed (Figure 1a).^[11] Compounds **2–9** include all possible stereo- and regioisomers derived from the carbohydrate template. The isomer **2** had 5- to 10-fold higher agonist activity ($EC_{50} \approx 10$ nM) relative to the commonly used agonist 1-oleoyl LPA ($EC_{50} \approx 50$ – 100 nM). The other isomers **3–9** were 10- to 500-fold less potent than **2**. Specifically, the natural (2*R*)-oleoyl LPA analogue **2** was 50-fold more active than the non-natural 2*S* analogue **3** ($EC_{50} > 500$ nM).^[12]

Next, we investigated the effect of individual recognition motifs on LPA₃ activation (compounds **10–14**) by fixing the stereochemistry to match that of the superior agonist **2**. Analogues **10**, **11**, and **12** did not show any agonist activity, which indicated the essential role of the oxygen atom in the six-membered ring as a hydrogen-bond acceptor.^[10] On the other hand, the activity of the thiophosphate analogue **13** was approximately 100- to 500-fold higher ($EC_{50} \approx 0.5$ nM) than 1-oleoyl LPA. Thus, **13** is one of the most potent LPA₃ agonists reported to date.^[13] Moreover, **13** had only very weak agonist activity for LPA₁ (500- to 1000-fold weaker than 1-oleoyl LPA, Figure 1b), and no agonist activity for LPA₂.^[10,14] Thus, **13** is a highly potent LPA₃-selective agonist that can be used as a biological tool.^[15]

We next targeted the discovery of LPA₁-selective agonists. An LPA₁-selective agonist would be highly desirable, because of the importance of this receptor. No agonist has been reported that can selectively activate the LPA₁ receptor in the

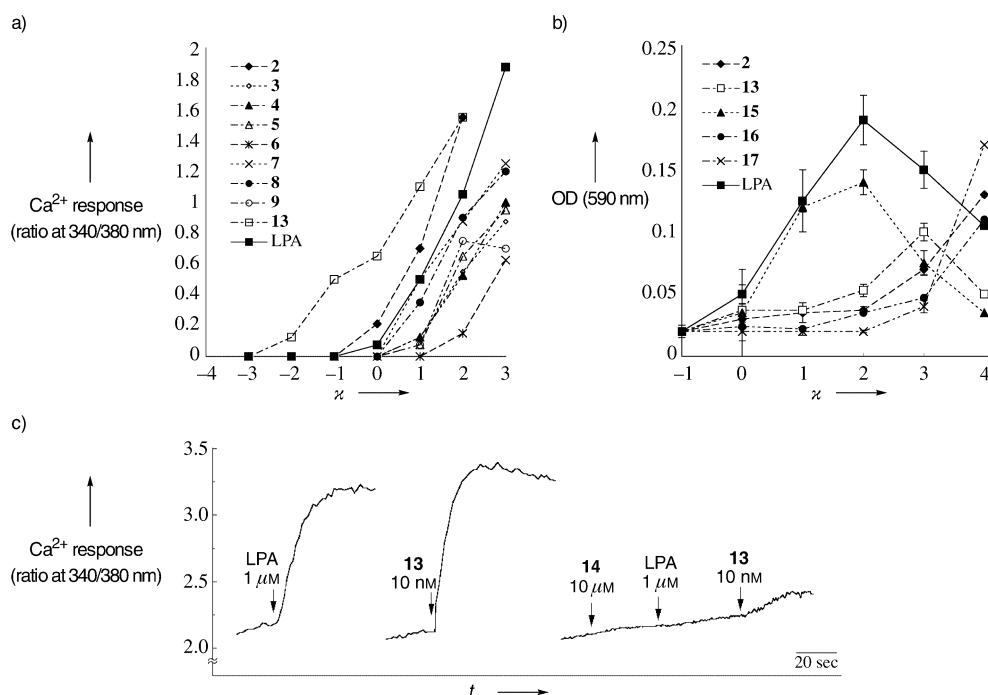


Figure 1. a) LPA₃-agonist activities. Assays were performed at least three times; representative data are shown. The change in the intracellular concentration of Ca²⁺ ions (Ca²⁺ response; y axis) upon activation by the agonists was determined by the emission ratio of Fura-2 AM fluorescence at an excitation wavelength of 340 nm (Ca²⁺-bound form) and 380 nm (Ca²⁺-free form); $x = \text{dose}$ (10⁶ nM). b) LPA₁-agonist activities. The numbers of migrated cells upon activation by the agonists was determined by measuring the optical density (OD) at 590 nm (y axis). c) LPA₃-antagonist activity of 14.

presence of the LPA₂ receptor. As compounds **2–14** had no significant agonist activity for LPA₁, we attempted to introduce a substituent on the six-membered ring to adjust the relative position of the recognition motifs for LPA₁ through fine-tuning of the ring conformation. Thus, we synthesized **15–17** and found that **15** had equivalent or stronger potency as an agonist for LPA₁ relative to 1-oleoyl LPA (Figure 1b).^[14,16] As **15** did not activate LPA₂,^[10] it is the first compound that can be used to distinguish between LPA₁- and LPA₂-agonist activity.^[15,17]

The subtype-selective agonist activity of **2**, **13** (LPA₃-selective), and **15** (LPA₁-selective) might be partly rationalized based on the hypothesis that a specific LPA receptor distinguishes a specific three-dimensional arrangement of the recognition motifs. To test this hypothesis, we determined the ring conformation in a solution state by using NMR techniques. All NOE data and coupling constant values suggested that **2** exists in a skewed-boat conformation with both the phosphate and oleoyl groups in pseudoequatorial positions (Figure 2a). On the other hand, **15** exists in a chair conformation with the methyl and oleoyl groups in equatorial positions and the phosphate group in an axial position (Figure 2b).^[18] The observed recognition-motif arrangements in **2** and **15** might correspond to the active binding structures of flexible 2-oleoyl LPA to LPA₁ and LPA₃, respectively, and the arrangements might be recognized selectively by each receptor.

Finally, we found that the analogue **14**, which contains a sulfate instead of a phosphate group, inhibited LPA₃ (Figure 1c).^[10,19] No response was observed upon activation of

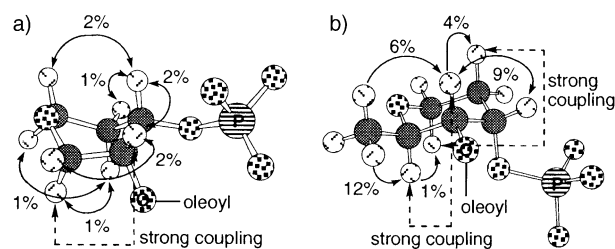


Figure 2. Observed conformations of **2** (a) and **15** (b) in solution in CD₃OD.

LPA₃ with 1-oleoyl LPA after pretreatment with **14** (10 μM), and only weak activation was observed even with the highly potent agonist **13**. Moreover, **14** did not antagonize the 1-oleoyl LPA induced migration of LPA₁-expressing cells (MDA, PC3, and 203g), nor the 1-oleoyl LPA-induced mobilization of Ca²⁺ ions in LPA₂-expressing cells (HT29). Thus, the antagonist activity of **14** is LPA₃-selective. Although the inhibitory activity was not very strong, **14** can be used as a lead LPA₃-selective antagonist for further structural optimization.

In conclusion, potent and subtype-selective agonists (**2**, **13**, and **15**) for LPA₁ and LPA₃ were developed by using carbohydrates as a core structure. The basic concept for ligand discovery was the selective extraction of active three-dimensional recognition-motif arrangements from conformationally flexible 2-oleoyl LPA. The concept allowed the discovery of a lead compound **14** for subtype-selective antagonists. To our knowledge, this is the first example of

receptor subtype-selective recognition by an array of small molecules by changing the relative three-dimensional arrangement of pharmacophores attached to a carbohydrate core. These compounds can be synthesized on a gram scale, and are stable for at least several months at -20°C . Physiological studies on these subtype-selective agonists, as well as studies toward the development of potent and selective LPA antagonists are in progress.

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Keywords: agonists · biological activity · carbohydrates · conformation analysis · receptors

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- [17] Compound **15** activates the LPA_3 receptor with comparable efficacy to 1-oleoyl LPA; see Supporting Information.
- [18] The ring in compound **16** should occupy the same conformation as that in **15**; however, **16** might not fit into the binding pocket of LPA_1 as a result of the bulky substituent.
- [19] Only **14** showed inhibitory activity among compounds **2–17**.

Heterogeneous Catalysis

MOCVD-Loading of Mesoporous Siliceous Matrices with Cu/ZnO: Supported Catalysts for Methanol Synthesis**

Ralf Becker, Harish Parala, Frank Hipler,
Olga P. Tkachenko, Konstantin V. Klementiev,
Wolfgang Grünert, Hagen Wilmer, Olaf Hinrichsen,
Martin Muhler, Alexander Birkner, Christof Wöll,
Sven Schäfer, and Roland A. Fischer*

The Cu/ZnO system is the basis of industrial methanol synthesis^[1] and an important component of fuel-cell technology.^[2] Furthermore, it is a prototype for the exploration of synergistic metal-support interactions in heterogeneous catalysis.^[3] Recently, high-resolution in situ TEM studies revealed dynamic structural changes of copper nanocrystallites (2–3 nm) deposited onto ZnO, which depended on the redox potential of the gas phase.^[4] The reducing conditions (H₂/CO) of methanol synthesis lead to a flattening of the copper particles which increases the coverage of the ZnO support. A positive correlation between the microstructural strain of ZnO-supported copper nanoparticles and catalytic activity has been described.^[5] The promotion of Cu(111) surfaces with zinc reveals that the formation of Cu/Zn alloys is also of importance.^[6] A common motif in discussion is the presence of CuZnO_x species at the Cu/ZnO interface, which is in agreement with recent theoretical studies.^[7] These findings inspired us to look for new strategies in the synthesis of the catalysts, which allow a molecular control of the Cu/ZnO interface and offer new possibilities to its maximization.

Owing to their high specific surface area and precisely controllable pore structures in the lower nm range, periodic mesoporous silicates (PMS), such as MCM-41, MCM-48, SBA-15, have proven to be excellent supports for numerous

catalytically active species. In particular the Cu/PMS and CuO_x/PMS have been studied extensively.^[8] Less is known about Cu/ZnO/PMS catalysts.^[9] In addition to conventional aqueous impregnation/calcinations procedures, metal-organic chemical vapor deposition (MOCVD) techniques can be considered for the preparation of catalysts.^[10]

Treating a sample (350 mg) of freshly calcined MCM-41^[11] ($\phi_{\text{BJH}} = 2.7 \text{ nm}$, $S_{\text{BET}} = 712 \text{ m}^2 \text{ g}^{-1}$) under static vacuum (0.1 Pa) with the vapor of the blue-violet copper precursor [Cu(OCHMeCH₂NMe₂)₂] (**1**, 1.0 g)^[12] at 340 K in a sealed Schlenk tube, causes the original white siliceous support to turn light blue. Comparison of the IR data of loaded MCM-41 with that of pure **1** reveals that **1** is adsorbed intact (see Supporting Information). The adsorption is strong, since **1** cannot be desorbed even at elevated temperatures (373 K) in dynamic vacuum (0.1 Pa, 24 h). The interaction of **1** with the pore walls is clearly through hydrogen bonds, this is indicated by the absence of the bands at 3745 cm⁻¹, which stem from free silanol groups in the untreated material. Treating the support, which is coated with **1**, with diethyl zinc vapor (this is done by placing both samples in a Schlenk tube and sealing it after evacuation (0.5 g ZnEt₂, 300 K, 0.1 Pa)), leads to a slow change in color from light blue to red brown. The powder X-ray diffraction (XRD) pattern of a sample prepared under inert atmosphere shows a weak, broad reflection at $2\theta = 44.70^\circ$ which is assigned to the reflection of the (111) crystal plane of small copper particles (Figure 1 and 2). Solid-state ¹³C NMR spectroscopic studies reveal that [Zn(OCHMeCH₂NMe₂)₂] (**2**) is formed as a byproduct (see Supporting Information). The conversion taking place in the nanotubes of the PMS corresponds to the solution-phase reaction outlined in Scheme 1, in which metallic copper precipitates (XRD), the zinc alkoxide **2** stays in solution (NMR spectroscopy), and gaseous butane is evolved (GC-MS). It is observed, that reducing the reactivity of the zinc alkyl used, for example, by employing highly bulky alkyl groups, such as C(SiMe₃)₃, does

[*] Dr. R. Becker, Dr. H. Parala, Dr. F. Hipler, Prof. Dr. R. A. Fischer
Inorganic Chemistry II—Organometallics & Materials Chemistry
Ruhr-University Bochum, 44870 Bochum (Germany)
Fax: (+49) 234-321-4174
E-mail: roland.fischer@ruhr-uni-bochum.de

Dr. O. P. Tkachenko, Dr. K. V. Klementiev, Prof. Dr. W. Grünert,
Dr. H. Wilmer, Priv.-Doz. Dr.-Ing. O. Hinrichsen, Prof. Dr. M. Muhler
Engineering Chemistry, Ruhr-University Bochum, 44870 Bochum
(Germany)

Dr. A. Birkner, Prof. Dr. C. Wöll
Physical Chemistry I, Ruhr-University Bochum, 44870 Bochum
(Germany)

Dipl.-Chem. S. Schäfer
Inorganic Chemistry I—Cluster and Coordination Chemistry, Ruhr-
University Bochum, 44870 Bochum (Germany)

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

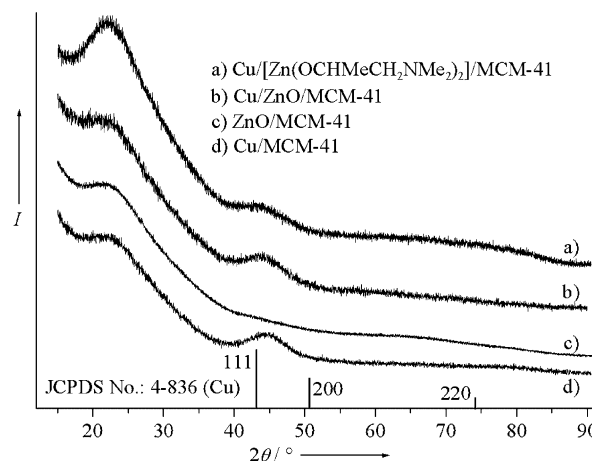


Figure 1. X-ray powder diffractograms of the samples: a) the precursors Cu/[Zn(OCHMeCH₂NMe₂)₂]/MCM-41 obtained at room temperature, b) the catalytically active sample Cu/ZnO/MCM-41 and the reference samples c) ZnO/MCM-41, and d) Cu/MCM-41. The (111), (200), and (220) reflections of polycrystalline copper are marked at the abscissa.

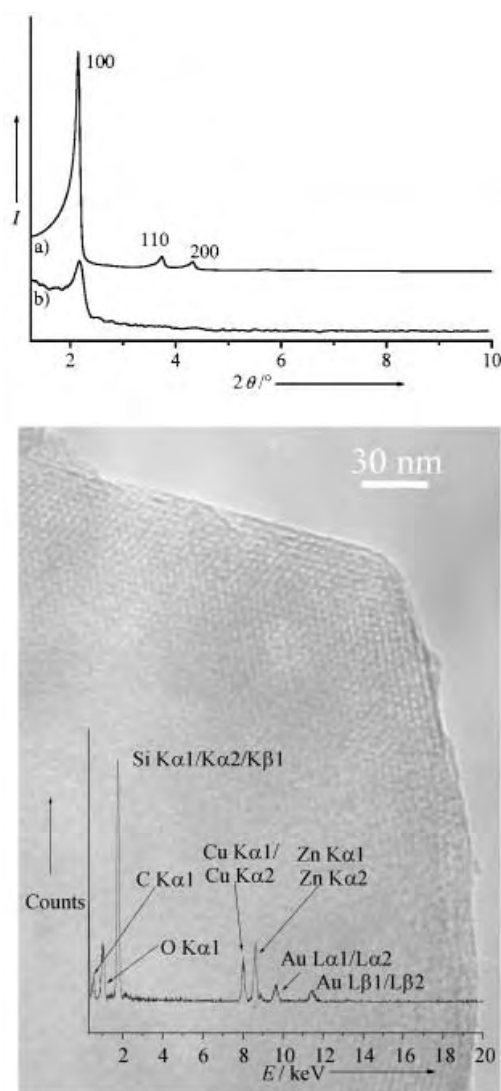
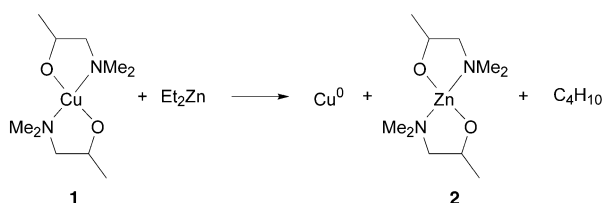


Figure 2. Low-angle powder X-ray diffractograms of a) empty calcined MCM-41 and b) Cu/ZnO/MCM-41. The characteristic reduction of the intensity of (b) compared to (a) is an indication for the loading of the pores.^[11] Inset: TEM image of Cu/ZnO/MCM-41 which clearly reveals that the pore structure is intact, and that owing to their small size the copper or zinc oxide particles can not be seen. The presence of copper and zinc is verified by the corresponding EDX spectrum.



Scheme 1. Reaction of 1 with diethyl zinc in the nanotubes of the MCM-41 support.

not lead to an alkyl/alkoxide metathesis and bimetallic alkyl zinc/copper alkoxide complexes can be isolated and structurally characterized. To date, only microcrystalline and catalytically inactive Cu/ZnO materials were obtained by the solid-state pyrolysis of these bimetallic complexes.^[13]

Careful annealing of the Cu/[Zn(OCHMeCH₂NMe₂)₂]/PMS sample at 623 K in dynamic vacuum (0.1 Pa, 2 h) provides a material which is free from CH_x contamination, but still exhibits a very broad Cu(111) reflection. On the basis of XRD there is no evidence for the formation of ZnO nanocrystallites (Figure 1). The specific copper surface area of the Cu/ZnO/PMS samples is 5–6 m²_{Cu} g^{−1}_{cat} both before and after the catalytic tests (Table 1).^[14] The methanol synthesis performances, determined to be 19 to 130 μmol g^{−1}_{cat} h^{−1}, are in the same range as those of binary Cu/ZnO catalysts, prepared by conventional coprecipitation/calcination techniques. Because of its three dimensional (3D) pore structure, MCM-48 offers more efficient diffusion than MCM-41. Thus, the highest values in the test runs were obtained using MCM-48 as the support. The reduction (with H₂) of samples that were fully oxidized (disappearance of the Cu(111) reflection) by storage under ambient conditions regenerated both the initial activity and the copper surface area. The two samples Cu/MCM-41 (10–12 wt. %; 5–7 m²_{Cu} g^{−1}_{cat}) and ZnO/MCM-41 used for comparison were synthesized by annealing [M(OCHMeCH₂NMe₂)₂]/MCM-41 (M = Zn, Cu), or by treating MCM-41 with diethyl zinc vapor followed by a calcination step and were catalytically not active. Freshly prepared Cu/ZnO/PMS samples exhibited remarkable high “apparent” Cu surface areas of 50–60 m²_{Cu} g^{−1}_{cat} during the first oxidation/reduction cycle (N₂O/H₂), which decreased to the characteristic level of 5–6 m²_{Cu} g^{−1}_{cat} within the next cycle. Clearly, this discrepancy is not due to a sintering of the copper particles, but is a result of the O–Zn–C₂H₅ groups (solid-state NMR, see Supporting Information) bound to the pore walls of PMS. In accordance with Scheme 1, these groups represent an excess of Zn species and are oxidized by N₂O.

EXAFS spectra (Figure 3) of Cu/ZnO/MCM-41 confirm the existence of very small Cu aggregates. The calculated coordination number of 5.8 and the relatively high Debye–Waller factor of the first metal shell are indications of a high amount of disorder. Assuming spherical, mono dispersed particles, would lead to a particle diameter of 0.7 nm, which corresponds to a cluster of 13 copper atoms.^[15] Even though an underestimation of the particle size has to be considered because of the correlation of coordination number and Debye–Waller factor, the typical dimension of the particles must be below the pore diameter of 2–3 nm as higher coordination shells are absent. There appears to be a particle size distribution, hence X-ray diffraction can detect larger particles (around 2 nm). The Cu–Cu separation of 2.50 Å, calculated from the Cu(111) reflex at 2θ = 44.35°, resembles that obtained from the EXAFS data (2.51 Å) and differs from the Cu–Cu separation in the bulk phase of 2.56 Å. This decrease in Cu–Cu separation is a typical effect of small particles.^[16] The origin of the Cu–O coordination cannot be explained unambiguously. Because of the medium particle size it seems reasonable to assume that oxygen atoms from the pore wall can be detected in the EXAFS spectrum.^[17] However, it cannot be excluded, that a small amount of copper exists as Cu⁺. There is no similarity between the Zn_K spectra of the sample and those of ZnO and Zn (Figure 3a). The intensity of the first oxygen sphere is too weak and the second sphere (Zn) is almost completely missing. By consid-

Table 1: Relevant catalytic properties of the Cu/ZnO/PMS samples.

Sample	Rate of production ^[a] in [$\mu\text{mol}_{\text{MeOH}} \text{g}_{\text{cat}}^{-1} \text{h}^{-1}$] at			$\mu\text{mol}_{\text{MeOH}} \text{h}^{-1} \text{m}_{\text{Cu}}^{-2}$	Weight % Cu	Weight % Zn
	$4.6 \text{ m}_{\text{Cu}}^2 \text{g}_{\text{cat}}^{-1}$	$6.2 \text{ m}_{\text{Cu}}^2 \text{g}_{\text{cat}}^{-1}$	$5.8 \text{ m}_{\text{Cu}}^2 \text{g}_{\text{cat}}^{-1}$			
Cu/ZnO ^[b]	71	86	80	13.8	10–90	10–75
Cu/ZnO/Al ₂ O ₃ ^[b]	167	184	172	29.7	30–50	20–40
Cu/ZnO/MCM-41	19			4.1	6.85	10.44
Cu/ZnO/MCM-41		36		5.8	9.30	15.57
Cu/ZnO/MCM-48			130	22.4	10.62	21.95
Cu surface area						
Cu/MCM-41		$5\text{--}7 \text{ m}_{\text{Cu}}^2 \text{g}_{\text{cat}}^{-1}$		n.i. ^[c]	10–12	–
ZnO/MCM-41		–		n.i. ^[c]	–	20.75

[a] The copper surface area was determined using the N₂O-RFC (reactive frontal chromatography) technique.^[14] After a pretreatment with diluted H₂ atmosphere (2 vol %), the catalyst was kept in flowing N₂O (1 vol % N₂O in He, 300 K) and the copper surface area was calculated from the produced nitrogen (density of surface Cu atoms: $1.47 \times 10^{19} \text{ m}^{-2}$). The methanol-synthesis activity was examined under normal pressure at a temperature of 493 K. The synthesis gas used contained of a mixture of 72 % H₂, 10 % CO, 4 % CO₂, and 14 % He. The listed data were taken after a reaction time of 2 h. Because of the low turnover at ambient pressure, only methanol could be identified as a product. [b] Specific catalytic data of classically produced catalysts, for which methanol-synthesis performances have been determined under the same conditions as those used for the Cu/ZnO/PMS samples. By using regression analysis (rate of production dependent on the Cu surface area of catalysts of different metal loading), the production rates of the classical systems were calculated according to the Cu surface area of the Cu/ZnO/PMS samples (interpolation for Cu/ZnO, and extrapolation for Cu/ZnO/Al₂O₃). Data taken from ref. [21]. [c] not identified.

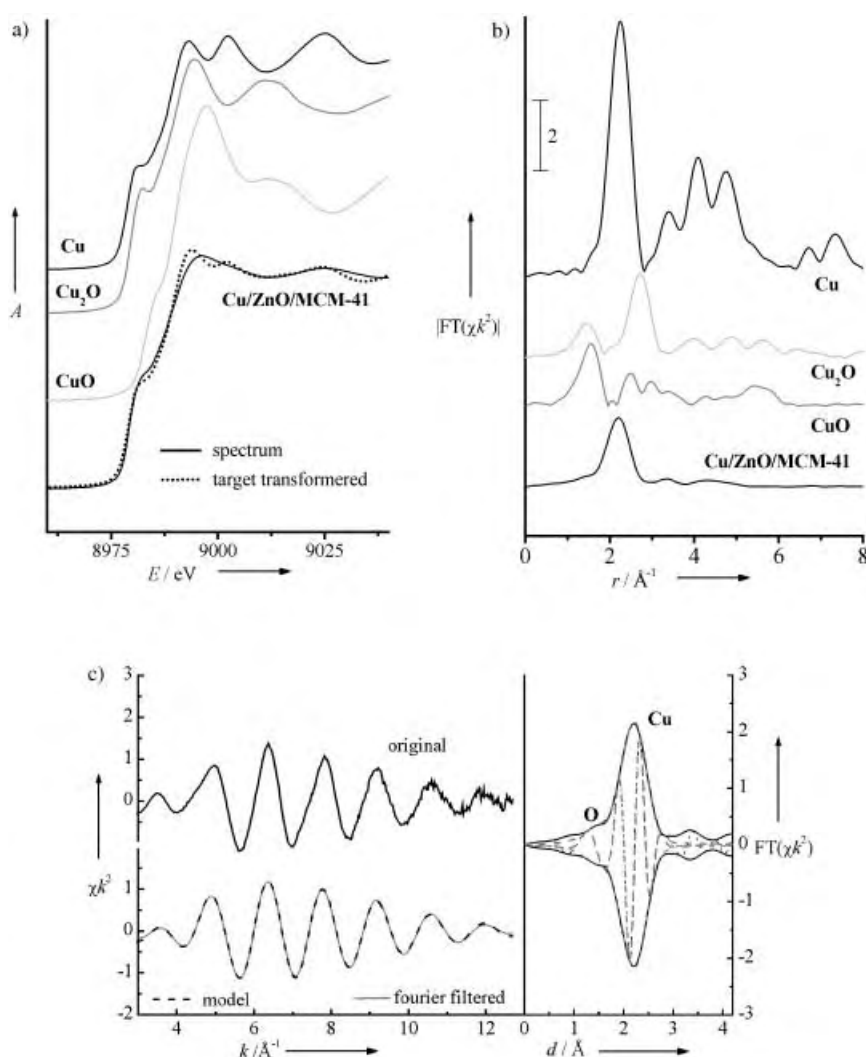


Figure 3. a) Zn_K and b) Cu_K EXAFS spectra (absolute value of the Fourier transform) of Cu/ZnO/MCM-41 and reference samples, c) analysis of the Cu/ZnO/MCM-41 spectrum; model parameters: neighbor Cu separation (d) = $2.512 \pm 0.002 \text{ Å}$, coordination number (N) = 5.8 ± 0.3 , Debye–Waller factor (σ^2) = $(9.6 \pm 0.4) \times 10^{-3} \text{ Å}^2$, neighbor O: $d = 1.86 \pm 0.04 \text{ Å}$, $N = 0.3 \pm 0.1$, $\sigma^2 = (7 \pm 0.11) \times 10^{-3} \text{ Å}^2$.

ering Si and Zn as next-but-one neighbors, we obtained a good alignment, which supports the absence of free silanol groups (solid-state NMR and IR spectroscopy) and indicates coverage of the pore walls with ZnO. Therefore the degree of aggregation of the ZnO component is infinitesimal. ZnO/PMS materials that have been synthesized by aqueous impregnation/calcination techniques exhibit similar properties.^[9] However, unequivocal evidence for CuZnO_x species, or Cu–O–Zn coordination could not be obtained from the data.

Copper-based catalysts for methanol synthesis can be divided into three groups: the binary systems Cu/Al₂O₃ (**I**) and Cu/ZnO (**II**), and the ternary Cu/ZnO/Al₂O₃ (**III**). Classically prepared materials show a linear correlation between synthesis activity and the copper surface area, which increases from **I** to **III**.^[18] The activity of our Cu/ZnO/MCM-48 sample of $130 \mu\text{mol g}_{\text{cat}}^{-1}$ is much higher than the expected value for a binary Cu/ZnO catalyst of same copper surface area and is found in the range of the ternary systems **III**. Interactions of copper particles with the siliceous matrix can be neglected, since the Cu/PMS control sample was not active and the walls of the active Cu/ZnO/PMS sample appear to be covered with ZnO (see above). It seems as if the simultaneous high dispersion of the Cu and the ZnO components leads to a new, positive effect. An aggregated (nano)crystalline ZnO phase, which is inevitably obtained from coprecipitation/calcination methods, is not necessary for the synergistic effects. This observation corresponds to the cited formation of Cu/Zn alloys, and to our finding,

that a classically prepared Cu/Al₂O₃ catalyst can be efficiently promoted by a short pulse of diethyl zinc vapor, which leads to a catalyst with an activity higher than the ternary system.^[18] Using a special, large surface-area, high-defect, nanodispersed ZnO support (153 m² g⁻¹), synthesized from [(Me₃SiO)ZnMe]₄ by solid-state pyrolysis, a surprisingly active, binary Cu/ZnO catalyst can be obtained.^[18]

The perspectives for an MOCVD loading of PMS supports to generate Cu/ZnO materials are clear. Not only a variation of the dimension and framework of the pores (e.g. MCM-41 vs. MCM-48), but also the exploitation of the precursor chemistry offers a possibility to control the Cu/ZnO interface. The MOCVD loading of PMS supports with the ternary Cu/ZnO/Al₂O₃ system using appropriate Al₂O₃ precursors is an option. There is in principal no limit to the simultaneous maximization of the specific copper surface area and the Cu/ZnO interface area (or the ZnO dispersion) and thus to an increase of the catalytic activity beyond that which is currently possible.

Experimental Section

Cu/ZnO/MCM-41: Freshly synthesized,^[11] calcined and dried MCM-41 (350 mg) and a sample of **1** (ca. 1.0 g) were placed inside a Schlenk tube in separate glass receptacles and evaporated in static vacuum (0.1 Pa) to 340 K for 2 h. The blue product (200 mg) and diethyl zinc (ca. 0.5 g) are placed together in a Schlenk tube in separate glass receptacles and evaporated in static vacuum (0.1 Pa) over a period of 2 h at room temperature. The variation of time of vaporization, temperature, initial amounts of the compounds, and differences in the PMS material leads to different loadings (Table 1). Heating a sample of Cu/[Zn(OCHMeCH₂NMe₂)₂]/MCM-41 to 623 K (2 h) in dynamic vacuum (0.1 Pa) provided ZnO. Other PMS-based materials, such as MCM-48, were treated in the same manner.

Cu/MCM-41 and ZnO/MCM-41: Heating a sample of [Cu(OCHMeCH₂NMe₂)₂]/MCM-41 in dynamic vacuum (0.1 Pa) at 523 K (20 min) produced Cu/MCM-41. Pyrolysis of [Zn(OCHMeCH₂NMe₂)₂]/MCM-41 at 623 K (0.1 Pa, 2 h), with **2** as a ZnO precursor, gave ZnO/MCM-41. [Zn(OCHMeCH₂NMe₂)₂]/MCM-41 was prepared by impregnation of MCM-41, in a solution of **2** (1 g)^[19] in pentane (40 mL), followed by several washing cycles of the isolated, solid material. Alternatively, ZnO/PMS can be synthesized by treating the supports with diethyl zinc vapor and a subsequent calcination step.

Characterization: (see also Supporting Information) The powder X-ray diffractograms (PXRD) were measured using a D8-Advance Bruker AXS diffractometer equipped with a position sensitive detector with Cu K_α radiation (λ = 1.5418 Å) in θ-2θ geometry (capillary technique, inert gas). All diffractograms have been fitted with the Profile Plus 2.0.1 software applying a pseudo-Voigt function. TEM analyses were performed on a Hitachi H-8100 instrument at 200 kV with a tungsten filament (preparation under exclusion of air, gold grids Plano, vacuum transfer holder). X-ray absorption spectra were collected at the Hasylab (DESY, Hamburg) at station X1 using a Si(311) double crystal monochromator in transmission (software VIPER^[20]). Nitrogen-adsorption measurements were performed using a Quantachrome Autosorb-1 MP apparatus. The pore diameter was calculated according to the Barrett-Joyner-Halenda (BJH) method. The specific surface area (S_{BET}) of empty, calcined MCM-41 and that of CuO_x/MCM-41 was obtained from the linear part of the BET graph (p/p₀ = 0.05–0.35).

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Chirality

Bis(fluoroformyl)trioxide, $\text{FC(O)OOOC(O)F}^{**}$

Holger Pernice, Michael Berkei, Gerald Henkel,
Helge Willner,* Gustavo A. Argüello,
Michael L. McKee, and Thomas R. Webb

Dedicated to Professor P. J. Aymonino
on the occasion of his 75th birthday

“How long can you make an oxygen chain?” or “How stable are molecules containing long oxygen chains and what are their structures?” are frequently asked questions.^[1–4] There is no question that compounds of the type RO_xR are less stable and less known with increasing x . To date there are no chainlike polyoxides with $x > 3$ and only a few trioxides are well characterized. Only for the trioxide $\text{CF}_3\text{OOOCF}_3$, which is stable at room temperature, is the structure known.^[5]

In the family of compounds of the type $\text{FC(O)O}_x\text{C(O)F}$ with $x = 0–3$ the species with $x = 0$ ^[6,7] and 2 ^[8,9] have been isolated and long since studied. After the first IR spectroscopic evidence for FC(O)OOOC(O)F and FC(O)OC(O)F by photolysis of oxalyl fluoride in the presence of oxygen in a long-path IR cell,^[10] we were able to prepare the more stable fluoroformic acid anhydride on a preparative scale and to characterize it comprehensively.^[11]

Herein we report the synthesis, characterization, and structure of bis(fluoroformyl)trioxide, which can be viewed as the anhydride of the unknown peroxyfluoroformic acid. It is the second, but most simple example of a chainlike acyl

trioxide after $\text{CF}_3\text{OC(O)OOOC(O)OCF}_3$, whose structure is still unknown.^[12]

We observed the formation of FC(O)OOOC(O)F during the synthesis of FC(O)OOC(O)F in molar scale by treating CO with F_2 in the presence of O_2 according to the literature method.^[13] The gas-phase pyrolysis of FC(O)OOC(O)F yielded FCO_2 radicals and allowed the measurement of their rotational spectrum.^[14]

During vacuum transfer of the crude FC(O)OOC(O)F product, the formation of oxygen was observed. At the same time in the IR gas-phase spectra, weak IR bands at 530 and 798 cm^{-1} disappeared while the bands of the other products remained nearly unchanged. Finally the new compound was isolated by continuous sublimation of the more volatile FC(O)OOC(O)F from the product mixture at -95°C in vacuo.

After fast evaporation of a small sample from the residue into an evacuated IR-gas cell, a 1.5-fold pressure increase was observed. Simultaneously at room temperature the IR spectrum changed with a half-life of 70 s. As the only IR-active compound, pure FC(O)OOC(O)F was left behind as a decomposition product in the gas cell according to Equation (1).



These observations suggest the presence of FC(O)OOOC(O)F . With the aid of a reference spectrum the gas-phase IR spectrum of pure FC(O)OOOC(O)F was obtained by subtraction of the peroxide contribution to the spectrum (Figure 1). In Table 1 the observed fundamentals are com-

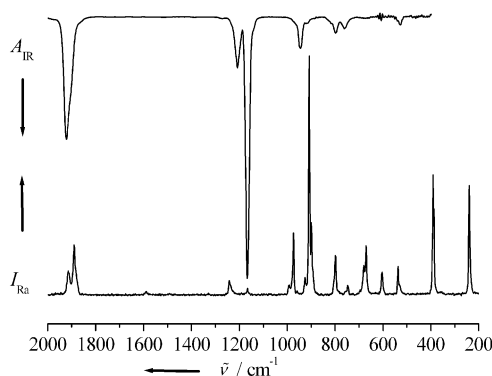


Figure 1. Top: IR gas spectrum of FC(O)OOOC(O)F in absorbance ($p = 67\text{ Pa}$, $T = 25^\circ\text{C}$, optical path length 195 mm, 0.5 mm Si wafer as windows). Bottom: low-temperature (-196°C) Raman spectrum of the solid compound.

pared with the calculated (DFT) band positions and intensities. Of the 21 possible fundamentals 18 could be assigned. The bands at around 800 and 900 cm^{-1} are characteristic of the O–O stretching vibrations. The excellent agreement between observed and calculated band positions is further evidence for the existence of FC(O)OOOC(O)F . In addition it was possible to produce pure trioxide along with OCF_2 and CO_2 by treating CO/ O_2 mixtures with O_2F_2 at -120°C .

[*] Dipl.-Chem. H. Pernice, Dr. M. Berkei, Prof. Dr. G. Henkel, ++
Prof. Dr. H. Willner+
Fakultät 4, Anorganische Chemie
Gerhard-Mercator-Universität Duisburg, Lotharstrasse 1
47057 Duisburg (Germany)
E-mail: willner@uni-wuppertal.de

Prof. Dr. G. A. Argüello
INFIQC, Universidad Nacional de Córdoba
5000 Córdoba (Argentina)

Prof. Dr. M. L. McKee, Prof. Dr. T. R. Webb
Department of Chemistry
Auburn University, AL 36849-5312 (USA)

[+] Current address: Fachbereich C, Anorganische Chemie
Bergische Universität Wuppertal
42119 Wuppertal (Germany)
Fax: (+49) 202-439-2901

[++] Current address: Department Chemie und Chemietechnik
Fakultät Naturwissenschaften
Universität Paderborn
33098 Paderborn (Germany)

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Table 1: Vibrational wavenumbers [cm⁻¹] for the fundamentals of the lowest-energy rotamer of FC(O)OOOC(O)F (C₂) and their assignments.

IR (Gas, 25 °C)	<i>I</i> ^[a]	Calcd ^[b]	<i>I</i> ^[b]	Raman (solid, -196 °C)	Assignment	Approximate description
1921.7	43.9	1954	89.7	1915 m	B, ν_{12}	$\nu_{as}(\text{C=O})$
		1960	5.8	1890 s	A, ν_1	$\nu_s(\text{C=O})$
1207.1	18.3	1196	21.8	1243 w	A, ν_2	$\nu_s(\text{C-F})$
1167.1	100 ^[c]	1155	100 ^[d]	1166 vw	B, ν_{13}	$\nu_{as}(\text{C-F})$
1139 sh	4.3				ν_{13}	$\nu_{as}^{(13)}(\text{C-F})$
969 sh	2.1	992	0	974 s	A, ν_3	
944.9	11.4	948	17.8	926 m	B, ν_{14}	$\nu_{as}(\text{C-O})$
918.5	2.4	936	0.2	909 vs	A, ν_4	$\nu_s(\text{O-O-O})$
797.2	5.9	818	14.2	798 s	B, ν_{15}	$\nu_{as}(\text{O-O-O})$
761.8	4.4	752	5.0		A, ν_5	$\gamma_s(\text{oop, ip, FC(O)O})$
755 sh		747	1.9	748 w	B, ν_{16}	$\gamma_{as}(\text{oop, op, FC(O)O})$
		671	0	680 m	A, ν_6	$\delta_s(\text{COO, OC(O), OOO})$
678.0	0.5	670	0.8	670 s	B, ν_{17}	$\delta_{as}(\text{COO, OC(O)})$
		600	0	604 m	A, ν_7	$\delta_s(\text{OOO})$
530.6	3.0	527	4.1	537 m	B, ν_{18}	$\delta_{as}(\text{C(O)F})$
		380	0	391 vs	A, ν_8	
		376	0.2	387 s	B, ν_{19}	$\delta_{as}(\text{COO})$
		218	0.1	241 vs	A, ν_9	$\delta_s(\text{COO})$
		158	0	177 w	B, ν_{20}	τ
		69	0		A, ν_{10}	$\tau(\text{C-O})$
		63	0.2		B, ν_{21}	τ
		50	0.1		A, ν_{11}	$\tau(\text{O-O})$

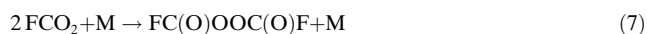
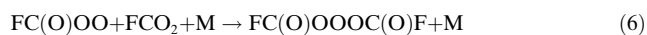
[a] Relative intensities. [b] B3 LYP basis set 6-31+G(d). [c] Absorption cross sections at 1167.1 cm⁻¹: 685 × 10⁻²⁰ cm²/molecule. [d] Corresponds to 852 km mol⁻¹.

For the formation of the trioxide the following reaction sequence is assumed, taking into account the kinetic measurements of the CO reaction with O₂/F₂ mixtures^[15] (M = third body collision partner):

In the mixture CO/O₂/F₂, the rate-determining step in the starting reaction is given by Equation (2), and, if CO/O₂ is treated with O₂F₂ the rate-determining step is given by Equation (3).



The subsequent reactions [Eq. (4)–(7)] proceed very fast.



The ratio of the products trioxide [Eq. (6)] to peroxide [Eq. (7)] is strongly dependent on the temperature, with an increasing yield of trioxide with decreasing temperature. Thermally, the trioxide decomposes unimolecularly according to the reverse of Equation (6) as shown by matrix isolation experiments.^[16] Pure trioxide reacts with equimolar amounts of NO₂ at low temperature (slow thawing) to form the known FC(O)OONO₂.^[17]

For further characterization of the trioxide, NMR, UV, and mass spectra were recorded, and an X-ray diffraction

study was performed. The NMR spectroscopic data fit very well with the data of the related compounds FC(O)O_xC(O)F, *x* = 0–3 (Table 2). The couplings in the AA'XX' spin system decrease between the FC(O) groups with increasing O_x-chain length and the signals of the trioxide appear as an AX spin system. The UV spectrum is very similar to that of FC(O)OOC(O)F^[18] but with somewhat higher absorption cross sections overall. The 70 eV mass spectrum with the fragments F₂CO⁺(3), FCO₂⁺(4), FCO⁺(59), CO₂⁺(100), O₂⁺(32), CO⁺(28) is also consistent with FC(O)OOOC(O)F.

On storage of the trioxide in evacuated glass ampoules on dry ice, over several months single crystals of mm size were formed. On further cooling to -196 °C the crystals shatter as the result of a phase transition. Therefore for the structure determination small crystals were collected in a -85 °C nitrogen gas stream on a copper trough under a microscope.^[19] Suitable crystals were wedged in glass capillaries, flame sealed, transferred to a diffractometer and measured at -123 °C.

Table 2: ¹⁹F and ¹³C NMR data of FC(O)O_xC(O)F, *x* = 0–3.

	δ (¹⁹ F)	δ (¹³ C)	$^1J(\text{CF})$	$ ^{(x+3)}J(\text{FF}) $	$^{(x+2)}J(\text{CF})$
FC(O)C(O)F ^[a]	+23.8	+143.2	-366.3	50.6	+102.8
FC(O)OC(O)F ^[a]	-10.6	+136.0	-293.8	34.6	+12.6
FC(O)OOC(O)F ^[a]	-34.1	+142.3	-301.1	3.8	-
FC(O)OOOC(O)F ^[b]	-30.6	+143.8	-308.6	-	-

[a] Ref. [11]. [b] In CD₂Cl₂ at 243 K. Chemical shifts in ppm relative to internal CFCl₃ (¹⁹F) and CD₂Cl₂ at 53.7 ppm (¹³C), coupling constants in Hz.

In the crystal (unit cell see Figure 2) the fragment FC(O)OO_{0.5} forms the asymmetric unit and the molecule is present in the most stable conformer with C₂ symmetry. In the studied crystal only one of the two possible (left- and right-hand thread) of the *trans-syn-syn* conformers (the C–O bonds *trans* and both C=O bonds *syn* with respect to the O₃ plane) is present and it belongs to the chiral space group *P*4₃2₁. However, similar compounds, such as CF₃OOOCF₃,^[5] CF₃S_xCF₃,^[20] *x* = 2, 3, and CCl₃S₇CCl₃^[21] crystallize as racemic mixtures of left- and right-hand threads in nonchiral space groups. The observed bond lengths and bond angles are generally in good agreement with the calculated data (Table 3). Only the COOO dihedral angle is clearly wider than calculated, presumably caused by packing effects in the crystal. In Figure 3 and the Supporting Information the chiral left-handed C–O–O–O–C chain can be seen. In this case as well as in the structure of CF₃OOOCF₃ the theoretically postulated helical structure of the oxygen framework is found.^[3] Sulfur chains with similar features are present in the compounds CF₃S_xCF₃, *x* = 2, 3, and CCl₃S₇CCl₃.^[20,21]

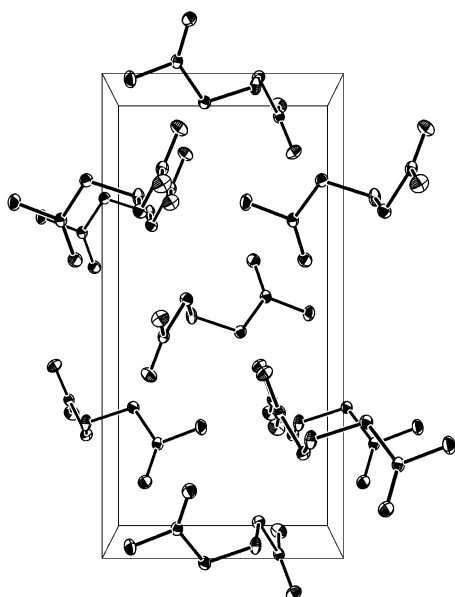


Figure 2. X-ray structure of FC(O)OOOC(O)F at low temperature. View of the unit cell along [100].

Table 3: FC(O)OOOC(O)F, *trans-syn-syn*: bond lengths [Å] and angles [°].

Bond	FC(O)OOOC(O)F		F ₃ COOCF ₃
	Crystal	Calculated ^[a]	Crystal ^[b]
C–F	1.314(2)	1.334	1.315(2)
C=O	1.162(2)	1.181	
C–O	1.364(2)	1.376	1.389(2)
O–O	1.440(1)	1.430	1.437(2)
F–C=O	125.8(1)	126.2	
O–C–O	130.1(1)	129.9	
F–C–O	104.1(1)	103.9	
O=C–O–O	–2.1(1)	2.9	
C–O–O–O	–99.0(1)	–90.7	95.9(8)
O–O–O	104.0(1)	106.4	106.4(1)

[a] B3LYP basis set 6-31+G(d). [b] Ref. [5].

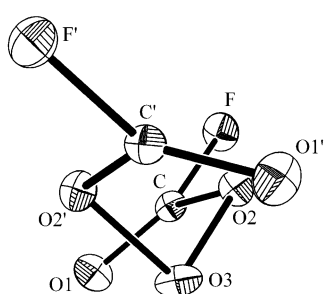


Figure 3. Structure of FC(O)OOOC(O)F (Thermal ellipsoids set at 25 % probability). The left-handed chiral thread can be recognized.

According to DFT calculations^[22] FC(O)OOOC(O)F should be present at room temperature in a mixture of the *trans-syn-syn* rotamer with *C*₂ symmetry (see Figure 3) and the 4 kJ mol^{–1} energetic higher *trans-syn-anti* rotamer of *C*₁ symmetry (see Supporting Information). Indeed there are

indications for the presence of more than one rotamer in the IR matrix spectrum and the low-temperature Raman spectrum.

Experimental Section

Caution: The mixture of reagents for the synthesis of FC(O)OOOC(O)F and FC(O)OOOC(O)F and the peroxide and trioxide are potentially explosive, especially in the presence of oxidizable materials. They must be handled with appropriate safety precautions.

Similar to the described method,^[13] an oxygen (80 cm³ min^{–1}) and a fluorine gas stream (25 cm³ min^{–1}) were mixed in a stainless steel T-joint piece (Swagelok). In a following stainless steel T-joint piece a stream of CO (10 to 15 cm³ min^{–1}) was introduced into the O₂/F₂ mixture. Subsequently the reaction mixture passed through a 500-mL glass reactor at 25 °C followed by two cold traps held at –78 °C (dry ice) and –183 °C (liquid oxygen). Residual O₂, CO, and F₂ were released in the hood through a bubbler filled with perfluorinated oil. The contents of both traps were separated by trap-to-trap condensation in vacuum in traps held at –78, –110, and –196 °C. FC(O)OOOC(O)F and FC(O)OOOC(O)F were retained in the trap at –110 °C. The temperature was raised to –95 °C and under continuous pumping (ca. 10^{–3} mbar) the more volatile FC(O)OOOC(O)F is transferred to another trap until pure FC(O)OOOC(O)F is left behind at –95 °C. After work up of approximately 70 g FC(O)OOOC(O)F about 3 g FC(O)OOOC(O)F were obtained (yield 4 % relative to the peroxide and 1 % relative to CO).

The samples were flame sealed in glass ampoules and stored under liquid nitrogen. By using a special device^[23] ampoules can be opened at the vacuum apparatus, the desired amount can be taken out, and the ampoule flame sealed again.

¹⁹F- or ¹³C NMR: Bruker Avance 300, 282.40 MHz or 75.47 MHz, –30 °C, CFCl₃ and CD₂Cl₂ as internal standard and lock. Vibrational spectra: Bruker FTIR 66v, FT Raman FRA 106.

Crystal structure analysis: Siemens P4RA 4-cycle diffractometer, rotating anode, MoK_α radiation (λ = 0.71073 Å) with graphite monochromator scintillation counter, T = 150 K, empiric absorption correction (Ψ-scan), direct methods, least-square refinements (full matrix) on F², all atoms anisotropic, one extinction parameter, one scaling factor, C₂F₂O₅, M_r = 142.02 g mol^{–1}, tetragonal, a = 6.108(2), c = 12.295(5) Å, V = 458.7 Å³, space group P4₃2₁2, Z = 4, ρ = 2.057 Mg m^{–3}, λ(MoK_α) = 0.252 mm^{–1}, transmission range 0.763–0.731, 2θ_{max} = 48°, ω-scan, crystal size ca. 0.60 mm × 0.35 mm × 0.22 mm, 501 symmetry independent reflections, RI (wR₂) = 0.0285 (0.0577) for 447 reflections with I > 2σ(I), 42 variables. CCDC 224327 (C₂F₂O₅) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

Six conformers of FC(O)OOOC(O)F were found at the B3LYP6-31+G(D)+ZPC level (including thermal corrections)^[22] with the *trans-syn-syn* species at lowest energy (see Supporting Information). The calculated (B3LYP6-31+G(D)) IR band positions and intensities supported the assignment of the observed spectra.

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Keywords: chirality · crystal structure · density functional calculations · IR spectroscopy · oxygen · radical reaction

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Intramolecular C–H Activation in Complexes with Mo–Bi Metal Bonds**

Stefan Roggan, Christian Limberg,* Burkhard Ziemer, and Marcus Brandt

Dedicated to Professor Helmut Werner
on the occasion of his 70th birthday

The unique property of $n\text{MoO}_3/\text{Bi}_2\text{O}_3$ phases to act as catalysts for the allylic oxidation of propene remains a subject of intense discussion,^[1] that stimulates research with respect to the element combination Mo/Bi on the molecular level.^[2–5] Among the known compounds containing these two metals one class exhibits direct Mo–Bi metal bonds, and, bearing in mind the oxygenating properties of bismuthmolybdate phases, it seems interesting to investigate the behavior of such linkages on exposure to oxidants. Such a study, however, is only possible, if the residual complex is not affected by the oxidizing-agent employed, and until recently all known compounds with Mo–Bi units (e.g. $[\text{ClBi}\{\text{Mo}(\text{CO})_3\text{Cp}\}_2]$ ^[3] and $[\text{Mo}_2(\text{CO})_4(\text{MeCp})_2(\mu\text{-}\eta^2\text{-Bi}_2)]$ ^[4] ($\text{Cp} = \text{C}_5\text{H}_5$, $\text{MeCp} = \text{C}_5\text{H}_4\text{Me}$) as well as certain others^[5]) contained ligands that are not inert under oxidizing conditions. We have recently found a synthetic route leading to compounds with both Mo and Bi centers in oxidatively robust coordination spheres and with the Mo centers in comparatively high oxidation states: For instance treatment of $[\text{Cp}_2\text{MoH}_2]$ with $[\{\text{Bi}\{\text{OCH}(\text{CF}_3)_2\}_3(\text{thf})\}_2]$ provided $[\text{Cp}_2\text{Mo}(\text{Bi}\{\text{OCH}(\text{CF}_3)_2\}_2)]$ (**1**), which functions as an effective epoxidation precatalyst.^[6] Herein we describe the investigation of the system $[\text{MeCp}_2\text{MoH}_2]/\text{Bi}(\text{OtBu})_3$ that revealed a remarkable C–H activation process.

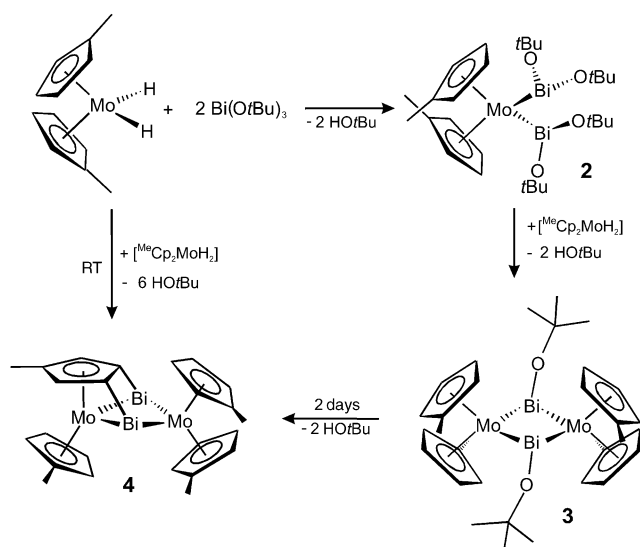
The reaction between $[\text{MeCp}_2\text{MoH}_2]$ and $\text{Bi}(\text{OtBu})_3$ in a 1:2 ratio (Scheme 1) yields $[\text{MeCp}_2\text{Mo}\{\text{Bi}(\text{OtBu})_2\}_2]$ (**2**), the replacement of the electron-poor hexafluoroisopropyl residues employed in the synthesis of **1** by the electron-rich *tert*-butyl residues does not alter the outcome of the reaction. The molecular structure^[7] of **2** is depicted in Figure 1. While the Bi–Bi separation of 3.487 Å is too long to be interpreted in terms of a Bi–Bi bond, the Bi–Mo–Bi angle of 75.27° is even smaller than the H–Mo–H angle in $[\text{Cp}_2\text{MoH}_2]$ (75.5°)^[8] which means that there are no significant repulsive forces between the two $\text{Bi}(\text{OR})_2$ moieties bound to the Mo center. Unlike the

[*] S. Roggan, Prof. Dr. C. Limberg, Dr. B. Ziemer, M. Brandt
Humboldt-Universität zu Berlin
Institut für Chemie
Brook-Taylor-Strasse 2, 12489 Berlin (Germany)
Fax: (+49) 302-093-6966
E-mail: christian.limberg@chemie.hu-berlin.de

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



Scheme 1. Reactions within the system $[\text{MeCp}_2\text{MoH}_2]/\text{Bi}(\text{OtBu})_3$.

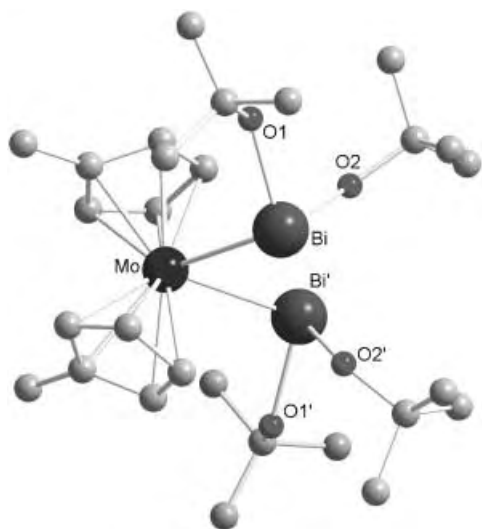


Figure 1. Molecular structure of **2**; all hydrogen atoms are omitted for clarity. Selected bond lengths [Å] and angles [°]: Mo–Bi 2.8551(9), Bi–O1 2.159(5), Bi–O2 2.117(4), Bi–Bi' 3.487(2); Bi–Mo–Bi' 75.27(3), O(1)–Bi–O2 89.5(2), Mo–Bi–O1 102.9(2), Mo–Bi–O2 94.0(2).

reaction leading to **1**, the synthesis of **2**, as followed visually, as well as indicated by its yield proved to be very sensitive to the reaction conditions. This observation gave rise to the following thoughts: from its bond lengths and angles, **2** is an unstrained molecule and should undergo a condensation reaction with excessive molybdocenedihydride, under elimination of two further equivalents of *tert*-butyl alcohol to give **3** (Scheme 1); this should lead to Mo–Bi–Mo angles of approximately 105°, that is, angles that are common to unstrained bismuthanes. Hence, the synthesis of **3** was attempted by adjustment of the stoichiometry of the two reagents $[\text{MeCp}_2\text{MoH}_2]$ and $\text{Bi}(\text{OtBu})_3$ in Scheme 1 from 1:2 (required for the formation of **2**) to 1:1. These experiments were successful but only when they were carried out in petroleum ether from

which **3** precipitates directly as black crystals suitable for an X-ray crystal-structure analysis (Figure 2).^[7] Recently, a dibismuthene complex was described containing a $\text{Me}_3\text{SiCH}_2\text{Bi}=\text{BiCH}_2\text{SiMe}_3$ ligand coordinated to two

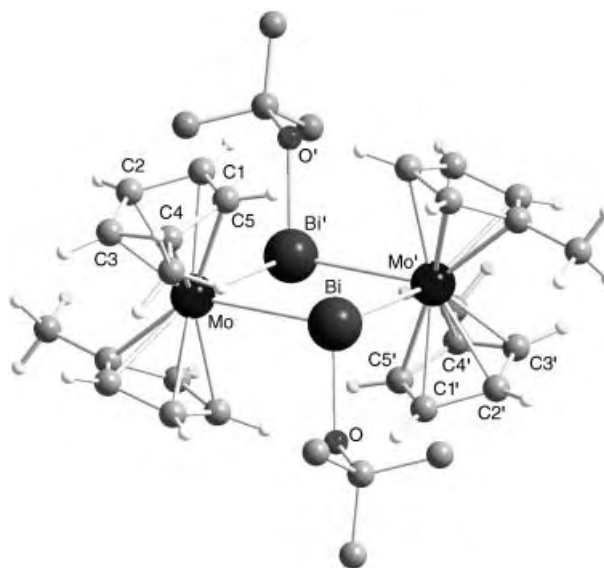


Figure 2. Molecular structure of **3**; the butyl hydrogen atoms are omitted for clarity. Selected bond lengths [Å] and angles [°]: Mo'–Bi 2.960(2), Mo'–Bi' 2.924(1), Bi–Bi' 3.6333(9), Bi–O 2.215(5); Bi–Mo–Bi' 76.3(1), Mo'–Bi–Mo 103.74(2), Mo'–Bi–O 106.6(2), Mo–Bi–O 101.9(2).

$\{\text{W}(\text{CO})_5\}$ moieties in a $\mu_2\text{-}\eta^2\text{:}\eta^2$ fashion^[9] but the Bi–Bi separation in **3** is too long (3.6333 Å) for a corresponding bismuthene complex. The Mo–Bi bonds (2.960, 2.924 Å) are somewhat longer than those in **2** (2.8551 Å) as are the Bi–O bonds. Bi–Mo–Bi and Mo–Bi–Mo angles of 76.3° and 103.74°, respectively, are indicative of an unstrained structure (see above). During the NMR spectroscopic characterization of **3** in C_6D_6 it became clear why all attempts to isolate **3** had failed when its synthesis was pursued in benzene or toluene, solvents in which it is soluble. Compound **3** decomposes in solution within a few hours to give two diamagnetic compounds **4** and **4'** (ratio 1:0.8) with rather complex ^1H NMR spectra. Subsequent investigations showed that **4** and **4'** can be obtained on a preparative scale when an equimolar solution of $[\text{MeCp}_2\text{MoH}_2]$ and $\text{Bi}(\text{OtBu})_3$ in toluene is allowed to react at room temperature for 2 days. Cooling of such a toluene solution provides pure **4** in form of black crystals, and a single crystal X-ray diffraction study^[7] finally revealed its constitution and deviation from **3**: the $\eta^5\text{-MeCpMo}\{\text{Bi}(\text{OtBu})\}_2$ moiety in **3** undergoes two intramolecular *tert*-butyl alcohol eliminations leading to a—to our knowledge—hitherto unprecedented bonding situation, where a still almost planar $\mu_3\text{-}\eta^5\text{:}\eta^1\text{:}\eta^1\text{-Cp}$ ligand bridges three metal centers, which are additionally linked by metal bonds (Scheme 1, Figure 3). This unusual arrangement features Bi–C bonds that are bent by about 40° out of the mean plane defined by the C_5 unit. This large bending angle combined with a nonetheless only slight distortion of the Cp ring, which consequently retains its

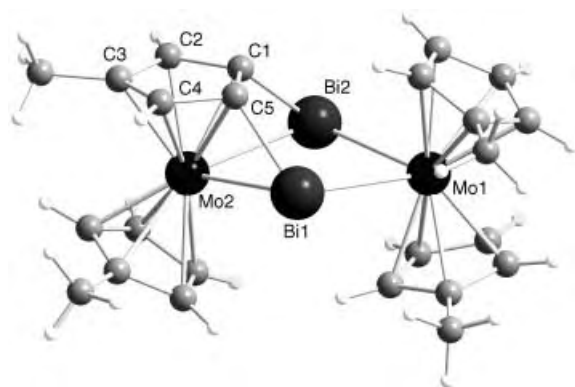


Figure 3. Molecular structure of **4**. Selected bond lengths [Å] and angles [°]: Mo1–Bi1 2.927(2), Mo1–Bi2 2.942(2), Mo2–Bi1 2.880(2), Mo2–Bi2 2.895(2), Bi1–Bi2 3.558(1), Bi1–C5 2.24(2), Bi2–C1 2.26(2), C4–C5 1.44(2), C5–C1 1.42(3), C1–C2 1.48(2), C2–C3 1.43(3), C3–C4 1.45(2); Bi1–Mo1–Bi2 74.65(3), Bi1–Mo2–Bi2 75.90(3), Mo1–Bi1–Mo2 103.57(3), Mo1–Bi2–Mo2 103.06(3), Mo2–Bi1–C5 50.3(3), Mo2–C5–Bi1 80.1(4), Bi1–C5–C1 118.4(7), C2–C1–C5–C4 0.7(12), C3–C4–C5–C1 0.5(13).

aromatic character, are strongly indicative of nonlinear orbital overlap between the Bi and the C atoms.

Comparable “bent bonds” have been suggested for some dinuclear transition-metal compounds (first in $[\text{Cp}(\text{CO})\text{Mo}(\mu\text{-}\eta^5\text{-}\eta^1\text{-C}_5\text{H}_5)\text{Mn}(\text{CO})_4]^{[10]}$), and DFT/Bader calculations^[11] support the nonlinear orbital overlap for **4**: Bond critical points between Bi and C atoms (Figure 4) are strong



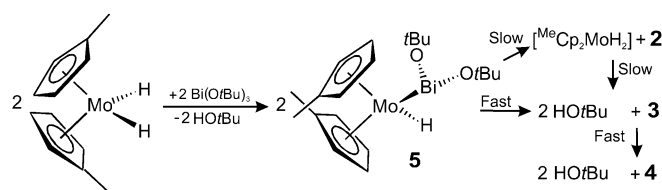
Figure 4. Molecular graph of **4** obtained from DFT/Bader calculations showing bond critical points and bond paths.

indications that there are in fact bonds between these atoms. The high level of ellipticity at these bond critical points indicates a nonlinear orbital overlap (despite almost linear bond paths; see Figure 4). Additionally the calculations suggest that the electron density belonging to each Bi–C bond is shifted in the direction of the C atom, that is, the unit can be regarded as a carbanion stabilized by a Lewis acid (Mo_2Bi^+).

The structure of **4** as shown in Figure 3 is retained in solution as evidenced by ^1H , TOCSY, and NOESY NMR investigations, which in combination, allowed an assignment

of all the signals to the individual protons in **4**. These methods additionally identified **4'** as an isomer of **4** differing from it in that the methyl group of the bridging MeC_5H_2 ligand is not located at C3 (Figure 3) but at C2. The two isomers are not interconvertible under moderate conditions: warming to 60 °C did not alter the spectra significantly.

The reaction between $[\text{MeCp}_2\text{MoH}_2]$ and $\text{Bi}(\text{OrBu})_3$ in the ratio 1:1 was monitored at room temperature by means of ^1H NMR spectroscopy. This study revealed, that after mixing of the reactants all the $\text{Bi}(\text{OrBu})_3$ is immediately consumed to give a mixture consisting of $[\text{MeCp}_2\text{MoH}_2]$, **2**, and the novel monohydride complex $[\text{MeCp}_2\text{Mo}\{\text{Bi}(\text{OrBu})_2\}\text{H}]$ (**5**) in a ratio of 1:1:5. Complex **5** proved to be the most reactive species in the mixture decomposing with a half-life of approximately 15 min through two pathways (Scheme 2): in one, two



Scheme 2. Intermediate formation and reactions of **5**.

molecules of **5** eliminate *tert*-butyl alcohol to give **3**, while the other is a much slower process, in which two molecules of **5** form the metathesis products $[\text{MeCp}_2\text{MoH}_2]$ and **2**. After complete conversion of **5** the concentration of **3** rapidly decreases since its subsequent reaction to **4** and *tert*-butyl alcohol is much faster than its formation from $[\text{MeCp}_2\text{MoH}_2]$ and **2**. When the concentration of **3** is negligible the composition of the mixture changes only slowly and the conversion of $[\text{MeCp}_2\text{MoH}_2]$ and **2** into **4** and **4'** takes days to get near completion (it can be accelerated by warming).

Naturally the question arises, how the intramolecular alcohol eliminations within **3** which lead to **4** can proceed, bearing in mind that they require the cleavage of a non-activated C–H bond, which would not normally be considered as being acidic enough to be susceptible to an attack by an alkoxide function. However, the crystal structure of **3** reveals that for each alkoxidic oxygen atom there is one cyclopentadienyl hydrogen atom (namely H_{C_1} for O' and $\text{H}_{\text{C}_1'}$ for O) whose position is calculated to be only 2.161 Å away from it (the corresponding $\text{O}\cdots\text{C}$ separations amount to 3.014 Å). This finding indicates that in dissolved **3** the C–H bonds of its MeCp rings might be activated by “complex-induced proximity effects” facilitating elimination of alcohol. For the formation of **4** this requires a sequential process:^[12] After the first Bi–C bond formation the resulting molecule has to rearrange by an inversion of the configuration of the second Bi atom, so that both Bi atoms are linked to the same C_5 ring.

Experimental Section

All manipulations were carried out under an argon atmosphere using dried solvents.

2: A solution of $[\text{MeCp}_2\text{MoH}_2]$ (0.200 g, 0.78 mmol) in petroleum ether (10 mL) was added to a stirred suspension of $\text{Bi}(\text{OrBu})_3$ (0.669 g,

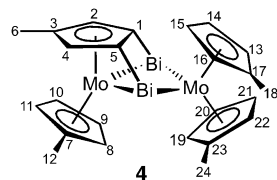
1.56 mmol) in petroleum ether (10 mL). The resulting clear red solution was stirred for 1.5 h at room temperature, and eventually became cloudy. Concentrating the solution and cooling it to -30° yielded overnight 0.651 g (0.68 mmol; 86 %) **2** in form of red–orange crystals. ^1H NMR (300 MHz, C_6D_6): δ = 4.85 (m, 4H, Cp), 4.57 (m, 4H, Cp), 1.56 (s, 6H, Me_{Cp}), 1.38 ppm (s, 36H, $\text{OC}(\text{CH}_3)_3$); ^{13}C NMR (300 MHz, C_6D_6): δ = 97.66, 88.99, 82.69 (Cp), 70.74 ($\text{OC}(\text{CH}_3)_3$), 35.48 ($\text{OC}(\text{CH}_3)_3$), 14.57 ppm (Me_{Cp}).

3: A solution of $[\text{MeCp}_2\text{MoH}_2]$ (0.200 g, 0.78 mmol) in petroleum ether (10 mL) was added to a stirred solution of $\text{Bi}(\text{OtBu})_3$ (0.334 g, 0.78 mmol) in petroleum ether (10 mL). A red solution was formed, which turned black after a few minutes. The reaction mixture was filtered and the filtrate stored at room temperature without further stirring. After 1 h black needles of **3** precipitated, and after 12 h the solution is removed by filtration. The crystals are washed with petroleum ether repeatedly to yield 184 mg (0.17 mmol; 43 %) of pure **3**. ^1H NMR (300 MHz, C_6D_6): δ = 5.03 (m, 4H, Cp), 4.12 (m, 4H, Cp), 3.98 (m, 4H, Cp), 3.64 (m, 4H, Cp), 1.74 (s, 12H, Me_{Cp}), 1.37 ppm (s, 18H, $\text{OC}(\text{CH}_3)_3$).

4: Toluene (4 mL) was added to a solid mixture of $[\text{MeCp}_2\text{MoH}_2]$ (0.100 g, 0.39 mmol) and $\text{Bi}(\text{OtBu})_3$ (0.167 g, 0.39 mmol). Shaking the mixture resulted in a red solution, that turned black within 5 min. The

reaction mixture was allowed to stand at room temperature for 2 days, then petroleum ether (2 mL) was added. After cooling the mixture to -30° for 3 days

0.059 g (0.064 mmol; 33 %) **4** were isolated in form of black crystals. ^1H NMR (300 MHz, C_6D_6): δ = 6.21 (s, 2H, H2,4), 4.89 (m, 2H, H14,15), 4.84 (m, 2H, H9,10), 4.32 (m, 2H,



H8,1), 4.25 (m, 2H, H13,16), 3.76 (m, 2H, H20,21), 3.24 (m, 2H, H19,22), 2.00 (s, 3H, H6), 1.68 (s, 6H, H12,18), 1.62 ppm (s, 3H, H24); MS (EI, 70 eV): m/z (%): 924 (30) $[\text{M}^+]$, 715 (12) $[\text{M}-\text{Bi}]^+$, 670 (50) $[\text{M}^+-(\text{MeCp})_2\text{Mo}]$ or $\{(\text{CH}_3)_5\text{C}_5\text{H}_4\}\{(\text{CH}_3)_5\text{C}_5\text{H}_4\text{MoBi}_2\}^+$, 506 (10) $[\text{M}^+-2\text{Bi}]$, 418 (20) $[\text{Bi}_2^+]$, 254 (80) $\{[(\text{CH}_3)_5\text{C}_5\text{H}_4]\{(\text{CH}_3)_5\text{C}_5\text{H}_4\text{Mo}\}^+\}$, 79 (100) $[\text{MeCp}^+]$; HR-MS for $^{12}\text{C}_{24}^{1\text{H}}_{26}^{92}\text{Mo}_2^{209}\text{Bi}_2$ calcd: 915.97784, found: 915.97679 u.

Correct elemental analyses (C,H) were obtained for all three complexes.

NMR data for **5**: ^1H NMR (300 MHz, C_6D_6): δ = 4.56 (m, mixture of Cp signals arising from **2** and **5**), 4.53 (m, 2H, Cp), 4.43 (m, mixture of Cp signals arising from **5** and $[\text{MeCp}_2\text{MoH}_2]$), 4.17 (m, 2H, Cp), 1.73 (s, 6H, Me_{Cp}), 1.48 (s, 18H, $\text{OC}(\text{CH}_3)_3$), -11.17 ppm (s, 1H, MoH).

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Keywords: alkoxides · bismuth · C–H activation · heterobimetallic complexes · molybdenum

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[7] Data for the X-ray structure analyses: **2**: $\text{C}_{28}\text{H}_{50}\text{Bi}_2\text{MoO}_4$, M_r = 964.58, monoclinic, space group $C2/c$, a = 13.038(4), b = 16.364(6), c = 16.435(5) Å, β = 112.67(3) $^{\circ}$, V = 3235.4(18) Å ^{3} , Z = 4, T = 180(2) K, F_{000} = 1832, μ = 11.261 mm $^{-1}$, Θ = 2.10–22.33 $^{\circ}$, reflections collected 7318, independent reflections 2013 [R_{int} = 0.0528], GoF = 1.018, R = 0.0265, wR_2 = 0.0580, largest diffraction peak and hole 0.653/–1.316 e Å $^{-3}$; **3**: $\text{C}_{32}\text{H}_{46}\text{Bi}_2\text{Mo}_2\text{O}_2$, M_r = 1072.53, monoclinic, space group $P2_1/n$, a = 11.7096(16), b = 9.3332(16), c = 14.852(2) Å, β = 90.023(17) $^{\circ}$, V = 1623.1(4) Å ^{3} , Z = 2, T = 180(2) K, F_{000} = 1008, μ = 11.590 mm $^{-1}$, Θ = 2.21–25.25 $^{\circ}$, reflections collected 9139, independent reflections 2791 [R_{int} = 0.0855], GoF = 0.969, R = 0.0355, wR_2 = 0.0716, largest diffraction peak and hole 1.711/–1.440 e Å $^{-3}$; **4**: $\text{C}_{24}\text{H}_{26}\text{Bi}_2\text{Mo}_2$, M_r = 924.29, monoclinic, space group $P2_1/n$, a = 7.4941(12), b = 21.093(5), c = 14.192(2) Å, β = 91.56(2) $^{\circ}$, V = 2242.6(7) Å ^{3} , Z = 4, T = 180(2) K, F_{000} = 1680, μ = 16.747 mm $^{-1}$, Θ = 2.41–25.25 $^{\circ}$, reflections collected 14593, independent reflections 3990 [R_{int} = 0.0910], GoF = 1.056, R = 0.0514, wR_2 = 0.1333, largest diffraction peak and hole 4.311/–2.580 e Å $^{-3}$. All data were collected on a Stoe IPDSI using MoK_{α} radiation, λ = 0.71073 Å, and the structures were solved by direct methods (program: SHELXS-97), refined versus F^2 (program: SHELXL-97) with anisotropic temperature factors for all non-hydrogen atoms. All hydrogen atoms were added geometrically and refined by using a riding model. CCDC 225730 (**2**), CCDC 225731 (**3**), CCDC 225732 (**4**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

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[11] The B3LYP[LanL2dz; pseudo = LanL2 for C,H] (Stuttgart RSC 1997, ECP (Mo) cc-pVDZ-PP (Bi)) level of theory (G98) proved to reproduce the crystal structure of **4** reasonably well, and this result was used as a basis for a calculation with the AIM 2000 program. The most recent investigations concerning the stem complex of **4** (lacking the methyl groups) show that the use of ECPs does not lead to significant alterations in comparison to an all-electron treatment; a detailed theoretical investigation will be reported elsewhere.

[12] Indeed the above mentioned NMR experiment revealed the occurrence of intermediates on the way from **3** to **4**.

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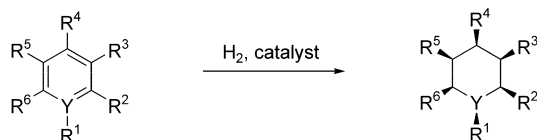
[5] Compare reference 10 in reference [6].

Efficient Asymmetric Hydrogenation of Pyridines**

Frank Glorius,* Nick Spielkamp, Sigrid Holle, Richard Goddard, and Christian W. Lehmann

Dedicated to Professor H. Martin R. Hoffmann on the occasion of his 70th birthday

Catalytic asymmetric hydrogenation has become a key technology in academic research^[1] and in industry^[2] owing to its generally unrivalled efficiency and selectivity.^[3] Nevertheless, no satisfactory solution for the asymmetric hydrogenation of aromatic or heteroaromatic substrates according to Scheme 1 exists,^[4] although these compounds are readily



Scheme 1. Stereoselective hydrogenation of (hetero)aromatic compounds ($YR^1 = CR^1, N$).

available and the reaction has the potential for the simultaneous creation of multiple stereocenters. Here we describe an efficient and unprecedented auxiliary-based method for the asymmetric hydrogenation of substituted pyridines ($YR^1 = N$),^[5] which enables the stereoselective formation of piperidines^[6] with up to four new chiral centers in a single operation (Scheme 2a).

The heterogeneous catalytic hydrogenation of pyridines is usually performed in acidic media.^[7] Protonation not only activates the pyridines for hydrogenation, it also suppresses catalyst poisoning by the resulting piperidines. We reasoned that single-point attachment of chiral oxazolidinones **4**^[8] for ease of introduction of the auxiliary in the 2-position of the pyridine would be ideal (Scheme 2a). Moreover, it occurred

to us that whereas conformation **2** should be strongly preferred for unprotonated pyridines due to dipole-moment minimization, upon protonation hydrogen bonding between the pyridinium and the oxazolidinone moiety would favor conformation **5**, in which the auxiliary is oriented coplanar with the pyridine ring but rotated by 180°. Indeed, on hydrogenation the *i*Pr substituent shields one of the diastereotopic π -faces and selective hydrogen transfer to the opposite side leads to aminal **6** (Scheme 2b).^[9,10]

Substrates **2** can be readily synthesized from oxazolidinones and the corresponding 2-bromo- or chloropyridines **1** by copper catalysis (Scheme 2a).^[13] Gratifyingly, hydrogenation of pyridine **2d** in acetic acid under a hydrogen atmosphere of 100 bar with PtO_2 as the catalyst led to the formation of (*S*)-3-methyl piperidine (**3d**) in 85% *ee*. Different catalysts were screened, and $Pd(OH)_2/C$ was identified as the optimum catalyst, providing **3d** in 98% *ee* (Table 1, entry 4).^[14] Importantly, the reaction does not stop at aminal **6d**, but leads directly to piperidine **3d** and oxazolidinone **4**. Evidently traceless^[15] cleavage of the auxiliary occurs under the reaction conditions, thereby combining chirality transfer from and release of the auxiliary into a single operation. We were pleased to find that after treatment of the crude reaction mixture with hydrochloric acid, separation and purification of the less soluble piperidine hydrochloride **3d** and the more soluble auxiliary could be achieved efficiently by simple extraction with ether/hexanes mixtures. The piperidinium hydrochloride **3d** was obtained in 90% yield (98% *ee*) and **4** was recovered unchanged (93% yield, >99% *ee*), allowing the recycling of the auxiliary.

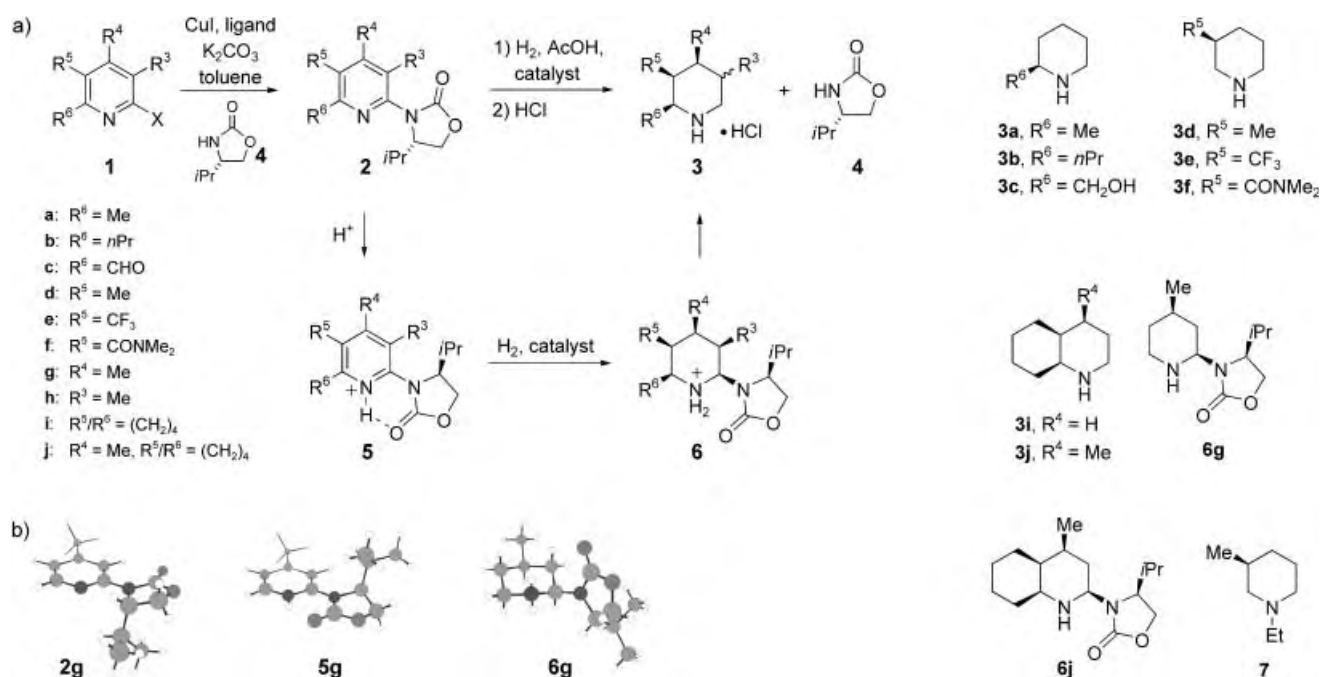
This method for the stereoselective synthesis of piperidines has been applied successfully to a large number of substrates, whereby the oxazolidinone with a *t*Bu group often resulted in slightly improved *ee* values relative to those obtained with the *i*Pr group (Table 1).^[12] Substituents in the 4-, 5- or 6-position of the 2-oxazolidinone-substituted pyridine can be used to create stereocenters at the corresponding positions (entries 1–7). Even multiple stereocenters can be generated, as exemplified by the stereoselective formation of di- and trisubstituted piperidines in near-quantitative yield and excellent enantioselectivities (entries 9, 10). As far as we know this is the first highly asymmetric hydrogenation of an aromatic compound that selectively generates three stereocenters. Under milder conditions we even succeeded in the stereoselective synthesis (>95:5) of aminal **6j**, which bears four new chiral centers (entry 11). Furthermore, functional groups on the pyridine ring are well tolerated (entries 3, 5–6). The versatility of the process can be increased still further, since hydrogenation of **2d** in the presence of acetaldehyde or acetic anhydride results in the formation of the corresponding (*S*)-*N*-ethylpiperidine **7** (entries 12, 13).^[16] The only present limitation concerns 3-substitution of the pyridine ring. A methyl substituent in the 3-position leads to a less reactive substrate, presumably because the oxazolidinone is rotated out of the plane of the pyridine ring thereby shielding both π -faces. Hydrogenation of **2h** results in a nearly racemic product (entry 8). Finally, we were pleased to find that our method gives easy access to coniine (**3b**), the poisonous hemlock alkaloid, in excellent yield and enantiomeric excess (entry 2).

[*] Dr. F. Glorius, N. Spielkamp, S. Holle, Dr. R. Goddard,⁺ Dr. C. W. Lehmann⁺
Max-Planck-Institut für Kohlenforschung
Kaiser-Wilhelm-Platz 1, 45470 Mülheim an der Ruhr (Germany)
Fax: (+49) 208-306-2994
E-mail: glorius@mpi-muelheim.mpg.de

[⁺] X-Ray structure analyses.

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



Scheme 2. a) Synthesis and asymmetric hydrogenation of oxazolidinone-substituted substrates **2** ($X = \text{Br}, \text{Cl}$). b) DFT-generated structures **2g** and **5g**^[11] and X-ray structure **6g** (chloride anion omitted for clarity).^[12]

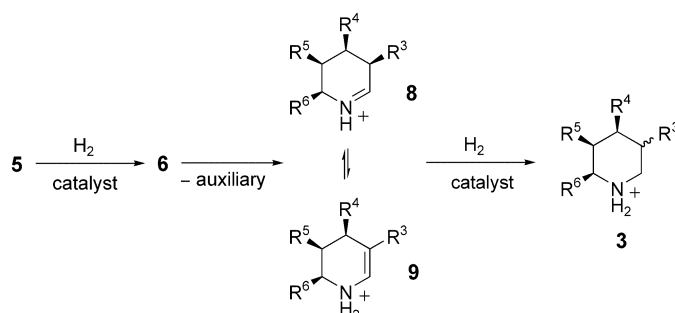
Table 1: Asymmetric hydrogenation of oxazolidinone-substituted pyridines **2** according to Scheme 2.^[a]

Entry	2	Prod. (HCl salt)	Cat.	T [°C]	t [h]	Yield [%]	$ee^{[b]}$ [%]
1	a	3a	$\text{Pd}(\text{OH})_2/\text{C}$	50	22	93	91
2	b ^[n]	3b	$\text{Pd}(\text{OH})_2/\text{C}$	40	22	95	95
3	c ^[c, n]	3c	$\text{Pd}(\text{OH})_2/\text{C}$	25	20	64 ^[d]	97
4	d	3d	$\text{Pd}(\text{OH})_2/\text{C}$	25, 65 ^[e]	24, 3 ^[e]	90	98
5	e ^[n]	3e	$\text{Pd}(\text{OH})_2/\text{C}$	25	26	93	95
6	f ^[n]	3f	$\text{Pd}(\text{OH})_2/\text{C}$	45	22	92	85
7 ^[f, g]	g	6g	PtO_2	25	20	87	> 95:5 ^[h]
8	h	3d	Rh/C	25	72	100 ^[i]	4
9	i ^[n]	3i	$\text{Rh}/\text{Pd}/\text{C}$	40	22	94 ^[k]	96
10 ^[f]	j	3j	Rh/C	40, 75	24, 18	92 ^[k]	94
11	j	6j	$\text{Rh}/\text{Pd}/\text{C}$	40	40	63	> 95:5 ^[h]
12 ^[l]	d	7	$\text{Pd}(\text{OH})_2/\text{C}$	25	22	87	93
13 ^[m]	d	7	$\text{Pd}(\text{OH})_2/\text{C}$	25	22	81	95

[a] General conditions: 2-mmol scale, AcOH (0.13 M), 100 bar H_2 .

[b] Enantiomeric excess determined by GC analysis of the *N*-trifluoroacetamide derivatives of the crude reaction products. [c] $R^6 = \text{CHO}$. [d] Product purified as *N*-Boc derivative. [e] 25 °C for 24 h, then 65 °C for 3 h. [f] 150 bar H_2 . [g] Conc. hydrochloric acid (6 equiv) and MeOH used as solvent. [h] Diastereoselectivity determined by NMR. [i] Conversion, determined by GC-MS. [k] *syn/anti* > 100:1. [l] MeCHO (4 equiv) added. [m] Ac_2O (4 equiv) added. [n] In these cases, the catalyst **4** with a *t*Bu group instead of the *i*Pr group was used.

A plausible mechanism is depicted in Scheme 3. Saturation of the pyridinium ring in **5** presumably results in the stereoselective formation of amination **6**. Disintegration of **6** into the oxazolidinone and iminium salt **8**,^[9] which is in equilibrium with the respective enaminium salt **9**, is followed by hydrogenation of the resulting C–N or C–C double bond leading to the observed product **3** in high optical purity. The



Scheme 3. Probable mechanism for the asymmetric hydrogenation giving piperidinium salts **3**.

involvement of achiral **9h** in the hydrogenation of **2h** might explain the observed formation of nearly racemic product (entry 8).

In summary, we describe a conceptually novel, practical, and efficient synthesis of optically active piperidines, an important substructure of many biologically active compounds. This process is distinguished by the fact that piperidines with multiple stereocenters can be formed in very good yields and excellent optical purities. To the best of our knowledge, this transformation unites for the first time highly selective chirality transfer and nondestructive and traceless cleavage of the chiral auxiliary in one reaction. In addition, the piperidinium hydrochloride and the auxiliary can be separated easily by extraction and the auxiliary recycled.

Experimental Section

Typical experimental procedure: No special care was taken to exclude air or moisture. A mixture of wet 20% $\text{Pd}(\text{OH})_2/\text{C}$ (w/w, 140 mg),

substrate **2b** (524 mg, 2 mmol), and acetic acid (15 mL) was stirred in an autoclave under a hydrogen atmosphere (100 bar) at 40 °C for 22 h. The mixture was filtered through a short pad of Celite, which was subsequently washed with MeOH (15 mL). Hydrochloric acid (333 µL, 4.0 mmol) was added, and the solvent was removed by rotary evaporation until no acetic acid was left. The *ee* value of **3b** was determined to be 95% by GC analysis of the *N*-trifluoroacetamide derivative of the crude reaction product. The remaining white solid was washed repeatedly with methyl *tert*-butyl ether/hexanes to yield hydrochloride **3b** (310 mg, 95%, 96% *ee*) as a white solid. Evaporation of the organic phase left behind (*S*)-*t*Bu-oxazolidinone (251 mg, 88%) as a white solid. All new compounds were fully characterized. The sources and types of catalysts used are given in the Supporting Information.

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- [12] The absolute stereochemistry of the products was determined unequivocally by comparison of optical rotation data with literature values (**3a**, **3b**, **3d**; *N*-Boc derivative of **3c**), by X-ray-analysis of **3i** (CCDC-230263), **6g** (CCDC-230265), **6j** (CCDC-230266), and **7** (CCDC-230267). CCDC 230262–230267 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK; fax: (+44)1223-336-033; or deposit@ccdc.cam.ac.uk).
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Chirality

Determination of the Absolute Chirality of Adsorbed Molecules**

Roman Fasel,* Joachim Wider, Christoph Quitmann,
Karl-Heinz Ernst,* and Thomas Greber

Pasteur's separation of the optical antipodes of ammonium sodium tartrate tetrahydrate in 1848 and the concept of the asymmetrically substituted carbon atom, introduced by van't Hoff and le Bel, marked the birth of stereochemistry.^[1] Although the Fischer–Rosanoff convention based on D-(+)-glyceraldehyde got general acceptance,^[2] it was not possible to link the optical rotary power to the absolute configuration of chiral molecules. In 1951, however, Bijvoet and co-workers determined the absolute configuration of (+)-sodium rubidium tartrate through anomalous X-ray scattering and showed that the arbitrary D–L-convention matches reality.^[3] Increasing computational power and improved quantum mechanical codes nowadays allow to connect chiroptical spectra with absolute configuration,^[4] but this approach is still limited to relatively small or rigid molecules. Other methods for determining the absolute configuration, such as wetting or enantioselective adsorption on polar crystals^[5] and measurements of the electric polarization in ferroelectric liquid crystals,^[6] have been reported.

The conformation of chiral molecules adsorbed on a surface plays an important role in biomineralization^[7] and in stereoselective heterogeneous catalysis.^[8] For example, tartaric acid serves as chiral modifier for the enantioselective hydrogenation of β -ketoesters over supported nickel catalysts.^[9] To gain deeper insight into the mechanisms of enantioselective surface chemistry and biomineralization, a detailed analysis of the local adsorbate structure is necessary.^[10]

The absolute chirality of molecules adsorbed on surfaces has been inferred from scanning tunneling microscopy (STM)

images.^[11] However, in most cases of molecular adsorption it is very difficult to correlate the observed electron densities with the absolute atomic positions, and modeling of STM images requires extensive theoretical and computational effort.^[12]

Herein we demonstrate that the absolute configuration of chiral molecules adsorbed on single-crystal surfaces can be determined in a straightforward fashion by means of angle-scanned X-ray photoelectron diffraction (XPD),^[13] a method delivering direct real-space structural information. For our study we chose the classic tartaric acid molecule adsorbed on the Cu(110) surface. This adsorbate system has been studied in great detail.^[14] At low coverages and after activation at 405 K, tartaric acid becomes doubly deprotonated, with the resulting bitartrate species^[15a] forming long-range ordered chiral structures on the surface.^[15b] Based on IR spectroscopy results, a local C_2 symmetry for the bitartrate adsorbate complex has been proposed.^[14]

Figure 1 illustrates the basic principles of the XPD experiment for the case of the adsorbed bitartrate species.

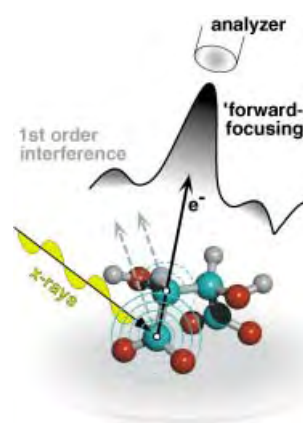


Figure 1. Illustration of the general principle of the angle-scanned XPD experiment. Angle-resolved photoelectron intensities from a particular emission line (e.g. C1s) are collected over the complete hemisphere above the sample surface. At sufficiently high electron kinetic energies, the scattering of the outgoing electron wave (emanating from the C atoms in the present case) from the neighboring ion cores is strongly anisotropic. Most of the photoelectron intensity is scattered into the forward direction, giving rise to the so-called forward-focusing maxima in the angular distribution. Since these maxima occur in directions of nearby atoms, they allow a direct determination of the molecular structure.

When a molecule is illuminated by X-rays, photoelectrons are emitted from the atoms within the molecule and scattered by the surrounding atomic cores. At electron energies above about 500 eV, the scattering amplitude is mostly directed in the forward direction, which results in a strongly enhanced electron flux along the emitter–scatterer axis. This so-called forward-focusing effect allows a simple geometrical interpretation of the diffraction pattern where prominent intensity maxima can be identified with near-neighbor directions. The angular distribution of the photoelectrons therefore is, to a first approximation, a projected image of the atomic structure

[*] Dr. R. Fasel, Dr. K.-H. Ernst*
Swiss Federal Laboratories for Materials Testing and Research (EMPA)
Überlandstrasse 129, 8600 Dübendorf (Switzerland)
Fax: (+41) 1-823-4034
E-mail: roman.fasel@empa.ch
karl-heinz.ernst@empa.ch

Dr. J. Wider, Dr. T. Greber
Physik-Institut, Universität Zürich
Zürich (Switzerland)
Dr. J. Wider, Dr. C. Quitmann
Swiss Light Source, Paul-Scherrer Institute
Villingen (Switzerland)

[†] Current address: University of Washington
Department of Bioengineering
Seattle, WA 98195 (USA)

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around the photoemitters. Electrons from a particular emission line have their origin at the atoms selected by the choice of the emission line in the spectrum. This chemical sensitivity allows the corresponding XPD pattern to be associated with the local geometrical environment of the particular atom type chosen, for example, the local structure around the C atoms. Note that the source of the diffracted electron wave carrying structural information is within the molecule itself, and XPD therefore does not require long-range ordered structures, but is very sensitive to local order. This, together with its chemical selectivity, makes XPD to a powerful technique for surface structural investigations, which has been successfully applied to a variety of adsorbate systems.^[16] However, only a few larger molecules, such as heptahelicene ($C_{30}H_{18}$)^[17] and the fullerenes C_{60} and C_{70} ^[18] have been investigated. In particular, the low core-level photoelectron yield for submonolayers of the low atomic number atoms C, O, and N and their weak electron scattering power make XPD experiments on such molecular systems challenging. For the submonolayer coverage bitartrate/Cu(110) system herein, a conventional standard laboratory X-ray source did not yield C 1s XPD patterns with sufficient signal-to-noise ratio even at prolonged counting times. To overcome this limitation, an endstation for full-hemispherical XPD experiments has been set up at a third-generation synchrotron radiation source^[19] providing a high flux of monochromatic and polarized photons at energies well suited for XPD in the forward-focusing regime.

Figure 2 shows the experimental C 1s XPD patterns from the (*R,R*)- and (*S,S*)-bitartrate enantiomers adsorbed on the Cu(110) surface. The patterns are in a stereographic projection, where the center of the plot corresponds to normal emission of electrons (perpendicular to the surface) and the

outer circle marks emission along the surface plane. The anisotropy of the patterns, that is, the difference between maximum and minimum intensity divided by maximum intensity, is of the order of 30%. The orientation of the Cu(110) substrate surface as determined from a substrate core-level XPD pattern (not shown) is indicated. Ten prominent intensity maxima are observed in the XPD patterns, and it is clear that the patterns from the two enantiomers are mirror images of each other. Furthermore, the chirality of each tartaric acid enantiomer is directly reflected in the corresponding XPD patterns which exhibit twofold rotational symmetry, but no mirror symmetry. As the strong intensity maxima correspond to emitter-scatterer directions and the emitters are known to be the carbon atoms, the conformation of the carbon backbone and the positions of the OH groups relative to the carbon skeleton are easily determined from the XPD patterns by applying purely geometrical arguments (Figure 2c and d).^[20] By triangulation of the interatomic directions derived from the emission angles of the prominent forward-focusing peaks 1–5, molecular conformations are determined directly for the *R,R* and the *S,S* enantiomers (Figure 2c,d). Peak 1 is due to electrons emitted from a carboxylate carbon atom and forward focused along the C–C bond. Peak 2 corresponds to emission from the same carbon atom but scattering by the β carbon atom. Most important, peaks 3, 4 and 5 are fingerprints of the absolute configuration of the respective molecule: they are due to scattering from the hydroxy oxygen atoms, and locate the position of these oxygen atoms with respect to the carbon skeleton, hence they directly reflect the absolute configuration.

In addition to the absolute configuration, detailed structural parameters can be determined by comparing the experimental XPD pattern to those obtained from calculations, systematically optimizing the structural parameters until best agreement is achieved. The relatively simple and efficient single-scattering cluster (SSC) formalism^[13] has proven adequate in most cases, and in particular for molecular adsorbate systems.^[17,18] Figure 3a defines the structural parameters α , β , and γ of the (*R,R*)-bitartrate molecule that we have varied in the SSC analysis. The initial bond lengths and bond angles were those typically found for the bitartrate ions in alkali-metal salts.^[21] In a series of calculations we have varied the structural parameters in small steps and compared the resulting SSC diffraction patterns to the experimental XPD pattern from the (*R,R*)-enantiomer (Figure 2a). The agreement between simulated and experimental diffraction patterns was quantified using the reliability factor R_{MP} .^[22] A color-coded contour-plot of the resulting *R*-factor values as well as a top view of the best-fit molecular structure are shown in Figure 3c and b, respectively. The *R*-factor minimum of 0.078 is found at values of $21 \pm 4^\circ$ for the carbon backbone torsion angle α and $24 \pm 4^\circ$ for the deviation γ of the C2–C3 bond direction away from the [001] direction. The dependency of the *R*-factor from the carboxy group rotation angle β is too weak to allow a determination of β . This result is not unexpected since the oxygen atoms O1, O2, O5, and O6 binding to the Cu surface are located below the carbon photoemitters and thus do not contribute to the observed

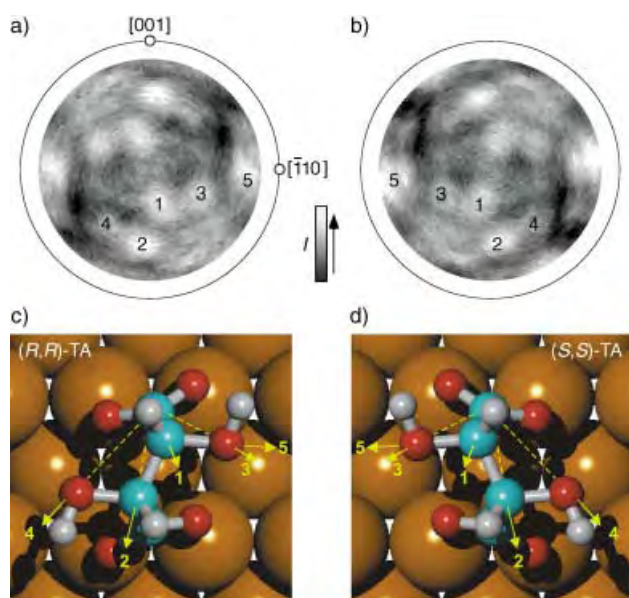


Figure 2. a), b): Experimental C 1s XPD patterns from the (*R,R*)- and (*S,S*)-bitartrate species on Cu(110). The patterns are shown in stereographic projection and in a linear gray scale with white corresponding to maximum intensity. c), d): Molecular conformations deriving from a purely geometrical evaluation of the positions of the prominent forward-focusing maxima labeled 1–5 in (a) and (b).

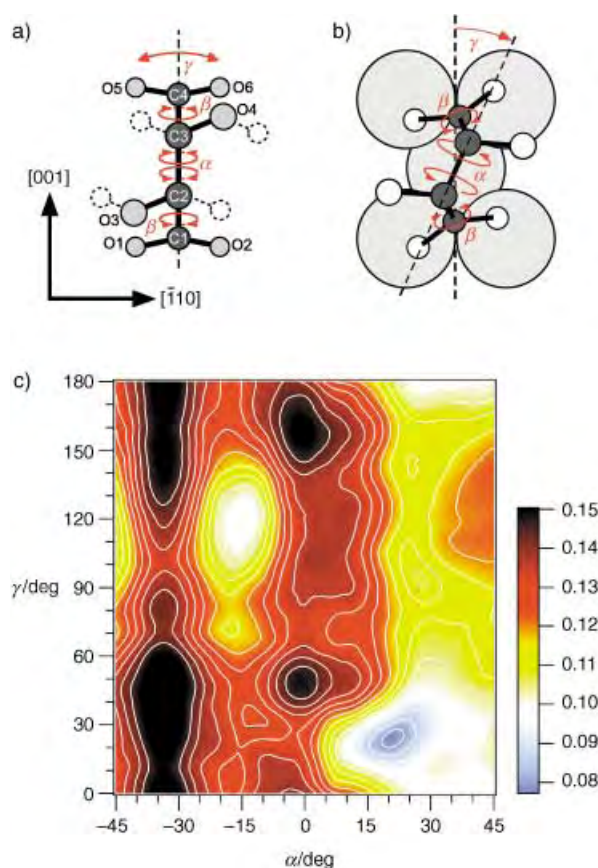


Figure 3. Determination of detailed structural parameters for the *R,R* enantiomer by means of SSC calculations. a) Schematic representation of the molecular geometry assumed as a starting point for the calculations. The two angular parameters α and β as well as the azimuthal orientation γ of the C2–C3 bond are varied. Hydrogen atoms are shown as broken circles since they do not contribute to the XPD anisotropy, and hence are not taken into account in the calculations. b) Top-view of the best-fit molecular conformation. c) Contour plot of the dependency of the *R* factor on the carbon-skeleton torsion angle α and the molecule azimuthal orientation γ . The *R* factor changes by 0.005 between contour lines. Best agreement between calculation and experiment (lowest *R*-factor) is found for $\alpha = 21 \pm 4^\circ$ and $\gamma = 24 \pm 4^\circ$.

forward-focusing pattern.^[23] For the geometry shown in Figure 3b all the carboxy group oxygen atoms are located at an equal distance above the Cu surface, as previously concluded from IR spectroscopy studies,^[14] which leads to a calculated β angle of 47.5° for the structure shown in Figure 3b.

This best-fit structure (Figure 3b) is identical to the structural model derived from the purely geometrical arguments (Figure 2c), thus yielding a consistent picture of the molecular conformation. Finally, the experimental (*R,R*)-tartaric acid C1s XPD pattern and the pattern obtained for the best-fit calculation are in excellent agreement (Figure 4). The calculation does not only reproduce the prominent forward-focusing maxima but also most of the fainter features of the intensity distribution. The remaining minor differences, in particular with respect to relative intensities, must be assigned to the known limitations of the SSC model.^[24]

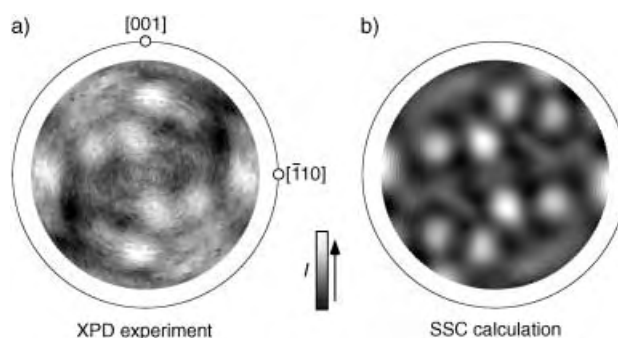


Figure 4. Comparison between experimental XPD pattern for the (*R,R*)-bitartrate (a) and best-fit calculation for the optimized molecular conformation shown in Figure 3 b (b). The simulation accurately reproduces the experimental XPD pattern.

The conformation of the (*R,R*)-bitartrate enantiomer on Cu(110) as predicted from density functional theory (DFT) calculations is qualitatively similar to the one determined here, but with significantly smaller angular distortions.^[25] The bitartrate model based on the above-mentioned IR investigation had all four carbon atoms positioned in a plane perpendicular to the surface.^[14] A highly strained bitartrate, however, was proposed for the adsorbate complex on Ni(110).^[26] On the other hand, enantiomorphous domains have been observed for the achiral bisuccinate species (OOC-CH₂-CH₂-COO) on Cu(110).^[27] It must therefore be concluded that it becomes chiral upon adsorption, which can only be explained by an angular distortion of the molecular frame, as found here for bitartrate. Such conformational changes upon adsorption are driven by the energy balance between bonding to preferred substrate sites, intermolecular interactions, and the stability of the bonds in the adsorbate. Investigations on the change of molecular structure upon adsorption, as presented herein, therefore have the potential to give detailed insight into the mechanisms of molecular chemisorption and provide a stringent test for results obtained from DFT calculations. The only drawbacks of the XPD method are the need for a single crystalline substrate to align the majority of the molecules, and an ultrahigh vacuum environment to provide the mean free path for the photoelectrons.

In conclusion, we have demonstrated that angle-scanned XPD allows the handedness of adsorbed chiral molecules to be determined, and the enantiomers to be distinguished and identified in a straightforward way. The C1s XPD patterns from tartaric acid adsorbed on Cu(110) exhibit pronounced forward-focusing maxima owing to intramolecular scattering that directly yield the molecular conformation without the need for complex calculations. Using SSC calculations, the molecular distortions upon adsorption have been determined to a high degree of accuracy. With further increasing brilliance and stability of new synchrotron radiation sources it will become possible to achieve additional chemical sensitivity in XPD by selecting a specific chemically shifted core-level emission line. This opens the opportunity to selectively investigate the local geometrical environment of an element in different chemical surroundings within a molecule.

Experimental Section

(2*R*,3*R*)- and (2*S*,3*S*)-tartaric acid (Aldrich 99%) were evaporated from a Knudsen cell at 120°C in ultrahigh vacuum (UHV) onto a Cu(110) crystal held at 405 K. The formation of the ordered bitartrate structure was confirmed by low-energy electron diffraction (LEED) and photoelectron spectroscopy (XPS). The XPD experiments were performed at the Surface and Interface Microscopy Beamline of the Swiss Light Source using linearly polarized synchrotron X-ray radiation of 920 eV. The C 1s ($E_{\text{kin}} = 633$ eV) XPD patterns were collected with the sample held at room temperature.

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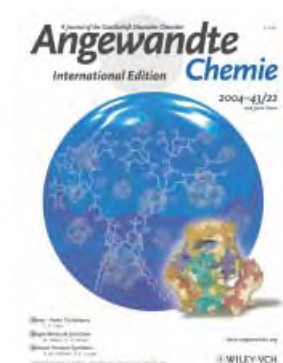
Keywords: absolute configuration · chirality · molecular adsorption · photoelectron diffraction · surface chemistry

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Cover Picture

Michael Kellermann, Walter Bauer, Andreas Hirsch,* Boris Schade, Kai Ludwig, and Christoph Böttcher*

The exceptional structure of the first reconstructed micelles is formed by the self-assembly of amphiphilic dendrocalixarenes in aqueous solution. A. Hirsch, C. Böttcher, and co-workers report in their Communication on page 2959 ff. that the relatively rigid T-shaped structure of the molecule allows efficient backfolding and thus the formation of aggregates with high curvature. Structural determination of the nanocontainers permits characterization of the micellar architecture at the molecular level.



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P. A. Wender,* N. M. Deschamps, T. J. Williams:
Intermolecular Dienyl Pauson–Khand Reaction
 DOI: 10.1002/anie.200454117
 Published online: May 13, 2004

M. Köhn, R. Breinbauer*:
The Staudinger Ligation—A Gift to Chemical Biology
 DOI: 10.1002/anie.200401744
 Published online: May 12, 2004

T. K. Maji, K. Uemura, H.-C. Chang, R. Matsuda, S. Kitagawa*:
Expanding and Shrinking Porous Modulation Based on Pillared-Layer Coordination Polymers Showing Selective Guest Adsorption
 DOI: 10.1002/anie.200453923
 Published online: May 12, 2004

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Internet-Based Spectroscopy Tools:
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M. Schäfer and N. Schlörer

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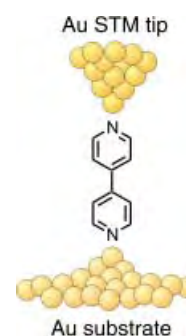
Highlights

Molecular Electronics

M. Mayor,* H. B. Weber* — 2882–2884

Statistical Analysis of Single-Molecule
 Junctions

Establishing contact: Recent progress in the investigation of single-molecule contacts established in an electrochemical scanning tunneling experiment are described (see picture). The setup allows for the successive formation of many hundreds of junctions and a statistical analysis of the voltage dependence of the electrical current. This method is a new reliable tool to characterize molecular junctions and contributes to elucidating the potential of molecular electronics.





Snapshots of excited-state molecular structures in solution during chemical reactions serve as the basis for understanding reaction intermediates. The method of choice for obtaining such structures is photo-chemical excitation by a laser pulse and subsequent analysis by time-resolved X-ray absorption spectroscopy. (X-rays generated by third-generation synchrotron sources; see picture of the Advanced Photon Source of the Argonne National Laboratory, Illinois).

Reviews

Pump-Probe Techniques

L. X. Chen* — 2886 – 2905

Taking Snapshots of Photoexcited Molecules in Disordered Media by Using Pulsed Synchrotron X-rays

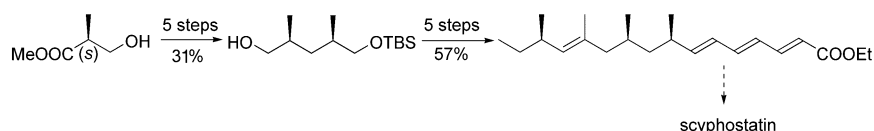
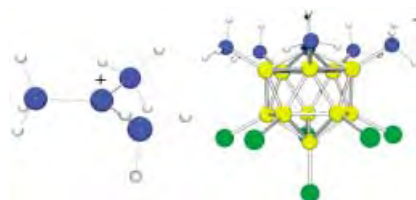
Communications

Carbocations

T. Kato, C. A. Reed* — 2908 – 2911

Putting *tert*-Butyl Cation in a Bottle

Usually viewed as intermediates that become long-lived at low temperatures in superacidic media, simple alkyl carbocations such as the *tert*-butyl cation can be isolated as stable salts at room temperature when partnered with inert carborane anions. The X-ray crystal structures of *tert*-butyl (depicted), *tert*-pentyl, and methylcyclopentyl cations have been determined with $\text{CHB}_{11}\text{Me}_6\text{X}_6^-$ counterions ($\text{X} = \text{Br}$ or Cl). C blue; H white; B yellow; X green.



A decrease in the number of linear synthetic steps and an increase in efficiency have been realized in the synthesis of the side chain of scyphostatin. An efficient and selective synthesis of α,ω -difunctional reduced polypropionates

from methyl 3-hydroxy-2-methylpropionate through the use of a Zr-catalyzed asymmetric carboalumination has been developed (see scheme, TBS = *tert*-butyldimethylsilyl).

Synthetic Methods

Z. Tan, E.-i. Negishi* — 2911 – 2914

An Efficient and General Method for the Synthesis of α,ω -Difunctional Reduced Polypropionates by Zr-Catalyzed Asymmetric Carboalumination: Synthesis of the Scyphostatin Side Chain

For the USA and Canada:

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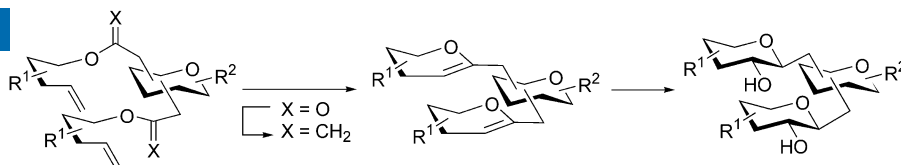
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Carbohydrate Synthesis

M. H. D. Postema,* J. L. Piper,
V. Komanduri, L. Liu — 2915 – 2918

A Double Ring-Closing Metathesis
Approach for the Synthesis of
 β -C-Trisaccharides



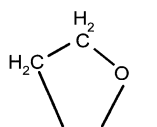
Good things come in threes: A variety of β -C-trisaccharides have been successfully synthesized by double ring-closing metathesis, which provides the products in excellent overall yield after functionaliza-

tion of the newly formed double bonds (see scheme). The precursor olefin–enol ethers were prepared by Takai methylenation of the appropriate diesters.

Surface Chemistry

S. Linic, H. Piao, K. Adib,
M. A. Barteau* — 2918 – 2921

Ethylene Epoxidation on Ag: Identification
of the Crucial Surface Intermediate by
Experimental and Theoretical Investiga-
tion of its Electronic Structure



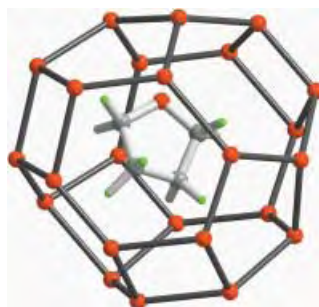
Intermediate success: A combination of high-resolution X-ray photoelectron spectroscopy and density functional

theory allows the electronic structures of adsorbates to be probed to an unprecedented level of detail. By using these techniques, the adsorption of ethylene oxide onto an Ag(111) surface has been shown to result in the formation of an unsymmetrical surface oxametallacycle structure (see picture).

Host–Guest Chemistry

A. V. Kurnosov,* V. Yu. Komarov,
V. I. Voronin, A. E. Teplykh,
A. Yu. Manakov — 2922 – 2924

New Clathrate Hydrate Structure: High-
Pressure Tetrahydrofuran Hydrate with
One Type of Cavity

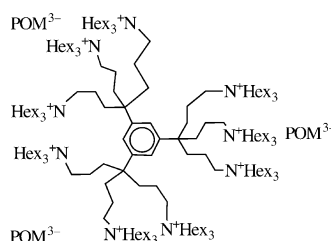


Water cages: A water framework consisting of uniform space-filling polyhedral cages containing tetrahydrofuran molecules has been detected in a new clathrate hydrate formed under a pressure of 0.8 GPa. Topologically the cage represents a space-filling tetradehedron with four tetra-, four penta-, and six hexagonal faces (see picture).

Dendrimers

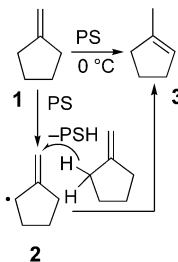
L. Plault, A. Hauseler, S. Nlate, D. Astruc,*
J. Ruiz, S. Gatard,
R. Neumann — 2924 – 2928

Synthesis of Dendritic Polyoxometalate
Complexes Assembled by Ionic Bonding
and Their Function as Recoverable and
Reusable Oxidation Catalysts



Regenerable and reusable dendritic catalysts have been synthesized by the ionic assembly of polyammonium dendrimers and polyoxometalate (POM) tri-anionic units. These dendrimers (see example depicted, in which the POM is $[\text{PO}_4\{\text{WO}(\text{O}_2)_2\}_4]^{3-}$) are found to catalyze, at ambient temperature, the quantitative epoxidation of olefins by H_2O_2 in water/ CDCl_3 and the selective and quantitative oxidation of thioanisole to its sulfone.

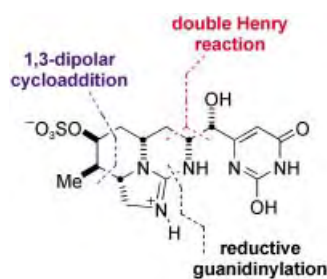
A **radical intermediate** is generated when methylenecyclopentane (**1**) is isomerized by SO_2 into 1-methylcyclopentene (**3**) through the formation of a polysulfone polymer (PS), which first abstracts a hydrogen atom from the alkene. The allyl radical intermediate **2** abstracts a hydrogen atom from another alkene molecule **1**, to yield the isomerized alkene and regenerating the allyl radical. Polysulfones are organic catalysts.



Alkene Isomerization

D. Marković, P. Vogel* — 2928 – 2930

Polysulfones: Catalysts for Alkene Isomerization

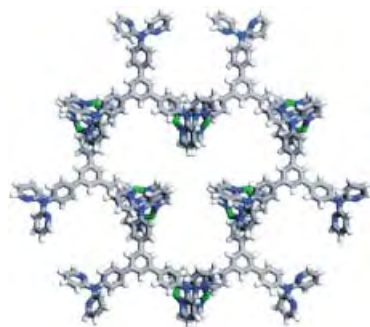


Born from a simple amino acid ... the potent hepatotoxic cyanobacterial alkaloid 7-epicyclindrospermopsin (see picture) has been synthesized through a concise asymmetric eighteen-step route. An intramolecular 1,3-dipolar cycloaddition and a nitroaldol reaction are key steps in the construction of the natural product from a precursor with a single stereogenic center.

Natural Product Synthesis

R. E. Looper,
R. M. Williams* — 2930 – 2933

A Concise Asymmetric Synthesis of the Marine Hepatotoxin 7-Epicyclindrospermopsin



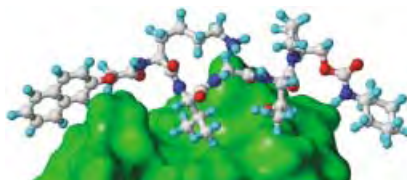
The impact of the **anion** on the assembly of extended 2D structures has been demonstrated by two distinct luminescent 2D lattices. Shown here is the macrocyclic cloverleaf-shaped cavity obtained from the reaction of a starburst ligand with $\text{Ag}(\text{NO}_3)$. Use of the triflate salt instead results in a honeycomb-shaped cavity.

Coordination Networks

C. Seward, W.-L. Jia, R.-Y. Wang,
G. D. Enright, S. Wang* — 2933 – 2936

Luminescent 2D Macrocyclic Networks Based on Starburst Molecules: $[\{\text{Ag}(\text{CF}_3\text{SO}_3)\}_{1.5}(\text{tdapb})]$ and $[\{\text{Ag}(\text{NO}_3)\}_3(\text{tdapb})]$

Barring infection: A new CD4-binding peptidomimetic (see ball-and-stick structure; CD4 is shown in green) has been designed and synthesized as an entry inhibitor of the HIV virus. The binding affinity of the peptidomimetic for CD4 is $40\ \mu\text{M}$, which is 150-fold better than that of the lead peptide NMWQKVGTPPL. The mimetic also has a lower molecular weight and a higher proteolytic stability than the lead peptide.



HIV Inhibition

A. T. Neffe, B. Meyer* — 2937 – 2940

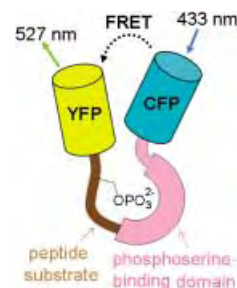
A Peptidomimetic HIV-Entry Inhibitor Directed against the CD4 Binding Site of the Viral Glycoprotein gp120

Phosphorylation Reporters

C.-W. Lin, A. Y. Ting* — 2940 – 2943

A Genetically Encoded Fluorescent Reporter of Histone Phosphorylation in Living Cells

An increase in FRET indicates phosphorylation of histone H3 at serine 28. The protein-based reporter (see picture) responds to phosphorylation through intramolecular complexation between a substrate domain derived from histone H3 and a linked phosphoserine-recognition domain. The reporter is also effective inside living mammalian cells. FRET = fluorescence resonance energy transfer.

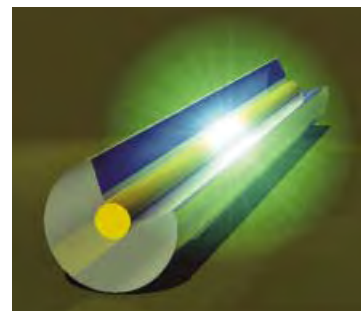


Dendrimeric Structures

W.-S. Li, D.-L. Jiang,*
T. Aida* — 2943 – 2947

Photoluminescence Properties of Discrete Conjugated Wires Wrapped within Dendrimeric Envelopes: “Dendrimer Effects” on π -Electronic Conjugation

Third-generation dendrimers have been used to enable the synthesis and isolation of a series of spatially isolated, discrete conjugated wires G_3-n (where n is the number of repeating monomer units, see schematic representation), including 147-nm long G_3-64 , which contains 192 aromatic rings and 256 triple bonds in the backbone. Photochemical studies show there is enhanced π -electronic conjugation in these systems.

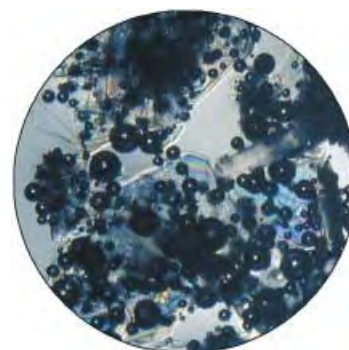


Host–Guest Systems

J. L. Atwood,* L. J. Barbour,*
A. Jerga — 2948 – 2950

A New Type of Material for the Recovery of Hydrogen from Gas Mixtures

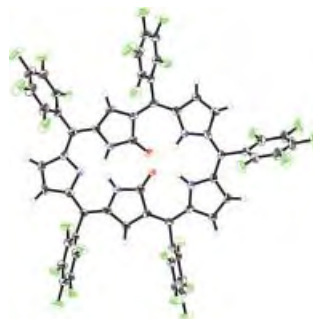
Molecular gas works: *p*-tert-Butylcalix[4]-arene crystals possess large interstitial cavities when grown by sublimation and, although nonporous, are able to absorb and retain gases such as O_2 , N_2 , and CO_2 under near-ambient conditions. H_2 is not retained and thus the material can be used to effect the separation of H_2 from gas mixtures. The gas can be removed by evacuation or immersion of the crystals in nitrobenzene (see picture).



Porphyrin Derivatives

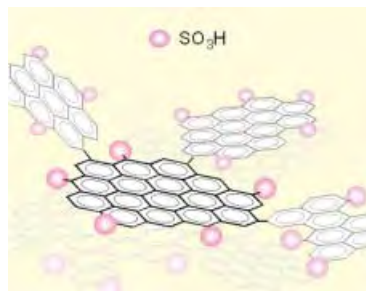
A. Srinivasan, T. Ishizuka, H. Maeda,
H. Furuta* — 2951 – 2955

Doubly N-Confused Pentaphyrins



Adding to the confusion: [3+2] coupling of confused tripyrrane and confused dipyrromethane dicarbinol, followed by oxidation, results in the synthesis of *meso*-pentafluorophenyl-substituted, doubly N-confused, oxo-substituted pentaphyrins (see X-ray crystal structure; green: F, blue: N, red: O). The oxopyrrole moieties have been shown to readily form intra- and intermolecular hydrogen bonds.

Incomplete carbonization of sulfoaromatic hydrocarbons results in a carbon material that consists of small polycyclic aromatic carbon sheets with attached SO_3H groups (see picture). This insoluble solid acid exhibits remarkably high activity in the formation of ethyl acetate, the hydrolysis of cyclohexyl acetate, and the hydration of 2,3-dimethyl-2-butene.



Heterogeneous Catalysis

M. Hara,* T. Yoshida, A. Takagaki,
T. Takata, J. N. Kondo, S. Hayashi,
K. Domen _____ 2955 – 2958

A Carbon Material as a Strong Protonic Acid

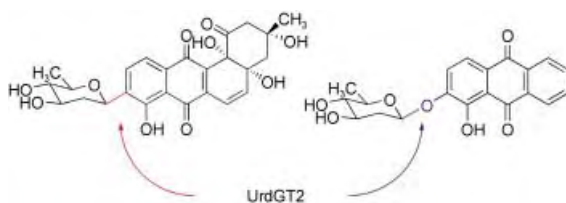


Defined micelles: A completely uniform and structurally precise micelle spontaneously formed by exactly seven amphiphilic dendro-calixarene molecules in aqueous solution has been determined by cryo-TEM and 3D reconstruction techniques. The picture shows seven calixarene head groups, which have been fit visually into the reconstructed density map of the micelle.

Micelles

M. Kellermann, W. Bauer, A. Hirsch,*
B. Schade, K. Ludwig,
C. Böttcher* _____ 2959 – 2962

The First Account of a Structurally Persistent Micelle



Sweet success: A natural product glycosyltransferase, UrdGT2 from *Streptomyces fradiae* Tü2717, is the first glycosyltransferase able to catalyze both C-glycosidic and O-glycosidic sugar transfers. These

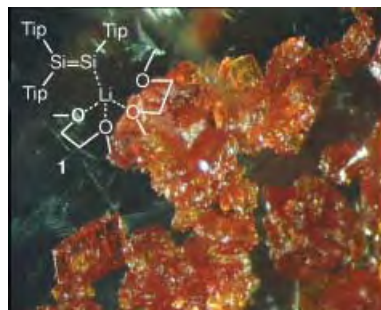
results shed new light on the mechanisms of C-glycosylation of aromatic compounds, which is significant for chemoenzymatic drug lead derivatization as well as for enzymology.

Bioorganic Chemistry

C. Dürr, D. Hoffmeister, S.-V. Wohlerl,
K. Ichinose, M. Weber, U. von Mulert,
J. S. Thorson, A. Bechthold* _____ 2962 – 2965

The Glycosyltransferase UrdGT2 Catalyzes Both C- and O-Glycosidic Sugar Transfers

The reduction of $\text{Tip}_2\text{SiCl}_2$ by finely dispersed lithium powder directly affords the disilenide **1** (see picture), the structure of which could be characterized by X-ray crystallography. The reaction of **1** with Me_3SiCl leads to the corresponding asymmetrical disilene. The ready availability of **1** is expected to open new possibilities for the synthesis of disilenes in general. $\text{Tip} = 2,4,6\text{-}i\text{Pr}_3\text{C}_6\text{H}_2$



Organosilicon Chemistry

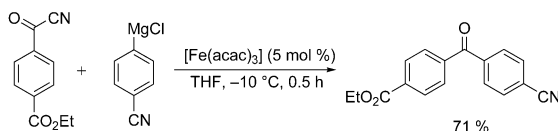
D. Scheschkewitz* _____ 2965 – 2967

A Silicon Analogue of Vinylolithium:
Structural Characterization of a Disilenide

Organomagnesium Reagents

C. Duplais, F. Bures, I. Sapountzis,
T. J. Korn, G. Cahiez,
P. Knochel* _____ **2968–2970**

An Efficient Synthesis of Diaryl Ketones by
Iron-Catalyzed Arylation of Aroyl Cyanides



An alternative to acyl chlorides: Iron(III)-catalyzed arylation of aroyl and hetero-aryyl cyanides with aryl magnesium reagents (see example in scheme)

provides an easy and mild approach to polyfunctionalized diaryl ketones in yields up to 98%.



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Statistical Analysis of Single-Molecule Junctions

Marcel Mayor* and Heiko B. Weber*

Keywords:

electron transport · molecular electronics · scanning probe techniques · single-molecule studies · structure–property relationships

The integration of molecules into electronic circuits has been a scientific challenge for many years. The research is driven by both scientific interest as well as the technological vision of employing molecules as functional building blocks in future nanoelectronics. This idea is today termed “molecular electronics”.^[1] However, it originates in the late 1960s when Hans Kuhn introduced his vision of “molecular engineering”.^[2]

In early studies, theoretical predictions^[3] and attempts to investigate molecular multi-^[2,4] and monolayer^[5] architectures based on Langmuir–Blodgett films were reported. But the enormous improvements in nanoscale engineering in recent years, driven by the invention of scanning-tunneling microscopy (STM) techniques by Binnig and Rohrer,^[6] revitalized the idea of wiring an individual molecule in an electronic circuit. Several experiments with immobilized nanoscale single molecules, in particular conjugated π systems,^[7] coordination compounds,^[8] and DNA molecules,^[9] in contact with electrode pairs have been reported. The observed current-transport characteristics vary between the different investigations because the, on the atomic scale, poorly comparable microscopic conditions in different experiments affect the results strongly. To date, most experimental studies carried out with STM, mechanically controlled break junctions, and electromigration-fabricated nanoelec-

trodes were limited to a small number of junctions. However, when instead of a large statistical ensemble, the single-molecule level is probed, both reproducibility and comparability are intrinsically limited. Hence, a statistical evaluation of a large ensemble of single-molecule contacts is desired. This approach has already proven to yield additional relevant information in metallic nanojunction experiments^[10] and in junctions which are bridged by hydrogen molecules.^[11] The histograms of the appearing conductance values were generated and allowed the detection of “typical” contact configurations. Further, scanning-probe investigations were carried out on junctions based on single molecules embedded in self-assembled monolayers (SAMs) and capped by gold clusters.^[12,13] The statistical analysis of these results showed that many curves could be identified as multiples of a common “parent curve” and hence correspond to an integer number of contacted molecules.^[12] Also the occurrence of variations in physical parameters, such as, the peak position of negative differential resistance (NDR) effects, have been characterized with histograms.^[13,14]

Xu and Tao recently applied such a statistical approach to investigate single-molecule junctions in an electrochemical experiment by means of STM.^[15] The gold tip of the STM is repeatedly brought into contact with the gold substrate and pulled off again, thus successively forming hundreds of contacts. On pulling the tip from the surface, the formation of gold contacts with cross sections of just a few atoms is observed, which is detected by a stepwise decrease of the conductance (the conductance of each step is close to multiples of the

conductance quantum $G_0 = 2e^2/h$, as has been thoroughly investigated in vacuum experiments).^[10] Data collected for a large number of contacts can be plotted in histograms, which display a distinct accumulation of contacts with one, two, or three conductance quanta (Figure 1 b).

Whereas these results are very similar to the findings in vacuum experiments,^[10] qualitatively new effects are observed when molecules are added to the solution. Of particular interest are molecules that are expected to bridge the gap between both electrodes. Therefore, Xu and Tao investigated molecular wires with terminal groups that bind to gold surfaces, such as alkane thiol or pyridine derivatives.^[15]

The most convincing and significant set of data was collected with an electrolyte solution containing 4,4'-bipyridine. This is a rodlike aromatic system with two terminal nitrogen atoms each bearing a lone pair for binding to the gold surface of the electrodes. As the STM is retracted from the surface, the current is monitored. At a distance corresponding to the length of the 4,4'-bipyridine molecule a new set of current plateaus evolves at conductance values two orders of magnitude smaller than before (Figure 1 c). This feature is assigned to the formation of molecular junctions between the gold of the substrate and the tip. The histogram underscores the statistical significance of this observation (Figure 1 d). Pronounced peaks at multiples ($1\times$, $2\times$, and $3\times$) of $G' = 0.01 G_0$ have been observed, pointing to the formation of junctions of one, two, and three molecules between the electrodes. These current plateaus have not been observed without the 4,4'-bipyridine molecule (Figure 1 e and f)

[*] Dr. M. Mayor, Dr. H. B. Weber
Forschungszentrum Karlsruhe GmbH
Institute for Nanotechnology
P. O. Box 3640, 76021 Karlsruhe (Germany)
Fax: (+49) 7247-82-5685
E-mail: marcel.mayor@int.fzk.de
heiko.weber@int.fzk.de

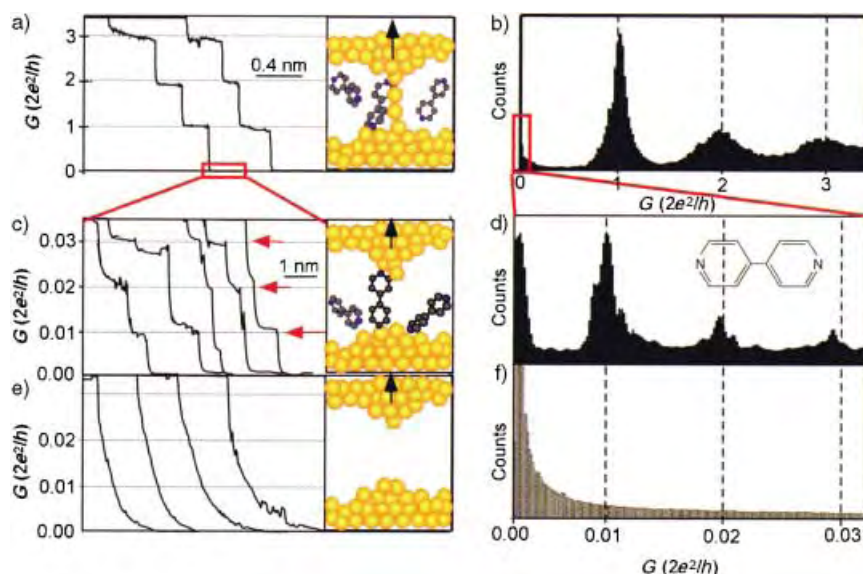


Figure 1. Left: Conductance patterns as a function of distance when the STM tip is retracted from the surface: conductance steps indicate discrete reorganizations of the contact. The color image illustrates the attributed microscopic configuration: a) metallic contacts, c) molecular contacts, and e) featureless conductance patterns in the absence of molecules. Right: The histograms evaluate hundreds of such conductance patterns. b) An accumulation at $G \approx (2e^2/h)$ can be clearly seen which indicates contacts with atomic diameter. d) At larger distance, a peak at $0.01 (2e^2/h)$ is attributed to a single-molecule junction. f) In the blind experiment such peaks are absent. Modified with permission from ref. [15]. Copyright 2003 AAAS.

or with 2,2'-bipyridine, which lacks the terminal pyridine nitrogen atoms that are required to form bonds to the gold surface. The experimental setup also allows the potential of the electrolyte solution to be varied with respect to the potential of the substrate. Thereby the ability to form the molecule–gold bond can be switched on/off electrochemically, as shown by the appearance/disappearance of the conductance peaks. Still under discussion is whether the pronounced reproducibility of the conductance is related to the electrochemical environment, which may efficiently screen the undesired influence of the electrostatic environment and provide better-controlled conditions.

The histogram technique allows for a new type of measurement: the current values I_{Peak} at the histogram peaks are plotted as a function of the bias voltage V , and thus current/voltage (I/V) graphs can be prepared, in which each point is a statistical value for many repeatedly formed junctions. Consequently, this curve does not represent the characteristics of a single-molecule contact, but corresponds to the I/V behavior of a large statistical ensemble of junctions. This technique can be compared to the

more familiar optical spectroscopy on a large number of molecules dispersed in a solid, which has proven to be different from single-molecule optical spectroscopy with its variations from sample to sample.^[16]

However, there is a remarkable difference between single-molecule experiments in molecular electronics and in optics. Whereas in optical spectroscopy a large statistical ensemble is rather simple to characterize, a large ensemble of molecular junctions is not easy to investigate simultaneously: In a molecular-film device, for example, where many metal–molecule–metal junctions are in parallel, the best-conducting contacts (or an unintended short-circuit) will dominate the conductance behavior, whereas the worse-conducting parts of the ensemble remain undetectable.

Following the above-mentioned method, I/V plots of the positions of the first, second, and third current plateaus against the applied voltage have been generated. When the corresponding I/V s are rescaled by a factor of one, two, three, respectively, all three curves collapse onto a single curve, which corroborates the expectation that

the first peak corresponds to single-molecule junctions, the second to a double-molecule junction and so on.

In the same paper Xu and Tao, apply this method to measure transport properties of alkane dithiols.^[15] This class of molecules provides a simple test system which is currently investigated by other groups because it allows systematic comparisons of different methods.^[12]

In conclusion, Xu and Tao^[15] have developed a new experimental method to investigate electronic transport properties of individual molecules between two electrodes in an electrolyte. With this method they have overcome the drawback of having very limited experimental data that is associated with single-molecule experiments. They have also demonstrated the applicability of their technique for two different types of molecular rods. The method has more recently been used to investigate the redox-state dependence of single-molecule conductance.^[17] This type of large-number study is a new tool to collect facts in the still puzzling field of molecular electronics and hopefully help further clarification of both the prospects and the limitations of the concept of molecular electronics.

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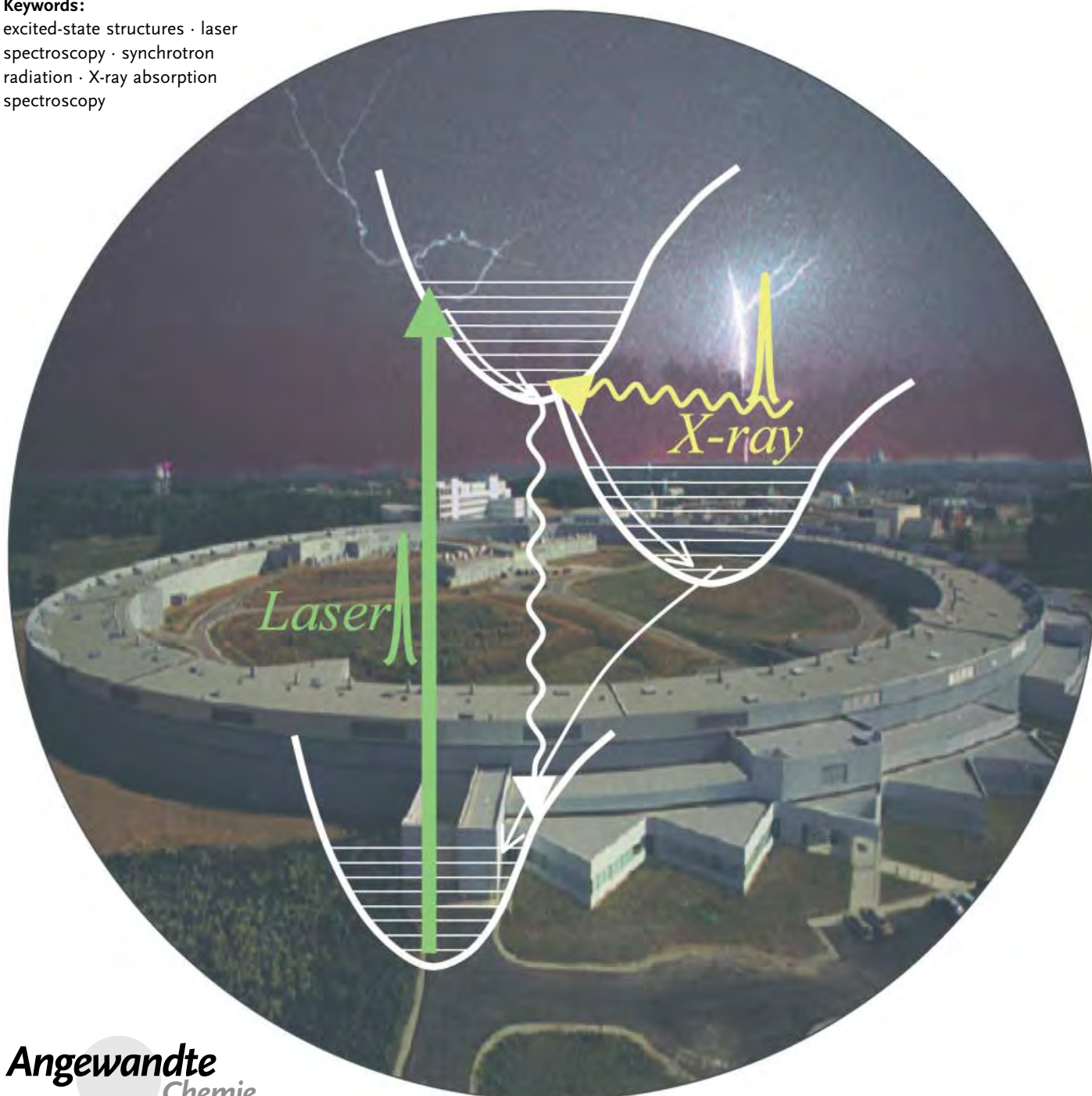
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Pump–Probe Techniques

Taking Snapshots of Photoexcited Molecules in Disordered Media by Using Pulsed Synchrotron X-rays

*Lin X. Chen****Keywords:**

excited-state structures · laser spectroscopy · synchrotron radiation · X-ray absorption spectroscopy



Angewandte
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Photoexcited molecules are quintessential reactants in photochemistry. Structures of these photoexcited molecules in disordered media in which a majority of photochemical reactions take place remained elusive for decades owing to a lack of suitable X-ray sources, despite their importance in understanding fundamental aspects in photochemistry. As new pulsed X-ray sources become available, short-lived excited-state molecular structures in disordered media can now be captured by using laser-pulse pump, X-ray pulse–probe techniques of third-generation synchrotron sources with time resolutions of 30–100 ps, as demonstrated by examples in this review. These studies provide unprecedented information on structural origins of molecular properties in the excited states. By using other ultrafast X-ray facilities that will be completed in the near future, time-resolution for the excited-state structure measurements should reach the femtosecond timescales, which will make “molecular movies” of bond breaking or formation, and vibrational relaxation, a reality.

1. Introduction

1.1. Importance of Excited State Structural Information

When a molecule absorbs a photon with energy ranging from ultraviolet to near infrared, it may undergo electronic transitions that redistribute valence electrons, and becomes an excited-state molecule.^[1] Nuclei of the molecule in such an excited state (referred to as the Franck–Condon (FC) state) experience a new field while retaining the same configuration as the ground state (Figure 1).^[2] Highly unstable in nature, the nuclei in an FC state will structurally relax themselves to

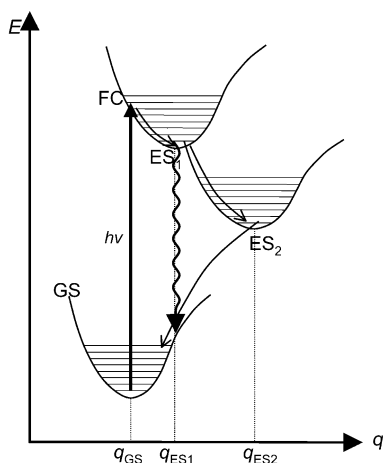


Figure 1. Illustration of the interaction between light and a molecule. GS: ground state; FC: Franck–Condon state; ES₁ and ES₂: the first and the second thermally equilibrated excited states. q is a generalized molecular coordinate. While FC has identical coordinates as GS, ES₁ and ES₂ may have distinctively different coordination from that of GS. The purpose of the pump–probe XAFS on a 100 ps time scale is to capture the coordinates for ES₁ and ES₂ rather than follow the time evolution of the coordinates from FC to ES₁, which will be accomplished by femtosecond X-ray techniques.

accommodate the new field from within tens of femtoseconds to a few picoseconds.^[3] Consequently, the excited state is equilibrated to a new structure (q_{ES1}) that is no longer identical to the ground state (q_{GS}). Although light absorption in general could populate different excited states, the upper excited states generally decay rapidly to the lowest excited state with another set of nuclear positions (e.g., q_{ES2}). Because of these changes in both electronic and nuclear configurations, a molecule in its lowest excited state is a different species from the ground state and has additional energy, different atomic coordinates, and different vibrational and electronic spectra. Such a highly energetic species has a different chemistry referred to as photochemistry. Hence, excited-state molecules are quintessential reactants in photochemical reactions.^[4]

Some of the most important photochemical and photo-physical processes that involve excited-state molecules are depicted in Figure 2. The photoinduced energy transfer (EnT) refers to a process in which an excited state of one molecular entity (the donor D*) is deactivated to a lower-lying state by transferring energy to a second molecular entity (the acceptor A), which is thereby raised to a higher-energy state. The photoinduced EnT proceeds by coulombic or exchange mechanisms in which the relative orientations of the transition dipoles of D* and the acceptor A (μ_D and μ_A), as well as their separation R_{DA} will determine rate constant k_{EnT} (Figure 2.).^[5] The photoinduced electron-transfer (ET) process results also from an excited state of the electron donor D*, produced by the resonant interaction of electromagnetic radiation with matter. The rate constant for a photoinduced

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[*] L. X. Chen
Chemistry Division
Argonne National Laboratory
Building 200, 9700 South Cass Avenue, Argonne, IL 60439 (USA)
Fax: (+1) 630-252-9289
E-mail: lchen@anl.gov

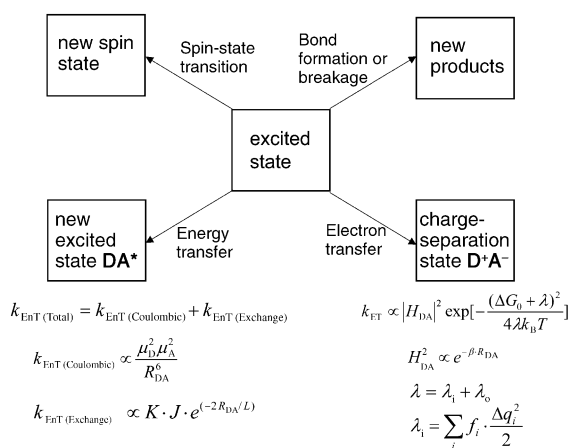


Figure 2. Roles of the excited-state molecules in various photochemical and photophysical processes. D and A are donor and acceptor respectively. DA* is the photoinduced energy-transfer product, the energy of excited D* is transferred to create the excited state A*, and D+A[−] is the photoinduced electron transfer product, or the charge separation state where an electron is transferred from D* to A. k_{EiT} and k_{ET} are rate constants for photoinduced energy- and electron-transfer reactions respectively.

ET process k_{ET} is a function of the wavefunction overlap H_{DA} between D* and the acceptor A and their separation distance R_{DA} , as well as the reorganization energy λ (defined as the Gibbs energy dissipated when a system that has undergone “vertical” electron transfer relaxes to the equilibrium state for its new charge distribution).^[6] The total λ is the sum of an inner contribution (λ_i) and an outer contribution (λ_o) attributed to nuclear reorganization of the redox partners and their environment (solvent), respectively. λ_i is directly related to the coordinate changes Δq from D*A to D+A[−]. Examples of photoinduced EnT and ET processes can be found in natural and artificial photosynthesis in which the light energy is collected by an array of chromophores and is transferred to a protein at a reaction center or electron donor–acceptor complexes through EnT, respectively, and in which sequential ET processes take place within a few picoseconds to milliseconds to produce charge-separation states at which the chemical potentials are generated.^[7] The photoexcitation of the molecule also induces the spin-state transition via the excited states on which the structural changes take place, because of the rearrangement of electrons among different

orbitals (Figure 2). Moreover, intermediates that participate in bond formation or breakage are often excited states.^[7] Extensive studies, both experimental and theoretical, have been carried out to investigate the influence of various factors on rate constants, such as driving force, donor–acceptor distance, reorganization energy, and reaction media.^[8–15] Detailed kinetic information for photochemical and photophysical reactions has become readily available from time-resolved optical spectroscopic studies. However, there is only limited information on excited state structures mainly due to the lack of suitable techniques for obtaining information on transient molecular structures. The most important questions are: How do molecular coordinates respond to photoexcitation? Which vibrational modes are most responsible for the photochemical reactions? Although resonance Raman measurements attempt to address this issue,^[16–20] the structural information obtained from those measurements is indirect. While μ_D , μ_A and R_{DA} can be obtained by measuring steady-state structures of the reactants in photoinduced EnT and ET reactions,^[21] these measurements may not be feasible for fast and reversible processes in most of the photochemical and photophysical reactions when the excited state adopts a different structure from that of the ground state.

1.2. Why is Direct Molecular Structural Information on Excited States So Sparse?

Although knowing ground-state structures of reactants allows us to predict energetics and pathways for some photochemical reactions, such knowledge on short-lived excited-state molecules remains largely unattainable because of the lack of suitable X-ray sources. The birth and development of synchrotron facilities provided X-ray photon fluxes that were several orders of magnitude higher than X-rays generated by a rotating anode. However, the probing of excited state molecules in disordered media remained elusive until the third generation of synchrotron facilities were available, which provided an unprecedented X-ray photon flux within a single X-ray pulse. Early studies by using older generation synchrotron sources were carried out mainly by one of three approaches: 1) gating detectors or chopping an X-ray pulse to gain the time resolution,^[22–30] 2) cryogenic trapping of excited-state species for a long period of time so that they can be examined by the steady-state methods,^[31–35] and 3) by using energy dispersive X-ray absorption, which captures the excited-state structure within the detector read-out time.^[36–41]

However, these early works were carried out on microsecond time resolution, which excludes many excited states, especially singlet excited states, with shorter lifetimes. During the past decade, three new types of pulsed X-ray sources have been developed, 1) the third generation synchrotron sources^[42] (Figure 3), 2) ultrafast laser-generated-plasma X-ray sources,^[43–50] and 3) ultrafast laser-generated high-harmonic soft X-rays.^[51–55] Consequently, a new era of time-resolved X-ray studies has begun, as the intrinsic time resolution is provided by pulse durations of X-rays ranging from tens of femtoseconds to 100 ps.^[56] As X-ray pulses become shorter, it



Lin X. Chen received her Ph.D. in physical chemistry in 1987 under Graham R. Fleming from the University of Chicago. After doing postdoctoral research with Herbert L. Strauss and Robert G. Snyder at the University of California at Berkeley, she joined the Chemistry Division at Argonne National Laboratory near Chicago in 1989. Her research interests are mainly on solar-energy conversion and storage, but also excited-state dynamics and structures in photoinduced electron- and energy-transfer reactions by using ultrafast laser and X-ray techniques, and molecular modeling.



Figure 3. Aerial view of the Advanced Photon Source at Argonne National Laboratory in Illinois, USA, one of the third-generation synchrotron facilities (Courtesy of Argonne National Laboratory).

is possible to follow the coherent atomic displacements after excitation as a function of time. However, on the time scale of 100 ps provided by the current third-generation synchrotron X-ray pulses, only thermally equilibrated excited-state structures (i.e., q_{ES} in Figure 1) can be determined, which is the focus of this review.

The third-generation-synchrotron sources are currently major facilities that provide X-ray pulses^[42] with a duration of 30–100 ps and about 10^6 photons per pulse (based on an undulator beamline with Si 220 monochromator at 10 keV at the sample), which is 10^3 to 10^4 times higher than that from a bending magnet beamline of a second generation synchrotron (Figure 4). The X-ray photon flux in each pulse is crucial in obtaining analyzable structural information within a reasonable data-acquisition time. This is the main reason why the example experiments included in this review could not be carried out in the second-generation synchrotrons and why direct excited-state structures have been so sparse.

1.3. Pump-probe Laser and X-ray Spectroscopy

Pump-probe laser spectroscopy has been one of the most frequently used approaches in detecting ultrafast events in photochemical processes. A laser pulse initiates a photochemical process or creates a perturbation in the system, and another laser pulse with lower intensity probes the optical responses of the system as a function of the delay time between the pump and the probe, as well as the probe wavelength.^[57–61] Thus, dynamics of the wave packets in the excited states,^[62–64] the excited-state population, and energetics can be obtained.^[65–68] The perturbation created by the pump pulse is generally reversible, hence the pump-probe cycle can be repeated until the data satisfy a desired signal-to-noise ratio. An important advantage of this technique is that the intrinsic time resolution of the measurement is only limited by the pulse duration of the pump or probe pulse, whichever is longer.^[69] Ideally, the kinetics of each excited state could be monitored by detecting changes in the optical

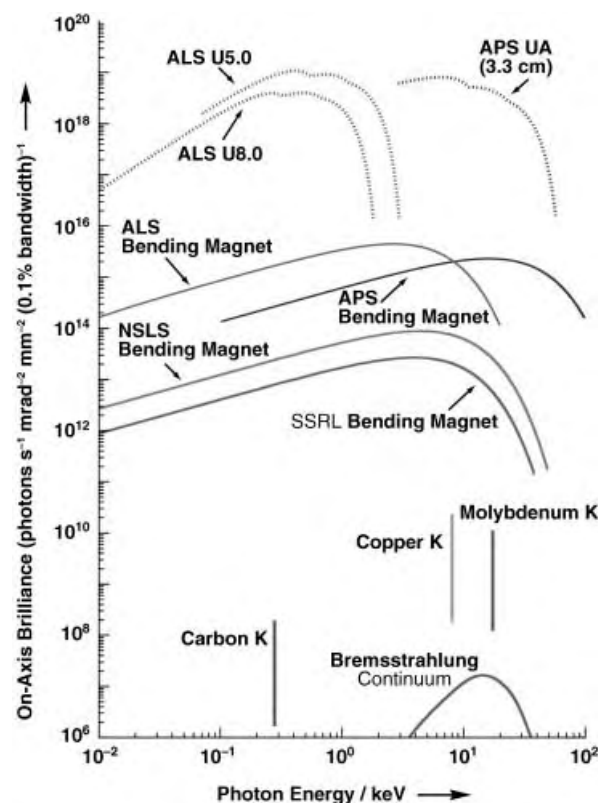


Figure 4. The brilliance of different X-ray sources versus photon energy (Courtesy of Argonne National Laboratory). ALS: Advanced Light Source; APS: Advanced Photon Source; NSLS: National Synchrotron Light Source; SSRL: Stanford Synchrotron Radiation Laboratory; U5.0, U8.0, UA: Different beamlines at ALS and APS.

absorption as a function of time at a particular wavelength corresponding to a characteristic transition. Correlations between excited-state kinetics probed at different wavelengths should reveal the coherence between excited states. However, pump-probe laser spectroscopy offers little structural information on excited states. To obtain atomic coordinates of excited-state structures, it is necessary to extend the pump-probe approach into the X-ray regime.

Figure 1 also demonstrates two distinctively different types of excited-state dynamics, 1) coherent atomic displacements in vibrational relaxation from the FC state to the first thermally equilibrated excited state, which take place on the time scale of subpicosecond within a single potential surface, and 2) incoherent atomic displacements that take place on a much longer time scale and involve barrier crossing of one or more potential surfaces. The coherent motion of atoms can be followed by femtosecond X-rays pulses at different delay times relative to the pulse of the pump laser. In contrast, the incoherent motion of atoms cannot be followed in real time but statistically averaged and thermally equilibrated excited-state structures that reside in the bottoms of different potential surfaces at different stages of the reaction can be probed. On the time scale beyond the initial coherent atomic motion due to the photoexcitation, excited state dynamics are just incoherent population decays of one or more excited states. Hence, for the purpose of structural determination beyond the dephasing time, only one snapshot for each

discrete excited state at its optimal concentration is necessary, because the population-decay kinetics of the excited state can be followed by the transient optical spectroscopy much more easily.

1.4. Scope of the Review

This review focuses mainly on the use of pulsed X-rays to resolve structures of photogenerated excited states or other transient states in disordered media during photochemical reactions. The enormous breakthroughs by using time-resolved X-ray-diffraction methods on crystalline materials, proteins, and surfaces deserve separate reviews. Moreover, this review emphasizes only recent studies and assumes familiarity with earlier work. For the second part of the review, challenges in laser-pulse pump X-ray pulse-probe experiments will be discussed with emphasis on the use of synchrotron X-rays. The third section is devoted to recent examples related to photochemistry, excluding those in condensed phase physics, materials science, and engineering. The fourth section lists recent work by using X-ray techniques other than X-ray absorption spectroscopy (XAS). Finally, perspectives in future time-resolved X-ray studies in condensed phase photochemistry will be briefly described.

2. Challenges in Laser-pump, X-ray probe XAS

2.1. Background

In principle, X-rays techniques for solving steady-state structures, such as diffraction, scattering, and absorption, can be applied directly to solving excited-state structures. The X-ray diffraction is based on coherent interference of scattered X-rays from ordered lattices, and determines atomic coordinates with the resolution up to one thousandth of angstroms in single crystals or powders that are either macroscopically or microscopically ordered lattices.^[70] X-ray scattering originates from pair distributions of electrons in an entity that varies from molecules to bulk materials.^[71] X-ray absorption spectroscopy, including X-ray absorption fine structure (XAFS) and X-ray absorption near edge structure (XANES), arises from resonant absorptions that result from electronic transitions from core levels to the continuum.^[72–75]

Following Fermi's golden rule, XAS measures dipole-mediated transitions of an electron in a deep core state $|i\rangle$ into an unoccupied state $|f\rangle$. The absorption $\mu(E)$ is proportional to $\sum |\langle f | \mathbf{e} \cdot \mathbf{r} | i \rangle|^2 \delta(E)$, in which $\mathbf{e} \cdot \mathbf{r}$ is an electronic-transition dipole operator, and E is the energy of the X-ray photon.^[76] XAFS results from the interference between the outgoing photoelectron wave from the X-ray absorbing central atom and the back-scattered photoelectron waves from neighboring atoms (Figure 5).^[72–75] The X-ray absorption cross section of the atom is modulated by the interference of the two waves as a function of E , and is determined by the number of neighboring atoms and their distances to the central X-ray absorbing atom. The pioneering work by Sayers, Stern, and Lytle laid the foundation for modern XAFS

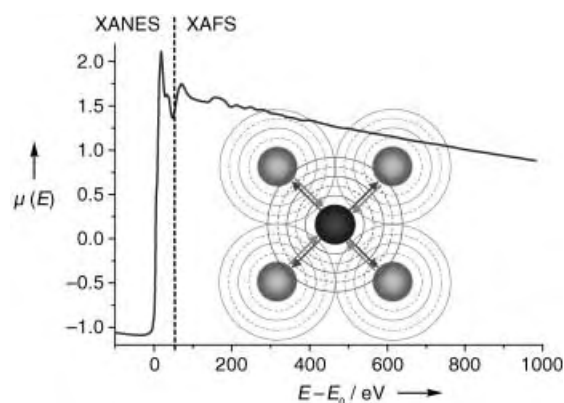


Figure 5. The interference between out-going and back-scattered X-ray photoelectron waves is the foundation of XAFS. The normally referred XANES and XAFS regions are marked. E_0 is the energy of the transition edge for the element.

analysis,^[72–75] in which they translated the modulations on the absorption cross section of the atom into its local structure by Fourier transform, which converts the spectrum from energy space to distance space. The resulting spectrum displays multiple peaks that correspond to different distances from the central X-ray absorbing atom to its neighboring atoms. The correlation between the structural parameters and the modulation frequencies can be described by Equation (1):

$$\chi(k) \propto \sum_j N_j F_j(k) e^{-2\sigma^2 k^2} e^{-\frac{2r_j}{\lambda(k)}} \frac{\sin[2kr_j + \delta_{ij}(k)]}{kr_j^2} \quad (1)$$

in which j is the index for the neighboring shells of atoms that surround the central X-ray absorbing atom, $F(k)$ is the back-scattering amplitude, N is the coordination number, r is the average distance, σ is the Debye–Waller factor due to thermal vibration and static disorder, λ is the electron mean-free path, δ is the phase shift of the photoelectron wave, and k is the wavevector, $k = [2m(E - E_0)/\hbar^2]^{1/2}$ (m is the electron mass and E_0 is the threshold energy for the transition edge). Due to a limited range of the free-electron path and the Debye–Waller-factor damping from the heterogeneity in distance between the central atom to neighboring atoms, XAFS is essentially for local structure determination with high precision and without restriction on the form of the samples.^[77]

XANES refers to the X-ray absorption near edge structure, including the pre-edge, the transition edge, and 30–50 eV above the edge (Figure 5). The pre-edge bands are attributed to electronic transitions from the core level to empty bound states.^[77] Hence, they follow selection rules for the dipolar transitions, and are directly related to the density of the states (DOS) and the occupancy of these states. The pre-edge bands are sensitive to electronic structural changes related to molecular orbital energies and occupancies. The transition-edge position is responsive to the oxidation state of the element because of the charge-shielding effect on the ionization threshold for the core electrons, and to the coordination geometry because of the variation of the molecular-orbital energy in different coordination geometries.

Although XAS is element specific, it represents an overall average for atoms of the same element in the sample. Hence, it is a challenge to determine the molecular structures for different species of the same element. The total X-ray absorption $\mu(E)$ at X-ray photon energy E can be described by $\sum_j f_j \mu_j(E)$, in which, f_j and $\mu_j(E)$ are the fraction and absorption, respectively, of j th species in the sample. The X-ray absorption spectrum of a laser-pumped sample is often a mixture of the ground and the excited states, because the photoexcitation rarely converts all of the ground-state molecules into the excited state. Therefore, if the time dependence of f_j and $\mu_j(E)$ are included, the X-ray absorption spectrum of a laser-pumped sample can be described as Equation (2):

$$\mu(E, t) = [1 - \sum_j f_{ES_j}(t)] \mu_{GS}(E) + \sum_j f_{ES_j}(t) \mu_{ES_j}(E, t) \quad (2)$$

$\mu(E, t)$ is the total absorption at X-ray photon energy E and time t , $f_{ES_j}(t)$ is the fraction of the j th excited state at t , and the subscripts GS and ES are the ground and excited states, respectively. The complexity of Equation (2) is attributed to $\mu_{ES_j}(E, t)$ and $f_{ES_j}(t)$. The former term accounts for the atomic movements as a function of t and the latter for population change in the same excited state with t . Details of time-dependent XAFS spectra with wavepacket dynamics and the atomic motions were described by Brown et al.^[78] To monitor the fast coherent atomic motions as mentioned earlier, $\mu_{ES_j}(E, t)$ must be followed by femtosecond X-ray probe pulses as a function of the delay time from the pump-laser pulse, while $f_{ES_j}(t)$ within the dephasing time would be a constant. If the time resolution of the experiment only allows the statistically averaged and thermally equilibrated excited-state structure to be probed, $\mu_{ES_j}(E, t)$ can be viewed as static for a particular excited state at a delay time of the probe when the optimal concentration of the excited state appears, and described by $\mu_{ES_j}(E)$. As the barrier crossing between different potential surfaces occurs (Figure 1), the fraction of a particular excited state $f_{ES_j}(t)$ is proportional to the population decay of that state. Thus, Equation (2) can be approximated to Equation (3):

$$\mu(E, t) = [1 - \sum_j f_{ES_j}(t)] \mu_{GS}(E) + \sum_j f_{ES_j}(t) \mu_{ES_j}(E) \quad (3)$$

Further simplification can be made if only one thermally equilibrated excited state exists when the excited-state structure is probed. In this case, the delay time between the laser-pump and the X-ray probe pulses is set to probe the excited-state structure at its optimal concentration, normally at the nominal time $t = 0$. Equation (3) can be simplified to Equation (4) as:

$$\mu(E) = [1 - f_{ES}(0)] \mu_{GS}(E) + f_{ES}(0) \mu_{ES}(E) \quad (4)$$

2.2. Limitation on Repetition Rates of the Laser-Pump Pulse

In general, both $\mu_{ES}(E)$ and $f_{ES}(0)$ in Equation (4) are unknown, which imposes a great uncertainty in extracting the structure for even one excited state. Both $f_{ES}(0)$ and the excited state lifetime can be obtained through optical transient spectroscopy simultaneously conducted with

pump–probe XAFS or separately under the same conditions used for the photoexcitation. $f_{ES}(0)$ obtained from such measurements is used in data analysis to divide two simultaneously existing-ground and excited-state structures. On the other hand, $\mu_{ES}(E)$ can be estimated sometimes when prior knowledge of the excited structure is available, especially in the XANES region.^[79,80] Such an effort in deducing $\mu_{ES}(E)$ or $f_{ES}(0)$ makes Equation (4) solvable.

Because XAS is a nonzero-background method, a certain value of $f_{ES}(0)$ would be required to extract the excited-state structure, depending on the actual spectral differences between the two states. In general, the larger the difference between the two, the lower value of $f_{ES}(0)$ is required. It is difficult to have a definitive value of the minimum $f_{ES}(0)$, but about 20–30% is reasonable based on our experimental results.^[79] To attain such a $f_{ES}(0)$ value, sufficient laser photons are required in each laser pump pulse. The required laser-pump pulse energy for a certain $f_{ES}(0)$ value can be calculated based on the Beer–Lambert law for light absorption, ($I_t = I_o 10^{-\epsilon l C}$), in which I_t and I_o are transmitted and incident light intensities, respectively, ϵ is the molar extinction coefficient ($\text{M}^{-1} \text{cm}^{-1}$), l is the thickness of the sample (cm) and C is the concentration of the ground-state molecules (M). Light intensity absorbed by the sample $I_a = I_o(1 - 10^{-\epsilon l C})$. Assuming that the laser pulse energy is P_o (in J), the number of absorbed photons from each pump laser pulse is $N_a = P_o / h\nu (1 - 10^{-\epsilon l C})$, and $h\nu$ is the energy per quanta of the laser photon. $f_{ES}(0)$ can be obtained by solving Equation (5):

$$f_{ES}(0) = \frac{P_o Q \{1 - 10^{-\epsilon l C_{\text{total}} [1 - f_{ES}(0)]}\}}{h\nu C_{\text{total}} a l A} \quad (5)$$

a is the sample area illuminated by the laser-pump pulse, A is the Avogadro constant, and Q is the quantum yield for generating a particular excited state. Equation (5) correlates $f_{ES}(0)$ with experimental parameters, such as the quantum yield of the excited-state generation, laser beam size, sample thickness, molar extinction coefficient, total concentration of the sample, and the pump pulse energy as depicted in Figure 6. Figure 6a demonstrates that it is difficult to create a large value of $f_{ES}(0)$ if a) ϵ is small (i.e., $< 1000 \text{ M}^{-1} \text{cm}^{-1}$), or b) the sample concentration is too high (Figure 6b). Thus, it is desirable to have high laser-pulse energy for creating a significant fraction of the excited state, which imposes a limit on the repetition rate of the laser. Currently, most lasers used in pump–probe XAS are running at repetition rates of one to a few kHz, which is three orders of magnitude lower than that of the X-ray pulses from a third-generation-synchrotron source in normal operating modes. Such a mismatch in the repetition rates between the pump and the probe pulses causes prolonged data acquisition in current pump–probe XAS experiments. The situation can be improved by a higher flux of X-ray photons with a smaller X-ray beam, so the laser beam can be further focused to create the same fraction of the excited with less photons per pulse, and hence a higher repetition rate can be used.

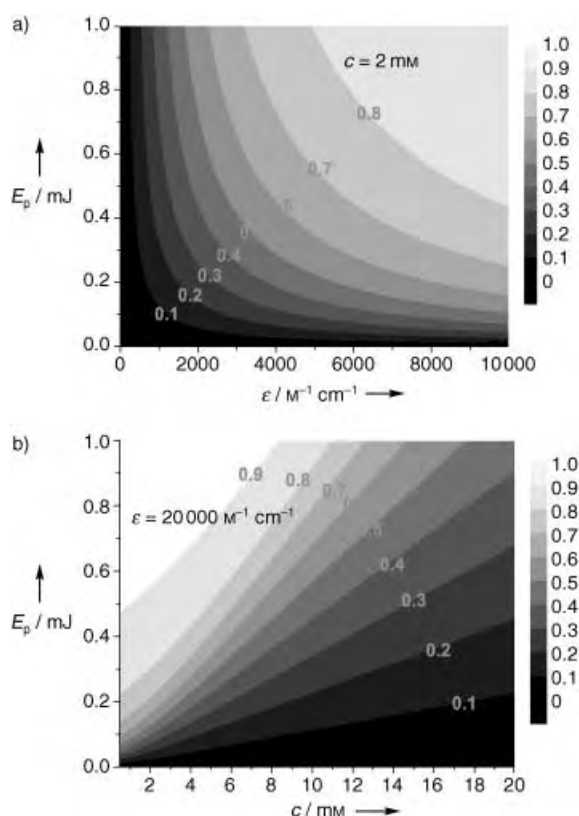


Figure 6. a) The calculated excited-state yield as a function of molar extinction coefficient ϵ at 2 mm concentration; b) The calculated excited-state yield as a function of sample concentration at $\epsilon = 20000 M^{-1} cm^{-1}$ (see text). E_p = energy of the pump pulse.

2.3. Absorption Coefficients for Laser and X-ray Photons

The discrepancy between molecular absorption coefficients for laser and X-ray photons is another difficulty encountered in laser-pump, X-ray probe experiments. Molar extinction coefficients for a majority of molecules in the UV/Vis region range from 1000 – $100000 M^{-1} cm^{-1}$ (and higher), which is 100 to 1000 times higher than that in the X-ray region. For example, zinc tetraphenylporphyrin, [Zn(tp)], in toluene has an absorption coefficient for the Q band at 560 nm at $100 cm^{-1}$ at 5 mm, while its absorption coefficient at 10 keV is $2 cm^{-1}$ (the majority of the absorption is from the solvent). The discrepancy in the absorption coefficients widens at higher concentrations (Figure 7). At a certain thickness for laser excitation, the majority of the X-ray photons are not absorbed by the atoms of interest in the sample. On the other hand, when most of the X-ray photons are absorbed by the sample, the concentration is too high for a laser pulse to create a significant fraction of the excited-state molecules. Improvement can be made by using a sample geometry that the path for the X-ray is many times longer than of the laser light, but such an approach results in only limited improvement in practice.^[81,82] Frequently, a dilute sample in favor of laser excitation is opted for and fluorescence detection is employed.^[81,83–85]

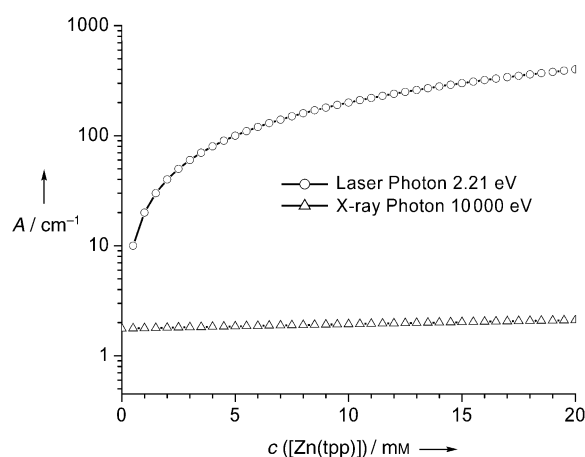


Figure 7. Discrepancy of the absorption coefficients between the X-rays and the laser light as a function of the sample concentration for [Zn(tp)] in toluene.

2.4. Sample Concentration Preferences for XAS and Photochemistry

A majority of photochemical processes prefer isolated excited-state species that are free from interference of others nearby. Consequently, most of the photochemical studies in ultrafast optical spectroscopic studies are conducted in dilute solutions to ensure isolation between excited species. However, desired concentrations for transmission XAFS measurements are at minimum of tens of mm, which is unattainably high for a large number of compounds. Even when the solubility is not the concern, high concentrations only permit a very small fraction of the molecules to be excited according to Equation (5). Furthermore, the high concentration also promotes excited-state quenching and exciton–exciton annihilation, which significantly shortens the lifetimes of excited states and reduces excitation efficiency.^[86] In many cases, the ideal sample concentration for photochemistry is lower than a few mm, whereas the ideal concentration for XAFS measurements through transmission detection is 10–100 times higher.^[80] This mismatch on the concentration requirements prompted the need for an alternative XAFS detection, such as X-ray fluorescence. X-ray fluorescence-detected XAFS has been applied to many dilute samples, such as metalloproteins.^[87,88] The details of its application in pump–probe XAS has been reported recently.^[83]

2.5. Response Times of Detectors and Desired Time-resolution

The statement that the intrinsic time resolution of the pump–probe XAFS is determined by the duration of the pump or the probe pulse is only correct if the pump–probe cycle is much longer than the detector recovery time. Unfortunately, the time separation between X-ray pulses from synchrotron sources ranges from tens to a few hundreds of nanoseconds, which is significantly shorter than the recovery times for existing solid-state germanium X-ray fluorescence detectors. Therefore, the key to obtaining a

time resolution limited only by the duration of the synchrotron X-ray pulses (30–100 ps) is being able to isolate the signal due to the probe X-ray pulse from those of adjacent pulses. Three approaches have been implemented: a) use of an X-ray shutter to block unwanted X-ray pulses and allow only the probe X-ray pulse to illuminate the sample;^[89–91] b) use of a hybrid fill of electron bunches in the storage ring with a hybrid timing mode to allow a sufficient recovery time of the germanium solid-state X-ray fluorescence detector;^[92] and c) use of a combination of diffraction based X-ray optics and photomultiplier tube (PMT) detectors with electronic signal gating to extract signals only from the probe X-ray pulse.^[93–95]

The available X-ray shutters that are capable of extracting single X-ray pulses from the pulse train are mainly based on either rotating blades^[89,91,96] or a rotating Si single crystal,^[97] both of which have been successfully locked in phase with synchrotron X-ray pulses. The former method has the capability of varying the repetition rate of opening from 10–897 Hz with an opening time of around 100 ns (Figure 8).^[96] Alternatively, another mechanical shutter is

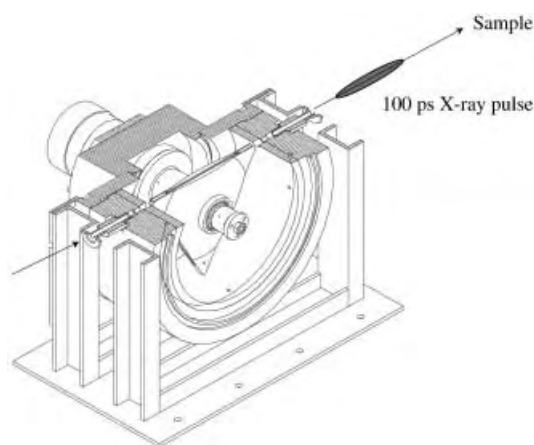


Figure 8. The chopper for single X-ray pulse selection at the European Synchrotron Radiation Facility. The rotor is synchronized with the synchrotron electron-bunch clock. (from reference [96]).

based on a narrow slit imbedded in a cylindrical rotor.^[98] The X-ray shutter based on the diffraction of X-rays on a rotating crystal is relatively simple to use for monochromatic diffraction or scattering experiments,^[97] but it needs positional adjustment for XAFS measurements, during which the energy of the X-ray, and therefore the diffraction angle changes.

The second approach is filling the storage ring with a hybrid timing mode in which a single electron bunch is separated from other bunches in the storage ring. Such an operating mode enables simultaneously a sufficient time window for single X-ray pulse extraction (for time-resolved studies) and a high average X-ray photon flux for steady-state studies.^[92] Such a time separation allows a solid-state germanium-detector array for the fluorescence XAS to recover before signals from adjacent X-ray pulses enter the detector. Thus, signals from X-ray probe pulses can be extracted by electronically gating the detector without an X-ray shutter.^[83] The concerns of this approach are 1) sample damage due to

exposure to unused X-ray photons, b) insufficient time window for recovery of the charge coupled device (CCD) detector commonly used in diffraction and scattering measurements, and c) still insufficient recovery time for the Ge detector used in synchrotrons with small storage ring sizes.

The third approach has been explored by either Laue diffraction from bent thin crystals^[93,94] or diffractions from multilayer coated Si crystal arrays.^[95] The general idea is to take advantage of the directionality in X-ray diffraction to energy discriminate against strong elastic scattering signals from entering the detector, while allowing only weak fluorescence signals from a dilute sample to be collected by PMT detectors with a recovery time of less than 100 ns. With a combination of gated integrators, signals from a single X-ray probe pulse can be extracted without using the hybrid timing-mode operation of the storage ring and without the X-ray shutter.

3. Photoexcited Molecular Structure Determination in Disordered Media by Using Pulsed X-rays from Synchrotron Sources

The advances in technology for the generation of X-rays in recent years, including both synchrotron and table-top laser sources, have made it possible for the first time to directly determine photoexcited molecular structures with the laser pulse pump, X-ray pulse–probe approach on a time resolution limited only by the duration of the pump or the probe pulses. The following are recent examples that make use of synchrotron X-ray sources.

3.1. Photodissociation Intermediate Structure of $[\text{Ni}(\text{tpp})\text{L}_2]$ ^[99]

Structural similarities between metalloporphyrins and heme or chlorophylls lead to their heavy involvement in modeling photoinduced energy and electron transfer in photosynthesis,^[100] oxygen transportation,^[101] and molecular devices.^[102] Properties of the excited states of porphyrins can be manipulated by modification of the side groups or the porphine macrocycle, metallation, and axial ligation.^[103] Metalloporphyrin excited states such as Nickel(II) tetraphenylporphyrin, $[\text{Ni}(\text{tpp})]$, are examples of how, through photoinduced electron redistribution, the coordination geometry of the metal center is altered.

According to previous studies^[104,105] (Figure 9), the ground-state of square-planar $[\text{Ni}(\text{tpp})]$ (with $3d^8$ configuration of the Ni^{II} center) adopt a singlet spin state $^1A_{1g}(3d_{z^2})^2$ (the first superscript indicates the spin state and the second superscript designates the occupation of $3d_{z^2}$ orbital) in a noncoordinating solvent. In ground-state octahedral $[\text{Ni}(\text{tpp})\text{L}_2]$ (L, axial ligand) a triplet spin state, $^3B_{1g}(3d_{x^2-y^2}, 3d_{z^2})(L_2)$, is adopted in coordinating solvents. For L = piperidine, a laser-pump pulse induces an electronic transition from the ground state, $^3B_{1g}(3d_{x^2-y^2}, 3d_{z^2})(L_2)$, to a triplet excited state, $^3T_{1g}(L_2)$ (Figure 9), which undergoes vibrational relaxation (1 ps) and conformational relaxation (10–20 ps) accompanied by intersystem crossing to an excited singlet state,

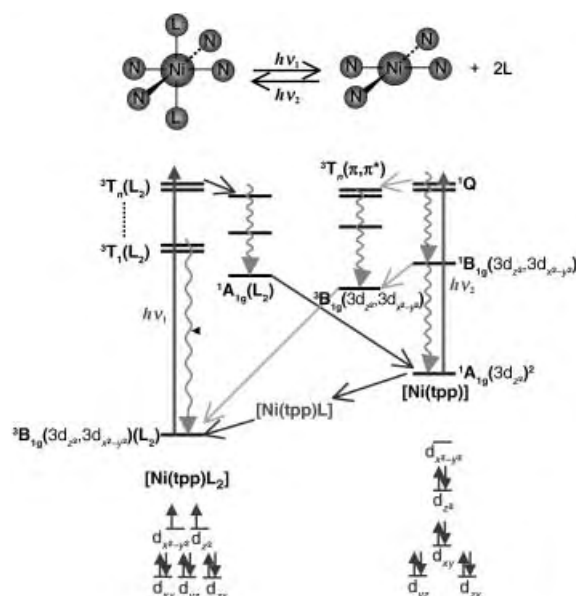


Figure 9. Energy diagram and reaction pathways for [Ni(tpp)] photo-dissociation/association reaction.^[104]

$^1A_{1g}(L_2)$, from which the two axial piperidine ligands are ejected to produce $^1A_{1g}(3d_z)^2$ with a square-planar geometry.^[104] In a strongly coordinating solvent, the $^1A_{1g}(3d_z)^2$ state is less stable than the $^3A_{1g}(L_2)(3d_{x^2-y^2}, 3d_z)$ state, so it returns to the ground state in > 20 ns by the recoordination of two axial ligands. The pathways for this recoordination process were considered either simultaneous ligation, or a two-step recombination with [Ni(tpp)L] as an intermediate. Because of the ambiguity in [Ni(tpp)L] and [Ni(tpp)L₂] optical absorption spectra, transient optical absorption of [Ni(tpp)] alone is insufficient to identify the intermediates.

By using the pump-probe XAFS technique, the photodissociation intermediate structure of [Ni(tpp)L₂] (L = piperidine) in piperidine solution was measured at the Advanced Photon Source under the hybrid fill operating mode, which provided a sextuplet electron bunch cluster with a total duration of 14 ns separated by > 1.5 μ s from other bunches in the storage ring. A laser pulse, with a 5 ps duration at 351 nm and coincident with the X-ray pulses, induced a π - π^* transition in the porphyrin macrocycle. Hence, the X-ray probe was applied to the sample at the time delay between the pump and probe when the photodissociated species had the maximum population (30% according to Equation (5) and results from transient absorption measurements). Figure 10 shows XANES spectra for [Ni(tpp)L₂] with and without the laser, as well as ground-state [Ni(tpp)] in noncoordinating toluene. A distinct shoulder at 8.340 keV in the XANES spectrum of ground-state [Ni(tpp)] is attributed to the $1s \rightarrow 4p_z$ transition in a square-planar transition-metal coordination geometry,^[106] which is otherwise absent or severely attenuated in an octahedral or a square-pyramid geometry. This shoulder emerged again in the XANES spectrum of the laser-illuminated sample with lower amplitude, and disappeared when the laser beam was blocked.

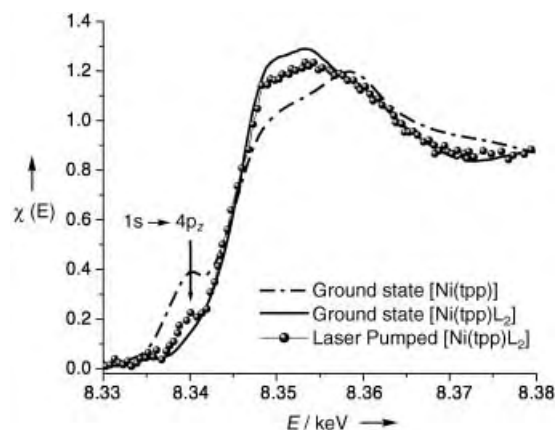


Figure 10. XANES spectra of [Ni(tpp)] in toluene.

The XAFS spectrum of the laser-pumped sample (not shown) also exhibits differences from the spectra of both the ground-state octahedral [Ni(tpp)L₂] and square-planar [Ni(tpp)].^[99] The spectra of the ground-state [Ni(tpp)] and [Ni(tpp)L₂] fit one Ni–N distance model with coordination numbers of 4 and 6, and the average bond lengths of 1.91 ± 0.02 and 2.09 ± 0.02 Å. These structural parameters agree reasonably well with their respective crystal structures.^[107,108] In contrast, the laser-illuminated sample could be best described by two bond lengths of 1.92 ± 0.02 Å and 2.10 ± 0.02 Å with a relative ratio of 1:3.2, corresponding to about 30% square-planar photodissociated intermediate and 70% octahedral ground-state [Ni(tpp)L₂], as seen in the XANES spectrum. The Ni–N bond length obtained for the photodissociated intermediate is very similar to that of ground-state [Ni(tpp)].

A previous study on the structure of Ni bacterial chlorophyll with an identical nearest-neighbor coordination of [Ni(tpp)] showed that the Ni–N bond length increased from 1.95 to 2.04 Å as the Ni coordination changed from a square-planar to a square-pyramidal geometry.^[109] If a substantial amount of [Ni(tpp)L] was present after the photodissociation on the time scale of the experiment, the nearest-neighbor distances would not resemble so closely those for the ground states of [Ni(tpp)] and [Ni(tpp)L₂], and the shoulder due to the $1s \rightarrow 4p_z$ transition at 8.340 keV would not be enhanced as much as it is. The most convincing evidence for the square-planar intermediate is based on the fact that the fraction of the [Ni(tpp)] intermediate obtained from the XANES spectrum agrees well with the coordination numbers obtained from the two-distance model in the XAFS analysis. Therefore, the photoinduced intermediate formed during the first 14 ns after the laser pulse is primarily the square-planar [Ni(tpp)] with an average nearest-neighbor distance that is almost identical to the ground-state [Ni(tpp)]. This experiment was the first pump-probe XAFS experiment that used the pulsing ability of the synchrotron X-rays with nanosecond resolution in a solution.

3.2. Metal-to-Ligand Charge-Transfer (MLCT) Excited State of Transition Metal Complexes

The intense colors of metal complexes with π -conjugated ligands originate from $d \rightarrow \pi^*$ transitions that move electrons from the metal to the ligand, which causes a transient change in the oxidation state of the metal (MLCT) and produces both singlet and triplet states. Owing to their photoluminescence and electron-donating capabilities, many such metal complexes are involved in photoinduced electron- or energy-transfer processes, which generally start with the MLCT excited states. Because of the oxidation-state dependence of the XAS transition-edge energy for metal atoms, the pump-probe XAS provides a sensitive and sometimes unique tool for determining transient oxidation-state changes on the metal atom during the MLCT transition. Consequently, atomic displacements due to the change in oxidation state of the metal could be detected by the pump-probe XAFS technique.

3.2.1. The MLCT State Structure of $[\text{Ru}^{\text{II}}(\text{bpy})_3]^{2+}$

As a metal complex that has a high yield of luminescence at room temperature, a long excited-state lifetime, and the ability to perform photoinduced energy and electron transfer, ruthenium(II) tris-2,2'-bipyridine, $[\text{Ru}^{\text{II}}(\text{bpy})_3]^{2+}$, has attracted a lot of attention for use in solar-energy conversion and storage,^[110–113] and photocatalysis.^[114–117] $[\text{Ru}^{\text{II}}(\text{bpy})_3]^{2+}$ and its derivatives are among the most extensively studied metal complexes. The photoexcitation of $[\text{Ru}^{\text{II}}(\text{bpy})_3]^{2+}$ results in the formation of a FC singlet excited state, $^1(\text{MLCT})$, which undergoes subpicosecond intersystem crossing and vibrational relaxation to a long-lived triplet excited state, $^3(\text{MLCT})$,^[118] with near-unity quantum yield. During this process, the charge transferred from Ru^{II} center to the ligands is initially delocalized among three bpy ligands and then quickly localized to one of the ligands to form $[\text{Ru}^{\text{III}}(\text{bpy})_2(\text{bpy}^-)]^{2+}$ in the $^3(\text{MLCT})$ state.^[119] An XANES study conducted at the Advanced Light Source in the Lawrence Berkeley Laboratory investigated the transient oxidation state of Ru at the long-lived $^3(\text{MLCT})$ state with a lifetime of 300 ns at Ru L_{III} and L_{II} edges by using single X-ray pulses.^[80] The ground states of Ru^{II} and Ru^{III} complexes exhibit pronounced differences in their L -edge XANES^[120] around the Ru L_{III} edge (Figure 11), because $2p \rightarrow 4d/5s$ transitions attributed to the edge are dipole allowed. An XANES band at 2.841 keV characterizes the bivalent compounds due to the $2p_{3/2} \rightarrow 4d_{3/2}$ (e_g) transition,^[120] and another weaker band at 2.851 keV due to the $2p_{3/2} \rightarrow 5s_{1/2}$ transition. The vacancy in the $4d_{3/2}$ (t_{2g}) orbital in $[\text{Ru}^{\text{III}}(\text{NH}_3)_6]^{3+}$ species prompts an additional absorption due to the allowed $2p_{3/2} \rightarrow 4d_{5/2}$ (t_{2g} ; A' band) in addition to an oxidation-state-induced shift.^[120] Thus, if the MLCT takes place after the photoexcitation, which removes an electron from Ru^{II} to the ligand to yield an effective Ru^{III} , the A'/B' doublet and a transition-edge-energy shift occurs, as seen in the spectrum of $[\text{Ru}^{\text{III}}(\text{NH}_3)_6]^{3+}$.

Electron bunches with a special fill pattern were in operation with several bunches spaced 2 ns apart to generate an approximately 550 ns long “superbunch”, followed by an

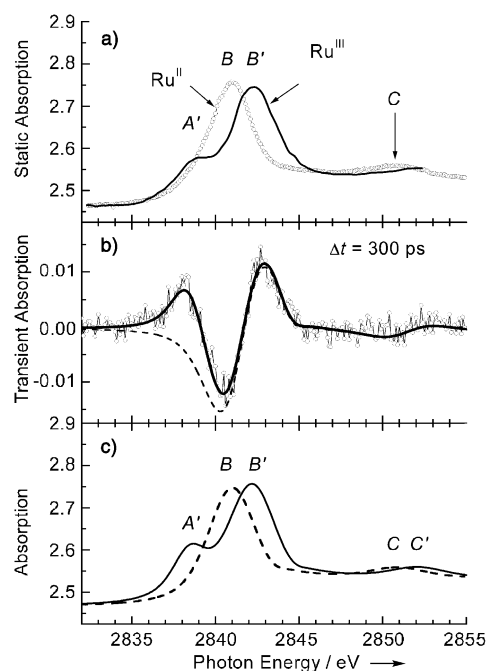


Figure 11. a) Static XANES spectra at the Ru L_{III} edge of aqueous $[\text{Ru}^{\text{II}}(\text{bpy})_3]^{2+}$ (open circles), and of $[\text{Ru}^{\text{III}}(\text{NH}_3)_6]\text{Cl}_2$.^[120] b) Transient XANES spectrum of photoexcited aqueous $[\text{Ru}^{\text{II}}(\text{bpy})_3]^{2+}$ ions (open circles) recorded 300 ps after the laser-pump pulse. The dashed curve is a fit accounting for the blueshift of the static XANES due to the photo-induced increase of oxidation state, and the solid fit curve includes the appearance of the A' band for the reaction intermediate. c) Absorption spectrum of the Ru^{II} reactant state (dashed) fitted from the experimental data in (a), and intermediate compound absorption spectrum (solid curve) constructed from the reactant state and the transient absorption fit (solid curve in (b)). The labeled bands A' , B' , C' , denote the allowed transitions of Ru^{III} and B , C of Ru^{II} centers (from reference [80]).

approximately 100 ns dark section, in which one single electron bunch (the “camshaft” pulse) was placed. The timing structure of the X-ray pulses derived from this fill pattern is similar to that of the Advanced Photon Source, but the timing window for the single X-ray probe pulse is about 100 ns instead of about 3000 ns, because the storage ring of the Advanced Light Source is only approximately one sixth of the Advanced Photon Source. In their XANES measurements, the transmission detection was used with a suitably fast (and sensitive) avalanche photodiode detector (APD) that had nanosecond response and recovery time. The amplified femtosecond laser was synchronized to the X-ray probe pulse that originated from the “camshaft” electron bunch at 1 kHz repetition rate and at a fixed time delay. The single X-ray pulses were recorded at twice the laser repetition rate (i.e., at 2 kHz), with and without the laser pulse illumination. Therefore, the transient XAS in transmission mode could be obtained after subtraction of adjacent X-ray pulse intensities. This detection scheme improved sensitivity to nearly the shot-noise limit of the X-ray source.^[121] In this study, 80 mm aqueous solution jet of $[\text{Ru}^{\text{II}}(\text{bpy})_3]^{2+}$ was used. The X-ray and the laser beams were both focused to 0.3 mm diameter on to the solution jet that had a thickness of 0.1 mm. The pump-laser pulses (0.3 mJ, 400 nm) were from the frequency-

doubled, amplified Ti-Sapphire femtosecond laser system. This example demonstrated the importance of tightly focused laser and X-ray beams at a thin sample. If the diameter of the beam size was 1 mm and the thickness of the solution jet was 0.5 mm, as in the earlier example, then the excited state population would have been only about 2%.

Figure 11 shows the difference XANES spectrum recorded at 300 ps after the laser excitation (open circles). This transient spectrum $T(E, t)$ can be expressed through Equation (6):

$$T(E, t) = f(t) (P(E, t) - R(E)) \quad (6)$$

$R(E)$ is the ground state absorption spectrum, $P(E, t)$ is the $^3(\text{MLCT})$ state absorption spectrum at the time t after photoexcitation. $f(t)$ is the fraction of the excited state at time t . To determine both $f(t)$ and $P(E, t)$, Saes and co-workers first fit the experimental data to the transient spectrum at L_{II} edge at which distinct spectral features corresponding to the $2p_{1/2} \rightarrow 4d_{3/2}$ (e_g) transition for Ru^{II} and Ru^{III} at different energies were observed. By adjusting the shift as well as the $f(t)$, they were able to fit the experimental data and obtained an energy shift of 1.2 eV for the spectrum of the $^3(\text{MLCT})$ state relative to the ground state 300 ps after the pulse of the pump laser, and 9% for $f(300 \text{ ps})$.^[80] In addition, the spectral shift obtained from the data of the Ru L_{II} edge was used in the reconstruction of the L_{III} edge. By varying $f(300 \text{ ps})$, they obtained a fit with the experimental data shown in Figure 11 c. The overall fit gave an edge energy shift of about 1.2 eV and $f(300 \text{ ps}) = 11\%$, which is very close to the results at the L_{II} edge. The resulting curve (dashed in Figure 11 b) matches the observed transient perfectly, except in the lower energy region. The solid curve in Figure 11 b now describes the measured transient XAS. The product state XANES $P(E, t)$, constructed through Equation (6) with this transient fit curve, is shown in Figure 11 c (solid curve). To date, no XANES exists for the intermediate species $[\text{Ru}^{\text{III}}(\text{bpy}^-)(\text{bpy})_2]^{2+}$ (solid curve in Figure 11 c), but Saes and co-workers^[80] compared the spectrum with the static XANES of the isoelectronic species $[\text{Ru}^{\text{III}}(\text{NH}_3)_6]\text{Cl}_3$ shown in Figure 11 a. Indeed, the spectra are very similar in $A'-B'$ splitting (3.8 eV) and A'/B' relative intensities (ca. 2.5), which confirms the $+3$ oxidation state of the central Ru atom. In addition, the time evolution of the signal near B was also monitored (not shown) in which the time response of the signal matches the X-ray pulse duration, thus indicating that the rate of formation of the MLCT state is faster than the X-ray pulse from the Advanced Light Source can resolve, as seen in femtosecond optical studies,^[118,119] which paves the way for the future application of femtosecond X-rays.

3.2.2. The MLCT State Structures of $\text{Cu}^{\text{I}}(\text{dmp})_2^+$ —the “Exciplex”^[79,122]

The MLCT excited states of cuprous diimine compounds are potential-energy and electron donors,^[123–128] and functional moieties in molecular devices, because of their room temperature luminescence and structural reorganization upon $\text{Cu}^{\text{I}}-\text{Cu}^{\text{II}}$ interconversion triggered electrochemically

or photochemically.^[129,130] Among these compounds, $[\text{Cu}^{\text{I}}(\text{dmp})_2]^+$ ($\text{dmp} = 2,9\text{-dimethyl-1,10-phenanthroline}$) is one of the most extensively studied, in which the photo-induced MLCT transition can be shown as, $[\text{Cu}^{\text{I}}(\text{dmp})_2]^+ + h\nu \rightarrow [\text{Cu}^{\text{II}}(\text{dmp}^-)(\text{dmp})]^{+*}$ (Figure 12 a).^[123–128] The ground

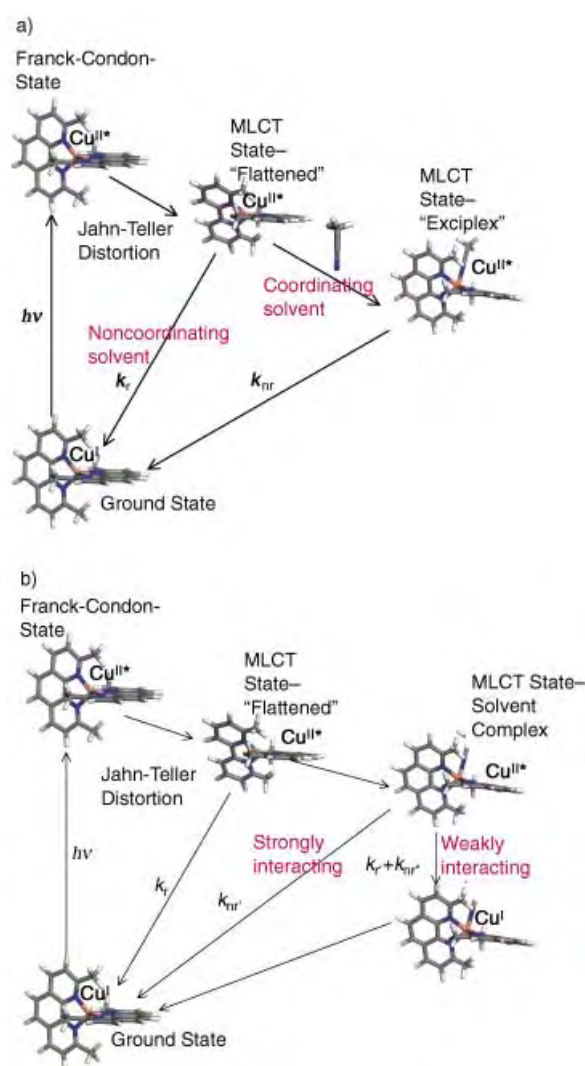
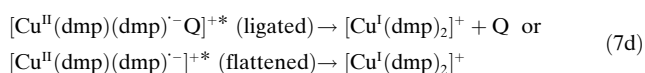
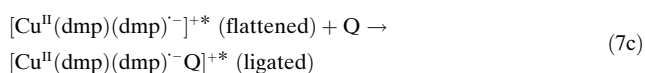
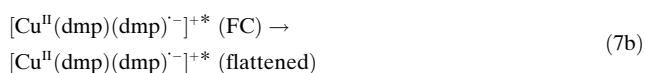
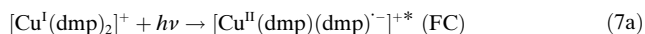


Figure 12. a) Previously accepted mechanism in literature for photoinduced MLCT excitation of $[\text{Cu}(\text{dmp})_2]^+$ in solution,^[123–128] and b) the reaction mechanism modified by the results from the XAFS study.

state $[\text{Cu}^{\text{I}}(\text{dmp})_2]^+$ has a pseudotetrahedral coordination geometry, with two dmp planes almost orthogonal to each other.^[131] Absorption of a visible-light photon promotes an electron from Cu^{I} to one of the dmp ligands, which results in intramolecular charge separation in a FC MLCT state with a $\text{Cu}^{\text{II}*}$ center coordinated to one reduced and one neutral dmp ligand. The initial FC MLCT state has the identical pseudotetrahedral coordination geometry as the ground state, but the $\text{Cu}^{\text{II}*}$ center, with a d^9 electronic configuration, is susceptible to Jahn–Teller distortion resulting in a MLCT state with flattened tetrahedral coordination (Figure 12 a). The property of this MLCT state was strongly solvent dependent. In noncoordinating solvents, the MLCT state is

luminescent at room temperature and has lifetimes ranging from several tens to over 100 ns. In contrast, the MLCT state is almost nonluminescent and has lifetimes as short as a few nanoseconds at room temperature in strongly coordinating Lewis base solvents. The large “Stokes-like” shift observed between the absorption and emission is consistent with significant structural changes in the MLCT state that either returns to the ground state through a radiative decay pathway in noncoordinating solvents, or forms a pentacoordinate complex with strong Lewis basic solvents, resulting in the exciplex quenching.^[132–142] Based on previous studies, four events could take place after the photoexcitation: [Eq. (7a–d)]



Equation (7a): intramolecular charge separation and intersystem crossing to yield an excited state that is formally $\text{Cu}^{\text{II}*}$ in the ground state geometry, the FC MLCT state; Equation (7b): Jahn–Teller distortion to produce a flattened tetrahedral geometry, the flattened MLCT state; Equation (7c): the coordination of a solvent molecule to yield a five-coordinate compound, the ligated MLCT state; Equation (7d): the flattened or ligated MLCT state decays to the ground state. Q is either a solvent molecule or a counterion that binds the $\text{Cu}^{\text{II}*}$.^[133, 134, 139, 141, 143]

Despite extensive studies on $[\text{Cu}^{\text{I}}(\text{dmp})_2]^+$ in various solvents, dynamics for the initial charge-transfer and structural rearrangements as well as the MLCT state structures of $[\text{Cu}^{\text{I}}(\text{dmp})_2]^+$ have not been fully explored until recently. In a recent study, ultrafast MLCT state dynamics were investigated by femtosecond pump–probe optical transient spectroscopy, and the MLCT state structure was studied by subnanosecond and nanosecond pump–probe XAFS, in acetonitrile and in toluene.^[79, 122]

The femtosecond optical pump–probe transient spectra were obtained in acetonitrile (Figure 13b) and in toluene (Figure 13c). These spectra display ground-state bleaching (GB) below 480 nm and excited-state absorption (EA) above 500 nm as a function of time after the photoexcitation. In the same EA spectral region, the well-documented double-peak band, assigned to absorption by $\text{dmp}^{\cdot-}$ ions,^[139, 144] developed about 10–20 ps later as a result of a blue shift and spectral narrowing from the red side of the absorption peak from 570–600 nm. This change was followed by the decay of the entire absorption band with time constants of 1.7 ns for acetonitrile and 100 ns for toluene solutions. Assuming first-order processes, the kinetics at different probe wavelengths can be described by triexponential functions, $f(t) = \sum A_i \exp(-t/\tau_i)$, ($i=1-3$), with time constants $\tau = 0.2-0.8$ ps,

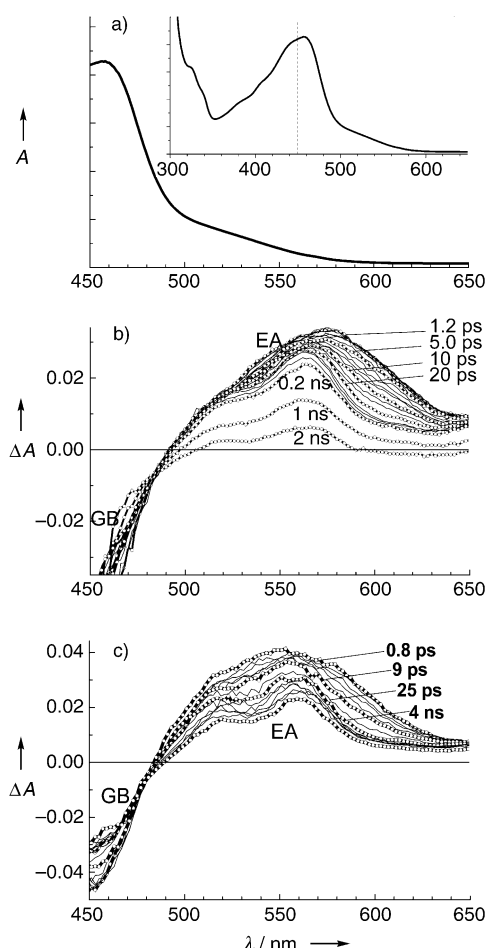


Figure 13. a) The ground state UV/Vis absorption spectrum of $[\text{Cu}(\text{dmp})_2]^+$ in acetonitrile; b) femtosecond transient absorption spectra of $[\text{Cu}(\text{dmp})_2]^+$ in acetonitrile; and c) in toluene.

10–20 ps and 1500–1700 ps for acetonitrile solution. In toluene solution, the first two high time constants are similar to those in acetonitrile, but the third time constant is 100 000 ps.

A recent study on dynamics of the MLCT state of $[\text{Ru}^{\text{II}}(\text{bpy})_3]^{2+}$, which has a similar MLCT transition and ground-state spectrum, supports the hypothesis that the initial subpicosecond time constant in the MLCT dynamics of $[\text{Cu}^{\text{I}}(\text{dmp})_2]^+$ is due to the formation of the MLCT triplet state.^[118, 119] Naturally, the flattening of the tetrahedral coordination geometry [Equation (7b)] and binding the fifth ligand [Equation (7c)] would occur on a slower time scale than the initial formation of the triplet MLCT state, and may have the 10–20 ps time constant. The flattening motion was also shown by a time-dependent density function theory (TD-DFT) calculation.^[122] More interestingly, the calculation confirmed that the energy differences between the lowest and upper triplet levels that correspond to the 500–600 nm region for the EA followed a trend with the smaller dihedral angle associated with the larger transition energy, which agrees with the blue shift in EA spectra observed within 10–20 ps of excitation. The pump–probe optical transient absorption study demonstrated that only the thermally

equilibrated MLCT state after the reactions of Equation (7b) and (7c) could be investigated by X-ray pulses from a synchrotron source with 100 ps resolution.

Differences between XANES spectra with and without the laser for $[\text{Cu}^{\text{I}}(\text{dmp})_2]\text{PF}_6$ in acetonitrile and $[\text{Cu}^{\text{I}}(\text{dmp})_2]\text{-BARF}$ (BARF, tetrakis(3,5-bis(trifluoromethyl)phenyl)borate) in toluene are clearly visible in Figure 14a. The intensity of

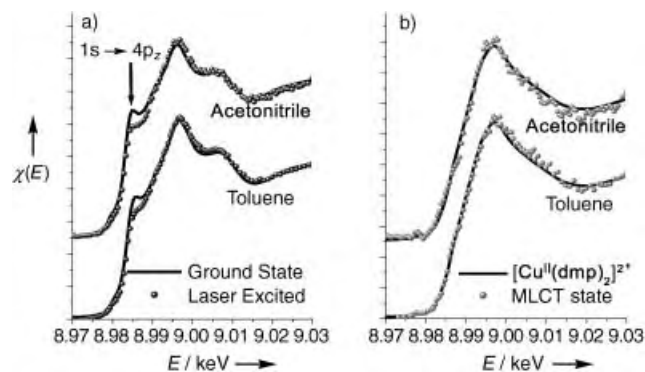


Figure 14. a) XANES spectra of $[\text{Cu}^{\text{I}}(\text{dmp})_2]^+$ in solutions with and without laser illumination. b) XANES spectra of the MLCT state in solutions generated by subtraction of the ground-state contributions to the spectra of the laser illuminated sample followed by normalization to 100% MLCT state (see text). $\chi(E)$ is the interference function defined as $[\mu(E) - \mu_0(E)]/\Delta\mu_0(E)$, $\mu(E)$ and $\mu_0(E)$ are absorption coefficients of $[\text{Cu}^{\text{I}}(\text{dmp})_2]^+$ and isolated copper atoms, respectively.

the shoulder at 8.985 keV was reduced upon laser excitation, accompanied by a slight increase of the peak intensity at 8.996 keV. To extract the spectrum for the MLCT state in each case, the XANES spectrum from the remaining ground state $[\text{Cu}^{\text{I}}(\text{dmp})_2]^+$ had to be subtracted from the spectrum of the laser excited sample in which both states were present. (Figure 14b) According to calculations based on the Beer–Lambert law for the optical absorption, about 20% of the ground state $[\text{Cu}^{\text{I}}(\text{dmp})_2]^+$ is converted to the MLCT state. In both solvents, the shoulder for ground-state $[\text{Cu}^{\text{I}}(\text{dmp})_2]^+$ is missing in the MLCT state, and the transition edge is shifted by about 3 eV to the same edge energy as that of the ground state $[\text{Cu}^{\text{II}}(\text{dmp})_2]^{2+}$ obtained by in situ electrolysis of $[\text{Cu}^{\text{I}}(\text{dmp})_2]^+$ in acetonitrile. The shoulder at 8.985 keV is due to the $1s \rightarrow 4p_z$ transition that is pronounced in $[\text{Cu}^{\text{I}}(\text{dmp})_2]^+$ and missing from $[\text{Cu}^{\text{II}}(\text{dmp})_2]^{2+}$. This is a typical observation for transformation from four-coordinate to five- or six-coordinate geometry, as the $4p_z$ orbital is localized in the former, giving a sharp band, but delocalized in the latter, giving a broad, smooth band.^[106,145]

The copper coordination number and the Cu-to-ligand bond lengths for the MLCT state of $[\text{Cu}^{\text{I}}(\text{dmp})_2]^+$ were extracted from the XAFS data. Figure 15 displays XAFS and Fourier-transformed XAFS spectra for ground-state and laser-excited $[\text{Cu}^{\text{I}}(\text{dmp})_2]^+$. The Cu–N peaks in Figure 15b for the laser excited $[\text{Cu}^{\text{I}}(\text{dmp})_2]^+$ are higher in both solvents, which suggests an increase in the coordination number of copper in the thermally equilibrated MLCT state. Interestingly, the Cu–N peak shifts to a shorter distance in acetonitrile, but to a longer distance in toluene, compared to the

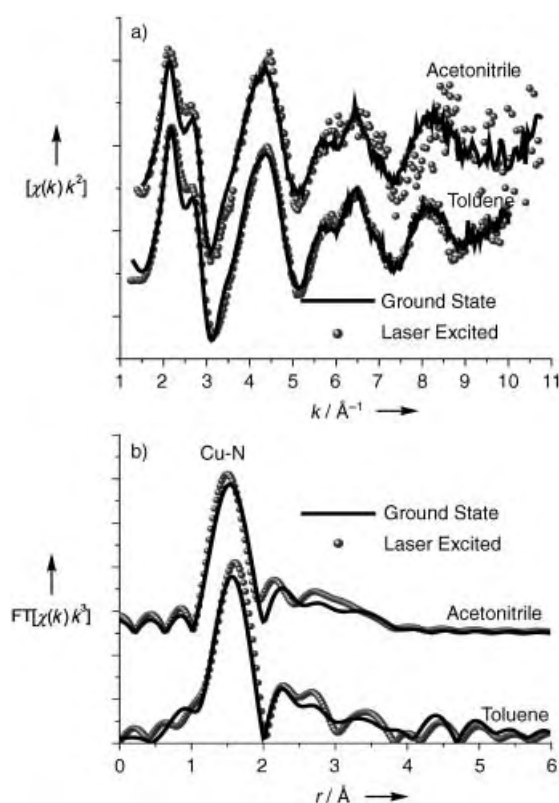


Figure 15. a) XAFS spectra of ground-state and laser-illuminated $[\text{Cu}^{\text{I}}(\text{dmp})_2]^+$ ions in solutions. b) Fourier-transformed XAFS spectra of (a). Each peak represents one average distance between the copper atom and its neighbors. R is the distance, in Å, from the central copper atom. The peak corresponding to the nearest neighbors is labeled. The shift of the peak and the amplitude change reflects structural changes. The spectra are not corrected with phase factors, so the peaks appear at distances smaller than the real distances.

average Cu–N distance of 2.07 Å for the ground state. The average Cu–N distance for the laser-excited sample in acetonitrile fit to two distances of 2.07 and 2.03 Å, whereas that in toluene fit to two distances of 2.06 and 2.11 Å while the relative ratio of the two distances is kept at 4:1. Clearly, the first Cu–N distance is from the ground state and the second is from the MLCT state. The results of the experiment demonstrated: 1) light excitation generated a formally $\text{Cu}^{\text{II}*}$ center; 2) the inner-sphere reorganization changed the coordination number of the MLCT state from four to five in toluene, which is presumed to be noncoordinating; 3) the average Cu–ligand bond lengths lengthened in the MLCT state in toluene, but shortened in acetonitrile.

The XANES spectra provided strong evidence for the pentacoordinate $\text{Cu}^{\text{II}*}$ center in both acetonitrile and in toluene. These results are in accordance with the fact that $\text{Cu}^{\text{II}*}$ in the MLCT excited state is formally a 17-electron d^9 system prone to the addition of a fifth ligand.^[132] It is well-known that MLCT excited states in general^[146,147] follow the Jortner energy-gap law that the nonradiative rate constant increases exponentially with decreasing energy separation between the ground state and the excited state.^[140,148] The formation of the exciplex stabilizes the MLCT state, thus

reducing the energy gap between the ground state and the MLCT state. As the energy gap becomes small enough owing to the strong interaction of the Cu center with the ligand, the nonradiative decay dominates the relaxation pathway and no emission is detected, as in the case with the acetonitrile solution. However, the above arguments do not explain the solvent dependency of the behavior of the MLCT state.

The XANES results for $[\text{Cu}^{\text{I}}(\text{dmp})_2]^+$ in both toluene and acetonitrile solutions support the formation of a pentacoordinated $\text{Cu}^{\text{II}*}$ center in the MLCT state based on the attenuation of the shoulder in the transition K edge of copper in the spectra. However, a broad range of the Cu-to-ligand bond lengths for the fifth ligand could smooth the transition edge, as seen in previous studies.^[106] The respective elongation and the shortening of the average Cu–N bond lengths for the MLCT state of the $[\text{Cu}^{\text{I}}(\text{dmp})_2]^+$ in toluene and in acetonitrile signal the difference in the interactions of the copper with the fifth ligand. Such variation was also seen by a broad range of the MLCT state lifetimes in various Lewis base solvents with different electron-donating capabilities.^[141] Toluene (or BArF anion) is bulkier and has less electron-donating capability compared to acetonitrile, hence it could only act as the fifth ligand with a longer and less-stable bond with the $\text{Cu}^{\text{II}*}$ center in the MLCT state of $[\text{Cu}^{\text{I}}(\text{dmp})_2]^+$. Consequently, the MLCT state of the $[\text{Cu}^{\text{I}}(\text{dmp})_2]^+$ –toluene complex will not be as stable as the corresponding acetonitrile complex, and will not lower the energy of the MLCT state to such an extent that nonradiative decay becomes the sole path for the MLCT state to return to the ground state. The interaction between the MLCT state of $[\text{Cu}^{\text{I}}(\text{dmp})_2]^+$ with toluene or the BArF anion is so weak that the complex retains room-temperature luminescence and has the rate constant for radiative and nonradiative decays of $k_r = 1.14 \times 10^4 \text{ s}^{-1}$ and $k_{nr} = 1.02 \times 10^7 \text{ s}^{-1}$, compared to $k_{nr} = 6 \times 10^8 \text{ s}^{-1}$ in acetonitrile. Since the fifth ligand coordinates to the $\text{Cu}^{\text{II}*}$ center in the MLCT state of $[\text{Cu}^{\text{I}}(\text{dmp})_2]^+$ in both toluene and acetonitrile and only the latter shows excited-state quenching, the exciplex previously inferred is more appropriate for cases in which strong excited-state adducts completely quench the excited state. The variation of the structure of the MLCT state in different solvents confirmed that the origin of distinctly different behavior of the excited states is due to the formation of MLCT state–solvent complexes with different electronic interactions. When the interaction is strong, the lifetime of the MLCT state is shortened significantly and the luminescence is quenched, which has been characterized by the exciplex formation reported previously.^[123–127] Therefore, the pump–probe XAFS measurements in the time domain provide new insights into the fundamental aspects of structural factors that may influence photoinduced electron and energy-transfer processes (Figure 11 b).

4. Examples of Other Pulsed X-ray Techniques

Other X-ray techniques, such as diffuse scattering and powder diffraction, have been used in laser pulse–pump, X-ray pulse–probe structural measurements for light-generated transient structures in photochemical reactions in disordered

media. Below are examples from the European Synchrotron Radiation Facility (ESRF) in Grenoble.

4.1. Photodissociation of $\text{I}_2^{[149]}$ and $\text{HgI}_2^{[150]}$

The intermediate structures in photodissociation of I_2 and HgI_2 in solution were investigated with laser pump X-ray probe diffuse scattering by using single X-ray pulses from the synchrotron.^[96,149,151,152] These two reactions were studied by ultrafast laser spectroscopy decades ago,^[153,154] but the intermediate structures were only obtained by indirect optical spectroscopy without atomic resolution. The potential energy surfaces versus I–I bond length for I_2 in CCl_4 solution are shown in Figure 16.^[96] The ground-state molecule was excited

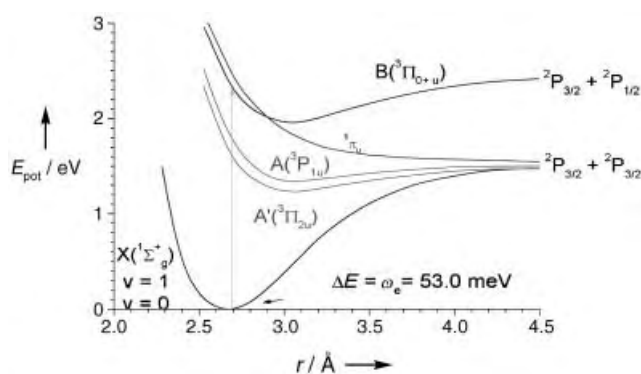


Figure 16. The potential-energy surface for different wavefunctions X, A/A' etc. of I_2 molecule as a function of I–I bond length. The molecule was photodissociated by a 150 fs pulse at 530 nm that excited the molecule to the B state (arrow). (From reference [96]).

vertically to the B state, which is crossed with the potential surface of the $^1\pi_u$ state, by using a laser pulse at 530 nm. Therefore, photoexcited I_2 in CCl_4 could either thermally equilibrate on the potential surface of the B state, which has a longer I–I bond length than that of the ground state, or completely dissociate on the potential surface of the $^1\pi_u$ state. The FC factor ratio of the two states is 5.18:1 in favor of the B state. At the point of crossing between B and $^1\pi_u$ states, the photoexcited I_2 could either cross to $^1\pi_u$ state and continue to expand I–I bond length until it reaches the liquid boundary at 4.5 Å, or remain on the B state surface after the first passage of curve crossing. After 1–2 ps oscillation at the crossing point, the B state is depleted. The solvent molecules form a cage that surrounds the excited I–I, which prevents it from escaping and forces the highly energetic photodissociation intermediate to recombine diffusively within microseconds.

The recombination intermediates of I_2 in CCl_4 were probed by single X-ray pulses, but with time resolution of 2 ns limited by a nanosecond laser pulse at 530 nm. In this study, the X-rays were delivered by an undulator beamline at 16.45 keV with a 2.37% bandwidth. In other words, the X-rays were not monochromatic, but had a finite spectral range, which increased the photon flux by a factor of 460 in

comparison with a monochromatic beam with a Si(111) crystal monochromator. With 100 s accumulation for each delay time relative to the pulse of the pump laser, the time dependence of the diffuse X-ray scattering signals was collected (Figure 17). The results revealed that 78 % of I_2

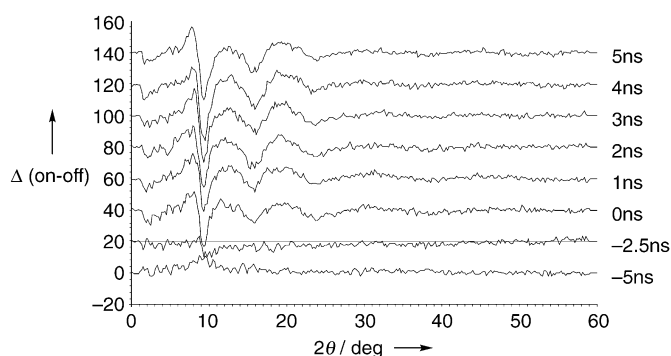


Figure 17. Difference maps of X-ray scattering signals (Δ (laser on—laser off) in analogue–digital units (ADU) per pixel per 100 s) of I_2 as a function of the diffraction angle for X-ray probe time delays between -5 ns to 5 ns relative to the laser pump. The exposure time was 100 s and the integral flux: 3×10^{11} photons (From reference [96]).

recombined into the A/A' state with a bond length of $3.14 \text{ \AA}$ while 22 % of the molecules escaped from the solvent cage irreversibly.^[96,149,152] This experiment demonstrates the feasibility of using pump–probe diffuse scattering in the determination of photoexcited intermediate structures with a time resolution limited by the duration of the single X-ray pulse.

At the same facility, the other time-resolved diffuse-scattering measurement was carried out on the photodissociation of HgI_2 .^[96,150] The molecule was to be fragmented upon laser excitation to HgI and I species. Similar to photodissociated $I-I$, photodissociated HgI undergoes gemination recombination with an I atom on a microsecond time scale owing to the diffusion-limited process. Geis et al. observed a qualitative difference between partial and total photodissociation ($\rightarrow Hg + I + I$).^[150] The most important advantages of the diffuse-scattering measurements are that it covers a broader range of atomic distances than XAFS, and its universal measurements of the pair distribution functions (PDF), so it is not limited to measuring interatomic distances only to X-ray-absorbing heavy atoms in the molecule. This technique has great potential in becoming one of the most-useful structural tools in determining excited-state molecular structures, solvent reorganization in photoinduced electron-transfer and structural rearrangements in supermolecules, dendrimers, and complexed molecular aggregates.

4.2. Photoinduced Structural Change in Organic Solids^[155]

The most accurate and complete structural information is obtained from X-ray diffraction of a single crystal or powder. The time-resolved diffraction by using X-ray pulses from synchrotron and table-top terawatt (T^3) laser sources has been carried out on solids in laser-induced melting and lattice

distortion^[156–167] and on proteins during light-triggered photochemical reactions.^[89,168–170] A pioneering investigation by Techert et al. on *N,N*-dimethylaminobenzonitrile (DMABN) by the time-resolved powder-diffraction technique revealed changes on the molecular structure arising from crystal relaxation after photoexcitation (Figure 18).^[155] The inversion motion of CH_3 around N and torsional motion of CH_3 are likely to be distinguished by using time-resolved powder diffraction.

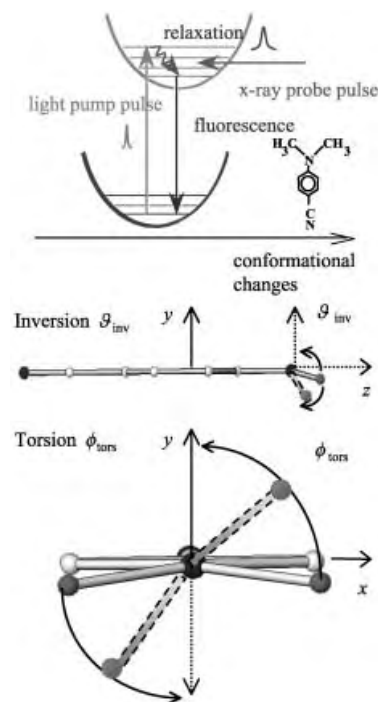


Figure 18. Top: principle of time-resolved X-ray diffraction on the excited-state potential-energy surface of DMABN crystals. Bottom: the intramolecular degrees of freedom, which contribute to the relaxation process (inversion θ_{inv} and torsion ϕ_{tors}). C atoms of the phenyl moiety are open, N atoms are black, and the amino C atoms are grey. H atoms are not shown, as they do not contribute to the X-ray diffraction signal. (From reference [155]).

Purified, recrystallized, and crushed samples were pressed into $30 \mu\text{m}$ thick gaskets and these samples were optically transparent. After excitation of the sample at 337 nm , the resulting fluorescence was biexponential with lifetime constants of 1.71 and 0.53 ns . The powder-diffraction data were collected at the same beamline in ESRF, mentioned in Section 4.1, at 16.5 keV . The monochromatic X-rays were selected by an Si(111) crystal and had a flux of $8 \times 10^7 \text{ photons s}^{-1}$ at the sample after the pulses had been selected with an X-ray chopper (Figure 19).^[171] Figure 20 depicts the results from this study in terms of difference spectrum of the powder diffraction between 80 ps after the photoexcitation and the ground state. The results showed that after absorbing an optical photon, DMABN molecules in the powder were excited to the repulsive part of the potential-energy surface of the excited state. These molecules reorganize quickly by

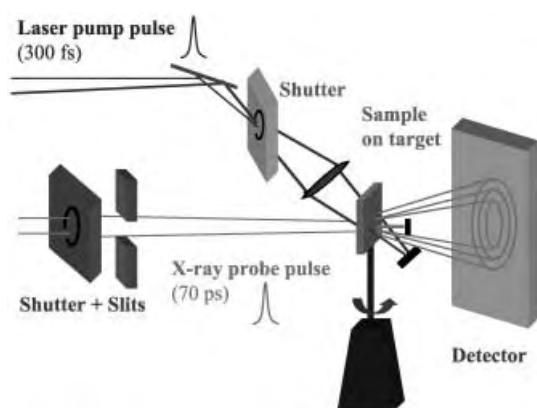


Figure 19. Scheme of the time-resolved X-ray-diffraction setup at ID09b following a pump-probe. (From reference [171]).

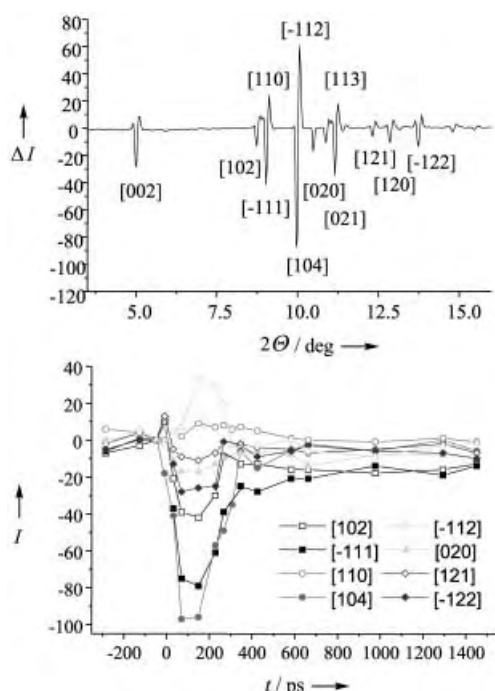


Figure 20. Top: intensity-difference map of the powder pattern at $t = 80$ ps and -240 ps. Bottom: change of the integrated Bragg intensities as a function of time. The intensity change ranges between 1–10% of the total signal. Selected Bragg reflections are marked by their Miller indices (From reference [155]).

changing the inversion angle (θ_{inv} in Figure 18) from 13° to 3° , thus bringing the molecule to a quasiplanar configuration. Meanwhile, the CH_3 groups are rotated out of plane of the benzyl group. The structure after photoexcitation resembles the low-temperature structure, indicating that the DMABN was transiently “cooled down” as the result of relaxation from the excited state. The success of this experiment opened a series of investigations on similar types of systems for structural information in photochemical processes, such as the topochemical reaction of *p*-formyl-*trans*-cinnamic acid crystals,^[171] and light-induced ferroelectric structural order in an organic charge-transfer crystal.^[172] These studies revealed changes on molecular structure and crystal packing as well as

the kinetics associated with different steps of the reaction. In contrast to previously mentioned photochemical reactions, these photochemical solid-state processes in *p*-formyl-*trans*-cinnamic acid crystals were irreversible starting from light-induced phonon motion that led to a pairlike metastable state in 70 ps, which resulted in the formation of a dimer. Because of severe lattice deformation, the crystal transformed into smaller domains and then reached a complete amorphisation in 30 min.^[171] Photoinduced phase transition in tetrathiafulvalene-*p*-chloranil solid, a charge-transfer molecular material, was studied recently by using time-resolved powder diffraction with single X-ray pulses as the probe from the ESRF.^[172] A 300 femtosecond laser pulse triggers the material from a neutral to an ionic state within 500 ps, and generates self-organized long-range order with different molecular orientations from the ground-state neutral species. The time-resolved X-ray diffraction confirmed that the change at the molecular level is the origin for such a phase transition.^[172]

5. Summary and Outlook

The initial success of the laser pump, X-ray probe time-resolved XAS by using single X-ray pulses from the third-generation synchrotron sources has begun to produce results that address the structural origins of the photochemical properties of the photoexcited-state molecules. While this technique has great potential in many studies of excited-state structure, it faces further challenges in terms of improving limited time resolution. For example, the initial flattening of the ligands around Cu^{II} , the excited state structural change of [Ni(tp)] during photodissociation, and the change of the interatomic distance in excited HgI_2 and I_2 are all on much faster time scales than currently available synchrotron pulses can provide. Therefore, application of laser pump, X-ray probe time-resolved X-ray techniques on an ultrafast time scale to the excited state or reaction intermediate structures determination is still in its infancy. Significant progress and breakthroughs are predicted in the near future as two types of ultrafast X-ray sources, based on accelerators^[155,165,173–183] and T^3 laser systems,^[43,46,48,160,166,184–190] become increasingly available. Since many photochemical processes have been investigated extensively by ultrafast laser spectroscopy and are well-documented, we expect prolific scientific results with structural information on the ultrafast time scale to be obtained by using ultrafast X-ray sources. Much insight into the fundamental photochemistry should be generated from these studies. This technical evolution is analogous to that of lasers from nanosecond to femtosecond, and attosecond. As shown in Figure 1, the X-ray sources with different pulse widths can be applied to study structures present in photochemical reactions on different time scales, as seen today by the coexistence of ns and femtosecond lasers for different applications.

On the forefront of the accelerator based X-ray sources, femtosecond pulse slicing from a 100 ps synchrotron X-ray pulse has been realized^[180] at the Advanced Light Source, in which a portion of the electron bunch (< 100 fs) is extracted

by interactions between an electron bunch with a strong electromagnetic field generated by a femtosecond laser pulse. X-ray pulse-narrowing techniques based on acoustic and optical phonon is being implemented at the Advanced Photon Source, respectively.^[191] The on-going construction of the Sub-Picosecond Photon Source (SPPS) and its foreseeable evolution into the Linear Coherent Light Source (LCLS) at Stanford has generated international interest in ultrafast structural studies.^[179, 192–194] On the other forefront of T³ based X-ray sources, several outstanding sources have now become operational for the XAS measurements.^[46, 47, 184, 195–199] The scientific foresight, as well as the pioneering work of the late Professor Kent Wilson and his group, has made great contributions in the ultrafast X-ray field.^[49, 78, 200–206] Application of these ultrashort X-ray pulses in photochemistry have been carried out on some preliminary work on the photodissociation of iron pentacarbonyl in solution,^[47] on the photoionization of I[−],^[121] and on photodissociation of Br₂ by soft X-ray photoelectron spectroscopy.^[53] Hence, taking snapshots and movies during the course of photoinduced bond breaking and formation will become a reality in the foreseeable future.

Parallel to experimental investigation, several theoretical investigations have also been extended to the ultrafast X-ray regime.^[78, 152, 204, 207] So far, the X-ray signals for the excited-state molecules at each probe delay time have been considered to originate from a “static” structure, meaning time dependence of the signals is assumed due to the change of the population (with exception to proteins). Such a scenario is no longer true now that the time scale of the experiments is on femtosecond regime, as described in recent works. In addition, modeling of XANES and XAFS spectra with fundamental theory in solid-state physics has made tremendous advances through the work from the FEFF project.^[208–212] Such modeling will be extended to molecules and to more precise inclusion of the potentials of the central atoms from quantum-mechanical calculations.^[213–215] Furthermore, as femtosecond X-ray pulses become intense enough, nonlinear X-ray phenomena may be observed, which may result in the expansion of the entire field of nonlinear optics into an unexplored territory of the X-ray regime.^[207, 216–219] Therefore, great impacts are expected in the future in ultrafast X-ray research.

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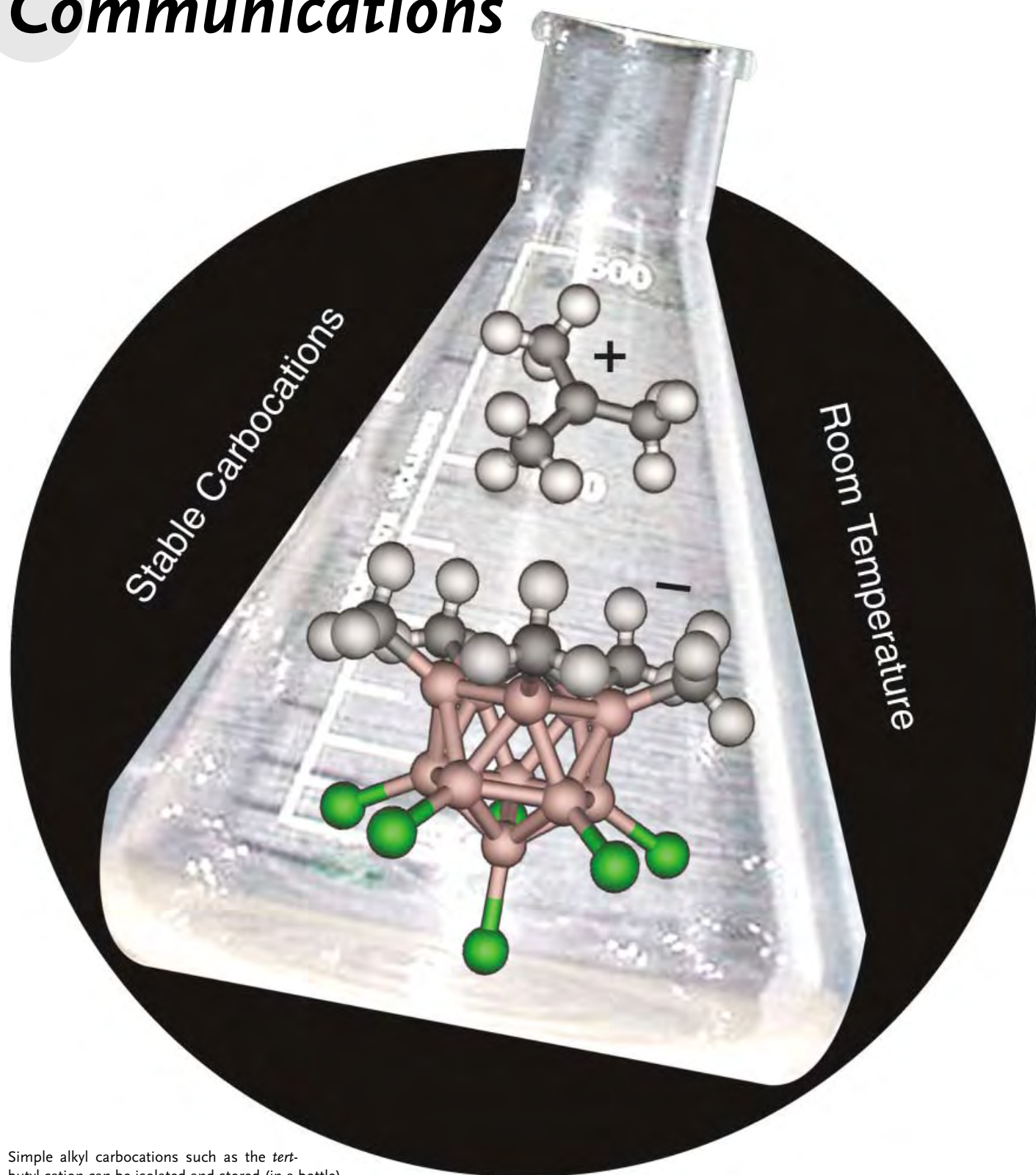
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Communications



Simple alkyl carbocations such as the *tert*-butyl cation can be isolated and stored (in a bottle) as stable salts at room temperature when partnered with inert carborane anions. The “Me⁺” reagents CH₃(CHB₁₀H₅X₆), X = Cl or Br, are sufficiently powerful electrophiles that they can abstract hydride from hydrocarbons at or below room temperature. For more information, see the Communication by C. A. Reed and T. Kato on the following pages.

Carbocations

Putting *tert*-Butyl Cation in a Bottle**

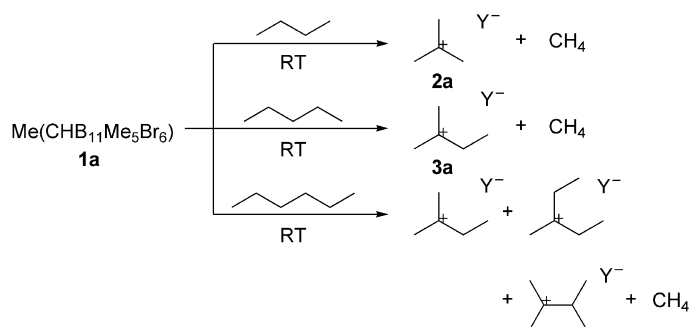
Tsuyoshi Kato and Christopher A. Reed*

The *tert*-butyl cation has a special place in the history of organic chemistry. Tracking its existence from a reactive intermediate postulated on the basis of kinetic measurements to its isolation and characterization at low temperatures in superacidic media is a Nobel Prize winning story.^[1] The hexafluoroantimonate salt has been isolated at low temperatures,^[2] and solutions containing the *tert*-butyl cation in magic acid (HFSO₃/SbF₅) are reportedly stable up to 150 °C.^[3] In 1993 a crystal of [(CH₃)₃C][Sb₂F₁₁] suitable for X-ray crystal-structure determination was isolated at –30 °C from an amyl precursor.^[4] A secondary alkyl cation stabilized by Sn hyperconjugation,^[5] and a vinyl cation stabilized by Si hyperconjugation,^[6] have recently been isolated and structurally characterized. The bridgehead adamantyl cation is stable at room temperature as a fluoroantimonate salt.^[2] We now report that *tert*-butyl and other simple tertiary alkyl cations can be isolated at room temperature and their X-ray structures determined.

The external conditions necessary to stabilize alkyl cations are: a) a counterion that is too weakly nucleophilic to form a covalent bond with the cationic carbon atom, and b) high acidity to prevent deprotonation of the carbocation to form an alkene, leading to subsequent oligomerization of the cation. Since icosahedral carborane anions of the type [CHB₁₁R₅X₆][–] (R = H, CH₃; X = halide) have been shown to be the least-coordinating anions towards silylium ions R₃Si⁺,^[7] they should be excellent candidates for noncoordinating anions with analogous R₃C⁺ carbenium ions. The conjugate acids of carborane anions, H(CHB₁₁H₅X₆), are stronger than the strongest oxy superacids (such as HFSO₃, CF₃SO₃H),^[8,9] which suggests that carborane anions might be sufficiently weak bases not to deprotonate carbocations. The third consideration is a suitable synthetic route.

The synthetic route of choice is hydride abstraction from a hydrocarbon since highly electrophilic “Me⁺” reagents are known to produce carbocations by methane elimination.^[3] In their reactivity towards benzene, methyl carboranes have been shown to be much stronger methylating reagents than methyl triflate.^[10] Indeed, they are such strong methyl⁺

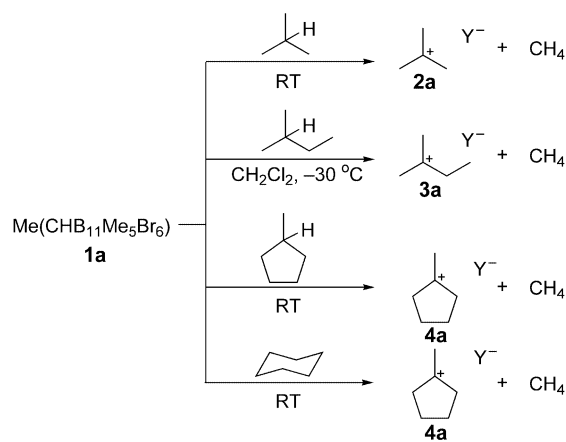
sources that they can abstract a hydride from hydrocarbons at or below room temperature (Scheme 1).



Scheme 1. Reactivity of **1a** with linear alkanes (Y = CHB₁₁Me₅Br₆).

The reactions were carried out by stirring solid CH₃(CHB₁₁H₅Br₆), **1a**, in liquefied alkane at room temperature for a few days. As expected, linear hydrocarbons led to branched-chain products reflecting the high thermodynamic stability of tertiary carbocationic centers over primary or secondary, and the facile hydrogen or methyl 1,2 shifts that occur readily in carbenium ions.^[11] The colorless microcrystalline [R₃C][carborane] products are stable indefinitely if kept dry and free of nucleophiles. The success of the synthesis probably derives from the very low solubility of the salts in the hydrocarbon solvent. With *n*-heptane as reactant and solvent, a material is obtained that has broad ¹H NMR resonances in the alkane region and no ¹³C resonances in the carbocationic region. This suggests the formation of an oligomerized product. We surmise that heptyl cations are sufficiently soluble in heptane during the reaction that deprotonation to heptenes can occur, followed by intermolecular reaction of these heptenes with heptyl cations.

Reactions with branched-chain alkanes or cyclic alkanes also give the expected tertiary carbocation products **2a–4a** (Scheme 2). When a typical laboratory mixture of hexanes is used, the methycyclopentyl cation **4a** is exclusively produced, which suggests that the methycyclopentane isomer of hexane undergoes hydride abstraction much faster than the



Scheme 2. Reactivity of **1a** with tertiary and cyclic alkanes.

[*] Dr. T. Kato, Prof. Dr. C. A. Reed
Department of Chemistry
University of California
Riverside, CA 92521-0403 (USA)
Fax: (+1) 909-787-2027
E-mail: chris.reed@ucr.edu

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

other isomers. As indicated in Scheme 2, some reactions were carried out in CH_2Cl_2 at -30°C to grow crystals suitable for X-ray crystallography.

Methane was identified as the by-product of these reactions by condensing the head gas from the reaction mixture into an NMR tube and observing in the corresponding spectra the appearance of the ^1H signal at $\delta = 0.158$ ppm due to dissolved methane in C_6D_6 .^[12] Proof that the methane originated exclusively from the methyl carborane reagent was obtained from a ^{13}C labeling experiment. When 99% enriched $^{13}\text{CH}_3(\text{CHB}_{11}\text{Me}_5\text{Br}_6)$ was treated with hexanes, the only ^1H signal showing observable and complete ^{13}C coupling was the methane signal ($J = 125.8$ Hz). Not incidentally, this experiment also shows that the mechanism of hydride abstraction does not involve a five-coordinate carbonium ion since such cation formation would allow scrambling of methyl groups. A μ -hydridobridged cation may be involved.^[13]

The carbocations were characterized by ^1H and ^{13}C NMR spectroscopy^[14] by dissolution of the crystalline products in liquid SO_2 at -60°C . The products of Scheme 1 showed clean and quantitative formation of **2a** and **3a**. In the case of hexane, a very similar spectrum to the one previously reported by Olah and Lukas^[3] was obtained, indicating formation of an equilibrium mixture of isomers of hexyl cations. The diagnostic downfield carbocation resonances appear in the ^{13}C spectrum at about $\delta = 335$ ppm. Similarly, the cations of Scheme 2 were characterized as single products.

Crystals of the *tert*-butyl- and *tert*-pentyl-cation salts **2a** and **3a** prepared according to Scheme 1 showed unacceptable crystallographic disorder under X-ray examination of their structures. However, single crystals of a *tert*-butyl-cation salt **2b**, without disorder in the cation or anion, could be grown by the reaction of the corresponding hexachloro carborane reagent, $\text{CH}_3(\text{CHB}_{11}\text{Me}_5\text{Cl}_6)$ **1b**,^[10] with *n*-butane in CH_2Cl_2 at -30°C . The same conditions gave good single crystals of **3a** and a manageably twinned crystal of **4a**, both with the hexabromo carborane anion.

Figure 1, Figure 2, and Figure 3 show the crystal structures of $[\text{tBu}][\text{CHB}_{11}\text{Me}_5\text{Cl}_6]\cdot\text{CH}_2\text{Cl}_2$ (**2b**· CH_2Cl_2), $[\text{t-C}_5\text{H}_{11}][\text{CHB}_{11}\text{Me}_5\text{Br}_6]\cdot\text{CH}_2\text{Cl}_2$ (**3a**· CH_2Cl_2), and $[\text{c-MeC}_5\text{H}_8][\text{CHB}_{11}\text{Me}_5\text{Br}_6]$ (**4a**).^[15] As judged by the sum of the C–C⁺–C angles being $360.0 \pm 0.1^\circ$, the carbocationic centers are planar within experimental error, as expected for sp^2 hybridization. The lattice structures are typical of well-defined salts with separated cations and anions. Halogen atoms from the anions do not approach the carbocationic carbon centers closer than van der Waals separation. Indeed, the closest C⁺...X approach is actually shorter for Br than Cl (3.42 vs 3.49 Å) even though Br is the larger atom. This suggests that electrostatic stabilization of the cation through anion approach to the central carbocationic C atom is not as important in carborane salts as in earlier studies with smaller fluoroantimonate anions.^[16] On the other hand, there are many close C–H...X interactions. For example, each methyl group of **2b** has one or more H...Cl distances < 2.85 Å, the shortest being 2.71 Å (with a C–H...Cl angle of 161°). Similarly, all methyl or methylene groups adjacent to carbocationic centers in the structures of **3a** and **4a** show

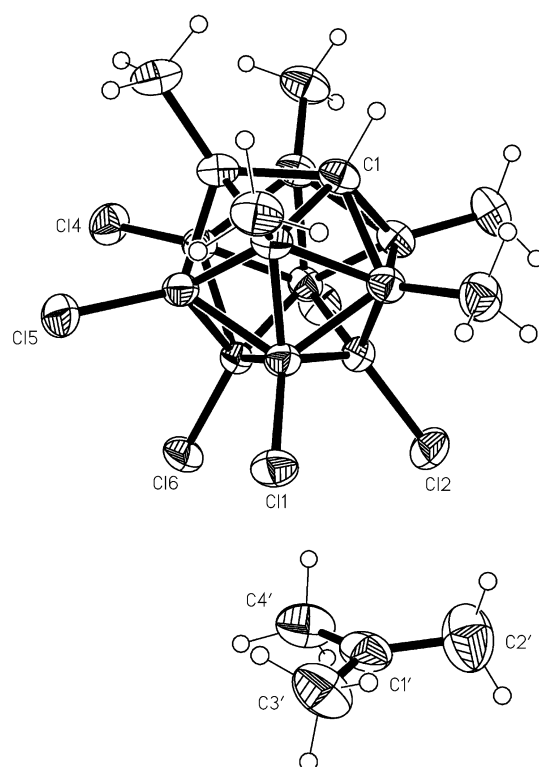


Figure 1. Crystal structure of **2b**· CH_2Cl_2 . Thermal ellipsoids are shown at the 50% probability level. The disordered solvent molecule CH_2Cl_2 is omitted for clarity. Selected bond lengths [Å] and angles [$^\circ$]: C1'–C2' 1.429(4), C1'–C3' 1.438(4), C1'–C4' 1.459(4); C2'–C1'–C3' 120.3(3), C2'–C1'–C4' 120.0(3), C3'–C1'–C4' 119.7(3).

one or more close H...Br approaches in the range 2.80–3.08 Å. The shortest Br approach is found to a methyl group in the cation with the fewer C–H bonds adjacent to the carbocationic center, namely the methylcyclopentyl cation, **4a**. These C–H...X interactions are consistent with the survey of C–H hydrogen bonding in the crystallographic database.^[17]

These hydrogen-bonding interactions are reflected in the IR spectra of the salts in which $\nu\text{C-H}$ bands from the carbocations are markedly shifted to low frequencies, even lower than those observed in fluoroantimonate salts.^[18] The lowest frequency (2695 cm^{-1}) appears in the methylcyclopentyl salt. This may correlate with the shortest C–H...Br distance (2.80 Å), observed for this salt. Together with the X-ray data, these IR indications of significant C–H...X hydrogen bonding to the carborane anions are taken as palpable structural evidence for the very high protic acidity, indeed superacidity, of carbocations.^[14,16] Similar IR effects and short C–H...Br distances were observed for the highly acidic protons of the benzenium ion, C_6H_7^+ , in recently isolated carborane salts.^[8]

Perhaps the most interesting new structure is that of **3a**, because the conformation of the C⁺– CH_2 – CH_3 moiety gives information about the relative importance of C–C versus C–H bond hyperconjugative stabilization of the carbenium-ion center. Theory at the MP4(SDTQ)/6-31G**//MP2(FU)/6-31G* + ZPE level finds a negligible energy difference between the conformer having the C–C bond aligned with the

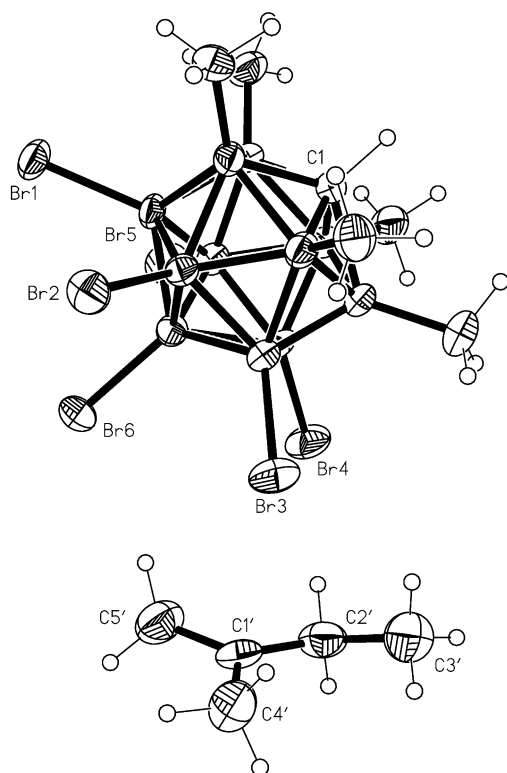


Figure 2. Crystal structure of **3a**·CH₂Cl₂. Thermal ellipsoids are shown at the 50% probability level. The disordered solvent molecule CH₂Cl₂ was omitted for clarity. Selected bond lengths [Å] and angles [°]: C1'-C2' 1.431(7), C1'-C4' 1.448(7), C1'-C5' 1.440(7), C2'-C3' 1.485(8); C2'-C1'-C4' 120.4(5), C2'-C1'-C5' 120.2(5), C4'-C1'-C5' 119.3(5), C1'-C2'-C3' 121.3(5).

vacant p orbital relative to that having it perpendicular.^[19] Preference for the aligned conformation (i.e., having C–C bond hyperconjugation) was made on the basis of better congruence of the computed ¹³C NMR chemical shifts with those determined experimentally in solution. However, in the X-ray crystal structure (Figure 2), the 25.8° dihedral angle between the carbocationic plane and the C(1)–C(2)–C(3) plane indicates the near absence of C–C bond hyperconjugation. This is consistent with the C(1)–C(2)–C(3) angle of 121.3°, which is close to the value of 119.5° calculated for no C–C bond hyperconjugation (dihedral angle = 0°) and much greater than the 101.7° value calculated for maximum C–C bond hyperconjugation (dihedral angle = 90°). Evidently the torsional potential in the C⁺–CH₂–CH₃ moiety is very soft and crystal-packing forces probably dictate the observed orientation, favoring C–H over C–C bond hyperconjugation.

As observed in the X-ray crystal structure of [*t*Bu][Sb₂F₁₁] by Laube and Hollenstein,^[4] the C(sp²)–C(sp³) distances in the present three tertiary carbocation structures are all short, thus reflecting partial-double-bond character from substantial C–H bond hyperconjugation. The C⁺–C bond lengths in the three cations all lie in the narrow range 1.429(4)–1.459(4) Å. The average in **2b** is 1.442(4) Å, identical to that in [*t*Bu][Sb₂F₁₁]. This indicates similar overall influences of the carborane and [Sb₂F₁₁][–] ions on *tert*-butyl cation, even though the carborane favors interactions with C–H bonds

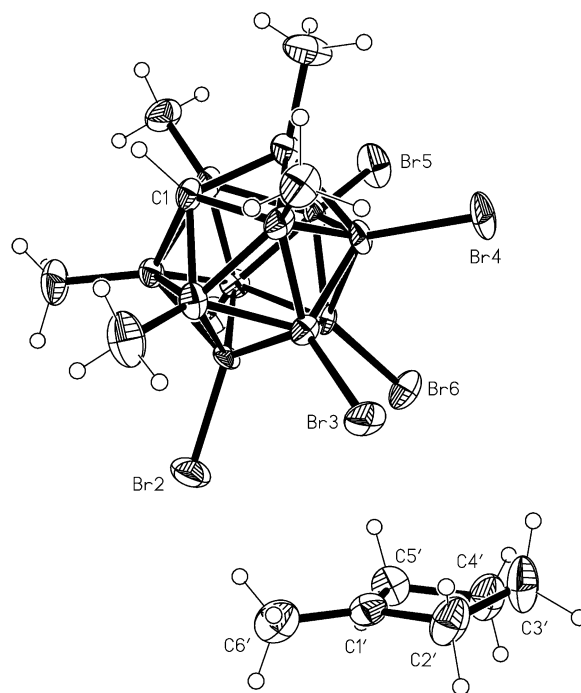


Figure 3. Crystal structure of **4a**. Thermal ellipsoids are shown at the 50% probability level. Selected bond lengths [Å] and angles [°]: C1'-C2' 1.450(11), C1'-C5' 1.450(10), C1'-C6' 1.457(11), C2'-C3' 1.516(12), C3'-C4' 1.525(12), C4'-C5' 1.525(11); C2'-C1'-C5' 109.9(7), C2'-C1'-C6' 124.9(7), C5'-C1'-C6' 125.2(7), C1'-C2'-C3' 106.0(6), C1'-C5'-C4' 106.8(7), C2'-C3'-C4' 104.2(7), C3'-C4'-C5' 102.5(7).

rather than the C⁺ center. Theory gives a calculated value of 1.458 Å for the C–C bond length in an isolated *tert*-butyl cation.^[20] The high level of congruence between calculated and observed bond lengths and angles reflects the intrinsic stability of tertiary carbocations and the relatively insignificant perturbation of their structures by external effects. Their isolation at room temperature simply required shutting down pathways to chemical decomposition.

In summary, we have shown that Me⁺ reagents, **1a** and **1b**, based on carborane anions are sufficiently powerful electrophiles that they can abstract hydride from hydrocarbons at or below room temperature. As long as the resulting tertiary carbocation salt is insoluble, it can be synthesized and stored at room temperature. This has allowed the accurate structure determinations of **2b**, **3a**, and **4a** and opens the way for simple tertiary carbocations to be used as reagents, something that has not been practical with carbocations generated in SbF₅-containing superacidic media.

Supporting Information for this article (synthetic procedures, NMR and IR spectra, complete X-ray data) is available for this paper.

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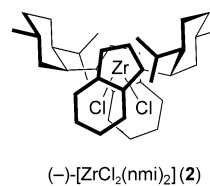
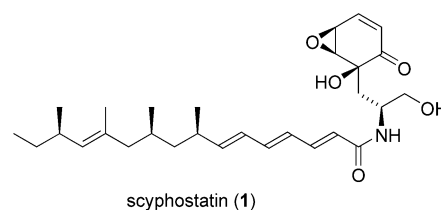
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Synthetic Methods

An Efficient and General Method for the Synthesis of α,ω -Difunctional Reduced Polypropionates by Zr-Catalyzed Asymmetric Carboalumination: Synthesis of the Scyphostatin Side Chain**

Ze Tan and Ei-ichi Negishi*

We recently reported an efficient and general method for the synthesis of reduced polypropionates with a single hetero-function^[1] through the application of Zr-catalyzed asymmetric carboalumination.^[2,3] In view of the large number of complex natural products that are of medicinal and biological interest, such as scyphostatin (**1**),^[4] ionomycin,^[5] dolicolide,^[6]

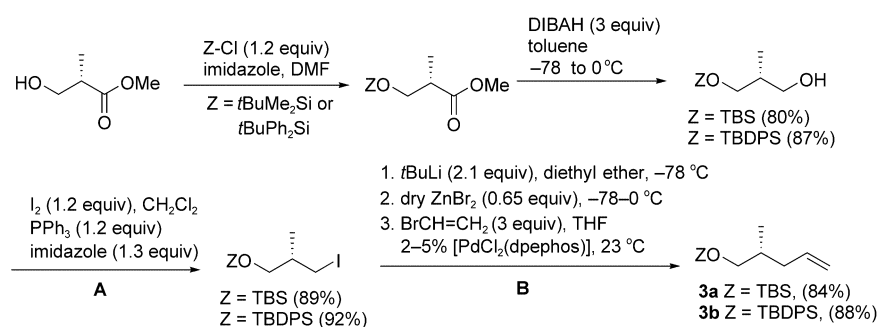


and borrelidin,^[7] we thought it worthwhile to search for a related method for the synthesis of terminally differentiated α,ω -difunctional reduced polypropionates through the use of Zr-catalyzed asymmetric carboalumination.^[1-3] We report herein one such method involving 1) just one relatively inexpensive and enantiomerically pure ($\geq 99\%$ *ee*) methyl (*R*)- or (*S*)-3-hydroxy-2-methylpropionate and 2) a catalytic and reagent-controlled asymmetric carbometalation^[1-3] with Me₃Al and either enantiomer of [ZrCl₂(nmi)₂] (nmi = 1-neomenthylindenyl; see **2**).^[8] We also report the application of this method to the synthesis of the scyphostatin side chain.

Preparation of **3** (Scheme 1) started with protection of methyl (*S*)-3-hydroxy-2-methylpropionate ($\geq 99\%$ *ee*; TCI,

[*] Dr. Z. Tan, Prof. E.-i. Negishi
Herbert C. Brown Laboratories of Chemistry
Purdue University
560 Oval Drive, West Lafayette, IN 47907-2084 (USA)
Fax: (+1) 765-494-0239
E-mail: negishi@purdue.edu

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Scheme 1. Synthesis of TBS- or TBDPS-protected (*R*)-2-methyl-4-penten-1-ol (**3**) from methyl (*S*)-3-hydroxy-2-methylpropionate. TBS = *tert*-butyldimethylsilyl, TBDPS = *tert*-butyldiphenylsilyl, DMF = dimethylformamide, DIBALH = diisobutylaluminum hydride, THF = tetrahydrofuran, dpephos = bis[2-(diphenylphosphanyl)phenyl] ether.

Tokyo, Kasei Kogyo Co., Ltd) by treatment with either TBSCl or TBDPSCl in the presence of imidazole and DMF (ca. 95 % yield). Reduction by treatment with DIBALH, followed by iodination led to the TBS- or TBDPS-protected (*S*)-3-iodo-2-methyl-1-propanol. This compound was converted into the corresponding zinc reagent in situ by treatment with *t*BuLi (2.1 equiv) in diethyl ether at -78 °C^[9a] and then with dry ZnBr₂ (0.65 molar equiv) at -78–0 °C. The organozinc intermediate was vinyllated with BrCH=CH₂ (3 equiv) in THF/diethyl ether^[9b–d] in the presence of [PdCl₂(dpephos)]^[10] (2–5 mol %) at 23 °C to give either **3a** (Z = TBS) or **3b** (Z = TBDPS) in a yield of 84 or 88 %, respectively. The overall yields of **3a** and **3b** from methyl (*S*)-3-hydroxy-2-methylpropionate over four steps were 57 % and 67 %, respectively.

Treatment of **3a** with Me₃Al (3 equiv), methylaluminumoxane (MAO; 1.2 equiv), and (+)-[ZrCl₂(nmi)₂] (5 mol %) in CH₂Cl₂ at 0 °C for 20 h produced, after oxidation with O₂, a 13:1 mixture of (2*S*,4*R*)- and (2*R*,4*R*)-**4** in 75 % combined yield (Table 1). The observed diastereomeric ratio (d.r.) of 13:1 suggests that the enantioselectivity at C2 would be 93 %, were it not for the pre-existing asymmetric carbon center at C4. This value, which is somewhat higher than the 85–90 % enantioselectivity range observed with achiral alkenes, suggests a modest level of favorable internal asymmetric induction. The corresponding reaction in the presence of

(-)-[ZrCl₂(nmi)₂] led to the formation of a 1:8 mixture of the same two isomers, with a combined yield of 72 %. This d.r. value of 1:8 corresponds to 89 % stereoselectivity at C2. The observed modest preference for the formation of *syn*-2,4-dimethyl-1-alkanols over that of *anti*-2,4-dimethyl-1-alkanols appears to be a general trend in Zr-catalyzed asymmetric carboalumination, although the corresponding reaction of the TBDPS-protected **3b** did not show any clear sign of internal asymmetric induction (Table 1). To probe the extent of internal asymmetric induction, we treated **3a** and **3b** with Me₃Al in the presence of [ZrCl₂(ind)₂] (ind = indenyl). Yields of 73 % and 78 % were obtained from **3a** and **3b** after oxidation, and the products exhibited 2*S*,4*R*/2*R*,4*R* ratios of 1.12:1 and 1.1:1,

respectively, which suggests that there is indeed a minor preference for the formation of the *syn* isomer. Chromatographic separation of the minor isomer (2*R*,4*R*)-**4** from (2*S*,4*R*)-**4** was readily achieved in one operation on silica gel (EtOAc/hexanes, 1:50). (2*S*,4*R*)-**4a** (Z = TBS; d.r. ≥ 40:1) and (2*S*,4*R*)-**4b** (Z = TBDPS; d.r. ≥ 37:1) were obtained in yields of 55 and 61 %, respectively, based on the starting alkene **3**. These yields correspond to 73 % and 77 % recovery in the chromatographic separation. Since d.r. values of 13:1 and 10:1 indicate maximum possible recovery rates of 93 % and 91 %, respectively, there seems to be room for further improvement in the recovery in view of the ease of diastereomeric separation in these cases.

The high enantiomeric purity of **3** (≥ 98 % *ee*) guarantees an overall *ee* value at least this high, regardless of the extent of stereoselection in the conversion of **3** into **4**. Statistical enantiomeric amplification, which is nothing more than a manifestation of the kinetic mass action law, would further elevate the overall *ee* value after each additional asymmetric stereogenic step. For example, the overall *ee* values established on the basis of the mass action law for asymmetric reaction of compounds of 98.0 % *ee* in the absence of internal asymmetric induction may reliably be predicted as shown in Table 2. The overall *ee* value of the products **4a** and **4b** may be estimated from Table 2 to be 99.6 % or more. HPLC analysis of the urethanes derived from **4a** and (+)- and (-)-1-

Table 1: Conversion of **3** into 5-siloxy-2,4-dimethyl-1-pentanol (**4**).

		1. Me ₃ Al (3 equiv) 5% [ZrCl ₂ (nmi) ₂] MAO (1.2 equiv) CH ₂ Cl ₂ , 0 °C, 20 h		2. O ₂			

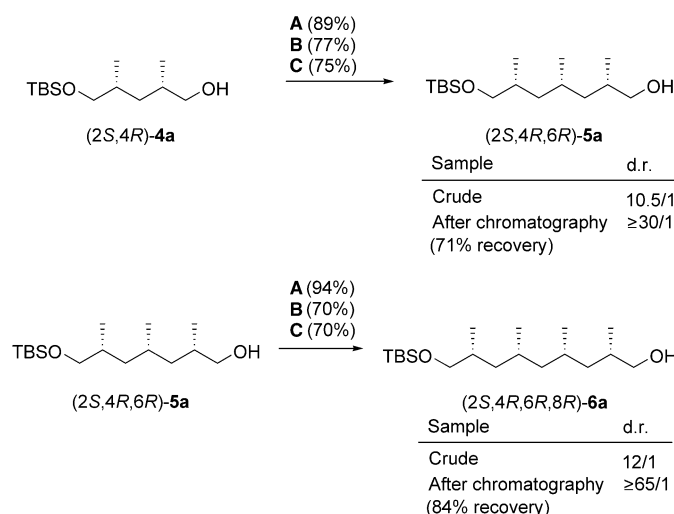
Table 2: Estimation of overall *ee* values and maximum attainable yields for asymmetric reactions of chiral compounds of 98.0% *ee*.

Asymmetric induction <i>R/S</i> or <i>S/R</i> ^[a]	<i>de</i> [%] (d.r.)	Max. possible yield [%]	Overall <i>ee</i> [%] ^[b]
50/50	00.0 (1:1)	50.0	98.0
80/20	58.8 (3.9:1)	79.4	99.5
85/15	68.6 (5.4:1)	84.3	99.6
90/10	78.4 (8.3:1)	89.2	99.8
95/05	88.2 (9.4:1)	94.1	99.9
100/0	100.0 (∞)	100.0	100.0

[a] The *R/S* or *S/R* ratio at the stereogenic center. [b] Statistically estimated.

(α -naphthyl)ethyl isocyanates provides experimental evidence of *ee* values of more than 99%. In these cases, however, the maximum possible product yields are in practice much more important. If yields of at least 80% are assumed to be desirable for practical purposes, asymmetric reactions with stereoselectivities at the second stereogenic center of 80% or higher would suffice to attain this practical goal. Such a stereoselectivity corresponds to over 4:1 d.r. and the generation of chiral compounds of over 99% *ee*. In short, we have developed an efficient, selective, and practical five-step protocol for the synthesis of 2,4-dimethyl-1-alkanols that is catalytic in chiral auxiliaries (Table 1). Synthesis of (2*S*,4*R*)-**4a** and (2*R*,4*R*)-**4b** from methyl (*S*)-3-hydroxy-2-methylpropionate was achieved with total yields over five steps of 31% and 41%, respectively, 97–98% diastereomeric purity, and at least 99.6% *ee* (estimated).

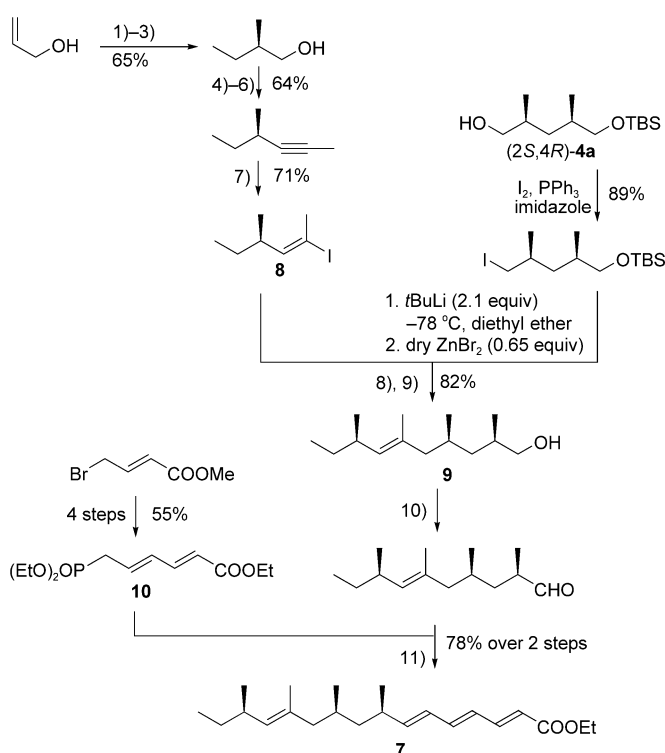
Conversion of 2,4-dimethyl-1-alkanols into 2,4,6-trimethyl-1-alkanols and higher reduced polypropionates can be achieved by application of an iterative three-step protocol^[1] consisting of 1) iodination (**A**), 2) Pd-catalyzed vinylation (**B**), and 3) Zr-catalyzed methylalumination (**C**, Scheme 2). (2*S*,4*R*)-**4a** was converted into (2*S*,4*R*,6*R*)-**5a** (d.r. > 30:1) in 36% combined yield over three steps, and iteration of the same three-step protocol converted


Scheme 2. Synthesis of trimethyl- and tetramethyl-1-alkanols from (2*S*,4*R*)-**4a** through application of a three-step protocol. **A**, **B**, and **C** are defined in Scheme 1 and Table 1.

(2*S*,4*R*,6*R*)-**5a** into (2*S*,4*R*,6*R*,8*R*)-**6a** (d.r. > 65:1) in 40% combined yield over three steps. The diastereomeric ratios observed for the products of the Zr-catalyzed asymmetric carboalumination itself (step **C**) were 10.5:1 and 12:1 (Scheme 2).

Scyphostatin (**1**), a potent inhibitor of neutral sphingomyelinase,^[4a] was first reported in 1997 but its total synthesis does not appear to have been reported.^[11] The structure of this compound consists of a C₂₀ carboxylic acid side chain and a bicyclic amine core fragment. The side chain has been synthesized once before in the form of ethyl ester **7** in nine steps (six isolation steps) from (2*S*,4*R*)-**4b** and in 15% yield.^[4b] However, the preparation of (2*S*,4*R*)-**4b** from diethyl 2-methylmalonate and ethyl α -bromoisobutyrate by enzyme-catalyzed monoacetylation of *meso*-2,4-dimethyl-1,5-pentanediol required seven additional steps and was achieved in a mere 4% yield. Thus, the linear 16-step synthesis of **7** proceeded in 0.6% total yield. Use of the 5-step synthesis of (2*S*,4*R*)-**4b** (d.r. ≥ 37:1) in 41% total yield (Table 1) would alone amount to a tenfold increase in the yield of **7**.

We sought a convergent synthetic scheme to make the synthesis of **7** even more efficient. We developed a synthesis whose longest linear sequence is 11 steps (Scheme 3). In


Scheme 3. Convergent synthesis of the scyphostatin side chain (**7**) by Zr-catalyzed asymmetric carboalumination. 1) TBDPSCl (1.2 equiv), imidazole, DMF; 2) a) 5 mol % (–)-[ZrCl₂(nm)₂], MAO (1.2 equiv), Et₃Al (3 equiv), 0 °C; b) aq HCl; 3) TBAF (1.5 equiv), THF; 4) Swern oxidation; 5) PPh₃ (2 equiv), CBr₄ (2 equiv), Zn dust (2 equiv), CH₂Cl₂, 0 °C; 6) a) *n*HexLi (2.05 equiv), –78–0 °C; b) MeI (3 equiv); 7) a) [HfZrCp₂Cl] (1.5 equiv), THF, 60 °C; b) I₂ (1.5 equiv), 0 °C; 8) 5 mol % [PdCl₂(dpephos)], vinyl bromide (3 equiv), 23 °C; 9) TBAF (1.5 equiv); 10) cat. TPAP, NMO, CH₂Cl₂; 11) LDA, 0 °C. TBAF = tetrabutylammonium fluoride, TPAP = tetrapropylammonium perruthenate, NMO = 4-methylmorpholine *N*-oxide, LDA = lithium diisopropylamide.

addition to (2*S*,4*R*)-**4a**, (2*E*,4*R*)-2-iodo-4-methyl-2-hexene (**8**) and the previously employed 6-*P*-substituted (2*E*,4*E*)-hexa-2,4-dienoic acid ethyl ester^[4b] (**10**) were used as key intermediates. The preparation of **8** started with protection of allyl alcohol with TBDPSCl. Asymmetric ethylaluminumation of the TBDPS-protected allyl alcohol with Et₃Al (3 equiv), MAO (1 equiv), and 5 mol % (–)-[ZrCl₂(nmi)₂], followed by protonolysis and desilylation by treatment with TBAF gave (*R*)-2-methyl-1-butanol in 92 % *ee* and 65 % combined yield over three steps. No enantiomeric separation was carried out. The crude (*R*)-2-methyl-1-butanol was 1) oxidized under Swern conditions,^[12] 2) subjected to the Corey–Fuchs reaction^[13] to give (*R*)-1,1-dibromo-3-methyl-1-pentene, and 3) converted into (*R*)-4-methyl-2-hexyne. Hydrozirconation/iodinolysis of this compound provided (2*E*,4*R*)-2-iodo-4-methyl-2-hexene (**8**) in more than 98 % isomeric purity and 71 % yield. The total yield of **8** from allyl alcohol over seven steps (six isolation steps) was 30 %.

Iodination of (2*S*,4*R*)-**4a** produced the desired iodide in 89 % yield. This iodide was treated first with *t*BuLi (2.1 equiv) in diethyl ether at –78 °C and then with dry ZnBr₂ (0.65 molar equiv). The alkyl zinc derivative thus generated was cross-coupled with **8** in the presence of [PdCl₂(dpephos)] (5 mol %).^[10] The coupling proceeded in 94 % yield based on the amount of iodide used. After desilylation with TBAF, column chromatography on silica gel (ethyl acetate/hexanes, 1:25) provided the desired compound **9** in more than 98 % isomeric purity and 87 % yield. Compound **9** was thus synthesized in 25 % overall yield and the longest linear synthetic sequence consisted of nine steps starting with allyl alcohol. An additional six steps were needed for the synthesis of the TBS-protected 5-iodo-2,4-dimethyl-1-pentanol in 37 % overall yield. Since the previously reported synthesis of **7** employed **9** as a key intermediate, a formal synthesis of **7** was complete at this point. For a variety of reasons, we nevertheless decided to complete the synthesis of **7** by following the reported procedure involving 1) oxidation of **9** with TPAP and NMO and 2) olefination of the resultant aldehyde with **10**, which was prepared from methyl (*E*)-4-bromocrotonate in 55 % yield over four steps.^[4b] These two processes were completed in 78 % combined yield. Our convergent and efficient synthesis of **7** therefore occurred in 19 % yield. The longest linear sequence of the synthesis involved 11 steps. The ¹H and ¹³C NMR spectra of the product were not only consistent with the assigned structure but also agreed well with those reported by Hoye and Tennakoon.^[4b]

In conclusion, 1) an efficient and potentially general method for the synthesis of α,ω-difunctional reduced polypropionates through the use of Zr-catalyzed asymmetric carboalumination has been developed, 2) catalysis with respect to a chiral auxiliary for asymmetric C–C bond formation, coupled with high overall efficiency makes the method reported herein unique among the known methods for the synthesis of α,ω-difunctional reduced polypropionates, 3) the use of commercially available and relatively inexpensive methyl (*R*)- and (*S*)-3-hydroxy-2-methylpropionates with *ee* values of at least 98 % makes enantiomeric separation unnecessary for the synthesis of reduced polypropionates of more than 98 % *ee*, and 4) a convergent and efficient synthesis

of the scyphostatin side chain in the form of its ethyl ester **7** was achieved in 11 linear steps (nine isolation steps) with 19 % overall yield. These results should prove to be useful for exploitation of **7** in the total synthesis of scyphostatin.

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Keywords: carboalumination · enantioselectivity · homogeneous catalysis · synthesis design · zirconium

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Carbohydrate Synthesis

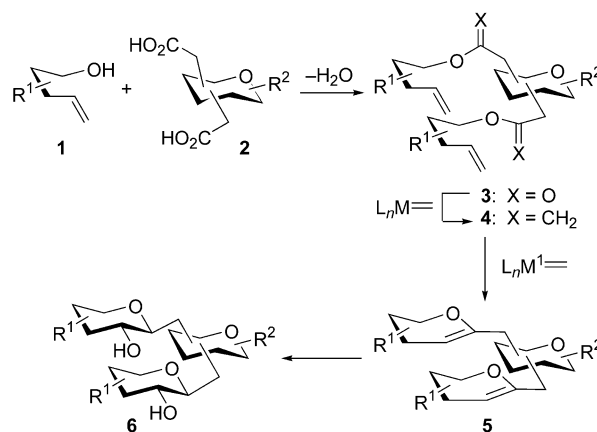
A Double Ring-Closing Metathesis Approach for the Synthesis of β -C-Trisaccharides**

Maarten H. D. Postema,* Jared L. Piper,
Venu Komanduri, and Lei Liu

The synthesis of C-glycosides, compounds in which the interglycosidic oxygen atom has been replaced by a carbon atom, has received considerable attention from both the synthetic^[1] and biological^[2] point of view. They comprise an important class of stable carbohydrate mimics and the debate regarding their validity as conformational mimics of the parent O-glycosides is ongoing.^[3]

Although there have been many interesting and unique approaches to the synthesis of C-glycosides,^[4] the preparation of C-saccharides,^[5] whether they are C-disaccharides or higher homologues, has been considerably more challenging. The first C-trisaccharide synthesis by Kishi and co-workers^[6] clearly showed that these compounds could be prepared and, since that time, several other research groups^[7] have targeted these carbohydrate-based compounds for synthesis. There are two main challenges associated with the synthesis of C-saccharides. The first challenge is the difficulty associated in functionalizing one carbohydrate ring (or open chain) followed by its subsequent attachment to the anomeric center of a second (or more) monosaccharide unit(s). To address this, several approaches to the synthesis of a variety of differentially linked C-saccharides^[5] have been developed and we have recently published a unified and versatile strategy for a convergent and efficient synthesis of (1 $\rightarrow$ 6)- β -C-disaccharides^[8] and a variety of differentially linked β -C-disaccharides.^[9] Our ring-closing-metathesis (RCM)^[10] approach is flexible enough to deliver a wide variety of C-glycoside-type structures.^[11] We now report that our metathesis-based approach can be used to efficiently synthesize a variety of β -C-trisaccharides through a highly efficient double enol ether–olefin RCM cyclization^[12] that provides the products in excellent overall yield after functionalization of the newly formed double bonds.

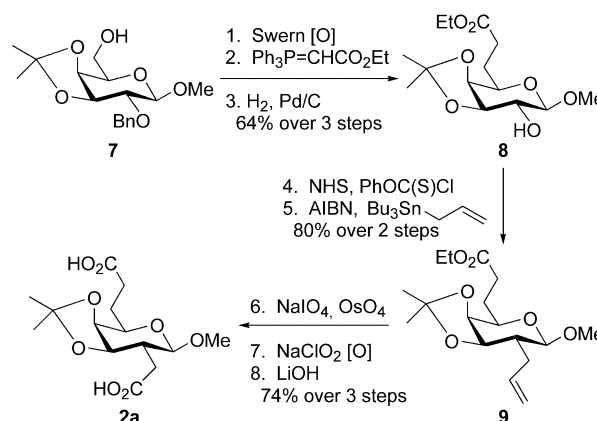
The general approach begins with dehydrative coupling of olefin alcohol **1** with a suitable carbohydrate-based diacid such as **2** to give diester **3** (Scheme 1). Methylenation of **3** is



Scheme 1. Double RCM approach to C-trisaccharides. L = ligand, M = metal.

followed by a double RCM reaction to give bis-C-glycal **5**. Functionalization of the bis-glycal double bonds then delivers the β -C-trisaccharide **6**.

In our previous C-disaccharide work^[8,9] the installation of only one acetyl group onto the pyranose ring was needed, whereas in this case the preparation of a diacetyl derivative was required. We permitted the nature of the diacid to dictate the type of chemistry that would be used for its preparation. Scheme 2 shows the use of both Wittig- and Keck-type



Scheme 2. Preparation of diacid **2a**. Bn = benzyl, NHS = N-hydroxysuccinimide, AIBN = azobisisobutyronitrile.

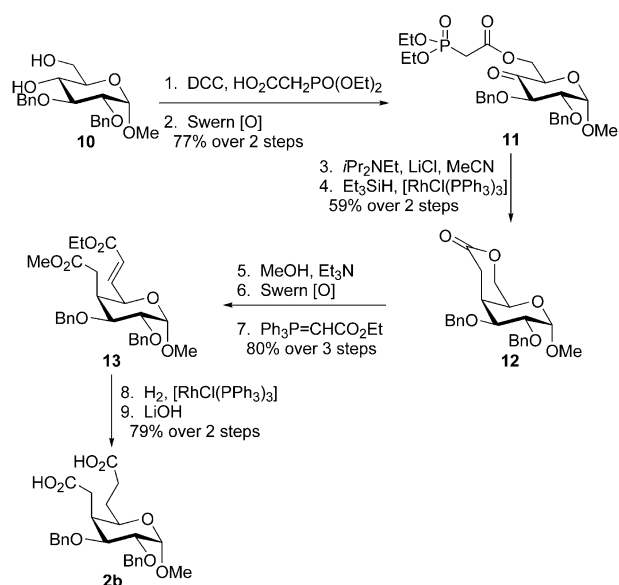
allylation chemistry for the synthesis of diacid **2a**. The primary alcohol on **7**^[13] was oxidized, olefinated, and hydrogenated, which served to reduce the double bond and cleave the benzyl group, to furnish **8**. The Robins-based^[14] radical precursor was then installed by using the N-hydroxysuccinimide method^[15] and Keck allylation^[16] then delivered compound **9** as the sole isomer. Two-step oxidative cleavage of the olefin in **9** to an intermediate monoacid was followed by saponification to give diacid **2a**.

We relied upon a slightly different set of reactions to prepare acid **2b** (Scheme 3). Diol **10** was selectively esterified to provide the stable keto-monophosphonate **11**, after Swern

[*] Prof. M. H. D. Postema, J. L. Piper, V. Komanduri, L. Liu
Department of Chemistry
Wayne State University
Detroit, MI 48202 (USA)
Fax: (+1) 313-577-2554
E-mail: mpostema@chem.wayne.edu

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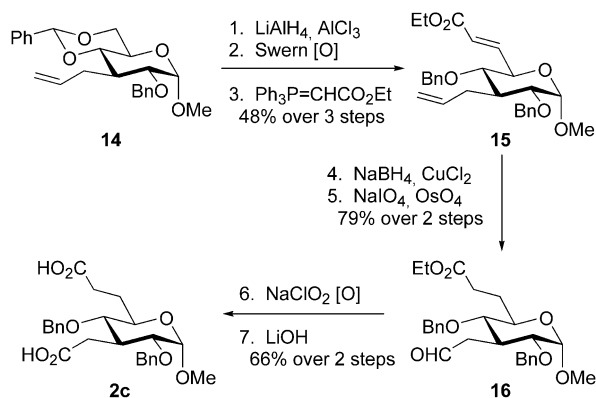
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Scheme 3. Preparation of diacid **2b**. DCC = *N,N'*-dicyclohexylcarbodiimide.

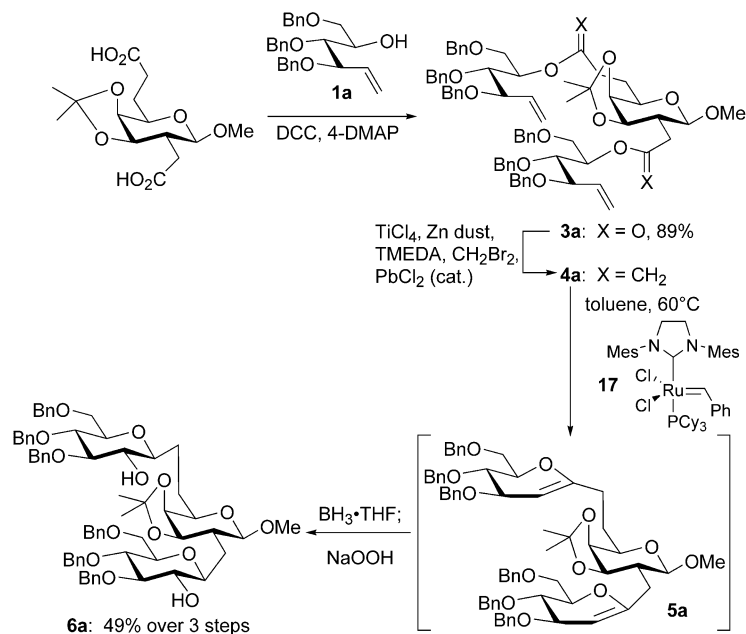
oxidation of the remaining secondary alcohol. Masamune–Roush olefination^[17] gave an intermediate unsaturated lactone that, under a variety of different reduction conditions, gave exclusively lactone **12** possessing the *galacto* configuration at the C4 position. Methanolysis of **12**, according to the conditions of Corey et al.,^[18] was followed by oxidation and Wittig reaction to form **13**. Reduction of the olefin was followed by bis-saponification to deliver the 4,6-diacid **2b** in good overall yield (Scheme 3).

Diacid **2c** was prepared by a slightly different strategy, as shown in Scheme 4. Known olefin **14**^[9] was converted into α,β -unsaturated ester **15** in three steps, and conjugate reduction^[19] was followed by oxidative cleavage of the remaining double bond to afford **16**. Oxidation and saponification of **16** then delivered the target diacid **2c** in good overall yield (Scheme 4).



Scheme 4. Preparation of diacid **2c**.

The stereochemistry of all the diacids, esters, and/or allylated derivatives was rigorously established by NOE, proton-decoupling and two-dimensional NMR spectroscopy experiments. In theory, the esters could be differentiated and distinctive olefin alcohols installed sequentially^[20] but, in the present work, we chose to install the same olefin alcohol on each acid function. DCC-mediated coupling of diacid **2a** with two equivalents of alcohol **1a** gave diester **3a** in good yield (Scheme 5). Methylenation^[21] of **3a** with an excess of the Takai reagent and subsequent RCM with the second-generation Grubbs catalyst **17**^[22] (35 mol %) gave the intermediate

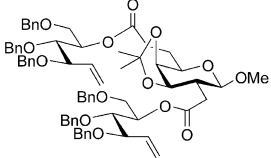
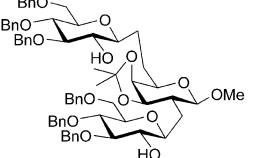
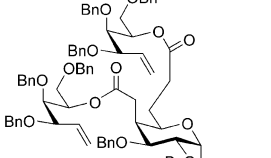
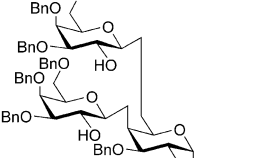
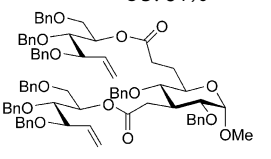
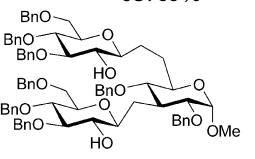
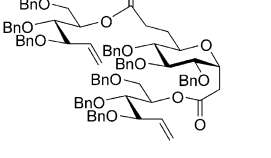
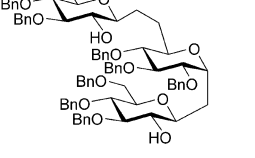
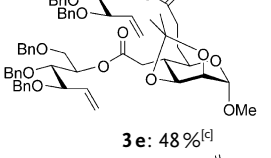
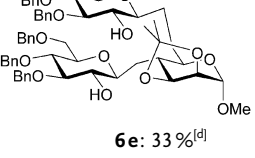
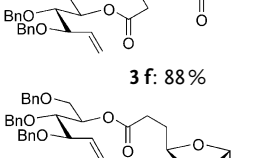
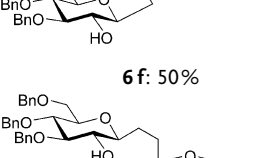
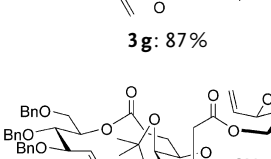
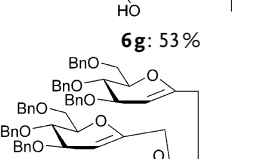
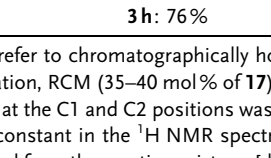
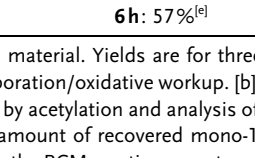


Scheme 5. Double RCM synthesis of β -C-trisaccharides. 4-DMAP = 4-dimethylaminopyridine, TMEDA = *N,N,N',N'*-tetramethylethylenediamine, Mes = β -morpholinoethanesulfonic acid, Cy = cyclohexyl, THF = tetrahydrofuran.

bis-*C*-glycal **5a**. The bis-*C*-glycal was not isolated but instead was directly subjected to hydroboration by $\text{BH}_3\cdot\text{THF}$.^[23] Subsequent oxidative workup then afforded the *C*-trisaccharide **6a** in 49% yield over three steps.^[24]

Table 1 shows the examples that have been prepared thus far. Ester formation (**1** + **2** $\rightarrow$ **3**), mediated by DCC and 4-DMAP, proved to be routine, except for the case shown in entry 5 (Table 1). A large excess of the methylenating reagent was required for the methylenation (**3** $\rightarrow$ **4**) reactions to be driven to completion. In all the cases, 35–40 mol % of the RCM catalyst **17** was needed to achieve a complete reaction. It is noteworthy that the three-step protocol works quite well even when both groups to be cyclized are on the same side of the pyranose ring, as in **3b** (entry 2, Table 1). No evidence of other cyclized products was noted by thin-layer chromatography (TLC) or crude NMR spectroscopy of the crude reaction mixtures. In one case (entry 8), we isolated the bisglycal **5h**, since hydroboration gave a mixture of two inseparable products.

Table 1: Synthesis of β -C-trisaccharides by double RCM.^[a]

Entry	Diester 3	C-Trisaccharide 6 ^[b]
1	 3 a: 89 %	 6 a: 49 %
2	 3 b: 84 %	 6 b: 55 %
3	 3 c: 74 %	 6 c: 57 %
4	 3 d: 88 %	 6 d: 59 %
5	 3 e: 48 % ^[c]	 6 e: 33 % ^[d]
6	 3 f: 88 %	 6 f: 50 %
7	 3 g: 87 %	 6 g: 53 %
8	 3 h: 76 %	 6 h: 57 % ^[e]

[a] Yields refer to chromatographically homogeneous material. Yields are for three steps: methylenation, RCM (35–40 mol % of **17**), and hydroboration/oxidative workup. [b] Stereochemistry at the C1 and C2 positions was determined by acetylation and analysis of the H2 coupling constant in the ¹H NMR spectra. [c] A fair amount of recovered mono-1,6-ester was isolated from the reaction mixture. [d] In this case, the RCM reaction was stopped early and the bis-C-glycal was isolated, purified (48%, unoptimized), and then subjected to hydroboration (66%, unoptimized). [e] In this case, the yield is for two steps (methylenation and RCM) since hydroboration gave an inseparable mixture of two isomers.

The above results show that our double RCM approach for C-trisaccharide synthesis is viable and efficient. Application of this methodology to other congeners is currently underway and will be reported in due course.

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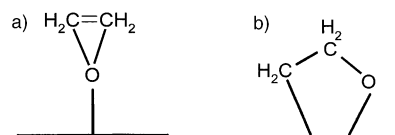
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Ethylene Epoxidation on Ag: Identification of the Crucial Surface Intermediate by Experimental and Theoretical Investigation of its Electronic Structure**

Suljo Linic, Hong Piao, Kaveh Adib, and Mark A. Barteau*

An important issue regarding the mechanism of heterogeneous epoxidation of ethylene on silver (Ag) is related to the formation of C–O bonds.^[1–3] There are two possible ways for the C–O bonds to form: It is conceivable that the dominant pathway would proceed through either a concerted addition of surface oxygen to the C=C bond of ethylene or by sequential formation of the two C–O bonds by a creation of an asymmetric surface intermediate (Scheme 1).



Scheme 1. Possible intermediates in ethylene epoxidation on Ag: a) in the concerted addition of ethylene to oxygen and b) in the sequential formation of C–O bonds (surface oxametallacycle).

It has been shown previously in temperature-programmed desorption (TPD) experiments that a surface intermediate formed on Ag(111) upon adsorption of ethylene oxide (EO) at about 250 K under ultrahigh vacuum (UHV) conditions reacts at about 310 K to reform EO plus small amounts of other products, including ethylene.^[4] High-resolution electron energy loss spectroscopy (HREELS) was used to identify this surface intermediate as the asymmetric structure shown in Scheme 1, namely a surface oxametallacycle.^[4] This work

[*] Dr. S. Linic, Prof. Dr. M. A. Barteau
Center for Catalytic Science and Technology (CCST)
Department of Chemical Engineering
University of Delaware
Newark, DE 19716 (USA)
Fax: (+1) 302-831-8201
E-mail: barteau@che.udel.edu
Dr. H. Piao, Dr. K. Adib
Brookhaven National Laboratory (BNL)
Upton, NY 11973 (USA)
Dr. S. Linic
Fritz-Haber Institut der Max-Planck-Gesellschaft
Faradayweg 4–6, 14195 Berlin (Germany)
and
Department of Chemical Engineering
University of Michigan
Ann Arbor (USA)

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provided important evidence that the surface oxametallacycle can react on the Ag surface to form EO, which is consistent with the hypothesis that sequential formation of C–O bonds is the dominant pathway in ethylene epoxidation. Subsequent theoretical and microkinetic modeling studies have bolstered the conclusion that the surface oxametallacycle is the key intermediate in this process.^[5–8] The surface oxametallacycle was identified on the basis of the vibrational fingerprint obtained in experiments in which it was quite difficult, because of thermodynamic constraints, to obtain a high surface concentration of this intermediate and therefore a high-quality vibrational spectrum.^[4] We have thus explored additional tools to conclusively identify the relevant surface intermediate. The identification of this intermediate is crucial, since many aspects of ethylene epoxidation, most importantly selectivity, are governed by the surface chemistry of the intermediate.^[2,6,8] Also, a thorough understanding of the nature of C–O bond formation could have a significant impact on other epoxidation processes, such as the potential development of stable and efficient heterogeneous catalysts for propylene epoxidation.

Herein, surface intermediates formed upon adsorbing EO onto Ag(111) at various temperatures are examined by a combination of high-resolution X-ray photoelectron spectroscopy (HRXPS) and density functional theory (DFT). The work presented herein is significant not only because it reinforces the link between the existence of surface oxametallacycles and EO formation on Ag, but also because it represents a clear illustration of a potential utility of the combined use of HRXPS and DFT to investigate interesting surface intermediates by probing the electronic structures of atoms that form them. High-resolution chemical information obtained in HRXPS experiments is sufficient to conclusively identify intermediates and even to distinguish among those which are fairly similar.

Ethylene oxide was adsorbed onto a clean Ag(111) crystal at various adsorption temperatures and the C 1s X-ray photoelectron spectra were measured as a function of the adsorption temperature. Figure 1 shows the C 1s HRXPS spectrum after exposure of Ag(111) to EO at temperatures of 100 and 130 K. Measurements obtained after adsorption of EO at 100 K reveal that the ethylene oxide exists in two different states. The peak at 287.1 eV is assigned to multilayers of ethylene oxide, while the peak at 286.2 eV is assigned to molecular EO that is weakly bound to the surface. These assignments are corroborated by the observation that the peak at 287.1 eV corresponding to EO multilayers disappears after flash heating the sample to 157 K. It is also observed that the weakly bound molecular EO is almost completely desorbed after flash heating the sample to 204 K. Figure 1 also shows the C 1s HRXPS spectrum after EO was adsorbed onto Ag(111) at 130 K. It is observed that at this temperature the adsorbate is the weakly bound molecular EO, as indicated by the peak at 286.2 eV. This species desorbed after the sample was flash heated to 202 K, as evidenced by the disappearance of the C 1s peak. Peak-fitting of the HRXPS spectra after low-temperature adsorption of EO also indicated that the two C atoms are chemically

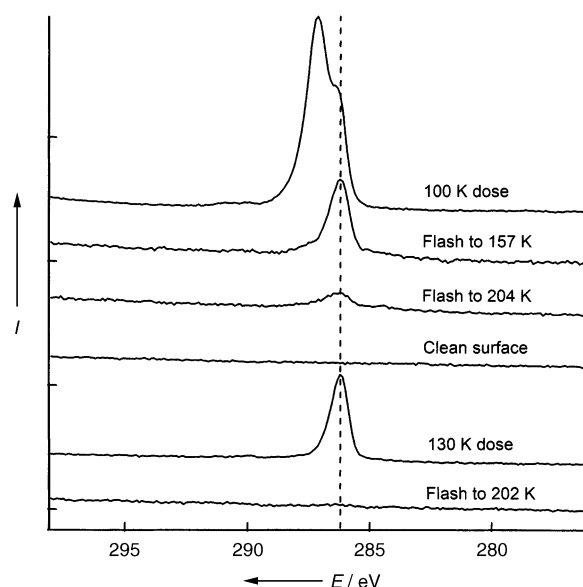


Figure 1. Measured C 1s spectra after EO was adsorbed onto the Ag(111) crystal plane at 100 K or 130 K, and subsequently heated to the temperatures indicated. States corresponding to multilayers and molecularly adsorbed EO are observed.

indistinguishable in molecularly adsorbed EO, that is, the symmetric framework of the EO molecule is preserved.

In separate experiments, EO was adsorbed onto the Ag(111) surface at 225 and 250 K. The corresponding C 1s HRXPS spectra are shown in Figure 2. The spectrum measured after adsorption of EO at 225 K is characterized by two peaks. One peak is centered at 286.5 eV and assigned to weakly bound molecular EO. It is observed that there is a

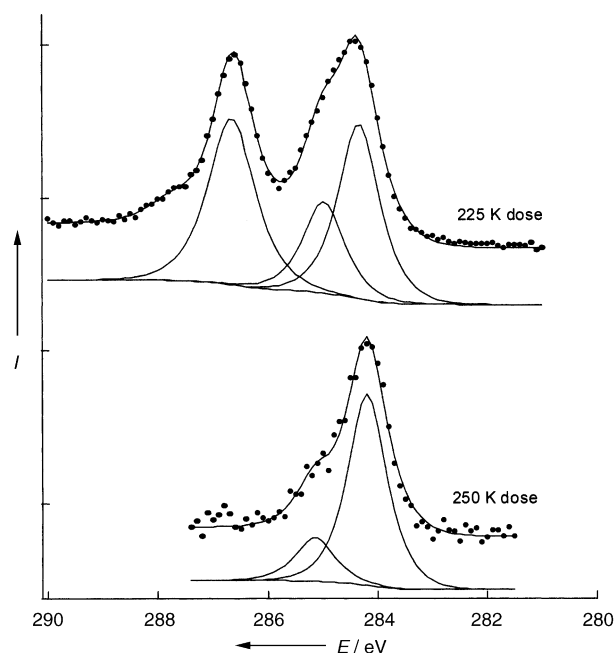


Figure 2. Measured C 1s spectra after EO was adsorbed onto the Ag(111) crystal plane at 225 K or 250 K. The lower lines represent peak-fits to the measured spectra. Multiple states are observed.

slight shift (from 286.2 to 286.5 eV) in the binding energy of the C 1s state compared to that in the low-temperature adsorption. This shift is a consequence of variation in the adsorbate coverage as a function of adsorption temperature and also a result of a different binding environment; molecular EO is not the only species present on the surface. The two effects lead to a change in the surface work function which becomes evident as a small shift in the binding energy. The adlayer adsorbed at 225 K is also characterized by an additional peak at lower binding energy (284.3 eV) that was not observed after adsorption at lower temperatures. The C 1s spectrum indicates that the species associated with this peak is chemically different from molecular EO. Furthermore, the peak-fit of the 284.3 eV peak suggests that there are two distinct carbon atoms contributing to the signal in this region, that is, an asymmetric surface intermediate is formed. The spectrum measured after EO was adsorbed at 250 K is characterized by only a broad peak in the 284–285 eV region corresponding to an intermediate with two distinct carbon atoms; the peak at 286.5 eV assigned to molecular EO is not observed. It is important to reiterate that the intermediate formed on Ag(111) after adsorption of EO at 250 K reacts at 310 K to reform EO, as shown in TPD experiments.^[4] The formation of this intermediate is an activated process—it does not occur for adsorption at 100–130 K, as demonstrated by the results of Figure 1 and previous TPD experiments.^[4] We utilize below computational tools, mainly DFT, to assign the spectra shown in Figure 2 to a particular species on the surface.

When comparing experimentally measured electronic spectra to theoretical predictions from DFT it is often fruitful to focus on shifts in electron binding energies with respect to the binding energy of some standard.^[9] In these experiments we have used the C 1s state of molecular EO as a standard. We are interested in shifts in the C 1s states as EO reacts to form a stable surface intermediate after it is adsorbed onto Ag(111) at approximately 250 K. The intermediate that has been proposed on the basis of its vibrational spectrum^[4] is the surface oxametallacycle shown in Scheme 1.^[4–7,10,11] The presence of two distinct carbon atoms in the asymmetric surface oxametallacycle structure is consistent with the peak-fit of the 284.3 eV HRXPS peak in Figure 2.

Figure 3a shows the experimental HRXPS spectra after adsorption of EO at 225 K onto the Ag(111) surface with energies shifted to utilize the C 1s peak of molecularly adsorbed EO as a reference. All-electron DFT calculations have been used to calculate the energy levels of the C 1s electrons in molecular EO and the surface oxametallacycle. The calculations were performed with only initial state effects,^[12] that is, the reported energies represent the energies needed to remove electrons from the Kohn–Sham eigenvalues of the particular core state, the C 1s orbital. These calculations were done for the geometry of the surface oxametallacycle that was computed to be stable on a Ag₁₅ cluster. The identical geometry was computed in pseudo-potential plane-wave calculations with the slab representation of the Ag(111) surface.^[5,6] The peak shapes are simulated by assuming a Gaussian distribution with standard deviation values and peak heights measured in the exper-

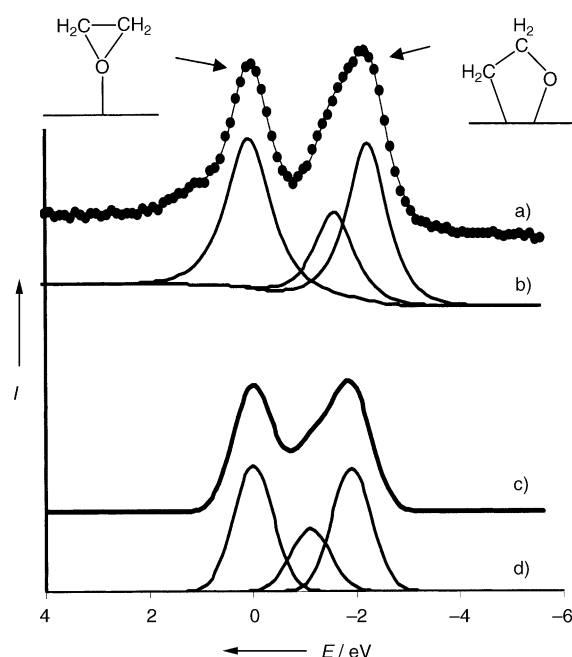


Figure 3. a) Measured C 1s spectra after EO was adsorbed onto the Ag(111) crystal plane at 225 K. The energy of 0 eV is assigned to molecular EO. b) Peak-fit for the measured spectrum. c) DFT-predicted C 1s spectra calculated from electronic structure calculations of EO and the surface oxametallacycle. This spectrum is computed as a superposition of the calculated C 1s peaks, shown in (d). d) DFT-calculated C 1s spectra for carbon atoms of molecular EO and the surface oxametallacycle. Experimental peak widths and heights are used.

imental studies. The agreement between the experimental results and the theoretical predictions of C 1s chemical shifts is quite remarkable (Figure 3).

From these experimental and theoretical studies it is concluded that after adsorption of EO onto the Ag(111) surface at 225 K there are two adsorbate states on the surface: weakly bound molecular EO and the surface oxametallacycle formed by opening of the EO ring. As the adsorption temperature is increased to 250 K the only species that remains on the surface is the oxametallacycle. The measured C 1s XPS spectrum of the surface oxametallacycle and chemisorbed EO are characterized by C 1s binding energies lower than those of physisorbed EO. The directions of these shifts are not surprising and are a probable consequence of charge transfer at the adsorbate. This process is driven by an alignment of the adsorbate electronic states with respect to the Fermi level of the substrate. It is also observed that the C 1s spectrum of the surface oxametallacycle formed after adsorption of EO at 250 K is characterized by peaks that are at lower binding energies than peaks assigned to molecular EO. This shift is measured to be approximately 1.5 eV for the central carbon atom of the oxametallacycle and approximately 2.3 eV for the terminal carbon atom. These shifts are calculated in DFT calculations to be 1.1 and 1.9 eV for the respective carbon atoms (Figure 3). The shifts for the surface oxametallacycle can be explained in terms of atomic charge distributions in the adsorbates. Even though the assignment of atomic charge is somewhat arbitrary, both Vernoi and

Mulliken analysis of the charge distribution illustrate that the C atoms of the surface oxametallacycle are less negatively charged than C atoms of molecular EO. The higher binding energies of the C 1s eigenvalues assigned to molecular EO compared to the surface oxametallacycle are a consequence of the extra Coulombic interactions between C 1s electrons and the ion cores of its more positively charged C atoms.

Ethylene oxide adsorbed on Ag(111) at 200–250 K undergoes ring opening to produce a stable surface intermediate. The experimental and theoretical studies on the electronic structure of the intermediate support the identification of this species as an oxametallacycle. This intermediate reacts at about 310 K to reform EO,^[4] thus providing an important link between ethylene epoxidation on Ag and the surface oxametallacycle. The combination of HRXPS and DFT calculations to probe electronic structures of adsorbates permits the identification of important surface intermediates, including elusive species such as the oxametallacycle, to an unprecedented level of detail.

Experimental Section

High-resolution X-ray photoelectron spectroscopy (HRXPS) experiments were performed in a UHV chamber on the U12A beamline at the Brookhaven National Laboratory. The chamber was equipped with turbo-molecular and cryo pumps. This pumping system was able to achieve base pressures of 1×10^{-10} Torr. The chamber was also equipped with a UTI 100C mass spectrometer for TPD experiments. XPS spectra were collected in the fixed pass energy mode using a concentric hemispherical analyzer. The setup allowed for resolution better than 0.3 eV. Binding energies were calibrated by setting the instrument work function to fit the position of the Ag3d_{5/2} line, which was used as a standard.

The Ag(111) single crystal was mounted on a 0.25 mm Ta wire loop which was spot-welded to two additional Ta wires. The sample could be resistively heated to over 900 K or cooled to 110 K by controlling the temperature of two Ta wires to which the sample was attached. The sample temperature was monitored by a K-type thermocouple spot-welded to the Ta wire loop. The sample was cleaned by successive cycles of Ar ion bombardment followed by annealing at 900 K. The surface cleanliness was confirmed by the absence of O 1s and C 1s signals in the XPS spectrum.

The EO used in the experiments (99 %, Aldrich) was transferred to the chamber from a lecture bottle through a short stainless-steel tube. Dosing was accomplished in a direct fashion by bringing the Ag single crystal to a distance of about 1 cm from the outlet of the stainless-steel tube.

The Amsterdam density functional (ADF) program was implemented to calculate fully optimized geometries of molecular gaseous EO and surface oxametallacycles adsorbed on Ag₁₅ clusters, as previously described.^[6] The same program was used to calculate the energetic positions of C 1s orbitals in these structures. The program represents molecular orbitals as linear combinations of Slater-type atomic orbitals. All calculations were spin-unrestricted and employ Becke^[13] and Perdew^[14] gradient approximations for the exchange and correlation energies, respectively. Double-zeta basis sets with polarization were employed in all computations.

The XPS spectra were calculated assuming that the binding energy “seen” by the photoelectron leaving a core level is identical to that before ionization, hence all other electrons are in the same state before and after the photoionization event. Possible relaxation effects are not taken into account.^[12] This assumption means that the screening contribution from the valence electrons in the response to a created hole is identical for excitation from both C 1s states and that

the electron self-interactions are identical for both respective electrons.

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New Clathrate Hydrate Structure: High-Pressure Tetrahydrofuran Hydrate with One Type of Cavity**

Alexander V. Kurnosov,* Vladislav Yu. Komarov,
Vladimir I. Voronin, Alexander E. Teplykh, and
Andrej Yu. Manakov

Since the last decade interest in clathrate hydrates has increased mainly because of the finding of immense deposits of hydrocarbon gas hydrates (clathrates) in the Earth's crust.^[1,2] For scientists, interest is augmented by prospective investigations into the types of water arrangement in supra-molecular structures (water being the most abundant chemical compound on earth). Herein we report on a new type of polyhedral clathrate framework, which was detected in a high-pressure $[D_8]$ tetrahydrofuran hydrate. Its peculiarity consists in there being only one type of framework-building polyhedral cavity.

Clathrate hydrates are inclusion compounds where the host is a polyhedral framework built of hydrogen-bonded water molecules in which guest molecules are trapped. Host–guest interactions are usually van der Waals in nature. Structures of clathrate hydrates are classified by the type of water framework, which is considered to be a three-dimensional packing of polyhedral cages with tetra-, penta-, and hexagonal faces, oxygen atoms as vertices, and hydrogen bonds as edges. The cages can act as hosts to a variety of guest molecules, from inert gases to large organic molecules (including those with hydrophilic groups). The shape and size of the guest molecules define the structural type of hydrate formed. Among the clathrate hydrates which arise under ambient pressure, three structural types are prevalent: cubic structures I and II, and hexagonal structure III (structure H), and these have been considered in detail in the literature.^[3–5] Reports have appeared on bromine hydrate, which was shown to have a structure previously found only in semi-clathrate hydrates,^[6] and the hydrate of dimethyl ether, which was found to have a quite new structure.^[7] For a long time the question of the effect of pressure on the structure of the clathrate hydrate formed remained open. It was known that under a pressure of hundreds of MPa, high-pressure phases existed in almost all the water–guest systems studied, though

information on their structures was lacking.^[8–12] Systematic studies of clathrate hydrate structures carried out in situ under high pressures were developed only during the last few years. In 2002, two new structural types of clathrate hydrates were identified in water–methane and water–argon systems under high pressure.^[13,14] Hydrates of the structural type first found in methane hydrate were then also found in water–nitrogen and water–argon systems.^[15,16] The hydrate of the structural type first found in argon hydrate was then also found in the water–nitrogen system.^[15] The characteristic feature of the structures under ambient pressure was the presence of several types of polyhedral cages packed in the water framework without any noticeable distortion of the lengths and angles of the hydrogen bonds. In contrast, the new high-pressure structures are built from one type of space-filling polyhedral cage (in methane hydrate they can be considered as a system of one-dimensional channels). Such polyhedrons contain several strained (neither pentagonal nor hexagonal) faces, as a consequence of significant distortion of the angles between the hydrogen bonds. Thus, a new unusual class of high-pressure hydrate frameworks was found, which was built of uniform space-filling polyhedrons. Herein we report on a new structural type of this class, which was found in a high-pressure tetrahydrofuran hydrate.

Investigators have repeatedly used the water–tetrahydrofuran system as a model system containing a bulky hydrophobic/hydrophilic guest. In 1957 Stackelberg and Meuthen showed that the hydrate formed in this system under ambient pressure had cubic structure II.^[17] Sixteen years later Dyadin et al. found two high-pressure hydrates in the same system.^[12] In 1994, Zakrewsky, Klug, and Ripmeester examined the structure of the second hydrate which existed in the pressure range of 0.18–0.53 GPa and determined that it had the known cubic structure I.^[18] The stoichiometry of the third hydrate was only deduced approximately (tetrahydrofuran·5H₂O) from the maximum of the liquidus on the T_x section of the phase diagram.^[17] The data on heat conductivity and capacity implied that this hydrate had to be assigned not as the clathrates, but as an ordinary crystal phase.^[19] Our recent study of its Raman spectra infers, however, just the opposite conclusion, and proves the clathrate nature of the hydrate.^[20] Moreover, the water framework of the hydrate was shown to consist, most probably, of one type of space-filling polyhedral cage. We thus decided to study this phase by a neutron-diffraction method to resolve the ambiguity, and the results of the study are presented herein.

Examination of the hydrate by neutron diffraction was performed in situ under a pressure of 0.8 GPa. The powder neutron diffraction pattern (Figure 1) allowed us to determine the syngony and unit-cell parameters and, thus, to realize that we were dealing with a previously unknown structural type of clathrate hydrate. For further corroboration we checked the idealized water framework of all known semiclathrate hydrates. None of these structures corresponded to our experimental data. Structure determination by conventional methods proved to be impossible; therefore, we decided to simulate possible structures. Evaluation of the possible cage volume and information on the uniformity of cages led to the proposal that a space-filling 14-hedron was

[*] A. V. Kurnosov, V. Yu. Komarov, Dr. A. Yu. Manakov
A.V. Nikolaev Institute of Inorganic Chemistry SD RAS
Lavrentieva avenue 3, Novosibirsk, 630090 (Russia)
Fax: (+7) 3832-344-489
E-mail: kurnosov@ngs.ru

Dr. V. I. Voronin, Dr. A. E. Teplykh
Institute of Metal Physics UrD RAS
S. Kovalevskoi street 18, Yekaterinburg, 620219 (Russia)

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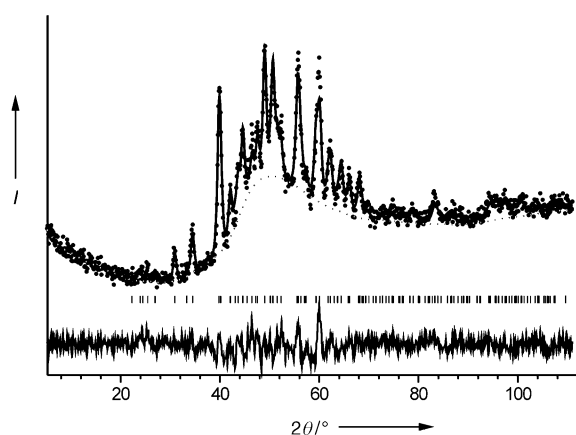


Figure 1. Neutron diffraction pattern of $[D_8]$ tetrahydrofuran hydrate ($P=0.8$ GPa, $T=293$ K). Circles: experimental data, line: calculated pattern; bottom trace: difference between the experimental and calculated data. The baseline of the experimental data is represented by a dotted line.

the most probable type of polyhedron. The first stage of the study resulted in the generation of ten types of space-filling 14-hedrons with tetra-, penta-, and hexagonal faces, and twenty possible ways for their spatial packing. The symmetry and atomic coordinates were then determined for each, including the arrangement of a tetrahydrofuran molecule in a cage. The details of this part of the work will be given in future publications. Simulation of the neutron diffraction patterns for all the models allowed the determination of the single structure that fitted the neutron diffraction pattern obtained experimentally.

The structure of the high-pressure $[D_8]$ tetrahydrofuran hydrate which represents a new structural type of clathrate hydrate was an orthorhombic structure with the space group $Pnma$ and unit-cell dimensions of $a=12.54$, $b=11.44$, $c=6.60$ Å.^[21] The water framework forms 14-hedral cages of a new type (Figure 2) that is able to pack three-dimensionally without the need for other types of polyhedrons. The cage consists of four tetra-, four penta-, and six hexagonal faces ($4^45^46^6$). The stoichiometry of a unit cell can be presented as $4T_3 \cdot 24D_2O$, where T_3 is a $4^45^46^6$ cage. Figure 2 also shows the projection of the structure along the b axis.

When refining the structure, the tetrahydrofuran molecule was assigned as a rigid model. We failed to find a correct model which took into account the full set of possible orientations and conformational changes of the guest molecule, because of the low ratio of the number of parameters under refinement relative to the number of measured reflections in the neutron diffraction pattern. We settled on the model of a rigid tetrahydrofuran molecule disordered over four positions. The simulated neutron diffraction pattern is shown in Figure 1, together with the experimental one. The R factor could be improved significantly (to $R=6\%$) if the rigid model was abandoned when the guest position was refined. The topology of the tetrahydrofuran molecule is then not retained, though all its atoms remained within the free volume of the cage. This situation argues for a complex disordering of the guest molecule in a cage, and our data are not sufficient to study this. The length of the hydrogen bonds

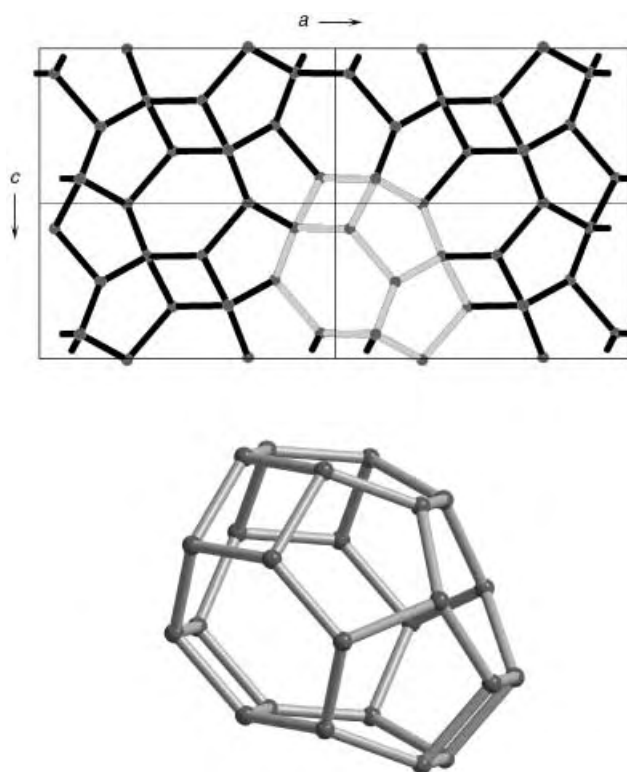


Figure 2. Packing and schematic view of the new space-filling polyhedron.

in the water framework vary from 2.67 to 2.99 Å and the angles between the hydrogen bonds vary from 87.4 to 123.3°. The angles differ from ideal tetrahedral (109.5°), but they are consistent with the range of values found in high-pressure phases of ices and clathrate hydrates.^[13,14]

The structure of the tetrahydrofuran hydrate studied represents a new structural type of clathrate hydrate in which the water framework consists of uniform space-filling polyhedral cages. This type of space-filling polyhedron (and the derived structure) has so far not been reported for clathrate hydrates and topologically related compounds (semiclathrate hydrates, clathrasils, framework aluminosilicates). The new polyhedron represents a typical example of the large group of space-filling 14-hedrons with tetra-, penta-, and hexagonal faces. This class of structural types made up of such polyhedrons is probably limited to the twenty structures modeled. Besides the structure reported, this class includes two known structures: the cubic structure of some semiclathrate hydrates based on 4^66^8 cavities^[3] and the argon hydrate structure mentioned above.^[14] We also expect the revelation of some hydrate structures based on other polyhedrons of this group, with the structure depending on the shape and size of the guest molecule. It also seems probable that similar structures will occur in other compounds whose framework includes tetrahedrally coordinated junctions.

Experimental Section

The work was carried out using an IVV-2M reactor (Yekaterinburg),^[22] $\lambda=2.4236$ Å. The design of the high-pressure cell used in the

neutron-diffraction studies under pressures up to 1 GPa is described elsewhere.^[23] The diffraction pattern was recorded at ambient temperature and a pressure of 0.8 GPa. Refinement of the structure was carried out using the FullProf program,^[24] with the tetrahydrofuran molecule described as a rigid model. The baseline was fitted using 39 points connected by a cubic spline. The large hump in the baseline occurred because of the presence of excess liquid D₂O (this was taken into account when the structure was refined as shown in Figure 1). The hydrate was synthesized directly in the high-pressure cell from deuterated liquid tetrahydrofuran (hydrogen content not exceeding 0.2%) and powdered low-pressure hydrate; the [D₈]tetrahydrofuran:D₂O ratio was 1:17. The ratio of the reagents was chosen to keep the [D₈]tetrahydrofuran:D₂O ratio in the system at 1:6. The high-pressure cell was charged with reagents at 250 K, then the pressure was raised to 0.8 GPa, and after that the temperature was increased to room temperature.

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Dendrimers

Synthesis of Dendritic Polyoxometalate Complexes Assembled by Ionic Bonding and Their Function as Recoverable and Reusable Oxidation Catalysts**

Lauriane Plault, Andreas Hauseler, Sylvain Nlate, Didier Astruc, Jaime Ruiz, Sylvain Gatard, and Ronny Neumann*

*Dedicated to Professor David Carrillo
on the occasion of his 65th birthday*

Dendrimers^[1] are known for a number of potential applications in supramolecular chemistry,^[2] nanosciences,^[3] biology, and medicine,^[4] and many of their transition-metal complexes^[5] are especially promising in catalysis.^[6] Whereas covalent-based metallodendrimers have been shown to act as catalysts in numerous cases, the ionic-bonding approach has only recently been mentioned.^[7] We now wish to report a

[*] L. Plault, Dr. A. Hauseler, Dr. S. Nlate, Prof. D. Astruc, Dr. J. Ruiz
Nanosciences and Catalysis Group
LCOO, UMR CNRS N° 5802
Université Bordeaux I
351 Cours de la Libération, 33405 Talence Cedex (France)
Fax: (+33) 5-4000-6646
E-mail: d.astruc@lcoo.u-bordeaux1.fr
Dr. S. Gatard, Prof. R. Neumann
Department of Organic Chemistry
Weizmann Institute of Science
76100 Rehovot (Israel)

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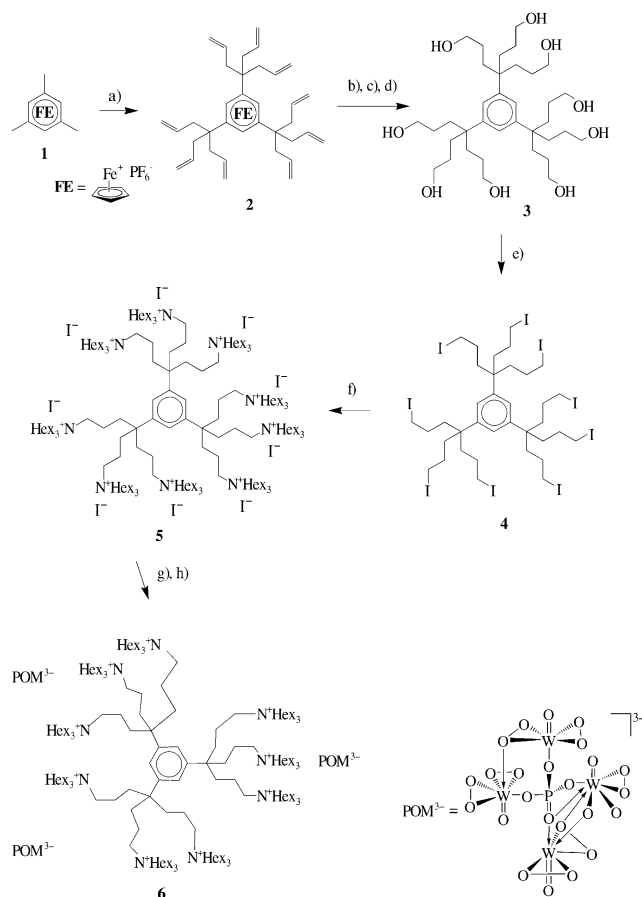


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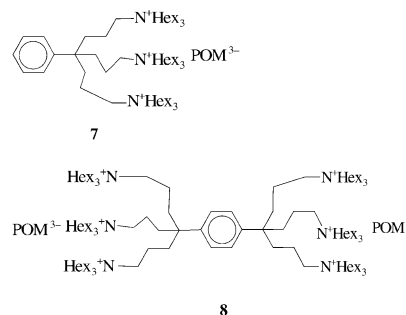
new series of five metallodendrimers based on ionic bonding between dendritic polyammonium polycations and trianionic polyoxometalates (POMs) and their use as recoverable and reusable catalysts in oxidation reactions. POMs are the source of fascinating architectures^[8] and very rich redox chemistry^[9] that provides the basis for their catalytic activity in oxidation reactions.^[10] There is only one report of catalysis by using a dendritic-type framework that contains POMs: the oxidation of tetrahydrothiophene to its sulfoxide by *t*BuOOH and H₂O₂ was catalyzed by a compound consisting of four POM units covalently bonded to a tetradirectional core.^[11]

Among the POM oxidation catalysts, we have selected the peroxophosphotungstate $[\text{PO}_4\{\text{WO}(\text{O}_2)_2\}_4]^{3-}$, which is known for its catalytic properties in olefin epoxidation and alcohol oxidation.^[11,12] To build the supramolecular assembly of the dendritic oxidation catalysts in which each POM trianion is connected to a trication, we have designed tricationic tripods that consist of triammonium groups. For this purpose, our synthetic strategy involves polymethyl arenes in which each methyl group can be functionalized by three allyl groups by using the temporary complexation/activation by the 12-electron fragment $[\text{CpFe}]^+$ (see Scheme 1, the example is mesitylene).^[13] Regioselective hydroboration of the nona-

alcene **2**, which was produced from **1**, and oxidation of the nona-borane to give the primary nona-ol **3** followed by treatment with $\text{SiMe}_3\text{Cl}/\text{NaI}$ provided the nona-iodo compound **4**.^[14] Alkylation of tri-hexylamine by using **4** gave the nona-ammonium salt **5** as an iodide that cannot be used as such for oxidation chemistry, because of facile oxidation of the iodide anion.^[15] Treatment of **5** with AgBF_4 in ethanol gave the BF_4^- ammonium salt. The latter was transformed into the desired nona-ammonium salt **6** of $[\text{PO}_4\{\text{WO}(\text{O}_2)_2\}_4]^{3-}$ in dichloromethane by treatment with an aqueous solution of the commercial heteropolyacid $\text{H}_3\text{PW}_{12}\text{O}_{40}$ and H_2O_2 . A peroxotungstate product, $[\{\text{WO}(\text{O}_2)_2(\text{H}_2\text{O})\}_2\text{O}]^{2-}$, was easily removed, as it remained in the aqueous phase.^[12d] This procedure was also applied to toluene and *p*-xylene providing the mono and bis(POM)-triammonium salts **7** and **8**. Standard



Scheme 1. Synthesis of the tris(POM) complex **6**. a) KOH, $\text{CH}_2=\text{CH}-\text{CH}_2\text{Br}$, DME, -50°C to RT. b) PPh_3 , CH_3CN , RT, 12 h $h\nu$ (Hg lamp). c) BH_3 , isobutene, THF, RT, 12 h. d) aq NaOH, 3 M, H_2O , 30% H_2O_2 , 50°C , 12 h. e) NaI, CH_3CN , Me_3SiCl , 50°C , 12 h. f) NHHex_3 , CH_3CN , 80°C , 12 h. g) AgBF_4 , EtOH, RT. h) $\text{H}_3\text{PW}_{12}\text{O}_{40}$, H_2O_2 , H_2O , CH_3Cl .



characterization techniques, such as ^1H and ^{31}P NMR spectroscopies and elemental analysis, were applied to the dendrimers, the results of which are reported in the Supporting Information. AFM characterization of the tris(POM) dendrimer is shown in Figure 1, and the height of the complex recorded by this technique, 2.9 nm, indicates the presence of

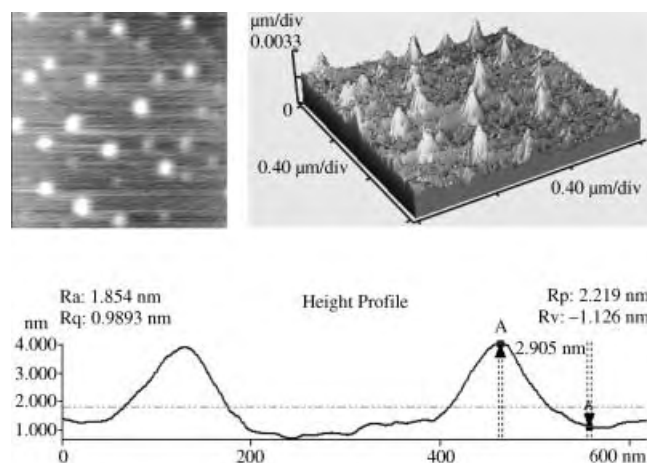
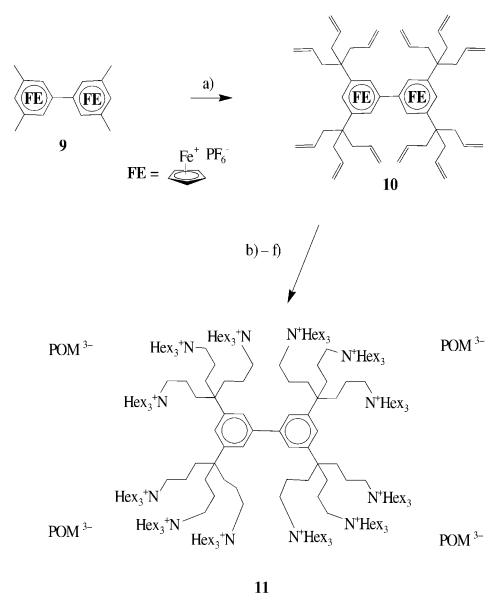


Figure 1. AFM images of the tris(POM) dendrimer **6** on graphite HOPG support shown in two dimensions (left) and three dimensions (right). The height is reproducibly 2.9 nm (bottom) indicating a monolayer of monomeric tris(POM) dendrimer **6**. AFM was operated in the tapping-mode with a resonance frequency of around 200 MHz. The tip has a radius of 10 nm.

monomeric species, a feature that is evidenced by the good solubility properties.

Since the trifunctionalization of polymethyl arenes used here requires that each methyl group be free of its methyl neighbor in the arene structure, thus it cannot be extended to more than three methyl groups on a single arene ring. To extend the arene system to four methyl groups, we used the dinuclear tetramethylbiphenyl complex **9**, which was recently shown to provide the dodeca-allyl derivative **10** by treatment with KOH/DME (DME = 1,2-dimethoxy ethane) and allyl bromide at room temperature.^[16] This synthetic strategy was successfully employed and also provides the tetra(POM) polyammonium compound **11** according to Scheme 2.



Scheme 2. Synthesis of the tetra(POM) complex **11**. a) $t\text{BuOK}$, $\text{CH}_2=\text{CHCH}_2\text{Br}$, THF, 3 d, b) PPh_3 , CH_3CN , RT, 12 h $h\nu$ (Hg lamp). c) BH_3 , isobutene, THF, RT, 12 h. d) aq. NaOH , 3 M, H_2O , 30% H_2O_2 , 50 °C, 12 h. e) NaI , CH_3CN , Me_3SiCl , 50 °C, 12 h. f) NHHex_3 , CH_3CN , 80 °C, 12 h. g) AgBF_4 , EtOH, RT, h) $\text{H}_3\text{PW}_{12}\text{O}_{24}$, H_2O_2 , H_2O , CH_3Cl .

We also wished to compare the stability, solubility, recovery, and reuse properties within a series of ionic dendrimers that contain between 1 and 6 POM units. Thus, we designed a new pathway to synthesize a hexa(POM) ammonium compound. Therefore, we used the new $[\text{CpFe}]^+$ -induced hexa-alkenylation of the C_6Me_6 ligand of **12** to obtain **13** (Scheme 3),^[17] followed by the analogous synthesis of the hexa-ol **14** and hexamesylate **15**. Then, the latter was allowed to react with the phenolate dendron $p\text{-O}^-\text{-C}_6\text{H}_4\text{C(allyl)}_3$,^[14,18] to give the 18-allyl dendrimer **16**, which was further converted to the octadecyl ammonium iodide, BF_4^- , and hexa(POM) salt **17** in a way similar to that described above for the mono-, bis-, and tris(POM). An important difference between this hexa(POM) salt **17** and the other salts (**6**, **8**, and **11**) however, is that it is insoluble in all common solvents, which indicates a polymeric form. Nevertheless, **17** has been characterized by its correct elemental analysis.

Epoxidation of cyclooctene by 35 % aq H_2O_2 in a biphasic water/ CDCl_3 system was carried out at room temperature, and was catalyzed by 0.3 % equivalents of POM embedded in the ionic dendrimer. Under these conditions, monitoring of the kinetics by plotting the ratio between the intensity of the disappearing ^1H NMR signal of cyclooctene at $\delta = 5.6$ ppm versus TMS and that of the rising peak of the epoxide at $\delta = 2.9$ ppm versus TMS showed that the reaction was quantitative after 5 h. A comparison of the homogeneous mono-, bis-, tris-, and tetra(POM) catalysts indicated that there was no measurable dendritic effect on the reaction kinetics within this series.

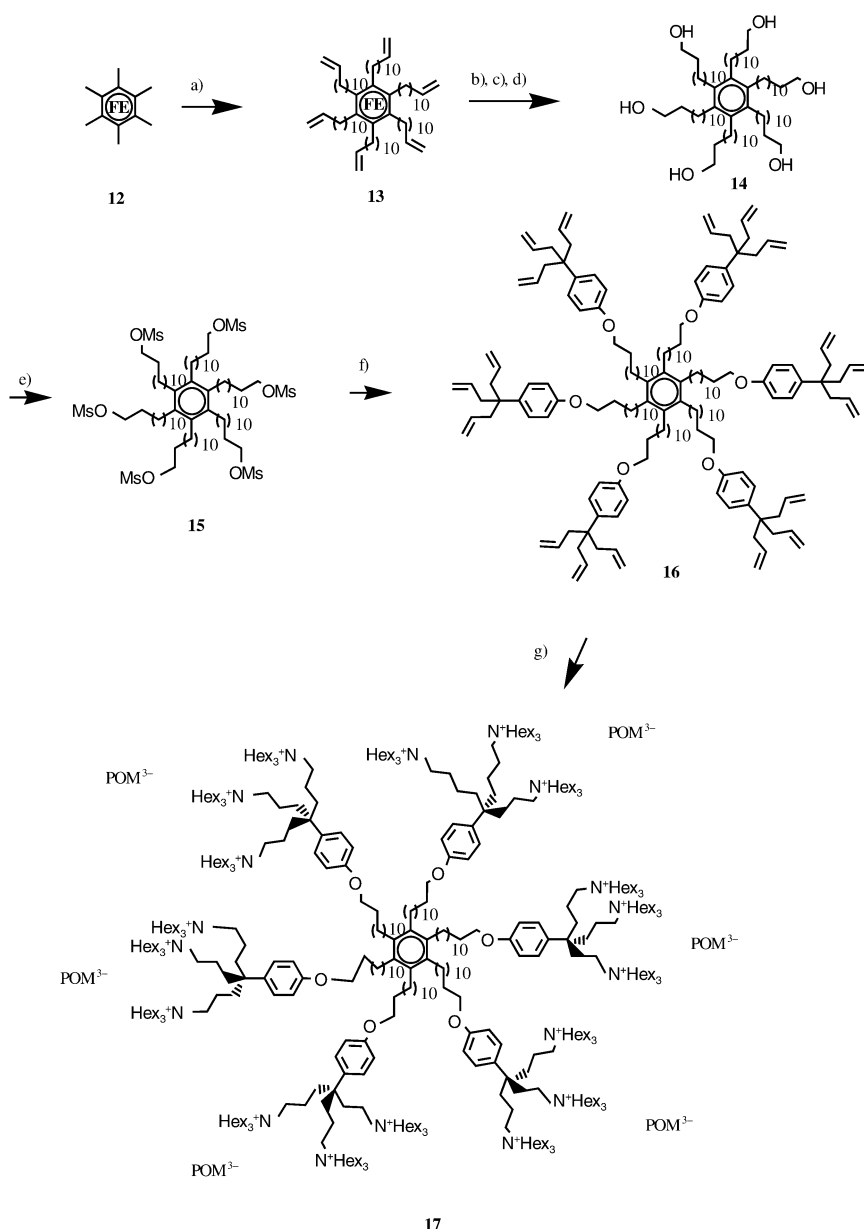
A dendritic effect, however, was noted in the recovery of the catalysts. The catalysts are precipitated from the organic phase by the addition of pentane leaving a clear solution in the case of the tris and tetra(POM) catalysts **6** and **11**, whereas precipitation of the mono(POM) catalyst **7** is tedious and leaves a white colloidal solution. Therefore, the recovery of the tri and tetra(POM) catalysts is easier than that of the mono(POM) catalyst. Out of a catalytic amount as low as 20 mg, the recovery of **6** by precipitation and filtration was between 80 and 85 % after each catalytic cycle, and the efficiency of this catalyst was reproducible. With **11**, the catalyst recovery reached 96 %. After catalysis, the ^{31}P NMR spectra of **6** and **11** were unchanged.

It was noticed however that, if the dendritic POM catalysts were left for several days in air, they became less soluble in CDCl_3 , and their ^{31}P NMR spectrum changed (peak shift from $\delta = 3.45$ to 1.11 ppm). Then, their catalytic activity was suppressed. Preliminary experiments indicate that the mono(POM) catalyst is more sensitive to this deactivation than the tris and tetra(POM) catalysts. If the POM catalysts are kept under an inert atmosphere, they are not the subjects of deactivation. Although this deactivation procedure was very slow with **6**, since two catalytic cycles with recovery in air could be performed without storing this catalyst under an inert atmosphere, it is best to store the POM catalyst under argon for prolonged periods of time. Indeed, storage of **6** for several months under an inert atmosphere allows further catalytic use without problem.

A successful catalysis experiment was also carried out by using the heterogeneous hexa(POM) catalyst **17**, which led quantitatively to epoxycyclooctane and to direct recovery of the catalyst by filtration.

The oxidation of thioanisole to the corresponding sulfone by H_2O_2 in the biphasic water/ CDCl_3 system could also be catalyzed by 0.1 % of the tris(POM) complex **6** and was selective and quantitative at room temperature after two hours. This reaction does not work in the absence of catalyst. The catalyst **6** was recovered analogously, as in the epoxidation experiments, and it was checked by ^{31}P NMR spectroscopy that it was unchanged after recovery.

In conclusion, we have designed and assembled nanosized ionic dendritic polyammonium POM oxidation catalysts **6**, **7**, **8**, **11**, and **17**, in which a peroxophosphotungstate trianion is bound to a tripod terminated by three trihexylammonium groups. A comparison of the homogeneous POM dendritic epoxidation catalysts showed that their efficiency (kinetics) were the same, but recovery of the soluble tris and tetra-



Scheme 3. Synthesis of the hexa(POM) complex **17**. a) KOH, 1-iodo-11-undecene, DME, 60 °C, 3 d. b) PPh₃, CH₃CN, *hν*, 16 h (Hg lamp), RT. c) BH₃, isobutene, THF, 1 d, RT. d) NaOH, 30% H₂O₂, H₂O. e) CH₃SO₃Cl, C₅H₅N, HCl, 4 h, −5 °C–0 °C. f) *p*-O[−]-C₆H₄C(allyl)₃, KCO₃, Me₂CO, reflux 2.5 days.^[14] g) BH₃, isobutene, THF, RT, 12 h, then aq NaOH, 3 M, H₂O, 30% H₂O₂, 50 °C, 12 h, then NaI, CH₃CN, Me₃SiCl, 50 °C, 12 h, then NHex₃, CH₃CN, 80 °C, 12 h, then AgBF₄, EtOH, RT, then H₃PW₁₂O₄₀, H₂O₂, H₂O, CH₃Cl. The locations of the counteranions are arbitrary.

(POM) catalysts **6** and **11** was complete, which was in contrast to that of the mono(POM) **7**, whereas the insoluble hexa(POM) **17** worked heterogeneously. Characterization of **6** by AFM confirms its monomeric structure, and it can be stored under an inert atmosphere for months without change for efficient reuse as a catalyst. Finally, this synthetic and catalytic study shows the usefulness of the ionic-dendrimer approach for the design and efficient use of fully recyclable oxidation catalysts. The large dendritic POM catalysts may also prove interesting in the near future for continuous epoxidation by using a membrane reactor.^[19]

Experimental Section

General procedure for the synthesis of dendritic POM complexes: The dendritic polyallyl, polyol, and polyiodo derivatives were synthesized according to procedures analogous to those described in references [14] and [16]. For the synthesis of the dendritic polyammonium polyiodide salts, a mixture of iodo compound and trihexylamine in CH₃CN was stirred for 12 h at 80 °C. After removal of the solvent under vacuum, the residue was washed with pentane and dissolved in about 2 mL of CH₃CN. Then, 30 mL pentane was added to precipitate the polyammonium polyiodide salt. The polyammonium BF₄[−] salt was prepared by adding AgBF₄ to an ethanol solution of ammonium iodide salt. AgI precipitated immediately and was removed by filtration, the ethanol was removed by evaporation, and the residue was extracted with CH₂Cl₂. The solvent was then removed under vacuum, yielding the ammonium BF₄[−] salt. The dendritic polyammonium POM salts were synthesized by adding H₂O₂ (35% in water) to a water solution of commercial heteropolyacid H₃PW₁₂O₄₀. The mixture was stirred at room temperature for 30 min. Then, a CH₂CH₃ solution of ammonium BF₄[−] salt was added, and the mixture was stirred for an additional hour. The CH₂Cl₂ layer was dried over sodium sulphate and evaporated under vacuum, thus providing the desired dendritic POM complex as a white solid.

General procedure for catalytic reactions and catalyst recycling: The POM catalyst **6**, **7**, **8** or **11** (4.68 mmol) and cyclooctene (284 equiv per POM unit) were dissolved in 3 mL CDCl₃, then H₂O₂ (35% in water, 773 equiv per POM unit) was added. The mixture was stirred at room temperature, and the reaction was followed by ¹H NMR spectroscopy. After completion of the reaction, the CDCl₃ layer was concentrated under reduced pressure to about 1 mL, and 5 mL of pentane was added producing a white precipitate. The solid was filtered over celite then redissolved with CH₂Cl₂, and the solution was dried over sodium sulfate. The solvent was then removed under vacuum, yielding the POM complex as a white solid that was analyzed by ¹H and ³¹P NMR spectroscopies, stored under nitrogen, and reused for a subsequent catalytic experiment.

Supporting Information available: Complete synthetic procedures and standard characterizations for all the dendrimers.

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Keywords: dendrimers · epoxidation · homogeneous catalysis · polyoxometalates · tungsten

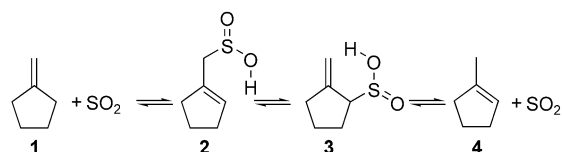
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Polysulfones: Catalysts for Alkene Isomerization**

Dean Marković and Pierre Vogel*

It has long been known that alkenes can be isomerized by sulfur dioxide. The process is generally explained by invoking an ene reaction between an SO₂ molecule and the alkene to give a β,γ-unsaturated sulfinic acid intermediate (e.g. **1** + SO₂ → **2**; Scheme 1), which then undergoes a [1,3] sigmatropic shift to form an isomeric β,γ-unsaturated sulfinic acid (**2** → **3**), followed by a retro-ene reaction that eliminates SO₂ (**3** → **4** + SO₂).^[1]



Scheme 1. General mechanism for SO₂-catalyzed isomerization of methylenecyclopentane (**1**).

When we treated methylenecyclopentane (**1**) with various amounts of SO₂ in CD₂Cl₂ at 0°C, isomerization into 1-methylcyclopentene (**4**) occurred within a few hours. However, in the presence of a small amount of CH₂I₂, the reaction was totally inhibited. As 1,1-diodoalkanes in the presence of SO₂ are suspected to generate iodine radicals,^[2] this striking observation suggests that the SO₂-promoted isomerization **1** → **4** does not follow the generally accepted mechanism **1** → **2** → **3** → **4**, but rather another mechanism that involves radical intermediates. This hypothesis was confirmed by the observation that TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy free radical) and Bu₃SnH both efficiently inhibit the SO₂-promoted isomerization **1** → **4**. In the absence of radical-scavenging agents, the kinetics of the SO₂-promoted isomerization **1** → **4** showed an induction period, the duration of which increased upon decreasing the SO₂ concentration. This period was followed by a zeroth-order reaction until about 70% conversion. During the induction period a white precipitate formed, the amount of which increased upon

[*] D. Marković, Prof. Dr. P. Vogel
Institute of Chemical Sciences and Engineering,
Swiss Federal Institute of Technology (EPFL),
BCH, 1015 Lausanne (Switzerland)
Fax: (+41) 21-693-9355
E-mail: pierre.vogel@epfl.ch

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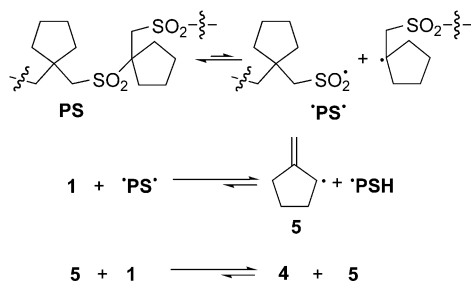
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increasing the proportion of SO_2 . Elemental analysis and IR spectra confirmed that the precipitate is a 1:1 copolymer of **1** and SO_2 , a polysulfone (**PS**). Titration of this polymer with aqueous NaOH (0.1M) neutralized the free sulfinic acid moieties and allowed us to evaluate an average molecular mass of 230 000.

The ESR spectrum of the polymer **PS** taken during its formation showed typical signals for carbon-centered (tertiary) and sulfonyl radicals.^[3] At 298 K, their estimated concentrations amount to approximately 10^{-6} M.

When freshly prepared **PS** or neutralized **PS** (with aqueous NaOH) was added to a solution of **1** in CD_2Cl_2 an immediate zeroth-order isomerization **1**→**4** occurred at 0°C. The reaction was inhibited efficiently with TEMPO and Bu_3SnH . Polysulfones are known to equilibrate with radicals upon heating (ceiling temperature of polysulfone formation).^[4,5] These radicals can abstract a hydrogen atom from **1** to give the allyl radical intermediate **5** (initiation step, Scheme 2.). Intermediate **5** then reacts with **1** to produce **4** during which allyl radical **5** is regenerated (propagation step).

Isomerization of **1**→**4** was also induced upon irradiation or heating (120°C) of **1** in the presence of a catalytic quantity of $(\text{PhSO}_2)_2$ (diphenyldisulfone);^[6] however, polymeric material was also formed. Therefore the $\text{PhSO}_2\cdot$ radical generated under these conditions is not as selective a catalyst as **PS** for the alkene isomerization, perhaps because of its ability to add to the alkene concurrently with hydrogen abstraction. All the experiments reported above are consistent with the radical chain mechanism shown in Scheme 2. Further proof was obtained in the following way.



Scheme 2. Radical mechanism for the polysulfone-catalyzed isomerization of alkenes.

Upon treatment with nondeuterated **PS** at 0°C, a 1:1.06 mixture of hexadeuterated methylidenecyclopentane (**6**)^[7] and **1** in CD_2Cl_2 (toluene, $[\text{D}_8]$ toluene as internal reference) was isomerized into a mixture of nondeuterated and partially deuterated 1-methylcyclopentenenes. The $^2\text{H}\{^1\text{H}\}$ -NMR spectrum of the mixture displayed three signals for the methyl group corresponding to CH_2D , CHD_2 , and CD_3 with relative integrations of 1.31:1.93:1 (Figure 1).^[8] This observation proves the intermolecular nature of hydrogen migration between the allylic methylene group in **1** (**6**) and the terminal carbon atom of the allyl radicals. Thus the signal for CHD_2 is due to a hydrogen atom transfer from **1** to the allyl radical $[\text{D}_5]\mathbf{5}$ (which arises from **6**), whereas the signal for CH_2D is a result of a deuterium atom transfer from **6** to the non-

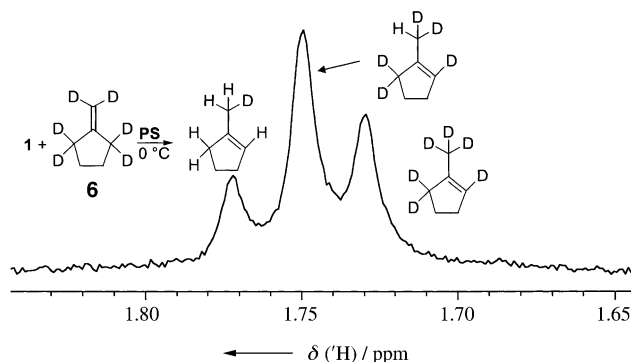


Figure 1. Partial $^2\text{H}\{^1\text{H}\}$ -NMR spectrum of 1-methylcyclopentene (methyl region) arising from the reaction **1** + **6**→**4** (CD_2Cl_2 , 0°C).

deuterated allyl radical **5**. At the end of the isomerization (20 more half-lives), the ^1H - and $^2\text{H}\{^1\text{H}\}$ -NMR spectra did not change, showing that the exchange between positions C3,4 and the methyl groups in 1-methylcyclopentenenes is much slower than the isomerization **1**→**4**.

The polysulfone **PS** catalyzed the isomerization of the methylidenecyclohexane into 1-methylcyclohexene, 2-methylbut-1-ene into 2-methylbut-2-ene, 2-ethylbut-1-ene into 2-ethylbut-2-ene, and 2-methylhept-1-ene into 2-methylhept-2-ene in CD_2Cl_2 at room temperature. All reactions were high-yielding, with no formation of polymeric material (^1H NMR spectroscopic analysis). This was not the case with the SO_2 -induced isomerizations, which were all accompanied by the formation of polymers.

In summary, we have uncovered a new mechanism for alkene isomerization induced by sulfur dioxide: The polysulfone polymers resulting from their co-condensation are organic catalysts for the alkene isomerization.

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Keywords: alkenes · isomerization · organocatalysts · polysulfones · radicals

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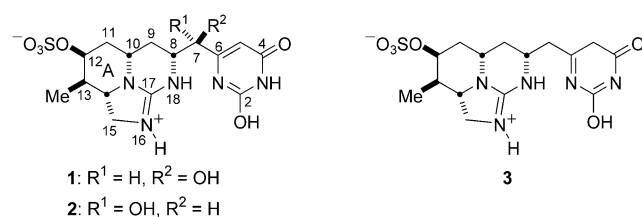
Natural Product Synthesis



A Concise Asymmetric Synthesis of the Marine Hepatotoxin 7-Epicylindrospermopsin**

Ryan E. Looper and Robert M. Williams*

The cyanobacterial toxin cylindrospermopsin (**1**) was isolated as the principal hepatotoxin from *Cylindrospermopsis raciborskii* in 1992 after suspicion of its involvement in an outbreak of hepatoenteritis that hospitalized 150 people on



[*] R. E. Looper, Prof. Dr. R. M. Williams
Department of Chemistry
Colorado State University
Fort Collins, CO 80523 (USA)
Fax: (+1) 970-491-3944
E-mail: rmw@chem.colostate.edu

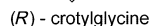
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Palm Island, Australia.^[1] It has since been isolated in Japan from *Umezakia natans* and in Israel from *Aphazinenon ovalisporum*.^[2] Following the discovery of the parent compound, 7-epicylindrospermopsin (**2**) was isolated from *A. ovalisporum* as a toxic minor metabolite.^[3] The natural congener 7-deoxycylindrospermopsin (**3**) was initially isolated from *C. raciborskii* and has recently been co-isolated with **1** in China from *Raphidiopsis curvata*.^[4] Cylindrospermopsin has been shown independently to be a potent hepatotoxin (LD₅₀ ~ 0.2 mg kg⁻¹ in mice), and 7-epicylindrospermopsin is equipotent with **1**, whereas **3** is nontoxic.^[1,4a,5] It is thought that the toxicity of these substances results from a general inhibition of protein synthesis, but it is unclear whether this action occurs at the ribosomal or transcriptional level by the interaction of oxidized metabolites with DNA.^[6,7] The threat posed to global public health by these molecules in drinking water and the isolation of *C. raciborskii* in several regions of the United States have prompted the National Toxicology Program (NTP) of the National Institutes of Health and the Unregulated Contaminant Monitoring Rule (UCMR) of the Environmental Protection Agency to elect **1** for toxicological and environmental evaluation.^[8] The paucity of understanding of the biological functions of these agents has prompted us to develop an efficient and flexible synthetic approach that will be adapted to the production of uracil analogues to be deployed for biomechanistic evaluation.

These highly polar compounds pose a significant synthetic challenge as they contain almost as many heteroatoms as carbon atoms, and their structures include a zwitterionic guanidinium sulfate, a rare tetrasubstituted tricyclic guanidine, and a uracil moiety. Not surprisingly, these natural products have attracted the attention of synthetic chemists for over ten years.^[9] Snider and co-workers completed the first racemic total synthesis of cylindrospermopsin in 20 steps eight years after its discovery.^[10] However, their studies failed to illuminate the misassigned stereogenic center at C7. The configuration at that center was corrected by Weinreb and co-workers in an elegant 30-step racemic, stereoselective synthesis of **2**, thus validating the illustrated structures.^[11] Shortly thereafter White and Hansen completed the first asymmetric total synthesis of **2** in 28 steps and confirmed the absolute stereochemistry as 7*S*, 8*R*, 10*S*, 12*S*, 13*R*, 14*S*.^[12]

We envisaged that the three contiguous stereogenic centers of the A ring of cylindrospermopsin could be constructed by a single intramolecular 1,3-dipolar cycloaddition of the nitron **4** to yield the tricyclic compound **5** (Scheme 1). At the onset of our work, the absolute stereochemistry of these natural products was not known. Retrosynthetic analysis suggested that the nitron **4** could be derived from a simple amino acid, namely (*R*)- or (*S*)-crotylglycine, so that **1** could be constructed from a precursor with a single stereogenic center, which would provide the inherent flexibility to produce either enantiomer of the natural product. The two remaining, remote stereogenic centers at C7 and C8 and their respective oxidation states define this family in terms of both structure and function. Considering this, it was anticipated that a nitroaldol (Henry reaction) strategy would permit access to both C7 epimers (**1** and **2**), and through reductive nitromethylation provide access to **3**.



Scheme 1. Retrosynthesis of the cylindrospermopsins. PMB = *p*-methoxybenzyl.

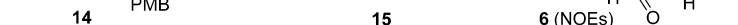
To this end, the protected crotylglycine derivative **11** was prepared from our oxazinone template **10** (Scheme 2).^[13] Thus, alkylation of **10** with (*E*)-crotyl iodide gave the



Scheme 2. a) KHMDS, (*E*)-crotyl iodide, THF, -78°C , 92%, $>99\%$ *ee*; b) Li, NH_3 , THF, EtOH, 68–87%; c) AcCl , MeOH, $0^{\circ}\text{C} \rightarrow \text{RT}$; d) LiAlH_4 , THF, 65% (2 steps); e) $\text{BrCH}_2\text{CO}_2\text{Ph}$, $i\text{Pr}_3\text{N}$, MeCN, 63–80%; f) MCPBA, Na_2HPO_4 , CH_2Cl_2 , -78°C , 84%; g) PhMe, 200°C (sealed tube), 78%. KHMDS = potassium hexamethyldisilazide, MCPBA = 3-chloroperoxybenzoic acid.

corresponding crotylated lactone in 92% yield with > 99% *ee*.^[14] Reductive removal of the chiral auxiliary with lithium in ammonia gave **11** in moderate to good yield. Acidic removal of the Boc group with concomitant methyl ester formation followed by reduction with lithium aluminum hydride gave the optically pure alcohol **12**. This substance was then transformed into the free morpholinone in a one-pot procedure by treatment with bromophenyl acetate.^[15] Oxidation of the secondary amine was most conveniently effected by treatment with purified MCPBA in dichloromethane to give the oxazinone *N*-oxide **4** in 84% yield.^[16] Exposure of **4** to elevated temperatures gave the tricyclic isoxazolidine **5** in 78% yield as a 10:1 mixture with the minor product assumed to arise from an *endo* approach of the alkene to the nitron.^[9i,17] We also observed that the treatment of **4** with scandium triflate at room temperature for up to three days generated **5** in an improved 12:1 mixture. The relative stereochemistry of **5** was confirmed by X-ray crystallography.^[9j]

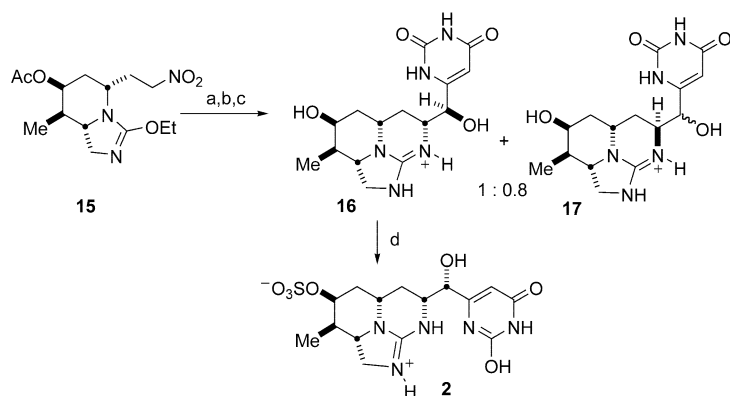
The installation of N16 proved difficult as it necessitated a simultaneous oxidation-state change at C15. It was eventually found that a two-step protocol in which the lactone carbonyl group was first reduced to the corresponding lactol with DIBAL-H fulfilled this requirement (Scheme 3). Tandem reductive amination/N–O bond cleavage gave a free diamine which was more readily handled as the urea **13** after in situ treatment with bis(*p*-nitrophenyl)carbonate.^[18] Selective oxidation of the primary alcohol with TEMPO gave the sensitive aldehyde **6** in 75 % yield.^[19] Most common oxidation protocols showed selectivity for the secondary hydroxy group or led to epimerization of the sensitive α -ureidoaldehyde. Eventually it was found that the addition of methanesulfonic acid (1 mol %) accelerated the reaction to give **6** without attendant epimerization, as evidenced by ¹H NMR NOE experiments. The aldehyde **6** underwent nitromethylation upon treatment with the lithium salt of nitromethane in THF. The resulting



Scheme 3. a) DIBAL-H, CH_2Cl_2 , -78°C , 87%; b) PMBNHN_2 , $\text{H}_2/\text{Pd/C}$, EtOAc, then $(p\text{-NO}_2\text{PhO})_2\text{CO}$, MeCN, 81%; c) TEMPO, $\text{PhI}(\text{OAc})_2$, MsOH (1 mol%), CDCl_3 , 75%; d) MeNO_2 , $n\text{BuLi}$, THF, room temperature, 84%; e) Ac_2O , DMAP, CH_2Cl_2 , then NaBH_4 , EtOH, 67%; f) TFA (neat), reflux, 80%; g) Et_3OBF_4 , Cs_2CO_3 , CH_2Cl_2 , 78%. DIBAL-H = diisobutylaluminum hydride, DMAP = 4-dimethylaminopyridine, MsOH = methanesulfonic acid, TEMPO = 2,2,6,6-tetramethylpiperidine *N*-oxide, TFA = trifluoroacetic acid.

nitroalcohol was peracetylated with acetic anhydride, and the nitroalkene was then reduced to afford the nitroalkane **14** in 67% yield. The PMB group was removed cleanly in TFA at reflux to give an 80% yield of the urea poised for alkylative activation.^[20] Attempts to methylate the oxygen atom of the urea gave an unstable compound that decomposed. Fortunately, *O*-ethylation with triethyloxonium tetrafluoroborate gave the satisfactorily stable isourea **15** (78% yield), which could be subjected to subsequent reactions.^[21]

Initial attempts to effect the nitroaldol reaction yielded essentially equimolar amounts of all four C7/C8 diastereomers.^[22, 23] The treatment of **15** and 2,6-dimethoxypyrimidine-4-carbaldehyde with 2 equivalents of tetra-*n*-butylammonium fluoride for short reaction times, followed by reductive guanidinylation of the nitro group, gave the best selectivities (Scheme 4).^[24, 25] These conditions afforded a kinetic 1:0.8 mixture in favor of the diastereomer required for the syn-



Scheme 4. a) 2,6-Dimethoxypyrimidine-4-carbaldehyde, TBAF (2.0 equiv), -15°C , THF; b) $\text{Pd}(\text{OH})_2$, H_2 , 5% AcOH/MeOH ; c) conc. HCl , reflux, 32% for **16**, 29% for **17** (3 steps); d) SO_3 ·pyridine, DMF, MS (3 Å), 59%. DMF = *N,N*-dimethylformamide, TBAF = tetra-*n*-butylammonium fluoride.

thesis of **2**. The other diastereomer in the mixture led to **17**, which is epimeric at C7 with a diastereomer described by Snider and co-workers (relative configuration not assigned).^[10] It was found that the nitroaldol reaction must be quenched with acetic acid and the products immediately subjected to reduction conditions. Isolation of the products after treatment with TBAF provided ~1:1:1:1 mixtures of the nitroalcohols, thus indicating that the reaction is indeed highly reversible. At this stage the diastereomeric dimethoxypyrimidines were inseparable. Acidic hydrolysis of the pyrimidines gave a separable mixture of **16** (32% yield from **15**) and **17** (29%).^[10,12] In our hands, the sulfonation of the C12 hydroxy group proved capricious under the conditions reported in the literature.^[10–12] The use of sulfur trioxide/pyridine complex in DMF with 3-Å molecular sieves gave **2** reproducibly in 59% yield,^[26] along with the corresponding bis(sulfate) (2:1 ratio by HPLC), as previously observed by others.^[11,12,27]

The asymmetric synthesis of **2** detailed herein represents the shortest successful route toward this family of natural products. 7-Epicylindrospermopsin has thus been obtained in only eighteen steps with few protecting-group manipulations from commercially available **10**. Investigations directed at controlling the diastereoselectivity of the nitroaldol process to afford cylindrospermopsin (**1**) are ongoing and will be reported in due course. The uracil moiety in **1** and **2** has been deemed essential for their hepatotoxicity.^[28] We believe that the incorporation of the uracil synthon at a late stage in the synthesis should render our strategy amenable to the production of uracil analogues for evaluation of their biological and hepatotoxic activity.

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- [26] Synthetic **2** had spectroscopic properties identical to those reported: $[\alpha]_{\text{D}}^{25} = -12.5$ ($c = 0.04$, H_2O); lit.:^[3] $[\alpha]_{\text{D}}^{24} = -20.5^\circ$ ($c = 0.04$, H_2O).
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Coordination Networks

Luminescent 2D Macrocyclic Networks Based on Starburst Molecules: $[\{\text{Ag}(\text{CF}_3\text{SO}_3)\}_{1.5}(\text{tdapb})]$ and $[\{\text{Ag}(\text{NO}_3)\}_3(\text{tdapb})]^{**}$

Corey Seward, Wen-Li Jia, Rui-Yao Wang,
Gary D. Enright, and Suning Wang*

Luminescent supramolecular architectures have recently attracted much research interest primarily because of their potential applications in optoelectronic devices and as fluorescent sensors and probes.^[1–7] One of the most effective and attractive approaches for the assembly of supramolecular architectures is the use of coordination bonds. It has been demonstrated extensively by many researchers that by

choosing appropriate metal ions and organic building blocks, it is possible to control and manipulate the structures and the resultant properties (electronic, photonic, magnetic, and catalytic) of the supramolecular materials.^[8–13] One way to induce large cavities in supramolecular structures based on coordination compounds is to employ a large organic linker that has well-defined shape and coordination geometry. Large starburst or star-shaped ligands are attractive candidates for this purpose because they form interesting and uncommon extended structures such as hexagonal or triangular macrocycles. Although a few triangle-shaped ligands have been reported as successful linkers for extended cyclic structures,^[7,14] examples of this class of supramolecular structures remain relatively scarce.

Recently we reported a class of triangular starburst molecules that contain three 2,2'-dipyridylamino chelating sites.^[15] We demonstrated that these molecules are efficient blue emitters for organic light-emitting diodes and are capable of binding to metal centers such as Zn^{II} and Pt^{II} through the pyridyl groups to form various discrete starburst metal complexes.^[16] One of the metal complexes, $[(\text{ZnCl}_2)_3(\text{tdapb})]$ ($\text{tdapb} = 1,3,5\text{-tris}(4\text{-(2,2'-dipyridylamino)}\text{-phenyl})\text{benzene}$), has been shown by our group to be a highly selective and sensitive fluorescent sensor for benzene. A crystal structural study revealed that the molecular shape and the extended structure of $[(\text{ZnCl}_2)_3(\text{tdapb})]$ in the solid state are responsible for its high affinity for benzene molecules and the consequent effective fluorescent quenching by the incorporated solvent.^[17]

Encouraged by this finding and intrigued by the possibility of using the tdapb ligand as a triangular linker to achieve novel extended luminescent structures, we investigated the coordination chemistry of tdapb with Ag^{I} ions. Although Zn^{II} and Ag^{I} ions have the same electronic configurations, the Ag^{I} ion has a much stronger tendency to display versatile and often irregular coordination geometry than the Zn^{II} ion. As a consequence, the Ag^{I} ion is much more accommodating toward the ligand and is expected to form extended structures more readily than the Zn^{II} ion.

Two different Ag^{I} salts, $\text{Ag}(\text{NO}_3)$ and $\text{Ag}(\text{CF}_3\text{SO}_3)$ were used in our investigation. The reactions of tdapb with $\text{Ag}(\text{CF}_3\text{SO}_3)$ and with $\text{Ag}(\text{NO}_3)$ in a 1:3 ratio under the same conditions resulted in the formation and isolation of two distinct 2D coordination polymers with the formulae $[\{\text{Ag}(\text{CF}_3\text{SO}_3)\}_{1.5}(\text{tdapb})]_n$ (**1**), and $[\{\text{Ag}(\text{NO}_3)\}_3(\text{tdapb})]_n$ (**2**), respectively. The crystals of both compounds are solvated by benzene, methanol, and dichloromethane molecules, and lose these solvent molecules rapidly when isolated from solution. Compounds **1** and **2** are air-stable.

Crystals **1** and **2** belong to the trigonal space group $R\bar{3}c$ and $R\bar{3}c$, respectively, as determined by X-ray diffraction analyses; the connectivity between the Ag^{I} ions and the tdapb ligands in **1** and **2** is shown. The local coordination environment around the Ag^{I} ions in both compounds is shown in Figure 1. In **1**, only one of the pyridyl groups from each of the 2,2'-dipyridylamino (dpa) units is coordinated to the Ag^{I} ion, whereas in **2** both pyridyl groups of the dpa unit are bound to Ag^{I} ions. In **1**, one Ag^{I} ion links two dpa units from two neighbouring tdapb ligands together, whereas in **2** the same

[*] C. Seward, Dr. W.-L. Jia, Dr. R.-Y. Wang, Dr. G. D. Enright, Prof. S. Wang
Department of Chemistry
Queen's University
Kingston, Ontario, K7L 3N6 (Canada)
Fax: (+1) 613-533-6669
E-mail: wangs@chem.queensu.ca
and
Stacie Institute for Molecular Sciences
National Research Council
100 Sussex Drive, Ottawa, K1A 0R6 (Canada)

[**] $\text{tdapb} = 1,3,5\text{-tris}(4\text{-(2,2'-dipyridylamino)}\text{phenyl})\text{benzene}$. We thank the Natural Sciences and Engineering Research Council of Canada for financial support.

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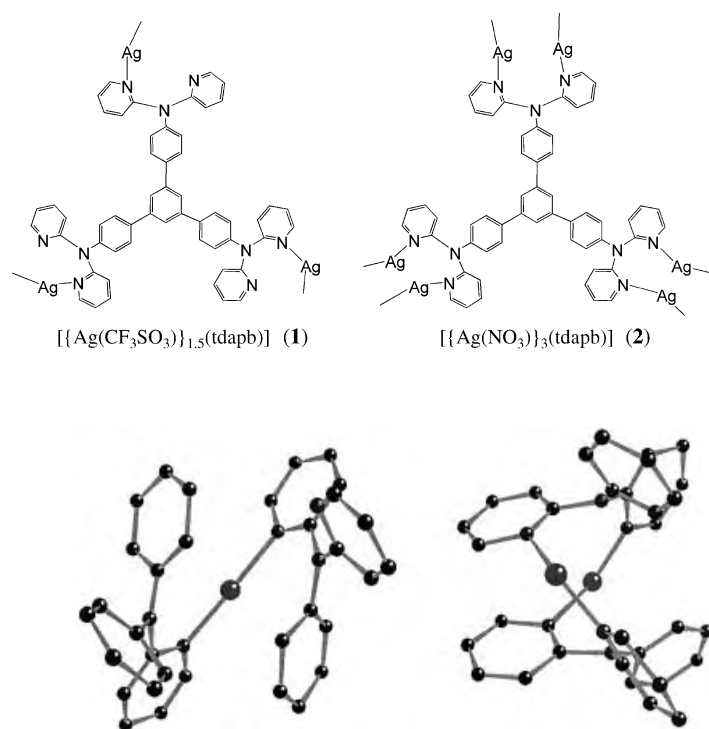


Figure 1. The coordination environment of Ag^{I} ions in **1** (left) and **2** (right).

linkage employs two Ag^{I} ions. Each Ag^{I} ion in **1** and **2** is bound by two nitrogen atoms with normal Ag–N bond lengths in an approximately linear fashion for **1** ($\text{N–Ag–N}' = 171.17(15)^\circ$) and in a bent manner for **2** ($\text{N–Ag–N} = 141.6(5)^\circ, 143.1(4)^\circ$). The anions CF_3SO_3^- in **1** and NO_3^- in **2** are weakly bound to the Ag^{I} ions through one oxygen atom with a long Ag–O bond ($\text{Ag–O} = 2.73(2) \text{ \AA}$ for **1**, $2.56(2) \text{ \AA}$ for **2**). As a consequence, the Ag^{I} ion in both compounds has a distorted three-coordinate geometry.

In **1**, six tdapb ligands are linked together by six Ag^{I} ions in an alternating *up* and *down* fashion to form an approximately honeycomb-shaped macrocycle as shown in Figure 2. The distance between the two opposite pairs of Ag ions on the macrocycle is $\sim 21 \text{ \AA}$. A benzene solvent molecule is located in the center of the macrocyclic cavity, and other solvent molecules such as CH_2Cl_2 and CH_3OH are also located inside the cavity along with the anions. The macrocyclic structure of **1** propagates along two dimensions, resulting in the formation of a 2D layer (Figure 2b). These 2D layers are arranged in the crystal in an *ABAB* fashion resembling that of hexagonal close packing, such that the macrocyclic cavity does not form extended channels. Solvent molecules are found in the space between the 2D layers. Crystals of **1** with a Star-of-David shape can be sheared readily into thin triangular plates by manipulating them with a needle, which is a manifestation of the 2D layered structure.

In **2**, six tdapb ligands are linked together by twelve Ag^{I} ions in a similar *up* and *down* fashion to compound **1**, to form a macrocyclic structure. However, in contrast to the honeycomb structure of **1**, the macrocycle of **2** has an

unusual cloverleaf shape as shown in Figure 3. As a result, the macrocyclic cavity of **2** is much smaller than that of **1** and no benzene solvent molecule is located inside the cavity; CH_3OH and CH_2Cl_2 solvent molecules along with the anions, however, are again found in the cavity as seen with **1**. There are no significant interactions between the solvent molecules and the host framework. The macrocycles of **2** propagate along two dimensions in the same manner as **1** to form a 2D macrocyclic network (Figure 3b). The 2D networks of **2** also stack in an

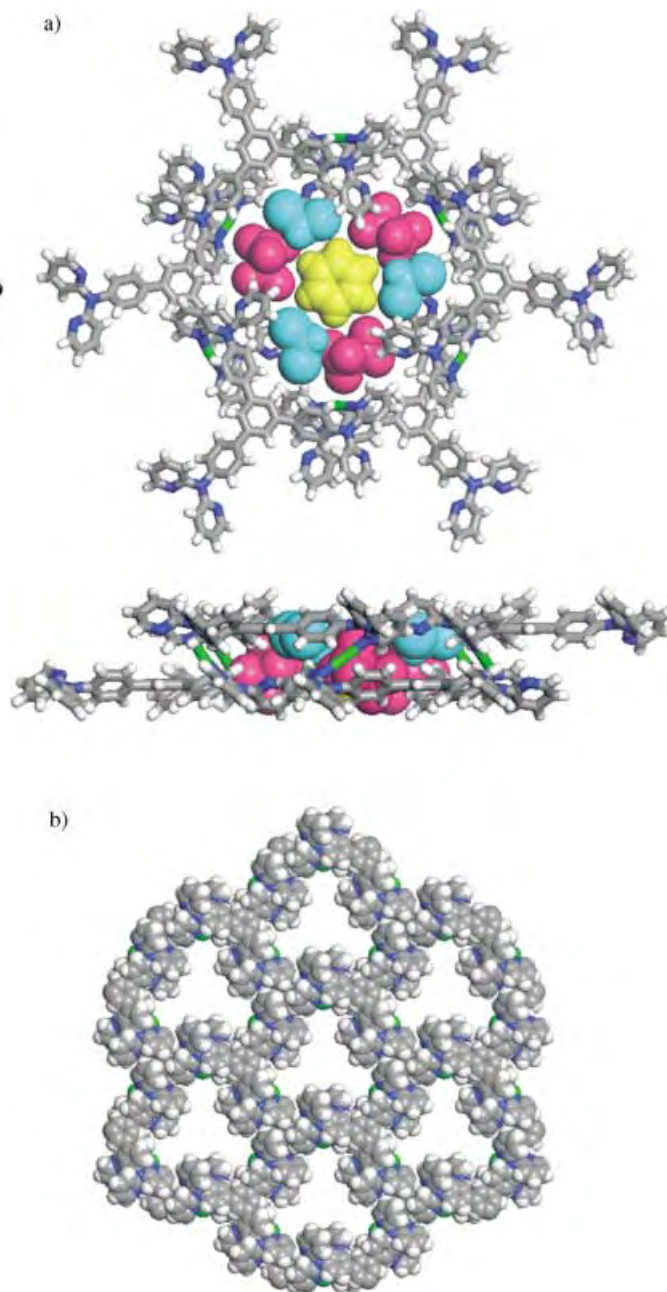


Figure 2. a) The single honeycomb-shaped cavity in **1** (top view and side view). Anions and solvent molecules are shown as space-filling models. Ag: green, N: dark blue, CF_3SO_3^- : purple, CH_2Cl_2 : light blue, C_6H_6 : yellow. Three methanol solvent molecules located above the benzene ring are omitted for clarity. b) The 2D extended framework of **1** shown as a space-filling model with anions and solvent molecules removed.

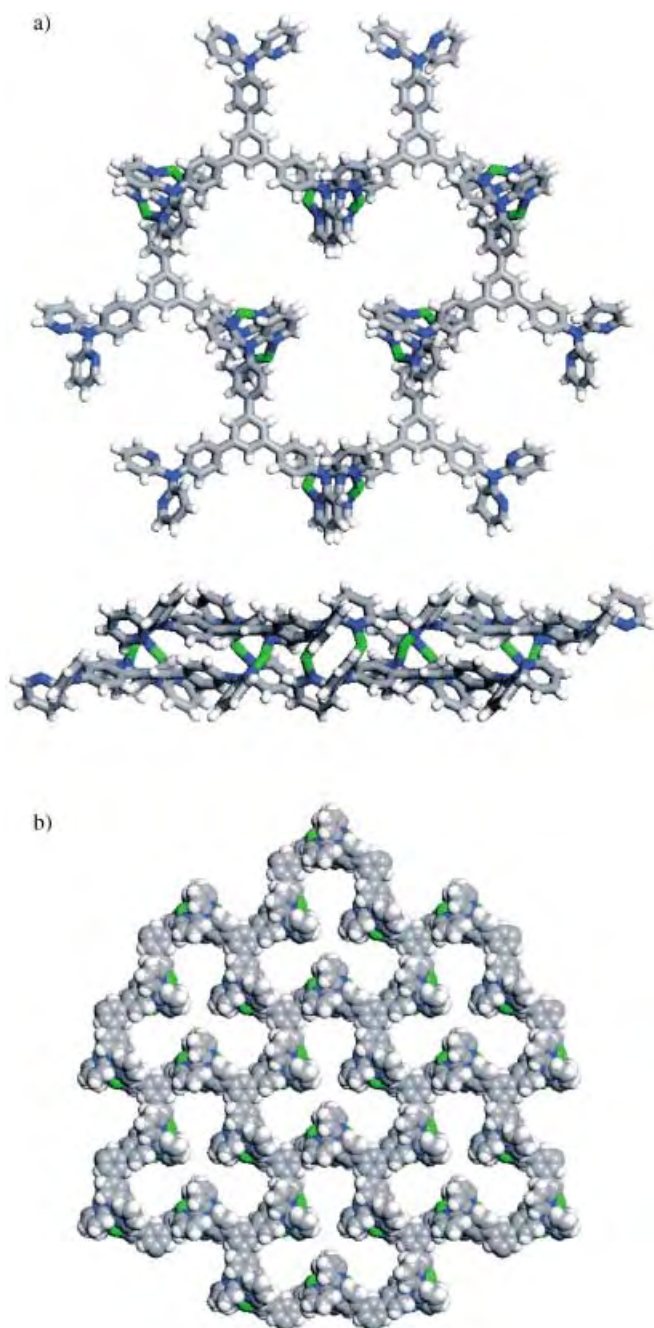


Figure 3. a) The single cloverleaf-shaped cavity in **2** (top view and side view). Anions and solvent molecules are not shown owing to disordering. Ag: green, N: dark blue. b) The 2D extended framework of **2** shown as a space-filling model with anions and solvent molecules removed.

ABAB fashion, resulting again in the absence of solvent channels perpendicular to the 2D layer.

The striking difference between the macrocyclic structure of **1** and **2** is clearly caused by the different number of Ag^I ions present in the framework. In the syntheses of **1** and **2**, the same 1:3 molar ratios of ligand to Ag was used, and yet from the reaction of Ag(CF₃SO₃) with tdapb, only the 1:1.5 product **1** was isolated consistently. The anion appears to have played

a key role in the formation of structures **1** and **2**. The NO₃[−] anion is a stronger donor than the CF₃SO₃[−] anion as indicated by the relatively short Ag–O (NO₃[−]) bond length and the bent N–Ag–N angle in **2**. This, along with the fact that the NO₃[−] anion is much smaller than CF₃SO₃[−], perhaps facilitates the binding of two Ag^I ions at each connecting point between the two tdapb ligands and the formation of a relatively small cavity in **2**. Anion-dependent extended structures have been observed frequently.^[18] However, the relationship between a given anion and the selective formation of a certain type of supramolecular structures is not yet well understood.

1 and **2** are insoluble in common organic solvents. They are slightly soluble in polar solvents such as DMSO, which appear to break down the extended framework and result in the formation of discrete molecular compounds, based on the similar NMR and emission spectra of **1**, **2**, and the free ligand in solution. When irradiated with UV light at ambient temperature, the free ligand tdapb displays bright blue luminescence with λ_{max} at ~400 nm in both the solution (CH₂Cl₂) and the solid states.^[15] Solid samples of **1** and **2** show no detectable luminescence at ambient temperature. However, an intense green emission (λ_{max} at 489 nm, 523 nm for **1**, λ_{max} at 489 nm, 527 nm for **2**) was observed upon excitation of both compounds at 77 K (Figure 4). The green

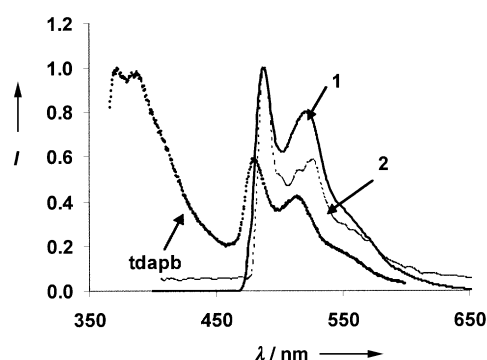


Figure 4. The emission spectra of the free ligand tdapb in CH₂Cl₂ solution at 77 K (excitation at 355 nm) and of **1** and **2** in the solid state at 77 K (excitation at 386 nm and 381 nm, respectively).

emission of the complexes was determined to be phosphorescence, based on their microsecond-order decay lifetimes (6–10 μs). At 77 K, the free ligand tdapb also displays a very weak phosphorescent emission band in the same region as that of **1** and **2** (Figure 4). Therefore, we believe that the green emission of the complexes at 77 K originates from the tdapb ligand.

The absence of detectable fluorescence emission from the complexes in the solid state could be attributed to quenching by the Ag^I ions and interlayer π–π stacking interactions. The Ag^I ion appears to enhance the phosphorescence emission of the ligand since the green emission of the complexes is much brighter (readily visible to the naked eye) than that of the free ligand. Phosphorescence enhancement through the heavy atom effect is a well-known phenomenon,^[19] and many examples have been reported previously.^[20] For example, a frozen solution of the tdapb–Pt^{II} complex [Pt₃Ph₆(tdapb)] in

CH₂Cl₂ displays a ligand-based blue-green phosphorescence band similar to those observed in the case of **1** and **2**.^[20e]

In summary, two novel 2D networks with nanoscale cavities based on a starburst molecule and coordination bonds involving Ag^I ions have been demonstrated. The formation of these unusual structures appears to be anion-dependent. These new networks have large cavities for solvent molecules such as benzene and CH₂Cl₂. The absence of luminescence at ambient temperature precludes their use as fluorescent sensors for organic molecules based on quenching. However, they may find use as sensors/probes based on guest-induced fluorescent enhancement, which is currently being explored in our laboratory.

Experimental Section

The ligand tdapb was synthesized according to published methods.^[1] All syntheses and recrystallizations were conducted in air, in a fumehood, in reaction vials covered with aluminum foil to prevent light-induced decomposition of the silver salts. Elemental analyses were performed by Canadian Microanalytical Services, Delta, BC, Canada. ¹H NMR spectra were recorded on Bruker Avance 300, or 400, or 500 MHz spectrometers. Fluorescent spectra were recorded on a Photon Technologies International Quantamaster Model C-60 spectrometer. UV/Vis absorption spectra were recorded on a HP8542A Diode Array Spectrophotometer.

1: Upon a solution of tdapb (50 mg, 0.0614 mmol) in CH₂Cl₂ (6 mL) were successively layered benzene (4 mL), methanol (4 mL), and a solution of Ag(CF₃SO₃) (47 mg, 0.184 mmol) in methanol (6 mL). The vial was covered in aluminum foil, and the solvents were allowed to diffuse slowly over several days to afford colorless crystals of **1** (68 mg, 92%). Elemental analysis: calcd for Ag_{1.5}C_{55.5}H₃₉N₉O_{4.5}·1.5 CH₂Cl₂: C 52.73, H 3.17, N 9.50; found: C 51.82, H 3.06, N 9.72.

2: Upon a solution of tdapb (50 mg, 0.0614 mmol) in CH₂Cl₂ (6 mL) were successively layered benzene (4 mL), methanol (4 mL), and a sonicated solution of Ag(NO₃) (31 mg, 0.184 mmol) in methanol (6 mL). The vial was covered in aluminum foil, and the solvents were allowed to diffuse slowly over several days to afford colorless crystals of **2** (70 mg, 84%). Elemental analysis: calcd for Ag₃C₅₄H₃₉N₁₂O₉·1.5 CH₂Cl₂: C 45.93, H 2.89, N 11.59; found: C 45.85, H 2.91, N 11.85.

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HIV Inhibition

A Peptidomimetic HIV-Entry Inhibitor Directed against the CD4 Binding Site of the Viral Glycoprotein gp120**

Axel T. Neffe and Bernd Meyer*

The first step in the infection of a human cell with HIV is interaction between the viral envelope glycoprotein gp120 and the human protein CD4.^[1] We used this molecular interaction as a template to design and synthesize a new peptidomimetic compound that has the potential to block the interaction. In HIV infection, binding is followed by a conformational change^[2] after which gp120 can interact with a co-receptor (CCR5 or CXCR4). This interaction finally leads to fusion of the membrane of the virus with that of a human T-cell. The fusion process is mediated by viral gp41 and leads to infection of the human cell. Compounds that inhibit this process are referred to as entry inhibitors.^[3] We present a new way to inhibit the gp120/CD4 interaction through blocking the binding site on the CD4 protein with a peptidomimetic. Other research groups have targeted the same interaction and have developed gp120- or antibody-related peptides,^[4] or gp120-binding molecules.^[5] In our approach, which involves the development of CD4 ligands, interference with the human immune system or viral resistance are potential problems. However, resistance of the virus to inhibitors that bind to CD4 would require large changes in the viral glycoproteins to allow the virus to utilize a different entry mechanism. Such resistance is therefore not as likely as the evasion of inhibitors of viral proteins whose binding can be circumvented by one or two mutations of the viral amino acids.

We started from the CD4-binding decapeptide NMWQKVGTPPL,^[6] which was derived from an X-ray structure of gp120.^[2b] The peptide has a low binding affinity to CD4 ($K_D = 6$ mM, determined by the surface plasmon resonance (SPR) and saturation transfer difference (STD) NMR spectroscopy protocols described herein) but has an antiviral activity that can be detected by a virus neutralization assay. The main targets of the design phase were to lower the molecular weight of the peptide, enhance its binding affinity, and incorporate nonnatural elements to increase proteolytic

stability. The docking of peptides generated by molecular modeling studies by substituting each amino acid of the lead sequence in turn with alanine (see the Supporting Information) supported the results of saturation transfer difference (STD) NMR epitope mapping.^[7] The docking study results suggest that the amino acids Asn, Met, and Gln do not contribute to the binding to CD4. Therefore, these inactive subunits were removed from the lead structure to reduce the molecular weight. Interactions of the aromatic ring of Trp and the hydrophobic side chain of Leu with CD4 could be replaced by the interaction of numerous other hydrophobic side chains without losing binding energy. Only the core peptide KVGTP was considered crucial for binding. The N and C termini were modified with various hydrophobic residues connected by nonpeptidic bonds. The candidate found from the molecular modeling studies to interact best with CD4 (Figure 1) was synthesized (Scheme 1).

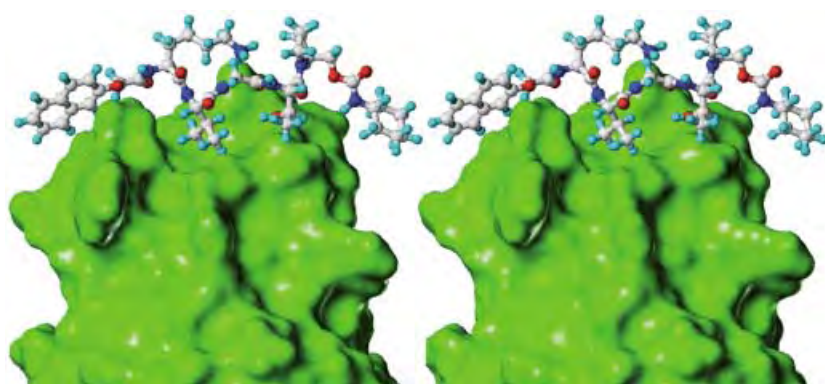


Figure 1. Stereoview of the peptidomimetic **4** (ball and stick) docked to CD4 (green surface).

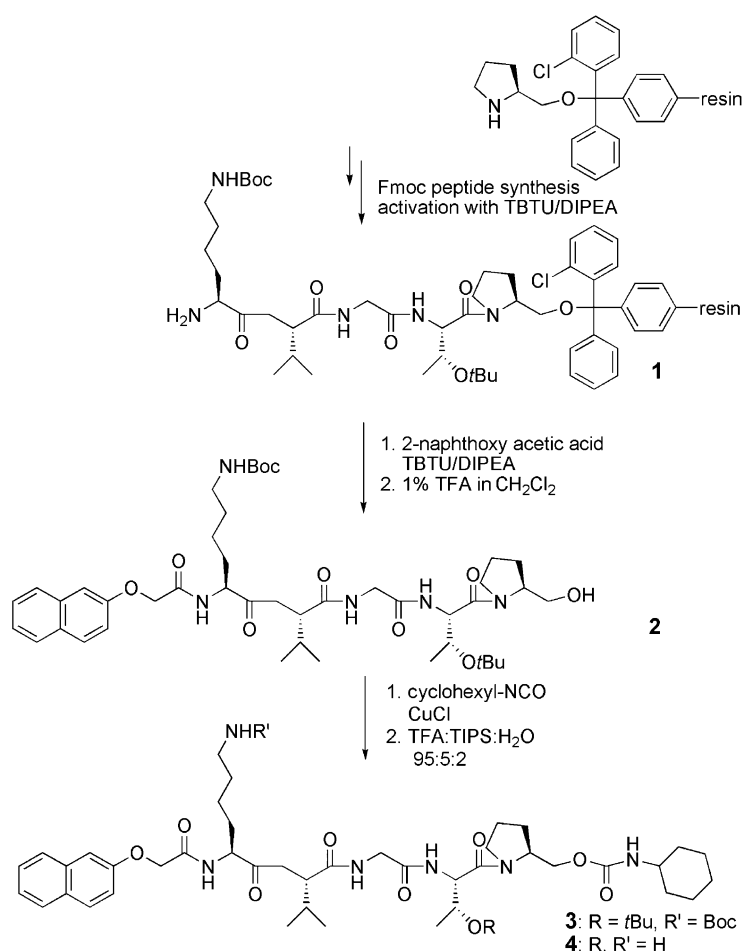
We synthesized the peptidomimetic from the amino alcohol L-prolinol, which was linked to a 2'-chlorotriptyl resin through the hydroxy functional group. The remaining amino acids of the sequence were coupled to the molecule by using standard peptide coupling protocols (activation by treatment with TBTU/DIPEA, Fmoc as an N-terminal protecting group, and Boc and *t*Bu as side-chain-protecting groups). Coupling of the terminal 2-naphthoxy acetic acid was accomplished in the same way through formation of a normal amide bond. The amide bond is stable to the subsequent reaction conditions and should not be cleaved by proteases. Mild acidic conditions (1% TFA in CH_2Cl_2) result in cleavage of the molecule from the resin to form the side-chain-protected free alcohol **2**. The alcohol group reacts with cyclohexylisocyanate under copper(I) catalysis to make a carbamate linkage, which results in **3**.^[8] Subsequent treatment with 95% TFA gives the unprotected peptidomimetic **4**. The overall yield of the product was 5% after HPLC purification. The free intermediates and the final product were characterized by MALDI-TOF MS. The structure of the peptidomimetic was determined from its 1D and 2D NMR spectra.

The MS and HPLC analyses indicate the presence of just one compound, but the NMR spectra revealed two signals for

[*] Dipl.-Chem. A. T. Neffe, Prof. Dr. B. Meyer
Institute for Organic Chemistry
University of Hamburg
Martin-Luther-King-Platz 6, 20146 Hamburg (Germany)
Fax: (+49) 40-42838-2878
E-mail: bernd.meyer@sg11.chemie.uni-hamburg.de

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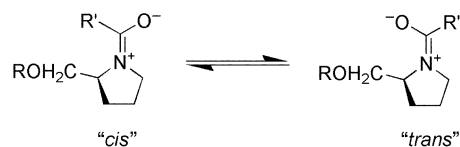
Supporting information for this article is available on the WWW under <http://www.angewandte.org> or from the author.



Scheme 1. Synthesis of the CD4-binding peptidomimetic **4**. The peptide and the 2-naphthoxy acetic acid residue were coupled on a solid support. Treatment with a mild acid cleaves **2** from the resin, while the side-chain-protecting groups are retained. Coupling of the isocyanate produces the carbamate **3**. The side-chain-protecting groups were finally removed by treatment with 95% TFA to yield **4**. Fmoc = 9-fluorenylmethoxycarbonyl, Boc = *tert*-butoxycarbonyl, TBTU = O-benzotriazol-1-yl-*N*-tetramethyluronium tetrafluoroborate, DIPEA = diisopropylethylamine, TFA = trifluoroacetic acid, TIPS = triisopropylsilyl.

each spin system, with large differences in the chemical shifts of some of the NH and H α protons (see the Supporting Information). Although the prolinol signals could not be individually assigned, we observed that the biggest differences in chemical shift occurred at the isocyanate residue and the shift differences decreased along the sequence. No differences could be measured at the naphthyl residue. An equilibrium between *cis* and *trans* rotamers at the Thr–prolinol amide bond is the likely explanation (Scheme 2). The ratio of the *trans*:*cis* isomers at 320 K is 2.3:1.

The binding specificity of compound **4** was determined by SPR studies on a Biacore instrument and by STD NMR spectroscopy. Compound **4** was passed over CD4 that had been immobilized on a Biacore CM5 chip. About 11 fmol CD4 was active after immobilization. A concentration-dependent binding assay was performed with 2, 4, 8, 16, and 32 μ M **4** in buffer. The results of the binding assays are shown in Figure 2. A regression analysis with a one-site binding model gave a binding constant of $K_D = 39 \mu$ M.



Scheme 2. *cis* and *trans* rotamers of the Thr–prolinol amide bond. About 70% *trans* and 30% *cis* rotamer was detected by ^1H NMR spectroscopy in $\text{H}_2\text{O}/\text{D}_2\text{O}$ (9:1) at pH 3 and 320 K.

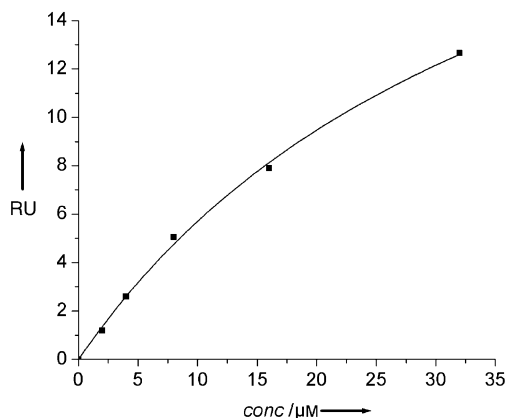


Figure 2. Determination of the binding properties of **4** by surface plasmon resonance on a Biacore 3000 instrument. CD4 was immobilized on a dextrane-coated chip. Ligand **4** in 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) buffered saline (150 mM, pH 7.4) was passed over the chip. The association curve is concentration-dependent and calculations with these data result in a dissociation constant K_D of 39 μ M. RU = response units from the Biacore instrument.

STD NMR experiments (Figure 3) can also be used to determine dissociation constants.^[7d] An 8.8 μ M solution of soluble CD4 in buffered D_2O was used in the presence of various concentrations of the peptidomimetic. Analysis of the dependence of the STD amplification factor on the concentration of **4** gives the K_D value (Figure 4). This analysis shows that the binding model fits the data very well. The resulting K_D values are 18 μ M (Val-H γ), 29 μ M (cyclohexyl-H3), and 45 μ M (naphthyl-H6/7), respectively. The binding epitope for the interaction of the peptidomimetic **4** with CD4 has almost identical interacting groups to those found in the original lead structure (Scheme 3).^[6] Digestion of the peptidomimetic **4** and NMWQKVGTP with pronase shows that **4** has a half life ($t_{1/2}$) 4.5-times longer than that of the lead peptide.

We have developed a CD4-binding peptidomimetic that has the potential to block HIV infection. The mimetic has a 170-fold higher K_D value ($K_D = \text{ca. } 35 \mu$ M) than the lead peptide ($K_D = \text{ca. } 6 \text{ mM}$). The peptidomimetic is also four to five times more stable to proteolysis. The biological activity of the lead structure was determined in earlier experiments. The similarity of the binding epitope of **4** to that of the lead peptide suggests that further optimization of the binding properties of **4** is possible. It remains to be shown in future experiments whether the increased binding affinity of the ligand is also operative in competition with the CD4/gp120 interaction in vivo.

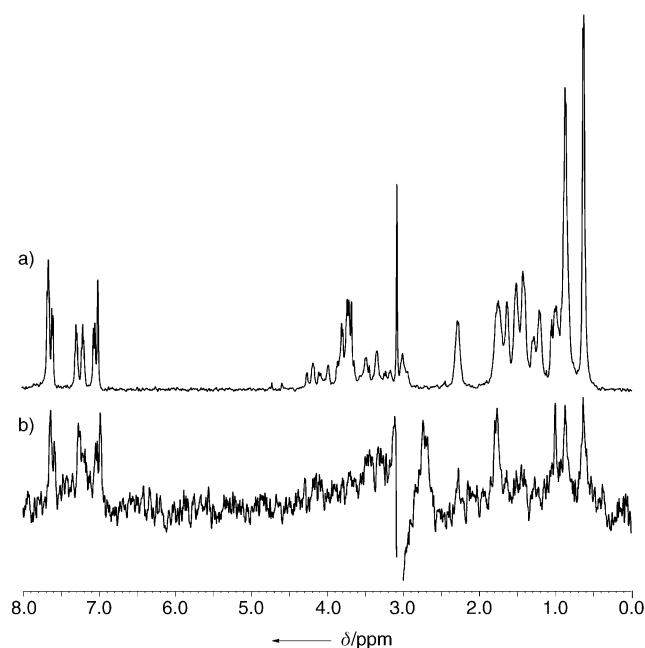


Figure 3. a) ^1H NMR spectrum of the peptidomimetic **4**. b) ^1H STD NMR spectrum of the peptidomimetic **4** (156 μM , 17.6-fold excess over soluble CD4, 15000 scans) in phosphate-buffered saline (150 mM) in D_2O solvent at pH 7.4. The ^1H STD spectrum shows very different relative intensities compared to those in the spectrum shown in (a). This information can be used to define the binding epitope of ligand **4** (see Scheme 3).

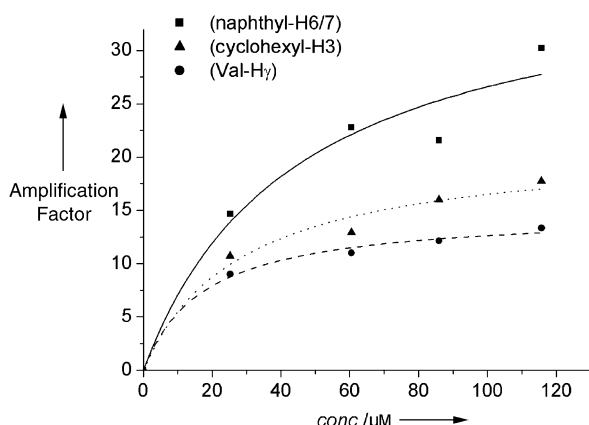
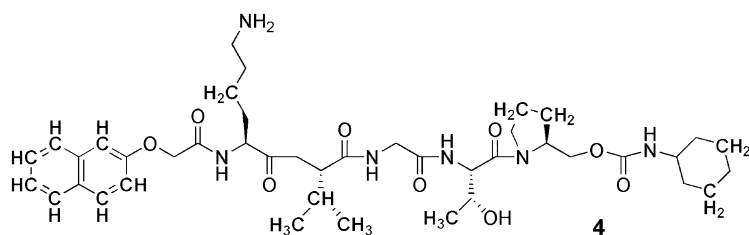


Figure 4. Determination of the binding properties from STD NMR titration data. The titration curves of three protons from different parts of the molecule (see Scheme 3b) are shown. The K_D values were determined from the STD NMR titrations by using a mathematical model of one-site binding. The resulting K_D value for **4** is $31 \pm 13 \mu\text{M}$.

Experimental Section

The solid-phase synthesis was performed on an ACT 496- Ω robot system. 2'-Chlorotriethyl resin (55 mg) bearing L-prolinol (50 μmol ; ACT) was shaken for 2 h with Fmoc-Thr(*t*Bu)-OH (4 equiv) and DIPEA/TBTU (5 equiv) in DMF. After washing the resin, this procedure was repeated twice. The resin was treated twice with 10% Ac_2O in DMF for 15 min. Cleavage of the Fmoc group was achieved by treatment with 20% piperidine in DMF for 2×10 min. The next amino acids and 2-naphthoxy acetic acid were coupled by using the same protocol. The resin was transferred to a glass frit and was shaken



Scheme 3. STD NMR epitope mapping of the peptidomimetic **4**. The protons in bold are in close contact to CD4.

with a freshly prepared solution of dichloromethane (1880 μL), TFA (20 μL), and TIPS (100 μL) for one hour. The mixture was filtered and the resin was washed with the same solution (1 equiv) then three times with dichloromethane. The combined solutions were washed three times with a 5% aqueous sodium acetate solution. The aqueous phase was re-extracted with dichloromethane and the combined organic phases were shaken with Amberlyst A-21 for one hour. The ion-exchange resin was filtrated and washed with dichloromethane, the organic phases were dried over MgSO_4 , and the dichloromethane was evaporated to give crude **2**. The alcohol **2** was dissolved in dry DMF and then a freshly prepared suspension of CuCl (50 μmol , 5 mg) in dry DMF (250 μL) and cyclohexylisocyanate (50 μmol , 6.3 mg) added. This mixture was shaken for one hour and then diluted with a 5% aqueous $(\text{NH}_4)\text{HCO}_3$ solution and extracted three times with dichloromethane. The organic phase was dried over MgSO_4 and the solvents were evaporated to give the protected carbamate **3**. This compound was dissolved in TFA (1.9 mL), TIPS (100 μL), and water (40 μL) and the solution was shaken for one hour. The solvents were evaporated and the product was purified by HPLC (solvent A: water/acetonitrile (95:5); solvent B: water/acetonitrile (5:95); both solvents contained 0.1% TFA; 80% A for 1 column volume (CV), then gradients of 1 CV to 50% A and 5 CV to 0% A; product elutes at 38.6% A on a preparative C18 substituted silica column). The pure product was lyophilized. Yield: 2 mg (2.5 μmol) white solid **4**; overall yield: 5%.

2: MALDI-TOF MS: m/z 827 ($[M+H]^+$), 849 ($[M+Na]^+$), 865 ($[M+K]^+$); **3:** MALDI-TOF MS: m/z 952 ($[M+H]^+$), 974 ($[M+Na]^+$), 990 ($[M+K]^+$); **4:** MALDI-TOF MS: m/z 796 ($[M+H]^+$), 818 ($[M+Na]^+$), 834 ($[M+K]^+$); NMR data for **4** are given in the Supporting Information.

The SPR protocol and details of the docking procedures can be found in the Supporting Information.

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Keywords: drug design · molecular recognition · NMR spectroscopy · peptidomimetics · viruses

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receptor (EGFR), Src kinase, and Abl kinase,^[3] while others have used this approach to develop reporters for protein kinase A,^[4] protein kinase C,^[5] and the insulin receptor.^[6] The phosphoserine/threonine-binding module in the reporter described herein, which represents the first for histones, is the signaling protein 14-3-3 τ ,^[7] which consists of 232 amino acids and naturally binds to the phosphorylated form of residues 615–644 of Cbl,^[8] a sequence similar to residues 1–30 of H3.

We constructed the reporter gene by standard cloning methods (GenBank accession no. AY422821), expressed the His₆-tagged recombinant protein in *E. coli*, and purified it by nickel affinity chromatography. In *in vitro* assays with purified Msk-1 kinase, which phosphorylates both serine 10 and 28 of H3,^[9] the reporter gave a 25 % increase in the YFP/CFP emission ratio (Figures 1 b and 2 a). Leaving out either ATP or

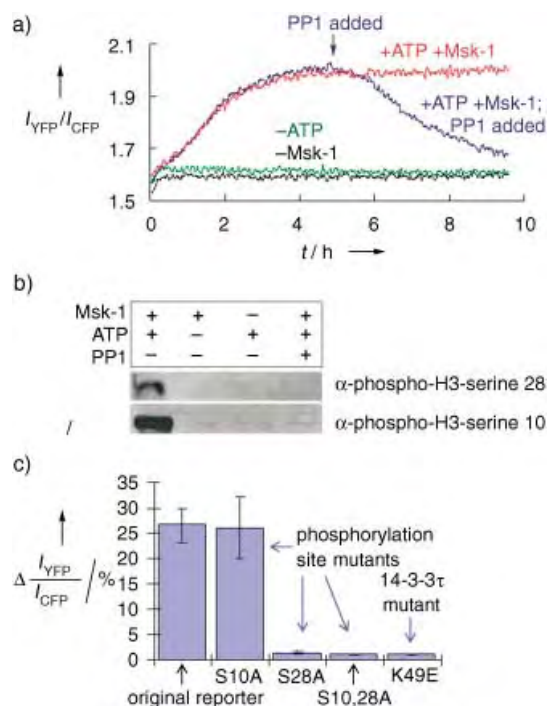


Figure 2. a) Change in the reporter YFP/CFP emission ratio over time under various reaction conditions. b) Immunoblot analysis of the four reactions in (a) after 10 h with anti-phospho-H3-serine 10 and -serine 28 antibodies. c) Maximal emission ratio changes observed in phosphorylation reactions with four reporter mutants. Each measurement was performed in triplicate.

the enzyme abolished the FRET change (Figure 2a). To examine reporter reversibility, we treated the phosphorylated reporter protein with PP1, a broad-specificity serine/threonine phosphatase.^[10] Over about 5 h, the FRET returned to its original level (Figure 2a). Reporter protein from each reaction in Figure 2a was also analyzed by an anti-phospho-H3-serine 10 and -serine 28 immunoblot. Figure 2b shows a good correspondence between the FRET level and this traditional method for determining the phosphorylation state of a protein. Lastly, the phosphorylation rate of the *in vitro* reporter is very similar to that of full-length H3 under

identical conditions (data not shown), which indicates that the former is a good mimic of the latter.

Because the N-terminus of H3 contains two potential phosphorylation sites (serine 10 and serine 28), we performed site-directed mutagenesis on the reporter to determine which site(s) were responsible for the FRET increase. Figure 2c shows that mutation of serine 10 to a phosphorylation-incompetent alanine had no effect on the reporter response, whereas mutation of serine 28 to alanine abolished the FRET change. These results indicate that the reporter specifically responds to serine 28 phosphorylation. Because we wondered whether this specificity was a consequence of the reporter geometry or reflected an inherent selectivity of the 14-3-3 τ domain for phosphoserine 28, we constructed another reporter identical to the original but with the 14-3-3 τ and histone peptide domains transposed. Interestingly, this reporter was responsive to phosphorylation at both the serine 10 and serine 28 positions (see Supporting Information), which suggests that geometrical constraints determine the specificity of the original reporter.

A charge-reversal mutation in the binding pocket of the 14-3-3 τ domain (K49E), expected to abolish the binding affinity of the 14-3-3 τ domain for phosphoserine,^[7] also eliminated the FRET response (Figure 2c). This is consistent with the designed mechanism for sensing of phosphorylation through FRET. We wondered, however, whether the known dimerization potential of 14-3-3 τ ,^[7] also demonstrated in a previous 14-3-3 τ -based reporter,^[4] would lead to dimerization of our reporter. Analytical ultracentrifugation confirmed that our reporter exists as a dimer in solution (data not shown). Therefore, the fluorescence response of our reporter might be due either to true intramolecular binding or to intermolecular binding across the dimer interface.

To test the histone phosphorylation reporter in living cells, we appended a nuclear localization sequence (PKKKRK) to the C-terminus of the reporter gene and introduced the plasmid DNA into HeLa cells by lipofection. The reporter was efficiently expressed after about 12 h. Because previous studies have shown that serine 28 phosphorylation levels transiently increase during the mitotic (M) phase of the cell cycle,^[11] we monitored the FRET level of our reporter in dividing HeLa cells over 26 h. As shown in Figure 3 and in the movie provided as Supporting Information, the reporter displayed a rapid increase in YFP/CFP emission ratio (15–25 %) that, in eight separate cells, closely preceded breakdown of the nuclear envelope (as measured by reporter distribution in the cell) by 5–15 min. The high FRET was sustained for several hours and then dropped slowly after cytokinesis, or separation of the cytoplasm into daughter cells. A control experiment with the S28A mutant of the reporter did not give a FRET change during cell division, demonstrating that the response of the reporter is not an artifact of changes in reporter concentration or cellular pH over the course of cell division (see Figure 3b).

Elevated serine 10 and 28 phosphorylation levels have both been linked to mitosis,^[12,13] but the precise molecular roles of these modifications have yet to be elucidated. Our study provides a high-resolution picture of serine 28 phosphorylation onset and disappearance in single living HeLa

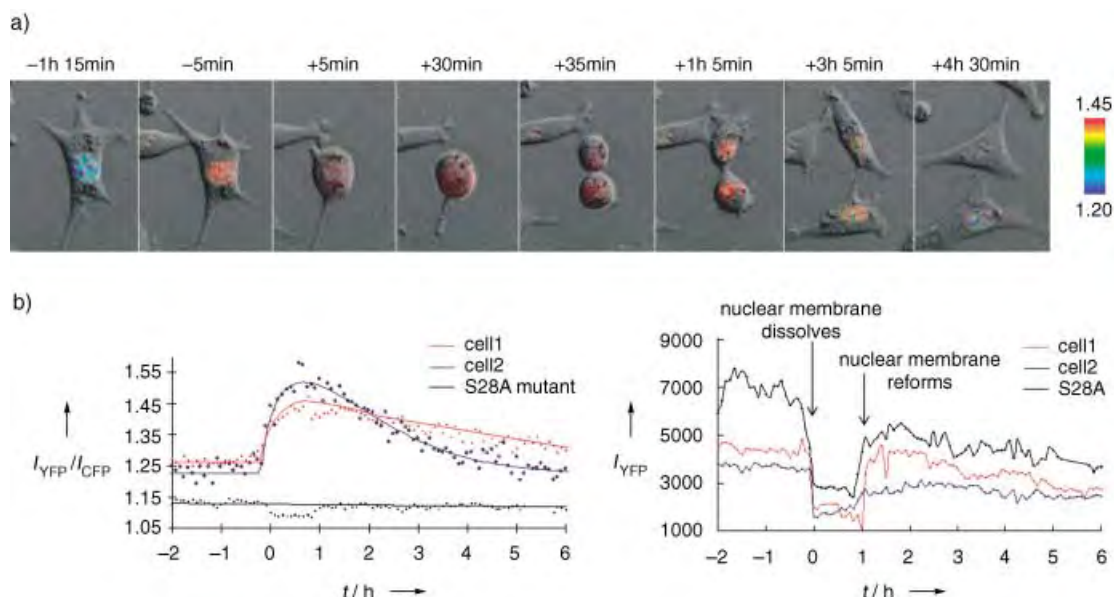


Figure 3. a) Reporter response during HeLa cell division. The bar on the right shows the correlation between YFP/CFP emission ratio and cell pseudocolor. The indicated times refer to hours/minutes after nuclear-membrane breakdown. b) Emission ratio and YFP emission intensity as a function of time after nuclear-membrane breakdown for two cells expressing the reporter and one cell expressing the S28A reporter mutant (t = time after nuclear-membrane breakdown).

cells during cell division. We find a previously unobserved correlation between serine 28 phosphorylation onset and nuclear-envelope breakdown, which may suggest a mechanism for coordinating changes in chromatin and cell morphology during the M phase. Future investigation will follow up on this intriguing possibility. In another avenue for future study, if the phosphorylation reporter were physically anchored to chromatin (for example, through genetic fusion to one of the four histone proteins), spatial resolution might be further increased.

The work described herein also demonstrates the capabilities and limitations of current methodology for the design of fluorescent reporters. Without a recognition element known a priori to bind the phosphorylated histone sequence, we developed a reporter for H3 serine 28. In fact, the 14-3-3 τ domain consensus recognition sequence is quite different from the peptide sequence flanking serine 28.^[14] Either 14-3-3 τ binding is significantly more promiscuous and context-dependent than previously thought, or our reporter design can accommodate recognition domains of only modest intermolecular affinity for the substrate peptide. On the other hand, the impressive, geometry-sensitive specificity of our reporter for H3 serine 28 over other phosphorylation-competent sites in the same substrate peptide indicates a keen dependence on reporter design elements such as spacing and orientation of the binding domain and the substrate peptide.

In conclusion, this reporter, and similar ones such as histone methylation reporters^[15] and acetylation reporters under development in our laboratory, should allow the study of transcriptional regulation in cells with greatly improved spatiotemporal resolution.

Experimental Section

The reporter gene was ligated into the pRSETB vector (Invitrogen, Carlsbad, CA) between the *Bam*HI and *Eco*RI sites, which installs a His₆ tag at the N-terminus. For protein expression, the plasmid was introduced into BL21(DE3) bacteria (Stratagene, La Jolla, CA) and the cells were grown in Luria Broth supplemented with ampicillin (100 μ g mL⁻¹) at 37°C until OD₆₀₀ 0.5. Isopropyl- β -D-thiogalactopyranoside (IPTG) was then added to a final concentration of 0.4 mM to induce reporter expression. The cells were grown for 3 h at 30°C and then harvested by centrifugation. Cells were lysed by sonication at 4°C (six 30-second pulses at half-maximal power with 1 min between each pulse) in lysis buffer (50 mM Tris pH 7.8, 300 mM NaCl, 4 mM PMSF, and 1/4 EDTA-free protease inhibitor cocktail tablet (Roche, Indianapolis, IN) per 10 mL of lysis buffer). The His₆-tagged reporter was purified from the lysate using a Ni-NTA agarose column (Qiagen, Valencia, CA) following the manufacturer's protocol. Fractions containing the reporter were pooled and transferred into TBS (140 mM NaCl, 3 mM KCl, 25 mM Tris pH 7.4) by two rounds of dialysis for storage in aliquots at -80°C. Typical yields were 100–500 μ g of protein per 0.5 L culture. Msk-1 kinase was prepared in an identical manner using the Msk-1-expression plasmid (His₆-tagged human Msk-1 in pET-11 vector (Novagen, Madison, WI)) that we received from Prof. Dr. Jiahuai Han.

Reaction conditions for the in vitro phosphorylation assays were as follows: 1–5 μ M reporter, 0.6 mM ATP, 20 mM Hepes pH 7.7, 10 mM MgCl₂, 0.1 mM EGTA, 0.5 mM DTT, and 4 μ M of Msk-1. Reactions were incubated at 30°C for the indicated times. The phosphorylation state of the reporter from the in vitro assays was analyzed by Western blot using 12% SDS-PAGE gel, anti-phospho-H3-serine 10 antibody (Cell Signaling Technology, Beverly, MA) or anti-phospho-H3-serine 28 antibody (Upstate Biotechnology, Charlottesville, VA) (both at 1/1000 dilution for 3 h at 25°C), and goat anti-rabbit-HRP conjugate or goat anti-mouse-HRP conjugate (1/1000 dilution for 2 h at 25°C) (Bio-Rad, Hercules, CA). The HRP was visualized by chemiluminescence (Supersignal West Pico, Pierce, Rockford, IL).

pcDNA3 (Invitrogen, Carlsbad, CA) plasmid containing the reporter gene between the *Bam*HI and *Eco*RI sites was introduced

into HeLa cells by transfection with Fugene 6 (Roche, Indianapolis, IN). Images were collected 8–24 h after transfection on a Zeiss Axiovert 200M inverted epifluorescence microscope with differential interference contrast (DIC). Cells were maintained in 10% fetal bovine serum in phenol red-free DMEM (Invitrogen, Carlsbad, CA) at 37°C under 5% CO₂ during imaging with an environmental control system (Solent Scientific, Portsmouth, UK) that housed the microscope stage. At each time point, four images were collected in rapid succession (automated, using OpenLab software (Improvision, Lexington, MA)): a CFP image (420DF20 excitation, 450DRLP dichroic, 475DF40 emission), a FRET image (420DF20 excitation, 450DRLP dichroic, 530DF30 emission), a YFP image (495DF10 excitation, 515DRLP dichroic, 530DF30 emission), and a DIC image (775DF50 emission). Fluorescence images were background corrected. Acquisition times ranged from 100 to 800 ms. The emission ratio image was generated by dividing the FRET image by the CFP image. The data shown in Figure 3a are a merge between the emission ratio image and the DIC image. The YFP intensity graph shown in Figure 3b was generated from the YFP images.

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Photoluminescence Properties of Discrete Conjugated Wires Wrapped within Dendrimeric Envelopes: “Dendrimer Effects” on π -Electronic Conjugation**

Wei-Shi Li, Dong-Lin Jiang,* and Takuzo Aida*

Conjugated molecular wires with discrete lengths are important for the exploration of physical properties related to the delocalization of π electrons, and have also attracted attention for their potential application in molecular electronics and photonics.^[1] Examples of discrete conjugated wires so far reported include derivatives of polyphenylene, polyacetylene, poly(phenylenevinylene), poly(phenyleneethynylene), polythiophene, and polyporphyrin.^[2] However, with the exception of only one example,^[2c] those oligomers are limited in length to tens of nanometers, because of their low solubility and strong tendency to aggregate. From a photochemical point of view, such a strong tendency to aggregate is a major drawback of “naked” nanowires, which results in collisional deactivation of photoexcited states and hinders their potential utilities. A promising approach to solving this problem is to design “isolated” nanowires bearing “insulating” shells. However, such insulated nanowires with discrete molecular lengths are unprecedented.^[3,4]

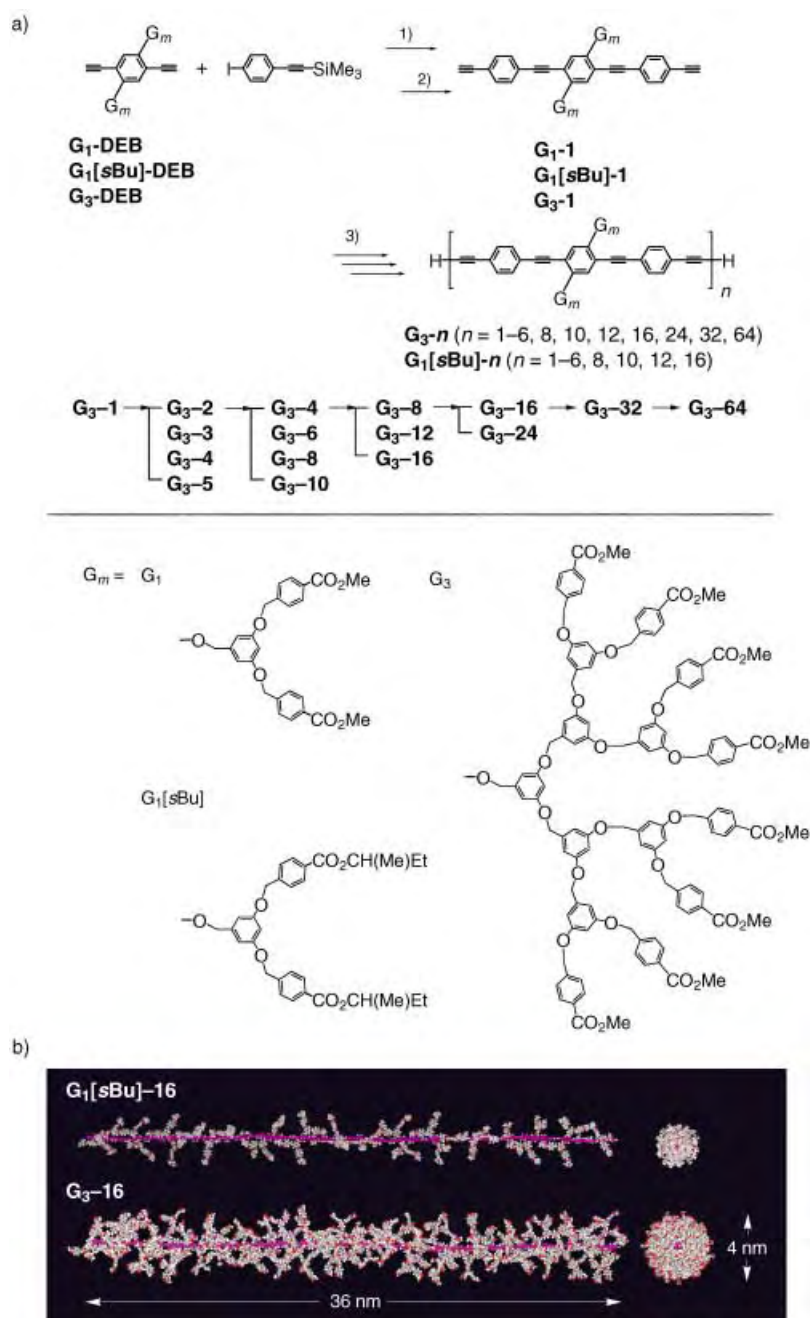
We report herein the first example of discrete conjugated wires wrapped in dendrimeric envelopes (Scheme 1 a, G_m - n ; m = generation number of dendrimeric wedges, n = number of repeating monomer units). Incorporation of large G3 poly(benzyl ether) dendrimeric wedges into the repeating units^[5] allowed us to overcome the solubility problem and to synthesize conjugated wires with a molecular length of up to 147 nm. This dendrimeric core-shell strategy^[6a] guarantees that only a single conjugated chain is integrated into the focal core, thereby representing a clear contrast to reported strategies with other nanoscopic architectures, such as zeolite channels, that incorporate bundles of conjugated polymers.^[6b,c] Herein, we highlight “dendrimer effects” on the photoluminescence properties of the conjugated focal core, with an emphasis on a possible effect of intramolecular interactions between the large dendrimeric wedges on the π -electronic conjugation of the backbone.

[*] Dr. W.-S. Li, Dr. D.-L. Jiang, Prof. T. Aida
Aida Nanospace Project
Exploratory Research for Advanced Technology (ERATO)
Japan Science and Technology Agency (JST)
2-41 Aomi, Koto-ku, Tokyo 135-0064 (Japan)
Fax: (+81) 3-5841-7310
Fax: (+81) 3-3570-9183
E-mail: jiang@nanospace.miraikan.jst.go.jp
aida@macro.t.u-tokyo.ac.jp

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



Scheme 1. a) Synthesis of **G₁-1**, **G₁[sBu]-n**, and **G₃-n**. Conditions: 1) [Pd(PPh₃)₄], CuI, *i*Pr₂NH, THF, reflux; 2) Bu₄NF, THF, RT; 3) Cu(OAc)₂, TMEDA, THF, 55 °C. THF = tetrahydrofuran, TMEDA = tetramethylethylenediamine. b) Computer-generated images of the molecular structures of **G₁[sBu]-16** and **G₃-16**.

Monomer **G₃-1** was synthesized by a Pd⁰/Cu^I-catalyzed coupling of dendritic 1,4-diethynylbenzene with 1-iodo-4-trimethylsilylbenzene in THF, followed by treatment with Bu₄NF.^[7] Coupling of **G₃-1** in the presence of a mixture of Cu(OAc)₂ and TMEDA in THF at 55 °C for 10 minutes^[8] afforded a mixture of dimer **G₃-2** (23 %), trimer **G₃-3** (15 %), tetramer **G₃-4** (10 %), and pentamer **G₃-5** (7 %), which were separated by recycling preparative gel permeation chromatography (GPC) with CHCl₃ as the eluent, and then further

oligomerized to give higher oligomers (Scheme 1 a). In contrast to the case with short-chain **G₃-1**–**G₃-8**, the coupling reaction of higher oligomers such as **G₃-16** and **G₃-32** proceeded rather sluggishly to afford only their dimerized products **G₃-32** (26 %) and **G₃-64** (15 %), respectively. Likewise, oligomers **G₁[sBu]-n** were synthesized as lower-generation reference compounds (Scheme 1 a; $n = 2-6, 8, 10, 12$, and 16) from **G₁[sBu]-1**, which bears CO₂sBu groups on its external surface, since the G1 monomer (**G₁-1**) with CO₂Me surface groups was barely soluble in common organic solvents. **G₃-n** and **G₁[sBu]-n**, thus prepared, were unambiguously characterized by analytical methods.^[7] A computer-aided molecular modeling study suggested that higher oligomers **G₃-n** ($n \geq 4$) adopt a rodlike morphology with a diameter of roughly 4 nm (Scheme 1 b; **G₃-16**). **G₃-64**, which contains 192 aromatic rings and 256 triple bonds at its focal core, was estimated to be 147 nm long,^[9] which is the longest discrete wire reported to date.^[2] The ¹H NMR spectrum of a solution of **G₃-1** in CDCl₃ at 30 °C displayed a set of signals at $\delta = 6.98$, 7.19, and 3.04 ppm corresponding to the aromatic (H^a and H^b) and acetylenic (H^c) protons, respectively, at the focal core. In contrast, the lower-generation **G₁[sBu]-1** showed the corresponding signals at slightly lower magnetic fields with δ values of 7.08 (H^a), 7.30 (H^b), and 3.13 ppm (H^c), respectively. Interestingly, the spin–spin relaxation times (T_2) of H^a and H^b, located in the proximity of the dendritic wedges, are dependent on the generation number m , whereas that of H^c is virtually unaffected (Figure 1):^[10] The T_2 values of H^a and H^b in **G₃-1** were 0.39 and 0.59 s, respectively, which were clearly smaller than those of **G₁[sBu]-1** (0.78 [H^a] and 1.20 s [H^b]). The relatively short T_2 values observed for the backbone of **G₃-1** indicate there are constrained conformational motions of the focal aromatic rings attached to the large G3 dendritic wedges.

Solutions of **G₃-n** in THF at 25 °C exhibited absorption bands in the visible region as a consequence of the conjugated backbone, as well as two absorption bands at 231.0 and 276.0 nm arising from the dendritic wedges (Figure 2 a). As the number of repeating units n increased, this absorption band was red-shifted from 379.8 nm for **G₃-1** to 428.7 nm for **G₃-64**, with saturation of the spectral change observed at around $n = 8$. Lower-generation **G₁[sBu]-n**, under identical conditions to the above, showed a similar spectral change profile upon increment of n , again with a saturation point around $n = 8$.^[7] However, one may also note that the absorption bands of higher-generation **G₃-n** are located at a longer wavelength than those of **G₁[sBu]-n**. For example, decamers **G₃-10** and **G₁[sBu]-10** showed absorption bands at 427.0 and 416.2 nm, respectively, thus the energy difference is as large as 608 cm^{−1} (Table 1). It is unlikely that the observed spectral differences between **G₃-n** and **G₁[sBu]-n** are caused by their surface groups, since the transformation of the CO₂Me groups on the exterior surface of **G₃-10** into CO₂sBu (**G₃[sBu]-10**) resulted in no substantial

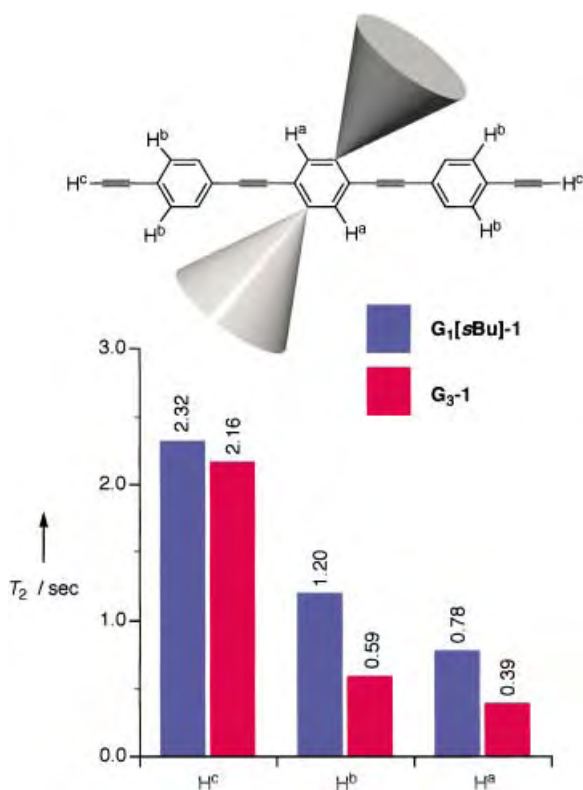


Figure 1. ^1H NMR spin–spin relaxation times T_2 of H^a , H^b , and H^c in $G_1[sBu]-1$ and G_3-1 recorded in CDCl_3 at 30°C .

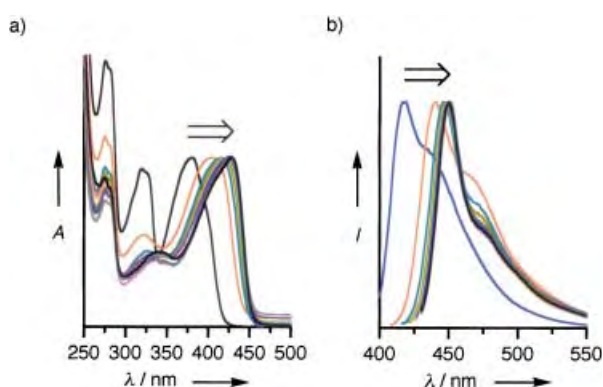


Figure 2. a) Electronic absorption and b) emission spectra (normalized) of G_3-n ($n=1-6, 8, 10, 12, 16, 24, 32$, and 64 ; from left to right) in THF at 25°C .

Table 1: Energy differences between G_3-n and $G_1[sBu]-n$ at the absorption and emission maxima of their conjugated backbones in THF at 25°C .

n	Energy differences [cm^{-1}] at absorption maxima	Energy differences [cm^{-1}] at emission maxima
1	266.1	155.6
2	353.0	182.5
4	501.1	201.5
6	558.6	200.2
8	608.3	200.0
10	607.7	209.9
12	594.8	214.4
16	602.8	209.6

change in the absorption spectral profile. Furthermore, G_3-10 and $G_1[sBu]-10$ showed only slight spectral changes when the THF solvent was replaced with 1,3-dimethoxybenzene, an analogue of the dendrimeric wedge building block. The shorter-chain oligomers also displayed smaller energy differences, as shown in Table 1. In relation to this observation, 1,4-diethynylbenzene derivatives bearing G_1 and G_3 dendrimeric wedges ($G_1\text{-DEB}$, $G_1[sBu]\text{-DEB}$, and $G_3\text{-DEB}$; Scheme 1a), which are devoid of any conformational diversity at the focal cores, all showed an absorption maximum at 335.0 nm , irrespective of the surface group and generation number of the poly(benzyl ether) dendrimeric wedges.^[7] Thus, the spectral differences between G_3-n and $G_1[sBu]-n$ are most likely related to conformational aspects of their conjugated backbones; namely, the conformation of the backbone of G_3-n allows better conjugation between the chromophore units than that in $G_1[sBu]-n$, although the effective conjugation lengths of G_3-n and $G_1[sBu]-n$ are almost identical to one another.

Dendrimeric compounds G_3-n and $G_1[sBu]-n$ emitted a blue fluorescence upon excitation of their conjugated backbones in THF at 25°C (Figure 2b). As we have already reported for a nondiscrete poly(phenyleneethynylene) with G_3 dendrimeric wedges,^[3a] the fluorescence quantum yields (Φ_{FL}) of G_3-n were all high (80–90%), irrespective of the number of the repeating units n (Figure 3).^[11] In sharp

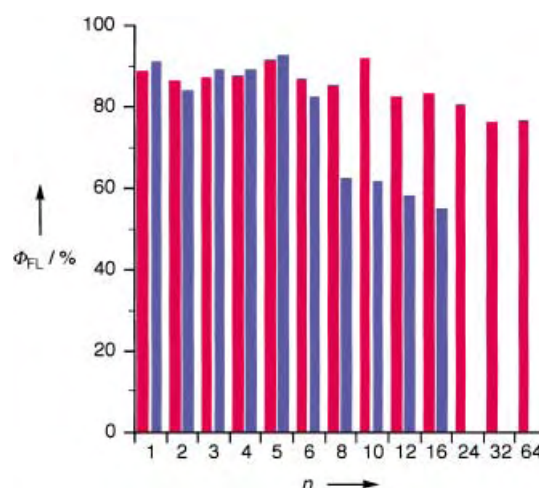


Figure 3. Fluorescence quantum yields (Φ_{FL}) of G_3-n (red bars) and $G_1[sBu]-n$ (blue bars) upon excitation at their absorption maxima ($A=0.1$) in THF at 25°C .

contrast, the Φ_{FL} values of lower-generation $G_1[sBu]-n$ displayed a tendency to drop when the n value was ≥ 8 , possibly because of an enhanced probability of collisional quenching of the singlet excited state. Higher-generation G_3-n are less-sensitive to concentration than $G_1[sBu]-n$. For example, when the absorbance of the solution was increased from 0.01 up to 0.24, the Φ_{FL} value of G_3-16 was preserved in a range of 80–85%, whereas a notable decrease in the Φ_{FL} value from 65% to 45% was observed for $G_1[sBu]-16$.^[7] Thus, the large dendrimeric envelope of G_3-n wraps around the conjugated backbone and prevents collisional deactivation of the excited

states.^[3a] A closer look at the luminescence properties of **G₃-n** and **G₁[sBu]-n** showed a dependence of the luminescence maximum on the “dendrimer size” (Table 1),^[7] analogous to that observed for the absorption spectral profiles. For example, a solution of **G₃-10** in THF at 25°C emitted a luminescence centered at 449.4 nm, which is red-shifted by 4.2 nm from that of lower-generation **G₁[sBu]-10** (445.2 nm), and the energy difference is calculated to be 210 cm⁻¹.^[12] In contrast, 1,4-diethynylbenzene derivatives **G₁-DEB**, **G₁[sBu]-DEB**, and **G₃-DEB**, which had no conformational diversity at the focal cores, displayed virtually identical fluorescence spectra to one another. On the other hand, not only **G₃-10** but also lower-generation **G₁[sBu]-10** showed only very small changes in the fluorescence spectra when the THF solvent was replaced by 1,3-dimethoxybenzene.

Since the conjugated backbone in **G₃-n** is spatially isolated by the thick G3 dendrimeric envelope, we investigated the fluorescence depolarization profiles of **G₃-n** ($n = 4, 8, 12, 16, 24, 32$, and 64), which are considered to reflect the photochemical events in the isolated wires. Suppression of Brownian motion in a viscous medium should result in the fluorescence depolarization occurring predominantly by exciton migration along the conjugated backbone.^[13] Here the degree of fluorescence depolarization (p) is defined as $p = (I_{\parallel} - GI_{\perp}) / (I_{\parallel} + GI_{\perp})$, where $I_{\parallel}$ and $I_{\perp}$ are the fluorescence intensities of parallel and perpendicular components relative to the polarity of the excitation light, respectively, while G is an instrumental correction factor. Excitation of the absorption maxima of viscous solutions of **G₃-n** in THF/polystyrene at 25°C gave fluorescence depolarization profiles (Figure 4)

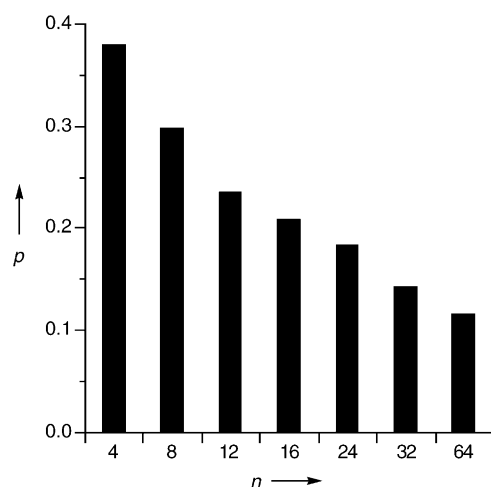


Figure 4. Fluorescence depolarization p of **G₃-n** upon excitation with a polarized light at their absorption maxima ($A = 0.1$) in THF/polystyrene (degree of polymerization (DP) = 1000–1400; 0.2 g mL⁻¹) as a viscous solvent at 25°C.

where the value of p gradually became smaller as the number of the repeating units n increased. For example, the p value for short-chain **G₃-4** was 0.38, which dropped to 0.21 and further to 0.11 when the n value was increased to 16 (**G₃-16**) and then to 64 (**G₃-64**). The absence of a saturation tendency up to a molecular length of 147 nm (**G₃-64**) is quite interesting, since previous studies on conjugated polymers without

dendrimeric side groups have shown that the exciton migration subsides within several nanometers.^[13,14]

Taking all the above results into account, it is likely that **G₃-n** bearing the large G3 dendrimeric wedges prefers a planar conformation of the conjugated backbone, which is good for electronic conjugation (Table 1)^[15] and may also allow preservation of fluorescence anisotropy in a long-range exciton migration (Figure 4). There are several examples of attractive van der Waals interactions between poly(benzyl ether) dendrimers in their self-organized structures.^[16] We assume that the dendrimeric wedges in **G₃-n** could similarly interact with one another intramolecularly. To support this hypothesis we investigated the dynamics of the conformational change of the dendrimeric wedges of **G₃-n**. The ¹H NMR spectrum of a solution of **G₃-1** in CDCl₃ at 30°C exhibited a doublet at $\delta = 7.97$ ppm which is attributed to the *ortho*-H of the outermost aromatic rings in the dendrimeric wedges.^[7] Dimer **G₃-2** showed, in addition to this signal, a new *ortho*-H doublet at a slightly higher magnetic field ($\delta = 7.93$ ppm). Furthermore, trimer **G₃-3** showed another new doublet at $\delta = 7.92$ ppm. When the degree of polymerization (n) of **G₃-n** was larger, the signal at $\delta = 7.92$ ppm was more intense. These characteristic signals were assigned as shown in Figure 5, where the signal at $\delta = 7.97$ ppm originates from the

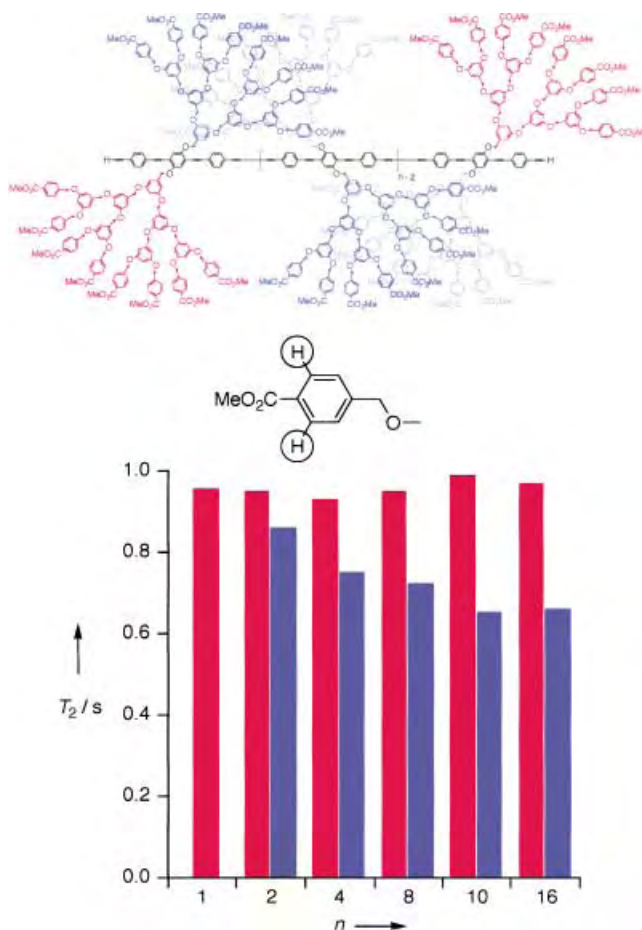


Figure 5. ¹H NMR spin–spin relaxation times T_2 of *ortho*-H in the outermost aromatic rings of the dendrimeric wedges of **G₃-n** in CDCl₃ at 30°C.

dendrimeric wedges located at both ends of the backbone (red), while the signals at $\delta = 7.93$ and 7.92 ppm are from the other dendrimeric substituents (blue). We measured spin–spin relaxation times (T_2) of these characteristic signals (Figure 5), and found that the T_2 value of the signal at $\delta = 7.92$ ppm is smaller when the degree of polymerization n is larger, and reaches a plateau at $n = 10$ (blue bars; the T_2 value of the signal at $\delta = 7.93$ ppm is shown for **G₃-2**). In sharp contrast, the T_2 value of the signal at $\delta = 7.97$ ppm, which arises from the dendron units at the edges of the backbone (red), is virtually unchanged by n (red bars). These contrasting results suggest that the molecular motions of the inner dendrimeric wedges, which are densely aligned along the rigid, conjugated backbone, are highly constrained as a consequence of intramolecular van der Waals interactions. In contrast, the T_2 values of the corresponding signals in lower-generation **G₁[sBu]-n** were only slightly dependent on n .^[7,17]

In summary, we have reported the first example of discrete conjugated wires **G₃-n** bearing large dendrimeric substituents. A great advantage of the dendrimeric architecture is that it allows for the synthesis and isolation of a 147-nm long discrete wire (**G₃-64**), in which the conjugated backbone consisting of 192 aromatic rings and 256 triple bonds is wrapped in a thick dendrimeric envelope. Comparative photochemical studies with lower-generation **G₁[sBu]-n** as reference compounds indicate that the conjugated backbone in **G₃-n** tends to adopt a planar conformation, most probably because of intramolecular van der Waals interactions between the large, densely aligned G3 dendrimeric wedges. The planar conformation of the backbone allows efficient electronic conjugation of the chromophores and low fluorescence depolarization in an exciton migration event. Application of such insulated nanowires to molecular electronics and photonics is one of the interesting subjects worthy of further investigations.

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Keywords: conjugation · dendrimers · exciton migration · luminescence · molecular wires

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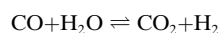
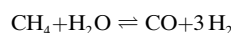
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A New Type of Material for the Recovery of Hydrogen from Gas Mixtures**

Jerry L. Atwood,* Leonard J. Barbour,* and Agoston Jerga

Sustained global dependence on fossil fuels as a primary source of energy is beset by several intractable problems which include dwindling reserves, increasingly unacceptable levels of pollution, and relatively low conversion efficiency.^[1] Of several proposed alternative technologies, hydrogen-based fuel cells have emerged as being particularly attractive, especially for mobile applications.^[2] The smooth transition to hydrogen as a viable and sustainable source of energy is, however, not without its own set of challenges. Much needs to be accomplished to realize the necessary infrastructure and technological advances that will ultimately lead to the production, purification, transport, storage, and conversion of hydrogen as an everyday consumer commodity.^[3]

In the short term, escalated production of hydrogen will most likely continue to rely on conventional technologies such as steam reforming of natural gas: followed by the water-gas shift reaction: The final step in the production process involves the purification of H₂ by the removal of impurities such as CH₄, CO, H₂O, and, primarily, CO₂.^[4]



Separation technology is critical to the deployment of hydrogen as a source of energy since the purity of hydrogen supplied to fuel cells affects their performance and longevity, and therefore their economic viability. Compact, durable, and inexpensive purification devices will be required if hydrogen is to fulfill its promise. New hydrogen plants are generally based on the use of pressure-swing adsorption (PSA) for the final purification of hydrogen. This technique utilizes the difference in adsorption properties of various molecules:^[5] components of a gas mixture are selectively adsorbed onto a solid matrix at high pressure, and subsequently desorbed by lowering the pressure.^[6] In recent years, both the design and operation of PSA processes have developed to the extent that

any notable further improvement in gas separation necessitates the discovery of a new adsorbent material.^[7] Although zeolites and activated carbon materials are currently employed to good economic effect, there is a continued demand for new designer materials that can be utilized for gas storage and separation.^[8] Although carbon nanotubes and metal-organic frameworks have undergone substantial scrutiny in this regard,^[9] molecular crystals have received little consideration since their constituent molecules generally pack with an efficiency deemed to preclude porosity.^[10] We now show that a nonporous organic molecular crystal can indeed be exploited for the purification of hydrogen gas.

We recently reported that a crystalline polymorph of pure *p*-*tert*-butylcalix[4]arene (TBC4), prepared by sublimation, forms a bilayer-type structure which contains large lattice voids of approximately 235 Å³ (Figure 1).^[11] Immersing these

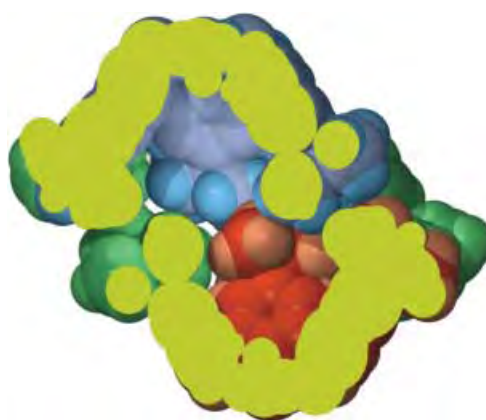


Figure 1. A section (yellow) through the TBC4 dimer (red and blue molecules) formed upon sublimation. The two empty calixarene cavities face one another in an offset arrangement, which yields a relatively large hourglass-shaped void with an estimated free volume of 235 Å³. Gaps between the *tert*-butyl groups at the equator of the dimer are blocked by *tert*-butyl groups (green) of neighboring calixarenes and there are thus no channels leading to the voids.

crystals in liquid vinyl bromide for a period of 15 minutes results in a single-crystal-to-single-crystal phase transition during which the vinyl bromide diffuses through the lattice to fill the vacancies. The phase transition is characterized by a lateral shift of the bilayers (by approximately 6 Å) relative to one another to allow guest diffusion, and to achieve a well-packed final structure that incorporates one molecule of vinyl bromide per TBC4 cavity. In our continued efforts directed at understanding the dynamics of guest transport through the TBC4 lattice, we noted that addition of sublimed TBC4 to liquid nitrobenzene caused numerous bubbles to emanate from the crystals (Figure 2). This chance observation implies that the crystals initially contain highly volatile molecules which are forced out of the lattice by incoming nitrobenzene. Since the pure host phase is crystallized under vacuum at 230°C, the possibility of having formed the lattice around small gas molecules can be ruled out.^[12] The most plausible explanation is that, upon removal from the sublimation

[*] Prof. J. L. Atwood, Dr. A. Jerga
Department of Chemistry
University of Missouri-Columbia
Columbia, MO 65211 (USA)
Fax: (+1) 573-882-2754
E-mail: atwoodj@missouri.edu
Prof. L. J. Barbour
Department of Chemistry
University of Stellenbosch
7602 Matieland (South Africa)
Fax: (+27) 218-083-849
E-mail: ljb@sun.ac.za

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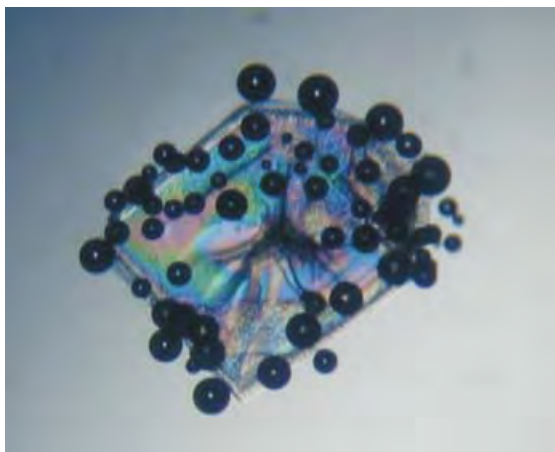


Figure 2. Photograph showing air escaping from platelike crystals of TBC4. After exposure of sublimed single crystals to the atmosphere for two days they were immersed in nitrobenzene. Air bubbles immediately began to emanate from the sides of the crystals as nitrobenzene enters the lattice, expelling the gas.

apparatus, the crystals absorb air while left exposed to the atmosphere prior to further handling.

The X-ray structure of a sublimed crystal was elucidated after its exposure to the atmosphere for two days. The host lattice is identical to that of the pure phase (that is, no lateral shift of the bilayers is apparent). Once the atomic parameters of the host molecule had been resolved, a significant level of residual electron density was located within the calixarene cavity. In contrast, structural analysis of a freshly sublimed crystal did not reveal any appreciable residual electron density at this site. Although a presumed combination of thermal motion, partial occupancy, and disorder precluded modeling the residual electron density as either O_2 or N_2 , it is reasonable to infer that this electron density within the calixarene cavity arises from the absorption of air from the atmosphere. This result raises the intriguing question of how a seemingly nonporous material might have the ability to absorb gas into its lattice under ambient conditions.^[13]

Sorption isotherms for air, N_2 , O_2 , and CO_2 were each recorded at 23 °C on freshly sublimed crystals (Figure 3a). In all cases, the initial pressure was approximately 700 torr (93.3 kPa) and the gas was absorbed, over a period of about 24 h or longer, until equilibrium was reached. Under the above conditions, it appears that the crystals are able to discriminate between the various gases in both their rate of uptake and amount absorbed. Indeed, since the composition of air is on the order of 78 % N_2 and 21 % O_2 ,^[14] the sorption rates for air, N_2 , and O_2 are approximately self-consistent. Of the three pure gases tested, CO_2 is absorbed far more rapidly and to a significantly greater extent than either N_2 or O_2 . Correspondingly, it is known that the rate of gas adsorption on activated carbon can be correlated with the condensability of the gas under consideration.

As anticipated, equilibrium was reached far more rapidly when the sorption experiments (Figure 3b) were repeated after the host crystals had been crushed by means of a mortar and pestle. However, the amount of gas absorbed appears to

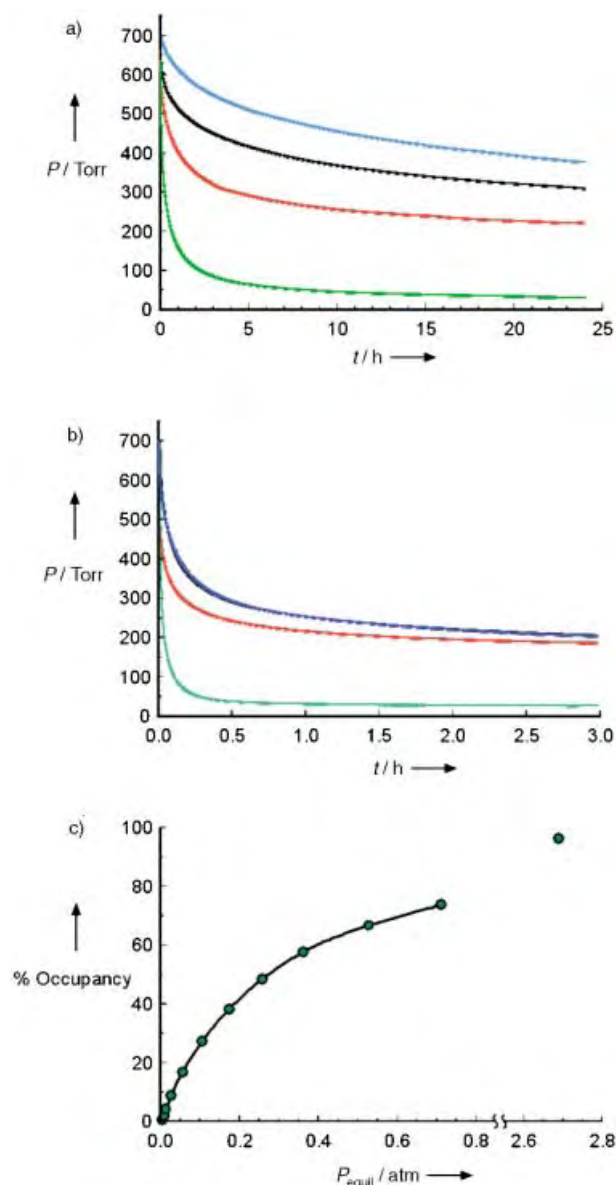


Figure 3. Gas sorption isotherms recorded at 23 °C. a) Isotherms for TBC4 sample (5.714 g; blue: N_2 , black: air, red: O_2 , and green: CO_2). The chamber was evacuated for at least 1 h between experiments to remove the absorbed gas from the sample. b) Crushing the crystals resulted in the rate of gas absorption increasing markedly while the equilibrium pressures were unchanged (note that the isotherms for N_2 and air are overlapped). c) Occupancy of the lattice voids by CO_2 (assuming a 2:1 molar ratio of TBC4: CO_2) versus equilibrium pressure. Full occupancy is obtained as the pressure approaches 3 atm.

be unaffected by grinding, which further supports our claim that the gas is assimilated into the lattice rather than adsorbed onto the surface of the particles. It is interesting to note that the selectivity between O_2 and N_2 is diminished markedly when the particle size is reduced. Figure 3c reveals a type I relationship^[15] between the equilibrium pressure and the percentage of calixarene cavities occupied by CO_2 . Since two calixarene molecules are required to form one discrete lattice void it appears that, on average, each lattice void can be

almost fully occupied by one gas molecule at approximately three atmospheres of CO₂ pressure. Indeed, the occupancy is almost 80% even at one atmosphere of pressure.

Unexpectedly, exposure of the material to hydrogen at pressures of up to 7 atm did not result in any discernable absorption of this gas. As a result of the large disparity between the affinity of the TBC4 substrate for CO₂ and H₂, coupled with the acute current commercial interest in separating these gases, we exposed the material to a 3:1 mixture of CO₂:H₂ at an initial pressure of 1 atm for 19 h. The composition of the gas mixture, both before and after exposure to TBC4, was determined by gas chromatography (Figure 4). This experiment illustrates rather dramatically

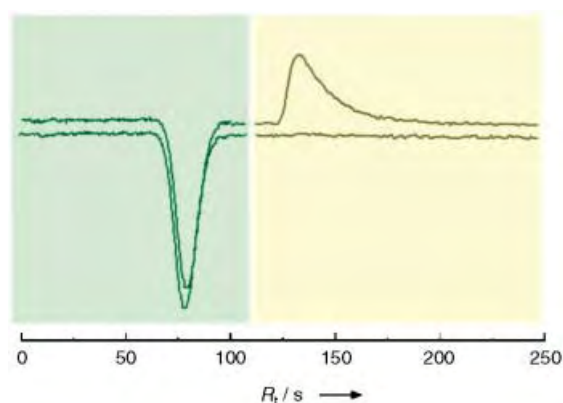


Figure 4. Gas chromatogram showing the composition of the supernatant CO₂/H₂ mixture prior and subsequent to the gas sample being subjected to TBC4. The intensity of the hydrogen peak (green box) does not change during the absorption experiment, while the carbon dioxide peak (yellow box) was entirely diminished, thus indicating that the solid selectively absorbs CO₂. Thermal conductivity detection of the gas mixture necessitated the use of nitrogen carrier gas to quantify the amount of hydrogen and, in a successive run, helium carrier gas to analyze the carbon dioxide content. ($T_{\text{column}} = -70^{\circ}\text{C}$; flow rate: 60 ml min⁻¹ nitrogen or helium; column: 120 × 0.5 cm; stationary phase: 20% DC200 Chromosorb PAWDMCS80/100 mesh).

that the substrate selectively sequesters CO₂ from the mixture, even at moderately low pressures, thus leaving the supernatant gas rich in H₂.

Guest species that normally exist as liquids or vapors are capable of appreciable intermolecular interactions that can provide the impetus for rearranging host molecules as part of a dynamic inclusion process. In contrast, volatile gases such as O₂, N₂, and CO₂ possess characteristically weak interactive capabilities, yet are still able to find and occupy the lattice voids without discernable disruption of the host lattice, or even the need for suitably sized pores. Thus, “soft” layered structures may employ as-yet unknown mechanisms that can allow the passive influx of gas molecules at rates comparable to those observed for porous materials. Moreover, the fate of the volatile hydrogen is clear: it is not absorbed. The hydrogen molecules may well visit the interior of the crystal, but if they do so, they are not retained. However, the classical

riddle of how the ship gets into the bottle remains, at least for now, a subject for speculation.

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Keywords: absorption · calixarenes · host–guest systems · hydrogen · inclusion compounds

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Doubly N-Confused Pentaphyrins

Alagar Srinivasan, Tomoya Ishizuka, Hiromitsu Maeda, and Hiroyuki Furuta*

In recent years, research on porphyrin analogues has received increased attention in regard to their structural diversity as well as their functionality.^[1] Among such porphyrinoids, N-confused porphyrin (NCP), a porphyrin isomer that contains a confused pyrrole ring connected through its α, β' -positions in the macrocycle, exhibits unusual physical and chemical properties which greatly differ from those of normal porphyrins.^[2] The further confused isomer, doubly N-confused porphyrin (N_2CP , *cis* and *trans*), also displays unusual coordination chemistry and properties, such as stabilization of unusual oxidation states of metals, existence of different tautomeric forms, efficient photosensitization of singlet oxygen, and formation of supramolecular hydrogen-bonding networks.^[3] Thus, the introduction of one or more confused pyrrole rings in the porphyrin macrocycles, which we call the confusion approach method, would be a useful strategy to

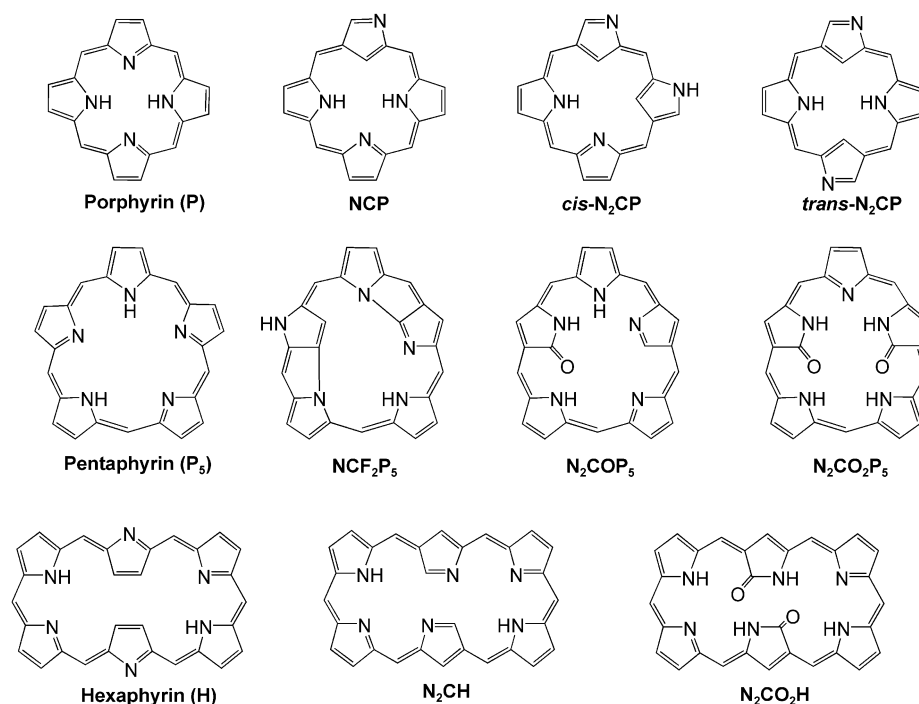
explore the hitherto unknown novel porphyrinoids. In fact, we have successfully demonstrated that the doubly N-confused hexaphyrin (N_2CH) and its dioxo (N_2CO_2H) derivatives preferably bound bismetal ions in the core.^[4] Very recently, we also identified N-confused, doubly N-fused pentaphyrins (NCF_2P_5), which contain two fused tripentacyclic rings of confused and normal types in the core, during an attempt to synthesize N-confused pentaphyrin.^[5] At this point, it was really a question whether introduction of further confusion into such a macrocycle would lead to a doubly N-confused isomer of $N_2CF_2P_5$ or a further fused one. Interestingly, the doubly N-confused pentaphyrin obtained was not fused, but existed in an oxo-substituted form similar to N_2CO_2H .^[4] Herein, we report the syntheses and the structural characterization of the doubly N-confused oxopentaphyrin (N_2COP_5) and doubly N-confused dioxopentaphyrin ($N_2CO_2P_5$). The mono-oxopentaphyrin forms a self-assembled dimer in the solid state through hydrogen-bonding interactions, while the dioxopentaphyrin exists in a monomeric form as a result of intramolecular hydrogen-bonding interactions within the core.

The synthetic method followed here is basically a [3+2] acid-catalyzed condensation, which has already been used for the synthesis of doubly N-fused pentaphyrins (NCF_2P_5).^[5] Stirring a mixture of a solution of N-confused

tripyrane (**1**)^[4,5] and N-confused dipyrromethane dicarbinol (**2**)^[6] in the presence of *p*-toluenesulfonic acid (*p*-TSA), followed by oxidation with *p*-chloranil afforded two isomeric, partially oxidized products **3a** and **3b** in yields of 23 and 12 %, respectively, together with a trace amount of **4** (Scheme 1a). The FAB mass spectra of both **3a** and **3b** showed a signal for the molecular ion at m/z 1222, which suggested the presence of the pentaphyrin framework. The ¹H NMR spectra of **3a** and **3b** in CDCl₃ were similar and the signals appeared between δ = 13.00 and 5.00 ppm. The NH proton of the confused pyrroles (rings A and B) resonated at δ = 8.56 ppm and the α - and β -CH protons resonated at 8.18 and 5.58 ppm, respectively, for **3a**, while those protons appeared at δ = 8.62, 8.17, and 5.75 ppm, respectively, for **3b** (Table 1). In

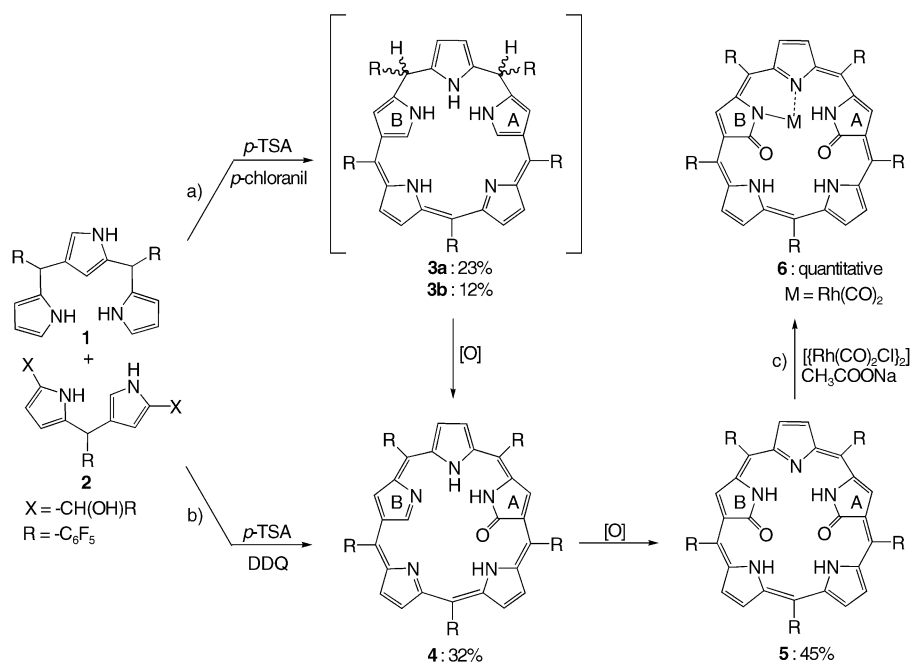
addition, the two *meso*-CH protons were observed as a singlet at δ = 5.78 ppm and a NH proton was observed in a downfield region at δ = 12.54 ppm as a result of the intramolecular hydrogen-bonding interactions. The ¹H NMR spectra suggested that **3a** and **3b** were both symmetrical isomers of the pentaphyrin intermediates shown in Scheme 1. Both the compounds were rather unstable and easily oxidized to aromatic **4** in quantitative yield.

The doubly N-confused oxopentaphyrin (N_2COP_5 , **4**) was obtained as a greenish solid in 32 % yield by changing the



[*] Dr. A. Srinivasan, Dr. T. Ishizuka, Dr. H. Maeda, Prof. Dr. H. Furuta
Department of Chemistry and Biochemistry
Graduate School of Engineering
Kyushu University
Fukuoka 812-8581 (Japan)
Fax: (+81) 92-651-5606
E-mail: hfuruta@cstf.kyushu-u.ac.jp
Prof. Dr. H. Furuta
PRESTO, JST

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



Scheme 1. Syntheses of doubly N-confused mono- and dioxopentaphyrins (**4**, and **5**) and the Rh^I complex (**6**).

Table 1: Selected physical data for **3–6**.

3a: m.p. > 300 °C (decomp); ¹H NMR (CDCl₃, 300 MHz): δ = 12.54 (brs, 1 H, NH), 8.56 (s, 2 H, NH), 8.18 (s, 2 H), 7.64 (s, 1 H, NH), 6.41 (d, <i>J</i> = 6 Hz, 2 H), 6.19 (d, <i>J</i> = 6 Hz, 2 H), 5.82 (d, <i>J</i> = 3 Hz, 2 H), 5.78 (s, 2 H), 5.58 ppm (s, 2 H); FABMS: <i>m/z</i> 1222 [M]⁺; UV/Vis (CH₂Cl₂): λ_{max} = 349, 391, 644 nm.
3b: m.p. > 300 °C (decomp); ¹H NMR (CDCl₃, 300 MHz): δ = 12.52 (brs, 1 H, NH), 8.62 (s, 2 H, NH), 8.17 (s, 2 H), 7.46 (s, 1 H, NH), 6.41 (d, <i>J</i> = 6 Hz, 2 H), 6.19 (d, <i>J</i> = 6 Hz, 2 H), 5.76 ppm (m, 6 H); FABMS: <i>m/z</i> 1222 [M]⁺; UV/Vis (CH₂Cl₂): λ_{max} = 349, 390, 492, 643 nm.
4: m.p. > 300 °C (decomp); ¹H NMR (CDCl₃, 300 MHz at –40 °C): δ = 9.19 (s, 1 H), 8.78 (s, 1 H), 8.38 (d, <i>J</i> = 6 Hz, 1 H), 8.31 (d, <i>J</i> = 6 Hz, 1 H), 8.17 (m, 3 H), 7.99 (d, <i>J</i> = 3 Hz, 1 H), 3.65 (s, 1 H, NH), 2.44 (s, 1 H), 1.42 (s, 1 H, NH), 1.10 ppm (s, 1 H, NH); FABMS: <i>m/z</i> 1232 [M]⁺; UV/Vis (CH₂Cl₂): λ_{max} (ε [mol^{–1} dm³ cm^{–1}]) = 388 (31 000), 544 (81 000), 676 (9600), 723 (6600), 861 nm (2600).
5: m.p. > 300 °C (decomp); ¹H NMR (CDCl₃, 300 MHz at –40 °C): δ = 10.04 (s, 2 H), 8.84 (m, 6 H), 0.85 (s, 2 H, NH), –0.54 ppm (s, 2 H, NH); FABMS: <i>m/z</i> 1251 [M]⁺; UV/Vis (CH₂Cl₂): λ_{max} (ε [mol^{–1} dm³ cm^{–1}]) = 341 (10 000), 393 (20 000), 446 (8500), 542 (77 000), 585 (87 000), 716 (7200), 801 (12 000), 875 nm (1900).
6: m.p. > 300 °C (decomp); ¹H NMR (CDCl₃, 300 MHz at –40 °C): δ = 10.22 (s, 1 H), 9.63 (s, 1 H), 8.83 (d, <i>J</i> = 6 Hz, 1 H), 8.70 (d, <i>J</i> = 6 Hz, 2 H), 8.57 (d, <i>J</i> = 3 Hz, 1 H), 8.30 (m, 1 H), 8.23 (d, <i>J</i> = 3 Hz, 1 H), 0.90 (s, 2 H, NH), –4.26 ppm (s, 1 H, NH); FABMS: <i>m/z</i> 1351 [M]⁺; UV/Vis (CH₂Cl₂): λ_{max} (ε [mol^{–1} dm³ cm^{–1}]) = 354 (28 000), 406 (41 000), 582 (96 000), 627 (87 000), 778 (13 000), 818 (14 000), 922 nm (9700).

oxidizing agent from *p*-chloranil to 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (Scheme 1b). The FAB mass spectrum of **4** showed a molecular ion signal at *m/z* 1232, which indicated the presence of a pentaphyrin skeleton as

well as an oxo group. In fact, the presence of a C=O group was confirmed by the presence of a band at $\tilde{\nu}$ = 1716 cm^{–1} in the IR spectrum. The ¹H NMR spectrum of **4** recorded in CDCl₃ at –40 °C showed the three different NH signals at δ = 3.65, 1.42 (CONH), and 1.10 ppm. The β-CH proton of the inverted oxo-substituted pyrrole (ring A) resonated at δ = 9.19 ppm while the α- and β-CH (ring B) protons of the confused pyrrole were observed at δ = 2.44 and 8.78 ppm, respectively, which proved that the product was a 22π electron aromatic compound (Table 1). Further support for this aromaticity came from the electronic absorption spectrum, which displayed a Soret-like band at 544 nm (Figure 1). However, the compound was relatively unstable and gradually oxidized in CH₂Cl₂ at room temperature over seven days into doubly N-confused dioxopentaphyrin (N₂CO₂P₅, **5**) in 45 % yield.

The FAB mass spectrum of **5** showed the molecular ion signal at *m/z* 1251, which suggested the presence of two oxo groups in

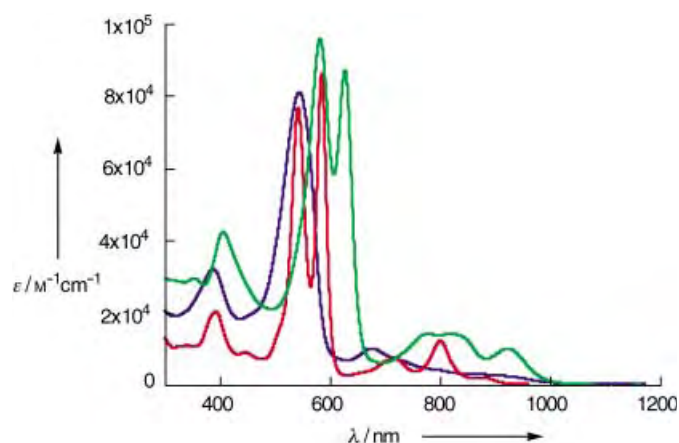


Figure 1. Absorption spectra of **4** (blue), **5** (red), and **6** (green) in CH₂Cl₂.

the pentaphyrin skeleton. The presence of a C=O group was confirmed by a band at 1710 cm^{–1} in the IR spectrum. The ¹H NMR spectrum of **5** recorded in CDCl₃ at –40 °C showed only four signals, thus indicating the symmetrical structure of the aromatic compound **5**. As expected, the α-CH proton of ring B observed in the spectrum of **4** disappeared and a set of two signals for the NH and β-CH groups in ring A and B appeared at δ = –0.54 and 10.04 ppm, respectively, while the remaining two NH signals were observed at δ = 0.85 ppm (Table 1). The aromatic structure of **5** was also suggested from the absorption spectrum, which displayed an intense, split Soret-like band at 542 and 585 nm as well as Q-bands from 700 to 900 nm (Figure 1).

The explicit structural details of **4** and **5** were revealed by X-ray single-crystal analyses (Figure 2a and b).^[7] Consistent

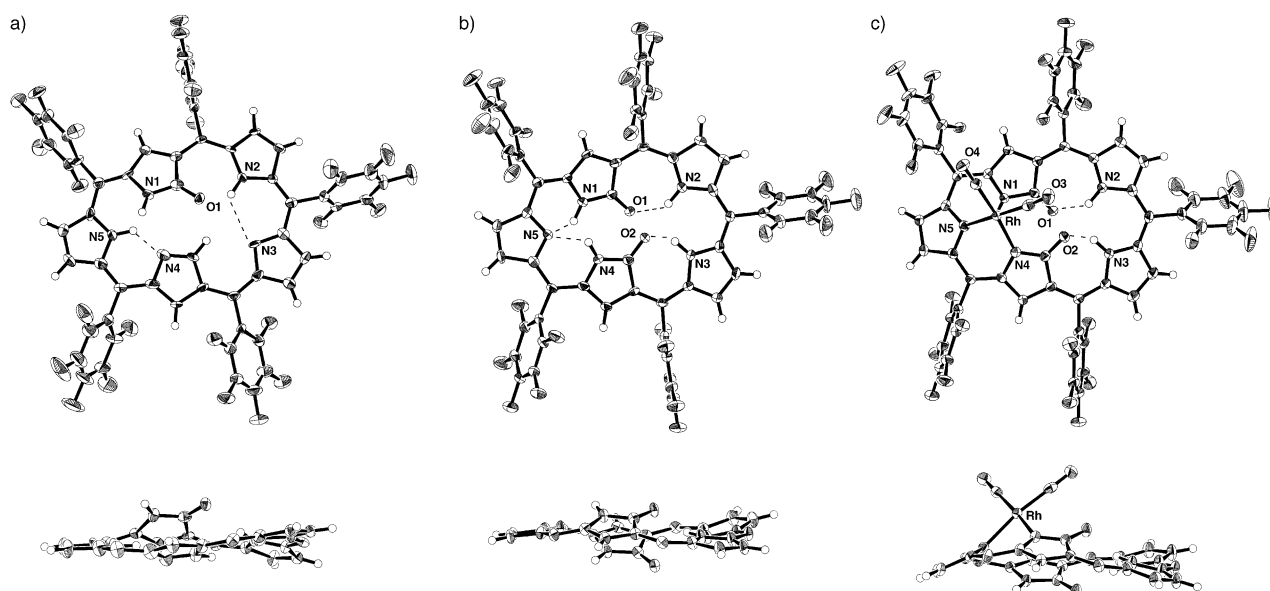


Figure 2. X-ray structures of a) **4**, b) **5**, and c) the Rh^I complex of **5** (**6**), top view (upper) and side view. (lower). *meso*-Aryl groups are omitted for clarity in the side view. Hydrogen-bonding interactions are indicated by broken lines.

with the above observations were the findings that both the confused pyrrole rings are inverted and one oxygen atom (O1) is attached at the inner α -carbon atom in **4**, while two oxygen atoms (O1, O2) are present in **5**. The bond lengths between the carbon and oxygen atoms are in the range of a C–O double bond, that is, 1.25(1) Å for **4**, and 1.224(8) and 1.231(7) Å for **5**. The pentaphyrin structure of **4**, which consists of the core 30 atoms, is highly distorted from the least-square plane, with a mean deviation of 0.32(1) Å and a tilting angle of the oxygen-substituted pyrrole (ring A) from the core plane of 146.0(4)°. The dihedral angles $\angle\text{CNC}$ clearly indicate that there are three inner NH protons located at N1, N2, and N5. Namely, these angles are larger than 110°, while those of N3 and N4 are smaller than 106°. [8] Two of the three NH protons (at N2 and N5) form intramolecular hydrogen bonds with the neighbors (N2–H···N3 and N4–H···N5, with lengths of 3.00(1) Å for N2···N3 and 2.55(1) Å for N4···N5), while the remaining N1–H proton forms an intermolecular hydrogen bond with the N1'–H group in another molecule to form a dimer (Figure 3). The distance between the N1 and

O1' atoms in the dimer is 2.84(1) Å and each molecule is arranged in a parallel fashion at an intermolecular distance of 4.3(2) Å. [7a] The CONH units present in **5** do not form such hydrogen-bonding interactions to form a dimer or oligomer, instead, four intramolecular hydrogen bonds are observed between O1···H–N2, O2···H–N3, N1–H···N5, and N4–H···N5 with lengths of 2.639(5), 2.567(6), 2.587(7), and 2.509(7) Å for O1···N2, O2···N3, N1···N5, and N4···N5, respectively. The intramolecular hydrogen-bonding interactions result in the pentaphyrin core of **5** being less distorted (0.270(7) Å) than **4** (Figure 2b). [7b]

The metal coordination chemistry of the stable compound **5** was examined using a Rh^I salt. Treatment of **5** with $[\text{Rh}(\text{CO})_2\text{Cl}]_2$ in the presence of sodium acetate in refluxing CH_2Cl_2 resulted in the formation of the Rh^I complex **6** in quantitative yield (Scheme 1c). [9] The unsymmetrical structure of the complex was suggested by the appearance of two different pyrrole β -CH (rings A and B) signals at $\delta = 10.22$ and 9.63 ppm in the ^1H NMR spectrum of **6**. The remaining pyrrole NH protons and the significantly shielded oxopyrrole NH (ring A) proton resonated at $\delta = 0.90$ and -4.26 ppm, respectively, which confirms that only two pyrrolic nitrogen atoms are coordinated to the Rh^I center in the cavity (Table 1). The electronic absorption spectrum of **6** displayed a split Soret-like band at 582 and 627 nm and the longest wavelength Q-bands appeared at 922 nm, which are 40 to 46 nm (Soret and Q-bands, respectively) bathochromic shifted relative to **5** (Figure 1). [10]

The structure of the Rh^I complex **6** was confirmed by single-crystal X-ray analysis (Figure 2c). [7c] As predicted from the above observations, there is only one Rh atom coordinated to the pentaphyrin skeleton in **6**. One imino (N5) and one amino (N4) nitrogen atom of the macrocycle are coordinated to the Rh^I ion and the other two coordination sites are occupied by the carbonyl ligands. The Rh atom is

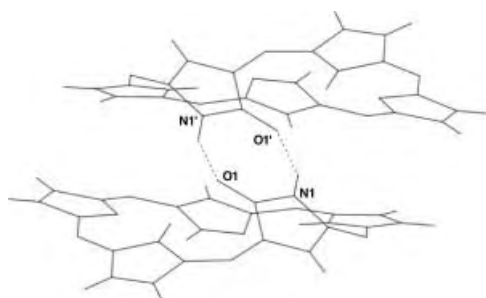


Figure 3. Hydrogen-bonded dimer of **4**. *meso*-Aryl groups are omitted for clarity. Hydrogen-bonding interactions are indicated by broken lines.

located above the mean plane of the macrocycle and the geometry around the metal center is close to square-planar (N4-Rh-N5, 80.2(2)°). The angle between the plane containing the Rh center and its coordinated atoms and the pentaphyrin plane is 54.64(9)°. Similar to **5**, intramolecular hydrogen bonds are formed between O1...H-N2 and O2...H-N3 in **6** and the atom distances of O1...N2 and O2...N3 are 2.643(5) and 2.557(6) Å, respectively. The pentaphyrin plane of **6** is highly distorted from the core plane with a mean deviation of 0.362(5) Å, while the intermolecular Rh...Rh distance is 3.3405(5) Å (see Supporting Information), which is considerably longer than a Rh^I–Rh^I single bond (2.617–2.705 Å).^[11]

In conclusion, we have synthesized doubly N-confused oxopentaphyrin and doubly N-confused dioxopentaphyrin as well as demonstrating the coordination chemistry of the latter compound. It has been clearly shown that the introduction of one more confused pyrrole ring into the macrocycle framework resulted in the remarkable structural diversity. Although the oxo-substitution mechanism is not clarified yet, the doubly N-confused oxopentaphyrins shown here are the first examples of a stable nonfused, *meso*-aryl-type pentaphyrin.^[5,12,13] The present study exploited the interaction of Rh^I ions, but other metal ions are likely to interact with the macrocycle. Similar to doubly N-confused dioxohexaphyrins (N₂CO₂H), efforts are currently underway to prepare a variety of bismetal complexes.

Experimental Section

3: A mixture of **1** (150 mg, 0.27 mmol) and **2** (190 mg, 0.27 mmol) in CH₂Cl₂ was stirred in a nitrogen atmosphere for 15 min at room temperature. *p*-TSA (7.7 mg, 0.040 mmol) was then added to the mixture and the solution was stirred for a further 1 h in the dark. The reaction mixture was then passed through a column of silica gel using CH₂Cl₂ (200 mL) as the eluent. *p*-Chloranil (199 mg, 0.81 mmol) was added to the combined solution and the mixture refluxed for a further 1 h, then stirred at room temperature for 2 days. After removal of the solvent, the crude product was purified by column chromatography, first on basic alumina and then on silica gel. A blue fraction was eluted with CH₂Cl₂/hexane (35:65), which gave **3b** (40 mg, 12 %) as a blue solid after removal of the solvent. Another blue fraction was eluted with CH₂Cl₂/hexane (50:50) and afforded **3a** (76 mg, 23 %) as a blue solid.

4: DDQ (183 mg, 0.81 mmol) instead of *p*-Chloranil was added to the mixture in the synthesis of **3**. The solution was stirred for 1 h at room temperature. After removal of the solvent, the crude product was purified by column chromatography, first on basic alumina and then on silica gel. A pink fraction was eluted with CH₂Cl₂/hexane (45:55), which gave **4** (106 mg, 32 %) as a greenish solid after removal of the solvent.

5: Compound **3** (50 mg, 0.041 mmol) was dissolved in CH₂Cl₂ (15 mL) and the solution stirred at room temperature. The reaction was monitored by TLC (silica gel) after every 10 h. The color of the solution gradually changed from pink to purple over 7 days. The solvent was evaporated, and the residue was purified by column chromatography on silica gel. A purple fraction was eluted with CH₂Cl₂/hexane (30:70) which gave **5** (23 mg, 45 %) as a greenish solid. The remaining product was identified as starting material **4**.

6: Compound **5** (25 mg, 0.02 mmol) was dissolved in CH₂Cl₂ (50 mL). Anhydrous sodium acetate (16 mg, 0.2 mmol) and [[Rh(CO)₂Cl]₂] (16 mg, 0.04 mmol) were added to the solution and the mixture was stirred under reflux for 3 h. After removal of the

solvent, the residue was purified by column chromatography on silica gel. A blue fraction was eluted with CH₂Cl₂/hexane (40:60), which gave **6** (quantitative) as a green solid after removal of the solvent.

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Keywords: aromaticity · macrocyclic ligands · oxidation · porphyrinoids · rhodium

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Heterogeneous Catalysis

A Carbon Material as a Strong Protonic Acid**

Michikazu Hara,* Takemi Yoshida, Atsushi Takagaki,
Tsuyoshi Takata, Junko N. Kondo, Shigenobu Hayashi,
and Kazunari Domen

Solid acids are conventional materials that have wide applications in chemical production, separation/purification, and polymer–electrolyte fuel-cell (PEFC) technologies, and the chemical industry is currently searching for a highly active and stable solid acid to improve the environmental safety of the production of chemicals and energy. Over 15 million tons of sulfuric acid is annually consumed as “an unrecyclable catalyst”—which requires costly and inefficient separation of the catalyst from homogeneous reaction mixtures—for the production of industrially important chemicals, thus resulting in a huge waste of energy and large amounts of waste products. The “green” approach to chemical processes has stimulated the use of recyclable strong solid acids as replacements for such unrecyclable “liquid acid” catalysts.^[1–4]

[*] Prof. M. Hara, T. Yoshida, A. Takagaki, Dr. T. Takata, Dr. J. N. Kondo, Prof. K. Domen

Chemical Resources Laboratory

Tokyo Institute of Technology

4259 Nagatsuta, Midori-ku, Yokohama 226-8503 (Japan)

Fax: (+81) 45-924-5282

E-mail: mhara@res.titech.ac.jp

Prof. K. Domen

Core Research for Evolutional Science and Technology (CREST)

Japan Science and Technology co.

4-1-8 Honcho, Kawaguchi, Saitama, 332-0012 (Japan)

Dr. S. Hayashi

Institute for Materials & Chemical Process

National Institute of Advanced Industrial Science and Technology (AIST)

Central 5, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8565 (Japan)

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Thermostable strong solid acids would have genuine applications in PEFCs as proton conductors, for improving fuel efficiency, and for reducing the use of noble-metal catalysts by increasing the working temperature.^[5] However, a major obstacle to such progress is the lack of a solid acid that is as active, stable, and inexpensive as sulfuric acid.

An ideal solid material for the applications considered here should have high stability and numerous strong protonic acid sites. It is essential for the solid acid to maintain strong acidity even in water since water participates in fuel-cell reactions and many industrially important acid-catalyzed reactions. While organic acid/inorganic solid oxide hybrids and strong acidic cation-exchangeable resins, including perfluorosulfonated ionomers (for example, nafion), have been studied extensively as promising approaches for the construction of desired solid acids or proton conductors,^[6] such materials are expensive and the acid activities are still much lower than that of sulfuric acid.^[3] These drawbacks have limited their practical utility. Herein, we report the synthesis of a carbon-based solid acid with a high density of sulfonic acid groups (SO₃H) and discuss its performance as a novel strong and stable solid acid. Here, a new strategy is adopted for the development of new types of solid acid: a carbon material is obtained by incomplete carbonization of sulfoaromatic hydrocarbons and consists of small polycyclic aromatic carbon sheets with attached SO₃H groups. This approach is simple and allows for the use of sulfoaromatic hydrocarbons—strong, stable solvent-soluble acids (for example, benzene sulfonic acid and naphthalene sulfonic acid)—as insoluble solid acids.

Such carbon-based solid acids can be readily prepared by heating aromatic compounds such as naphthalene in sulfuric acid at 473–573 K.^[7] In this synthesis, the sulfonation of the aromatic compounds is the first stage of the reaction. The resulting sulfonated aromatic compounds are incompletely carbonized, which results in the formation of a solid with a nominal sample composition of CH_{0.35}O_{0.35}S_{0.14}. The total yield of the product based on carbon is about 55 % by this method. The resulting black powder is insoluble in the tested solvents (water, methanol, ethanol, benzene, and hexane), even at boiling temperatures. A pellet formed from the powder has a high resistance of over 30 MΩ.

The powder X-ray diffraction (XRD) pattern and ¹³C magic angle spinning (MAS) nuclear magnetic resonance (NMR) spectrum of the sample are shown in Figure 1 a and b. The XRD pattern exhibits two broad, weak diffraction peaks (2θ = 10–30°, 35–50°) attributable to amorphous carbon composed of aromatic carbon sheets oriented in a considerably random fashion.^[8] The two peaks (δ = 141 and 130 ppm) in the ¹³C MAS NMR spectrum are assigned to aromatic carbon atoms with and without SO₃H groups.^[9] A neutralization titration gave 4.9 mmol g^{−1} SO₃H attached to 11 % of the aromatic carbon atoms (Table 1). This finding means that the effective density of the acid sites in the sample is about five times that of nafion, which is a highly active and stable perfluorosulfonated ionomer. These results indicate that this material is amorphous carbon consisting of polycyclic aromatic carbon sheets with attached SO₃H groups that functions as a strong solid acid with a high density of acid sites.

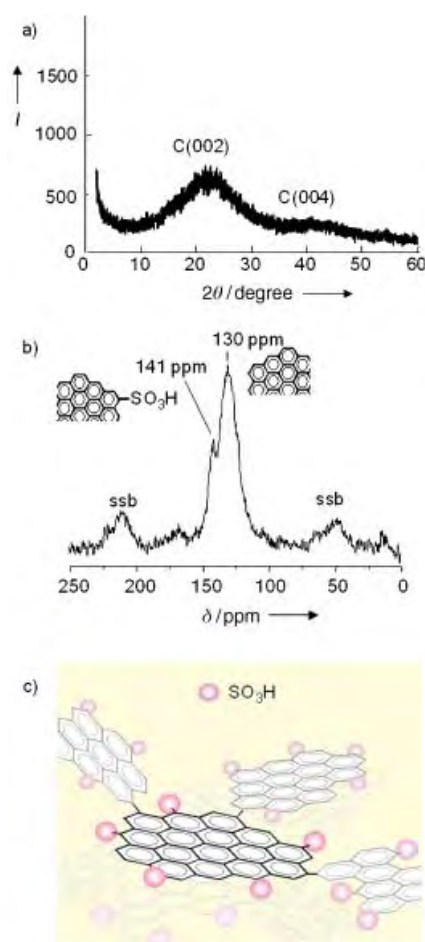


Figure 1. a) Powder XRD pattern, b) ^{13}C MAS NMR spectrum measured at 50.3 MHz, and c) proposed schematic structure of the carbon-based solid acid ($\text{CH}_{0.30}\text{O}_{0.33}\text{S}_{0.16}$). The carbon-based solid acid was evacuated at 180°C for 1 h prior to ^{13}C -MAS NMR measurement. Spinning sidebands (ssb) appear at $\delta = 50$ and 210 ppm. The material is amorphous carbon composed of small sulfopolycyclic aromatic carbon rings oriented in a random fashion. SO_3H groups are attached to 11% of the aromatic carbon atoms.

Table 1: Effective densities of the acid sites and catalytic activities of the tested samples in the formation of ethyl acetate.

Acid	Acid density [mmol g $^{-1}$]	Surface area [m ^{2} g $^{-1}$]	Catalytic activity [mmol min $^{-1}$]
carbon-based solid acid ($\text{CH}_{0.35}\text{O}_{0.35}\text{S}_{0.14}$)	4.90	24	1.3
protonated nafion (NR50)	0.93	< 0.1	0.2
H_2SO_4 -treated carbon materials			
carbonized resin	0.08	19	–
glassy carbon	0.05	26	–
activated carbon	0.15	1250	–
natural graphite	0.05	25	–
H_2SO_4	20.4	–	1.9

This carbon-based solid acid is not an electron conductor and contains relatively high densities of H and SO_3H groups, which suggests that the material is composed of sulfopolycy-

clic aromatic hydrocarbons as illustrated in Figure 1c, rather than aromatic carbon sheets as seen in conducting amorphous carbon materials.

The thermal stability of the material was examined by thermogravimetric analysis (TGA) and temperature-programmed desorption (TPD) under a flow of air (Figure 2).^[10] The sample weight decreased with increasing temperature in the TGA (Figure 2a), and reached a plateau

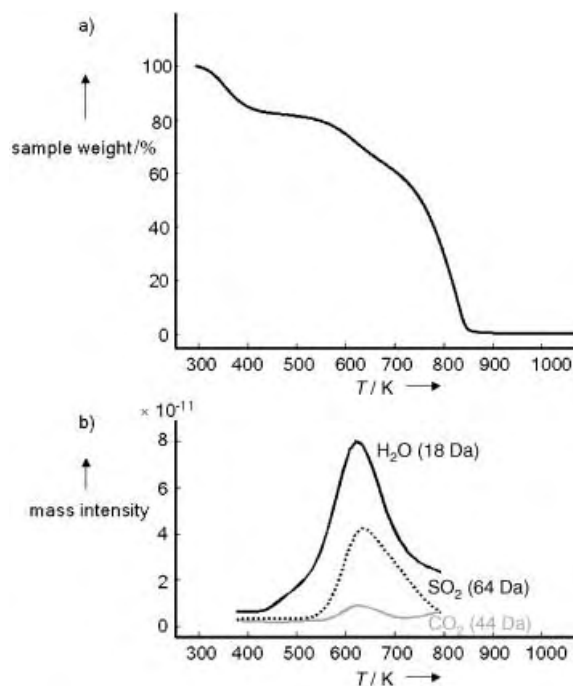


Figure 2. a) TGA of the carbon-based solid acid; b) TPD curves for the carbon-based solid acid. The sample was heated at 10 K min^{-1} .

at $390\text{--}420\text{ K}$. Heating the sample above $500\text{--}550\text{ K}$ resulted in a decrease in weight. TPD (Figure 2b) revealed that H_2O evolution ($M_r = 18\text{ Da}$) from the sample increases rapidly with temperature above 520 K , at which both SO_2 ($M_r = 64\text{ Da}$) and CO_2 ($M_r = 44\text{ Da}$) begin to evolve. No SO_3 ($M_r = 80\text{ Da}$) evolution was detected from the sample. No significant differences in the ^{13}C MAS NMR spectra or sample composition were observed before and after heating at 473 K . Heating the sample at 523 K caused a gradual reduction in the resonance of the sulfonated aromatic carbon atoms ($\delta = 144\text{ ppm}$) in the ^{13}C MAS NMR spectrum and a decrease in the H, O, and S compositions with increasing heating time. These results clearly indicate that the solid acid functions as such at temperatures up to $500\text{--}520\text{ K}$, thus the material is not at all inferior to perfluorosulfonated ionomers in chemical and thermal stability.

The strength and density of the acid sites of the material were demonstrated through the esterification of acetic acid (343 K), hydrolysis of cyclohexyl acetate (353 K), and hydration of 2,3-dimethyl-2-butene (343 K).^[11] Figure 3 shows the rates of formation of ethyl acetate (Figure 3a), cyclohexanol (Figure 3b), and 2,3-dimethyl-2-butanol (Figure 3c) on the carbon-based solid acid (0.2 g). For comparison, the results

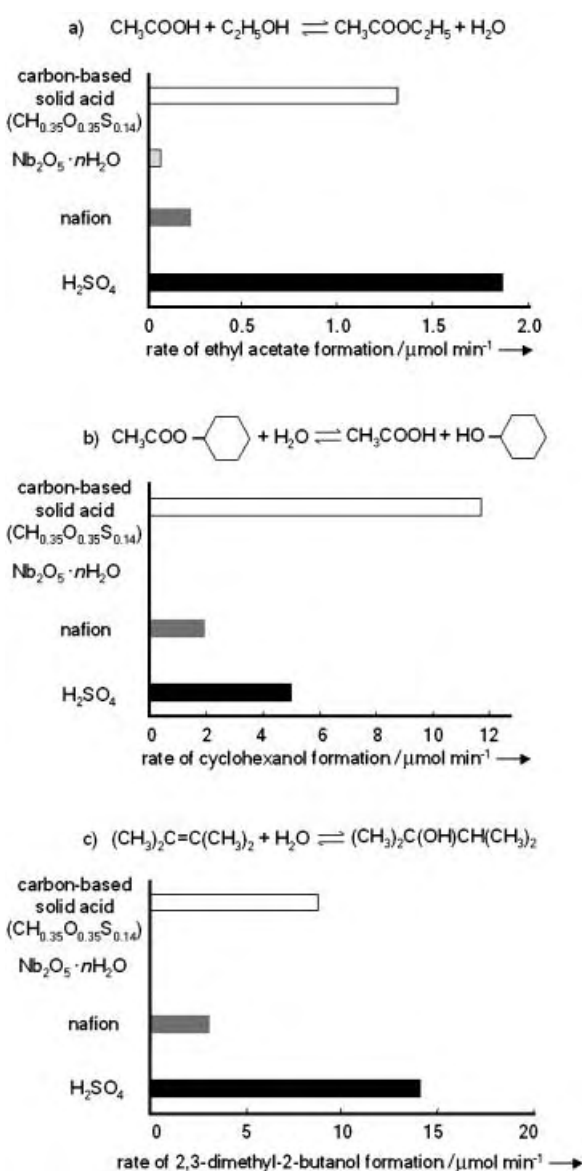


Figure 3. Acid-catalyzed liquid-phase reactions: a) Rate of formation of ethyl acetate during the esterification of acetic acid (343 K); b) rate of formation of cyclohexanol in the hydrolysis of cyclohexyl acetate (353 K); c) rate of formation of 2,3-dimethyl-2-butanol (343 K). Rates were measured in the early stage of the reaction (1 h).

for 0.2 g of concentrated sulfuric acid (>96%), protonated nafion (NR50), and niobic acid ($\text{Nb}_2\text{O}_5 \cdot n\text{H}_2\text{O}$) are also shown. Niobic acid is a rare inorganic solid acid that exhibits only marginal acidity in the presence of water, whereas protonated nafion, which is used as a proton conductor in PEFC, exhibits very high activity for reactions involving thermostable solid acids.^[3,6] The carbon-based solid acid exhibited a remarkably high activity for the formation of ethyl acetate: the activity is much higher than those of conventional solid acids and is comparable to that of sulfuric acid. After the reaction had reached equilibrium (7–10 h), the carbon-based solid acid was simply recovered by decantation and recycled for further reaction. It was confirmed that both the activity for

the formation of ethyl acetate and the sample composition ($\text{CH}_{0.35}\text{O}_{0.35}\text{S}_{0.14}$) remained unchanged, even after the sample had been recycled for a fourth time. Figure 3 b and c also show that the carbon-based solid acid rivals sulfuric acid in the hydrolysis of cyclohexyl acetate and the hydration of 2,3-dimethyl-2-butene, and also attacks hydrophobic reactants in excess water to the same extent as sulfuric acid. Since many industrial important chemicals such as alcohols, esters, ethers, and acetals are produced by such reactions in the presence of a sulfuric acid catalyst, the comparable performance of the carbon-based solid acid as indicated by the present results demonstrates that it has the potential to replace the sulfuric acid catalyst in such applications.

The carbon-based solid acid has clear potential for use as a replacement for the sulfuric acid catalyst. Furthermore, the SO_3H groups act as proton carriers, which means that the material with a high density of SO_3H groups is also useful as a proton conductor. A pellet formed from the sample powder ($\text{CH}_{0.35}\text{O}_{0.35}\text{S}_{0.14}$) exhibited a protonic conductivity of 0.11 S m^{-1} at 323 K (humidity: 100%), which is comparable to that of nafion (0.1 S m^{-1} , 353 K).^[12] The conductivity in the current case is considered to be limited by the irregular shape of particles in the pellet, which prevents rapid protonic conduction. In addition, the high density of SO_3H groups in the material suggests that the carbon-based material itself displays much higher protonic conductivity than the measured value.

The same carbon-based solid acids can also be synthesized from various polycyclic aromatic hydrocarbons, such as anthracene ($\text{C}_{14}\text{H}_{10}$), perylene ($\text{C}_{20}\text{H}_{12}$), and coronene ($\text{C}_{24}\text{H}_{12}$), in coal tar. This suggests that coal tar, petroleum pitch, and asphalt, which are composed predominantly of polycyclic aromatic hydrocarbons, are also potential strong solid acids. However, stable and active solid acids could not be prepared by sulfonation of an incompletely carbonized resin, amorphous glassy carbon, activated carbon, or natural graphite. Heating these carbon materials in sulfuric acid resulted in carbon materials with only a low density of SO_3H groups,^[13] and these H_2SO_4 -treated carbon materials did not act as an acid catalyst in the esterification of acetic acid. Effective acid site densities, surface areas, and catalytic activities of the tested samples are summarized in Table 1. There was no difference in the surface areas of the carbon materials before and after treatment with H_2SO_4 . The SO_3H groups on the carbon atoms were preserved in water at room temperature, but all were removed under the reaction conditions used or in warm water above 323 K. Apparently, there is a definitive distinction between the stability and SO_3H density of H_2SO_4 -treated carbon materials and carbon-based solid acids, even though the SO_3H groups are bonded to aromatic carbon atoms in both materials. Thus, it is difficult to introduce large amounts of active functional groups into carbon materials,^[14] but these results suggest that highly active and stable carbon materials with high densities of functional groups can be readily prepared by incomplete carbonization of aromatic compounds in sulfuric acid.

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- [11] Esterification of acetic acid (343 K), hydrolysis of cyclohexyl acetate (353 K), and hydration of 2,3-dimethyl-2-butene (343 K) were carried out in a mixture of ethanol (1.0 mol) and acetic acid (0.1 mol) and distilled water (3.3 mol) containing cyclohexyl acetate (4.4 mmol) or distilled water (0.22 mol) containing 2,3-dimethyl-2-butene (0.22 mol) in an Ar atmosphere. All catalysts tested except sulfuric acid were evacuated at 373–453 K for 1 h prior to reaction, and 0.2 g of each catalyst was used in the reactions. The liquid phase was analyzed during the reaction by gas chromatography using capillary columns.
- [12] The proton conductivity of the carbon-based material was obtained by an alternating current impedance method. A pellet (5 mmφ, 1 mm thickness) between carbon papers was set in a measurement cell in an atmosphere of H₂ and saturated H₂O vapor (total pressure: atmospheric pressure).
- [13] In a typical preparation, carbon powder (20 g; carbonized resin, glassy carbon, activated carbon, and natural graphite) was heated in concentrated sulfuric acid (> 96%, 200 mL) at 523 K for 15 h under a flow of N₂. The resulting carbon material was washed repeatedly with water at room temperature until sulfate ions were no longer detected in the wash water. Carbonized resin was obtained by incomplete carbonization of polymerized furfuryl alcohol. Furfuryl alcohol was polymerized in the presence of acid. The resulting polymer was heated at 543 K for 72 h in a flow of Ar. The carbonized resin was a considerably amorphous carbon material (CH_{0.35}O_{0.35}S_{0.14}).
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Micelles

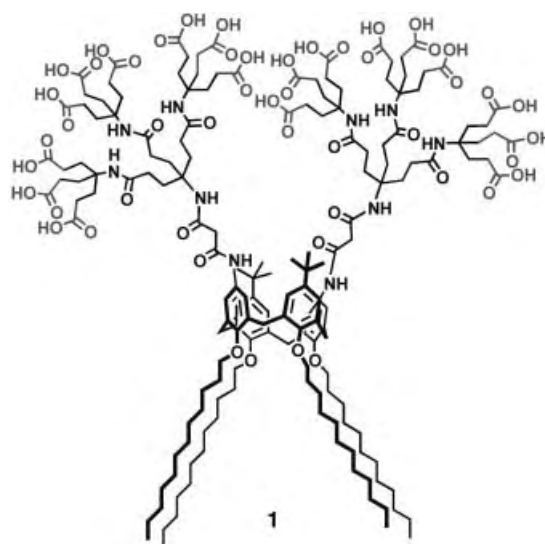
The First Account of a Structurally Persistent Micelle**

Michael Kellermann, Walter Bauer, Andreas Hirsch,*
Boris Schade, Kai Ludwig, and Christoph Böttcher*

Micelles are loose, mostly spherical aggregates spontaneously formed by amphiphilic molecules when they exceed their critical micelle concentration (cmc) in water or organic solvents.^[1] The formation of micelles is a result of a complex interplay between weak nondirectional repulsive forces and solvophobic effects. Micellar aggregates are short-lived dynamic species, which rapidly disassemble and reassemble. Only the average shape and aggregation numbers (n) of micelles can therefore be determined.

The controlled self-assembly of specific amphiphilic molecules, leading to precisely defined and stable micelles, not only represents an enormous challenge for fundamental research, but also has many potential nanotechnological applications, such as the development of high-performance drug-delivery capsules.^[2] The key to attaining this goal is the proper design of corresponding amphiphilic building blocks, since the information determining their specific supramolecular assembly must be encoded in their molecular architecture. It has been predicted that very small aggregates of high curvature require cone-shaped amphiphiles that involve sterically demanding and charged head groups and a considerably smaller hydrophobic part.^[3]

These structural requirements are combined in the amphiphilic dendro-calixarene **1**, which was obtained in a multistep synthesis from readily available starting materials (see Supporting Information). We report here for the first time the assembly of completely uniform and structurally precise micelles, which are spontaneously formed by exactly seven amphiphilic dendro-calixarene molecules of **1** in aqueous solution and are nondeformable upon drying. The



structure was determined at 12 Å resolution by cryo-TEM and 3D reconstruction techniques. This system represents a new prototype of chemically modifiable nanocontainers, which are uniform and stable but can also be reversibly locked to enclose a defined hydrophobic core volume.

Calixarene **1** was isolated in satisfactory yields as a yellow-ochre powder. Complete characterization was carried out by NMR and UV/Vis spectroscopy, and by FAB mass spectrometry. The very pronounced amphiphilic character of **1** is due to the presence of four C₁₂ chains attached to the lower rim and two convergently synthesized^[4] dendritic nona-carboxylates bound in opposite positions of the upper rim. Apart from **1** only one other amphiphilic calix[4]arene dendrimer containing peripheral carbohydrate moieties has been reported.^[5] At neutral pH most of the carboxylic groups are deprotonated, leaving **1** as an oligoanionic amphiphile. It readily dissolves in water (23 mg mL⁻¹ at pH 7.2) at room temperature and in DMSO at 40 °C. The solubility in organic solvents such as methylene chloride and tetrahydrofuran is very low. Lowering of the pH to 4 causes precipitation.

The aggregation behavior of **1** was investigated by pulse-gradient spin-echo (PGSE) NMR spectroscopy, by encapsulation experiments, and by cryo-TEM. PGSE NMR spectroscopy revealed only one type of aggregate, which had a diameter of approximately 6 nm (Figure 1). Similar particle sizes were recently found for amphiphilic calix[4]-resorcarene glycoclusters used for the assembly of artificial viruses.^[6] PGSE NMR spectroscopy carried out at pH 11 showed disassembly of the aggregates.

The micellar behavior of **1** in aqueous solution was also demonstrated by the fact that it is able to encapsulate very nonpolar hexane-soluble molecules such as porphyrin **2** or the fullerene derivative **3** and transfer them to the water phase. In a typical encapsulation experiment, porphyrin **2** was dissolved in hexane and added to an aqueous solution of **1**. The mixture was treated by ultrasonication for twelve hours. After this time the water subphase turned red, whereas the hexane subphase bleached. The absorption of **2** within the hexane subphase almost disappeared, as demonstrated by UV/Vis

[*] M. Kellermann, Prof. W. Bauer, Prof. A. Hirsch
Institut für Organische Chemie
Universität Erlangen-Nürnberg
91054 Erlangen (Germany)
Henkestrasse 42
Fax: (+49) 9131-85-26864
E-mail: andreas.hirsch@organik.uni-erlangen.de

Dr. B. Schade, Dr. K. Ludwig, Dr. C. Böttcher
Forschungszentrum für Elektronenmikroskopie
Institut für Chemie
Fabeckstrasse 36a
Freie Universität Berlin
14195 Berlin (Germany)
Fax: (+49) 30-838-56589
E-mail: bottcher@chemie.fu-berlin.de

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

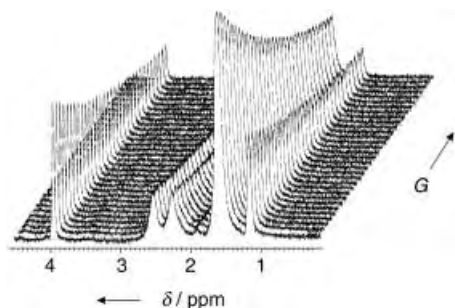


Figure 1. Stacked plot of ^1H PGSE (DOSY) NMR spectra of **1** at pH 7.2 in water, obtained on a JEOL Alpha500 spectrometer at 303 K and a diffusion delay of 100 ms. From front to rear the strength G of the gradients in the DOSY pulse sequence is increased, leading to an exponential decay of the signals. Small molecules yield a rapid decay, large molecules yield a slow decay of signal intensity I . A subsequent plot of $\ln(I/I_0)$ versus G^2 yields a straight line, whose slope is proportional to the diffusion constant D . By substituting D into the well-known Stokes–Einstein equation, the hydrodynamic radius R of a molecule can be obtained. The maximum gradient strength in this experiment was 180 Tm^{-1} .

spectroscopy. Definite proof for encapsulation was obtained from NMR and UV/Vis spectroscopy of the water subphase, which showed the characteristic spectra of **2**. Comparable treatment of **1** with **3** led to similar results.

Spherical micelles formed by conventional ionic detergents were directly visualized for the first time as recently as 1988 by the use of the cryo-TEM technique.^[7] Except for their approximate radius and spherical shape, structural details of these micelles could not be resolved. However, cryo-TEM micrographs of **1** (Figure 2a) revealed a very defined internal structural pattern, which was even amenable for advanced image-processing procedures (alignment, classification), as well as 3D reconstruction, which nowadays is a well established method for the 3D structure determination for example, of proteins.^[8] Extracted images of individual single aggregates corresponding to identical spatial orientations (identical Euler angles) could thus be identified, extracted, aligned, and summed up to eventually yield class-sum images with an enhanced signal-to-noise-ratio. Figure 2b (top) shows a gallery of selected class-sum images out of a total of 180,

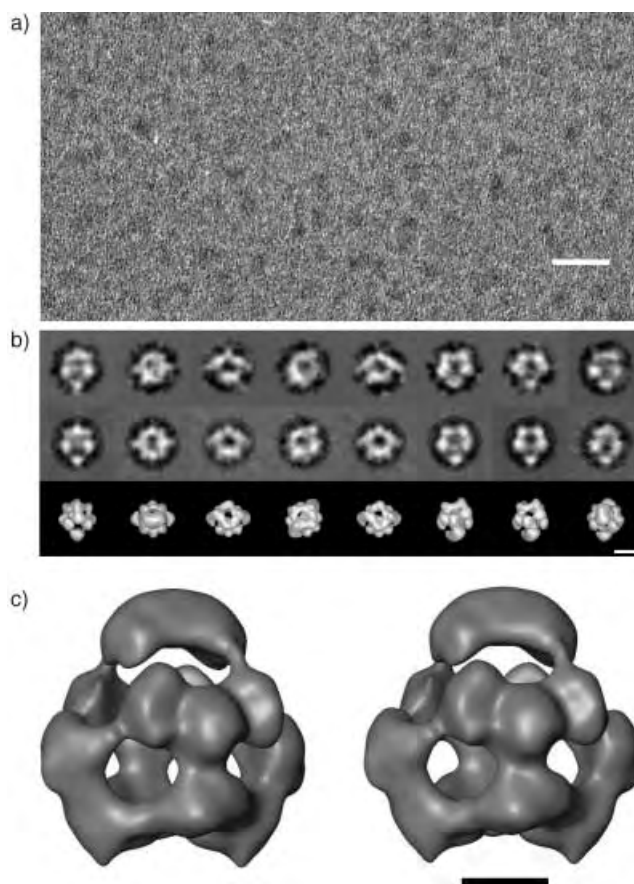
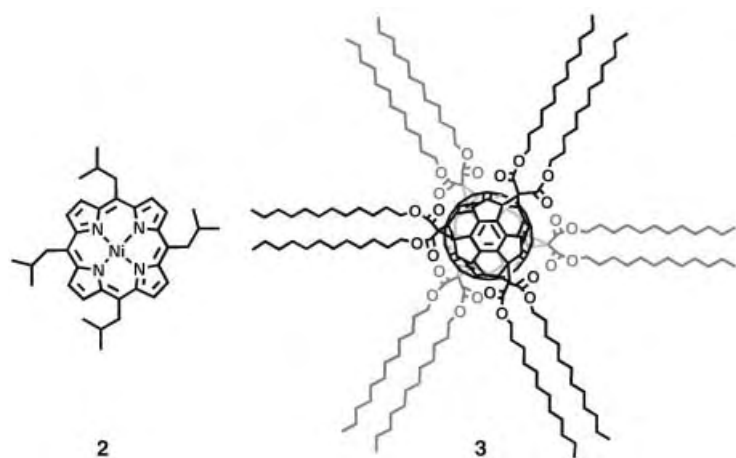


Figure 2. a) Representative electron micrograph of calixarene micelles embedded in vitreous ice (bar is 100 Å). b) Row 1 shows class averages^[9b] representing different spatial views of the micelles. Based on the assignment of corresponding Euler angles^[9a] 3D structure information can be retrieved at a resolution of 12 Å. Reprojections (row 2) into the 3D volume (row 3) at corresponding Euler angle directions illustrate the fit with the experimental data (bar is 50 Å). c) Stereo view of the isosurface rendered 3D structure (bar is 25 Å).

which were used for the subsequent assignment of Euler angles and a final 3D reconstruction.^[9] Successful 3D reconstruction at a resolution level of 12 Å confirmed the high degree of structural fidelity in the data set. The existence of smaller or bigger aggregates than those reported was not supported by the data. As the method is based on the mandatory precondition that all the aggregates used for processing represent different spatial orientations of one identical object, we can conclude that a micellar structure of unprecedented definition has been found.

The reconstruction appears as a hollow spherical cage of about 75 Å diameter with a specific pattern of surface penetrations (Figure 2c). A simple volume calculation points to aggregation numbers in the order of 12. Established micelle models^[10] propose a spherical hydrophilic corona formed by the fully hydrated polar head groups of amphiphilic molecules, which shield the apolar hydrocarbon chains from contact with the polar solvent. The headgroups are homogeneously distributed over the



micelle surface as a result of nonlinear repulsive forces. It has been proven that the hydrophobic chains of amphiphiles tend to be disordered in supramolecular aggregates.^[11] This fact might explain why density information in the reconstruction, corresponding to the interior hydrophobic core, is averaged out by the image processing procedure. Structural information from flexible nonisomorphic apolar hydrocarbon chains are therefore lacking in the reconstruction. The reconstructed structure information is therefore assumed to exclusively correspond to the spatially defined comparatively rigid head groups of the calixarenes. Moreover, the remarkable degree of monodispersivity and thus structural identity even allows for the assignment of pattern on the molecular scale. Visual 3D fitting by using the low-energy conformer of the calixarene headgroup (Figure 3) showed that exactly seven molecules can be precisely accommodated in the reconstructed

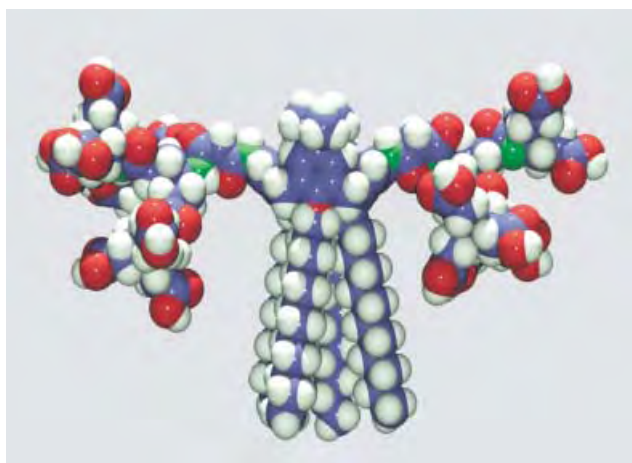


Figure 3. Space-filling model of the low-energy conformer of **1**.

density map (Figure 4).^[12] The best fit revealed a surprisingly unusual packing of seven molecules with C_2 symmetry: 2×2 molecules (green and blue in Figures 4c, d) are intercalated in such a way that one polar wing of the T-shaped molecule is located between the two polar wings of a second molecule. Two such “dimers” are almost exactly oriented opposite to each other. The distance corresponds to a typical bilayered arrangement (6.7 nm). Three more molecules (yellow, red, and purple) form additional cliplike connections in such a manner that the hydrophobic edges of the dimers are shielded.

Alternative model arrangements of seven molecules can be imagined. Figure 4b illustrates a variant with C_{2v} symmetry, which, based on chemical considerations, provides an even more plausible model since all molecules appear chemically equivalent. However, as can be seen, this arrangement fits less accurately into the reconstructed 3D density map. Other options such as C_{3v} or D_{5h} symmetry (see Figure 4a) provide a totally different density pattern (not shown), which is not consistent with the reconstruction and can therefore be excluded. The structure is so robust and stable that if the solvent is entirely removed from the sample before imaging, the structural pattern can be still reconstructed in exactly

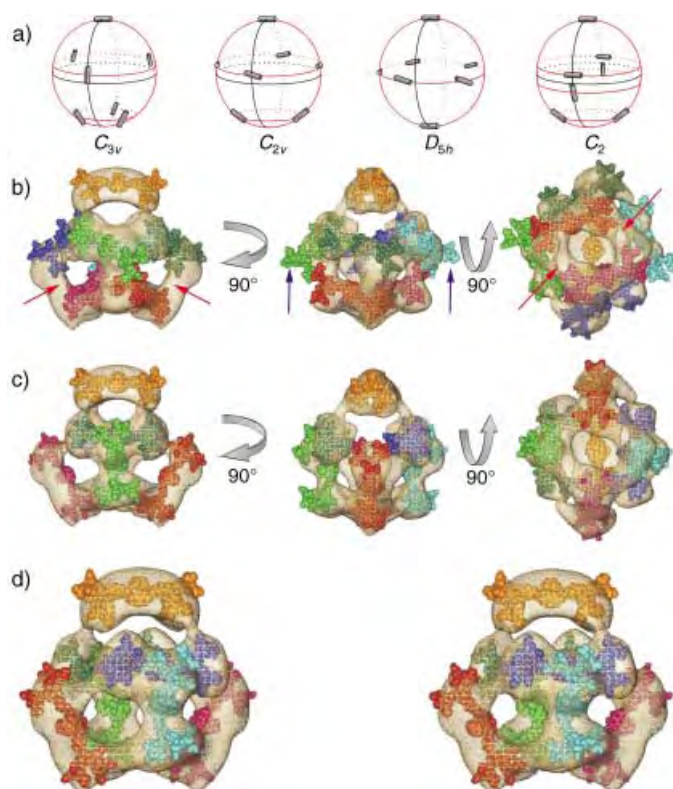


Figure 4. a) Some possible arrangements of seven molecules on a spherical shell. b), c) Views of the reconstruction including calixarene head groups. b) Chemical plausible arrangement of seven calixarene headgroups fitted into the EM density map. Misfitting arrays are indicated. c) The most satisfying spatial adjustment corresponds to a special C_2 arrangement of seven calixarene head groups. d) A stereo view of variant (c). Green and blue calixarene molecules are intercalated. Three more molecules (yellow, red, and purple) form additional cliplike connections.

same manner as described above (see Supporting Information).

In conclusion, we have synthesized the first amphiphile that self assembles into completely uniform and structurally persistent micelles. This in turn allowed the first structural characterization of a micelle on the molecular scale. The T shape of **1** induces a strong and specific back folding, leading to small and highly curved aggregates, which prevent the formation of larger assemblies of low curvature. At the same time, the specific arrangement of the partially intercalated charged head groups reduces flexibility within the aggregate for example, by hydrogen bonding, which is probably solvent-mediated. Specific directional interactions of the head groups thus stabilize a defined structural pattern with typical surface penetrations, in contrast to classical detergent micelles, in which nonlinear repulsion forces determine a fluidlike molecular organization. Nevertheless, a complete head-group hydration sphere, which does not contribute to the reconstructed density map, is thought to effectively exclude the hydrophobic core from the polar aqueous environment. Further investigations on the specific targeting of these uniform micelles and their use as vehicles for the delivery of apolar molecules are necessary, but we can already foresee a

variety of applications, some of which may still be in the realm of the imagination.

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The Glycosyltransferase UrdGT2 Catalyzes Both C- and O-Glycosidic Sugar Transfers**

*Clemens Dürr, Dirk Hoffmeister, Sven-Eric Wohler, Koji Ichinose, Monika Weber, Ursula von Mulert, Jon S. Thorson, and Andreas Bechthold**

*Dedicated to Heinz G. Floss
on the occasion of his 70th birthday*

The bioactivity of many natural products, among them valuable antibiotics and anticancer therapeutics, depends on regio- and stereospecifically attached sugar moieties.^[1] Although glycosylation methodology has vastly improved during the last decade, it is still exceedingly difficult to alter natural-product sugar patterns by chemical synthesis. Hence, the application of glycosyltransferases (GTs) in chemoenzymatic or in vivo approaches are of interest to develop complex natural-product-based drugs, such as glycopeptide and macrolide antibiotics.^[2]

In the majority of cases, natural-product GTs catalyze the transfer of a sugar, from a nucleotide diphosphate (NDP) sugar donor to the oxygen nucleophile of an acceptor substrate (typically, the hydroxy group of an aglycon or another sugar). A small number of GTs capable of forming C–C bonds and, in rare cases, C–N bonds have also been observed. The products of such C-GTs are of particular pharmaceutical interest as isosteric O-glycoside mimics that are stable against glycosidase degradation.^[3] However, while recent progress on O-GT structures^[4] provides increasing knowledge on the mechanism of O-glycosyl transfer, the mode of C-glycosyl transfer remains elusive. As an initial step towards further understanding this novel phenomenon we demonstrate, for the first time, that the urdamycin C-

[*] C. Dürr, Dr. D. Hoffmeister,† M. Weber, Dr. U. von Mulert, Prof. Dr. A. Bechthold
Institut für Pharmazeutische Wissenschaften
Albert-Ludwigs-Universität Freiburg
Stefan-Meier-Strasse 19, 79104 Freiburg (Germany)
Fax: (+49) 761-203-8383
E-mail: andreas.bechthold@pharmazie.uni-freiburg.de

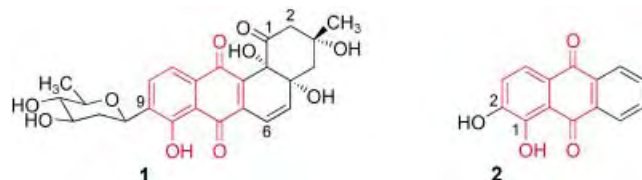
Prof. Dr. J. S. Thorson
School of Pharmacy, University of Wisconsin
777 Highland Avenue, Madison, WI 53705 (USA)
Dr. S.-V. Wohler
Combinature Biopharm AG
Robert-Rössle-Strasse 10, 13125 Berlin (Germany)
Prof. Dr. K. Ichinose
Graduate School of Pharmaceutical Sciences
The University of Tokyo, Bunkyo-ku, 113-0033 Tokyo (Japan)

[†] Current address:
School of Pharmacy, University of Wisconsin
777 Highland Avenue, Madison, WI 53705 (USA)

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glycosyltransferase (UrdGT2) displays the unusual ability to generate both C–C and C–O glycosidic bonds. The implications of this study in understanding the mechanism of enzymatic C-glycosyl transfer and potential applications for drug development are also discussed.

UrdGT2 is a well characterized C-GT, cloned from the soil bacterium *Streptomyces* (*S.*) *fradiae* Tü2717, which catalyzes the critical sugar-transfer step during the biosynthesis of the antitumor drug urdamycin. Specifically UrdGT2 transfers 2,6-dideoxy-D-glucose (D-olivose) from its NDP-donor to C9 of a benz[*a*]anthraquinone-derived polyketide, to yield the β -C-glycoside aquayamycin (Scheme 1, **1**).^[5,6] While UrdGT2



Scheme 1. Aquayamycin (**1**), the native product of UrdGT2 and 1,2-dihydroxyanthraquinone (**2**), the substrate mimic. The juglon-like acceptor motif is highlighted in red.

displays a remarkable intrinsic flexibility towards both the sugar-donor and acceptor substrate,^[7] regioselectivity (the sugar attachment takes place *ortho* to the phenol OH group) and stereoselectivity (inversion of anomeric configuration upon transfer) are strictly conserved in the UrdGT2-catalyzed reaction. The presence of an electron-donating substituent (the phenol hydroxy group) is essential for the regioselective C-glycosylation of the aromatic polyketide because aryl C-glycosylation occurs exclusively *ortho* and/or *para* to this critical substitution.^[8] A detailed sequence comparison of UrdGT2 with further natural product GTs revealed a strikingly high similarity to LanGT2 (50% identical amino acids), an O-GT of the landomycin biosynthesis.^[9] This remarkable sequence conservation prompted our investigation of UrdGT2's potential as an O-GT.

The substrate mimic selected was 1,2-dihydroxyanthraquinone (Scheme 1, **2**) which contains the minimal acceptor motif required for recognition by UrdGT2.^[5]

S. fradiae XKS, a strain specifically designed for bioconversion experiments,^[10] was selected as the host strain for this study.^[*] As a control, parallel bioconversion experiments utilized the *urdGT2*-deletion mutant *S. fradiae* BF-1-1.^[5]

After feeding *S. fradiae* XKS cultures with **2**, a new species with the characteristic UV/Vis spectra of the 1,2-dihydroxyanthraquinoid chromophore could be detected in the HPLC-UV/Vis analysis, with a retention time of 17.38 min compared to 20.38 min of **2** (Figure 1). ESI-MS analysis gave

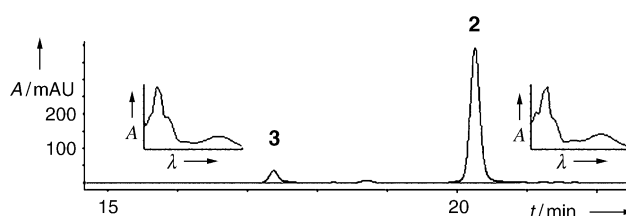
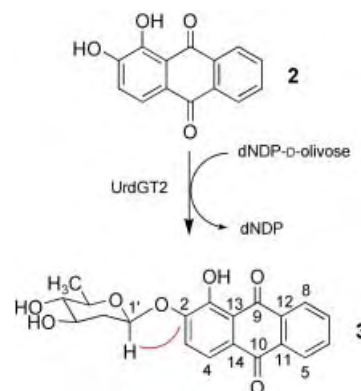


Figure 1. Feeding of **2** to *S. fradiae* XKS resulted in a new peak (**3**) in the HPLC trace with conserved UV-Spectra.

the molecular ion for the new derivative with m/z 369 [$M^+ - H$], consistent with the attachment of a dideoxyhexose (e.g. olivose) moiety to **2**. In contrast, analysis of the bioconversion of **2** by the control *S. fradiae* BF-1-1 revealed no conversion of **2** into this new species, a result consistent with UrdGT2 being required for the glycosylation of **2**.

To elucidate the precise structure of this substance, a 5.2-L fermentation provided 8.5 mg of the purified new product. High-resolution mass spectrometry indicated a molecular formula of $C_{20}H_{18}O_7$. The NMR spectroscopy data confirmed the aglycon structure of **2** within the new compound, with the proton and carbon signals consistent with the literature data.^[11] Further analyses of the one- and two-dimensional NMR data identified the sugar moiety attached to the aglycon as the 2,6-dideoxyhexose olivose, though the absolute configuration of the sugar moiety was not unambiguously elucidated. However, the 9.5 Hz transaxial coupling of the anomeric proton with 2'- H_a combined with the inverting GT mode of action in secondary metabolites (thus assigning α -configuration to L-sugars and β -configuration to D-sugars)^[12] strongly indicated the sugar to be D-olivose, β -glycosidically linked to the aglycon.^[13] Furthermore, the well characterized host strain *S. fradiae* Tü2717 is not known to produce L-olivose. Finally, the aglycon C2 regiochemistry was deduced from heteronuclear multiple bond correlation spectroscopy (HMBC) analysis (Scheme 2). Based upon these results, the structure of the new product is 2-O- β -D-olivosyl-1,2-dihydroxyanthraquinone (**3**).

For the formation of O-glycosidic sugar linkages it is proposed that a catalytic base abstracts a proton from the acceptor hydroxy group, followed by a nucleophilic attack on



Scheme 2. The conversion of **2** into 2-O- β -D-olivosyl-1,2-dihydroxyanthraquinone (**3**). The HMBC coupling of the D-olivosyl residue to the aglycone is displayed in red.

[*] This specific strain differs from wild-type *S. fradiae* Tü2717 by a 0.9 kb chromosomal deletion within the urdamycin biosynthetic gene locus which disrupts the genes for the urdamycin polyketide synthase (*urda-C*), yet retains the native urdamycin NDP-sugar biosynthetic and GT machinery. The disabled endogenous polyketide production in *S. fradiae* XKS eliminates any competition from the native urdamycin aglycon and thus greatly simplifies bioconversion analysis and the isolation of new derivatives.

the anomeric carbon of the activated dNDP-sugar.^[14] In contrast, at least two mechanisms can be put forth for C-glycosylation of aromatic polyketides. Mechanism I requires O-glycosylation of the phenol hydroxy group followed by an O–C rearrangement. Indirect support for this mechanism is twofold. First, as in the case of UrdGT2 and LanGT2, C-GTs and O-GTs often share significant sequence homology. Second, chemical models illustrate that Lewis acids promote facile rearrangement of O-aryl glycosides to the *ortho*-C-congeners but with the α -glycoside as the predominate product.^[15] However, no *para*-substituted variants were found in these model studies. Mechanism II is analogous, in a regiochemical sense, to a direct Friedel–Crafts aromatic substitution. Support for this mechanism derives solely from model studies in which $[\text{Cp}_2\text{ZrCl}_2]/\text{AgClO}_4$ ($\text{Cp} = \text{C}_5\text{H}_5$) promoted the efficient coupling of methoxynaphthalene derivatives with glycosyl fluorides to give both *ortho* and *para*-C-glycosides, with the β -glycoside predominating.^[16] The 1-hydroxyanthraquinoid systems typical of naturally occurring aryl C-glycosides would be appropriate substrates for this mechanism as the electron-donating effect of the 1-OH group, and thus the nucleophilicity of the *ortho* and *para* position, is also enhanced by hydrogen bonding to the nearby carbonyl group.

The observed UrdGT2-dependent biotransformation of **2** into **3** reveals the first natural product GT capable of constructing both C-glycosidic and O-glycosidic bonds. In addition, the UrdGT2-catalyzed production of **3** lends further indirect support for the C-glycosyltransferase mechanism II as the 1,4 O–O rearrangement required for mechanism I in forming **3** is disfavored and should terminate upon O-glycosylation of C1 of the aglycon. Alternatively, mechanism II would require an appropriate positioning of the activated sugar (dTDP-olivose) for direct attack (Scheme 3a). This attack could be further enhanced in enzymes by a catalytic base. In addition, the proximity of such base in mechanism II could also aid the analogous O-glycosylation of **2** (Scheme 3b).

In addition to the unique mechanistic consequences, this discovery may have relevance for applied pharmaceutical chemistry, since UrdGT2 could potentially be harnessed as both a C- and O-GT. For example, one potential application might be the conversion of the anthracenones anthralin and its 1,2-dihydroxy derivative into the corresponding 2-C- and 2-O-glycosides, to reduce the severe inflammatory side effects of these potent antipsoriatic agents.^[17]

In the context of highly effective enzymatic procedures for nucleotide sugar synthesis,^[18] the flexible glycosyltransferase

UrdGT2 should enhance the further development of glycosidic drugs.^[19] Further work on this unusual enzyme includes structure elucidation and targeted amino acid replacements to further explore the general potential of interconverting O- and C-GTs.

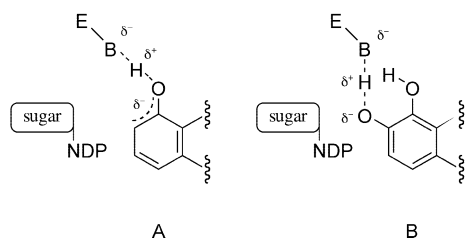
Experimental Section

For the feeding experiments, *Streptomyces fradiae* XKS and BF-1-1 were cultivated in NL111 V liquid medium (2% lab lemco meat powder, 1% CaCO_3 , 10% malt extract, pH adjusted to 7.2) dispensed into single baffled conical flasks and agitated at 27°C and 180 rpm. Main cultures (20 mL) were each inoculated with 24 h-old seed culture (1 mL). After 24 h of incubation a solution of 1,2-dihydroxyanthraquinone (40 μL , 25 mg mL^{-1} DMSO) was added. Samples were taken after 24, 48, and 96 h and submitted to LC/MS-analysis. For the scaled-up conversion 260 mg of substance were fed in four portions after 24, 36, 48, and 60 h to 5.2 L NL111 V main culture. The culture was harvested after 84 h.

Isolation of **3**: Crude extracts were obtained by extraction of culture (1 mL) with ethyl acetate (1 mL). The organic phase was evaporated to dryness under reduced pressure, the residue redissolved in MeOH and subjected to LC/MS analysis. The 5.2-L fermentation was filtered to separate mycelia from the aqueous phase. The aqueous phase was extracted three times with equal volumes of ethyl acetate. The organic phase was dried and redissolved in MeOH. The crude extract was adsorbed into a Varian RP-18 column and fractionated with a $\text{H}_2\text{O}/\text{MeOH}$ gradient. Fractions containing the new derivative were pooled and applied for further purification to a Sephadex LH-20 column (75 $\times$ 1.5 cm, solvent was MeOH). Analytical HPLC runs were performed on a Waters HPLC system equipped with a Waters Xterra C-18 column (3.5 μm particle size, 4.6 $\times$ 100 mm). Detection wavelength range of the UV/Vis detector was set to 190–500 nm. The gradient profile was: Solvent A: 0.5% acetic acid in H_2O , Solvent B: 0.5% acetic acid in CH_3CN . An initial hold for 3 min with 30% B and 70% A was followed by a step gradient from 30 up to 50% B within 13 min and from 50 up to 95% within 3 min and held at that relation for further 2 min. The solvent flow rate was 0.7 mL min^{-1} . LC/MS analysis was performed on an Agilent 1100 series LC/MS system by electrospray ionization (ESI) and detection in the positive and negative mode. The LC-system was equipped with a Hewlett Packard ZORBAX SB C-18 column (5 μm particle size, 4.6 $\times$ 150 mm), maintained at 24°C. Chromatographic conditions were as described for analytical HPLC.

Structure elucidation and characterization of **3**: Electrospray ionization (ESI-MS) and high resolution mass spectra (HR-ESI-MS) were acquired using a Micromass QTOF2 mass spectrometer. The structure was elucidated by using 1D spectra and 2D homo- and heteronuclear correlation experiments (^1H , ^{13}C , H,H-COSY , HSQC, HMBBC). ^1H NMR spectra were recorded using a Varian Unity 300 spectrometer and chemical shifts were referenced to TMS at 2.94 ppm (^1H) and 39.5 ppm (^{13}C). ^1H NMR (300 MHz [D_6]DMSO, 27°C, TMS): δ = 8.23 (1 H, m, H8), 8.16 (1 H, m, H5), 7.92 (1 H, m, H6), 7.91 (1 H, m, H7), 7.69 (1 H, d, J = 8.4 Hz, H4), 7.50 (1 H, d, J = 8.4 Hz, H3), 5.45 (1 H, dd, J = 9.5, 1.7 Hz, H1'), 3.51 (1 H, J = 10.0, 9.0, 4.9 Hz, H3'), 3.42 (1 H, dq, J = 9.0, 6.3 Hz, H5'), 2.85 (1 H, dd, J = 9.0, 9.0 Hz, H4'), 2.26 (1 H, ddd, J = 11.9, 4.9, 1.7 Hz, H2'e), 1.60 (1 H, ddd, J = 11.9, 10.0, 9.5 Hz, H2'a), 1.20 ppm (3 H, d, J = 6.3 Hz, $\text{CH}_3\text{6'}$); ^{13}C NMR (75 MHz [D_6]DMSO, 27°C, TMS): δ = 188.3 (C9), 180.9 (C10), 152.2 (C1), 150.7 (C2), 135.1 (C6), 134.2 (C7), 133.3 (C12*), 133.0 (C11*), 126.7 (C5), 126.5 (C8), 126.0 (C14), 120.6 (C3), 119.8 (C4), 116.3 (C13), 96.2 (C1'), 76.5 (C4'), 72.1 (C5'), 69.8 (C3'), 39* (C2'), 27.6 ppm (C6'); * assignment interchangeable; + only indirectly observed in 2D experiments due to overlap with solvent.

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Scheme 3. The putative mechanisms for C-glycosylation (A) and O-glycosylation (B) catalyzed by *S. fradiae* UrdGT2.

Keywords: drug design · glycosylation · glycosyltransferases · natural products · streptomycetes

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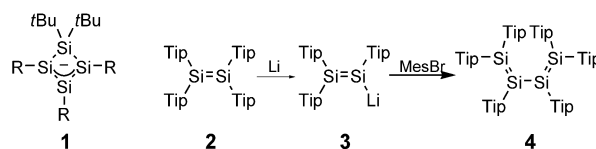
A Silicon Analogue of Vinyl lithium: Structural Characterization of a Disilenide**

David Scheschkewitz*

Dedicated to Professor Manfred Weidenbruch

Organosilyl anions are highly useful reagents in organic and inorganic synthesis. Although the structure and reactivity of lithiated derivatives have been studied in detail,^[1] information concerning unsaturated anionic silicon compounds is still scarce. In view of the rapid development of the chemistry of disilenes^[2] the availability of anionic derivatives would be highly desirable. In particular, the recently growing interest in conjugated homonuclear π systems of silicon justifies the need for new synthetic methods.

In this context cyclotetrasilene **1**, a silicon analogue of the allyl anion, has been recently described.^[3] In 1997 Weidenbruch et al. proposed that reduction of disilene **2** by lithium metal leads to disilenide **3**, which reasonably explained the formation of tetrasilabutadiene **4** upon treatment of **3** with mesitylbromide (Scheme 1).^[4] However, **3**



Scheme 1. R = Si(Me)*t*Bu₂; Tip = 2,4,6-*i*Pr₃C₆H₂; Mes = 2,4,6-Me₃C₆H₂.

could not be isolated nor characterized.^[5] The present work reports the isolation by means of a simplified synthetic protocol and the structural characterization of disilenide **3**.

Several approaches to the preparation of disilene **2** were reported,^[6] two of which employed reductive conditions. The original procedure by Watanabe et al. made use of the reductive dimerization of Tip₂SiCl₂ with a stoichiometric amount of lithium naphthalide.^[6a] In light of the observations made by Weidenbruch et al.,^[4] the assumption that the

[*] Dr. D. Scheschkewitz[†]
Eidgenössische Technische Hochschule Zürich
Department for Chemistry and Applied Biosciences
8093 Zürich (Switzerland)
E-mail: scheschkewitz@mail.uni-wuerzburg.de

[[†]] Present address:
Bayerische Julius-Maximilians Universität
Institut für Anorganische Chemie
Am Hubland, 97074 Würzburg (Germany)
Fax: (+49) 931-888-4623

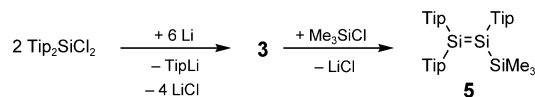
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Supporting information for this article is available on the WWW under <http://www.angewandte.org> or from the author.

disilenide **3** could be accessible by direct reaction of $\text{Tip}_2\text{SiCl}_2$ with the required amount of reduction equivalents seemed warranted.

Indeed the reduction of $\text{Tip}_2\text{SiCl}_2$ ^[7] with an excess of finely dispersed lithium powder in 1,2-dimethoxy ethane (DME) at 25 °C and subsequent crystallization from hexane leads to a uniform orange product in acceptable yield (51 %). The spectroscopic data of the thermally surprisingly stable compound (m.p. 121 °C) agree with the constitution of the disilenide **3** (Scheme 2).



Scheme 2.

The UV/Vis absorption of **3** in hexane at 417 nm is bathochromically shifted compared with that of **2** at 266 nm. Such a shift is typical for disilenes that bear electropositive substituents, for example, the highest wavelength absorptions of tetra(silyl)disilenes are found around 400 nm.^[8]

The ^{29}Si NMR spectrum shows two resonances at $\delta = 100.5$ and 94.5 ppm, both shifted downfield significantly with respect to that of **2** ($\delta = 53.4$ ppm)^[6a] Vinyl lithium exhibits a similar deshielding effect only for the α carbon atom ($\Delta\delta(\text{C}_\alpha) = +60.8$, $\Delta\delta(\text{C}_\beta) = +9.9$ ppm).^[9] The unusually strong effect of asymmetrical substitution on the shifts of the ^{29}Si NMR spectrum of disilenes has been discussed recently in a quantum-chemical study.^[10] The broadening of the signal at 100.5 ppm, which shows only a cross signal to one of the arene protons in the ^{29}Si - ^1H COSY spectrum of **3**, might arise from coupling of the Si atom to the coordinated ^7Li nucleus. However, even at -80 °C in toluene no quartet splitting of the signal was observed.

The structure of the disilenide **3** in the solid state as determined by X-ray diffraction analysis^[11] (Figure 1) shows the presence of a close-contact ion pair. The Si1-Li bond length of 285.3(3) pm, however, is long in comparison with those usually observed in silyllithium compounds, for example, 267 pm in $\text{Ph}_3\text{SiLi} \cdot 3 \text{THF}$.^[12]

The core of the disilenide **3** is visibly distorted from that of the planar uncharged disilene **2**. The Si1=Si2 bond of 219.2(1) pm is significantly longer (**2**: 214.4 pm),^[6a] despite the obvious relieve of steric strain. Ab initio calculations by Apeloig and Karni predicted a slight elongation of the Si=Si bond in the unsubstituted disilenide (**3u**) compared to $\text{H}_2\text{Si}=\text{SiH}_2$.^[13a] While both silicon atoms reside nearly in one plane with the *ipso*-carbon atoms C1, C16, and C31 (largest deviation: 6.6(1) pm for Si2), the distance of the lithium cation to this plane amounts to 54.7(3) pm. The angle formed by C1-Si1-Si2 (107.6(1)°) is noticeably smaller than the corresponding angles in **2** (120.8°, 121.6°). Conversely the Si1-Si2-C16 angle is strongly widened (140.3(1)°). Density functional theory (DFT) calculations by Schäfer III and co-workers on **3u** without the counterion even found an Si-Si-H angle of 90° at the negatively charged silicon atom, thus indicating the avoidance of hybridization at the central Si atom.^[13b]

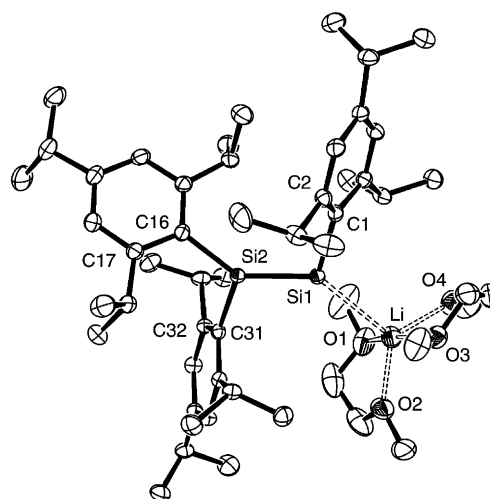


Figure 1. Structure of **3**:2DME in the solid state at 100 K. Protons are omitted for clarity. Thermal ellipsoids are set at 70 % probability. Selected distances [pm] and angles [°]: Si1-Si2 219.2(1), Si1-Li 285.3(3), Si1-C1 193.1(1), Si2-C16 191.5(1), Si2-C31 190.5(1), Si2-Si1-Li 131.7(1), C1-Si1-Li 119.0(1), C1-Si1-Si2 107.6(1), C16-Si2-Si1 140.3(1), C31-Si2-Si1 111.0(1), C31-Si2-C16 108.2(1), C31-Si2-Si1 111.0(1), C31-Si2-C16 108.2(1), C1-Si1-Si2-C31 174.9(1), Li-Si1-Si2-C31 10.4(1), Si1-Si2-C16-C17 -140.5(1), Si1-Si2-C31-C32 -109.5(1), Si2-Si1-C1-C2 70.1(1).

The structure of the solvent separated ion pair of **3** is therefore of considerable interest. In fact, addition of [2.2.1]cryptand to a THF solution of **3** and subsequent layering with hexane afforded red crystals, which, unlike those of **3**, were totally insoluble in hexane. The X-ray diffraction analysis left no doubt about the constitution as a solvent-separated ion pair of **3**. However, because of heavy disorder in the disilenide moiety the structure could not be refined satisfactorily. Attempts to improve the quality of crystals are underway.

The availability of pure **3** should be very useful for the synthesis of low-valent silicon compounds, for example, asymmetrically substituted disilenes, the preparation of which has so far been limited to only a couple of examples.^[14] The reaction of **3** with Me_3SiCl in toluene indeed affords the asymmetric disilene **5** (Scheme 2), whose structure is derived from spectroscopic data: the ^{29}Si NMR spectrum shows three resonances at $\delta = 97.7$, 50.9, and -8.3 ppm, which were unambiguously assigned on the grounds of the very well-resolved ^{29}Si satellite signals.^[15] The signal at $\delta = 50.9$ ppm exhibits two sets of satellites and is therefore attributed to the central sp^2 Si atom. The coupling constant of the signal at $\delta = 97.7$ ppm ($J(\text{Si},\text{Si}) = 116.3$ Hz) is more than 1.5 times larger than that of the signal at -8.3 ppm ($J(\text{Si},\text{Si}) = 75.1$ Hz). $J(\text{Si}=\text{Si})$ coupling constants for tetra(aryl)disilenes were found to be in the range of 155 to 158 Hz and have been interpreted in terms of higher s character of the Si=Si bond compared to single bonds.^[16b] Hence, the s character of the double bond in **5** is considerably lowered by the electropositive silyl substituent.

Reactions of the disilenide **3** with a variety of other electrophiles are currently under investigation.

Experimental Section

3: Thoroughly dried and degassed DME (40 mL) at 25 °C was added to a mixture of $\text{Tip}_2\text{SiCl}_2$ (7.50 g, 14.8 mmol) and finely dispersed lithium powder (0.38 g, 55 mmol). The reaction mixture instantly became intensely red and warm (ca. 40 °C). After it had cooled to ambient temperature stirring was maintained for 3 h. Excess lithium and precipitated salts were removed by filtration off and washed with 2×10 mL of DME. The combined filtrates were reduced to dryness under vacuum and hexane (80 mL) was added to the residue. This mixture was then heated to about 60 °C, thus dissolving most of the orange precipitate; the remainder was removed by filtration while hot. The solvent of the resulting red solution was removed under vacuum until about 20 mL remained. The copious amounts of an orange solid that precipitated were redissolved by heating to 60 °C and the solution was left at ambient temperature overnight. Large orange crystals were collected by decantation of the mother liquor and dried under vacuum: 3.25 g (51 %) **3** (mp 120–121 °C). ^1H NMR (300 MHz, $[\text{D}_8]\text{toluene}$, 25 °C, TMS): δ = 7.09, 7.08, 7.04 (each s, each 2H, *m*-H), 4.76 (sept., 4H, Me_2CH), 4.21 (br, 2H, Me_2CH), 2.99 (s, 12H, DME), 2.95 (s, 8H, DME), 2.86 (m, 3H, Me_2CH), 1.46, 1.39, 1.35, 1.29, 1.23, 1.19, 1.02 ppm (each d, overall 54H, Me_2CH); ^7Li NMR (97 MHz, $[\text{D}_8]\text{toluene}$, 25 °C, Li^+ aq): δ = 0.20 ppm; ^{13}C NMR (75 MHz, $[\text{D}_8]\text{toluene}$, 25 °C, TMS): δ = 155.5 (s), 154.1 (s), 149.5 (s), 148.6 (s), 147.9 (s), 145.9 (s), 145.2 (s), 142.6 (s), 121.8 (br d), 121.2 (d), 120.7 (d), 37.2 (d), 36.4 (d), 36.0 (br d), 35.7 (d), 35.5 (d), 35.3 (d), 27.4 (br q), 26.1 (br q), 25.5 (q), 25.4 (q), 25.2 (q), 25.0 ppm (q); ^{29}Si NMR (50 MHz, $[\text{D}_8]\text{toluene}$, 25 °C, TMS): δ = 100.5 (s, 1Si, SiLi), 94.5 ppm (s, 1Si, SiC_2); UV/Vis (hexane): $\lambda_{\text{max}}(\epsilon)$ = 417.2 (760) nm; IR (neat, 25 °C) = 2953 (s), 1456 (m), 1379 (w), 1358 (w), 1306 (w), 1244 (w), 1192 (w), 1162 (w), 1123 (m), 1082 (s), 1029 (m), 937 (w), 869 (s), 839 (m), 752 (m), 646 cm^{-1} (m).

5: Freshly distilled Me_3SiCl (0.1 mL, 85 mg, 0.78 mmol) was added by syringe at 25 °C to **3** (0.51 g, 0.597 mmol) in dry and degassed toluene (5 mL). After the reaction had been stirred for 30 min, all volatile products were removed under vacuum. Hexane (10 mL) was added to the orange residue. The resulting solution was filtered and reduced to dryness affording **5** (0.42 g, 95 %) as an orange oil. ^1H NMR (300 MHz, C_6D_6 , 25 °C): δ = 7.15, 7.09, 7.01 (each s, each 2H, *m*-H), 4.25, 4.07, 3.83 (each sept., each 2H, Me_2CH), 2.76, 2.75, 2.66 (each sept., each 1H, Me_2CH), 1.32 (d, 12H, Me_2CH), 1.28 (br., 12H, Me_2CH), 1.19, 1.18, 1.09 (each d, each 6H, Me_2CH), 0.96 (d, 12H, Me_2CH), 0.27 ppm (s, 9H, SiMe_3); ^{13}C NMR (75 MHz, C_6D_6 , 25 °C): δ = 155.9 (s), 155.1 (s), 154.8 (s), 151.1 (s), 150.5 (s), 150.2 (s), 135.9 (s), 135.5 (s), 131.7 (s), 122.3 (d), 122.1 (d), 121.7 (d), 37.8 (d), 36.8 (d), 34.8 (d), 34.7 (d), 34.4 (d), 25.2 (br), 24.6 (q), 24.2 (q), 24.1 (q), 23.9 (q), 3.0 ppm (q); ^{29}Si NMR (50 MHz, C_6D_6 , 25 °C): δ = 97.7 (s, 1Si, SiC_2), 50.9 (s., 1Si, SiSiMe_3), –8.3 ppm (s, 1Si, SiMe_3).

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Organomagnesium Reagents

An Efficient Synthesis of Diaryl Ketones by Iron-Catalyzed Arylation of Aryl Cyanides**

Christophe Duplais, Filip Bures, Ioannis Sapountzis, Tobias J. Korn, Gérard Cahiez, and Paul Knochel*

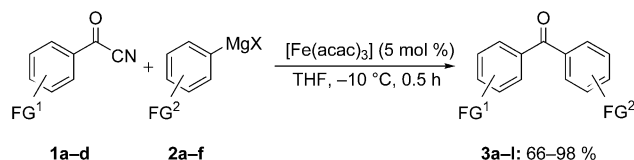
Dedicated to Professor Klaus T. Wanner on the occasion of his 50th birthday

The acylation of organometallic intermediates with acid chlorides is an important method for the preparation of polyfunctional ketones. This functionality is present in a great variety of pharmaceutical and material-science target molecules.^[1] Many organometallic reagents have been used for performing acylations, and organomanganese reagents have proved to be especially useful.^[2] Polyfunctional organozinc compounds have also been used frequently, and smooth acylations can be performed in the presence of stoichiometric amounts of CuCN·2LiCl^[3] or in the presence of a palladium catalyst.^[4] The preparation of functionalized arylzinc reagents is less straightforward^[5] and requires cobalt catalysis^[6] or the use of activated zinc powder (Rieke zinc).^[7]

Recently we reported a general method for preparation of polyfunctional arylmagnesium halides of type **2** using I/Mg- or Br/Mg-exchange reactions.^[8] We envisioned using these organometallics for the preparation of polyfunctionalized diaryl ketones by their reaction with acid chlorides. In preliminary experiments we treated benzoyl chloride with PhMgCl at various temperatures and obtained yields between 50–58%. We then turned our attention towards [Fe(acac)₃]-catalyzed reactions.^[9,10] The reaction of PhMgCl with PhCOCl in the presence of [Fe(acac)₃] (5 mol %) at 0 °C or 20 °C afforded benzophenone in only 38–53% yield. Extensive variation of the experimental reaction conditions (concentration, addition time, inverse addition) did not improve these results. This is in agreement with literature reports

which indicate that the preparation of benzophenone derivatives by the acylation of organometallics is only a moderately efficient reaction.^[1a]

We then examined the use of acyl cyanides **1** as the acylating agents^[11,12] and found that they readily react with various aromatic organomagnesium compounds of type **2** leading to polyfunctional benzophenone derivatives of type **3** (Scheme 1 and Table 1). Acyl cyanides are more powerful



Scheme 1. [Fe(acac)₃]-catalyzed reactions of functionalized magnesium reagents with acyl cyanides. For the functional groups (FG), see Table 1.

acylating agents than acid chlorides, since the cyano group enhances the reactivity of the adjacent carbonyl group. In contrary, the chlorine atom of acid chlorides plays the role of a donor by a mesomeric effect. The reaction of benzoyl cyanide (**1a**) and phenylmagnesium chloride (**2a**) without the iron catalyst furnished a higher yield than that obtained for the addition of phenylmagnesium chloride (**2a**) to benzoyl chloride (75% vs. 58% at 0 °C).

However, the use of catalytic amounts of [Fe(acac)₃] (5 mol %) was beneficial for the reaction of 4-ethoxycarbonylphenylmagnesium chloride (**2b**) with benzoyl cyanide (**1a**), increasing the yield from 58% to 80% at -10 °C.^[13] Thus, the reaction of PhMgCl (**2a**) with PhCOCN (**1a**) provided benzophenone (**3a**) in 84% yield (entry 1 of Table 1). Similarly, functionalized organomagnesium compounds **2b** and **2c** reacted in the presence of [Fe(acac)₃] (5 mol %) with PhCOCN (**1a**) at -10 °C within 0.5 h, furnishing the expected benzophenones **3b** and **3c** in 80 and 78% yield, respectively (entries 2 and 3). Functionalized acyl cyanides bearing a chlorine (**1b**), a methoxy (**1c**), or a ethoxycarbonyl group (**1d**) in *para* position, (entries 4–12) reacted with various arylmagnesium reagents (**2b–f**), leading to the diaryl ketones **3d–l** in good yields. Interestingly, an *ortho*-substituted arylmagnesium species like **2e** reacted as well, furnishing the benzophenone **3i** in 66% yield (entry 9). Also ketones bearing heterocyclic groups were prepared. The reaction of acyl cyanide **1c** with the heterocyclic Grignard reagent **2f** led to furyl ketone **3l** in 78% yield (entry 12). Heterocyclic acyl cyanides, like pyridine derivative **4**, reacted under our standard conditions (-10 °C, 0.5 h) with various aryl magnesium reagents, such as **2a**, **2b**, and **2d**, to give pyridyl ketones **5a–c** in 75–86% yields (Scheme 2).

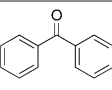
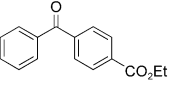
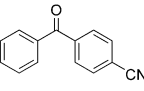
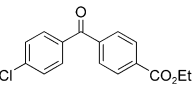
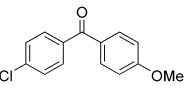
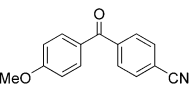
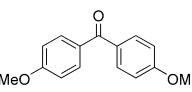
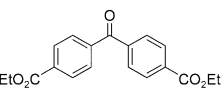
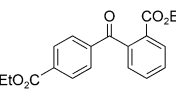
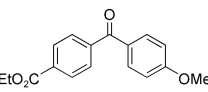
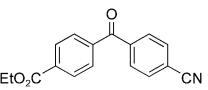
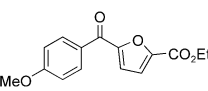
In summary, we have shown that the arylation of aryl and heteroaryl acyl cyanides with functionalized aryl and heteroaryl magnesium species is efficiently catalyzed by [Fe(acac)₃] (5 mol %), furnishing a range of new polyfunctional diaryl ketones.

[*] C. Duplais, Dipl.-Chem. F. Bures, Dipl.-Chem. I. Sapountzis, Dipl.-Chem. T. J. Korn, Prof. Dr. P. Knochel
Department Chemie
Ludwig-Maximilians-Universität München
Butenandtstrasse 5–13, Haus F
81377 München (Germany)
Fax: (+49) 089-2180-77680
E-mail: paul.knochel@cup.uni-muenchen.de
Prof. G. Cahiez
Laboratoire de Synthèse Organique Sélective et
Chimie Organométallique CNRS-UCP-ESCO
13, Boulevard de L'Hautail
95092 Cergy-Pontoise cedex (France)

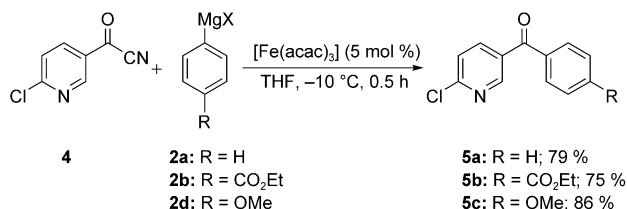
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Supporting information for this article is available on the WWW under <http://www.angewandte.org> or from the author.

Table 1: Ketones **3a–l** obtained by the Fe^{III} -catalyzed reaction of aryl acyl cyanides **1a–d** with aryl magnesium halides **2a–f** (Scheme 1).

Entry	Acyl cyanide	Grignard reagent	Product	Yield [%] ^[a]
1				84
2				80
3				78
4				74
5				89
6				84
7				98
8				68
9				66
10				83
11				71
12				78

[a] Yield of isolated, analytically pure product.



Scheme 2. $[\text{Fe}(\text{acac})_3]$ -catalyzed reactions of heterocyclic pyridyl acyl cyanide **4**.

Experimental Section

Typical procedure for the arylation of aryl cyanides.

3k (entry 11, Table 1): A dry and argon-flushed 10-mL flask equipped with a stirring bar and a rubber septum was charged with anhydrous THF (5 mL) and 4-iodobenzonitrile (374 mg, 2.4 mmol). The solution was cooled to -20°C and isopropylmagnesium chloride (1.9 mL, 1.4 M in THF, 2.6 mmol) was added slowly. The reaction mixture was stirred at this temperature until the exchange reaction was complete (30 min, checked by GC analysis of reaction aliquots). The resulting solution was then transferred dropwise over 25 min by cannula into a second 50-mL flask, which contained a solution of **1d** (406 mg, 2.0 mmol) and $[\text{Fe}(\text{acac})_3]$ (35 mg, 0.1 mmol) in anhydrous THF (10 mL) stirred at -10°C . At the end of the addition, the reaction mixture was quenched with aq. NH_4Cl (10 mL), diluted with water (25 mL), and extracted with Et_2O (3×25 mL). The combined organic layers were washed with aq. NaHCO_3 (10 mL), brine (2×20 mL), and dried (MgSO_4) and were concentrated in vacuo. The residue was purified by flash chromatography on silica gel (pentane/diethyl ether 4:1) yielding the diaryl ketone **3k** (397 mg, 71 %) as a colorless solid (m.p. 110 – 112°C).

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Keywords: acyl cyanides · homogeneous catalysis · iron · ketones · organomagnesium reagents

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- [13] Lower reaction temperatures like -20 , -40 , and -80°C increased the yield only slightly to 65, 70, and 68 %, respectively.

Cover Picture

**Jorge Sánchez-Quesada, Alan Saghatelian, Stephen Cheley,
Hagan Bayley, and M. Reza Ghadiri***

A single rotaxane structure created by threading and capturing a DNA–poly(ethylene glycol) (DNA–PEG) strand inside an α -hemolysin (α -HL) transmembrane pore protein is described by M. R. Ghadiri and co-workers on page 3063 ff. The DNA– α -HL rotaxanes exhibit a reversible two-state switching capacity that depends on the applied transmembrane potential, the orientation, and the method of threading and capture used. Functional rotaxanes might provide a means for critically assessing the feasibility of the pore-mediated single-molecule DNA sequencing hypothesis.



Angewandte EarlyView®

The following Communications are available online (in Wiley InterScience). You can find them, as well as forthcoming Reviews, Highlights, and Essays, at www.angewandte.org, under Early View.

C. Carbonera, A. Dei,* J.-F. Létard,* C. Sangregorio, L. Sorace:
Thermally and Light-Induced Valence Tautomeric Transition in a Dinuclear Cobalt–Tetraoxolene Complex
DOI: 10.1002/anie.200453944
Published online: May 19, 2004

R. Haiges,* J. A. Boatz, S. Schneider, T. Schroer, M. Yousufuddin, K. O. Christe*:
The First Binary Group 4 Azides [Ti(N₃)₄], [P(C₆H₅)₄][Ti(N₃)₅], and [P(C₆H₅)₄]₂[Ti(N₃)₆] and on Linear Ti–N–NN Coordination
DOI: 10.1002/anie.200454156
Published online: May 19, 2004

S. Jäger, M. Famulok*:
Generation and Enzymatic Amplification of High-Density Functionalized DNA Double Strands
DOI: 10.1002/anie.200453926
Published online: May 26, 2004

A. Krasovskiy, P. Knochel*:
A LiCl-Mediated Br/Mg Exchange Reaction for the Preparation of Functionalized Aryl- and Heteroaryl Magnesium Compounds from Organic Bromides
DOI: 10.1002/anie.200454084
Published online: May 26, 2004

B. Breit,* C. Herber:
Iterative Deoxypropionate Synthesis Based on a Copper-Mediated Directed Allylic Substitution
DOI: 10.1002/anie.200453990
Published online: May 26, 2004

B. Breit,* P. Demel, C. Studte:
Stereospecific and Stereodivergent Construction of Quaternary Carbon Centers through Switchable Directed/Nondirected Allylic Substitution
DOI: 10.1002/anie.200453991
Published online: May 26, 2004

Articles judged by the referees or the editor as being either very important or very urgent are immediately edited, proof-read, and electronically published once the manuscript has arrived in the editorial office in its final form. As long as there is no page number available these articles should be cited in the following manner:

Author(s), *Angew. Chem. Int. Ed.*, online publication date, DOI.

Meeting Reviews

The Peak of Stereochemistry: Bürgenstock 2004 _____ 2988 – 2989 R. Breinbauer

Books

Silicon Chemistry _____ 2990 Peter Jutzi, Ulrich Schubert reviewed by G. Linti

Electrokinetic Phenomena _____ 2990 Anurag S. Rathore, András Guttman reviewed by O. J. Schmitz

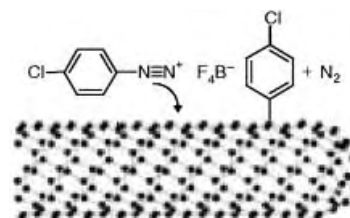
Highlights

Modifying Nanotubes

E. Joselevich* _____ 2992 – 2994

Chemistry and Electronics of Carbon Nanotubes Go Together

Chemistry makes the difference between metallic and semiconducting carbon nanotubes. It can also be used to turn metallic nanotubes into semiconducting ones, for example, by the addition of a simple organic fragment. This breakthrough has clear practical implications for nanotechnology. Moreover it begins to reveal the fundamental interplay between electronic structure and chemical reactivity (see scheme).





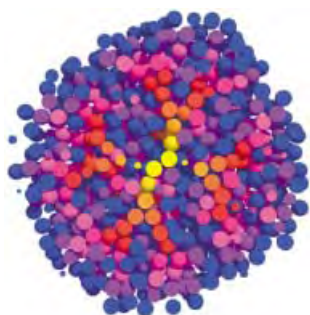
The magic of no metal: Enantiomerically pure α -hydroxy aldehydes and ketones as well as diols can be synthesized by a direct (S)-proline-catalyzed asymmetric nitroso-aldol reaction between the parent carbonyl compound and nitrosobenzene.

Further elaboration of the obtained adducts provides useful precursors for diversity-oriented synthesis. The one-flask transformation of 3-phenylpropanal into (R)-3-phenylpropane-1,2-diol (98% ee) is one example.

Asymmetric Catalysis

P. Merino,* T. Tejero — 2995 – 2997

Organocatalyzed Asymmetric α -Aminoxylation of Aldehydes and Ketones—An Efficient Access to Enantiomerically Pure α -Hydroxycarbonyl Compounds, Diols, and Even Amino Alcohols



Dense shell or dense core? Theoretical investigations have contributed considerably to the understanding of structures of dendrimers in solution. Computer simulations and scattering experiments show that dendrimers are flexible macromolecules that can adopt many possible conformations, in which back-folding of the end groups into the interior of the molecule plays an important role (see picture).

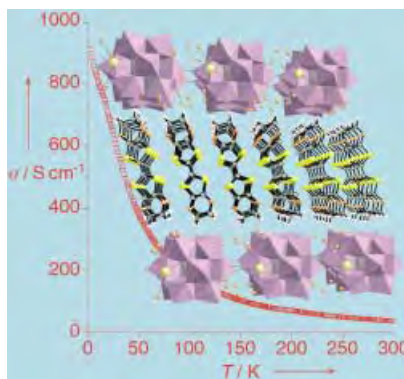
Reviews

Dendrimers

M. Ballauff,* C. N. Likos* — 2998 – 3020

Dendrimers in Solution: Insight from Theory and Simulation

A lengthy search in conducting hybrids: Molecular metals containing bulky and highly charged polyoxometalates can be prepared. The synthetic strategy is based on the combination of the bis(ethylene-dioxo)tetrathiafulvalene (BEDO-TTF) donor (see picture; ball-and-stick representation) with inorganic layers made of Keggin polyoxometalates (polyhedral representation) and K⁺ ions. This hybrid compound shows a conductivity of 910 S cm⁻¹ at 2 K.



Communications

Conducting Materials

E. Coronado,* C. Giménez-Saiz,*
C. J. Gómez-García,
S. C. Capelli — 3022 – 3025

Metallic Conductivity Down to 2 K in a Polyoxometalate-Containing Radical Salt of BEDO-TTF

For the USA and Canada: ANGEWANDTE CHEMIE International Edition (ISSN 1433-7851) is published weekly by Wiley-VCH PO Box 191161, D 69451 Weinheim, Germany. Air freight and mailing in the USA by Publications Expediting Inc. 200 Meacham Ave., Elmont, NY 11003. Periodicals

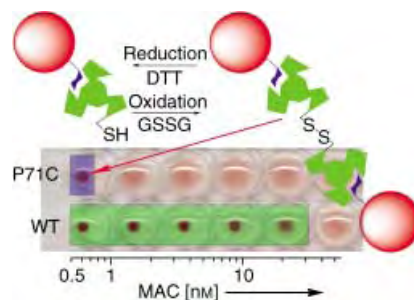
postage paid at Jamaica NY 11431. US POSTMASTER: send address changes to *Angewandte Chemie*, Wiley-VCH, 111 River Street, Hoboken, NJ 07030. Annual subscription price for institutions: Europe € 3430.00/3118.00; outside Europe US\$ 4499.00/4090.00 (valid for print and electronic/print or electronic delivery); for

individuals who are personal members of a national chemical society, or whose institution already subscribes, or who are retired or self-employed consultants, print only: Europe € 248.00/outside Europe US\$ 378.00. Postage and handling charges included. All Wiley-VCH prices are exclusive VAT.

Protein Engineering

R. E. McDonald, D. J. Hughes,
B. G. Davis* 3025 – 3029

Modular Control of Lectin Function:
Redox-Switchable Agglutination

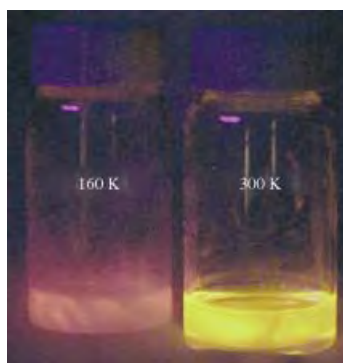


A specifically designed protein interface engineered into a model carbohydrate-binding lectin (GNAP71C) generates a blood-cell-binding activity that can sense ambient redox conditions (see picture; DTT = dithiothreitol, GSSG = oxidized glutathione, WT = wild-type protein, MAC = minimum agglutination concentration). The blood-cell binding exploits induced extended binding that can be varied by orders of magnitude with little change in the direct carbohydrate binding.

Antiquenching Luminescence

S. F. Wuister, A. van Houselt,
C. de Mello Donegá, D. Vanmaekelbergh,
A. Meijerink* 3029 – 3033

Temperature Antiquenching of the
Luminescence from Capped CdSe
Quantum Dots



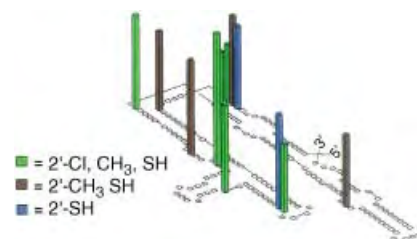
Actively passivated: The surprising recovery of the luminescence intensity between 200 and 300 K for CdSe quantum dots gives insight into the importance of the interaction between the capping layer and the nanocrystal semiconductor. The vial on the left is at 160 K while the brightly luminescing solution on the right is at 300 K. These results show that the capping layer does not merely passivate the surface but plays an active role in surface reconstruction.

RNA Folding

J. P. Schwans, N.-S. Li,
J. A. Piccirilli* 3033 – 3037

A Packing-Density Metric for Exploring the
Interior of Folded RNA Molecules

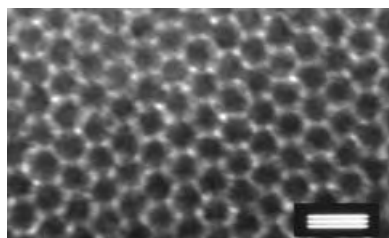
The intricate architectures of RNA molecules often contain expansive close-packed interfaces. The folding of the well-defined P4-P6 RNA domain was challenged by using a series of 2'-modified nucleotides with a narrow range of molecular volumes. The resulting folding interferences (see picture) reflect the spatial environment of the 2'-hydroxy groups and thereby define a packing-density metric.



Mesoporous Materials

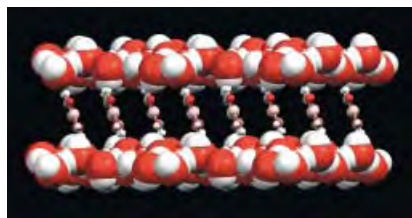
M. H. Bartl, S. P. Puls, J. Tang,
H. C. Lichtenegger,
G. D. Stucky* 3037 – 3040

Cubic Mesoporous Frameworks with a
Mixed Semiconductor Nanocrystalline
Wall Structure and Enhanced Sensitivity
to Visible Light



Highly ordered mesoporous films with a multicompositional nanocrystalline anatase titania/cadmium chalcogenide wall structure (see figure, scale bar is 20 nm) were synthesized by a fast and inexpensive one-pot single-precursor sol-gel-chemistry route based on supramolecular templating. These films show enhanced sensitivity to visible light in photocurrent generation experiments.

Chemistry that holds water: A honey-comblike water sheet having a hitherto unknown structure is described. The crystal lattice of bulk water in the presence of 1,4-phenylenediboronic acid (see picture) contains hexameric rings with chair and boat conformations. The hydrogen-bonding pattern is completely elucidated and gives two different motifs for each of the three independent hydrogen-bonded rings.

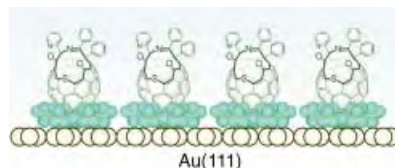


Water Chemistry

P. Rodríguez-Cuamatzi, G. Vargas-Díaz, H. Höpfl* _____ 3041–3044

Modification of 2D Water That Contains Hexameric Units in Chair and Boat Conformations—A Contribution to the Structural Elucidation of Bulk Water

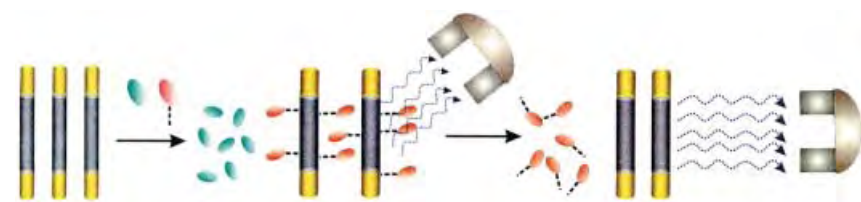
Aligning the layers: Immersing an Au(111) substrate successively into solutions of zinc(II) octaethylporphyrin and an open-cage C_{60} derivative in benzene results in the latter species exhibiting a well-defined electrochemical response as a consequence of the formation of a 1:1 supramolecular assembly (see picture). STM has been used to analyze the internal structure, orientation, and packing arrangement of the layers.



Surface Chemistry

S. Yoshimoto, E. Tsutsumi, Y. Honda, Y. Murata, M. Murata, K. Komatsu, O. Ito, K. Itaya* _____ 3044–3047

Controlled Molecular Orientation in an Adlayer of a Supramolecular Assembly Consisting of an Open-Cage C_{60} Derivative and Zn^{II} Octaethylporphyrin on Au(111)



Gold and nickel are the components of nanorods used to separate biomolecules. The rods can act as a high-surface-area scaffolding for biospecific recognition events in a variety of bioseparation

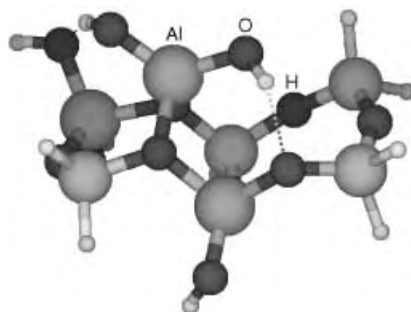
schemes. The magnetic Ni block not only provides specificity with respect to His-tagged proteins but also allows one to conveniently remove rods from a solution by using a magnetic field (see scheme).

Biomolecular Separation

K.-B. Lee, S. Park, C. A. Mirkin* _____ 3048–3050

Multicomponent Magnetic Nanorods for Biomolecular Separations

The effect of extra-framework aluminum (EFAL) species on the acidity of a HT_6 cluster ($T = Si, Al$) was studied by density functional calculations. A higher acid strength, compared to an isolated site, was only found with $Al(OH)_2^+$. The increase in acid strength was not due to Brønsted/Lewis acid synergism, as previously reported, but to the stabilization of the conjugated base through hydrogen bonding with the EFAL hydroxy groups (see picture).



Zeolites

C. J. A. Mota,* D. L. Bhering, N. Rosenbach, Jr. _____ 3050–3053

A DFT Study of the Acidity of Ultrastable Y Zeolite: Where Is the Brønsted/Lewis Acid Synergism?

Ionic Liquids

H. Lee, D. B. Kim, S.-H. Kim, H. S. Kim,*
S. J. Kim, D. K. Choi, Y. S. Kang,
J. Won ————— **3053 – 3056**

Zwitterionic Silver Complexes as Carriers for Facilitated-Transport Composite Membranes



X = NO₃, ClO₄, BF₄
n = 1 for R = Me, Bu; n = 2 for R = Ph

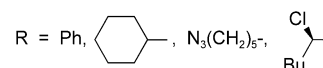
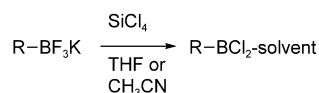
Carry on: Zwitterionic silver complexes were prepared from silver salts and zwitterionic-type sulfopropyl imidazolium compounds (see scheme), and their

performance as carriers for facilitated-transport composite membranes was investigated for the separation of a mixture of isoprene and *n*-pentane.

Boranes

B. J. Kim, D. S. Matteson* — **3056 – 3058**

Conversion of Alkyltrifluoroborates into Alkyldichloroboranes with Tetrachlorosilane in Coordinating Solvents



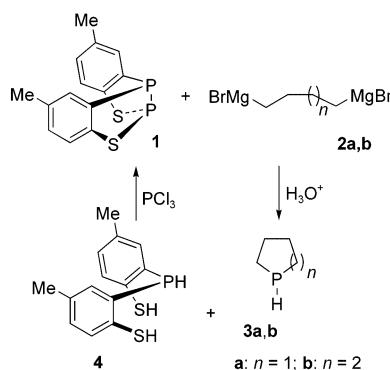
Organotrifluoroborate salts are converted rapidly by tetrachlorosilane in nucleophilic solvents such as THF or acetonitrile into organodichloroboranes coordinated with the solvent (see scheme). This reaction

completes a sequence from alkylboronic esters via the trifluoroborate salts to reactive alkyldichloroboranes under mild conditions.

Cyclic Phosphanes

G. Baccolini,* C. Boga,
M. Galeotti ————— **3058 – 3060**

Efficient One-Pot Synthesis of Secondary Cyclic Phosphanes with Easy Regeneration of the Phosphorus-Donor Reagent Used

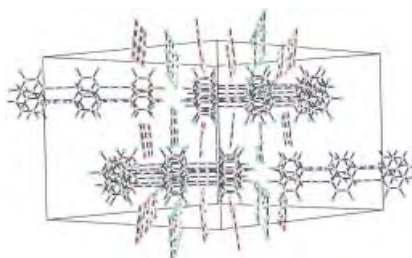


Phosphanes: No pain, all gain. A regenerable phosphorus donor is the key to an efficient and economic one-pot synthesis of five- and six-membered secondary cyclic phosphanes. The reaction, at room temperature, of **1** with 1 equivalent of bis(Grignard reagent) **2** followed by treatment with aqueous acid gives cyclic phosphanes **3** in 70–80% yield and the new compound **4**. Simply treating **4** with PCl₃ quantitatively regenerates the starting reagent **1**, which can be reused without further purification.

Crystal Engineering

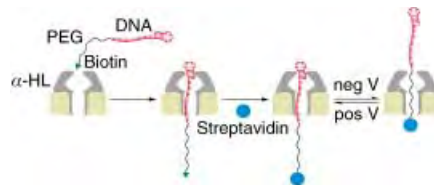
S. W. Watt, C. Dai, A. J. Scott, J. M. Burke,
R. L. Thomas, J. C. Collings, C. Viney,
W. Clegg, T. B. Marder* — **3061 – 3063**

Structure and Phase Behavior of a 2:1 Complex between Arene- and Fluoroarene-Based Conjugated Rigid Rods



The πs have it: An unusual 2:1 arene–perfluoroarene π complex is formed between 1,4-bis(phenylethynyl)benzene and its perfluorinated analogue, and this is the first example of a non-1:1 complex of this type. The interstack geometry (see picture; red H, green F) is also unusual, as is the complex mesomorphism of this system.

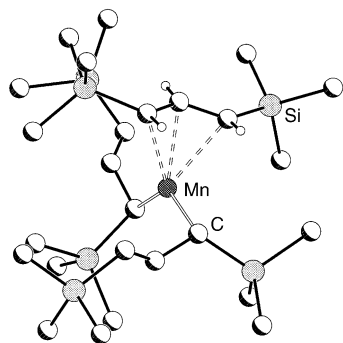
Threading a molecular needle: An α -hemolysin transmembrane pore can be threaded in a desired orientation by DNA–poly(ethylene glycol) (DNA-PEG) hybrid strands to yield functional rotaxanes (see figure). The single-molecule rotaxanes are stable and have a reversible two-state switching capacity that depends on the applied potential, orientation, and method of threading and capture used.



Supramolecular Chemistry

J. Sánchez-Quesada, A. Saghatelian, S. Cheley, H. Bayley, M. R. Ghadiri* 3063–3067

Single DNA Rotaxanes of a Transmembrane Pore Protein

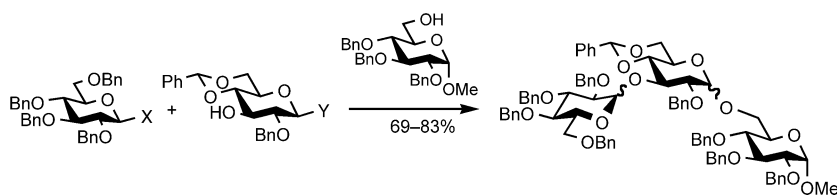


Lucky thirteen: The first example of a manganese(II) allyl complex, $[\text{Li}(\text{thf})_4][\text{Mn}\{\eta^3\text{-(Me}_3\text{Si)}_2\text{C}_3\text{H}_3\}\{\eta^1\text{-(Me}_3\text{Si)}_2\text{C}_3\text{H}_3\}_2]^-$, contains a tris(allyl)-manganate monoanion (depicted). The structure of the anion was determined and shown to contain both π - and σ -allyl ligands, resulting in an unusually low valence-electron count of thirteen.

Organometallic Compounds

R. A. Layfield,* S. M. Humphrey 3067–3069

A Manganese(II) Allyl Complex: Synthesis, Structure, and Magnetic Properties of $[\text{Li}(\text{thf})_4][\text{Mn}\{\eta^3\text{-(Me}_3\text{Si)}_2\text{C}_3\text{H}_3\}\{\eta^1\text{-(Me}_3\text{Si)}_2\text{C}_3\text{H}_3\}_2]^-$



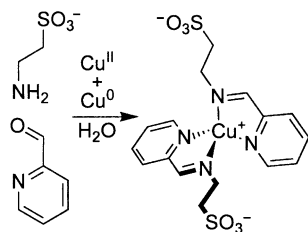
Donating with a difference: Thiazolyl (Taz) substituted thioglycosides have been successfully used in the synthesis of both 1,2-*trans*- and 1,2-*cis*-glycosides and also have found application in convergent

oligosaccharide synthesis. In addition, this study has demonstrated that STaz and SEt moieties are fully orthogonal (see scheme, Bn = benzyl; X = STaz, Y = SEt or X = SEt, Y = STaz).

Carbohydrate Synthesis

A. V. Demchenko,* P. Pornsuriyasak, C. De Meo, N. N. Malysheva 3069–3072

Potent, Versatile, and Stable: Thiazolyl Thioglycosides as Glycosyl Donors



Chemical symbiosis enables the quantitative formation of Cu^{I} -imine complexes from the mixture of Schiff base precursors with Cu^{II} and Cu^0 in water (see scheme), even though neither Cu^{I} nor imines are ordinarily stable in aqueous solution. The imine ligands undergo selective exchange with free amines, and the complex forms readily in the presence of a dynamic combinatorial library of nonchelating aldehydes.

Template Synthesis

J. R. Nitschke* 3073–3075

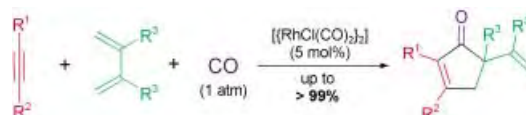
Mutual Stabilization between Imine Ligands and Copper(I) Ions in Aqueous Solution



Cycloaddition Reactions

P. A. Wender,* N. M. Deschamps,
T. J. Williams _____ 3076–3079

Intermolecular Dienyl Pauson–Khand
Reaction



Three-component [2+2+1] cycloadditions are facilitated by the use of dienes as activated two-carbon-atom components in a Rh^I-catalyzed intermolecular variant of the Pauson–Khand reaction (see scheme). The enhanced reactivity of the

dienes allows efficient access to a variety of alkenyl cyclopentenones from commercially or readily available starting materials. R¹, R² = alkyl, silyl, carbonyl; R³ = Me, Bn.



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Authors _____ 3083

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Sister Journals** _____ 3080–3081

Preview _____ 3085

Apologies

In the Communication “Cyclophane-Based Highly Active Late-Transition-Metal Catalysts for Ethylene Polymerization” by D. H. Camacho, E. V. Salo, J. W. Ziller, and Z. Guan (*Angew. Chem.* **2004**, 116, 1857–

1861; *Angew. Chem. Int. Ed.* **2004**, 43, 1821–1825, DOI 10.1002/anie.200353226), the authors neglected to update reference [12] in the galley proof and so failed to cross-reference their related communica-

tion in *Org. Lett.* **2004**, 6, 865–868. The latter publication reported the methodology for synthesizing cyclophanes which have nitrogen intra-annular functionalities. The authors apologize for this oversight.

In the Communication “Metal-Mediated Cross-Linking in the Generation of a Marine-Mussel Adhesive” by J. J. Wilker and co-workers (*Angew. Chem.* **2004**, 116, 454; *Angew. Chem. Int. Ed.* **2004**, 43, 448, DOI 10.1002/anie.200352759), three relevant citations discussing atomic force microscopy (“Adhesion of *Mytilus edulis* Foot Protein 1 on Silica: Ionic Effects on Biofouling”, B. P. Frank, G. Belfort, *Bio-technol. Prog.* **2002**, 18, 580–586), iron

binding to the Mefp-1 protein (“Ferric Ion Complexes of a DOPA-Containing Adhesive Protein from *Mytilus edulis*”, S. W. Taylor, D. B. Chase, M. H. Emptage, M. J. Nelson, J. H. Waite, *Inorg. Chem.* **1996**, 35, 7572–7577) and hydrolyzed peptides thereof (“Polarographic and Spectrophotometric Investigation of Iron(III) Complexation to 3,4-Dihydroxyphenylalanine-Containing Peptides and Proteins from *Mytilus edulis*”, S. W. Taylor,

G. W. Luther III, J. H. Waite, *Inorg. Chem.* **1994**, 33, 5819–5824) should have been included. The authors apologize for this oversight and point out that the novelty of their work lies in comparing spectroscopic data from live mussel glue to those of a protein-based adhesive film and peptide models of potential cross-links, thereby providing direct characterization of marine adhesive bonding.



The Peak of Stereochemistry: Bürgenstock 2004

Rolf Breinbauer

The Bürgenstock is a peak 500 m above picturesque Lake Lucerne in Switzerland. The ensemble of hotels steeped in tradition at the top provides a splendid setting for one of the most prestigious conferences in chemistry: the Bürgenstock Conference on Stereochemistry, which took place in April for the 39th time.

A peculiarity of this conference is that the program is not announced in advance. The speakers are invited by the conference president—this year Herbert Waldmann (Dortmund, Germany)—and the Switzerland-based organizing committee: Hans-Beat Bürgi (Universität Bern), François Diederich (ETH Zürich), Ernst Peter Kündig (University of Geneva), and Klaus Müller (Roche, Basel). The more than 130 participants from academic institutions and industry the world over were informed about the schedule after registration at the hotel reception.

The scientific program began on Sunday with a talk by Roger S. Goody (Max-Planck-Institut (MPI) Dortmund). In his lecture he presented structural and mechanistic aspects of the regulation of vesicular transport by Rab GTPases. By using a combination of kinetic studies, chemical biological tools, and X-ray crystal-structure analysis of protein complexes, his research group has been able to elucidate several important elements of vesicular trans-

port. Ilme Schlichting (MPI Heidelberg) followed with a discussion on how the reactivity of heme proteins is controlled through their molecular architecture. By using time-resolved protein X-ray crystallography she could characterize several intermediates in the catalytic oxidation cycles of cytochrome P450 (Figure 1) and nitric oxide synthases (NOS). The evening lecture was presented by Michel Rohmer (University Louis Pasteur, Strasbourg), whose studies have shown that isoprenoids are not only biosynthesized through the mevalonate pathway, but that in chloroplasts, green algae, and several eubacteria an alternative methyl erythritol phosphate (MEP) pathway exists.

On Monday morning, the participants were greeted by heavy snowfall and an informative talk by Jonathan Ellman (University of California (UC), Berkeley) on the use of *tert*-butylsulfinyl imines as powerful reagents for the preparation of substituted amines through C–C bond formation. Furthermore, he described how heteroatom-directed C–H activation can be carried out enantioselectively. Matthias Beller (Leibniz-Institut für Organische Katalyseforschung, Rostock, Germany) reported on a variety of new or optimized catalytic processes, such as amidocarbonylation, Ar–X activation, hydroamination, cyanation, and tandem isomeriza-

tion–hydroamination reactions. Nicholas Leadbeater (University of Connecticut, Storrs, USA) gave an introduction on “orgaNOmetallic” reactions. Suzuki and Sonogashira reactions can be carried out even in the absence of a metal, as careful mechanistic studies have proven. Milton R. Smith III (Michigan State University, East Lansing, USA) showed that an Ir–phosphane complex enables the *meta*-selective substitution of even substituted arenes with boronic acid moieties in high yields and high catalyst turnovers through C–H activation. In the final lecture of the day Matthew D. Shair (Harvard University) presented an elegant synthesis of longithorone A, which was inspired by nature and features inter- and intramolecular Diels–Alder reactions and an enyne macrocyclization. His research group has also successfully carried out a biomimetic enantioselective aldol reaction starting from malonic acid equivalents.

On Tuesday Atsuhiko Osuka (Kyoto University) demonstrated that porphyrin derivatives can be made to undergo oxidative oligomerization in a controlled manner to form micrometer-long entities. These materials could not only be characterized but also possess exciting electronic and optical properties. Harry Anderson (Oxford University, UK) showed that π systems can be

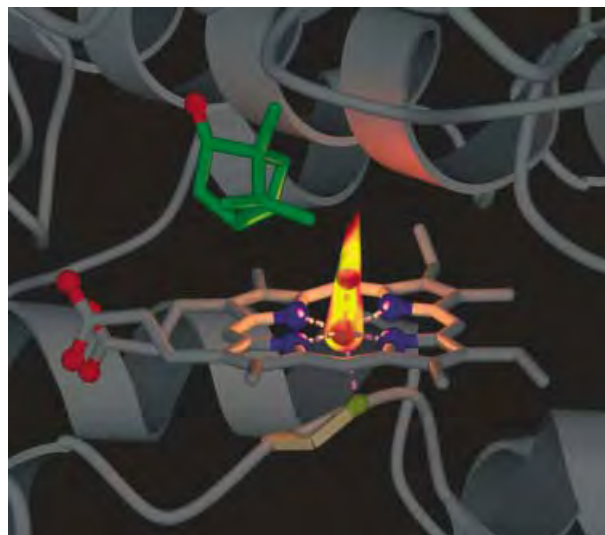


Figure 1. The active center of cytochrome P450 monooxygenases resembles a blowtorch. The heme-bound active oxygen species is able to hydroxylate even non-activated hydrocarbons. The figure shows the active site of cytochrome P450cam, which catalyzes the 5-*exo*-hydroxylation of camphor (green). Kindly made available by Daniel Peisach, Department of Biological Chemistry, University of Michigan, Ann Arbor, USA.

[*] R. Breinbauer

Max-Planck-Institut für molekulare Physiologie und Universität Dortmund (Germany)
E-mail: rolf-peter.breinbauer@mpi-dortmund.mpg.de

insulated by rotaxane formation. For example, a cyclodextrin/azo dye/rotaxane species does not undergo photo-bleaching, whereas the unprotected dye does.

On the following day Kazunari Taira (University of Tokyo) described several important applications of ribozymes. For example, he discussed the design of allosterically controllable ribozymes, the identification of genes by hybrid ribozymes, and a new type of RNA interference (shRNA), whereby even long RNA strands fail to induce an immune response. Michael Famulok (Universität Bonn, Germany) explained how intracellular aptamers ("intramers") can be used as a tool in chemical genetics. With a highly selective intramer he could differentiate between the biological functions of the highly homologous proteins cytohesin 1 and 2. Kevan Shokat (UC, Berkeley and UC, San Francisco) presented an ingenious approach for deciphering kinase signal transduction, whereby small-molecule compounds were used to control genetically modified proteins. With this method he could demonstrate that kinases act synergistically to produce

unique phenotypes that do not occur with monoinhibition.

The last day of the conference started with a talk by Henk Hiemstra (Amsterdam University) on the progress his research group has made towards the total synthesis of solaneclepin A. This natural product features a complex arrangement of three- to seven-membered rings, many of which have already been assembled to advanced fragments by his group. Larry Overman (UC, Irvine) presented elegant solutions to one of the most difficult problems in organic synthesis: the asymmetric construction of quaternary carbon stereocenters. Methodology based on Heck and diastereoselective dialkylation reactions enabled his research group to synthesize a series of challenging polypyrrolidinoindoline alkaloids. The privilege of holding the last lecture was given to an inorganic chemist: Karl Wieghardt (MPI Mülheim, Germany) explained in his talk entitled "Coordination chemistry of life" how his research group prepared a variety of inorganic models of the Cu-tyrosinyl enzyme galactose oxidase and ultimately discovered a catalyst

that mediates the oxidation of primary alcohols by oxygen. He also described how inorganic models of the Mn cluster in photosystem II have helped in the characterization of several catalytic intermediates in the water oxidation reaction. Klaus Müller closed the conference with his traditional witty review of events: a summary of the science presented, spiced up with some personal observations.

The balanced schedule of the conference and the indescribable beauty of its location were conducive not only to extensive scientific discussions, but also to social networking and sporting activities. Supported by their national societies, 20 young European scientists were able to participate in this conference and present their work at the two poster sessions. The 40th Bürgenstock conference (April 16–22, 2005) will be chaired by the new president Alain Krief (Namur, Belgium), whose duty this year as vice president was "to take care of the weather". His masterful accomplishment of this important task raises expectations for the next conference.

Chemistry and Electronics of Carbon Nanotubes Go Together

Ernesto Joselevich*

Keywords:

chemical reactivity · electronic structure · molecular electronics · nanotechnology · nanotubes

One of the critical issues for the application of single-wall carbon nanotubes (SWCNTs) in nanoelectronics is the control of their electronic properties, which can be either metallic or semiconducting in their pristine form, depending on their diameter and chirality.^[1] Since all known preparative methods yield mixtures of metallic and semiconducting nanotubes, extensive research has been devoted to the modification of the nanotube electronic structure and to the separation between metallic and semiconducting carbon nanotubes. Recently, metallic and semiconducting nanotubes have been physically separated by electric fields, based on their different dielectric constants.^[2] Recently, two groups simultaneously reported how chemical reactions involving covalent bonds can modify the electronic structure of single-wall carbon nanotubes,^[3] and have a high selectivity for metallic versus semiconducting carbon nanotubes,^[4] thus opening a potential avenue to their complete separation by chemical manipulation. Beyond the practical implication of this breakthrough for nanotechnology, the fundamental interplay between electronic structure and chemical reactivity in carbon nanotubes begins to be revealed.

Previously, the electronic properties of carbon nanotubes were modified by ionic doping,^[5] and recent advances have enabled significant discrimination

between metallic and semiconducting nanotubes to be made, based on differences in physical properties, such as their dielectric response to electric fields and electrical breakdown,^[2,6,7] or their selective ability to adsorb surfactants and DNA.^[8–11] What is new in the work of Kamaras et al.^[2] and Strano et al.^[4] is that they directly exploit the connection between the chemical reactivity and the electronic structure of metallic and semiconducting carbon nanotubes, which are treated as molecular species.

The effect of covalent modification on the electronic structure of single-wall carbon nanotubes is demonstrated by Kamaras et al.,^[3] who treated the nanotubes with dichlorocarbene (Figure 1 a). This covalent sidewall functionalization is found to convert metallic nanotubes into semiconducting ones, as shown by a strong decrease in intensity in the far-infrared absorption spectrum, assigned to intraband transitions near the Fermi level, and a simultaneous increase in the intensity in the visible region. The effect is opposite to that of ionic doping, which turns semiconducting nanotubes into conducting (metallic) nanotubes, by injecting electrons or holes into the valence or conduction bands, respectively. Covalent chemistry, on the other hand, breaks up the all-conjugated system into a series of smaller condensed aromatic systems by introducing saturated sp^3 carbon atoms in the nanotube backbone. The concomitant electronic localization and loss of translational symmetry open a gap at the Fermi level of the metallic carbon nanotubes, turning them semiconducting (the Fermi level is the chemical potential of the electrons, in metals it is the topmost filled level at zero temperature, whereas in semiconductors

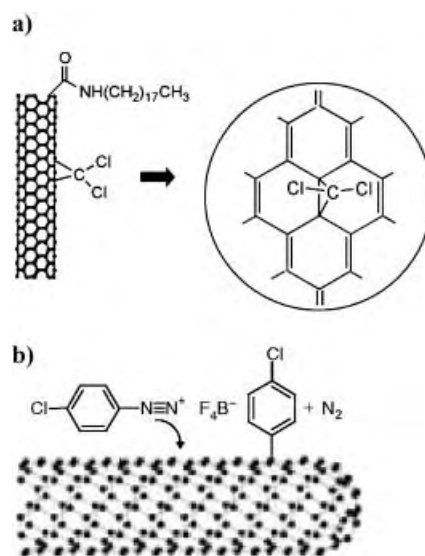


Figure 1. Two covalent reactions of single-wall carbon nanotubes. a) Result of the reaction with dichlorocarbene, which turns a nominal double bond into a cyclopropane ring (circled). b) Reaction with 4-chlorobenzenediazonium tetrafluoroborate, which releases nitrogen and adds a 4-chlorophenyl group to the nanotube.

it lies within the band gap, where no states are allowed).

The effect of electronic structure on chemical reactivity, on the other hand, is demonstrated by Strano et al.,^[4] who treated single-wall carbon nanotubes with diazonium reagents (Figure 1 b), while monitoring spectral changes. UV/Vis-NIR spectra indicates that 4-chlorobenzenediazonium reacts preferentially with metallic nanotubes, while Raman spectroscopy spectra provide details about the electronic structure of specific nanotubes under reaction. Low-wavenumber Raman spectra, associated with radial breathing modes, allow for the full

[*] Dr. E. Joselevich
Department of Materials and Interfaces
Weizmann Institute of Science
Rehovot 76100 (Israel)
Fax: (+972) 8-934-2350
E-mail: ernesto.joselevich@weizmann.ac.il

structural assignment of a few metallic and semiconducting nanotubes, and show that the reaction is completely selective for the metallic nanotubes, leaving the semiconducting ones intact. Raman peaks associated with tangential stretching modes show a decrease in intensity, because the reaction disrupts the resonance that gives rise to enhanced Raman scattering. On the other hand, the intensity of peaks associated with a symmetry-forbidden transition sharply increases, because the reaction breaks the translational symmetry that forbade the transition, and then decreases, along with the tangential stretching peaks, owing to the same loss of resonance. In addition to this metallic/semiconducting selectivity, the reactivity of the metallic nanotubes is inversely related to their diameter.

Although the electronic band structure of carbon nanotubes has been recognized from the time of their discovery, previous discussions on the chemical reactivity of carbon nanotubes have attributed a major role to local strain, which arises from curvature-induced pyramidalization and misalignment of π orbitals.^[11] The diameter selectivity found by Strano et al. can be correctly predicted by this formalism. However, the metallic/semiconducting selectivity underscores the role of the electronic band structure in determining the chemical reactivity of the single-wall carbon nanotubes. To account for this selectivity, Strano et al. propose a reaction mechanism in which the diazonium reagent forms a charge-transfer complex at the nanotube surface. Electron donation stabilizes the transition state that leads to the decomposition of the diazonium functional group and the covalent attachment of the aryl group to the nanotube. The extent of electron donation correlates with the density of states at the Fermi level, which is larger for metallic nanotubes.

The role of electronic structure in determining the chemical reactivity of molecules is central to organic-chemistry reaction mechanisms. One of the difficulties in rationalizing the chemical properties of carbon nanotubes in terms of their electronic structure is that the electronic structure is usually represented by band structure diagrams in reciprocal space, which are better suited for

describing materials properties than molecular reactions. We have recently shown a chemist's view of carbon nanotubes that merges the solid-state physics description with the molecular orbitals picture of chemical reactions, by applying traditional concepts of organic chemistry, such as aromaticity, orbital symmetry, and frontier orbitals.^[12] Metallic nanotubes are only slightly less aromatic than semiconducting ones in terms of overall resonance energy.^[13] However, from a frontier-orbitals perspective, the kinetic stability of polycyclic aromatic molecules is more related to their HOMO–LUMO energy gap,^[14] than to their resonance energy, which determines their thermodynamic stability. The selectivity towards addition found by Strano et al. underscores the role of the HOMO–LUMO energy gap in determining the reactivity of single-wall carbon nanotubes.

A more detailed perspective that could be useful to describe the π chemistry of carbon nanotubes, is Roald Hoffmann's frontier-orbitals picture of chemisorption.^[15] Molecule-surface two-orbital interactions are classified according to the total number of electrons in the two orbitals involved. These include the usual HOMO–LUMO and LUMO–HOMO two-electron interactions that normally dominate the overall interaction between two molecules, but also interactions between two occupied orbitals (four-electron) or between two empty orbitals (zero-electron), which are usually repulsive or ineffective but become attractive after charge transfer to or from the surface, respectively.

Charge transfer depends on the relative position of the Fermi level with respect to the mixed molecule–surface LUMO. This picture, when applied to carbon nanotubes in a system such as the one reported by Strano et al. (Figure 1b), could be represented as in Figure 2. In this case, a metallic nanotube (Figure 2a) can establish attractive interactions with the molecule that a semiconducting nanotube (Figure 2b) cannot. As in heterogeneous catalysis, chemisorption weakens the inner bonds inside the molecule and activates it towards chemical reaction.

In conclusion, the results of Strano et al.^[3] and Kamaras et al.,^[4] could be summarized in the familiar terms of organic chemistry: A higher reactivity for smaller diameter carbon nanotubes towards the addition reactions can be attributed to increased steric strain, while a higher reactivity for metallic versus semiconducting nanotubes is related to the large differences in their HOMO–LUMO gap. In addition, metallic nanotubes can act as redox catalysts by providing a reservoir of electrons. Upon reaction, the all-conjugated system of each metallic nanotube breaks into smaller polycyclic aromatic systems with larger HOMO–LUMO gaps. The road is now open for the manipulation and integration of carbon nanotubes into functional devices by chemical strategies. On the theoretical front, new models of carbon-nanotube chemistry will have to take into account both the localized and delocalized electronic structure, and blend together the quantitative power of the solid-state physics

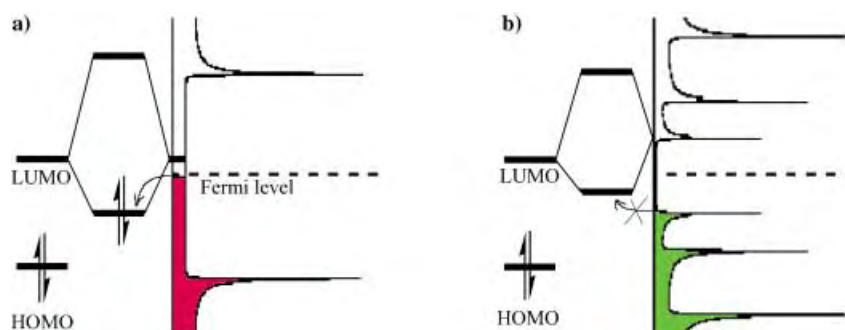


Figure 2. Frontier-orbital representation of zero-electron two-orbital interactions between a molecule and a metallic (a) or a semiconducting (b) single-wall carbon nanotube. The molecule frontier orbitals are designated as HOMO and LUMO, and the nanotube orbitals are represented by the density of states plots.

framework with the rich traditional knowledge of organic chemistry.

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Organocatalyzed Asymmetric α -Aminoxylation of Aldehydes and Ketones—An Efficient Access to Enantiomerically Pure α -Hydroxycarbonyl Compounds, Diols, and Even Amino Alcohols

Pedro Merino* and Tomas Tejero

Keywords:

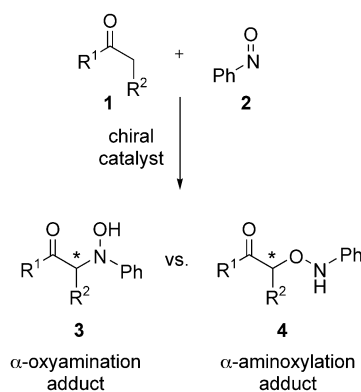
aldehydes · asymmetric catalysis · ketones · oxidation · proline

The asymmetric installation of a hydroxy group at the position α to a carbonyl function is an important C–O bond-forming process in organic synthesis.^[1] The resulting optically active α -hydroxy carbonyl compounds are of general interest since they provide direct routes to a vast number of biologically significant compounds including carbohydrates, antibiotics, alkaloids, and terpenes. The most widely used direct procedure for preparing optically active α -hydroxy carbonyl compounds is the oxidation of enolates with an oxidizing reagent.^[2] Asymmetry is induced by using either chiral nonracemic enolates with achiral reagents or prostereogenic enolates and enantiomerically pure oxidizing reagents, typically *N*-sulfonyloxaziridines.^[3] Alternative to these chemical methods a variety of α -oxy-functionalized carbonyl compounds, including α -hydroxy, α -hydroperoxy, and α -acetoxy derivatives, can also be prepared in enantiomerically pure form by biocatalytic methods.^[4]

Despite the emergence during the two last decades of new efficient and convenient enantioselective catalytic processes,^[5] not many methods are known for the catalyzed asymmetric

addition of electrophiles to enolates.^[3,6] The asymmetric α -amination of aldehydes and ketones provides a useful reference point for the reaction described in this account.^[7]

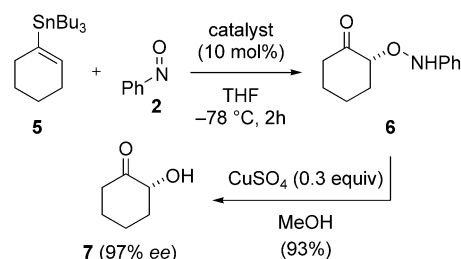
Among the electrophiles that can be used to introduce a heteroatom at the α position of a carbonyl group, nitroso compounds^[8] are particularly attractive because of the presence of two different electrophilic centers (N and O atoms) that could lead to either N- or O-alkylation, provided the regioselectivity of the reaction can be controlled (Scheme 1).



Scheme 1. N- vs. O-alkylation of carbonyl compounds in the reaction with nitrosobenzene.

Yamamoto and co-workers approached this problem by using lithium and tin enolates, in the absence of any catalyst, to generate the corresponding α -hydroxyamino carbonyl compounds 3.^[9] The regiochemical control of the

reaction illustrated in Scheme 1 resulted from the Lewis acid catalyst; the best results were obtained with Et_3SiOTf .^[10] Under these conditions both silyl and tin enolates provided the α -aminoxy carbonyl compounds 4. In order to introduce asymmetry in the reaction it was conducted in the presence of (*R*)-binap (binap = 2,2'-bis(diphenylphosphanyl)-1,1'-binaphthyl) and 10 mol % AgOTf . Complete O-selectivity with 91 % *ee* was observed, and of the various silver salts surveyed, the AgOTf and AgClO_4 complexes gave the best results in terms of both enantio- and regioselection (Scheme 2).^[11] The reaction was tested with various tin enolates and, in all cases, O-regioselectivity and enantioselectivity were maintained. The further transformation of α -aminoxy ketone 6 into α -hydroxy ketone 7 was smoothly



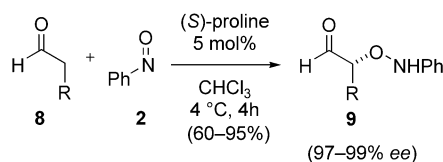
Catalyst	Yield [%]	ee [%]
(<i>R</i>)-binap/ AgOTf	88	91
(<i>R</i>)-binap/ AgClO_4	95	95
(<i>R</i>)-binap/ AgBF_4	64	54
(<i>R</i>)-tolbinap/ AgOTf	92	91
(<i>R</i>)-tolbinap/ AgClO_4	93	92

Scheme 2. Asymmetric, catalyzed nitroso-aldol reaction of tin enolate 5 with nitrosobenzene.^[11]

[*] Dr. P. Merino, Dr. T. Tejero
Laboratorio de Síntesis Asimétrica
Departamento de Química Orgánica
Facultad de Ciencias
Universidad de Zaragoza
50009 Saragossa, Aragon (Spain)
Fax: (+34) 976-762-075
E-mail: pmerino@unizar.es

carried out with a catalytic amount (0.3 equiv) of CuSO₄ in methanol. The selectivity found for ketone **7** was 97% *ee*.

During the past five years there has been a dramatic upsurge of interest in asymmetric non-transition-metal-catalyzed reactions,^[12] particularly those concerning aminocatalysis in which a carbonyl group is activated by a chiral amine.^[13] In this context, (*S*)-proline, which can serve as a ligand in asymmetric transition-metal-catalyzed reactions, has been also found to be an excellent catalyst by itself^[14] for reactions of carbonyl compounds,^[15] including the α -amination of aldehydes and ketones.^[7] Last year several reports appeared in which α -aminooxylated aldehydes and ketones **4** were prepared in enantiomerically pure form from the parent carbonyl compounds and nitrosobenzene with (*S*)-proline as the catalyst (Scheme 1). The advantages of this organocatalyzed transformation are clear: 1) the asymmetric reaction is carried out by a metal-free catalyst, and 2) no enolates must be preformed. The groups of MacMillan^[16] and Hayashi^[17] and also Zhong^[18] reported almost simultaneously the direct (*S*)-proline-catalyzed enantioselective α -aminooxylation of aldehydes (Scheme 3).

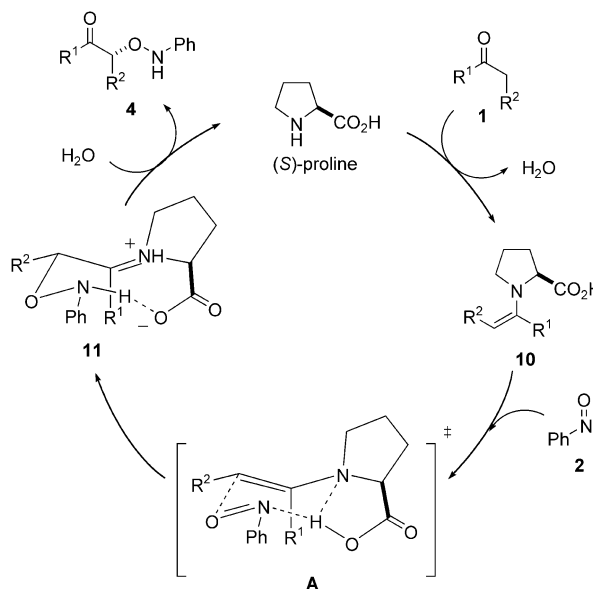


Scheme 3. Enantioselective (*S*)-proline-catalyzed α -aminooxylation of aldehydes. TIPS = triisopropylsilyl.^[16]

In contrast to other proline-catalyzed asymmetric reactions,^[15] which require up to 47 mol % catalyst,^[19] the reaction outlined in Scheme 3 is efficiently catalyzed even with catalyst loadings as low as 0.5 mol % as MacMillan et al. demonstrated.^[16] The use of 2 mol % is, however, more advisable in order to maintain expedient reaction times.

Almost as interesting as the high enantioselectivity observed for **9** is the preferred O-regioselectivity. In principle,

trends similar to those observed for tin enolates in the absence of any catalyst (N-alkylation) might be expected for the reaction of the intermediate enamine (a masked enolate), which is formed upon activation of the aldehyde. However, only O-alkylation is observed in all cases. This means that additional directing features are needed to ensure attack at the oxygen atom. Proline meets such a requirement due to the presence of the carboxyl group which may favor a transition state **A** with an intramolecular hydrogen bond stabilized by the enhanced Brønsted basicity of the nitrogen atom (Scheme 4). Model **A** has been proposed essentially in the same, but independent, way by the groups of MacMillan,^[16] Hayashi,^[17] and Zhong,^[18] and it follows the previously reported mechanistic considerations for (*S*)-proline-catalyzed reactions



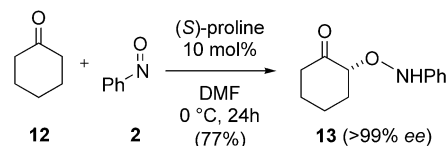
Scheme 4. Catalytic cycle for the (*S*)-proline-catalyzed α -aminooxylation of carbonyl compounds.

of ketones and aldehydes with electrophiles.^[7,13] It is important to emphasize that while model **A** is consistent with experimental data collected to date, it still needs a firmer foundation. Of course, our understanding of the details of proline-catalyzed reactions will change with time and experience.

The reaction appears to be quite general since both aliphatic and aromatic aldehydes gave good results. Even the presence of several functional groups,

such as a C=C bond, Si-protected hydroxy groups, and N-Boc amino groups, and variation in the steric demand of the aldehyde did not affect the reaction in terms of efficiency or enantioselectivity.

A considerable broadening of the scope of the reaction has been recently achieved with the extension to ketones. Cordova and co-workers^[20] as well as Hayashi and co-workers^[21] reported simultaneously the organocatalyzed asymmetric α -aminooxylation of ketones (Scheme 5). Condensation of an excess



Scheme 5. Asymmetric α -aminooxylation of ketones.

(2.0 to 10.0 equiv) of cyclohexanone **12** with 1.0 equiv nitrosobenzene in a highly polarized solvent such as DMSO or DMF and in the presence of a substoichiometric amount (5–30 mol %) of (*S*)-proline provided opportunities to examine the behavior of the reaction under a variety of experimental conditions.^[20,21] Although excellent enantioselectivities were observed for compound **13**, low chemical yields and considerable amounts of the corresponding α,α' -bis(aminooxylated)

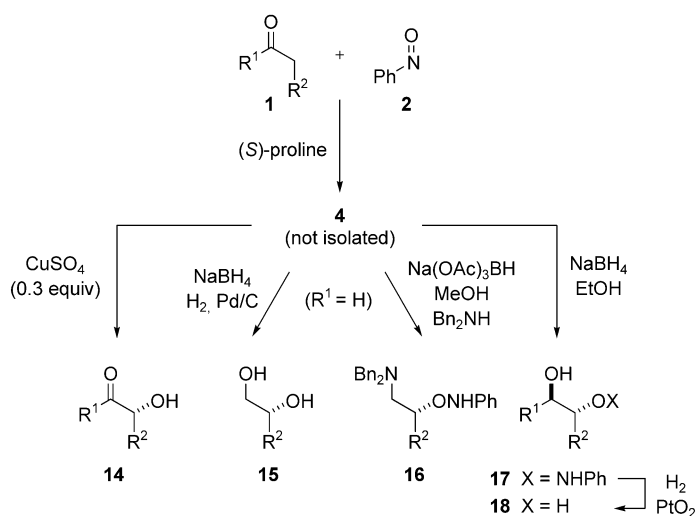
product were also obtained. Synthetically useful chemical yields and complete α -monoaminooxylation were possible by slow addition (syringe pump) of nitrosobenzene to the reaction mixture. The extension to other cyclic ketones resulted in excellent yields as well as almost complete regio- and enantioselectivities. Unfortunately, acyclic ketones gave unfavorable mixtures of O- and N-alkylated products; in all cases, with the exception of 3-butenyl methyl

ketone, the O-alkylated adduct was the major product.

α -Aminoxylation of carbonyls can be transformed readily into the corresponding α -hydroxy carbonyls and then reduced to diols without any loss in optical purity.^[11] The reduction of the carbonyl moiety can also be made in situ prior to cleavage of the N–O bond (NaBH_4)^[20] or in a simultaneous way (NaBH_4 , Pd/C, H_2)^[16] thus providing an alternative, synthetically attractive solution, in the case of α -hydroxy aldehydes (Scheme 6, $\text{R}^1 = \text{H}$), to the asym-

metry of the reaction are impressive advantages that should facilitate the synthesis of many useful small molecules possessing not only the α -hydroxy-carbonyl unit but also other functionalities such as diols or amino alcohols. Undoubtedly, all these possibilities should provide synthetic tools for the asymmetric synthesis of collections of molecules with high levels of diversity directed to the recently proposed “diversity-oriented synthesis”.^[22]

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Scheme 6. Synthetic applications of the asymmetric organocatalyzed α -aminoxylation of aldehydes and ketones.

metric dihydroxylation of terminal olefins. Scheme 6 illustrates the potential of the enantioselective α -aminoxylation of aldehydes and ketones for the synthesis of diols and immediate precursors of amino alcohols like **16**.

The asymmetric organocatalyzed α -aminoxylation of aldehydes and ketones is a powerful, metal-free, direct method for the synthesis of α -hydroxy aldehydes and ketones. The reaction with nitrosobenzene forms the α -phenylaminoxy carbonyl both regio- and enantioselectively in good to excellent yields. The directness of the approach to α -hydroxy carbonyls, the generality of the proline-mediated nitrosoaldol reaction, which permits great flexibility in selection of aldehydes and ketones, and the versa-

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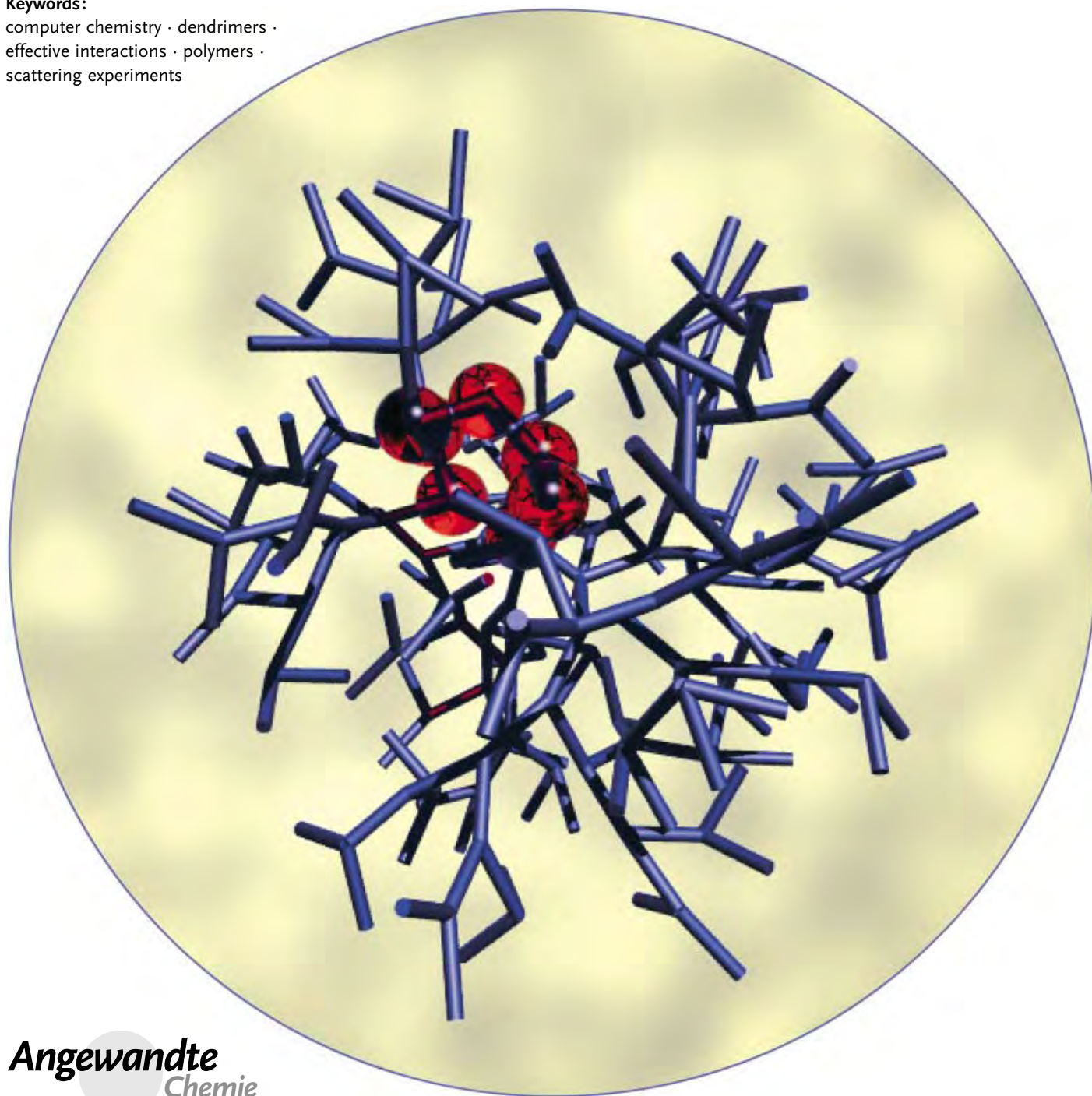
Dendrimers

Dendrimers in Solution: Insight from Theory and Simulation**

Matthias Ballauff and Christos N. Likos**

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A variety of experimental and theoretical approaches show that, akin to linear polymers, dendrimers in good solvent conditions are best described as flexible macromolecular aggregates with a dense core and fluctuating monomer groups. We present theoretical and simulational evidence of how the shape and inner structure of dendrimers depends on the generation number as well as the effective interactions that exist between dendrimers in solution. These approaches based on simplified dendritic structures show there is a tunable and ultrasoft interaction between the centers of the solubilized dendrimers. Results from small-angle neutron scattering data confirm the theory and indicate that dendrimers are model systems of ultrasoft colloids that bridge the gap between polymers and hard spheres. Dendrimers can form a class of materials analogous to the related systems of star polymers and block copolymer micelles which exhibit special properties.

1. Introduction

Dendrimers are synthetic macromolecules with defined architectures that are synthesized by iterative controlled reaction steps. The formation of subsequent generations on a trifunctional monomer core results in a treelike structure. Hence, these macromolecules have been termed “dendrimers”—from the Greek word *dendron* for tree. The terminal groups may be modified by suitable end groups. Figure 1 shows a typical example of a fourth generation dendritic structure with defined end groups.

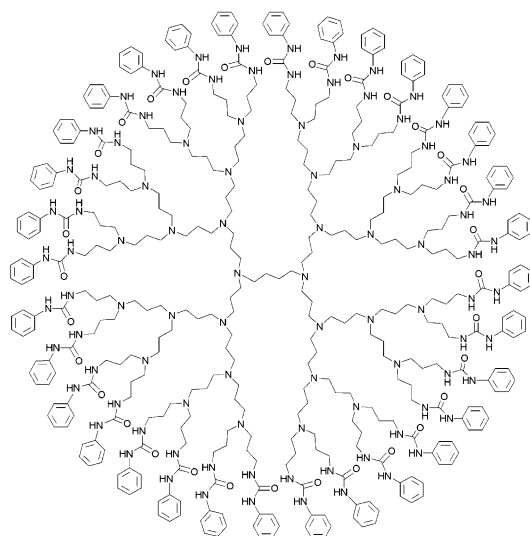


Figure 1. Structure of a fourth-generation dendrimer with defined end groups.

The first dendrimers were prepared in 1978 by Vögtle and co-workers,^[1] who termed these structures “cascade molecules”. This study, however, received little attention at that time. The term “dendrimer” was coined by Tomalia et al.,^[2] who also indicated a large number of possible applications of such molecules in subsequent publications. These investigations formed the basis of making research on dendrimers very

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popular in the last 20 years, and the number of publications on dendrimers has undergone an exponential increase

during this time. The number of chemical structures synthesized by this approach is currently very large and a number of review articles have been written.^[3–10]

Most of the aforementioned work was motivated by the idea that dendrimers possess a hollow core and a dense shell (Figure 1). The underlying assumption was that the segmental density increases from the center to the periphery, and thus it should be possible to use such molecules as carriers (dendritic boxes). This dense-shell picture has been strongly supported by a much-cited paper by de Gennes and Hervet in 1983^[11] in which they presented the first theoretical treatment of dendrimers and concluded that the density increases towards the periphery of the molecule. A survey of the literature dealing with the synthesis of dendrimers and possible applications shows that most studies are still based on this assumption. Lescanec and Muthukumar^[12] were the first to show, however, that a dendritic structure made up from flexible bonds should exhibit its maximum density at the center of the molecule (dense-core model). This pioneering work has been the starting point of a considerable number of theoretical studies. The subject of dendrimers has now become an important issue in the fields of polymer theory and physics of soft matter. All theoretical studies published so far support the early result of Lescanec and Muthukumar, thus firmly establishing the validity of the dense-core model. It seems, however, that these important results have not been

[*] Prof. Dr. M. Ballauff
Physikalische Chemie I
Universität Bayreuth
95440 Bayreuth (Germany)
Fax: (+49) 921-55-2780
E-mail: matthias.ballauff@uni-bayreuth.de

Prof. Dr. C. N. Likos
Institut für Theoretische Physik II
Heinrich-Heine-Universität Düsseldorf
Universitätsstrasse 1, 40225 Düsseldorf (Germany)
Fax: (+49) 211-81-12262
E-mail: likos@thphy.uni-duesseldorf.de

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fully accepted in the synthesis of dendrimers and their practical applications.

Small-angle neutron scattering (SANS) and small-angle X-ray scattering (SAXS) are in principle well-suited to determine the spatial structure of dissolved macromolecules. In 1992 Bauer et al. were the first to apply these methods to the problem of dissolved dendrimers,^[13] and this study was followed by that of Prosa et al. in 1997.^[14] A number of SANS and SAXS studies have been published since then, and the dense-core model has gained considerable support from this work (see, for example, Ref. [7] and references therein). The results published to date differ in a number of important details. Moreover, the number of investigations aimed at a direct comparison of the results of scattering experiments with theoretical predictions is scarce.

In this Review we give an overview of recent theoretical studies and compare these results to the present experimental data that originate from scattering experiments. The purpose of this Review is twofold: Firstly, we demonstrate that theory, simulations, and scattering experiments have now come to irrefutable conclusions concerning the radial structure of dendrimers. It has become evident from these studies that the dense-core model of dendrimers is correct and other models must hence be rejected. As a second point we demonstrate that theory and simulations have become so advanced in recent years that they may be used as direct guides in the course of the design and the synthesis of chemical nanostructures such as dendrimers. Chemical structures can be mapped directly onto simulations and the results thereof may be used in turn to decide which structures should be made. It is now possible to discuss the structural modifications necessary to go from the usual dense-core structure to a dendrimer having a dense-shell structure on the basis of theoretical considerations and simulations. We consider this an important development in the field of chemical nanostructures which will certainly have a major impact in the near future.

2. Isolated Dendrimers

2.1. Introduction

The investigation of the conformational properties of linear polymer chains and of the thermodynamic behavior of

concentrated polymer solutions has been one of the most active topics of theoretical physics research in the second half of the twentieth century. Dendrimers, on the other hand, present the theorist with a challenging problem: Despite their polymeric nature when looked upon as chemical substances, their peculiar, highly branched architecture clearly distinguishes them from linear chains and causes them, particularly at high generation numbers, to resemble rather compact, spherical colloids. Nevertheless, the typical monomer fluctuations of polymers are never completely lost, as demonstrated below. Thus, dendrimers build a natural bridge between colloids and polymers and bear some resemblance to the related system of star polymers, although some important differences to the latter also exist.

Looking at this problem from a fundamental point of view leads to the question of whether the principle of universality, which has been shown to be valid for sufficiently long chains, also applies to dendrimers. The term “universality” has a precise meaning in theoretical physics: it expresses the fact that certain properties of the system are, in some well-defined limit, independent of its microscopic details.^[17–20] For example, the typical size R of a polymer chain in a good solvent increases with the degree of polymerization N as $R = CaN^\nu$, where a is the monomer length, C is a nonuniversal dimensionless constant of the order of unity, whose precise value depends on the specific chain, and ν is the *universal* exponent. This so-called Flory exponent has the value $\nu = 0.588$ in three dimensions; this value was obtained by employing very accurate calculation of the renormalization group^[21] and simulations.^[22] For most practical applications the original result obtained by Flory, $\nu = 3/5$, is used. It is not at all clear, however, whether a similar universality principle applies to dendrimers. In the case of chain molecules, one can increase the degree of polymerization N to arbitrarily large values and it is precisely at the $N \rightarrow \infty$ limit that universality holds. This situation is not feasible with dendrimers, as the high degree of branching causes the number of monomers to grow exponentially with distance from the core, thus placing a limit on the maximum number of generations that can be practically achieved. One very important question, therefore, that has been the subject of computer simulations, is how sensitive the properties of dendrimers are to the interaction potentials acting between their constituent monomers. A particularly encouraging fact that has emerged from a number



Matthias Ballauff studied chemistry at the University of Mainz and received his PhD in 1981 in the group of Prof. B. A. Wolf at the Institute of Physical Chemistry. From 1981 to 1983 he was a postdoctoral researcher in Prof. P. J. Flory's group at Stanford University. He joined the Max-Planck-Institut für Polymerforschung in 1984 where he worked in the groups of Prof. G. Wegner and of Prof. E. W. Fischer. In 1990 he became a professor at the Polymer-Institut der Universität Karlsruhe. Since October 2003 he has been a professor at the Universität Bayreuth (Lehrstuhl Physikalische Chemie I).



Christos Likos obtained his Diploma in Electrical Engineering at the National Technical University of Athens in 1988. He obtained his Ph.D. in Physics with Prof. N. W. Ashcroft at Cornell University (1993). He was an Alexander von Humboldt Fellow at the LMU München, EU Fellow at the Università di Trieste, and a researcher at the Jülich Research Center. He obtained his Habilitation from Heinrich-Heine-Universität Düsseldorf (2000) and was a Heisenberg Visiting Scholar at the University of Cambridge (2003) before becoming Professor of Theoretical Physics at the Heinrich-Heine-Universität Düsseldorf.

of studies is that a certain type of universality indeed continues to hold, in the sense that the salient characteristics of the dendrimers' conformations are insensitive to the details of the microscopic interactions employed.

Additional theoretical questions that have been discussed in the last two decades range, in increasing order of complexity, from the influence of the architecture and the solvent quality on the sizes and conformations of isolated dendrimers, to the behavior of concentrated solutions of the dendrimers, and to the equilibrium and nonequilibrium dynamics of dendrimer solutions. In the following Section we present a review of these approaches and of the conclusions that have been drawn from them. Perhaps the most important result of many years of intensive research, which we want to state from the very beginning, is that a consensus now seems to be emerging regarding the conformations of isolated dendrimers in good solvents: the overwhelming majority of the studies have converged to the conclusion that dendrimers have a dense-core structure with a monomer density distribution, that is, high close to their centers and falls off at their periphery.

2.2. Characteristic Quantities

Let us consider a dendrimer branching from a single central segment of *functionality* f , which means that this element has f functional units on which additional monomers can be attached. Linear chains consisting of P bonds each are grown along each of the functional units; P is referred hitherto as the *spacer length* of the dendrimer.^[15] The central monomer together with the f initial chains forms the *zeroth-order generation* of the dendrimer, $g=0$. (Hitherto g will denote the generation index of a dendrimer with a total of G generations altogether, that is, $0 \leq g \leq G$.) At the end of each chain of length P is found a new functional unit, from which the dendrimer branches out anew, thereby forming the beginning of the next generation of monomers. Thus, every dendrimer can be characterized by three numbers: the functionality f , the spacer length P , and the number of generations G ; thus the special case of $G=0$ corresponds to star polymers with f chains of length P each. The total number of monomers N in a dendrimer of regular architecture is related to the other quantities through Equation (1).

$$N = 1 + fP \frac{(f-1)^{G+1} - 1}{f-2} \quad (1)$$

In the following discussion we are mainly going to focus on the most common experimentally realizable case of trifunctional units, $f=3$.

The study of the sizes and shapes of isolated dendrimers plays a key role in understanding not only the properties of the molecules themselves but the thermodynamics of concentrated dendrimer solutions as well. The typical quantities of interest are discussed below.

The overall size of the dendrimer is typically measured by its radius of gyration R_g . Denoting the instantaneous positions of the N monomers comprising the dendrimer ($i=1, 2, \dots, N$)

as $\mathbf{r}_i$ and the position of its center of mass by $\mathbf{r}_{\text{CM}}$ enables the gyration radius to be defined as Equation (2).

$$R_g^2 = \frac{1}{N} \left\langle \sum_{i=1}^N (\mathbf{r}_i - \mathbf{r}_{\text{CM}})^2 \right\rangle \quad (2)$$

The bracket $\langle O \rangle$ denotes the average of the measurable O value over all the microscopic states μ , each weighted with its Boltzmann factor $\exp(-\beta E_\mu)$. E_μ is the energy of the instantaneous configuration $\mu = \{\mathbf{r}_1, \mathbf{r}_2, \dots, \mathbf{r}_N\}$ and $\beta = (k_B T)^{-1}$ is the inverse temperature. Since the gyration radius is readily measurable by small-angle scattering experiments on dilute solutions, a direct comparison of simulations with experimental results is possible. Of particular interest is the dependence of the value of R_g on the number of monomers N , the spacer length P , and the generation number G , as well as on solvent quality, its pH value, and ionic strength. All of these quantities are experimentally controllable.

The conformation of the dendrimer is most conveniently expressed through the monomer density $\rho(\mathbf{r})$, which depends on the vector $\mathbf{r}$ connecting the dendrimer's center of mass (or the central functional unit) with the considered observation point. The quantity $\rho(\mathbf{r})d^3r$ is proportional to the probability of finding a monomer in a neighborhood d^3r of $\mathbf{r}$. The local density is formally defined as the average of the so-called one-particle-density operator $\hat{\rho}(\mathbf{r})$ [Eq. (3)],^[23,24] in which $\delta(\mathbf{x})$ is the Dirac delta function.

$$\rho(\mathbf{r}) = \langle \hat{\rho}(\mathbf{r}) \rangle \equiv \left\langle \sum_{i=1}^N \delta(\mathbf{r} - \mathbf{r}_i) \right\rangle \quad (3)$$

It is also useful to calculate generation-resolved density profiles, and particular attention has been paid to the distribution of the end groups $\rho_{\text{end}}(\mathbf{r})$. The density profiles become spherically symmetric if the average distribution is taken over all configurations, thereby demonstrating that the density profile depends solely on the magnitude r of the separation vector $\mathbf{r}$. Although the density profile is not directly measurable in experiments, it is possible under certain conditions to invert the scattering curves back into real space and also obtain indirect information about the monomer distribution from experiment.

The dendrimer's *form factor* is a very important quantity because it allows direct comparisons to be made between simulations and scattering experiments. Indeed, scattering from a dilute solution enables the intensity $I(\mathbf{q})$ to be obtained at scattering wave vector $\mathbf{q}$, whose coherent part is directly proportional to the form factor $F(\mathbf{q})$ of the macromolecule. This statement rests on the approximation that the correlations between scatterers (monomers) belonging to different dendrimers in dilute solutions are extremely weak and can thereby be ignored. Thus, the form factor arises entirely from intramolecular correlations and is defined as the Fourier transform of the pair correlation function $G(\mathbf{r})$ [Eq. (4)].

$$F(\mathbf{q}) = \int d^3r G(\mathbf{r}) \exp(-i\mathbf{q}\mathbf{r}) \quad (4)$$

The correlation function is a measure of the conditional probability density of finding two monomers i, j in the

dendrimer separated by the vector $\mathbf{r}$. If the instantaneous separation between the monomers' position vectors is set as $\mathbf{r}_{ij} = \mathbf{r}_i - \mathbf{r}_j$, the correlation function can be defined according to Equation (5).^[24]

$$G(\mathbf{r}) = \delta(\mathbf{r}) + \frac{1}{N} \left\langle \sum_{i=1}^N \sum_{j \neq i}^N \delta(\mathbf{r} - \mathbf{r}_{ij}) \right\rangle \quad (5)$$

The contribution from the term $i = j$ has been explicitly separated out in the equation. The combination of Equations (4) and (5) gives Equation (6).^[24–26]

$$F(\mathbf{q}) = 1 + \frac{1}{N} \left\langle \sum_{i=1}^N \sum_{j \neq i}^N \exp[-i\mathbf{q}(\mathbf{r}_i - \mathbf{r}_j)] \right\rangle \quad (6)$$

As for the case of the density profiles, the conformation average restores the angular isotropy and we obtain $G(\mathbf{r}) = G(r)$ and $F(\mathbf{q}) = F(q)$, with q denoting the magnitude of the scattering wave vector $\mathbf{q}$.

Although other quantities that additionally characterize the dendrimer's conformations can also be calculated or measured in a simulation, the above ones are the ones that have been most intensively investigated. We now proceed with an overview of the theoretical and simulational work performed along these lines.

2.3. Theory

2.3.1. Ideal Dendrimers

The simplest case is that of *ideal* dendrimers, namely, dendrimers that carry non-interacting monomers. Though somewhat unrealistic, this is still a useful reference system, since many interesting quantities can be calculated analytically and can thus serve as checks for the validity of simulation techniques. Additionally, the ideal model could be used to describe dendrimer conformations at the θ point, in analogy to the case of linear chains. The radius of gyration R_g is given by an analytical expression that has been derived by Carl.^[16] It is proportional to the size of a typical linear strand containing P monomers [Eq. (7)].^[27]

$$R_{g,\text{id}} \sim a(PG)^{1/2} \quad (7)$$

The density distribution around the center is a sum of Gaussian functions [Eq. (8)].^[16]

$$\rho(r) = f \sum_{i=0}^G (f-1)^i \sum_{j=1}^P \left(\frac{A_{ij}}{\pi} \right)^{3/2} \exp(-A_{ij}r^2) - f(f-1)^G \left(\frac{A_{GP}}{\pi} \right)^{3/2} \exp(-A_{GP}r^2) \quad (8)$$

A_{ij} is defined by Equation (9), where i and j are integer indices numbering the segments, $\sigma_{ij} = iP + j$, and a is the monomer length.

$$A_{ij} = \frac{3}{2a^2 \sigma_{ij}} \quad (9)$$

The monomer density is a sum of Gaussian functions with generation-dependent widths and has, evidently, a maximum at $r = 0$.

For the form factor of ideal dendrimers, an analytic expression was first derived by Hammouda;^[28] Boris and Rubinstein^[27] examined the special case where $P = 1$ and thereby derived explicit expressions for the number of distinct pairs of monomers separated by a *topological* distance L along the dendrimer backbone. Subsequently they calculated the overall form factor $F(q)$ as well as for the form factor $F_{\text{end}}(q)$ arising from scattering by the last generation only. We quote in Equation (10) the rather simple final result^[27] for $f = 3$ ($x = a^2 q^2$ and N_{end} is the number of end monomers).^[29]

$$F_{\text{end}}(q) = N_{\text{end}} \frac{1 + 2^G e^{-xG/3} - 2^{G+2} e^{-x(G+1)/3}}{(3 \cdot 2^G - 2)(1 - 2e^{-x/3})} \quad (10)$$

It can be determined for $G \gg 1$ that $\lim_{q \rightarrow 0} F_{\text{end}}(q) = N_{\text{end}}(3 \cdot 2^G - 1)/(3 \cdot 2^G - 2) \cong N_{\text{end}}$. Moreover, an expansion for small x values shows that the radius of gyration of the end monomers scales as $aG^{1/2}$ [Eq. (11)].

$$F_{\text{end}}(q) \cong N_{\text{end}} \left[1 - \frac{q^2 a^2 G}{3} \right], \quad (qa \rightarrow 0) \quad (11)$$

This, in conjunction with Equation (7) ($P = 1$), demonstrates that the terminal generation in ideal dendrimers has a spatial extension that is as large as that of the whole macromolecule.

2.3.2. Self-Avoiding Dendrimers

A much greater challenge is the problem of self-avoiding dendrimers that arise in good or athermal solvent conditions in which a further increase of temperature does not modify the solvent quality. Here, one must consider the steric, excluded-volume interactions between the monomers explicitly and it is expected that these will cause significant alterations in the configurations and sizes of real dendrimers with respect to their ideal counterparts. The first work on this subject was performed by de Gennes and Hervet.^[11] They considered flexible trifunctional monomers and very long spacers (large P) in an athermal solvent. By using a modified version of self-consistent field (SCF) method developed by Edwards^[30] they obtained a density profile with a global minimum at the center and a monotonic increase towards the periphery of the dendrimer. This is known as the hollow-center or dense-shell model. Implicit in the force-balance calculations of de Gennes and Hervet, however, was the assumption that each successive generation of monomers occupies a concentric shell of its own, which lies at a further radial distance from the center of the starburst than the preceding one. Hence, neither the finding of the hollow center nor the associated result of localization of end monomers at the outermost part of the molecule comes as a surprise. In addition, the size of the dendrimers was found to scale as $R_g \sim N^{1/5}$. This relationship yields an effective fractal dimension of $d_f = 5$ for the dendrimers and, since this exceeds the dimension of space, it follows that there is a limiting

generation number $G_{\max} \cong 2.88(\ln P + 1.5)$, beyond which no perfect dendrimers can be grown.

Although the findings of de Gennes and Hervet caused some excitement, since they identified the possibility of using the hollow dendrimers as hosts and delivery agents for smaller molecules, early simulations performed thereafter cast some doubt on the validity of the dense-shell model. Naylor et al.^[31] carried out microscopic molecular dynamics (MD) simulations using atomic force-fields of dendrimers up to $G = 7$ and found no indications of the concentric shell configuration of de Gennes and Hervet. Soon thereafter, Lescanec and Muthukumar^[12] performed the first extremely simplified simulations of dendrimers (see also Section 2.4). They used a kinetic growth algorithm of self-avoiding walks to build off-lattice dendrimers and they found, in contrast to Ref. [11], density profiles that decreased monotonically towards the edge of the molecule. This became known as the “dense-core model” of dendrimers. However, one significant shortcoming of the work^[12] is that the simulations were not run for long enough; thus the obtained structures were not relaxed to the maximum-entropy configurations. Since both the dense-shell and dense-core predictions suffered from different shortcomings, the issue of the equilibrium configurations of self-avoiding dendrimers became controversial. Although there now exists overwhelming evidence for the validity of the dense-core model, it took about a decade of theoretical and experimental work to reach this conclusion and settle the question.

2.3.3. Historical Aspects

The first theoretical work that supported the validity of the dense-core model was performed by Boris and Rubinstein.^[27] They carried out SCF calculations but, in contrast to de Gennes and Hervet,^[11] no prior assumptions about the distribution of the generations were put in. The probability density of finding a single branch of the dendrimer in the presence of the mean density of all the other branches was calculated by incorporating the excluded-volume interaction in the coupling to the self-consistent density field. The procedure is iterative in nature to enforce self-consistency. Boris and Rubinstein examined various generation numbers ($G = 2-8$), excluded-volume parameters ($v = 0.0001a^3 - 0.5a^3$), as well as dendrimers of varying spacer length. In all cases, the density profiles were found to be opposite to the predictions of the dense-shell model: they showed a monotonic decrease from the center towards the periphery, with increased spacer lengths causing the density distribution to spread further from the center. The end monomers were found to be distributed throughout the volume of the dendrimer, which is in direct contrast with the periphery-localization prediction of Ref. [11]. In addition, form factors were calculated that also showed a monotonic behavior as a function of q , with secondary maxima appearing at $qR_g \approx 5$ at larger generations ($G = 4-8$). The radius of gyration R_g was found to increase with generation number and excluded volume. A shortcoming of the SCF approach of Boris and Rubinstein is that it is a mean-field theory: all correlations along one branch are ignored and the spatial

density of the latter is calculated in the presence of a mean-density field that is pre-averaged from the configurations of all other branches. Nevertheless, the results were found to be in very good agreement with results from both simulation^[12,32,33] and experiment^[34] on poly(amidoamine) (PAMAM) dendrimers available at the time. The predictions of this theory have been confirmed qualitatively by a large number of simulations and experiments that followed (see Section 2.4). The strongest physical argument for the validity of the dense-core model, as formulated in Ref. [27], is: the configuration of the dendrimer is determined by the balance between the entropy and the excluded-volume interaction and none of those prefers a hollow-center configuration. Thus, it is to be expected that the dendrimers will assume a dense-core structure, at least in the absence of other interactions (such as electrostatic ones) that may enter as additional factors in the force-balance considerations.

2.3.4. Newer Developments

Very recently, Zook and Pickett^[35] revisited the original SCF calculation of de Gennes and Hervet^[11] and were able to pinpoint its major flaw. The method of de Gennes and Hervet tacitly rests on the reasonable yet incorrect assumption that a *single* conformation of the dendrimer (called “ground state” in the language of SCF methods) dominates all statistical averages within the limits of thermodynamics. The reality, though, is that there are *infinite* degenerate ground states contributing to the self-consistent potential. The situation is quite analogous to that of polymer brushes grafted on a planar surface: Whereas the so-called Alexander brush^[36] has a monomer density with the shape of a step function, the more accurate calculation of Milner, Witten, and Cates^[37] yields a parabolic profile instead. Zook and Pickett^[35] have shown, in analogy, that chains at each and every end-monomer position throughout the dendrimer corona contribute equally to the self-consistent field. Thus, a parabolic density profile was found in Ref. [35], thus demonstrating the validity of the dense-core model beyond doubt. It follows then that the end groups of the dendrimers are not localized at the periphery, but are distributed throughout the macromolecule; this feature was confirmed recently by SANS experiments.^[38]

An alternative approach was followed by La Ferla^[39] who applied the Rouse–Zimm discrete hydrodynamic model^[40,41] to starlike dendrimers to analyze their static and dynamical properties. Excluded volume effects were neglected, but stiffness of the bonds was considered explicitly. The procedure employed allows for the calculation of the overall size of the molecules and of the form factor, but not of the density profile. La Ferla reported a peculiar N -dependence of the radius of gyration of the dendrimer: $R_g \sim (\ln N)^{1/2}$ for the ideal dendrimer (freely jointed model) and $R_g \sim \ln N$ for the maximally extended radius (stiff chains). Since the latter behavior has not been seen in any simulations or experiments, we are led to believe that these results are rather an artifact of the theoretical approximations involved. It was shown by examining the evolution of the form factors with stiffness that extremely stiff dendrimers have very different conformations

than flexible ones, with the latter assuming compact shapes and the former open ones.

Ganazzoli, La Ferla, and Terragni generalized the approach of La Ferla to include excluded-volume effects.^[42,43] Here, the probability density of contact among pairs in the Gaussian approximation was introduced^[44] and the free energy arising from the excluded volume interactions was modeled on the basis of a second virial coefficient by making use of the aforementioned contact probability density. The free energy, including elastic and excluded-volume contributions, was thereby minimized with respect to all expectation values $\langle \mathbf{l}_i \mathbf{l}_j \rangle$, where $\{\mathbf{l}_i\}$ is the set of all bond vectors. They report a successive decrease in the angular correlations between neighboring segments at increasing generation indices, as well as an oscillatory structure for spacer length $P=2$ that is absent for $P=1$. The expansion factor of the dendrimers, $a_s^2 = R_g^2 (R_{g,id}^2)^{-2}$, was calculated to have a universal form and found to increase as the excluded volume interactions increased.^[43] However, no predictions for neither the N scaling of R_g nor for the monomer profile were reported in Ref. [42]. Ganazzoli and La Ferla also investigated the interchain θ temperature of dendrimers by introducing an additional three-body interaction between the monomers.^[45]

Timoshenko, Kuznetsov, and Connolly^[46] applied the Gaussian self-consistent (GSC) method to analyze the conformations of dendrimers with $G=1-7$ generations and spacer lengths $P=1-6$. An improved treatment of the problem was employed in this work, in comparison to the approach of Ref. [42], in the sense that it operates directly on a full Hamiltonian function without the need to resort to a virial expansion of the free energy (which is of questionable validity for high local monomer concentrations). The theoretical GSC approach was complemented by Monte-Carlo (MC) computer simulations. The connectivity of the dendrimers was modeled through harmonic springs whereas the intermonomer interactions were considered by means of a hard-sphere Lennard-Jones potential that allows the tuning of the strength of attractions and thereby for a change in solvent quality. In line with all theoretical approaches to date, neither detailed stereochemical interactions nor explicit solvent molecules were considered; instead, a simplified model that captures the salient dendrimer characteristics (architecture of the connectivity and steric monomer-monomer repulsions) was employed.

The MC simulations of Timoshenko, Kuznetsov, and Connolly^[46] confirm the dense-core model (see Section 2.4 for details). Whereas the object of interest in the SCF theory of Boris and Rubinstein is the monomer density $\rho(r)$,^[27] the main objects of the GSC approach are the mean-square distances between the monomers [Eq. (12)].^[47]

$$D_{ij} \equiv \frac{1}{3} \langle (\mathbf{r}_i - \mathbf{r}_j)^2 \rangle \quad (12)$$

Since, however, the above average is impossible to calculate for the true Hamiltonian function of the system, this is replaced by a reference Hamiltonian function that is a quadratic form of all $\mathbf{r}_i$ and $\mathbf{r}_j$ values. The coefficients of the quadratic form are determined in such a way that the

correlations $\langle \mathbf{r}_i \mathbf{r}_j \rangle$ in the original and the reference Hamiltonian are equal. The procedure can also be generalized to nonequilibrium situations. The gyration radius R_g was found to increase with both G and P , and a host of scaling laws was extracted from both the GSC approach and the MC simulation. The N dependence of R_g , induced by a change in P , for a fixed number of generations G in good solvent conditions has the form $R_g = a_G N^\nu$, with the usual Flory exponent $\nu = 0.588$, within simulation uncertainties and the known limitations of the GSC theory. When the generation-dependent coefficient a_G is taken into account, the scaling becomes $R_g \sim P^{1/4} N^{0.338}$. Thus, one obtains a scaling law for fixed P very close to $R_g \sim N^{1/3}$ which points to a fractal dimension of a compact object ($d_f = 3$), and is in agreement with the early predictions from the simulations of Murat and Grest.^[33] A shrinking of the molecule upon worsening of solvent quality was also predicted, which is in agreement with the results of the MC simulations performed by the same authors. The mean-square distances between radial nearest neighbors decay monotonically with generation index g for $P=1$, thus demonstrating that the springs from the core to the first monomers are the most stretched. An oscillatory structure, which has local maxima at the branching points, is superimposed on the general decreasing trend for dendrimers with $P > 1$. This result is similar to the findings in the above-mentioned Ref. [42], and is further corroborated by calculations of the local packing fraction that display maxima at the branching points: dendrimers with $P > 1$ are more congested locally at the terminal points of each generation than in the spacer domains. We emphasize that although the local packing fraction has, within the framework of the GSC theory of Ref. [46], a “hollow” structure as a function of the *topological* distance from the center (that is, as a function of the shell index), the density as a function of the *Euclidean* distance r from the center still has a dense-core configuration. There is no contradiction between the two findings: the reason lies in the back-folding of the terminal groups that distribute themselves throughout the volume of the dendrimer. Finally, the form factor $F(q)$ shows the development of oscillations for high q values with increasing generation number, which is a characteristic of the tendency of the molecules to be more compact, more spherical, and have sharper boundaries with increasing generation numbers. This result is again in agreement with the independent findings from the SCF theory of Boris and Rubinstein.^[27]

The methods and basic results from the various theoretical approaches are summarized in Table 1. Snapshots from the recent simulations of Götze and Likos (Figures 2, 3, and 4) provide a visualization of the dependence of the shapes of the dendrimers on G as well as of the location of the groups belonging to different generations.^[48] An increase in the terminal generation has the effect of making the dendrimer more compact and its boundary sharper. The loose outermost “blobs” that are visible for the G4 and G6 dendrimers have disappeared in the case of the G9 molecule. It is also interesting to point out that even the compact G9 dendrimer demonstrates a considerable amount of asphericity. This anisotropy has its roots in the particular architecture of the central unit (see Figure 1). The distribution of terminal

Table 1: Theoretical approaches applied to the problem of the conformation of isolated dendrimers.

Authors	Method	Density profiles	End group distribution	Overall size $R^{[a]}$
De Gennes and Hervet ^[11]	SCF ^[b]	dense shell	localized	$R \sim N^{0.2}$ (low N) $R \sim N^{1/3}$ (high N)
Boris and Rubinstein ^[27]	SCF	dense core	spread out	$R \sim G^{2/5} N^{1/5}$
La Ferla ^[39]	GVA ^[c] for ideal dendrimers and local stiffness	–	–	$R \sim (\ln N)^{1/2}$ (ideal) $R \sim \ln N$ (stiff)
Ganazzoli, La Ferla, and Terragni ^[42,43]	GVA, real dendrimers	–	–	Hill function
Timoshenko, Kuznetsov, and Conolly ^[46]	GSC ^[d]	dense core	spread out	$R \sim P^{1/4} N^{0.338}$
Zook and Pickett ^[35]	SCF	dense core	spread out	$R = \left \frac{15(2^G - 1)}{2\pi\omega(G)} \right ^{1/5} v_0^{1/5} N^{3/5 [e]}$

[a] N is the total number of monomers, P is the spacer length, and G is the total number of generations. [b] Self-consistent field. [c] Gaussian variational approximation. [d] Gaussian self-consistent theory. [e] The G dependence of the factor $\omega(G)$ is found in Ref. [35].

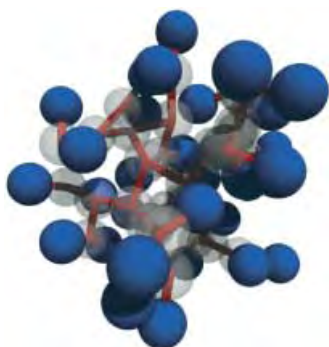


Figure 2. Simulation snapshot of a G4 dendrimer (bead–thread model of Equation (24)). The monomers are shown as semitransparent gray spheres of diameter σ and the end monomers are colored blue. The bonds are shown as cylinders. The spacer length here is $P=1$.^[48]

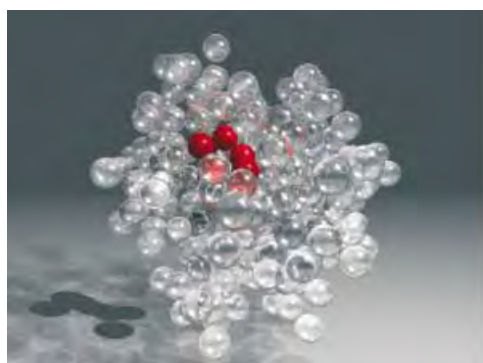
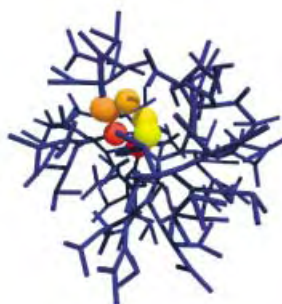


Figure 3. Simulation snapshot of a G6 dendrimer. Left: All monomers are shown as spheres of diameter σ . A single, back-folded branch has been selected and colored red, whereas all other monomers are shown as glass spheres. The shadowing assists in demonstrating the back-folding of the branch within the body of the dendrimer. Right: Only the monomers of the back-folded branch are shown as spheres and they are color-coded as follows: the end monomer is colored yellow and it is situated within the dendrimer. Starting from this, a path leads to one of the two innermost monomers, colored red. Increasing intensity of red denotes a closer approach to the aforementioned central monomer. All other monomers are omitted for clarity, but the bonds are shown as cylinders.^[48]



monomers throughout the whole of the molecule is evident. The following interesting feature can be discerned in the case of the G9 dendrimer: The need to accommodate the large number of monomers at the ninth and the eighth generation, forces the chains building the lower generations (up to $g=4$) to stretch and open up. There is strong localization of the low-

generation monomers, and the dendritic structure up to $g=4$ becomes clearly visible. This should be compared with the snapshot of a $G=4$ dendrimer (Figure 2): If the bonds were omitted, it would be hard to say that the spheres belong to a dendrimer, whereas in Figure 4 the dendritic architecture of the lowest generations is immediately recognizable and has a clear similarity with the “flattened out”, rigid chemical structure shown in Figure 1.

2.4. Simulations

2.4.1. Simulation Techniques

A great deal of progress in the understanding of the conformations of dendrimers in dilute solutions has been achieved through the application of computer simulations at

various levels of description, from the microscopic to the oversimplified ones. Here, the key quantity is the Hamiltonian function $\mathcal{H}(\{\mathbf{p}_i\}, \{\mathbf{r}_i\})$ of the system which describes all interaction potentials between the monomers and has the general form of Equation (13) which features the positions $\{\mathbf{r}_i\}$ and momenta $\{\mathbf{p}_i\}$, ($i=1,2,\dots,N$), of the N monomers each of mass m .

$$\mathcal{H}(\{\mathbf{p}_i\}, \{\mathbf{r}_i\}) = \sum_{i=1}^N \frac{\mathbf{p}_i^2}{2m} + U_{\text{pot}}(\mathbf{r}_1, \mathbf{r}_2, \dots, \mathbf{r}_N) \quad (13)$$

The first term in Equation (13) is the kinetic energy, while the potential energy function $U_{\text{pot}}(\mathbf{r}_1, \mathbf{r}_2, \dots, \mathbf{r}_N)$ includes various energy contributions between the interacting monomers: stereochemical, bonding, excluded-volume, and

dispersion, as well as (screened) Coulomb terms in the case of charged monomers. In the vast majority of cases, the solvent molecules are not explicitly treated; instead, the solvent enters the formulation as a continuous effective medium. Its effect is either to induce an effective excluded volume parameter v between the neutral monomers or to

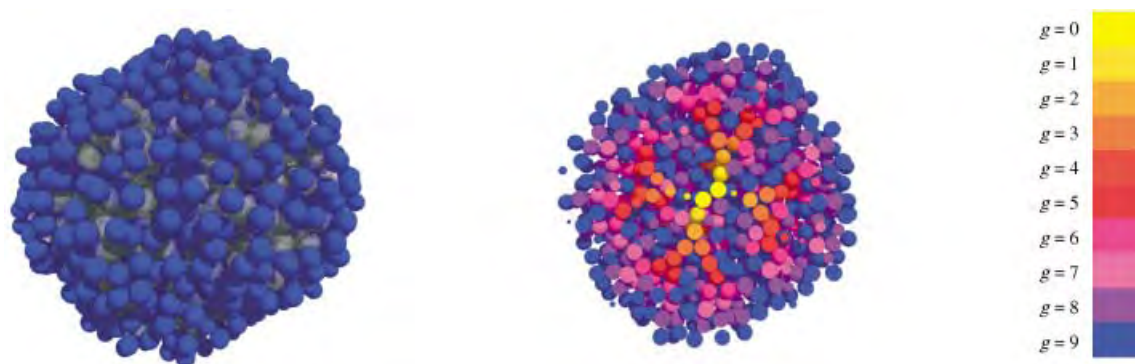


Figure 4. Simulation snapshot of a G9 dendrimer. Left: All the monomers are shown as spheres of diameter σ , with those belonging to the end generation colored blue and all others shown as gray semitransparent spheres. The outer surface is covered exclusively by monomers of $g=9$, but there are also many end monomers within the dendrimer that are somewhat obscured by the high concentration of semitransparent spheres. Right: A cross-section through the middle of the configuration on the left, which shows the coordination of the monomers belonging to various generations within the dendrimer.^[48]

screen the interaction between charged ones through its dielectric constant ϵ . Similarly, counterions or salt co-ions, if present in the solution, are usually modeled only through the introduction of a Debye inverse screening length κ which screens the bare Coulomb repulsion between the monomers, thus turning it into an exponentially decaying Yukawa potential with screening length κ^{-1} .^[49]

There are three types of simulation techniques: Monte-Carlo (MC), molecular dynamics (MD), and Brownian dynamics (BD).^[50,51] In the Monte-Carlo simulation sequential random configurations of the system under consideration are generated. The new state is produced from the old one by small random displacements of the particles within a prescribed range. Then, the energy of the old configuration E_{old} is compared with that of the new one E_{new} . If $E_{\text{new}} < E_{\text{old}}$, the new configuration is accepted automatically, but if not, it is only accepted with a probability $p_{\text{acc}} = \exp[-\beta(E_{\text{new}} - E_{\text{old}})] < 1$. The above relationship is known as the Metropolis algorithm. In this way a very large number of configurations is generated and interesting statistical quantities are calculated as averages over those. The particular criterion for rejecting a trial move guarantees that the final averages are weighted according to the Boltzmann factor $\exp(-\beta E_{\mu})$ of each configuration μ , and therefore canonical expectation values can be calculated. This way of taking averages corresponds to the statistical-mechanical notion of ensemble averaging.^[24]

In molecular dynamics, the instantaneous force $\mathbf{F}_i$ acting on monomer i is computed by Equation (14).

$$\mathbf{F}_i = -\nabla_{\mathbf{r}_i} U_{\text{pot}}(\mathbf{r}_1, \mathbf{r}_2, \dots, \mathbf{r}_N) \quad (14)$$

This Equation solves the N -coupled Newtonian equations of motion $m\ddot{\mathbf{r}}_i(t) = \mathbf{F}_i(t)$ of all monomers numerically. In this way the dynamic evolution of the system on the microscopic level is monitored and the various statistical quantities are calculated as averages along the run.

The goal in Brownian dynamics is to take into account the effects of the solvent molecules in a more realistic way, although still in a phenomenological fashion. The friction of a monomer that flows past the surrounding solvent is modeled

through the friction coefficient ζ . On the Brownian time scale the motion of the monomers is overdamped,^[52] hence it is not the acceleration $\ddot{\mathbf{r}}_i(t)$ of each monomer but rather its velocity $\dot{\mathbf{r}}_i(t)$ that is proportional to the force. The coupled equations give Equation (15).^[53]

$$\dot{\mathbf{r}}_i(t) = \sum_{j=1}^N \frac{1}{\zeta} \mathbf{H}_{ij} [-\nabla_{\mathbf{r}_i} U_{\text{pot}}(\{\mathbf{r}_j(t)\}) + \mathbf{w}_i(t)] \quad (15)$$

In this Equation, $\mathbf{H}_{ij}$ is the so-called hydrodynamic interaction tensor between monomers i and j ^[19,54] and is set equal to the identity matrix ($\mathbf{H}_{ij} = \mathbf{I}$) when hydrodynamic effects are ignored. Moreover, the total force acting on each monomer is supplemented by a Gaussian noise field $\mathbf{w}_i(t)$ that models the random collisions with the solvent molecules. The statistical properties of the noise are given through its average [Eq. (16)] and variance [Eq. (17)] with the inverse tensor $\mathbf{H}_{ij}^{-1}$ of $\mathbf{H}_{ij}$ ($\mathbf{H}_{ij} \mathbf{H}_{ij}^{-1} = \mathbf{I}$).

$$\langle \mathbf{w}_i(t) \rangle = 0 \quad (16)$$

$$\langle \mathbf{w}_i(t) \mathbf{w}_j(t') \rangle = 2k_B T \mathbf{H}_{ij}^{-1} \delta(t - t') \quad (17)$$

Thereby, N simultaneous generalized Langevin equations are solved. In a simplified treatment, hydrodynamic interactions can be at first ignored^[55] but the hydrodynamic tensor $\mathbf{H}_{ij}$ can also be included in some approximation.^[56] MD and BD differ only as to the type of dynamics that is considered, Newtonian in the former case and Brownian in the latter case. The averages calculated in MD and BD correspond to the notion of time averaging. As far as static (equilibrium) quantities are concerned, both techniques yield identical results that also coincide with those obtained from MC. Indeed, in the absence of ergodicity breaking, time- and ensemble-averaging are equivalent.^[24]

2.4.2. Simulations of Dendrimers

A considerable number of simulations have been dedicated to dendrimers in the last few years, and a summary of

these is shown in Table 2. The first simulational work on dendrimers was performed by Naylor et al.,^[31] who carried out atomic-scale MD simulations of dendrimers up to generation number $G=7$ by using atomic-level force fields. A transition from highly aspherical open structures for $G \leq 4$ to dense spherical ones for $G \geq 5$ was found, but neither the density profiles nor the radius of gyration were analyzed quantitatively. Unfortunately, the method requires very long equilibration times and thus it is not clear whether the generated conformations correspond to equilibrium structures. Lescañec and Muthukumar^[12] were the first to employ a simplified approach by modeling the dendrimers as self-avoiding walks and employing an off-lattice kinetic growth algorithm. They found the radius of gyration scaled as $R_g \sim N^{0.22} P^{0.5}$ and a monotonically decreasing bead-density profile, which is once again in contradiction with the predictions of the dense-shell model. Mansfield and Klushin^[32] performed Monte-Carlo simulations on the diamond lattice up to $G=8$ by employing a particular scheme for the generation of new conformations. They reported that the end groups of the dendrimers could be found everywhere in the molecule, thus contradicting the dense-shell picture of end groups localized at the molecule's periphery. Moreover, a significant degree of segregation of the different dendrons was found (see Section 2.4.3), which grew with increasing generation number. It

was later shown by Wallace, Buzza, and Read^[57] that the MC sampling technique of Mansfield and Klushin violates detailed balance,^[50,51] which is a necessary condition for equilibration. However, this violation turns out to have most impact on the results for ideal dendrimers, while having only a minimal effect on the findings for self-avoiding dendrimers.^[58,59]

The first numerical study that was free of doubts regarding equilibration was the BD-off-lattice simulation of Murat and Grest,^[33] which covered generation numbers $G=1-8$ and used a spacer length $P=7$. The potential energy function $U_{\text{pot}}(\{\mathbf{r}_i\})$ of Equation (13) was treated in a simplified fashion, namely, detailed interactions arising from electronic structure and torsional degrees of freedom were ignored, and only the bonding contribution $U_{\text{bond}}(r)$ and the general monomer–monomer interaction $U_{\text{mm}}(r)$ were taken into account [Eq. (18)].

$$U_{\text{pot}}(r) = U_{\text{mm}}(r) + U_{\text{bond}}(r) \quad (18)$$

Here, $r = |\mathbf{r}_i - \mathbf{r}_j|$ denotes the magnitude of the separation vector between the monomers i and j . $U_{\text{mm}}(r)$ was modeled using a Lennard–Jones potential, truncated, and shifted to the position r_c that can be freely chosen [Eq. (19)]. $\phi_{\text{LJ}}(r)$ is the standard Lennard–Jones interaction [Eq. (20)].

Table 2: A summary of the simulational approaches applied to the problem of the conformations of isolated dendrimers and of their main findings. For the meaning of the various abbreviations, see the text.

Authors	Simulation technique	Space	Level of description	Dendrimer type	Density distribution	Overall size R
Naylor et al. ^[31]	MD	continuous	atomistic	neutral	open ($G \leq 3$) dense ($G \geq 5$)	–
Lescañec and Muthukumar ^[12]	MC	continuous	simplified	neutral	dense core	$R \sim P^{0.5} N^{0.22}$
Mansfield and Klushin ^[32]	MC	discrete	simplified	neutral	dense core	not determined
Murat and Grest ^[33]	MD	continuous	simplified	neutral	dense core	$R \sim N^{1/3}$
Carl ^[16]	MC	continuous	simplified	neutral	dense core	not unique
Chen and Cui ^[64]	MC	continuous	simplified	neutral	dense core	$R \sim (PG)^{2/5} N^{1/5}$
Lue and Prausnitz ^[70]	MC	continuous	simplified	neutral	dense core	not determined
Scherrenberg et al. ^[71]	MD	continuous	atomistic	neutral	dense core	$R \sim N^{1/3}$
Cavallo and Fraternali ^[75]	MD	continuous	atomistic	neutral	dense core	$R \sim N^{0.29}$
Welch and Muthukumar ^[82]	MC	continuous	simplified	charged	dense core (low Q) dense shell (high Q)	See Ref. [82] ^[a]
Mansfield ^[76]	MC	discrete	simplified	neutral	dense core	not determined
Lue ^[87]	MC	continuous	simplified	neutral	dense core	not determined
Lyulin et al. ^[72]	BD ^[b]	continuous	simplified	neutral	dense core	not determined
Wallace et al. ^[57] and Giupponi and Buzza ^[58]	MC	discrete	simplified	neutral	dense core	$R \sim N^{1/3}$
Karatasos et al. ^[77]	MD ^[c]	continuous	simplified	neutral	dense core	$R \sim N^{1/3}$
Lee et al. ^[84]	MD ^[c]	continuous	atomistic	charged	dense core (low Q) dense shell (high Q)	not determined
Zacharopoulos and Economou ^[63]	MD ^[d]	continuous	atomistic	neutral	dense core	$R \sim N^{0.29}$
Mallamace et al. ^[68]	MD	continuous	atomistic	neutral	dense core	$R \sim N^{0.4}$ (low G) $R \sim N^{0.21}$ (high G) $R \sim (PG)^{2/5} N^{1/5}$
Sheng et al. ^[65]	MC	continuous	simplified	neutral	dense core	$R \sim P^{1/4} N^{0.338}$
Mansfield and Jeong ^[59]	MC	discrete	simplified	dense core	not determined	–
Timoshenko et al. ^[46]	MC	continuous	simplified	neutral	dense core	–
Harreis et al. ^[67]	MC	continuous	simplified	neutral	dense core	$R \sim N^{1/3}$ (low G)
Götze and Likos ^[48]	MC	continuous	simplified	neutral	dense core	$R \sim N^{0.24}$ (high G)

[a] For the case of charged dendrimers, the radius of gyration also depends on the amount of added salt and the Bjerrum length [see, for example, Eq. (28)]. [b] Including the simulation of dendrimers under shear. [c] With explicit simulation of the solvent molecules. [d] Including simulations in the melt.

$$U_{\text{mm}}(r) = \begin{cases} \phi_{\text{LJ}}(r) - \phi_{\text{LJ}}(r_c) & \text{for } r \leq r_c \\ 0 & \text{for } r > r_c \end{cases} \quad (19)$$

$$\phi_{\text{LJ}}(r) = 4\epsilon \left[\left(\frac{\sigma}{r} \right)^{12} - \left(\frac{\sigma}{r} \right)^6 \right] \quad (20)$$

The cutoff distance r_c allows for the modeling of solvents of varying quality. Selecting $r_c = 2^{1/6}\sigma$ renders the monomer–monomer interaction purely repulsive and thus suitable for an effective description of athermal solvents. Increasing r_c adds an attractive tail to the interaction. In this way, the value of the second virial coefficient B_2 of the interaction can be tuned and the θ value ($B_2 = 0$) and poor ($B_2 < 0$) solvents can be incorporated into the model. The connectivity was modeled by the so-called finite extensible nonlinear elastic (FENE) potential, first introduced by Grest, Kremer, and Witten for linear chains.^[60] The FENE potential acts only between a monomer and its bonded neighbors [Eq. (21), $R_0 = 1.5\sigma$ and $T = 1.2\epsilon/k_B$].

$$U_{\text{FENE}}(r) = \begin{cases} -15\epsilon \left(\frac{R_0}{\sigma} \right)^2 \ln \left[1 - \left(\frac{r}{R_0} \right)^2 \right] & \text{for } r \leq R_0 \\ \infty & \text{for } r > R_0 \end{cases} \quad (21)$$

The total potential for steric interactions and bonding between successive monomers is shown in Figure 5. As can be seen, the interaction becomes very high both for very close

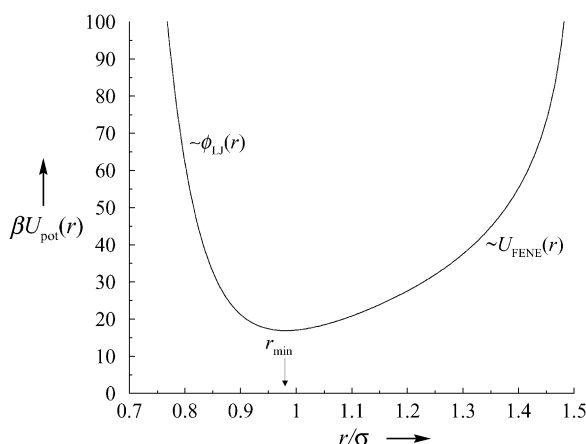


Figure 5. The total interaction potential $U_{\text{mm}}(r) + U_{\text{FENE}}(r)$ acting between two adjacent beads of the dendrimer [Eqs. (20) and (21)].

approaches (steric repulsion) and for distances nearing the length scale R_0 (stretching of the bonds of the FENE potential). This observation implies that the bonded monomers experience very strong restoring forces (given by the derivative of the interaction) every time they attempt to either come too close to one another or to fly apart. Most of the time they find themselves separated by a distance of $r_{\text{min}} \cong 0.97\sigma$, which corresponds to the minimum value of $U_{\text{pot}}(r)$ where the force vanishes.

The main findings in Ref. [33] can be summarized as follows: the monomer profiles of the dendrimers show a

region of very high density at the core, a local minimum at distances $r \cong 4\sigma$, followed by a broad plateau of almost constant density. The monomer profile eventually decays smoothly to zero at the periphery of the dendrimer. The width of this last zone was found to be almost independent of the generation number. The dendrimers become more compact as the solvent quality worsens, but the fractal dimension of all the dendrimers was calculated, within simulation errors, to be $d_f = 3$. Hence, dendrimers appear to be compact objects whose overall size scales as $R_g \sim N^{1/3}$. This is in direct contradiction with the dense-shell model predictions, but at the same time establishes dendrimers as macromolecular colloidal entities that are distinct from star polymers, whose density profile instead decays as $\rho(r) \sim r^{-4/3}$.^[61] End-group monomers were found to be almost uniformly distributed throughout the volume of the dendrimer.

2.4.3. Dendron Segregation

An additional quantity of interest that was investigated by Murat and Grest^[33] is the so-called segregation of dendrons in the molecule, which was originally introduced by Mansfield.^[62] To quantify the segregation of the dendrons, the space around the dendrimer's center is divided into cubic cells and $\Psi_b(i,j,k)$ is defined to be the number of monomers belonging to dendron b ($b = 1, 2, \dots, f$) within the cell (i,j,k) . With $\langle \Psi_b(i,j,k) \rangle$ being the average of this quantity measured during the simulation, the total overlap O_t is then defined as Equation (22).

$$O_t = \frac{\sum_{i,j,k} (\langle \Psi_1 \rangle \langle \Psi_2 \rangle + \langle \Psi_2 \rangle \langle \Psi_3 \rangle + \langle \Psi_3 \rangle \langle \Psi_1 \rangle)}{\sum_{i,j,k} (\langle \Psi_1 \rangle^2 + \langle \Psi_2 \rangle^2 + \langle \Psi_3 \rangle^2)} \quad (22)$$

In this definition O_t plays the role of an overlap order parameter; $O_t = 1$ means complete mixing of the dendrons, whereas $O_t = 0$ means complete segregation occurs. An increased segregation of the dendrons with increasing generation number was found in Ref. [33]. This finding is at odds with Mansfield's conjecture^[62] that the dendrons should intermix at high generations. On the other hand, Zacharopoulos and Economou^[63] defined the overlap parameter somewhat differently [Eq. (23)] in their own simulation study.

$$O_t = \frac{\sum_{i,j,k} (\langle \Psi_1 \Psi_2 \rangle + \langle \Psi_2 \Psi_3 \rangle + \langle \Psi_3 \Psi_1 \rangle)}{\sum_{i,j,k} (\langle \Psi_1^2 \rangle + \langle \Psi_2^2 \rangle + \langle \Psi_3^2 \rangle)} \quad (23)$$

In contrast to Equation (22), in which $\langle \Psi_\alpha \Psi_\beta \rangle \rightarrow \langle \Psi_\alpha \rangle \langle \Psi_\beta \rangle$, the definition of Zacharopoulos and Economou seems to make more sense, since one is interested in the *simultaneous* occupancy of a cell by monomers belonging to two different dendrons and the occupancy events are correlated; thus one should perform the average *after* carrying out the multiplication, not the other way around. As a result of this, it was found in Ref. [63] that the overlap increases (that is, the segregation decreases) with increasing generation number. This result is opposite that found by Murat and Grest,^[33] but in agreement with the conjecture of

Mansfield.^[62] Although the discrepancy is technically based on the two different definitions of the overlap parameters [Eqs. (22) and (23)], the results from Ref. [63] are more intuitive physically: indeed, as the generation number increases and the object becomes more compact, the different dendrons have to mix more to make use of the limited available space.

2.4.4. Alternative Simplified Approaches

Within the framework of simplified approaches, other options for the monomer–monomer and bonding interactions have also been made. It is of paramount importance to compare the results from these different models with one another to judge whether the results are insensitive to the details of the interaction potentials and hence to give an answer to the question of the universality of dendrimer properties.

Carl^[16] introduced a freely jointed bead–rod model (bead diameter σ , bond length d), in which the ratio σ/d is used as an additional parameter. Clearly, $\sigma/d=0$ corresponds to ideal dendrimers and in Ref. [16] bead diameters up to $\sigma=0.5d$ were examined. Not surprisingly, a swelling of the dendrimer with increasing σ value, was found and the density profiles were found to decay monotonically with r (dense-core configurations). The power-law dependence $R_g \sim N^{1/3}$ of Murat and Grest was not confirmed in this simulation study, since rather short spacers ($P=4$) were used and it was conjectured that the scaling limit had not been reached. Finally, the free energy of insertion $A(r)$ of a hard test particle of diameter $\tau=0.1d$ at a distance r from the dendrimer's center was measured in the simulation as a means of probing the existence of voids within the macromolecule. Indeed, if the dendrimer was densely packed, $A(r)$ would be expected to be a monotonically decaying function of r . On the other hand, the existence of voids able to accommodate the test particle would lead to oscillatory behavior of $A(r)$ for r values smaller than the dendrimer's size. A practically monotonic function was found for $G=2$, whereas weak oscillations develop for $G=3$ and 4. The result is quite interesting since it represents the first (and only, to the best of our knowledge) calculation of the effective interaction between a dendrimer and a much smaller colloidal hard sphere (see also Section 4). This is an issue that deserves more attention in the future because of the possibility of using dendrimers as selective agents for the absorption of molecules of narrowly distributed sizes.

The bead–rod model was also employed in the Monte-Carlo simulations of Chen and Cui.^[64] Though their simulation confirmed once again the dense-core model, the scaling law is at odds with the $R_g \sim N^{1/3}$ prediction of Boris and Rubinstein.^[27] A scaling of the form $R_g \sim (PG)^{2/5} N^{1/5}$ was conjectured instead. The discrepancy was resolved in the recent work of Sheng, Jiang, and Tsao,^[65] who employed a bead–thread model in which the bonding term in the potential energy is expressed by Equation (24), where $\Delta=0.4$.

$$U_{\text{bond}}(r) = \begin{cases} \infty & \text{for } r < \sigma \\ 0 & \text{for } \sigma < r < \sigma(1 + \Delta) \\ \infty & \text{for } \sigma(1 + \Delta) < r \end{cases} \quad (24)$$

They found that a scaling $R_g \sim N^{1/3}$ is obtained when R_g is considered as a function of N for fixed spacer length P (the monomer number N changing through a change of the final generation G). A different scaling $R_g \sim N^{3/5}$, identical to the known Flory law of linear polymers, is obtained if the generation number G is fixed and N varied through a change in the spacer length. However, if one wants to describe all the dependencies of R_g in a single scaling law, then the correct scaling is indeed $R_g \sim (PG)^{2/5} N^{1/5}$,^[65] as proposed by Chen and Cui.^[64] The contradiction between the various scaling laws is only partial, as can be understood by considering the fact that N , P , and G are, for a given functionality f , not independent quantities but rather connected through Equation (1). Indeed, at the limit of large values of P , where $N \propto P$, and thus for a fixed terminal generation G , the scaling law $R_g \sim (PG)^{2/5} N^{1/5}$ becomes $R_g \sim N^{3/5}$. Thus, Sheng, Jiang, and Tsao concluded that the $R_g \sim N^{1/3}$ law is apparently only a misleading fit of the simulation results.^[65] Only in poor solvent conditions does this law hold, since in this case the beads stick to each other and the dendrimer becomes compact.

Götze and Likos^[48] also applied the bead–thread model by using a fixed spacer length $P=1$ but varying the Δ value to model dendrimers with different degrees of internal flexibility. Simulation snapshots for dendrimers of generations $G=4$, 6, and 9 (Figures 2, 3, and 4) show that dendrimers are not hollow and that the sphericity and compactness of the molecule increases with generation number. Representative results for the density profiles are illustrated in Figure 6 (left picture) show that the density profile for $G=4$ is quite insensitive to the microscopic model employed in the simulation and the end groups are delocalized. The insensitivity on the specific model persists for $G=5$ (right). The monomer density develops a broad, almost constant plateau and the end monomers spread themselves more and more uniformly in the interior of the molecule.

The form factors are shown in Figures 7 and 8. As can be seen in Figure 7, the model reproduces very accurately the experimental results^[48] by using a single variational parameter, namely the hard-sphere diameter σ that was chosen to match the simulation radius of gyration with the value determined by experiment. With an appropriate rescaling of σ , all the form factor curves collapse onto one another. In addition, they are even identical with the form factor obtained in the MC simulation of Harreis, Likos, and Ballauff,^[67] in which the simplified model of Boris and Rubinstein^[27] was used. Thus, there is a broad, *quantitative* universality of the simplified dendrimer models: they all yield form factors that agree very accurately with the experimental results (within error limits), independent of their microscopic details. Figure 8, on the other hand, shows the development of the characteristic oscillations of the form factor for high q values as the generation number grows. This finding is in agreement with experimental results on PAMAM dendrimers^[68,69] and with the Gaussian self-consistent theory of Timoshenko, Kuznetsov, and Connolly^[46] mentioned in Section 2.3.

A number of additional simulations have confirmed the picture of dense-core dendrimers and corroborated the

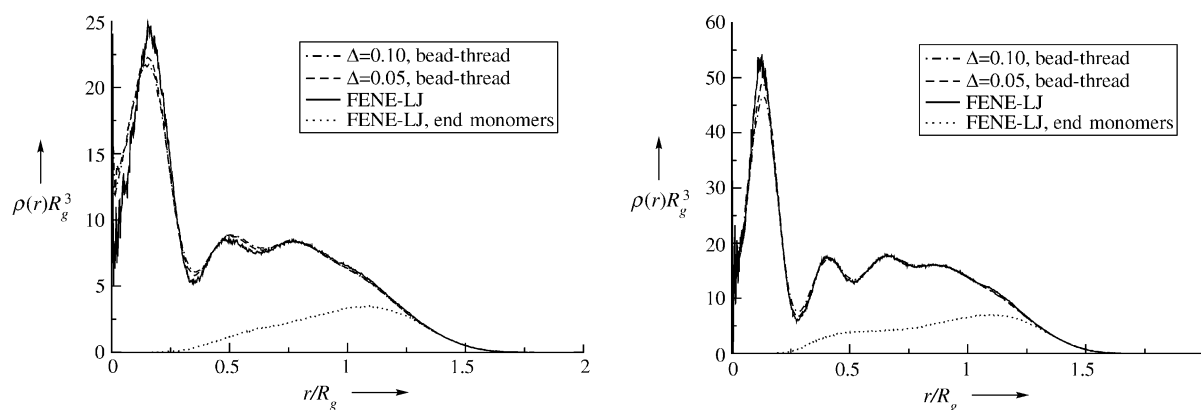


Figure 6. Left: Simulated density profiles of dendrimers with $G=4$ and $P=1$ as a function of the distance r from the molecule's center of mass. The solid line is the result from the FENE–Lennard–Jones model employed in Ref. [67]. The two broken lines are the result from the bead–thread model [Eq. (24), Ref. [48]] for two different values of the maximum thread length Δ . The minimum in the profiles at $r=0$ arises from the particular architecture of the simulated molecules, which grow from a central *bond* and not from a central *monomer* (see Figures 2–4). The dotted line is the density of the end groups. Notice the universality of the results when densities and distances are scaled with the radius of gyration R_g of the dendrimer. Right: Density profile for $G=5$ dendrimers. Notice the broadening of the density plateau and of the end-monomer profile compared with the $G=4$ case.^[48]

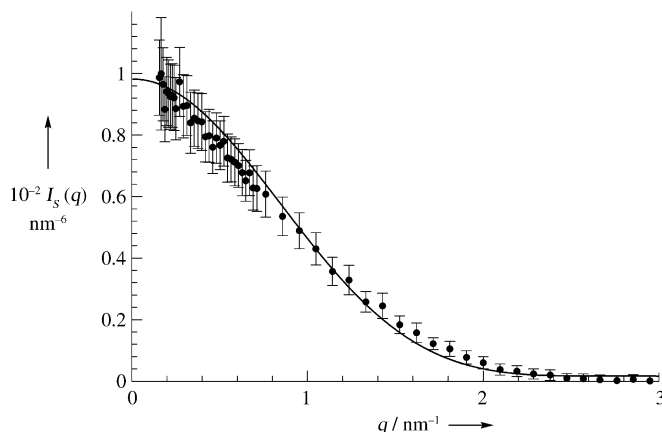


Figure 7. Comparison of the form factor of dendrimers with $G=4$, $P=1$ obtained from simulation of the bead–thread model (lines) and from SANS experiments (circles).^[48]

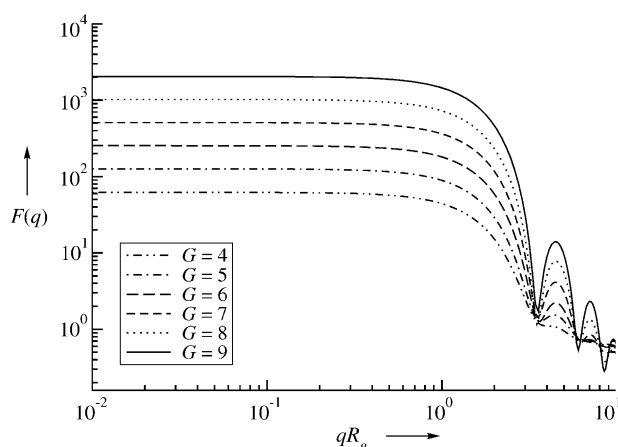


Figure 8. Form factors of dendrimers of various generations with $P=1$ as obtained through the Monte-Carlo simulation of the bead–thread model.^[48] Notice the development of oscillations and local minima with growing generation number, which is reminiscent of the form factor of colloidal hard spheres.^[48]

principle of the universality of the dendrimers' properties and conformations. Lue and Prausnitz modeled the connected monomers as tangential hard spheres,^[70] which corresponds to the model used in Equation (24) for the special case $\Delta=0$. This was also the first publication in which the *effective interaction* (potential of mean force) was calculated; this will be discussed in more detail in Section 4. Scherrenberg et al.^[71] carried out a combined experimental–simulational analysis. Two different force fields were used, which yielded results for the density profiles that differ slightly in their quantitative details but otherwise are like the dense-core model. In particular, it was found that the radius scales as $R_g \sim N^{1/3}$ and the conclusion was reached that the dendrimers are compact objects with a substantial degree of back-folding of the end groups, a constant density plateau, and a monotonic decrease in the monomer concentration toward the exterior of the molecule. This led Scherrenberg et al. to the conclusion that dendrimers have neither a dense core nor a dense shell. We prefer to adopt the view that a flat profile with a monotonic decrease at the molecule's periphery is much closer to a dense-core configuration than the dense-shell model, which predicts a monotonic increase in the density with distance r from the center.

Lyulin, Davies, and Adolf^[72] used a freely rotating bead–rod model in which the beads interacted by means of a Lennard–Jones potential, and performed BD simulations of dendrimers with $P=1$ and $P=2$ under shear flow. The density profiles show a general dense-core shape, with a shallow local minimum close to the center, then a broad flat region, and a smooth decay to zero at the periphery for a flow rate equal to zero. We emphasize that the shallow local minimum close to the origin arises from the correlation effects between the few monomers of the first generation; it is not a feature of a dense-shell model. This correlation minimum gradually disappears with increasing spacer length.^[72] The same authors found strong back-folding of the terminal groups. In a subsequent publication^[73] it was demonstrated that the end-group radius of gyration R_g^{end} can

even exceed the gyration radius of the whole molecule without this implying localization of the end groups at the periphery. This is an important point because it shows that experimental findings^[74] in which $R_g^{\text{end}} > R_g$ cannot be used as conclusive evidence for claiming localization of the end groups in the outermost shell. As a matter of fact, very recent state-of-the-art SANS experiments using labeled end groups in a fourth-generation dendrimer and including contrast variation have unambiguously shown that the end groups are distributed throughout the molecule.^[38]

Cavallo and Fraternali obtained very similar density profiles by performing atomic-level MD simulations.^[75] They found the end monomers distributed throughout the dendrimer and a monotonically decreasing overlap parameter O_i [Eq. (22)], in agreement with the simplified model used by Boris and Rubinstein.^[27] Thus, not only are all the simplified models in agreement with one another but, more importantly, they reproduce the results of atomistic simulations as well. Mansfield reached similar conclusions and also found form factors that developed oscillations for high generation numbers in his MC simulations on the diamond lattice,^[76] thus the salient characteristics of dendrimers do not even depend on whether they are modeled on a lattice or in continuous space.

The above findings remained unaffected by the modification of Mansfield's sampling procedure,^[58] which was introduced with the goal of remedying the violation of the detailed balance condition.^[57,58] It turns out that this violation mainly affects the ideal dendrimers and not the self-avoiding ones.^[58,59] In addition, Giupponi and Buzza^[58] found that the scaling of R_g with N is quite subtle. At a first look, the data seem to satisfy the $R_g \sim N^{1/3}$ law, but some deviations from this scaling become evident on plotting R_g^3/N against N . Whereas the ratio R_g^3/N should be a constant for all values of N if the $R_g \sim N^{1/3}$ law applied, it becomes clear that the scaling is violated from small ($N \lesssim 100$) and large ($N \gtrsim 1000$) values of the degree of polymerization. In particular, the exponent seems to be somewhat smaller than the "compact sphere" value $1/3$ for large values of N . Dense-core profiles were also obtained in the simulation study of Timoshenko, Kuznetsov, and Connolly^[46] which accompanied their theoretical work. A broad distribution of the end monomers and a shrinking of the molecule upon worsening of the solvent quality were shown to occur.

2.4.5. Atomistic Models

Up to this point, neither the torsional and bending degrees of freedom in the molecule nor the solvent molecules were considered explicitly. Limitations of the first kind were removed in the work of Zacharopoulos and Economou,^[63] and the second one in the work of Karatasos, Adolf, and Davies.^[77]

Zacharopoulos and Economou carried out MD simulations on $G = 2$ – 5 dendrimers employing a more sophisticated potential energy function that is based on atomistic force fields [Eq. (25)].

$$U_{\text{pot}} = \frac{1}{2} \sum_i k_b (b_i - b_i^0)^2 + \frac{1}{2} \sum_i k_\theta (\cos \theta_i - \cos \theta_i^0)^2 + \frac{1}{2} \sum_i k_\phi \{1 - \cos [n_i (\phi_i - \phi_i^0)]\} + \frac{1}{2} \sum_i \sum_{j \neq i} \epsilon \left[\left(\frac{\sigma}{r} \right)^{12} - \left(\frac{\sigma}{r} \right)^6 \right] \quad (25)$$

The first sum in Equation (25) runs over all the bonds, the second over all the bond angles, the third over all the torsional angles, and the last one over all the distinct pairs of monomers (bonded and nonbonded ones). As the first term shows, the bonding is modeled here through *harmonic* springs (compare with the anharmonic FENE potential employed in Refs. [33,67]). The second term accounts for bond bending and the third for dihedral angle rotation. The equilibrium values b_0 , θ_0 , ϕ_0 , the integer n_i , as well as the constants k_b , k_θ , k_ϕ , ϵ , and σ are parameters read off from fits of experimental and force-field data. This is a much more detailed model in comparison to the simplified ones, yet it reproduces all the results of the previous simulational works quantitatively, in particular the spreading of the end groups and the dense-core shape of the density profile. The radius of gyration was found to obey the approximate scaling $R_g \sim N^{1/3}$, although this power-law dependence could be misleading, as was shown in Ref. [65]

Karatasos, Adolf, and Davies performed MD simulations of $G = 3$ – 6 dendrimers in dilute solutions, in which they *explicitly* took account of the solvent molecules.^[77] The model employed by these authors falls into the general category of simplified approaches, in the sense that stereochemical details of the molecules were not taken into account. A freely jointed bead-spring model was employed instead, with harmonic springs connecting Lennard-Jones beads. The potential energy U_{pot} for the monomers is given here by the sum of the first and last terms of Equation (25). The same Lennard-Jones potential was employed to model monomer-solvent and solvent-solvent interactions, thus simulating a total number of molecules ranging from approximately 2000 (for $G = 3$) to 16000 (for $G = 6$). It was found that taking the solvent molecules explicitly into account does not modify the results obtained through an effective, solvent-mediated monomer-monomer interaction. The dense-core configuration, the $R_g \sim N^{1/3}$ -scaling, and the back-folding of the end groups were again confirmed in this approach. The finding that higher generation numbers result in the dendrimers developing sharper boundaries, which are of the order of several bond lengths and, thus, still broad, is interesting. Thus, the form factor of $G = 6$ dendrimers bears only a semi-quantitative resemblance with that of a hard colloidal sphere possessing the same radius of gyration.

2.4.6. Internal Density Fluctuations

A different question was examined in the Monte-Carlo simulation study of Harreis, Likos, and Ballauff.^[67] Here, the simplified model of Murat and Grest^[33] was applied to dendrimers of $G = 4$ and $P = 1$ with the aim of investigating the relevance of the fluctuation correlations within the

dendritic molecule. To quantify this concept, let us consider the local deviation $\Delta\rho(\mathbf{r})$ of the instantaneous monomer density from its equilibrium value $\rho(\mathbf{r})$ at the given point $\mathbf{r}$ in space; such thermal fluctuations occur continuously in a real system. The question is now whether two local deviations at the points $\mathbf{r}_1$ and $\mathbf{r}_2$ are correlated to one another, that is, whether the knowledge that a fluctuation of a certain amplitude at point $\mathbf{r}_1$ allows us to make the prediction that a similar fluctuation will be induced by the first one at point $\mathbf{r}_2$, or vice versa. Mathematically, this is described by the connected density–density autocorrelation function^[67] $C(r) = \langle \Delta\rho(\mathbf{r}_1)\Delta\rho(\mathbf{r}_2) \rangle$, where $r = |\mathbf{r}_1 - \mathbf{r}_2|$. If the fluctuations are completely uncorrelated at all length scales, then $C(r) = 0$. In general, though, $C(r)$ will attain the asymptotic value 0 when r exceeds the so-called *correlation length* ξ . If ξ is much smaller than the size of the molecule R_g , then one can treat the dendrimer as an approximate compact colloidal object and relate the coherent scattering intensity of the molecule to the square of the Fourier transform of the one-particle density $\rho(r)$.^[67,78] This is, for example, not the case for linear polymer chains. It turns out, though, that the correlation length for $G = 4$ dendrimers is of the order of the Kuhn segment of the molecule, thus the effects of the so-called blob scattering arising from the correlated fluctuations show up in Fourier space for values of the scattering wave vector $qR_g \approx 3.5$. This value is in excellent agreement with experimental results^[69] and translates into absolute wave-vector values $q \approx 2.5 \text{ nm}^{-1}$ for $G = 4$ dendrimers. For many practical purposes, therefore, dendrimers can be viewed as compact colloids. Inversion of the measured form factor back into real space yields a Gaussian density profile for dendrimers of the fourth generation and provides direct experimental evidence for the dense-core conformation of these macromolecules.^[66]

2.4.7. Charged Dendrimers

Up to now, the discussion has been limited to neutral dendrimers. An additional possibility for influencing the shapes of these macromolecules is by the introduction of charge. This can be achieved through variation of the pH value of the aqueous solution: at low pH values the monomer units of the dendrimer shown in Figure 1 are protonated and thus charged while at high pH values they are neutral. Moreover, the addition of salt influences the screening of the Coulomb interaction between the charged segments. Increasing the salt concentration leads to a corresponding increase in the Debye screening parameter κ [see Eq. (27)]. Therefore, in a heavily salted solution (high ionic strength), the Coulomb interactions between the charged units are so strongly screened that the dendrimers are expected to behave in the same way as neutral ones.

It is well-known, for example, from the cases of linear polyelectrolytes^[79] and polyelectrolyte stars^[80] that the addition of charge has the effect of *stretching* the macromolecule and inducing a transition from the coiled to the rodlike configuration. In comparison to neutral star polymers, polyelectrolyte stars have a considerably larger radius of gyration that scales *linearly* with the number of monomers per chain.^[81] The possibility of inducing conformational changes of a kind

similar to dendrimers is very exciting, because of its many potential applications. One can envision preparing dendrimers in aqueous solutions with low pH values and low salt concentrations. In such a system the molecules would assume a stretched, open configuration akin to the dense-shell model as a consequence of the strong Coulomb repulsions between the charged units. Smaller molecules (such as drugs) could then be introduced into the hollow host. Injection into a highly salted environment, such as human blood, would result in the Coulomb repulsions becoming screened, thus forcing the dendrimer to collapse back into the neutral, dense-core conformation and thereby to release the guest molecule into the living organism. It is precisely this scenario that makes dendrimers very exciting from a biophysical point of view, and this problem has recently attracted increased interest.

Welch and Muthukumar^[82] performed simplified Monte-Carlo simulations of dendritic polyelectrolytes in which every branching and end group carried a unit charge. The solvent, the counterions, as well as the salt counter- and co-ions were all treated effectively, not explicitly. In particular, the potential-energy function U_{pot} is expressed by Equation (26).

$$\beta U_{\text{pot}} = -K R^2 \sum_i \ln \left[1 - \left(\frac{l_i - l_0}{R} \right)^2 \right] + \beta \zeta \sum_{i=1}^N \sum_{i < j}^N \left\{ \left[e^{-\alpha(r_{ij} - d)} - 1 \right]^2 - 1 \right\} + \lambda_B \sum_{i=1}^{N_c} \sum_{i < j}^{N_c} \frac{e^{-\kappa r_{ij}}}{r_{ij}} \quad (26)$$

The first term in Equation (26) is a modification of the FENE potential [see Eq. (21)] and models the bonding; the sum is carried over all fluctuating bonds of length l_i and equilibrium length l_0 , whereas K is the spring constant and R an appropriately chosen length scale. The second term models the excluded-volume interactions between all N monomers, which are modeled by means of the so-called Morse potential with appropriately chosen parameters ζ , α , and d . Figure 9 shows a plot of the Morse potential for typical values of the parameters. The electrostatic interactions between the N_c charged monomers are taken into account in the framework of the linearized Poisson–Boltzmann (Debye–Hückel) theory, which predicts screening by the micro-ions and the formation of a Yukawa-type interaction. In the third term, $\lambda_B = e^2/(\epsilon k_B T)$ is the Bjerrum length, e the elementary charge, and ϵ the dielectric constant of the solvent, such that $\lambda_B = 7.1 \text{ Å}$ for water at room temperature. The inverse Debye screening length κ is given by Equation (27). The sum is carried over all types of micro-ions with concentrations c_i and valences z_i .

$$\kappa = \left[4\pi\lambda_B \sum_i c_i z_i^2 \right]^{1/2} \quad (27)$$

The simulations of Welch and Muthukumar^[82] revealed a spectacular change in the dendrimer conformation from a compact, dense-core conformation at high salt concentrations to a hollow, dense-shell conformation for low ones. This phenomenon was accompanied by a transition of the end groups that resulted in their localization at low salt concentrations. The sequence of conformational changes is *reversible*, hence this was termed a “smart” behavior of the

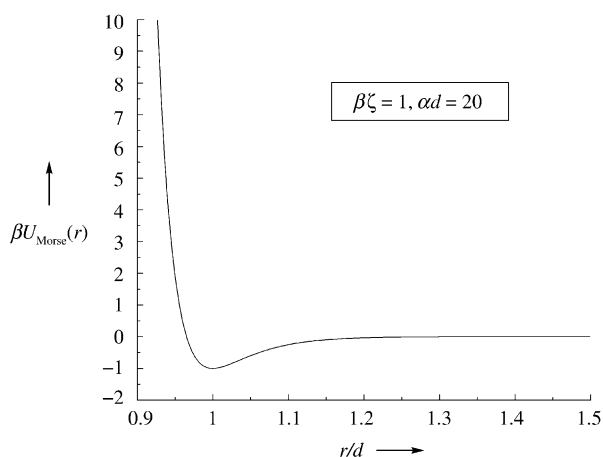


Figure 9. Plot of the Morse potential [second term in Eq. (26)] that models the van der Waals interactions between uncharged monomers in the study by Welch and Muthukumar.^[82] The range of the attractive part can be tuned by changing the parameter αd , thereby enabling the modeling of different solvent qualities.

dendrimers. A dramatic change in the radius of gyration was predicted to take place upon changing the parameter κ^{-1} from 3 to 300 Å. The overall gyration radius R_g was found to double, and R_g^{end} for the end groups to increase by a factor of 2.6. A simple law, based on scaling arguments, was given for R_g [Eq. (28)], where B_2 is the second virial coefficient of the short-range steric interaction potential.^[82]

$$\frac{R_g}{N^{1/4}} \propto \left[\left(B_2 + \frac{4\pi\lambda_B}{\kappa^2} \right) N^{5/4} \right]^{1/5} \quad (28)$$

Plots of $R_g/N^{1/4}$ against $(B_2 + 4\pi\lambda_B\kappa^{-2})N^{5/4}$ show a partial collapse of data from different dendrimers on one another, yet the $1/5$ power law dependence of the first quantity on the second one does not show up. In later work, the same authors used simulations and an analytical theory to study the complexation of charged dendrimers with polyelectrolytes.^[83]

Recently, Lee et al.^[84] carried out molecular dynamics simulations of dendrimers of generation $G=2$ at several pH values, and explicitly including water molecules. It was argued that simulations without explicit water may lead to uncertain results; however, since the explicit simulation of water is very expensive computationally and exceeds the capacities of current software and hardware, a different technique was adopted. Small $G=2$ dendrimers were simulated with and without explicit water; in the latter procedure phenomenological parameters, such as a distance-dependent dielectric constant $\epsilon(r)$, was included. Indeed, both the assumption of a continuous solvent, inherent in a single dielectric screening with constant ϵ , and the linear-screening Debye–Hückel theory for the counterions are bound to break down in the short distances between the charged units involved in dendrimers. By comparing the results with and without water for the small dendrimers, an optimal dielectric constant $\epsilon(r)$ was fitted, so as to maximize the agreement between the two approaches. This parameter was then used in simulations of larger dendrimers (up to $G=6$). A conformational change in the dendrimer from a compact object at high

pH values to an open one at low pH values was also reported. Nevertheless, there are some points in the simulation approach employed that cast doubt on its validity. Firstly, it is not mentioned whether the counterions, which screen the Coulomb interaction, are also simulated or not. Secondly, the simulation without water was performed with a dielectric constant $\epsilon=1$, which is not, in our opinion, the right way to compare the effective and the explicit approaches for the solvent. An effective description of the solvent does not mean that the latter must be replaced by vacuum. Thirdly, the distance-dependent dielectric constant $\epsilon(r)$ employed in this work was chosen simply as $\epsilon(r)=r$. This is highly unusual since the Coulomb potential becomes a r^{-2} power law for all distances. However, for large separations between the particles, the intervening medium should indeed behave as a continuum and the r^{-1} character of the Coulomb interaction should remain unaffected. Finally, the results after equilibration with explicit inclusion of water at high pH values are very different, and depend on the original conformation. This observation casts serious doubt as to whether the conformations were indeed equilibrated. To a lesser extent, this reservation also holds for the results at a neutral pH value.

The possible conformational change of the dendrimers upon charging and the possibility of controlling it externally through addition of salt are most certainly very promising areas of research. In their experimental study of $G=8$ dendrimers Nisato, Ivkov, and Amis^[85] observed *no change* of R_g whatsoever upon broad variations of the pH value ($4.7 < \text{pH} < 10.1$) and of the ionic strength (up to 3 M of NaCl). Whether this insensitivity is a result of the high generation numbers of the dendrimers employed, which indeed may leave no room for expansion because of very high steric crowding, or whether the phenomenon of charge-induced dendrimer swelling is absent altogether is a very interesting problem for future investigations.

3. Experimental Determination of the Form Factor of Isolated Dendrimers

In Section 2 we showed that the segment density of a dendrimer can be related to its form factor $F(q)$ in a straightforward manner. In this section the determination of $F(q)$ and $S(q)$ by small-angle neutron scattering (SANS) and small-angle X-ray scattering (SAXS) will be discussed. This discussion may appear superfluous at first since SANS and SAXS are well-established techniques in polymer and colloid physics, but there are a number of problems that do not exist for most polymeric or colloidal systems but exist for dendrimers.^[7]

1. SANS and SAXS probe the correlation of atomic or molecular scattering units on a length scale of $2\pi/q$. The resolution of these methods is therefore restricted by the maximum q value q_{max} that can be accessed experimentally. As shown above, the radius of gyration R_g can be directly taken as a measure of the spatial extension of the dendrimers. Reliable structural information necessary for a meaningful comparison of theory and experiment, however, can only be obtained if qR_g is considerably

larger than unity. In this case $2\pi/q$ is much smaller than R_g and the spatial resolution may be considered sufficient. In this way the present discussion parallels those well-established in optical or electron microscopy. The typical molecular weights of dendrimers are much smaller than the molecular weights of polymers and their spatial extensions are only of a few nanometers. Since the forward scattering scales linearly with the molecular weight, dissolved dendrimers lead to much smaller scattering intensities than, for example, dissolved polymers or suspended colloidal particles. This leads to poor statistics and a concomitantly diminished accuracy when collecting data at the higher scattering angles necessary for sufficient resolution.

2. An additional difficulty related to the foregoing problem is the incoherent part I_{incoh} of the scattering intensity which arises from the numerous hydrogen atoms in the chemical structure of typical dendrimers. If n is the number of scattering units, the coherent forward scattering, namely, the form factor $F(q)$ scales with n^2 , while the incoherent part scales directly with n . In the case of large objects, such as high-molecular polymers or colloidal particles, I_{incoh} is therefore a small if not negligible contribution to the measured scattering signal. For small objects, such as dendrimers, however, the coherent part is not a magnitude of order larger than I_{incoh} . This problem becomes very important at high scattering angles, where the coherent part $F(q)$ has very much diminished and may even become smaller than I_{incoh} . The incoherent part must be subtracted with utmost care.
3. Theory and simulations model dendrimers as spatial arrangements of scattering units that possess equal scattering power. Real dendrimers, however, are composed of chemically different scattering units that exhibit different scattering lengths. This leads to an inhomogeneity that is followed by an additional scattering contribution seen in SANS and SAXS but which is not included in the theoretical treatment. A meaningful analysis of SAXS and SANS data must at least assess the magnitude of this term, otherwise erroneous conclusions could be obtained.

This list shows that measurements done by small-angle scattering may be affected by several systematic errors that need special attention. This issues a clear warning against straightforward application of recipes that have been proved to be sufficient in the case of polymers or colloids.

The combination of SANS and the method of contrast variation can be used to solve the problems enumerated above. The method of contrast variation as applied to dendrimers has been repeatedly reviewed,^[6,7] and it hence suffices to give here only the main points of this analysis. The scattering of neutrons by the segments of the dendrimer may be coherent as well as incoherent and the magnitude of the both parts is given by the respective scattering lengths of the dendritic units. Only the coherent part, which contains all structural information, depends on the magnitude of the scattering vector q and can be expressed as Equation (29), where n is the number density of the dissolved dendrimers in

the scattering volume and $S(q)$ is the structure factor describing the mutual interaction of the solute molecules. The incoherent part I_{incoh} of the measured intensity does not depend on q . Hence, if measurements are extended to the highest scattering angles possible, the incoherent part I_{incoh} can directly be determined and subtracted from the experimental data. Technical details of this procedure can be found in Refs. [6, 25, 104]

$$I_{\text{coh}}(q) = nF(q)S(q) \quad (29)$$

In Section 4 we show that $S(q)$ is a function of concentration. Only at vanishing concentration is $S(q) = 1$ for all values of q . Since scattering experiments must often be done at rather high concentrations, the scattering data must be extrapolated to $n = 0$. The procedures employed for this extrapolation and a validation of $S(q)$ has been given recently.^[66]

After determining the scattering intensity extrapolated to infinitely small concentration, the next step consists of extrapolating the intensity to infinite contrast. This is necessary because the theory described in Section 2.3 refers to dendritic structures composed of segments of equal scattering length. Complete dendrimers, on the other hand, are made up of atoms and chemical groups that differ strongly with regard to their scattering power. As discussed recently,^[66] this fact leads to additional contributions that are not connected with the Fourier transform $F(q)$ related to $\rho(r)$. The measured scattering intensity is not directly related to the scattering length density within a dissolved dendrimer but to the difference in the scattering length density between the solute and the solvent. This difference is denoted contrast and can be changed systematically by use of mixtures of protonated and deuterated solvents. Two limiting cases are apparent: a) low contrast: here the differences between the scattering lengths of the different segments of the dendrimer become visible, and b) high contrast: the difference between the scattering length of different units is inconsequential and may be neglected. The dependence of $I_{\text{coh}}(q)$ on contrast thus obtained may be evaluated to yield $F(q)$, which refers to measurements carried out at infinitely high contrast. In this way, the form factor displayed in Figure 7 was obtained experimentally from SANS data.

The fact that deuterons scatter neutrons differently than hydrogen atoms may be used to mark certain parts of the molecule. This technique has been developed in the field of polymer physics and has become a standard technique for problems related to dense polymeric systems. Recently, the location of end groups within a fourth-generation dendrimer has been investigated by this method.^[38] The deuteration of end groups enabled a strong contrast between the end groups and the interior parts of the dendrimer. Subsequent analysis of the partially deuterated dendrimer by SANS then allowed the determination of the distribution of end groups within the dendritic scaffold. Figure 10 shows the key results of this study.^[38] Here the local scattering length density $\rho_p(r)$ of the partially deuterated dendrimer G4-D is plotted against the radial distance from the origin and the distribution is proportional to the density of the end groups. In this way, the

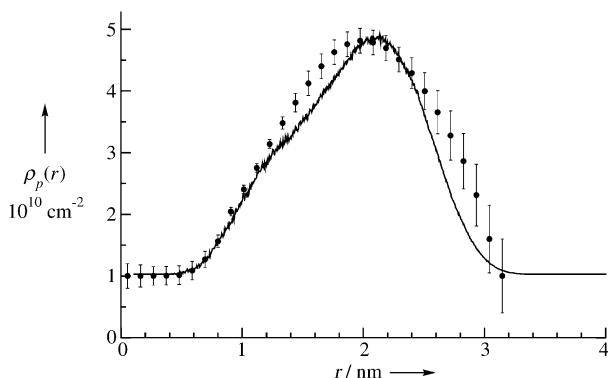


Figure 10. Distribution of the local scattering length density $\rho_p(r)$ of the partially deuterated dendrimer G4-D.^[38] This distribution is directly proportional to the distribution of end groups within the dendritic scaffold. The solid line shows the simulation result for the distribution of end groups.^[67] The vertical scale has been multiplied by an arbitrary constant, which arises from the scattering length contrast, so that the peak values from experiment and simulation coincide.

position of the deuterated end groups can be determined, since these marked end groups have a higher scattering length density. The distribution $\rho_p(r)$ is therefore directly proportional to the distribution of end groups and, as can be seen there, it compares favorably to the result obtained from simulations.

4. Interacting Dendrimers

4.1. Theory

Whereas the conformations of isolated neutral dendrimers are now well-understood, much less is known about both their conformations and effective interactions in concentrated solutions. Linear polymer chains are known to exhibit three concentration-dependent conformational regimes. Below the so-called overlap concentration c^* (dilute regime), they behave as self-avoiding walks and show the scaling behavior $R_g \sim N^\nu$, with the Flory exponent ν . Between the overlap concentration c^* and a second characteristic concentration $c^{**} \cong a^{-3}$ (semidilute regime), they shrink according to the law $R_g(c) = R_g(0)(c/c^*)^{1/8}$. For $c > c^{**}$ (concentrated regime), they behave like ideal chains because of the screening of the excluded volume interactions by the concentration, and the random-walk law $R_g \sim N^{1/2}$ holds.^[86] Similar scaling laws hold for star polymers.^[61]

A detailed comparison between linear chains and dendrimers regarding the sizes and the thermodynamic properties was performed by Lue.^[87] The model employed was that of tangential hard spheres, as in the earlier work of Lue and Prausnitz.^[70] Dendrimers of spacer length $P=2$ and of generation $G=0-5$ were examined in a concentrated solution that was characterized by a total packing fraction η of all the hard-sphere monomers.^[88] The evolution of their conformation with η as well as the total pressure $P(\eta)$ were investigated. A shrinking of the dendrimers with increasing concentration η was clearly observed, with the amount of

the reduction of the dendrimer's size depending on the generation number. Second-generation dendrimers reduce to about 60% of their size in dilute solutions at a monomer packing fraction $\eta=0.35$ (the highest examined in Ref. [87]), whereas for fifth-generation dendrimers they reduce to as much as 40% of their size in dilute solution. This difference stems from the fact that the dendrimers become more compact with increasing generation, hence the osmotic pressure they exercise on other dendrimer molecules in solution increases correspondingly. The shrinking of the molecules is also reflected in the evolution of their density profiles in which the core gets denser with increasing η values.

The results of Lue regarding the pressure of the dendrimer solution as a function of η are also very interesting. The existence of the semidilute regime was explicitly confirmed for linear chains in the sense that the $P(\eta)$ curve was found to be a universal function, independent of the degree of polymerization N of the chains.^[87] The pressure of a second-generation dendrimer (with $N=43$) is also described by the same universal curve in the semidilute regime, thus demonstrating that low-generation dendrimers behave very similarly to linear chains. The situation changes for higher generations. Indeed, $G=5$ dendrimers are compact objects with a large domain of constant density profile. Hence, whereas for low concentrations ($\eta \lesssim 0.2$) their pressure is lower than that of linear chains with the same N , for higher concentrations ($\eta 0.25$) the pressure is higher than the one given by the universal function. The extent of the "semidilute regime" in which the pressure is described by a universal function shrinks to zero for high-generation dendrimers. This leads to the conclusion that the semidilute regime does not exist for high-generation dendrimers and it could only be observed if dendrimers are synthesized with sufficiently long spacers, which could reintroduce a "linear-chain" component into these hyperbranched molecules.^[87]

Another quantity of major importance for analyzing the structural and thermodynamic behavior of concentrated colloidal solutions is the so-called effective interaction or potential of mean force $w(r)$.^[86] This is defined in the following way: One starts from the full Hamiltonian $\mathcal{H}$. Then, out of the L particles (in our case all monomers), one selects the M ones that are to be considered as "effective particles" (in this case the centers of the dendrimers) and holds them fixed in some prescribed configuration $\{\mathbf{R}_1, \mathbf{R}_2, \dots, \mathbf{R}_M\}$, where $\mathbf{R}_i$ is the position of the i th effective particle. Afterwards, the $L-M$ remaining particles are canonically traced out and the result of this integration is a constrained partition function $Q(\mathbf{R}_1, \mathbf{R}_2, \dots, \mathbf{R}_M)$. The effective Hamiltonian $\mathcal{H}_{\text{eff}}$ is defined by Equation (30), where $\beta = (k_B T)^{-1}$, T is the absolute temperature, and k_B is the Boltzmann constant.

$$\exp(-\beta \mathcal{H}_{\text{eff}}) = Q(\mathbf{R}_1, \mathbf{R}_2, \dots, \mathbf{R}_M) \quad (30)$$

The so-defined effective Hamiltonian has two useful properties:^[86] it preserves the overall thermodynamics of the system and it guarantees that the correlation functions of any order between any of the M remaining particles remain invariant, regardless of whether the expectation values are calculated with the original Hamiltonian $\mathcal{H}$ or with the

effective one $\mathcal{H}_{\text{eff}}$. Although the procedure of tracing out the $L-M$ degrees of freedom necessarily generates interactions of all orders between the M particles, in most cases it is sufficient to truncate those to pairs, thereby introducing the pair-potential approximation. This pair potential is precisely the above-mentioned quantity $w(r)$, which physically expresses the free energy needed to bring two macromolecules from infinite separation to a center-to-center distance r .

Effective interactions have been established for a long time and employed in the realm of charge-stabilized colloidal suspensions.^[89] Only recently were effective interactions quantitatively calculated for a number of polymeric systems. These include linear polymer chains,^[90–98] star polymers,^[99–101] polyelectrolyte stars,^[80,81] and dendrimers.^[87,102,103] The effective interaction is a very useful quantity for analyzing theoretically scattering data from concentrated solutions. Indeed, it can be shown that the coherent scattering intensity obtained, for example, in SANS experiments from concentrated solutions can be written in as a product of $F(q)$ and the structure factor $S(q)$ [Eq. (29)]. The latter quantity describing interparticle correlations between the entire macromolecules can be calculated for any density once the effective interaction $w(r)$ is known.^[24,86] Equation (29) holds provided the sample is monodisperse;^[26] it is valid throughout the whole concentration range only for scattering from hard colloidal particles that cannot deform, interpenetrate, or overlap. When scattering from soft, penetrable objects, such as polymer chains and dendrimers, there are three caveats that should always be taken into account when analyzing scattering data obtained above the overlap concentration c^* :

1. Since the macromolecules themselves deform, the zero-density form factor $F(q)$ no longer describes the conformation of the molecule. This restriction can be lifted in neutron-scattering experiments by labeling only a small part in the innermost of the molecule, which is expected to suffer minimal deformation upon an increase in the concentration.
2. The monomers from neighboring molecules overlap, hence the clear separation into inter- and intramolecular contributions to scattering, which is inherent in the factorization form of Equation (29), is no longer valid. Once more, the labeling technique offers a remedy.
3. It is expected that at high concentrations many-body potentials that are ignored in the pair approximation will become more and more significant, thus modifying the structure factor $S(q)$ accordingly. Although these forces are always present in principle, they have been demonstrated to be rather weak in the case of star polymer solutions^[105] and linear chains.^[91]

4.2. Comparison with Experimental Data

In a recent study Pötschke et al.^[104] employed SANS experiments at a series of different contrasts to measure the form factor of isolated dendrimers and to extract thereby the structure factor $S(q)$ for dendrimer solutions at low concentrations. This study revealed two important features: First, the

form factor $F(q)$ has a Gaussian form, a fact that leads to the conclusion that the shape function $T(r)$ of the dendrimer has itself a Gaussian shape.^[66,67,104] Since the latter is proportional to the density profile $\rho(r)$, these results constitute direct experimental confirmation of the dense-core model. Second, the extracted structure factors showed a reduction of the $q \rightarrow 0$ value, which is consistent with a decrease in the osmotic compressibility of the solution upon increasing the concentration, but at the same time they showed no significant correlation peaks at a finite wave vector. This unexpected finding contrasts the behavior that is known to be caused by the “usual” interactions in colloid chemistry and physics, such as the hard-sphere potential, Yukawa interactions, and Lennard–Jones potentials. There, a decrease of the compressibility is always accompanied by an enhancement of the structure factor peak, which indicates there is increased spatial correlations in the solution that can eventually lead to crystallization.

A theoretical explanation of these features was offered in the work of Likos et al.^[102,103] By utilizing the Gaussian density profiles of isolated dendrimers and putting forward a simple mean-field, Flory-type theory they calculated the effective interaction $w(r)$ to be of a Gaussian form [Eq. (31)].

$$w(r) = N^2 v k_B T \left(\frac{3}{4\pi R_g^2} \right)^{3/2} \exp \left(-\frac{3r^2}{4R_g^2} \right) \quad (31)$$

In this Equation, N is the number of monomers, R_g is the radius of gyration, and v the monomer–monomer excluded-volume parameter. A comparison between the theoretical results for $S(q)$ obtained by using Equation (31) and the experimental data is shown in Figure 11. The Gaussian effective interaction captures all the effects seen in the experiment. Unlike the hard-sphere potential that develops stronger and stronger correlation signals upon increasing the concentration, the Gaussian interaction delivers an “anomalous” structure factor with a weak signal that first increases with concentration until the overlap density is reached and then it disappears again at high densities.^[86,106,107] In Ref. [103] deviations between theory and experiment show up above the overlap concentration, which is in line with the restrictions mentioned above.

The effective interaction $w(r)$ has also been measured in simulation studies by Lue and Prausnitz^[70] as well as by Lue.^[87] In Ref. [70], $w(r)$ has been calculated for $G=2$ and 3 and for spacer lengths $P=2, 5$, and 10. Although the parameters are different than those in Refs. [102,103], and thus a direct comparison cannot be made, the results are in semiquantitative agreement with one another. Lue and Prausnitz also found an effective potential with a Gaussian-like form and with a maximum value of the order of $10 k_B T$.^[70] The potential of mean force becomes more repulsive on increasing the generation number to $G=5$,^[87] however, the value of $w(r)$ at zero separation is not shown in Ref. [87]. An important consequence of the Gaussian potential of Equation (31) is that it is too low at $r=0$ (of the order of $10 k_B T$) and thus not repulsive enough to cause crystallization of the system.^[106] It is a well-known fact that dendrimers are very difficult to crystallize; only anisotropic single dendrons have

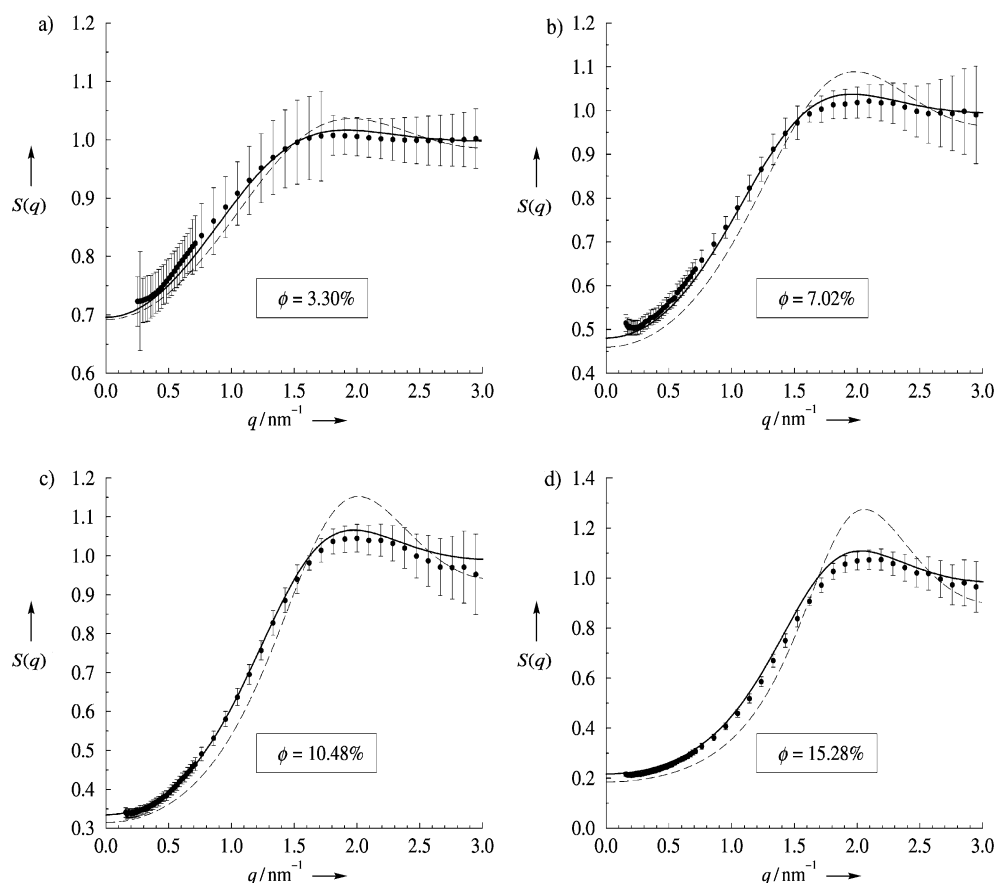


Figure 11. Experimental (points) and theoretical (lines) structure factors of concentrated solutions of fourth-generation dendrimers. The thick solid lines represent the results obtained with the Gaussian interaction of Equation (31) and the thin broken ones arising from a hard-sphere potential of diameter $\sigma_{HS} = 2R_g$. The volume fractions ϕ of the dissolved dendrimer are indicated on the plots.^[103]

been found to self-assemble into liquid-crystalline structures.^[108–110] Thus, other physical mechanisms, such as charge, need to be used to induce strong enough repulsions between the dendrimers so that they can then possibly sustain stable crystals. Fluid–fluid equilibria triggered by a worsening of the solvent quality is also a phenomenon that can be described by means of an appropriately defined effective interaction, in which the temperature-dependent excluded volume parameter v reflects the solvent effects. Although this line of research has not been followed up to now, an alternative, lattice-cluster theory^[111,112] has already led to predictions of dendrimer/solvent demixing that are in good agreement with experimental data.

The anomaly in the development of the structure factor has also been seen in the SANS studies of Ramzi et al.^[113] and of Topp et al.^[114] In Ref. [113] dendrimer volume fractions ϕ up to 60% were examined, thus the overlap concentration is greatly exceeded and the factorization approximation [Eq. (29)] breaks down. This could be the reason for the apparent, empirical scaling $q_{\max} \sim \phi^{0.55}$ found there.^[113] Since the dendrimers deform themselves, division of the scattering intensity by the inappropriate ($\phi \rightarrow 0$) form factor can lead to maxima of $S(q)$ at the wrong wave vectors. Nevertheless, the lack of pronounced maxima in $S(q)$ and even their virtual

disappearance upon increasing ϕ ^[113] is not seriously affected by this approximation. Another important finding of Ramzi et al. is the development of a strong repulsion between the dendrimers upon the introduction of charged monomers. The same effect has also been seen in the work of Nisato, Ivkov, and Amis.^[85] Topp et al.^[114] also measured what are termed “apparent” structure factors from concentrated solutions of two different types of dendrimers. The word apparent here refers once more to the fact that the factorization assumption of Equation (29) was made. The results reported in Ref. [114] display all the quantitative characteristics of the Gaussian model mentioned above, but they must be regarded with some reservation: parts of the data sets shown in Ref. [114] appear not to be appropriately normalized, as witnessed by the fact that $S(q)$ does not approach unity for high values of the wave vector.

5. Conclusions and Outlook

The foregoing review has demonstrated that dendrimers have become a subject of soft-matter physics within the last few years. A great number of simulations is now available and the analytical theory of dendritic structures has progressed considerably. The main results of all these studies may be briefly summarized as follows: flexible dendrimers can be treated successfully in the well-known framework of polymer physics. All simulations and analytical theories prove beyond doubt that these dendrimers have their maximum density in the center of the molecule (dense-core structure). The underlying physical reason for this occurrence is the flexibility of the dendritic scaffold. Indeed, under the conditions of fully flexible bonds, entropy drives the monomers of all generations to explore all the regions of conformational space that are compatible with the given excluded-volume interactions. The latter, for example, make it impossible for all monomers of the terminal generation to be localized at the periphery of the molecule (in particular for high-generation dendrimers) since the number of monomers grows exponentially with

generation number while the volume of the successive shells increases only as a power-law of the generation number. Thus, the end monomers are folded back into the molecule, thus making use of the space available through the stretching of the inner generations.

Concomitant with this fluctuating structure, the interaction of dendrimers in solution is soft and may be described by a Gaussian potential. Moreover, all the results obtained from theory can be directly proved by SANS data obtained in solution. In this way the investigations on dendrimers as outlined here show that the combination of simulations and analytical theory with scattering methods provides a powerful tool for the structural analysis of disordered supramolecular structures. We therefore believe that this combination will become indispensable in the field of supramolecular chemistry.

Future theoretical research on dendritic macromolecules can take various directions and a large number of open problems remain. The question of central interest is: Is it possible to tune the density profile of the dendrimer and induce conformational changes? From the point of view of synthetic chemistry, it is to be expected that conformations other than the ones discussed in this review can be caused by employing *stiff* spacers between adjacent generations. The effects of stiffness should already be visible in SANS experiments. On the one hand, the form factors of stiff dendrimers would show the characteristic high q oscillations associated with stiff molecules and, on the other hand, the interactions between stiff dendrimers are expected to be considerably stronger than Gaussian interactions, thus giving rise to distinct correlations in a concentrated dendrimer fluid.

Other possible mechanisms for inducing conformational changes in a dendrimer combine physical and chemical aspects. In particular, future attention should focus on the possibility of appropriately modifying the size and/or shape of the end groups during the preparation of the dendrimers and then on looking at the effects of dissolving these molecules in solvents whose compatibility with the end groups and the inner of the molecule varies. In this respect, a very promising approach for separating the end groups from the rest of the molecule is to use cylindrical units as terminal groups and examine the effects of a nematic solvent that will entropically tend to pull the end groups together, thus separating them from the rest of the dendrimer.

Finally, we believe that the effects of charge on dendrimers present a formidable challenge for theory, simulations, and experiments. Here, the very realistic prospect opens up that modifying the amount of uncompensated charge on the molecules will provide a controlled way for inducing conformational changes on the dendrimers. In view of the fact that the charges dealt with in the studies are separated by distances of atomic dimensions, the effects of the granularity of the solvent have to be taken into account, that is, the introduction of a simple dielectric constant ϵ does not provide an accurate description of the physics of the systems. From the point of view of simulations, therefore, it seems to be necessary to employ realistic models to simulate the solvent molecules explicitly, as in recent works dealing with transmission of water molecules through nanopores.^[115]

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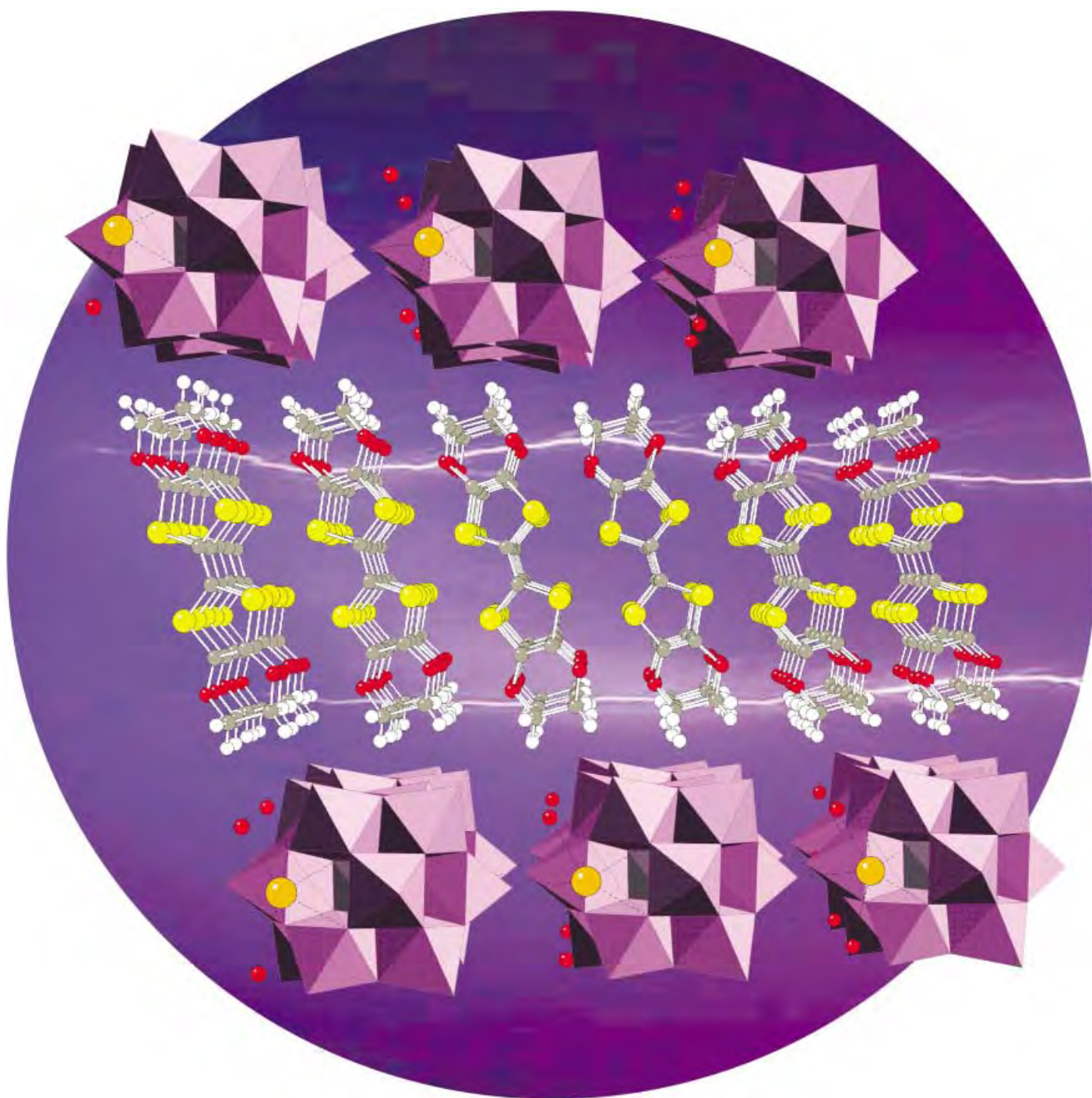
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Communications



The first polyoxometalate-containing molecular material that exhibits metallic behavior is formed by conducting layers of the radical bis(ethylenedioxy)tetrathiafulvalene separated by inorganic layers of Keggin polyoxometalates ($[\text{BW}_{12}\text{O}_{40}]^{5-}$) and K^+ ions. For more information see the Communication by E. Coronado, C. Giménez-Saiz et al. on the following pages.

Metallic Conductivity Down to 2 K in a Polyoxometalate-Containing Radical Salt of BEDO-TTF**

Eugenio Coronado,* Carlos Giménez-Saiz,*
Carlos J. Gómez-García, and Silvia C. Capelli

Owing to their structural and electronic versatility polyoxometalate anions have proven to be useful inorganic components for the design of novel hybrid molecular materials with functional properties.^[1] The application of these molecular metal-oxide clusters has been particularly pronounced in the field of molecular conductors. Typically molecular conductors are formed by segregated stacks of partially oxidized organic π -electron donor molecules of the tetrathiafulvalene (TTF) type interleaved by inorganic anions.^[2] The use of polyoxometalates in this area has provided the opportunity to examine the strong structural effects produced by these highly charged and large anions on the organic packing. On the other hand, as a result of the ability of these anions to coordinate magnetic ions or to act as electron acceptors, they have afforded interesting hybrid materials either with coexistence of localized spins and delocalized electrons,^[3] or with coexistence of delocalized electrons on both components.^[4] However, the observation of metallic properties in this class of conducting materials has remained an elusive challenge. In fact, most of these radical salts are semiconductors or insulators, for example, the salts based on Keggin anions and TTF-type donors.^[3–5]

In a few cases metallic-like properties have been observed but only at high temperatures. Two examples of this type are the series $[\text{BEDT-TTF}]_{11}[\text{P}_2\text{W}_{17}\text{MO}_{62}]$ ($M = \text{W}^{\text{VI}}, \text{Re}^{\text{VI}}$)^[6] and the compound $[\text{BEDT-TTF}]_5[\text{VW}_5\text{O}_{19}] \cdot 6\text{H}_2\text{O}$,^[7] which exhibit metallic behavior down to 240–260 K with room temperature conductivities between 10–14 Scm^{-1} . Below these temperatures broad metal-to-semiconductor transitions are observed. A significant improvement has been obtained in

the compounds $[\text{BEDT-TTF}]_5[\text{H}_3\text{V}_{10}\text{O}_{28}] \cdot 4\text{H}_2\text{O}$ ^[8] and $[\text{BEDT-TTF}]_6[\text{Mo}_8\text{O}_{26}] \cdot (\text{DMF})_3$ ^[9] which behave as metals down to 50 and 60 K with room temperature conductivities of 360 and 3 Scm^{-1} , respectively.

Herein we report the first example of a polyoxometalate-based radical salt that exhibits metallic behavior down to 2 K. The compound, formulated as $[\text{BEDO-TTF}]_6\text{K}_2[\text{BW}_{12}\text{O}_{40}] \cdot 11\text{H}_2\text{O}$ (**1**), is formed by the polyoxometalate ion $[\text{BW}_{12}\text{O}_{40}]^{5-}$ and the organic radical bis(ethylenedioxo)tetrathiafulvalene (BEDO-TTF). To our knowledge, in spite of its potential interest, no polyoxometalate-containing radical salt with the donor BEDO-TTF has been reported to date. Most radical salts containing the BEDO-TTF donor are molecular metals, including the superconductors, $[\text{BEDO-TTF}]_3[\text{Cu}_2(\text{NCS})_3]$ ^[10] and $[\text{BEDO-TTF}]_2[\text{ReO}_4] \cdot \text{H}_2\text{O}$.^[11] Moreover, as an oxygenated donor BEDO-TTF could establish stronger intermolecular interactions (of the type $\text{O} \cdots \text{O}$) with the metal-oxide clusters than other donors which do not contain oxygen, such as bis(ethylenedithio)tetrathiafulvalene (BEDT-TTF).

For these reasons, we have made numerous attempts to obtain radical salts containing the BEDO-TTF donor and Keggin polyoxometalates. However, whereas the donor BEDT-TTF has given rise to extensive series of crystalline radical salts with many different polyoxoanions possessing the Keggin structure, the synthesis of analogue salts based on BEDO-TTF has turned out to be much more difficult. In fact, the use of $[\text{R}_4\text{N}]^+$ acid salts of Keggin polyoxometalates as starting salts has not given any positive results, however, mixed $[\text{R}_4\text{N}]^+$ and K^+ salts afforded small single crystals of compound **1**. It seems that the K^+ ions, which enter into the structure, play an important role in stabilizing the salt and furthermore, they reduce the effective charge of the polyoxoanion and therefore the charge of the BEDO-TTF molecules.

The electrocrystallization method produced single crystals of compound **1** which were too weakly diffracting to perform a X-ray data collection on diffractometers equipped with conventional X-ray sources, but a reasonable diffraction pattern could be obtained using the high intensity of the synchrotron radiation produced at the Swiss-Norwegian Beamline at the European Synchrotron Radiation Facility in Grenoble (France). The structure of this radical salt^[12] consists of alternating layers of organic donors and inorganic polyoxometalates in the $[\frac{1}{2}, \frac{1}{4}, 1]$ direction (Figure 1).

The organic layers are made up of three crystallographically independent BEDO-TTF units (labeled as A, B, and C in Figure 2) which arrange forming very uniform stacks parallel to the *b* axis, following the sequence ...ABC... in one chain and the opposite one (...CBA...) in the adjacent chains (Figure 2).

The organic layers adopt the so-called β'' packing arrangement^[13] with stacks of BEDO-TTF molecules having numerous short intermolecular $\text{S} \cdots \text{S}$ and $\text{S} \cdots \text{O}$ separations which are shorter than the sum of the van der Waals radii (3.60 Å and 3.32 Å respectively, see Supporting Information).

Despite being crystallographically independent the three organic donors (A–C) all have the same geometry: they all have the same twisted, eclipsed conformation of their outer

[*] Prof. E. Coronado, Dr. C. Giménez-Saiz, Prof. C. J. Gómez-García
Institut de Ciència Molecular
Universitat de València
Dr. Moliner, 50, 46100 Burjassot (Spain)
Fax: (+34) 96-354-4859
E-mail: eugenio.coronado@uv.es
carlos.gimenez@uv.es

Dr. S. C. Capelli[†]
Swiss Norwegian Beamline (SNBL) at the
European Synchrotron Radiation Facility (ESRF)
6 Rue J. Horowitz, BP 220, 38000 Grenoble (France)

[[†]] Present address: ID11 Materials Science Beamline at ESRF

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

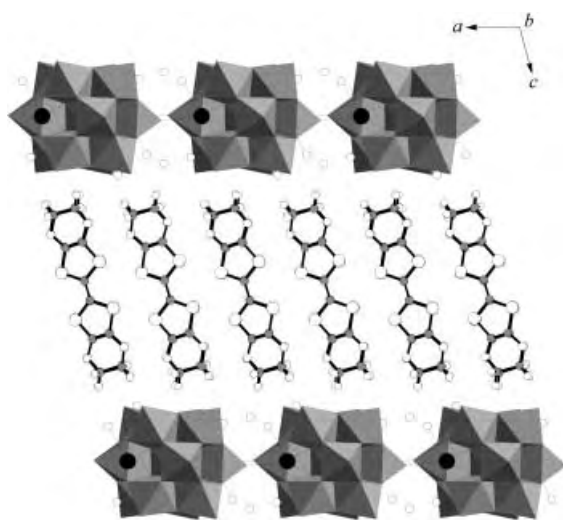


Figure 1. Structure of **1** showing the alternating layers of the polyoxoanions and the organic donors.

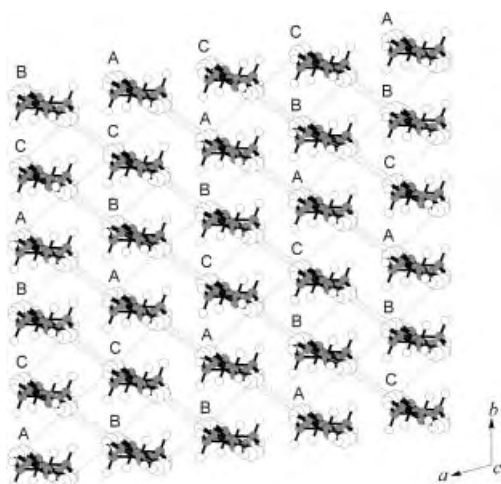


Figure 2. View of the organic layer showing the labeling of the three crystallographically independent BEDO-TTF molecules. Dotted lines represent S...S and S...O contacts shorter than the sum of the van der Waals radii.

ethylene groups, as well as similar intramolecular bond lengths and angles. This is a noteworthy difference with respect to the other radical salts of Keggin polyoxometalates with the related donors BEDT-TTF^[3] and BEDSe-TTF (bis(ethylenediseleno)tetrathiafulvalene),^[4] in which the organic molecules have very different conformations, geometries, and charges, and form very irregular and exotic stacks or even lose the typical 2D packings that these donors usually form when they are combined with simpler anions. This general feature of the polyoxometalate-containing radical salts has been attributed to the large charges and volumes of these anions and ultimately leads to charge localization and thus, to poor conducting properties.^[2]

In the present case the formation of very uniform organic stacks seems to be a result of the use of the oxygenated donor BEDO-TTF, which gives rise to many short intermolecular

C–H...O contacts that stabilize the formation of the eclipsed stacks characteristic of the β'' packing (typical for most BEDO-TTF-based organic metals). Figure 3 shows one stack

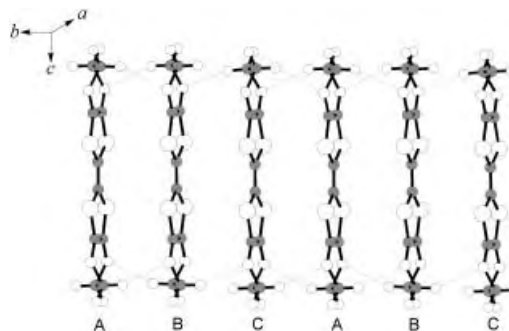


Figure 3. View of one eclipsed stack of organic donors. Dotted lines represent C–H...O contacts shorter than 2.72 Å (the sum of the van der Waals radii); A–C as in Figure 2.

of donor molecules in which the dotted lines represent intrastack C–H...O contacts shorter than the sum of the van der Waals radii (2.72 Å). The hydrogen atoms of the donors that do not establish short intrastack contacts with other donors, form C–H...O contacts with the oxygen atoms of the polyoxoanion or the water molecules, these C–H...O separations are between 2.37 and 2.94 Å.

The inorganic layers are formed by the bulky Keggin polyoxometalates and the K⁺ ions inserted between them. There is only one kind of K⁺ ion, it has a coordination number of 8 (K...O, 2.69–2.98 Å) and is coordinated to oxygen centers of three different polyoxoanions as well as two water molecules of crystallization which do not have short contacts with the ethylene groups of the organic donors.

The K⁺ ions inserted in the inorganic layers fill the cavities or voids existing in between the Keggin polyoxometalates. In the other Keggin-based radical salts the organic donors fill these voids by “docking” of the outer ethylene groups into the cavities provided by the polyoxometalate network. This feature often yields irregular stacks of the donors.^[2] In compound **1** the insertion of K⁺ ions is related to the formation of fully eclipsed stacks of BEDO-TTF units which can not fill the cavities created in the inorganic sublattice.

The analysis of the bond lengths of the three independent BEDO-TTF molecules suggest that they are partially oxidized with a charge of +0.5, in agreement with the anionic charge and the stoichiometry of the salt.^[14]

The thermal variation of the electrical conductivity (σ) of **1** along the best developed face of a single crystal is shown in Figure 4. The room temperature conductivity is about 37 Scm^{−1} and gradually increases as the temperature is decreased to reach a maximum value of 910 Scm^{−1} at 2 K. This behavior reveals a metallic character of this salt across the studied temperature range (300–2 K). The temperature dependence of the resistivity ($\rho = 1/\sigma$) shows a linear dependence from room temperature to 200 K, which is usually associated with electron–phonon scattering.^[9] Below this temperature the resistivity is proportional to T^2 (inset in

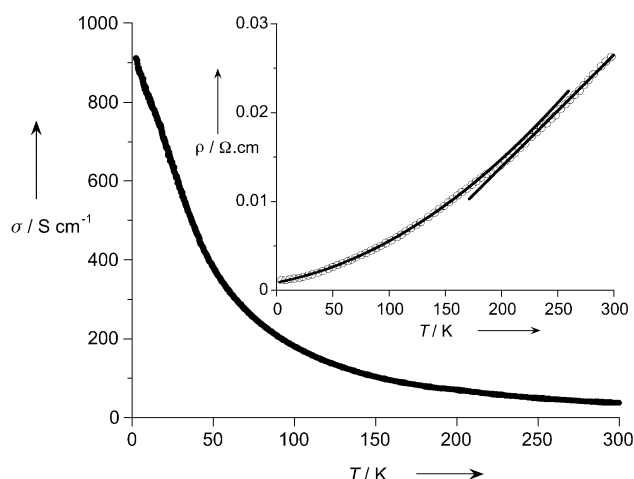


Figure 4. Thermal variation of the conductivity (σ) of **1** showing the metallic behavior down to 2 K. Inset: Plot of the thermal variation of the resistivity (ρ). Solid lines show the linear and quadratic dependencies of ρ above and below 200 K, respectively.

Figure 4), typical of metallic organic salts where electron–electron scattering is the dominant mechanism of conductivity.^[9]

The thermal variation of the molar magnetic susceptibility, χ_m , of **1** (not shown) has an almost constant value of $0.013 \text{ emu mol}^{-1}$ from room temperature to approximately 15 K. Below this temperature χ_m shows a continuous increase reaching a value of around $0.021 \text{ emu mol}^{-1}$ at 2 K. This behavior can be accurately reproduced with a simple model considering a Curie tail (C/T) with $C = 0.017 \text{ emu K mol}^{-1}$ plus a temperature-independent paramagnetism corresponding to the so-called Pauli paramagnetism of $0.013 \text{ emu mol}^{-1}$. This value is similar to those obtained in other synthetic metals and confirms the metallic character of this salt. The low C value indicates the presence of a very small paramagnetic impurity, already observed in many other radical salts and attributed to the presence of isolated donor molecules as the result of the ubiquitous crystal defects (less than 0.8% of isolated BEDO–TTF⁺ radicals). The room temperature ESR spectrum of **1** (not shown) has a single line centered at $g = 2.0059$ with a line width of 40 G, typical of this kind of radical salts.^[2] The high value of the line width suggests a marked 2D electronic character in **1**, as indicated by the electrical properties.

In conclusion, the organic/inorganic hybrid material presented herein is the first example of a polyoxometalate-containing radical salt that exhibits a metallic behavior down to 2 K. This result demonstrates that it is possible to use bulky and highly charged polyoxoanions as components of new radical salts that behave as a metal and opens up the possibility of synthesizing new molecular materials with coexisting or even coupling of conducting electrons and localized magnetic moments. The synthetic strategy simply consists in combining the BEDO–TTF donor with inorganic layers of magnetic Keggin polyoxometalates and K^+ ions. This would give rise to a new series of molecular materials, similar

to the reported series with the BEDT–TTF donor,^[3] but with the advantage of having a metallic behavior down to very low temperatures.

Experimental Section

Crystals of compound **1** were prepared by electrocrystallization of a 3:2 mixture of 1,1,2-trichloroethane/acetonitrile containing the soluble salt $(n\text{Bu}_4\text{N})_4\text{K}[\text{BW}_{12}\text{O}_{40}]$ (10^{-2} M), the organic donor BEDO–TTF ($2 \times 10^{-3} \text{ M}$), and some drops of distilled water. The donor was slowly oxidized at a constant current of $1.2 \mu\text{A}$. Under these conditions very thin, plate-like, brown single crystals were obtained on the platinum electrode of the electrochemical cell after 7 days. When dry conditions were used, no crystal growth was detected on the electrode after 20 days, indicating that water molecules are required for the stabilization of the crystal structure.

D.C. conductivity measurements over the range 2–300 K were performed using the four contacts method in several different single crystals, all the samples gave reproducible results. Contacts between the crystals and platinum wires (25 μm diameter) were made using graphite paste. The samples were measured in a Quantum Design PPMS-9 with a dc current of $1 \mu\text{A}$. The cooling and warming rate was 1 K min^{-1} and the results were, within experimental error, identical in the cooling and warming sweeps.

Variable temperature susceptibility measurements were carried out in the temperature range 2–300 K at a magnetic field of 0.1 T on a polycrystalline sample with a magnetometer (Quantum Design MPMS-XL-5) equipped with a SQUID (superconducting quantum interference device) sensor. The susceptibility data were corrected for the diamagnetic contributions as deduced by using Pascal's constant tables and for the sample holder. The room temperature ESR spectrum of the same polycrystalline sample was recorded at X-band with a Bruker E500 spectrometer.

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Protein Engineering

Modular Control of Lectin Function: Redox-Switchable Agglutination**

*Rhona E. McDonald, David J. Hughes, and Benjamin G. Davis**

Carbohydrate–protein interactions have been shown to be crucial in a range of biological events, including cell proliferation, signaling, and regulation.^[1] These interactions frequently involve lectins, which bind carbohydrates reversibly and specifically.^[2] Lectins typically contain two or more

[*] R. E. McDonald, Dr. B. G. Davis
Dyson Perrins Laboratory
Department of Chemistry, University of Oxford
South Parks Road, Oxford, OX1 3QY (UK)
Fax: (+44) 1865-275-674
E-mail: ben.davis@chem.ox.ac.uk

Dr. D. J. Hughes
Syngenta
Jealott's Hill International Research Centre
Bracknell, Berkshire, RG42 6EY (UK)

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carbohydrate recognition sites;^[3] hence, their interactions are dominated by potentially multivalent connections. This gives them the ability to interact with cells that have sugars on their surface, for example, erythrocytes, and to bind these sugars in single- or multipoint-attachment modes, often initiating cross-linking between cells in a process referred to as agglutination (Figure 1).^[4]

Indeed, lectin binding plays a critical role in the survival of platelets^[5] and agglutination of erythrocytes (hemagglutination) is a routine method of characterizing lectins as a result of the simplicity and low material requirements of the procedure.^[6] Another sensitive method of determining binding is the enzyme-linked lectin assay (ELLA),^[7] a variation on the ELISA. As a consequence of the near two-dimensional ligand and protein presentation that is used, ELLA results are typically not affected by cross-linking and they therefore provide a more direct measurement of binding,^[4] as opposed to cross-linking.^[8] These two methods, ELLA and agglutination, can therefore be used to probe the different phenomena of direct binding (Figure 1 a, b) and extended binding through cross-linked interactions (Figure 1 c, d), respectively.

Using these two contrasting assays as a guide, we have sought to engineer a novel lectin system in which extended binding, as measured by agglutination, may simply be switched on or off through redox control while maintaining similar levels of direct binding (Figure 1 e). Examples of lectin engineering are rare, partly as a result of some difficulties associated with establishing recombinant sources,^[9] and to the best of our knowledge this is the first example of such a modular control of lectin function through engineering. Critical sites in the model snowdrop lectin *Galanthus nivalis* agglutinin (GNA) were chosen that would allow us to introduce a cysteine residue capable of acting as a redox-active switch for covalent-bond formation across protein–protein interfaces. GNA, the first monocot mannose-binding lectin to be characterized,^[10] is a useful protein for this purpose. Investigation of the binding properties of GNA have shown that it is highly specific for α -mannosides and, in particular, for the linkages of Man(α -1,3) units.^[11] The 3D structure is known and reveals three mannose binding sites, I–III, (Figure 2).^[12,13] In this structure the strongest, primary

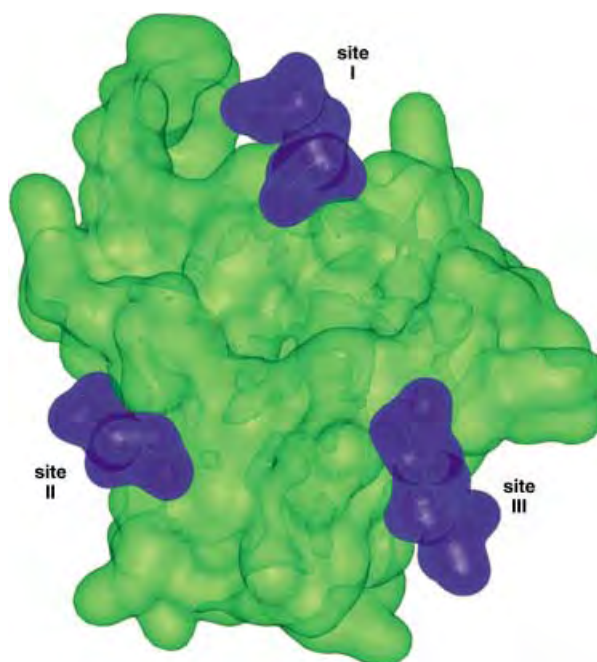


Figure 2. Representation of the GNA protein-surface (green) showing carbohydrate-binding sites occupied by a tri mannoside Man(1,6)-[Man(1,3)]Man (blue).

binding site, I, is the one that is most frequently occupied by carbohydrates; secondary and tertiary sites II and III show weaker binding (occupancies relative to site I of 0.7 and 0.6, respectively).^[12]

Accessibility is a critical parameter in establishing protein–protein interfaces.^[14] Analysis of the 3D structure of daffodil lectin *Narcissus pseudonarcissus* agglutinin (NPA),^[15] which has a strong sequence similarity to GNA and contains domain 1nplA0 that places it in the same homologous superfamily (CATH code 2.90.10.10.1^[16]) as representative GNA domain 1jpc00, revealed that a putative protein–protein interface is formed in NPA that is not present in the known 3D structure of GNA. This interface forms between different lectin units through the interaction and proximity of two

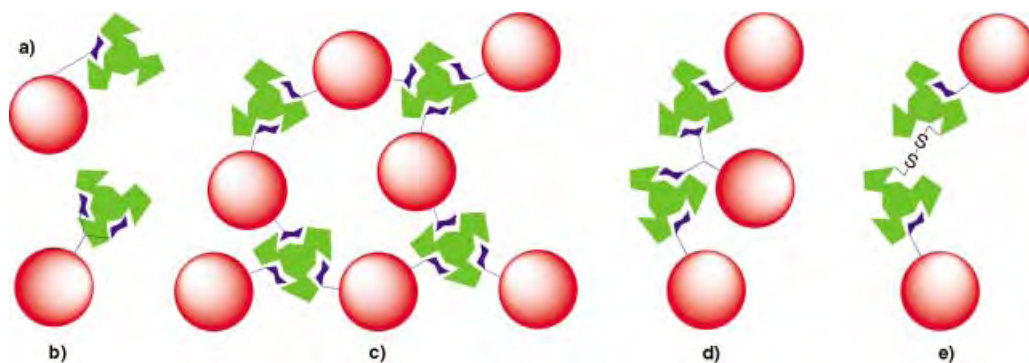


Figure 1. Binding modes of carbohydrate-displaying cell surfaces to lectins: a) single-point-attachment mode binding; b) multipoint-attachment mode binding; c) agglutination through cell-mediated cross-linking and single point attachment mode binding; d) agglutination through sugar-mediated cross-linking and multi/single point attachment mode binding; e) novel, engineered agglutination through disulfide-mediated cross-linking and single point attachment mode binding. (Here, the multipoint sugar-mediated cross-linking in (d) is simply replaced with a covalent disulfide linkage.)

residues, proline P71^[17,18] and asparagine N76, with the same residues in another NPA chain (Figure 3). These residues therefore seemed to be ideally accessible positions at which to introduce cysteine into GNA to design the key interface for our redox-active switch for extended binding.

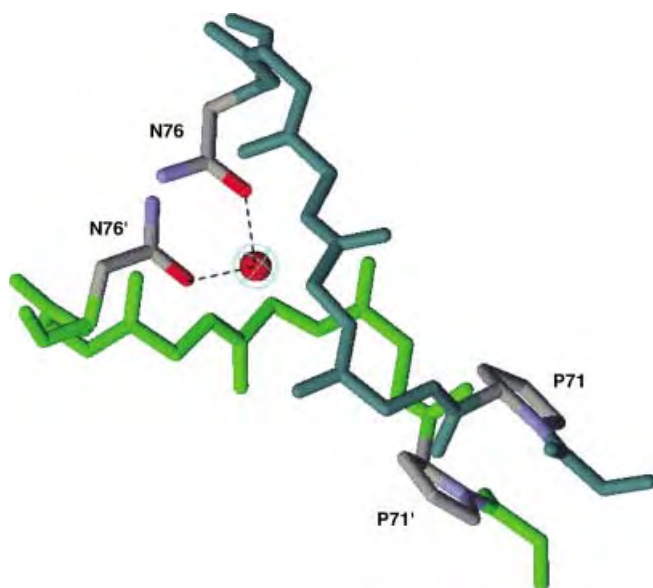


Figure 3. Representation of the protein–protein interface in daffodil lectin NPA highlighting the key role and close proximity of P71 and N76. The two interacting chains are shown in different shades of green while P71 and N76 side chains are colored according to element. The oxygen atom of a bridging water molecule is shown circled in blue.

To explore the potential for protein–protein interaction in GNA, the P71 and N76 residues of GNA, which occur on the edge of binding site II, were initially mutated to aspartic acid to create P71D, N76D, and P71DN76D mutants with the aim of introducing repulsive electrostatic effects across the interface.^[19,20] The effects of these mutations were analyzed by the agglutination assay (Figure 4) and ELLA (Figure 5). We were encouraged by the observation of an increase in the minimum

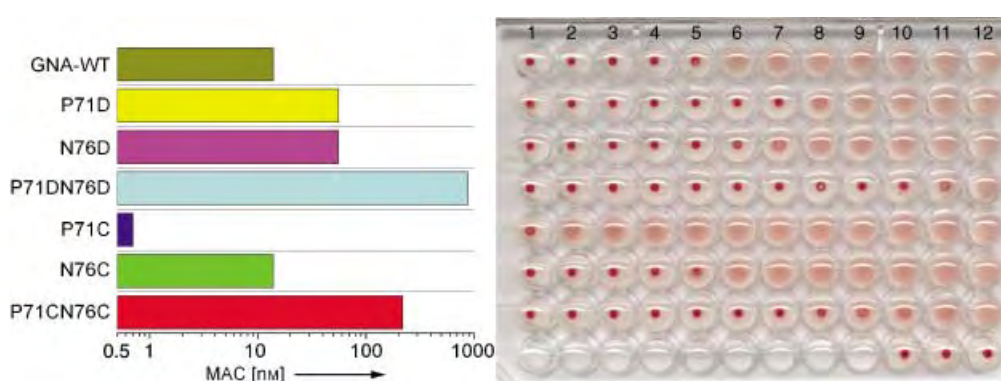


Figure 4. Comparison of the minimum agglutination concentration (MAC) of GNA wild type (WT) and mutants. Agglutination in the wells of the right-hand plate is indicated by the lack of a settled dark-red blood pellet. Protein concentrations [mg mL^{-1}] in the plate are as follows: Lane 1, 0.0061; 2, 0.012; 3, 0.024; 4, 0.049; 5, 0.097; 6, 0.20; 7, 0.39; 8, 0.78; 9, 1.56; 10, 3.13; 11, 6.25; 12, 12.5.

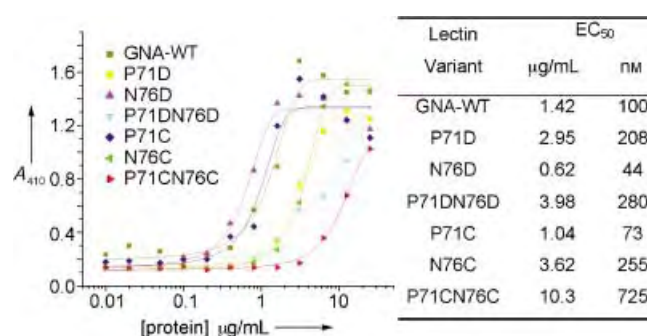


Figure 5. ELLA analysis of binding.

agglutination concentration values of up to 60-fold (MAC: GNA-WT 14 nM, P71D 56 nM, N76D 55 nM, P71DN76D 880 nM) whilst binding, as analyzed by ELLA, remained similar (EC_{50} : GNA-WT 100 nM, P71D 208 nM, N76D 44 nM (IC_{50}), P71DN76D 280 nM).^[21] Consistent with the putative model of introduced electrostatic repulsion, decreases in extended binding were greatest for the double-point P71DN76D mutant. This confirmed the critical nature of this site in primarily extended binding and not direct binding and demonstrated that for the first time extended and direct binding could be dissected by engineering a model lectin.

Next, the potentially redox-switchable P71C and N76C mutants were constructed and analyzed in the same manner (Figure 4, Figure 5). Excitingly, the P71C mutant showed a 20-fold increase in agglutination activity in comparison to the wild type (MAC: GNA-WT 14 nM, P71C 0.70 nM) whilst the binding analyzed by ELLA remained very similar (EC_{50} : GNA-WT 100 nM, P71C 73 nM (IC_{50})). This observation demonstrated that not only could extended binding be enhanced through engineering but that this could also be achieved without dramatic effect on direct binding. Interestingly, mutants containing the N76C mutation gave extended binding levels similar only to the GNA wild type (MAC: GNA-WT 14 nM, N76C 15 nM) and, given the strong similarity in the GNA and NPA structures, we attribute this to the poor ability of the covalent four-atom $\text{Cys}^{76}\text{CH}_2\text{SSCH}_2\text{Cys}^{76}$ interface cross-linking motif (ca. 6.0 Å) to replace the natural six-atom $\text{Asn}^{76}\text{CH}_2\text{-CONH}_2\text{NH}_2\text{COCH}_2\text{Asn}^{76}$ motif (ca. 9.3 Å).

To investigate whether the observed, enhanced extended binding was a result of disulfide-bond formation and to explore the usefulness of this interaction in a novel redox switch for such binding, the GNA wild type and the P71C mutant were exposed to varying concentrations of the reductant dithiothreitol (DTT). We hypothesized that the hemagglutination activity

of the P71C mutant would switch as the additional disulfide bond was reduced (Figure 6). Indeed, DTT had a dramatic effect on the P71C mutant and agglutination activity

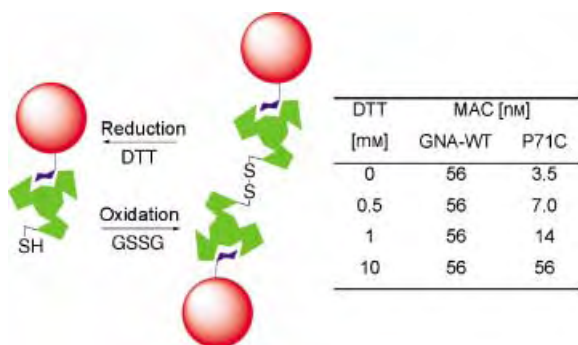


Figure 6. Redox switching of extended binding as judged by the minimum agglutination concentration.

decreased from a disulfide-enhanced level (MAC: 3.5 nM) for this blood sample set^[22] to the GNA-WT level (MAC: 56 nM; see the Supporting Information). In comparison, DTT had no effect on the GNA wild type, a result indicating that at these levels of DTT the internalized, less accessible C29–C52 disulfide bond in GNA was unaffected.^[23] Again, ELLA analysis showed that these results were a consequence of the modulation of extended binding alone rather than direct binding; the EC₅₀ values were all within about twofold of that for the GNA wild type. Hydrogen peroxide, oxidized glutathione (GSSG), oxidized DTT, and *S*-nitroso-*N*-acetyl-penicillamine (SNAP) were investigated for their ability to perform an oxidative “switch-back”. Although in some cases the detrimental effect (see the Supporting Information) of these oxidising agents in the blood cells proved to be a limiting factor with regard to the concentration used, pleasingly, the use of DTT followed by 1 mM GSSG (use above this concentration resulted in cell lysis) showed that it was possible to impair and then restore agglutination to a level approaching its initial prereduction level (within blood set:^[22] P71C prereduction 102 nM → P71C reduced 1300 nM → P71C GSSG switch-back 218 nM). This result demonstrated that it was indeed possible to switch extended carbohydrate binding on and off in this engineered lectin simply through the use of redox conditions.

To further confirm the key role of the free thiol group in our P71C model, we performed chemical modification on this residue by using the highly thiol-specific reagent methyl methanethiosulphonate^[24] to create the “methyl-capped” P71C-Me variant in which the formation of the critical interprotein cross-linking disulfide bond is prevented. Consistent with the need for a free thiol group at position 71, agglutination dropped approximately 20-fold from unmodified P71C to wild-type levels upon chemical modification to form the “methyl-capped” P71C-Me variant.

In summary, our results indicate that modular control of different modes of binding in a model carbohydrate-binding protein is possible through logical design. This work has included examples of engineered lectins in which extended

binding may be electrostatically deactivated and the first examples of redox-switchable extended binding that is controlled whilst maintaining direct binding. This switchable, condition-sensitive, covalently mediated protein-interface formation offers exciting possibilities in the design of protein systems that can sense and then manipulate cell–cell interactions differently according to the redox environment, as demonstrated here by redox-switchable red-blood-cell association. Indeed, key differences in redox conditions occur in certain disease states, such as during cancer cell proliferation,^[25] where such switchable cell–cell manipulation might prove useful. Interestingly, cross-interface disulfide-bond formation has recently been implicated in infectious prion protein propagation.^[26] We aim to investigate these possibilities, as well as the further modulation of multivalent interactions through variations in chemical modification in this and other systems.^[27]

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- [20] Excitingly, during the course of this work, structures of two similar lectins that show two contrasting dimerization modes at a weaker, secondary binding site interface have been described: one shows a sugar-mediated mode, the other shows a non-covalent, nonsugar mediated mode. These findings provide a natural, partial endorsement for the general engineering approach we describe here; see: M. Hayashida, T. Fujii, M. Hamasu, M. Ishiguro, Y. Hata, *J. Mol. Biol.* **2003**, 334, 551–565. Interestingly, initial indications in other systems suggest that a number of other lectins may also use a weaker, secondary carbohydrate binding site as a point for carbohydrate-induced protein–protein interface formation: H. Niwa, A. G. Tonevitsky, I. I. Agapov, S. Saward, U. Pfüller, R. A. Palmer, *Eur. J. Biochem.* **2003**, 270, 2739–2749. For an interesting example where it has been suggested that naturally occurring disulfide bridging might play a role in such multimerization, see: H. Kaku, N. Shibuya, *FEBS Lett.* **1992**, 306, 176–180. We suggest that this use of secondary sugar-binding sites in extended binding (rather than direct) and novel multimerization modes might be a common functional theme in certain lectin types. This, in part, further highlights the suitability of these interfaces for the type of engineering we have demonstrated here. Thus, although we have shown only a slight effect of manipulating such sites upon direct binding in the GNA system, interesting additional effects upon multivalency may also be engendered.
- [21] Lack of activity in control blood types in MAC analyses (chicken, horse, sheep, human) and control polysaccharide laminarin in ELLA analyses (see the Supporting Information) demonstrated the sugar-specific nature.
- [22] All MAC analyses were conducted on the same blood sample sets in triplicate. Up to fourfold variation in absolute MAC values was observed *between* different blood sample sets taken from different animals on different days. Variations *within* the same blood sample sets were $\leq 5\%$. Thus, whilst set-to-set comparisons should be made with caution, those within sets show high confidence levels ($p = 0.001$).
- [23] Analysis of the effects of different reductants upon the non-carbohydrate-induced, inherent multimerization of GNA showed that while the agglutination *and* noncarbohydrate-induced multimerization state of wild-type GNA is not affected by DTT under these conditions, the use of excess 2-mercaptoethanol instead under the conditions described in M. Longstaff, K. S. Powell, J. A. Gatehouse, R. Raemaekers, C. A. Newell, W. D. O. Hamilton, *Eur. J. Biochem.* **1998**, 252, 59–65 leads to denaturation and formation of monomeric wild-type GNA.
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Antiquenching Luminescence

Temperature Antiquenching of the Luminescence
from Capped CdSe Quantum Dots**

Sander F. Wuister, Arie van Houselt,
Celso de Mello Donegá, Daniël Vanmaekelbergh, and
Andries Meijerink*

Their efficient and tunable luminescence make semiconductor quantum dots (QDs) very promising for application in optoelectronic devices and as luminescent labels.^[1–3] Surface passivation with aliphatic capping molecules, such as trioctylphosphane (TOP), trioctylphosphaneoxide (TOPO) and hexadecylamine (HDA), plays a crucial role in obtaining luminescence quantum yields close to unity.^[4,5] Herein we demonstrate that the quantum yield of efficient TOP/TOPO/HDA-capped CdSe QDs gradually decreases above 20 K but

[*] S. F. Wuister, A. van Houselt, Dr. C. de Mello Donegá, Prof. Dr. D. Vanmaekelbergh, Prof. Dr. A. Meijerink
Chemistry and Physics of Condensed Matter
Debye Institute, Utrecht University
Princetonplein 5, 3584 CC Utrecht (The Netherlands)
Fax: (+31) 30-253-2403
E-mail: a.meijerink@phys.uu.nl

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increases sharply when the QDs are heated above a transition temperature of around 250 K. This observation is unique as it is the opposite of the commonly observed temperature quenching of luminescence. To understand the mechanism behind the temperature anti-quenching, the TOP/TOPO/HDA cap was exchanged with various alkylamines. We observe that the transition temperature shifts systematically to higher temperatures as the length of the alkyl chains increases. This indicates that a phase transition in the capping layer is involved and directly affects reconstruction of the CdSe surface. Surface reconstruction is required to remove surface (quenching) states.^[6] In addition, our results show how chemical exchange of the capping provides control over the temperature-dependent optical properties of QDs.

The influence of quantum confinement on the electronic and optical properties of semiconductor nanocrystals is of great fundamental and practical interest. Especially for direct bandgap II–VI semiconductors such as CdSe and CdTe, the increasing control over the size, shape, and surface chemistry has enabled the production of QDs with tunable (from blue to red) narrow-band luminescence and quantum yields close to unity.^[4,7,8] Research on these QDs continues to contribute to a better understanding of the influence of quantum size effects on the semiconductor core. The role of the semiconductor surface and its interaction with the passivation layer has not reached the same level of understanding. The capping layer is considered to be there merely to confine the charge carriers by providing a potential barrier and to passivate the dangling lone pairs of surface atoms. The large surface area makes nanocrystals ideal systems to investigate surface properties that cannot be studied in bulk systems. Even though the study of surface properties is recognized to be one of the important new areas of research in solid-state chemistry,^[9] only a few studies address this aspect of semiconductor nanocrystals. The present work provides insight into the role of the surface and the effect of its interaction with the aliphatic capping layer on the energy-level structure of CdSe QDs.

The most widely applied synthetic methods for strongly luminescent QDs are based on the growth of nanometer-sized crystallites of the semiconductor material inside a shell of coordinating molecules.^[4,7,8] The role of the ligands is twofold. First, inside a shell of coordinating ligands the semiconductor crystallites grow to a size that is determined by the kinetics of the binding and unbinding of the ligands. The second role of the ligands is to passivate dangling lone pairs to prevent nonradiative recombination at surface sites. The luminescence quantum yield is known to be very sensitive to subtle changes in the synthetic procedure, thus indicating that the surface structure is a key factor for the occurrence of band-gap states that quench the exciton luminescence.^[5,10]

For this study, high-quality CdSe QDs were synthesized by hot injection of the precursors $(\text{Cd}(\text{Me})_2)$ and Top–Se in TOP into a coordinating solvent mixture (TOPO/HDA).^[5,10] The suspension of QDs shows a bright orange exciton luminescence with a quantum efficiency of 60 % at room temperature. Luminescence decay curves are shown in Figure 1a at various temperatures between 4 and 300 K. At 4 K a biexponential decay is observed with a fast component due to emission from the singlet exciton state (spin-allowed) and a slow component

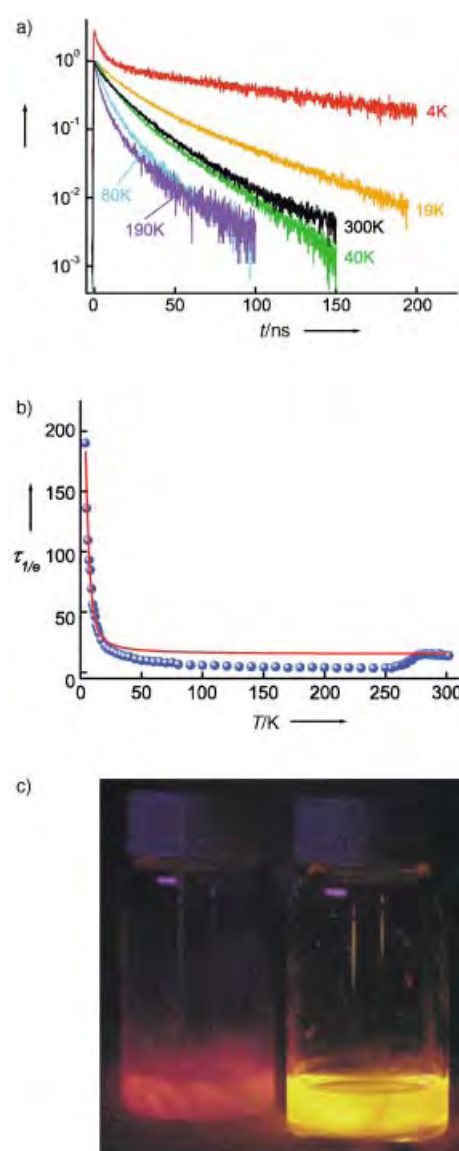


Figure 1. Temperature dependence of the exciton luminescence for TOP/TOPO/HDA-capped CdSe QDs. a) Luminescence decay curves are shown for TOP/TOPO/HDA-capped CdSe QDs at various temperatures between 4 and 300 K. Excitation is with a picosecond diode laser at 406 nm. b) The luminescence lifetime ($\tau_{1/e}$) of the exciton emission as determined from the curves in (a) is shown as a function of temperature. The drawn line is a fit to a three-level model with a lower-energy emitting triplet state and a higher-energy singlet state separated by 1.8 meV. c) The quenching of luminescence at low temperatures: Both vials contain a solution of TOP/TOPO/HDA-capped CdSe QDs and are illuminated with a UV lamp (360 nm). The vial on the left is cooled to 160 K while the brightly luminescing solution in the vial on the right is at 300 K. I = intensity, arbitrary units.

due to emission from the triplet state (spin-forbidden). As the temperature is raised, the radiative lifetime decreases due to thermalization between the triplet and singlet states similar to that previously reported.^[11–13] The temperature dependence of the lifetime is well described by a three-level model with decay rates for the triplet and singlet states of $5 \times 10^6 \text{ s}^{-1}$ and $1.25 \times 10^8 \text{ s}^{-1}$, respectively, and an energy difference of 1.8 meV between the two states. Above 20 K, the decay

curves become increasingly non-exponential (i.e., deviating from single-exponential decay) until a relatively fast ($\tau_{1/e} \approx 4$ ns) and strongly non-exponential decay curve is observed at 220 K. Luminescence life times ($\tau_{1/e}$) for the non-exponential decay curves are defined as the time in which the intensity drops to 1/e of the initial intensity. As the temperature is raised from 250 to 300 K the decay time becomes longer and the decay curves return to a single exponential with $\tau_{1/e} \approx 20$ ns. In Figure 1 b the $\tau_{1/e}$ times determined from the decay curves are plotted as a function of temperature. The drawn line represents the temperature dependence of the radiative lifetime, calculated by using the three-level model. The luminescence lifetimes are in good agreement with the measured $\tau_{1/e}$ values between 4 and 20 K and above 280 K. Between 20 and 280 K the luminescence decay is faster than that calculated, thus suggesting that nonradiative relaxation provides an additional recombination channel in this temperature regime. The non-exponential character of the decay curves shows that the nonradiative decay rates vary for different QDs. The observation that the luminescence intensity decreases between 20 and 250 K confirms the presence of nonradiative relaxation. Temperature quenching of luminescence is commonly observed and ascribed to thermally activated (phonon-induced) processes. Temperature quenching above 20 K has previously been reported for TOP/TOPO-capped CdSe QDs but without recovery of luminescence at higher temperatures.^[12]

Measurements of the intensity of luminescence between 250 and 300 K show that the lengthening of $\tau_{1/e}$ in this temperature range is accompanied by a recovery of the intensity. This is a striking observation; as nonradiative relaxation processes are usually thermally induced, it is unexpected to observe a rise in quantum efficiency with increasing temperature. The long (radiative) lifetime (≈ 20 ns), almost single exponential decay, and high quantum efficiency ($\approx 60\%$) for the CdSe QDs at room temperature show, however, that the temperature quenching of the luminescence between 20 and 250 K is reversed to temperature anti-quenching above 250 K. We conjecture that the striking recovery of purely radiative decay above the threshold temperature is due to subtle displacements of the semiconductor surface atoms induced by a phase transition in the capping layer. To verify if a phase transition is involved, the capping layer of the CdSe QDs was exchanged by using exactly the same procedure for alkylamines with linear chains varying in length between 6 and 18 carbon atoms. Capping exchange was carried out by mixing TOP/TOPO/HDA-capped QDs with a 20-fold excess of hexylamine (C_6), decylamine (C_{10}), dodecylamine (C_{12}), hexadecylamine (C_{16}), or octadecylamine (C_{18}), and stirring the mixture for 12 h at 70 °C. In Figure 2 luminescence decay curves and emission spectra are shown for CdSe QDs after capping exchange with hexylamine. Just as for the TOP/TOPO/HDA-capped QDs, the intensity of the exciton luminescence increases (Figure 2a) in the same temperature regime at which the decay curves evolve towards a single exponential while $\tau_{1/e}$ increases (Figure 2b). The temperature at which this transition takes place (≈ 235 K) is, however, considerably lower than that for the TOP/TOPO/HDA-capped QDs. In

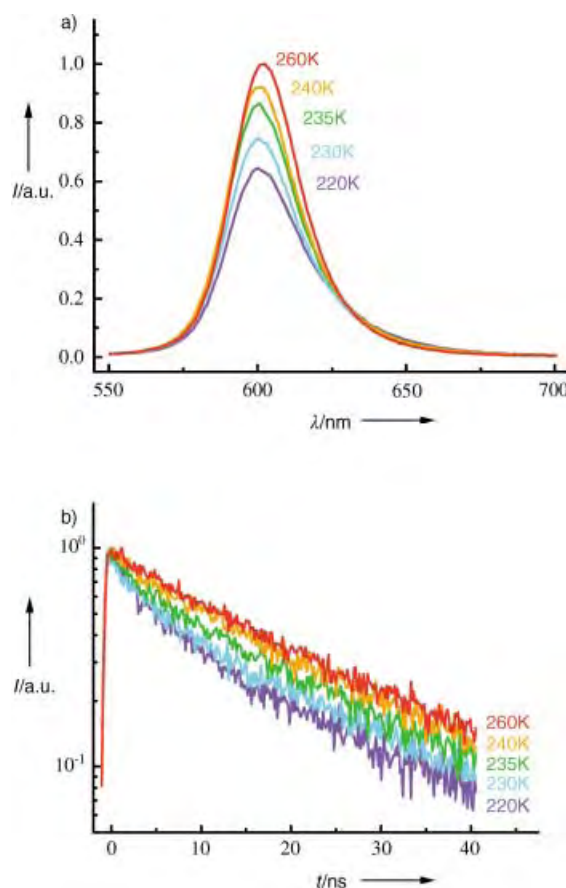


Figure 2. Luminescence of CdSe QDs after exchange of the capping molecules with hexylamine (C_6). a) The emission spectra show an increase in intensity of luminescence with increasing temperature around the anti-quenching temperature. b) Luminescence decay curves of the exciton emission at 604 nm for various temperatures around the anti-quenching temperature for the same hexylamine capped QDs. Excitation is with a picosecond diode laser at 406 nm.

Figure 3 the temperature dependence of the luminescence intensity and $\tau_{1/e}$ are plotted as a function of temperature for CdSe QDs after capping exchange with hexylamine, decylamine, dodecylamine, hexadecylamine, and octadecylamine. Figures 3 a–e show that for all types of capping layers, there is an excellent correlation between the temperature dependence of the lifetime and the luminescence intensity, which proves that the lengthening of the lifetime with increasing temperature corresponds to a decrease in the nonradiative relaxation rates. The decrease in intensity from 220 K to 240 K in Figure 3 c is due to the commonly observed quenching of the QD exciton emission.^[12] The anti-quenching temperature shifts to higher temperatures as the length of the alkyl chain increases. In Figure 3 f the anti-quenching temperature, defined as the temperature at which the intensity and luminescence lifetime have increased to 50 % of the total recovery, is plotted as a function of the number of carbon atoms in the capping alkylamine; the temperature effect is clearly shown.

With experimental evidence for the effect of a phase transition in the ligand shell on the luminescence of CdSe

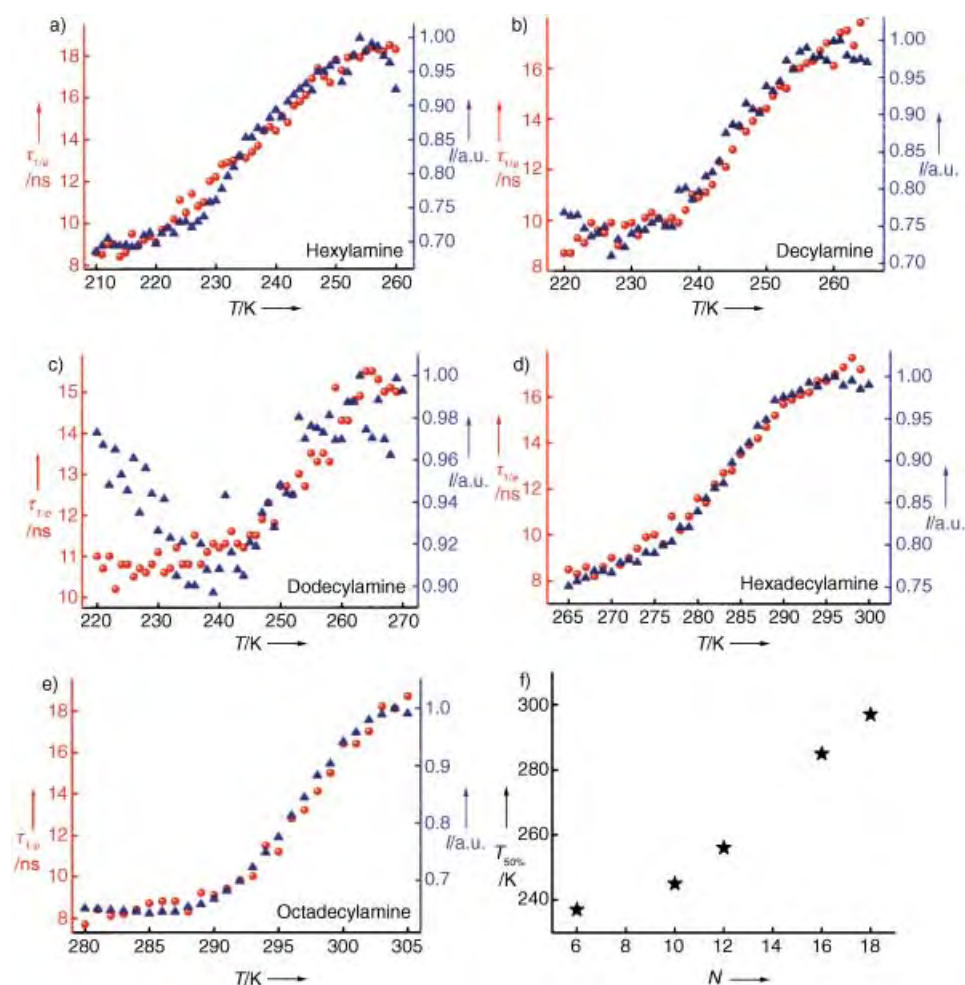


Figure 3. Influence of the capping molecules on the anti-quenching temperature for CdSe QDs. The temperature dependence of the luminescence lifetime ($\lambda_{em} = 604$ nm) and the integrated luminescence intensity (at 406 nm excitation) of CdSe QDs after capping exchange with a) hexylamine, b) decylamine, c) dodecylamine, d) hexadecylamine, and e) octadecylamine are shown. f) The anti-quenching temperatures ($T_{50\%}$) obtained from figures (a–e) are plotted as a function of the number of carbon atoms (N) in the alkyl chains of the passivating alkylamine capping molecules.

QDs, the next step was to understand the nature of the phase transition and how it affects the luminescence efficiency. Theoretical modeling of TOPO-capped CdSe QDs indicates that small displacements of the surface Cd and Se atoms are required to prevent surface (quenching) states with energies in the forbidden gap.^[6] If the capping layer of alkyl chains is in a frozen state then this surface relaxation may be hampered, which means that the luminescence-quenching centers remain active. The phase behavior of the alkylamine passivation layers on QDs has been studied and can be described by the same interactions as those in self-assembled monolayers (SAM).^[14] Experimental and theoretical work on the phase behavior of *n*-alkanes, *n*-alkylamines, and *n*-alkylthiols (SAM and bulk) shows a complex behavior with various phase transitions.^[15,16] A solid–liquid phase transition for long-chained alkylamines occurs above room temperature and cannot explain the observed changes below room temperature. At lower temperatures, phase transitions occur in the ordered phase. In the lowest temperature phase, the alkyl

chains are ordered by orientation. At higher temperatures (typically between 200 and 300 K for C_6 to C_{20} alkanes) a transition occurs to a phase in which rotational motion is possible (the locked rotator phase). At even higher temperatures a second phase transition takes place to the unlocked-rotator phase in which the tilt direction of the alkyl chains can fluctuate between nearest-neighbor molecules. Phase-transition molecular-dynamics simulations show that for SAMs of hexadecylamine, the transition temperature is around 275 K.^[15] This temperature is very close to the temperature at which the recovery of the luminescence intensity and lengthening of the decay time is observed for QDs capped with hexadecylamine. A transition to a phase in which neighboring ligand molecules can have different tilt angles is consistent with an onset of relaxation of those atoms bound to the ligands. As the tilt angle for neighboring alkyl chains can change, a vertical displacement of surface atoms bound to the polar head groups becomes possible. This surface reconstruction is not possible when the alkyl chains are ordered and have fixed tilt angles. Further evidence that supports the role of a phase transition is provided by an increase in temperature of between 10 to 15 K each time the alkyl chain length increases by two carbon atoms (Figure 3 f), which is very similar to increases previously reported.^[15,16] Room temperature X-ray scattering experiments have shown the outward displacement of surface Se

atoms and the inward displacement of Cd atoms in CdSe QDs at 300 K.^[17] Temperature-dependent X-ray-scattering experiments are expected to show that this surface reconstruction is hampered as the sample is cooled below the transition temperature of the capping layer.

The surprising observation of temperature anti-quenching of the luminescence from capped CdSe QDs gives insight into the importance of the interaction between the capping layer and the semiconductor core on the energy-level structure of QDs. It shows that the organic passivation layer not only passivates dangling lone pairs but also plays an active role in surface reconstruction. Chemical exchange of the capping molecules can be adapted to gain control over the temperature-dependent optical properties of QDs.

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complex architectures often contain coaxially stacked helices that adopt specific spatial arrangements through tertiary interactions between distinctly structured, complementary surfaces. These interactions result in the formation of expansive close-packed interfaces.^[1] Within these interfaces, atoms acquire distinct packing environments that together uniquely specify an RNA fold. To define these packing environments biochemically, we exploited a series of nucleotide analogues with 2'-substituents that span a range of molecular volumes (Figure 1). We used nucleotide analogue

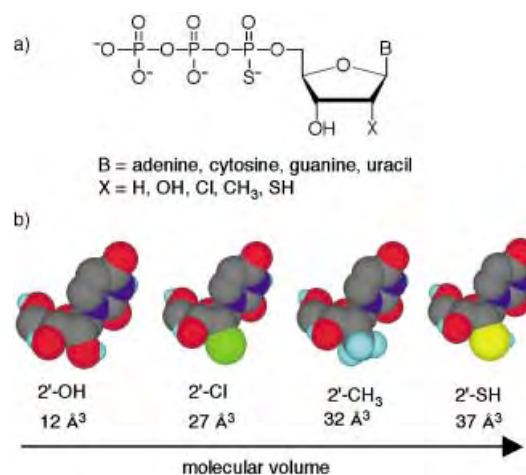


Figure 1. A series of nucleotide analogues that span a range of molecular volumes. A) Nucleotide- α -thiotriphosphates for NAIM analysis. B) Space-filling representations of the uridine analogues. The molecular volume of the 2'-substituent ranges from 12 to 37 Å³. Molecular volumes were calculated by using the approach described by Connolly^[4] and are consistent with volumes calculated from crystallographic data.^[5]

RNA Folding

A Packing-Density Metric for Exploring the Interior of Folded RNA Molecules**

Jason P. Schwans, Nan-Sheng Li, and
Joseph A. Piccirilli*

RNA molecules fold into intricate three-dimensional architectures that perform important biological functions. These

interference mapping (NAIM)^[2] to examine the effect of these volume changes at every residue within the structurally well-defined, independently folding P4-P6 RNA domain.^[3] Our results show that these analogues together provide a sensitive metric for the packing density of the atoms surrounding the 2'-hydroxy groups in an RNA molecule.

We synthesized 2'-chloro, 2'-methyl, and 2'-sulfanylnucleoside analogues of adenosine, cytidine, guanosine, and uridine and converted these analogues into the corresponding α -thiotriphosphates. We have reported the synthesis of 2'-sulfanylnucleotide analogues previously.^[6] The 2'-deoxy-2'-methylnucleoside analogues were synthesized by glycosylation of methyl 2- α -acetoxymethyl-3,5-di-*O*-(4-chlorobenzyl)-2-deoxy- α -D-ribofuranoside, followed by deoxygenation and deprotection.^[7] The procedure described by Verheyden and Moffatt served as a guide for the synthesis of 2'-chloro-2'-deoxyuridine.^[8] Displacement of the corresponding 2'- β -*O*-triflate esters with lithium chloride gave 2'-chloro-2'-deoxycytidine, adenosine, and guanosine.^[9] We prepared the α -thiotriphosphate derivatives of the chloro- and methylnucleosides as described previously for the sulfanylnucleosides.^[6]

[*] J. P. Schwans, N.-S. Li, Prof. J. A. Piccirilli
Howard Hughes Medical Institute
The University of Chicago
Department of Biochemistry and Molecular Biology
and Department of Chemistry
5841 S. Maryland Avenue, MC1028, Chicago, IL 60637 (USA)
Fax: (+1) 773-702-0271
E-mail: jpacciri@midway.uchicago.edu

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To establish a metric for RNA packing density, we used interference mapping^[2] to determine the influence of these analogues on the folding of Δ C209 P4-P6, an independently folding RNA domain derived from the *Tetrahymena* group I intron.^[3] This domain serves as an excellent model system with which to explore the physical basis of analogue interference. Crystallographic analyses have revealed the domain structure,^[3b,c] which consists of two sets of coaxially stacked helices that pack against each other to form a compact tertiary architecture. Biochemical analyses have allowed the thermodynamics and kinetics of folding of this domain to be defined.^[10] Divalent magnesium ions induce a two-state tertiary folding transition that renders the folded molecules more compact than the unfolded molecules.^[3a] Nondenaturing gel electrophoresis resolves these states and allows isolation (selection) of folded species from a population.^[6,11]

We incorporated each nucleotide α -thiotriphosphate into Δ C209 P4-P6 at random by in vitro transcription with Y639F T7 RNA polymerase.^[12] As described previously for the 2'-sulfanylnucleotides,^[6] the polymerase efficiently and evenly incorporates the 2'-chloro- and 2'-methylnucleotides into the RNA to generate populations with approximately 1–5 analogue substitutions per transcript. Folded species were selected from the population by nondenaturing gel electrophoresis. Treatment with I_2 induced cleavage of the RNA backbone at sites containing phosphorothioate linkages^[13] and thereby revealed the locations of the nucleotide analogues. Gaps in the I_2 cleavage ladder of the folded molecules compared to those of the unselected population indicate residues at which an analogue induces interference.^[14] To achieve maximum sensitivity to folding interference without compromising the signal, we conducted our experiments at 0.45 mM Mg^{2+} , the concentration corresponding to the midpoint of the Δ C209 P4-P6 folding transition.^[3c,6] Under these conditions, the selection assay detects interferences arising from as little as 0.2 kcal mol⁻¹ destabilization.^[6]

We observed little interference in simple duplex regions of Δ C209 P4-P6, which indicates that the minor groove of A-form helical RNA readily accommodates our atomic modifications. The interferences occur primarily in single-stranded regions and span the entire domain. When mapped onto the tertiary structure, the interferences cluster tightly in the three most densely packed regions of the molecule: the hinge region, the A-rich bulge/trihelical junction, and the tetraloop/receptor.^[6] The residues in these regions frequently engage in hydrogen-bonding interactions through their 2'-hydroxy groups, experience significant surface area burial, and/or exhibit strong deviations from A-form helical geometry.^[3b,c]

To explore the physical basis of these interferences, we obtained a 2'-deoxynucleotide (2'-H) interference map (Figure 2a) for comparison. Deoxynucleotide interference functionally distinguishes residues bearing dispensable (*nonfunc-*

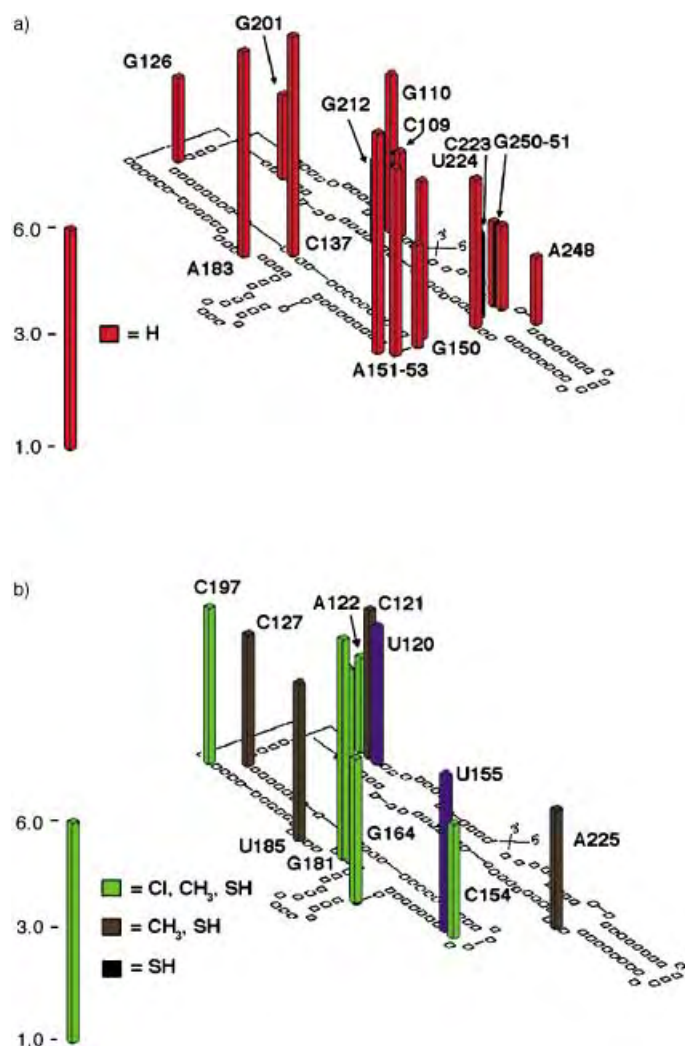


Figure 2. Interference histograms mapped onto the Δ C209 P4-P6 secondary structure. A) Deoxynucleotide interference distinguishes residues whose hydroxy groups engage in tertiary interactions from those that bear nonfunctional (dispensable) hydroxy groups. B) Superimposed interference maps for 2'-Cl, 2'-CH₃, and 2'-SH nucleotides. For clarity, the map shows only interferences at residues bearing nonfunctional hydroxy groups (no 2'-H interference). We conducted the folding selection at 0.45 mM Mg^{2+} , which corresponds to the midpoint of the Δ C209 P4-P6 folding transition,^[3c,6] and calculated interference values as described by Ryder et al.^[2e] For residues at which more than one analogue interferes, the histogram shows the maximum interference value observed. Residues that bear functional hydroxy groups generally exhibit the 2'-Cl/2'-CH₃/2'-SH interference profile. Sites at which one of the analogues interferes always also exhibit interference when an analogue of larger volume is present, such that three interference profiles emerge (green, brown, and blue bars). The data are summarized in Table 1.

tional) 2'-hydroxy groups from those bearing *functional* 2'-hydroxy groups that provide a significant energetic contribution to folding. All deoxynucleotide interferences in Δ C209 P4-P6 occur at residues whose 2'-hydroxy groups engage in hydrogen bonds, as inferred from the crystal structure.^[3b,c] The remaining residues in Δ C209 P4-P6 bear nonfunctional or dispensable 2'-hydroxy groups whose removal has little or no energetic effect on folding. Residues bearing functional hydroxy groups exhibit 2'-SH/2'-CH₃/2'-Cl interference, con-

sistent with our expectation that the analogues have little or no capacity to mediate tertiary interactions.^[15] The residues bearing nonfunctional hydroxy groups therefore provide the basis set of microenvironments with which to define a metric for packing density. Among these residues, the number of interferences increases as the analogue volume increases; the interferences induced by a given analogue comprise a subset of the interferences induced by an analogue of larger volume (Figure 2b, Table 1). These results suggest that residues bearing nonfunctional hydroxy groups in Δ C209 P4-P6 have a volume threshold above which RNA folding is destabilized.

Table 1: Δ C209 P4-P6 interference profiles at 0.45 mM MgCl_2 .

Residue	2'-Cl Int	2'-CH ₃ Int	2'-SH Int
A122	X	X	X
C154	X	X	X
G164	X	X	X
G181	X	X	X
C197	X	X	X
G200	X	X	X
C121	–	X	X
C127	–	X	X
U185	–	X	X
A225	–	X	X
U120	–	–	X
U155	–	–	X

We used the crystal structure of Δ C209 P4-P6^[3c] to determine the distances between the nonfunctional hydroxy groups and their nearest neighbors (Figure 3). Qualitatively, the interference profiles correlate with the number and closeness of neighboring atoms surrounding the hydroxy group. Residues for which all three analogues exhibit interference have the most densely packed hydroxy groups (Figure 3, left column); multiple close contacts usually occur with at least one atom residing within 2.65 Å of the hydroxy group. Residues that exhibit the 2'-SH/2'-CH₃ interference profile have less tightly packed hydroxy groups and usually only a single close contact within 3.1 Å (Figure 3, middle column). Residues that exhibit only 2'-SH interference have sparsely packed hydroxy groups whose closest atomic neighbors are between 3.2 and 3.5 Å away (Figure 3, right column). Residues that exhibit no interference have no close contacts. These observations suggest that the interference mapping volume thresholds reflect the spatial environment of the hydroxy groups in the folded state of Δ C209 P4-P6.

To define the space surrounding the hydroxy groups quantitatively, we introduced molecular volume changes at the hydroxy group sites and calculated the resulting change in van der Waals energy by using molecular mechanics.^[17] Figure 4 shows volume–energy dependencies for five representative positions in Δ C209 P4-P6. As the molecular volume increases, the calculated energy rises, which reflects the increasing van der Waals repulsion caused by steric overlap. Sites that exhibit 2'-Cl/2'-CH₃/2'-SH interference display the greatest sensitivity to molecular volume. Sites that exhibit

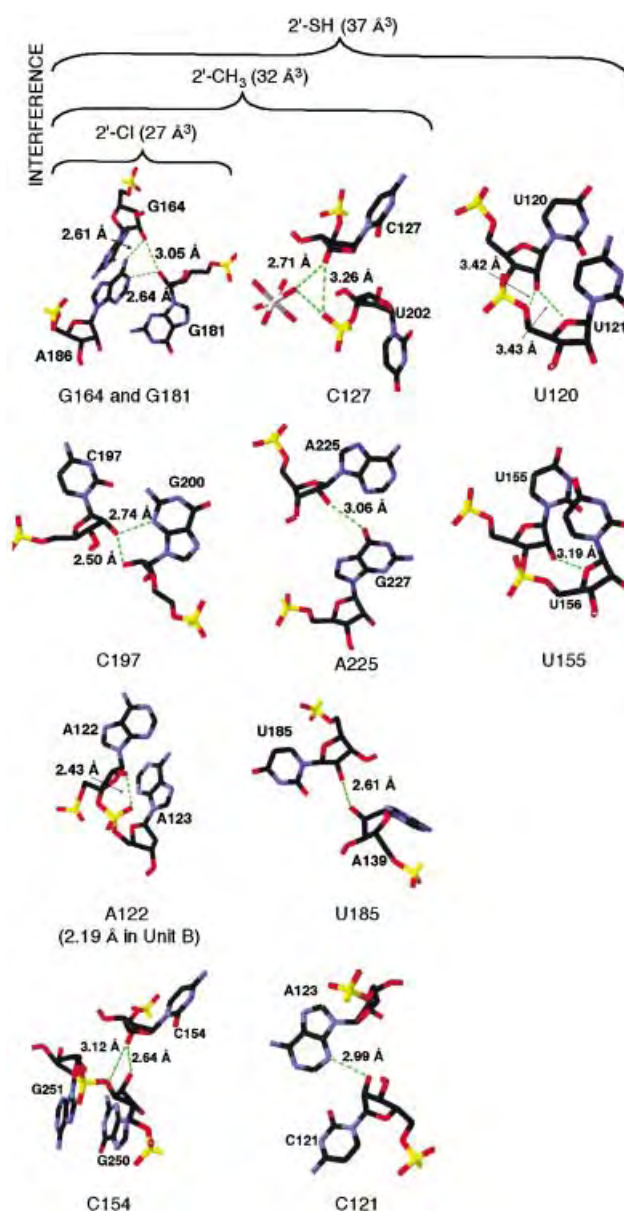


Figure 3. Contacts with neighboring atoms and interference profiles for nonfunctional 2'-hydroxy groups in Δ C209 P4-P6. Residues that exhibit 2'-SH interference have hydroxy groups whose closest atomic neighbors reside within 3.5 Å. Residues that exhibit interference in the presence of the intermediate-sized methyl group have hydroxy groups with closer neighbors (< 3.1 Å). Residues that exhibit interference caused by the smallest analogue (chlorine) have the most densely packed hydroxy groups with one or more close neighbors less than 2.65 Å away. We used Swiss-PDB Viewer software^[16] to determine interatomic distances within the Δ C209 P4-P6 crystal structure.^[3c] The atomic distances for unit A in the asymmetric unit are shown. Values for unit B are essentially the same as those for unit A, except that in unit B the 2'-oxygen atom of A122 is 2.19 Å from the phosphate oxygen atom of A123.

only 2'-SH interference have repulsive energy only after the molecular volume exceeds 35 Å³. Sites that exhibit 2'-CH₃/2'-SH interference display an intermediate sensitivity and exert significant repulsion only when the molecular volume exceeds

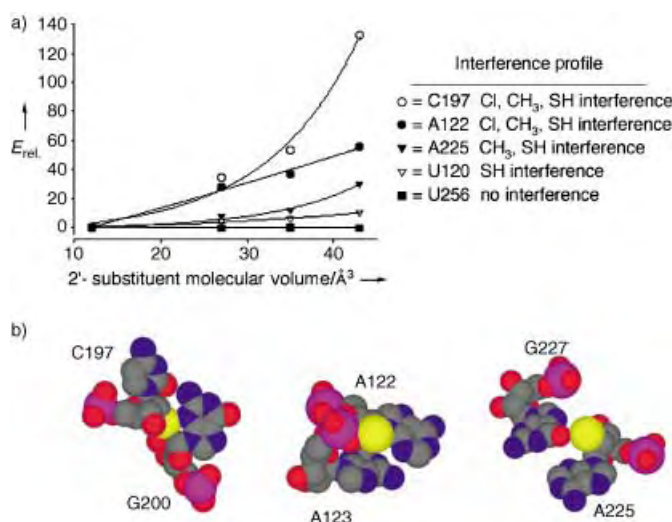


Figure 4. van der Waals repulsion determines the 2'-Cl/2'-CH₃/2'-SH interference profile. A) The dependence of van der Waals repulsion energy on molecular volume. The interference profile for a given residue matches its relative volume sensitivity. We used the Sybyl 6.9 molecular mechanics package^[17] to calculate the change in van der Waals energy upon introduction of variously sized atoms at the 2'-position (O, Cl, S, Br). The calculation included all force potentials but $\Delta C209$ P4-P6 was held rigid so that ΔE represents solely changes in van der Waals overlap. The smooth curves serve to guide the eye. B) Representative close-packed hydroxy groups and their local environment in space-filling representation. The models contain sulfur atoms (yellow) in place of the 2'-hydroxy groups.

30 $\text{\AA}^3$. These trends indicate that the interference profiles reflect the volume–energy dependencies.

The multifunctional hydroxy group exerts its influence on RNA structure and function in multiple ways, for example, by mediating tertiary interactions through hydrogen bonding or metal-ion coordination, or by serving as a scaffold for the integral hydration network associated with RNA.^[1] The 2'-hydroxy group may also make indirect contributions to structure and function as a consequence of its polar character and capacity to fill space, or its ability to withdraw electrons inductively or engender a ribonucleotide with a preference for the 3'-endo sugar conformation.^[18] These effects apparently have no energetic significance for folding at residues bearing dispensable 2'-hydroxy groups (that is, residues that do not interfere upon 2'-deoxynucleotide substitution). Nevertheless, the nucleotide analogues described herein can “induce” $\Delta C209$ P4-P6 folding interference when introduced at these locations. The analogues may act at several levels, which include, but are not necessarily limited to, 1) sugar conformational preferences, 2) hydrophobicity, and 3) molecular volume.

$\Delta C209$ P4-P6 can be used as a model system to define the influence of atomic modifications on folding.^[6] In addition to the analogues described herein, we have defined the influence of 2'-fluoro-, 2'-amino-, and 2'-methoxynucleotides on folding in the context of this RNA domain.^[6,19] Only the 2'-fluoronucleotide analogues induce interference in accordance with their strong preference for the 3'-endo sugar conforma-

tion by destabilizing the tertiary structure when located at residues that populate the 2'-endo conformation in the folded RNA.^[2d,19,20] We observe no link between interference and nucleoside analogues that preferentially populate the 2'-endo conformation.^[6,19] These sugar pucker preferences evidently have little energetic influence on the $\Delta C209$ P4-P6 tertiary folding transition, which was monitored by using the electrophoretic mobility shift assay. We did not observe a link between interference and the hydrophobic character of the introduced substituent, though we cannot rule out the possibility that hydrophobicity has some influence on tertiary folding stability.^[18] Overlapping molecular volumes exert the most significant energetic force in biology, since the repulsive energy between two atoms increases exponentially with the inverse of the distance between the centers of the two atoms.^[21] Our observation that $\Delta C209$ P4-P6 interferences correlate with molecular volume is in agreement with this relationship.

In summary, we have synthesized 2'-chloro-, 2'-methyl-, and 2'-sulfanylnucleoside- α -thiotriphosphates and have demonstrated that T7 RNA polymerase efficiently incorporates these compounds into RNA without sequence bias, which renders interference mapping analysis possible. These nucleoside analogues span a narrow range of molecular volume ($\Delta V = 10 \text{ \AA}^3$) and together provide a sensitive measure of the spatial environment (the packing density) of a 2'-hydroxy group within a structured RNA molecule. Many residues within RNA bear nonfunctional hydroxy groups that reside within the extensive packing interfaces formed by tertiary folding. Our packing density metric (PDM) defines these interfaces biochemically. PDM analysis may help to evaluate the functional validity of crystallographic and biochemical models of RNA structure and may reveal regions within RNA that undergo structural changes whilst carrying out their function.

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Cubic Mesoporous Frameworks with a Mixed Semiconductor Nanocrystalline Wall Structure and Enhanced Sensitivity to Visible Light**

Michael H. Bartl, Stefan P. Puls, Jing Tang,
Helga C. Lichtenegger, and Galen D. Stucky*

Since the discovery of mesostructured materials in the early 1990s^[1] a great deal of effort has been devoted to developing new structures, compositions, and functionalities.^[2] The ability to synthesize semiconducting single- or multicomponent metal-oxide mesostructured materials with controlled morphologies^[3] has been a major step towards functional materials with potential applications in sensing, catalysis, and optoelectronics.^[4] We describe herein the synthesis and properties of a new generation of ordered mesostructured composites with mesopore walls composed of integrated 3D arrays of different types of wide and narrow band-gap semiconductor nanocrystals including oxides, sulfides, and selenides. Such an integrated 3D coassembly of different functional units is of great importance for optoelectronic applications that rely on the transfer of electrons or holes between two or more active components. Examples of such systems are sensitized titania solar cells and photocatalysts, for which it has been shown that solar conversion efficiency can be strongly increased in the presence of sensitizing dye molecules or narrow band-gap semiconductor nanocrystals.^[5] Fabrication techniques previously developed for such functionalized composite titania materials are based on step-by-step methods in which organic molecules or premade semi-

[*] Dr. M. H. Bartl, S. P. Puls, Dr. H. C. Lichtenegger,
Prof. Dr. G. D. Stucky
Department of Chemistry and Biochemistry
University of California, Santa Barbara
Santa Barbara, CA 93106 (USA)
Fax: (+1) 805-893-4120
E-mail: stucky@chem.ucsb.edu
J. Tang, Prof. Dr. G. D. Stucky
Materials Department
University of California, Santa Barbara
Santa Barbara, CA 93106 (USA)
Dr. H. C. Lichtenegger
Institut für Werkstoffkunde und Materialprüfung
Technische Universität Wien
1140 Wien (Austria)

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conductor nanocrystals are grafted or deposited onto the surface of beforehand patterned titania film matrices.^[6] In contrast, the mixed nanocrystalline anatase titania/cadmium chalcogenide mesoporous films presented here are prepared by a fast and inexpensive single-run supramolecular templating route based on acidic sol-gel chemistry. By selectively using competing molecular self-assembly, phase transitions and stabilities as well as crystallization kinetics, we are able to combine synthesis, assembly, and patterning into a one-pot single-precursor dip-coating process followed by heat treatment of the samples with varying gas/vapor atmospheres.

The mesoporous composite films were prepared by dip-coating from a highly acidic solution containing titanium(IV)-ethoxide and cadmium chloride as precursors for the wide or narrow band-gap nanocrystalline semiconductors, respectively, and a non-ionic triblock copolymer as the mesostructure-directing species. It has been shown that under highly acidic conditions, the hydrolyzed titania nanoentities are not prone to cross-link and can be stabilized over an extended period of time. This gives the structure-directing surfactant enough time to assemble into its most stable thermodynamic phase, which is determined primarily by the temperature and the volume ratio of inorganic species to surfactant.^[3d,e] For the volume ratio of Cd/Ti to surfactant used here, a stable cubic mesostructural order was obtained by storing the dip-coated films at 10°C. The films were then subjected to an extended heat-gas/vapor treatment during which they changed from a liquid-crystalline hybrid organic-inorganic mesostructured composite to a mesoporous framework built up of integrated arrays of different semiconductor nanocrystals. The films were first kept in an oxidative high-temperature environment to remove the surfactant and promote condensation of the solubilized titania nanoentities, followed by nucleation and growth of semiconducting anatase nanocrystals out of the amorphous titania matrix. Simultaneously, the coassembled molecular cadmium species separates into cadmium oxide nanoclusters, which are evenly distributed in the titania framework in accordance with previous studies of sol-gel matrices doped with transition-metal ions.^[7] By changing the processing conditions to an S- or Se-vapor atmosphere diluted with an inert gas, these cadmium oxide nanoclusters are then selectively converted into crystalline semiconducting CdS or CdSe nanoparticles through a redox-coupled ion-exchange reaction mechanism.^[8] After only a few minutes of exposure to S or Se vapor, the films became yellow or orange (Figure 1 f), indicating the successful transformation of cadmium oxide into its nanocrystalline sulfide or selenide analogues.

The mesostructural order and wall composition of the final films were determined by high-resolution scanning electron microscopy (HRSEM) and transmission electron microscopy (TEM) in combination with energy dispersive X-ray spectroscopy (EDX). The body-centered-cubic (bcc) mesopore arrangement was confirmed by TEM imaging along the [100] and [111] crystallographic axes. Representative TEM micrographs demonstrate the high mesostructural order of both the titania-CdSe (Figure 1 a and b) and titania-CdS (Figure 1 c) composite films. Additionally, HRSEM imaging reveals a completely open-pore surface of the film

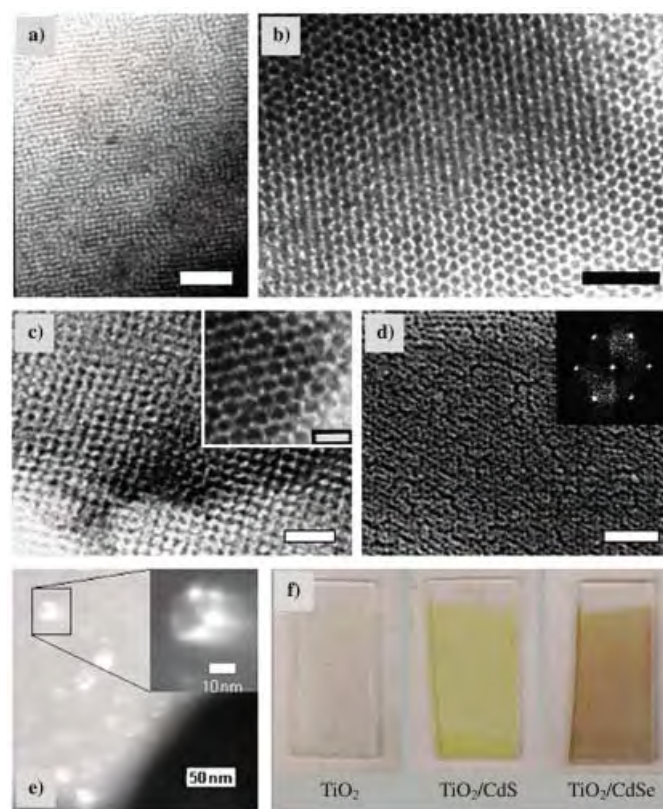


Figure 1. Electron micrographs and optical photographs of cubic mesoporous nanocrystalline anatase titania/cadmium chalcogenide composite films. a–c) TEM images of titania/CdSe, (a and b), and titania/CdS (c) thin films. Images shown in a) and c) were recorded along the [100] zone axis, whereas image b) and the inset of c) give a view along the [111] zone axis of the bcc symmetry. d) SEM image of the open-pore surface of a titania/CdS film (inset shows the Fourier transform of the open-pore symmetry). e) Bragg-contrast image in dark field TEM of a titania/CdS sample showing individual nanocrystals scattering in the off-centered objective aperture. f) Photographs of mesoporous pure titania and composite titania/cadmium chalcogenide films. The scale bars are 100 nm in Figure a) and d), 50 nm in Figure b) and c), and 20 nm in the inset of Figure c).

(Figure 1 d) with the [111] direction of the bcc mesopore symmetry running perpendicular to the film surface, as indicated by the hexagonal Fourier pattern (Figure 1 d inset). This image confirms that the continuously cubic ordered and interconnected mesoporous framework is extended throughout the film and thus fully accessible from the surface, which is a particular important property with respect to infiltration of the mesoporous framework with, for example, hole-conducting conjugated polymers.^[9] Furthermore, from quantitative EDX analysis we found a homogeneous cadmium distribution within the mesopore walls (Cd:Ti molar ratios are the same as in the respective precursor solutions) and a constant Cd:S or Cd:Se ratio of unity, thus indicating a quantitative oxygen-chalcogenide exchange during the S or Se vapor treatment.

We studied the crystallinity of the composite mesoporous framework by Bragg-contrast imaging in dark-field TEM and XRD analysis. Individual nanocrystals (3–6 nm in size) can be

imaged in Bragg contrast in the form of bright spots at various places within the mesoporous framework walls (Figure 1 e). The position of illuminated spots changes quickly upon tilting as different lattice planes of the various crystallites fulfill the diffraction condition and therefore diffract into the off-centered objective aperture. Both the type and structure of these nanocrystals were investigated by means of XRD. Figure 2 shows a comparison of the diffraction patterns of a nondoped titania mesoporous film (pattern A) and those doped with Cd and treated with either S or Se vapor (patterns B and C, respectively). While the diffraction peaks obtained from the nondoped films can be indexed according to an anatase phase, the patterns of the two composite films exhibit a number of additional diffraction peaks (Figure 2 a), which can all be assigned to reflections that are typical for a CdS and CdSe wurtzite structure (Figure 2 b). Due to the small size of the anatase, CdS, and CdSe crystallites, all of the diffraction peaks are strongly broadened. By applying the Scherrer formula to the peak widths of selected reflections (deconvoluted with standard peak fitting routines), we estimated the size of the crystallites to be between 4 and 7 nm.

To test for cooperative functionality of our nanocrystalline composite mesoporous films we measured their visible-

light photocatalytic activity. CdS nanocrystals are known to have a sensitizing effect on titania, since they combine a high absorption cross section for visible light with an energy-band structure that allows injection of the photoexcited electrons into the titania conduction band,^[6b,d,10] from where they are transferred along the mesoporous framework by a hopping mechanism, which is typical for nanocrystalline anatase titania composites.^[5a] We analyzed the solar sensitization efficiency of our nanocrystalline mixed anatase titania–CdS mesoporous composite films of 200 to 300 nm thickness by comparing their ability to generate a visible-light photocurrent (I_{ph}) with that of pure anatase titania mesoporous films. The setup for these measurements was a standard two-electrode photoelectrochemical cell working in non-regenerative mode as described elsewhere.^[5a] To compensate for small deviations from the sample and setup, such as film thickness and roughness or angle of incident light, all measurements were done in two steps. First we determined the I_{ph} response to UV and visible photons simultaneously (UV + vis) and used this value as maximum photoinducible I_{ph} under the given illumination, since in this case the whole film (anatase and CdS) is able to absorb light. The effect of visible-light sensitization expressed as the I_{ph} response to visible photons only (vis-only) was then determined with the same setup by blocking the UV photons with a 400 nm longpass filter. Typical experimental results for pure anatase titania and composite anatase titania/CdS (5 mol % CdS) mesoporous films are given in Figures 3 a and b, respectively. As expected, both samples exhibit a well-defined periodic on/off I_{ph} response to the light/dark modulated UV + vis illumination (left part of response curves). However, by switching from UV + vis to vis-only illumination (right part of

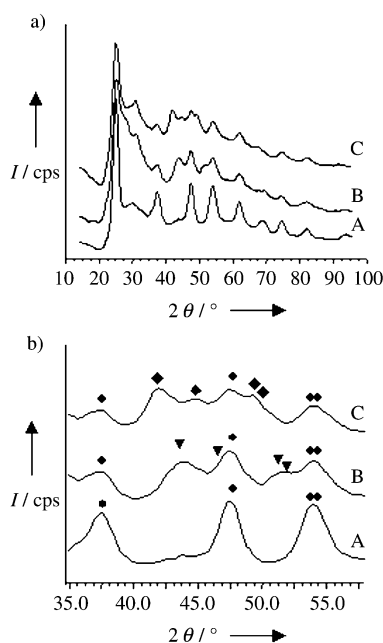


Figure 2. a) X-ray diffraction patterns of cubic mesoporous nanocrystalline pure anatase titania (pattern A) and composite nanocrystalline anatase titania/CdS (pattern B) and anatase titania/CdSe (pattern C) film samples. The diffracted intensities were plotted versus scattering angles $2\theta_{Cu}$ as would be observed with $Cu_{K\alpha}$ radiation, for better comparison with the literature. The initially obtained scattering angles $2\theta_{Mo}$ from the measurement with $Mo_{K\alpha}$ radiation were converted to $2\theta_{Cu}$ according to $\theta_{Cu} = \arcsin(\lambda_{Cu}/\lambda_{Mo} \sin\theta_{Mo})$, where λ_{Cu} and λ_{Mo} are the wavelengths of $Cu_{K\alpha}$ and $Mo_{K\alpha}$ radiation, respectively. b) Detailed comparison in the range 35 to 58° of $2\theta_{Cu}$ of the three diffraction patterns; dots indicate the (004), (200), and (105/211) reflections of the anatase nanocrystals; triangles and diamonds mark the (110), (103), (112), and (201) wurtzite reflections of the CdS and CdSe nanocrystals, respectively. Patterns are offset in X-ray intensity I .

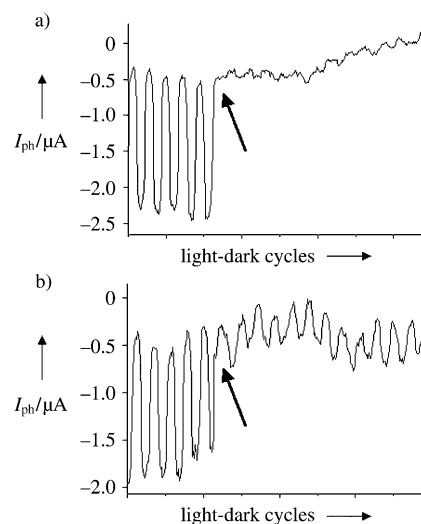


Figure 3. Periodic on/off photocurrent I_{ph} response curves of a) nanocrystalline pure anatase titania and b) nanocrystalline composite anatase titania/CdS cubic mesoporous films deposited on fluorinated tin oxide conducting glass electrodes and illuminated with a periodically light/dark modulated xenon lamp; arrows indicate the switching from illumination with UV and visible photons simultaneously (left side of the arrow) to illumination with visible photons only (right side of the arrow).

response curves), the pure anatase titania mesoporous films failed to produce a measurable I_{ph} , whereas the composite anatase titania–CdS mesoporous film was still able to generate a clear on/off I_{ph} response, which is around 25 % of the total UV+vis I_{ph} . It should be emphasized that the conservation of 25 % of the maximum photoinducible current under vis-only illumination is exceptionally high and points to an efficient intrinsic visible-light-sensitization effect, considering that under vis-only excitation only the 5 % CdS portion of the film contributes to the absorption process. These first results are very promising and we are currently exploring sensitization effects in a number of new mesoporous titania composites with integrated zinc-, lead-, and silver chalcogenide nanocrystals.

In this work we have presented a novel approach for the simultaneous synthesis and 3D organization of different optoelectronically active species. The procedure is fast, inexpensive, and suitable to be integrated into large area device fabrication techniques. Our cubic mesoporous films have a highly crystalline wall structure composed of integrated arrays of wide and narrow band-gap semiconductor nanocrystals, as determined by a combination of several analytical methods. Furthermore, we demonstrated the cooperative functionality of these composite nanocrystalline materials by successful proof-of-principle visible-light sensitization of a cubic mesoporous anatase titania film with cadmium sulfide nanocrystals acting as sensitizing integral part of the mesopore wall structure. Given the generality and flexibility of sol–gel self-assembly chemistry the fabrication approach presented here opens the field to a multitude of new cooperatively functional materials with custom-made nanostructures and compositions.

Experimental Section

Appropriate amounts of anhydrous CdCl_2 were dissolved in HCl (2.7 mL, 12.1 M) before titanium(IV)ethoxide (3.9 mL) was added and hydrolyzed while the mixture was cooled with ice/water and vigorously stirred for 5 minutes (ratio of Cd to Ti was varied between 1 and 10 mol %). This mixture was combined with a solution of poly(ethylene oxide)₂₀-poly(propylene oxide)₇₀-poly(ethylene oxide)₂₀ surfactant (1 g; Pluronic P123, BASF) dissolved in ethanol (12 g) and stirred for another 10 minutes before thin films were prepared by dip-coating onto cleaned glass slides at a speed of 1 mm s⁻¹. The films were dried at room temperature and then stored for at least 6 h at 10 °C. Calcination and sulfur or selenium vapor treatment was performed in a tube furnace. First the samples were heated in air to 400 °C at 1 °C min⁻¹ and kept there for 5 h. Then the temperature was reduced to 250 °C or 300 °C at 5 °C min⁻¹ and around 3 g of elemental sulfur or selenium were placed in the tube in front of the samples under a slow argon gas stream. After 10 to 30 minutes of vapor treatment the remaining sulfur or selenium was removed from the tube and the films were heated under argon at 5 °C min⁻¹ to 350 °C and held there for 15 minutes before they were allowed to cool down to room temperature at a rate of 10 °C min⁻¹.

A JEOL 2000FX electron microscope operating at 200 kV was used for TEM imaging and EDX spectroscopy. HRSEM images were taken on a JEOL 6340F electron microscope. XRD measurements were done on a SMART diffractometer (MoK_α radiation) equipped with a charge coupled device (CCD) detector. For the photocurrent I_{ph} measurements the mesoporous films with a thickness of 200 to 300 nm (as determined by cross-sectional SEM investigation) were

deposited on fluorinated tin oxide conducting glass electrodes and immersed in a sodium acetate electrolyte solution (0.1 M) with a platinum counter electrode. The films were excited on an area of 0.25 cm² by the chopped (≈ 0.25 Hz) emission of a broadband xenon lamp (150 W). The light/dark short circuit I_{ph} response under zero bias was recorded with a Philips amperemeter.

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Modification of 2D Water That Contains Hexameric Units in Chair and Boat Conformations—A Contribution to the Structural Elucidation of Bulk Water**

Patricia Rodríguez-Cuamatzi, Gabriela Vargas-Díaz, and Herbert Höpfl*

Given that water is the most abundant and cheapest solvent available for the development of new reagents and the study of chemical processes, it is not surprising that still the major part of research is related somehow to this substance. This fact is clearly also because water is nature's solvent, thus playing a fundamental role in almost all branches of natural sciences. In view of this relevance, it might be therefore somewhat surprising that there is still intensive research being carried out that concerns, among other aspects, the structure of this medium in the gaseous,^[1–3] liquid^[2–5] and solid state.^[6–8] There are two factors that make the study of water difficult. The first factor is that the hydrogen bond between water molecules is highly flexible so that they can aggregate into a variety of small and medium-sized clusters, which form the basic units for the construction of larger 1D, 2D, and 3D structures including clathrates.^[2–3,9] The second factor is that a series of low-energy, dynamic processes are occurring, at least in the gaseous and liquid phase.^[1–3] The need for detailed knowledge of the hydrogen bonding and the rearrangement dynamics between water molecules distributed in two and three dimensions arises from the fact that these processes rule the anomalous physical and chemical properties of liquid and solid water,^[3,4] the processes that occur at the ice–liquid, ice–air and liquid–air interfaces^[10–12] as well as the interaction between water and solutes.^[11]

From an experimental point of view, so far a significant progress has been made with respect to the structural characterization of discrete small^[1–3,9,13–15] and medium-sized^[16–18] water clusters (H_2O)_n ($n = 2–100$) as well as the characterization of 1D aggregates such as chains and tapes.^[19–21] However, only disperse information is available on 2D layers.^[9,19–29] Many of these layers are sandwich inclusion structures, in which the 2D water sheets are composed either of puckered edge-sharing pentameric clusters or result from the combination of cyclic tetrameric, pentameric, hexameric and/or (very rarely) larger clusters.^[9,21] Only a few structures that contain solely edge-sharing

hexameric rings have been characterized so far by X-ray crystallography and, interestingly, the conformations of the water layers have been different in each case, being all-chair,^[24] all-boat^[25] or an intermediate of them.^[26–29] Of these conformers, only that having an all-boat conformation^[25] has a structural motif similar to the one found in ice I_h (Figure 1).^[30] Although one of the structures has an all-chair conformation,^[24] it is apparent that its configuration (all-*cis*) is different from the one found in ice I_h (all-*trans*).

We report herein on the structure of a 2D sheet of water molecules: 1) that in close analogy with ice I_h contains two different types of hydrogen-bonded hexameric rings, one with a chair and the other with a boat conformation, in which 2) all water molecules are involved in hydrogen bonds only, and which 3) have a clearly defined hydrogen-bonding system. We consider that the description of this structure is fundamental

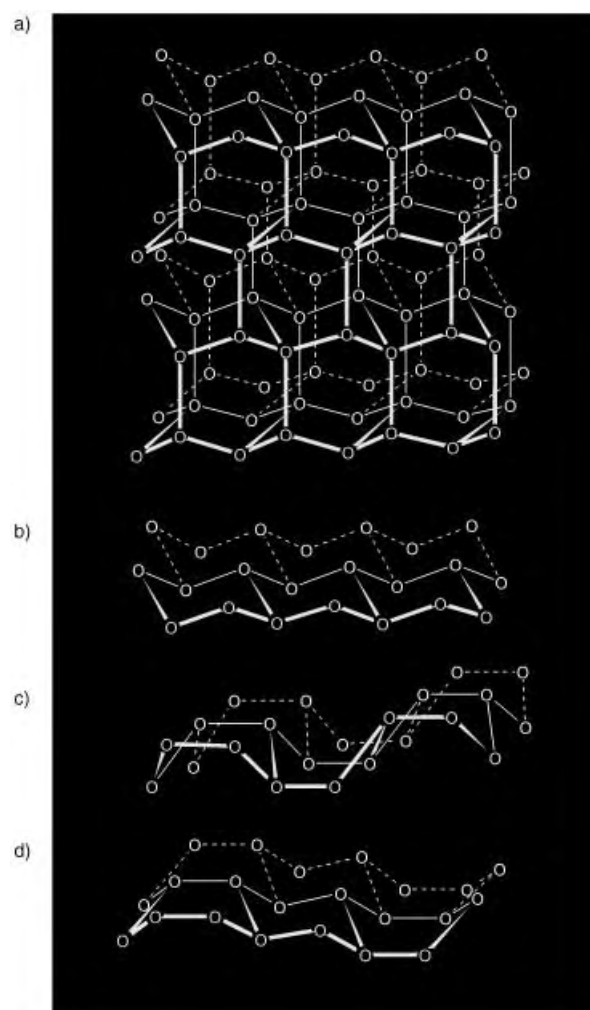


Figure 1. Comparison of the hydrogen bonding pattern found in 1,4-[B(HO)₂]₂C₆H₄·4 H₂O with that of hexagonal ice I_h : a) 3D structure of hexagonal ice I_h ; b) fragment of the structure of hexagonal ice I_h showing a 2D sheet with all hexameric rings in the chair conformation; c) fragment of the structure of hexagonal ice I_h showing a 2D sheet with all hexameric rings in the boat conformation (this pattern is also observed in the 2D water cluster described in reference [25]); d) 2D hydrogen-bonding pattern found for 1,4-[B(HO)₂]₂C₆H₄·4 H₂O with hexameric rings that have both chair and boat conformations.

[*] P. Rodríguez-Cuamatzi, G. Vargas-Díaz, Dr. H. Höpfl
Centro de Investigaciones Químicas
Universidad Autónoma del Estado de Morelos
Av. Universidad 1001, C.P. 62210 Cuernavaca (Mexico)
Fax: (+52) 777-329-7997
E-mail: hhopf@buzon.uaem.mx

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to understanding better the chemical processes that occur at the surface of water or at an interface, since it is known that cyclic hexameric water clusters are present in liquid water,^[3,4] participate in the solvation process of surfactants^[4] and are the only clusters found in the normal-pressure crystalline modifications of ice.^[30]

Crystals of 1,4-[B(OH)₂]₂C₆H₄·4H₂O were obtained after recrystallization of 1,4-phenylenediboric acid (pdba) in water.^[31] The IR spectrum of the crystals recorded on KBr showed two relatively sharp bands at $\tilde{\nu}$ = 3292 and 3408 cm⁻¹. To determine whether these bands represent the stretching modes of the water molecules, the stretching modes of the B–OH hydroxy groups, or both, a sample was heated at 250 °C for 2 h under vacuum to eliminate all water molecules from the crystal lattice. Since the IR spectra of the dried and hydrated samples were practically identical and considering that the stretching vibrations of hydrogen-bonded water molecules are generally weak and give rise to broad bands,^[29] it was concluded that the water stretching modes are hidden below the vibrational frequencies of the B–OH hydroxy groups.

The asymmetric unit of the triclinic crystal lattice contains one pdba molecule and four water molecules (space group *P* $\bar{1}$). The pdba molecules are oriented in such a way that supramolecular (pdba)_n chains are generated through point reflection at crystallographic inversion centers (Figure 2a). These polymeric chains are held together by pairs of BO–H··O(H)B hydrogen bonds that have a hydrogen-bonding pattern similar to that found in carboxylic acid dimers.^[32] This motif is commonly observed in molecules with phenylboronic acid groups.^[33] The two boronic acid moieties in each pdba

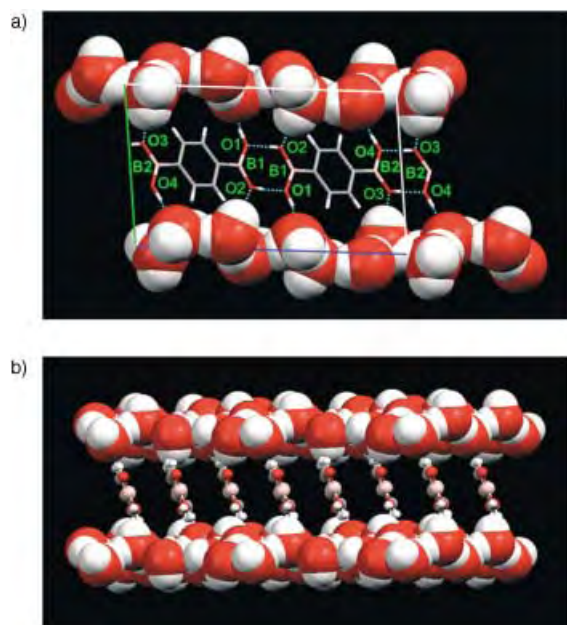


Figure 2. Fragment of the crystal lattice showing the intercalation of parallelly stacked supramolecular chains of pdba between two 2D-sheets of water: a) perspective view showing the dimeric units formed between the pdba molecules and the hydrogen bonding interactions to the water sheets; b) perspective view in direction of the supramolecular chains: grey, carbon; red, oxygen; pink, boron; white, hydrogen.

molecule are independent of each other, but have practically identical hydrogen-bonding geometries, O··O = 2.74 Å. The supramolecular chains are aligned parallel to each other, with neighboring chains being separated by a distance of 3.2 Å. To guarantee optimized π – π interactions, these chains are displaced with respect to each other. In the crystal-lattice layers of parallelly stacked diboronic acid chains are intercalated between 2D sheets of water molecules located in the *ac* plane of the crystal lattice (Figure 2b). The separation between the water layers is 8.5 Å. When viewed along axis *b*, the six-membered water rings are arranged in a distorted honeycomb-like manner (Figure 3a). Of the three independent cyclic water hexamers that are present in the crystal lattice (A, B, and C, Figure 3a), two of the rings (A and C) are each formed between only two crystallographically independent water molecules (O13 and O14 for ring A; O11 and O12 for ring C), and both rings have an inversion center and a chair conformation (Figure 3b). The third independent hexameric ring, B, is not located at a crystallographic symmetry center,

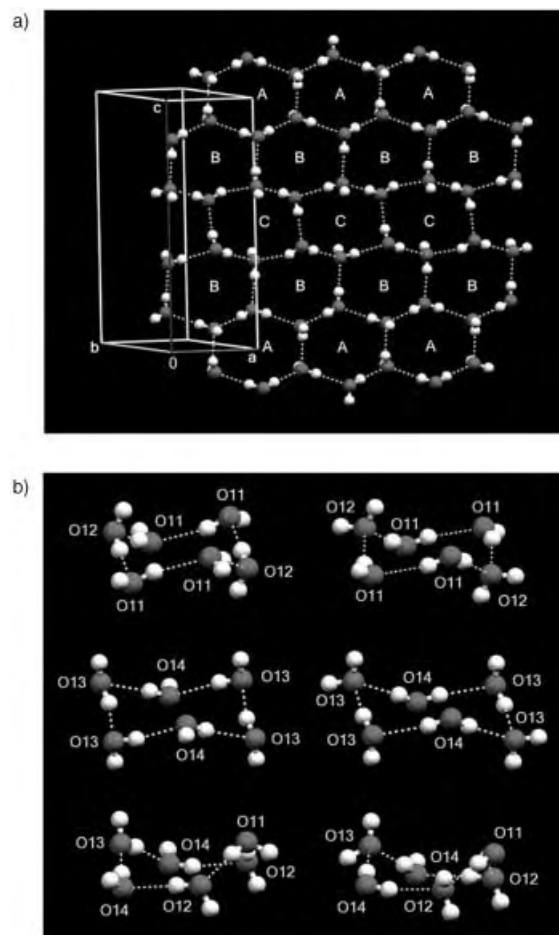


Figure 3. Ball-and-stick view of the 2D water sheet: a) presentation showing the overall hydrogen bonding interactions and the presence of three independent hexameric rings, which are arranged in tapes, which have alternately chair (A, C) and boat (B) conformation; b) view showing the conformations of the three independent hexameric rings with the two different hydrogen-bonding patterns observed in each. Top: rings C; Middle: rings A; Bottom: rings B. Gray, oxygen; white, hydrogen.

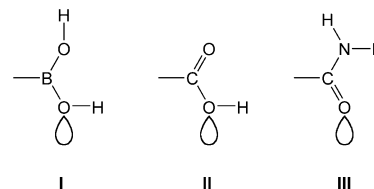
although it contains two O12 and two O14 oxygen atoms. Unexpectedly, this ring has a boat conformation, which can be seen also from Figure 3 b. The O...O distances in the hydrogen bonds found in this supramolecular structure of water range from 2.77–2.85 Å, with an average value of 2.81 Å. For comparison, the O...O separations in ice I_h ($T=60$ K) and liquid water are 2.75 and 2.85 Å, respectively.^[9,30] The O...O...O bond angles vary from 92.8 to 122.2°, thus deviating only up to 16.7° from the ideal tetrahedral angle found in ice I_h .^[30] The overall conformation of the 2D sheets of water molecules shown in Figure 1 d results from the alternate combination of hexameric-ring tapes that have all-chair and all-boat conformations. This motif is different from those found in ice I_h (Figure 1 a–c) and the water layers reported so far,^[19–29] and it may be therefore considered as a new phase of 2D ice.

Of the four independent water molecules present in the crystal lattice, each molecule is involved in a maximum of four hydrogen bonds. For the formation of the 2D water layer, each water molecule must participate in three hydrogen-bonding interactions. This means that for every oxygen atom, one further hydrogen bond is available for an interaction with one of the four B–OH groups, thus giving an extended 3D hydrogen-bonding system. The interactions between the intercalating 2D water sheets and the guest molecules occur simultaneously above and below the plane (Figure 2). The O...O separations from the water sheets to the pdba molecules are 2.72–2.83 Å; therefore, the hydrogen-bonding interactions are slightly stronger than those in the interior of the 2D sheet (see above). It is important to notice that like in the all-chair and all-boat conformations outlined in Figure 1 b and c, 50% of the interface water molecules can interact through a hydrogen bonding interaction with a substrate above the sheet and the other 50% with a substrate below that sheet. If one considers that the “ultimate” layer between bulk water and extended systems, such as hydrophilic and hydrophobic macromolecules or polymers, corresponds to the contact surface at which the chemical interactions between solvent and substrate take place, a detailed structural knowledge of these interfaces is essential for a more complete understanding of biochemical and pharmacological processes, surface chemistry, solvation, etc.^[10–12]

A detailed analysis of the hydrogen-bonding scheme in the water layers of 1,4-[B(OH)₂]₂C₆H₄·4H₂O shows that only the 2D hydrogen-bonding pattern shown in Figure 3 a is present. As required by the space-group symmetry ($P\bar{1}$), this pattern has inversion centers, which causes the neighboring hydrogen-bonded rings of the same type (A, B, or C) to have two different motifs (Figure 3 b). Because of these different motifs and since the inclined orientation of the pdba chains with respect to the 2D sheets causes a displacement of the hydrogen bonding patterns in neighboring water layers (by $|a|$ along axis a), in the final crystal structure, each water molecule is connected to three hydrogen atoms, of which the one directed to the B–OH group has an occupancy of 1.0 and the other two have an occupancy of 0.5.^[31]

Since the hydrogen bonding system in 2D water layers is highly flexible, the 2D water sheet described herein may represent one of the possible structures for the surface of

water or ice, especially in the presence of a macromolecular or polymeric system that has a spatial distribution of functional groups capable of hydrogen bonding in a similar way to pdba. This statement is not empty when one considers that the boronic acid moiety can be structurally related to biochemically important functional groups such as the carboxylic acid and carboxamide groups (Scheme 1).



Scheme 1. The spatial distribution of the functional groups capable of hydrogen bonding in the biochemically important carboxylic acid (II) and carboxamide (III) groups is similar to that of the boronic acid moiety (I).

Experimental Section

1,4-Phenylenediboronic acid was prepared according to a reported method.^[34] Crystals suitable for X-ray crystallography were grown from water.

X-ray diffraction studies of crystals mounted in a sealed capillary that contained some mother liquor were carried out on a BRUKER-AXS APEX diffractometer with a CCD area detector ($\lambda_{\text{K}\alpha} = 0.71073$ Å, monochromator: graphite).^[31] Frames were collected at $T = 293$ K by ω -rotation ($\Delta/\omega = 0.3^\circ$) at 10 s per frame.^[35] The measured intensities were reduced to F^2 and corrected for absorption with SADABS.^[36] Structure solution, refinement, and data output were carried out with the SHELXTL-NT program.^[37] Non-hydrogen atoms were refined anisotropically. C–H hydrogen atoms were placed in geometrically calculated positions by using a riding model. O–H hydrogen atoms were localized by difference Fourier maps and refined by fixing the bond length to 0.84 Å; the isotropic temperature factors were fixed to a value 1.5 times that of the oxygen atoms. Images were created with the MERCURY program.^[38] Hydrogen-bonding interactions in the crystal lattice were calculated with PLATON.^[39]

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Keywords: boron · hydrogen bonds · structure elucidation · supramolecular chemistry · water chemistry

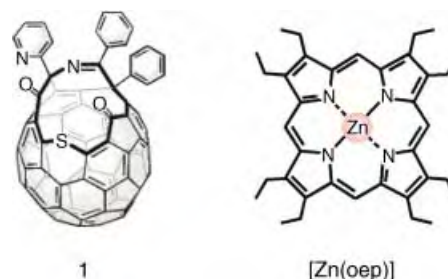
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Controlled Molecular Orientation in an Adlayer of a Supramolecular Assembly Consisting of an Open-Cage C₆₀ Derivative and Zn^{II} Octaethylporphyrin on Au(111)**

Soichiro Yoshimoto, Eishi Tsutsumi, Yosuke Honda, Yasujiro Murata, Michihisa Murata, Koichi Komatsu, Osamu Ito, and Kingo Itaya*

The supramolecular assembly of porphyrin–fullerene systems has been studied extensively because of interest in photo-induced energy- and electron-transfer processes.^[1–6] Fullerenes, in particular, are considered to be suitable building blocks for the formation of three-dimensional molecular architectures because of their ability to act as strong π -electron acceptors.^[1] Recently, several open-cage C₆₀ derivatives were synthesized^[7–9] and the unique ability of **1** to encapsulate a hydrogen molecule (H₂@**1**) was discovered.^[8a] The open cavity of this molecule is responsible for its unique



[*] Dr. S. Yoshimoto, E. Tsutsumi, Y. Honda, Prof. Dr. K. Itaya[†]
Department of Applied Chemistry
Graduate School of Engineering
Tohoku University
Aoba-yama 04, Sendai 980-8579 (Japan)
Fax: (+81) 22-214-5380
E-mail: itaya@atom.che.tohoku.ac.jp

Prof. Dr. O. Ito
Institute of Multidisciplinary Research for Advanced Materials
Tohoku University
Katahira 2-1-1, Aoba-ku, Sendai 980-8577 (Japan)

Dr. Y. Murata, M. Murata, Prof. Dr. K. Komatsu
Institute for Chemical Research
Kyoto University
Uji, Kyoto 611-0011 (Japan)

[[†]] Also at CREST-JST
Kawaguchi Center Building
4-1-8 Honcho, Kawaguchi, Saitama 332-0012 (Japan)

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functional properties. Although the π -conjugation system of **1** has been reduced by the addition of phenyl and pyridyl groups, as well as by rupture of the π system, the electronic properties of **1** are almost the same as C_{60} , as evident from electrochemical analysis.^[8b] Surface modification and further design of **1** is of interest because it has an unsymmetrical shape. Supramolecular assembly on metal surfaces has also been explored to control surface properties. Several interesting studies on the self-assembled monolayers (SAMs) obtained using the fullerene-porphyrin system were reported by the research group of Imahori and Fukuzumi.^[10] However, details on the orientation and arrangement of each moiety at the interface of the SAMs are still unclear. The control of molecular orientation at the nanoscale is of importance for the preparation of high-quality composite thin films through supramolecular assembly of donor-acceptor systems and for the development of new functional electrodes or devices.

Herein, we report a simple method for the construction of a 1:1 supramolecular assembled film of **1** and zinc(II) octaethylporphyrin [Zn(oep)] on an Au(111) surface and show that **1** has clear redox properties. Scanning tunneling microscopy (STM) also shows that highly ordered arrays of **1** constructed as a second layer on a well-defined [Zn(oep)] adlayer on Au(111) can be observed in 0.05 M H_2SO_4 .

Figure 1a shows typical cyclic voltammograms (CVs) recorded at a scan rate of 20 mV s^{-1} of a well-defined Au(111) (dotted line) layer and **1** directly attached to an

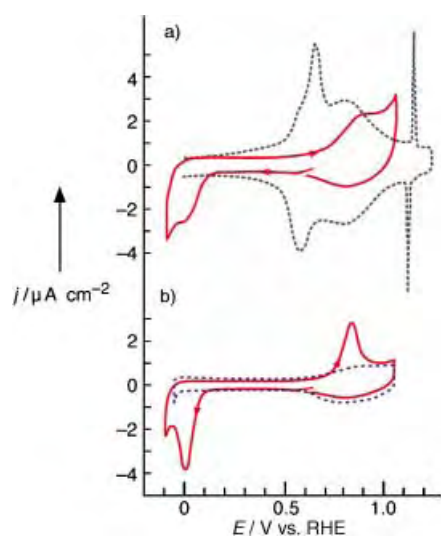


Figure 1. Typical cyclic voltammograms of a) bare Au(111) (dotted line) and **1** adsorbed onto Au(111) (solid line) and b) [Zn(oep)] (dotted line) and **1**/[Zn(oep)] adsorbed (red solid line) onto Au(111) electrodes in pure 0.05 M H_2SO_4 . The scan rate was 20 mV s^{-1} .

Au(111) electrode (red solid line) in 0.05 M H_2SO_4 . The voltammogram for bare Au(111) in Figure 1a in the double-layer potential region is identical to that reported previously,^[11] which indicates that well-defined Au(111) is exposed to the solution. A pair of spikes observed at 1.1 V results from the order-disorder phase transition of adsorbed sulfate

anions.^[11a,b] For the Au(111) electrode modified with **1**, a broad reduction peak was observed between 0.1 and -0.1 V , while a re-oxidation peak appeared at 0.85 V during the anodic scan. The dotted line in Figure 1b shows the CV of a [Zn(oep)]-modified Au(111) electrode in 0.05 M H_2SO_4 . No noticeable peaks were observed between 0 and 0.7 V . Subsequent immersion of the electrode into an approximately $50\text{ }\mu\text{M}$ solution of **1** in benzene for one minute resulted in a decrease in the double-layer charging current and a pair of characteristic redox peaks were clearly observed at 0 and 0.85 V during cathodic and anodic scans, respectively. This result suggests that **1** was attached on the [Zn(oep)]-modified Au(111) surface and that the carbonyl groups of **1** were oriented toward the solution phase. The CV profile drawn with a solid red line in Figure 1b is strongly associated with the electrochemical redox reaction of $>C=O$ to $>C^{\cdot-}OH$ (and $>C^{\cdot-}OH$ to $>C=O$) involving two carbonyl groups in each molecule of **1**. The amount of transferred electronic charge is estimated from the reductive peak area to be about $15.8\text{ }\mu\text{C cm}^{-2}$. If two-electron reduction occurs on the **1**/[Zn(oep)]-modified Au(111) electrode, this value corresponds to a surface concentration of $(7.9 \pm 0.7) \times 10^{-11}\text{ mol cm}^{-2}$. The electron-transfer process is very slow, as indicated by the CV profile shown in Figure 1b. When potential switching was carried out at potentials more negative than 0.85 V , no reduction peak at 0 V was observed. These redox peaks were not seen at C_{60} and C_{70} /[Zn(oep)]-modified Au(111) electrodes. Figure 2a shows a typical STM

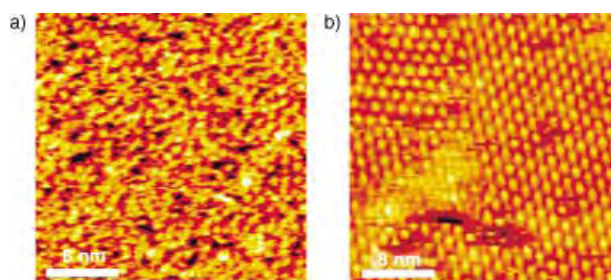


Figure 2. Typical large-scale STM images ($30 \times 30\text{ nm}^2$), acquired at 0.85 V versus RHE, of the **1** adlayer a) directly formed on Au(111) and b) on [Zn(oep)]-modified Au(111) in 0.05 M H_2SO_4 . The potential of the tip was 0.45 V . Tunneling currents were 0.5 nA and 0.3 nA , respectively.

image of an adlayer of **1** formed on Au(111) in 0.05 M H_2SO_4 . This STM image shows a completely disordered structure of **1** directly attached to the surface of the Au(111) layer. Individual molecules of **1** could not be distinguished under the conditions used. It is considered that **1** interacts sufficiently strongly with the Au surface to attach to the Au surface through the pyridyl and $C=O$ groups as well as the S atom on the rim of the orifice. Such an arrangement on the modified Au(111) surface was also consistent with the CV profile shown in Figure 1a. However, highly-ordered arrays consisting of bright round spots were seen in the STM image of the [Zn(oep)]-modified Au(111) layer (Figure 2b). Some domains were clearly seen on a terrace, which suggests that the highly ordered adlayer of **1** was not formed directly on the

Au(111) layer but on the [Zn(oep)]-modified Au(111) surface.

Further details on the internal structure, orientation, and packing arrangement of the supramolecular assembled layers of **1**/[Zn(oep)] on Au(111) are revealed in the high-resolution STM image of an area of $8 \times 8 \text{ nm}^2$ (Figure 3a). Intermolec-

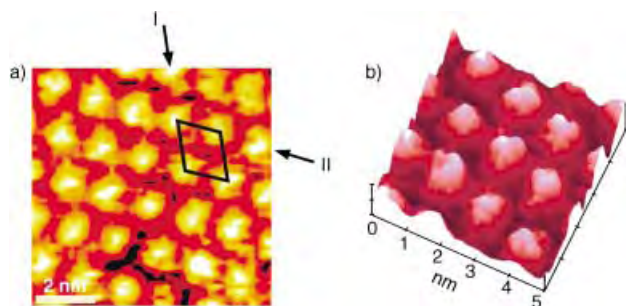


Figure 3. High-resolution STM images ($8 \times 8 \text{ nm}^2$) of a layer of **1**/[Zn(oep)] supramolecularly assembled on Au(111) in $0.05 \text{ M H}_2\text{SO}_4$; a) top view and b) height-shaded view ($5 \times 5 \text{ nm}^2$). The potential of the tip and the tunneling current were 0.46 V and 0.425 nA , respectively.

ular distances between the nearest neighbor molecules of **1** were found from a cross-sectional profile to be 1.68 ± 0.07 and $1.44 \pm 0.05 \text{ nm}$ for directions along arrows I and II, respectively. These distances are greater than that for C_{60} directly adsorbed on Au(111) (ca. 1.0 nm).^[12] The adlattice of **1** is superimposed as a black line in Figure 3a. The surface concentration was estimated to be $8.7 \times 10^{-11} \text{ mol cm}^{-2}$, which is in good agreement with that calculated from Figure 1b. The height of the corrugation of **1** was greater than that of the [Zn(oep)] layer on Au(111). Morphological details of each molecule are clearly displayed in the height-shaded view shown in Figure 3b. Protrusions were observed in each bright spot, thus showing the presence of phenyl and pyridyl groups located on the rim of the orifice. When C_{60} molecules were used as the second layer in the modification, a round spot could be seen in each molecule without any protrusions in the high-resolution STM image.^[13]

The STM image changed dramatically when the tunneling current was increased from 30 pA to 2.0 nA in the middle of the scan (Figure 4). The adlayer of **1** immediately disappeared and the underlying [Zn(oep)] layer on Au(111) was visible, thus indicating that a layer of molecules of **1** formed on the highly ordered [Zn(oep)] adlayer to form an exactly 1:1 supramolecular assembly. In fact, the top layer of **1** was easily removed with either tunneling currents higher than 0.5 nA or by using low bias conditions, and the underlying [Zn(oep)] layer was clearly visible with a tunneling current of 1.5 nA (Figure 5a). Careful inspection of Figure 5a allows two different orientations of [Zn(oep)] molecules to be distinguished in the molecular rows. Each [Zn(oep)] molecule can be recognized as a square with eight additional spots at the corners, which correspond to the eight ethyl groups. It is seen that the [Zn(oep)] molecules in the row marked by a red arrow have a different molecular orientation from those in the row marked by a blue arrow. The adlayer structure of

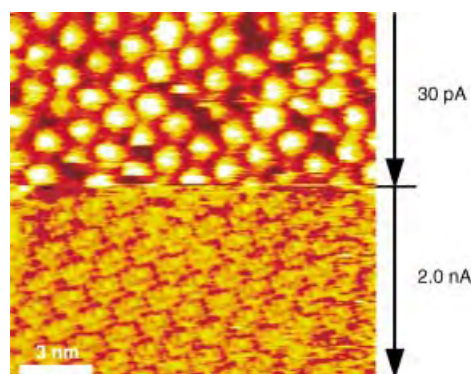


Figure 4. Composite STM image ($15 \times 15 \text{ nm}^2$), acquired at 0.85 V versus RHE, of a layer of the **1**/[Zn(oep)] supramolecular assembly on Au(111) in $0.05 \text{ M H}_2\text{SO}_4$. The potential of the tip was 0.35 V . Tunneling currents were 0.03 nA (upper part) and 2.0 nA (lower part), respectively.

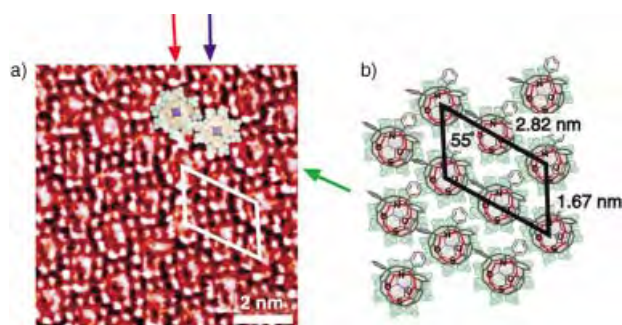


Figure 5. a) High-resolution STM image ($9 \times 9 \text{ nm}^2$) of the underlying [Zn(oep)] layer on Au(111) in $0.05 \text{ M H}_2\text{SO}_4$ and b) structural model of the supramolecularly assembled **1**/[Zn(oep)] layer on Au(111). The potential and the tunneling current were 0.46 V and 1.5 nA , respectively.

[Zn(oep)] on Au(111) was identical to that of [Co(oep)]^[14a] and [Fe(oep)]^[14b] on Au(111). Only one difference was found in the STM image between [Zn(oep)] and [Co(oep)] (or [Fe(oep)]), that is, the center of each [Zn(oep)] unit appeared as a dark spot, whereas that of [Co(oep)] (or [Fe(oep)]) was the brightest spot. This difference can be explained in terms of a difference in the d_{z^2} orbitals.^[15] Intermolecular spacings between the molecules in the rows marked by red and green arrows in Figure 5a were determined to be 1.67 ± 0.07 and $1.42 \pm 0.05 \text{ nm}$, which are in good agreement with the values observed in Figure 3a. The adlattice is superimposed in Figure 5a. The adlattice includes two [Zn(oep)] molecules with an included angle of $55 \pm 3^\circ$, which arises because neighboring [Zn(oep)] molecules alternately possess two different orientations for alignment in the direction indicated by the green arrow. In this system, the adlattice for the top layer of **1** is different from that of the underlying layer of [Zn(oep)], while the surface concentration of the top layer is the same as that of the underlying layer. A proposed structural model is shown in Figure 5b. The intermolecular distances between molecules of **1** are nearly equal to the distance between [Zn(oep)] molecules, a fact which indicates

that a molecule of **1** is located directly above the center of each [Zn(oep)] molecule. This structure was consistently observed between 1.0 and 0 V. Structural change of the layer of **1** through the redox reaction could not be identified by STM, even at 0 V. When a polycrystalline Au electrode such as a disk or a wire was used as the substrate, the redox reaction was barely seen, even with the **1**/[Zn(oep)] system. This result shows that the formation of a highly ordered [Zn(oep)] layer is a key factor in controlling the molecular orientation of **1**. Adlayers of C₆₀ and C₇₀ on [Zn(oep)]-modified Au(111) also behaved similarly as that of **1** when the adlayers were prepared in the same manner.^[13] The clearly enhanced redox peaks in the CV profile at the **1**/[Zn(oep)]-modified Au(111) layer and the high-resolution STM image strongly support the conclusion that the orientation of **1** is controlled by the [Zn(oep)] layer. Thus, the redox reaction shown in Figure 1b is assigned to a two-electron and two-proton reaction.

In conclusion, immersing an Au(111) substrate successively into solutions of [Zn(oep)] and **1** in benzene results in a well-defined electrochemical response of **1** as a consequence of the formation of a supramolecular assembly of **1** and [Zn(oep)]. This system has a wide peak separation, which may enable its application in electrochemical switching devices.

Experimental Section

Open-cage C₆₀ derivative **1** was synthesized by using the procedure described previously.^[8a] [Zn(oep)] was purchased from Aldrich and used without further purification. Benzene was obtained from Kanto Chemical Co. (spectroscopy grade). Au(111) single-crystal electrodes were prepared by the Clavilier method.^[16] Adlayers of **1**/[Zn(oep)] were formed by immersing an Au(111) electrode successively into a solution of [Zn(oep)] (ca. 100 μ M) in benzene for 10 s and into a solution of **1** (50 μ M) in benzene for 30–60 s, after annealing the Au(111) surface in a hydrogen flame and quenching it in ultrapure water (Milli-Q SP-TOC = 18.2 M Ω cm) saturated with hydrogen.^[14] The **1**/[Zn(oep)]-modified Au(111) layer was rinsed with ultrapure water and then transferred into an electrochemical STM cell and filled with 0.05 M H₂SO₄ (Cica-Merck, ultrapure grade). Cyclic voltammetry was carried out at 20°C using a potentiostat (HOKUTO HAB-151, Tokyo) and the hanging meniscus method in a three-compartment electrochemical cell in a N₂ atmosphere. Electrochemical STM measurements were performed in 0.05 M H₂SO₄ by using a Nanoscope E with a tungsten tip etched in 1 M KOH. Tips were coated with nail polish to minimize residual faradic currents. STM images were recorded in the constant-current mode with a high-resolution scanner (HD-0.5I). All potential values are referenced to the reversible hydrogen electrode (RHE).

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Multicomponent Magnetic Nanorods for Biomolecular Separations**

Ki-Bum Lee, Sungho Park, and Chad A. Mirkin*

Nanomaterials have been used extensively in the development of high sensitivity and high selectivity biodetection schemes.^[1–4] Designer particles, including noble-metal polyhedral structures, quantum dots, nanopatterns, and nanorods have found application in many forms of biological tagging schemes, including DNA and protein detection, cell sorting, and histochemical staining.^[5–12] Significant advantages over conventional molecule-based fluorophore strategies have been identified for several of these structures.^[13–15] Other applications for nanomaterials in biology, beyond diagnostics, include therapeutics and separations.^[9] A key area for researchers working with proteins involves separation and purification. Traditionally, nickel columns have been used in conjunction with histidine tagged proteins to separate such structures from a matrix of other undesirable biological elements. We and others have been working with nanorod structures prepared by the porous template synthesis approach pioneered by Martin and the group of Moskovits.^[16,17] This synthetic procedure allows one to prepare rods electrochemically with uniform diameters and with predefined block lengths of inorganic and organic materials with excellent control.^[18,19] We hypothesized that if nickel were introduced as one of the blocks in a single or multicomponent nanorod structure, it could be used as a magnetic nanosized affinity template for histidine tagged proteins and, therefore, an appropriately applied magnetic field could be used to effect separation of the protein–rod complex from a multicomponent solution. Herein, we demonstrate how one can use two-component triblock ≈ 330 nm diameter rod structures with gold end blocks and a nickel interior block as materials that can very efficiently separate His-tagged proteins from non-his tagged structures. This work builds on our work with the transport of His-tagged structures by dip-pen nanolithography (DPN) to bulk solid-state nickel oxide substrates and the work of others involving the generation of microscopic arrays of His-tagged proteins on bulk nickel oxide substrates.^[20,21]

Magnetic multisegment nanorods composed of nickel and gold blocks were synthesized by using the method of electro-deposition into a porous alumina membrane (experimental conditions are described in the Supporting Information). The gold portions of the three-component structure were used to prevent nickel domain etching during the silver dissolving procedure. Prior to use, the rods were repeatedly rinsed with distilled water until the pH of the solution was 7.

The multicomponent nanorods were washed with methanol and ethanol to remove contaminants from their surfaces. This was done by using a magnetic field (BioMag, Poly-sciences, Inc.) to pull the rods to the sidewalls of a plastic vial while rinsing them with the appropriate solvent. The gold portions of the nanorods were passivated with 11-mercaptoundecyl-tri(ethylene glycol)(PEG–SH) by incubating the rod samples in 1 mL, 10 mM ethanolic solution of the surfactant for 2 h followed by copious rinsing with ethanol and then nanopure water (Barnstead International, Dubuque, IA, USA). Others have shown that alkylthiols preferentially modify the gold surface in such two component structures.^[5] The gold surface was modified with PEG–SH for two reasons. First, the PEG–SH minimizes nonspecific binding of proteins to the nanorod structures.^[22,23] Second, it stabilizes the rods by minimizing bare gold surface–surface interactions.

The specific interaction of polyhistidine (His $\times$ 6) with bulk oxidized nickel surface is well known.^[20,21] Similarly, fluorescein-tagged poly-His (His $\times$ 6) binds specifically to the Ni portions of the substrate as evidenced by confocal fluorescence microscopy. In a typical experiment, Au–Ni–Au nanorods (10^9 – 10^{10}) were incubated in a 63 μ M fluorescein labeled poly-His solution (1 mL, 0.1M PBS(phosphate buffered saline), pH 7.4) for 12 h at room temperature (22 °C). Then the nanorods were vigorously rinsed with phosphate buffered saline (PBS) solution followed by nanopure water. During each rinsing step, the rods were separated from the supernatant by using magnetic force. Fluorescence imaging shows that the fluorescein-tagged polyhistidines efficiently bind to the nanorod structures (Figure 1b). This reaction between the poly-His and Au–Ni–Au rods can be monitored with the naked eye simply by watching the color of the solution decrease in intensity as a function of reaction time (Figure 1c). A quantitative analysis of the efficiency of poly-His adsorption was performed by preparing a standard calibration curve from the fluorophore-labeled poly-His over a range of concentrations starting with the experimental poly-His initial concentration of 63 μ M and going to 0.16 μ M (inset Figure 2). The fluorescence emission intensity of supernatant solution isolated from the reacted nickel nanorods shows that $\approx 90\%$ of the poly-His was captured by the rods from the starting solution (Figure 2). As a control experiment, pure Au nanorods (no Ni), passivated with PEG–SH, were incubated in the poly-His solution (63 μ M in 0.1M PBS, pH 7.4) under nearly identical conditions, and little interaction between the poly-His molecules and PEG–SH modified Au particles was observed (i.e., no detectable change in the emission of the fluoroscein as measured by fluorescence spectroscopy, data not shown).

The Au–Ni–Au rods can be used in a novel scheme for separating His-tagged proteins from structures without His-

[*] K.-B. Lee,† S. Park,† Prof. C. A. Mirkin
Department of Chemistry and
Institute for Nanotechnology
Northwestern University
2145 Sheridan Road, Evanston, IL 60208-3113 (USA)
Fax: (+1) 847-467-5123
E-mail: camirkin@chem.northwestern.edu

[†] These authors contributed equally to this work.

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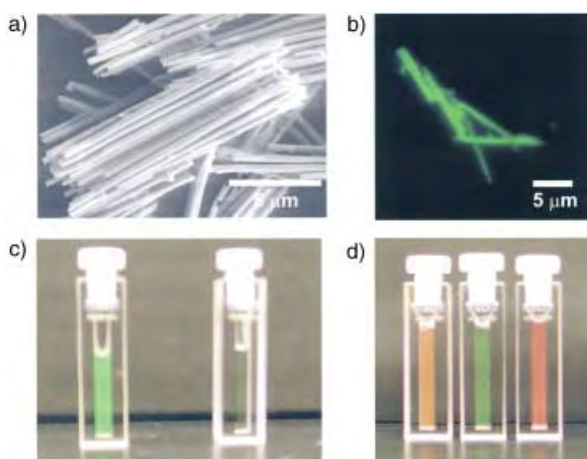


Figure 1. a) A field emission SEM image of Au-Ni-Au rods. b) A confocal fluorescence image of Au-Ni-Au rods after modification of Ni blocks with fluorescein-tagged poly-His. c) A picture of two cuvettes showing solutions of fluorescein-tagged poly-His before (left-hand side) and after (right-hand side) exposure to the Au-Ni-Au rods. d) Left: Orange solution containing dye-labeled His-tagged (Red, Alexa 568-tagged ubiquitin) and untagged (green, Alexa 488-labeled anti-rabbit IgG) proteins before exposure to nanorods. Middle: After exposure to the Au-Ni-Au rods, the solution is green, indicating removal of the red-dye labeled His-tagged ubiquitin. Right: The red solution formed after release of the red-dye labeled His-tagged ubiquitin in the eluent buffer.

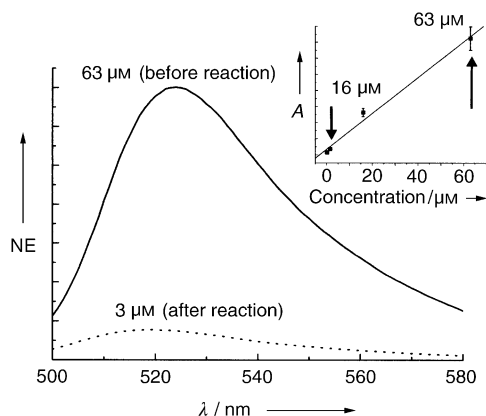
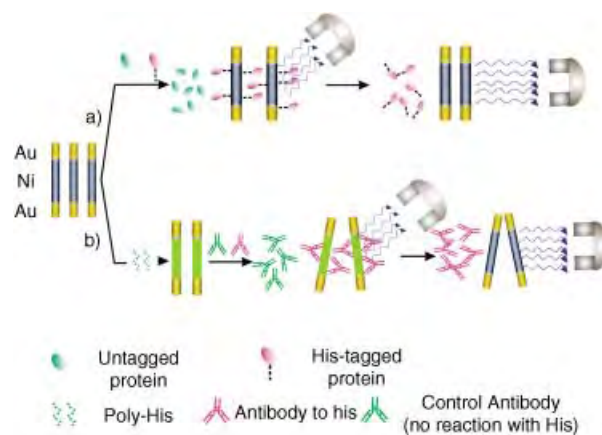


Figure 2. Fluorescence spectra corresponding to Figure 1 c, before and after separation of poly-His with Au-Ni-Au rods: Inset shows the standard curve and the arrows represent each concentration of poly-His before and after separation. NE is normalized emission, arbitrary units; A is the integrated area, arbitrary units.

tags (Scheme 1a). For example, a 1 mL solution of two different proteins with different dye labels (anti-rabbit IgG without a His tag but labeled with Alexa 488 and His-tagged ubiquitin labeled with Alexa 568; concentration of each protein = $100 \mu\text{g mL}^{-1}$, in 0.1M PBS, pH 7.4) were prepared (Figure 1d, far left, orange solution). The orange solution containing the mixture of proteins was added to a vial containing PEG-passivated nanorods (10^9 – 10^{10}). The solution was mechanically shaken for 24 h. Application of a magnetic field caused the nanorods to move to the side walls and the resulting green supernatant (Figure 1d, middle cuvette) could be collected and studied by fluorescence spectroscopy. Eluent buffer (pH 2.8, ImmunoPure IgG Elution Buffer, Pierce



Scheme 1. a) Separation of His-tagged proteins from untagged proteins. b) Separation of antibodies to poly-His (anti-poly-His) from other antibodies.

Biotech., Inc.) was added to the vessel containing the nanorods coated with His-tagged, Alexa 568-labeled ubiquitin. This addition results in the release of the His-tagged proteins from the nanorods and the formation of a red supernatant (Figure 1d, right). Each of the solutions discussed above was studied by UV/Vis and fluorescence spectroscopy and compared with each other. The spectrum of the mixture of proteins before the addition of nanorods shows two bands at $\lambda_{\text{max}} = 516 \text{ nm}$ and 600 nm that correspond to the two dye labels. After addition of the nanorods, there is an 86% decrease in the signal ($\lambda_{\text{max}} = 600 \text{ nm}$) for the dye label associated with the His-tagged protein and only 6% for the protein without the His tag ($\lambda_{\text{max}} = 516 \text{ nm}$). After adding the eluent buffer, which is at a pH 2.8 and results in the release of proteins from the Ni surface, we see a strong signal at $\lambda_{\text{max}} = 600 \text{ nm}$ associated with the His tagged proteins. Fluorescence spectroscopy shows that 56% of the original His-tagged ubiquitin has been effectively separated from the two component mixture in pure form (Figure 3).

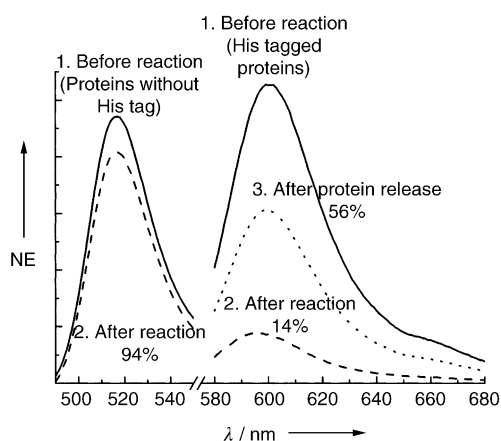


Figure 3. Fluorescence spectra corresponding to Figure 1 d. Solid lines represent a fluorescence spectrum of a mixture of dye labeled His-tagged and untagged proteins. Dashed traces show a spectrum of the supernatant after removal of His-tagged proteins with Au-Ni-Au rods. Dotted traces show a spectrum of the product generated by releasing the His-tagged proteins into eluent buffer.

Finally, Poly-His tagged Au-Ni-Au rods are bioactive and selectively react with antibodies for poly-His, Scheme 1b. Indeed, when a mixture of Alexa 488-labeled anti-human IgG and Alexa 568-labeled anti-poly-His IgG ($100 \mu\text{g mL}^{-1}$ in 0.1M PBS, pH 7.4) was introduced to a PBS solution (pH 7.4) of the Poly-His tagged Au-Ni-Au rods, the dye-labeled anti-poly-His IgG was very efficiently removed from the solution as evidenced by fluorescence spectroscopy (see Supporting Information). After 24 h, 70 % of the anti-poly-His ($\lambda_{\text{max}} = 600 \text{ nm}$) was removed from the solution while only 4 % of the signal ($\lambda_{\text{max}} = 516 \text{ nm}$) associated with the antihuman IgG is lost. The rods can be attracted to the side of the reaction vessel with a magnetic field allowing one to remove the supernatant. The anti-poly-His can be released from the rods by adding eluent buffer at pH 2.8. Acidic conditions are known to decrease the specific interaction between the antibody and poly-His.^[24]

We have demonstrated that Ni-containing nanorods can be used as novel materials for the efficient separation of mixtures of biomolecules by exploiting the chemical and physical properties of these nanostructures. The Ni portion of the nanostructure provides a docking site for His-tagged proteins and is ferromagnetic, allowing one to selectively and efficiently remove them from solution with an appropriately applied magnetic field. The gold ends are used to chemically protect the nickel interior and provide a second platform for introducing further chemical functionality (PEG, in this case, but in principle, many other thiol-rich molecules and materials). Finally, the nanorods provide increased surface area and are a pseudo-homogeneous system, compared to a bulk structure, thereby increasing the efficiency of the target binding and separation process. Histidine tagging of proteins has been broadly adopted throughout the molecular biology and biochemistry communities, and this novel and straightforward separation scheme provides an alternative to Ni chromatographic columns and potentially has a much greater degree of tailorability through choice of nanorod block compositions and surface coatings.

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Zeolites

A DFT Study of the Acidity of Ultrastable Y Zeolite: Where Is the Brønsted/Lewis Acid Synergism?***

Claudio J. A. Mota, Daniel L. Bhering, and
Nilton Rosenbach, Jr.*

Ultrastable Y zeolites (USY) are the main component of cracking catalysts. They are normally produced from a NH_4Y

[*] Prof. Dr. C. J. A. Mota, Dr. D. L. Bhering, N. Rosenbach, Jr.
Instituto de Química
Universidade Federal do Rio de Janeiro
Cidade Universitária CT, Bloco A
21949-900 Rio de Janeiro (Brazil)
Fax: (+55) 21-2562-7106
E-mail: cmota@iq.ufrj.br

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derivative, upon treatment with steam at 500–700 °C.^[1] Under these conditions, there is a partial loss of aluminium from the zeolite structure, which improves the thermal stability and catalytic properties of the zeolite. Nevertheless, the aluminum atoms released from the framework stay inside the cavities and channels as extra-framework aluminum species (EFAL). The nature of the EFAL species is not completely known, but it is postulated that oxoaluminum cations, such as AlO^+ , $\text{Al}(\text{OH})_2^+$, $\text{Al}(\text{OH})_2^{2+}$, and some neutral compounds such as AlOOH and hydrated Al_2O_3 , could account for some of the EFAL species.^[2]

The catalytic activity of USY zeolites is usually higher than the parent HY zeolite.^[3] As the framework Si:Al ratio goes up, the acid sites in USY becomes more isolated from each other, increasing the acid strength. However, the presence of EFAL species plays a more important role. Leaching out part of the EFAL of an USY zeolite reduces its catalytic activity.^[4] There are also ways to prepare a dealuminated zeolite without EFAL species. The most popular method is treatment with $(\text{NH}_4)_2\text{SiF}_6$ in aqueous solution.^[5] This procedure replaces the framework Al atoms by Si atoms of the SiF_6^{2-} anion, thus leaving no EFAL species, which remain dissolved in the aqueous solution and are subsequently washed away. Notwithstanding, the catalytic activity of the dealuminated Y zeolites prepared by this way is considerably lower^[6] than the catalytic activity of an USY zeolite, prepared by steaming. To explain these results, a Brønsted/Lewis synergism has been proposed, aroused upon

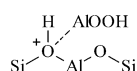


Figure 1. Proposed Brønsted/Lewis synergism in USY zeolites.

the interaction of the EFAL species (Lewis acid sites) with the framework hydroxy groups (Brønsted acid sites) as schematized in Figure 1.^[7]

Measurements of acid strength of the zeolite are difficult. Methods relied on Hammett bases are criticized, as many bases might be bulk enough to diffuse into the zeolite pores. Recent measurements, based on spectroscopic investigations,^[8] ranked the zeolites as strong acids, with acidity comparable to concentrated sulfuric acid solutions. Nevertheless, a study of the acid strength of dealuminated Y zeolites, with and without EFAL species, indicated virtually the same acidity values as those obtained by ammonia microcalorimetry.^[9]

On the other hand, theoretical methods have been used^[10] to study the acidity and catalytic properties of zeolites. We recently published a DFT study of the structure and coordination of some EFAL species on a zeolite Y cluster.^[11]

We present herein preliminary results of cluster calculations on the effect of the EFAL species on the zeolite acid strength. Our main objective is to investigate the interactions of the EFAL with the acid site, aiming to observe if the Brønsted/Lewis synergism really occurs on zeolites.

The calculations were performed on a T_6 cluster ($\text{T} = \text{Si}, \text{Al}$), which represents a real part of the zeolite Y structure. The same cluster was previously used in a study of the structure of EFAL species on zeolites.^[11] The border Si atoms were saturated with hydrogen atoms and the framework Al atoms with hydroxy groups, to avoid dangling bonds. The EFAL species studied were AlO^+ , $\text{Al}(\text{OH})_2^+$, $\text{Al}(\text{OH})_2^{2+}$, $\text{Al}(\text{OH})_3$, and AlOOH . To account for system neutrality, we used as many framework Al atoms as necessary. All calculations were performed at the B3LYP6-31G** level of theory by using the GAUSSIAN98 program.^[12] The acid strength was theoretically determined as the enthalpy difference for the deprotonation reaction. To better describe this process, single-point energy calculations were performed at B3LYP6-311++G**. The thermodynamic properties were obtained from the frequency calculations, scaled by 0.96. Calculations of the deprotonation energy for T_6 clusters with one and two framework Al atoms were also performed for comparison, as they represent models of Y zeolites without EFAL species.

Table 1 shows the results for the calculated systems. One can see that the cluster with one framework Al atom presented a lower deprotonation enthalpy than the cluster with two Al atoms. This is consistent with other calculations that show that the acid strength decreases as the sites become closer to each other. There is a drastic change in geometry upon deprotonation. The Al–O bond length shrinks from 1.95 Å in the acid form, to 1.77 Å in the deprotonated structure. The Si–O(H)–Al bond angle varies from 128.4° in the acid form to 147° in the anion. These results account for the HT_6 (1 Al), with one framework Al atom, but the trend is the same for the other calculated structures.

All the EFAL- HT_6 clusters studied, excepted the $\text{Al}(\text{OH})_2^+$, presented a higher deprotonation enthalpy, compared with the respective HT_6 clusters. Contrary to what is proposed in the literature, the EFAL reduced the acid strength of the framework hydroxy groups and no Brønsted/Lewis synergism was found. The optimization of the $\text{Al}(\text{OH})_3\cdot\text{HT}_6(1\text{Al})$, $\text{AlOOH}\cdot\text{HT}_6(1\text{Al})$ and $\text{AlO}^+\cdot\text{HT}_6(2\text{Al})$ led to an intramolecular proton transfer and all ended with the proton being bound to one of the oxygen atoms of the EFAL (Figure 2). Hence, deprotonation of these species

Table 1: Calculated deprotonation energies at 298.15 K.^[a]

Reaction ^[a]	ΔH [kcal mol ⁻¹]	B3LYP6-31G**		B3LYP6-311++G**//B3LYP6-31G**		
		ΔS [cal mol ⁻¹ K ⁻¹]	ΔG [kcal mol ⁻¹]	ΔH [kcal mol ⁻¹]	ΔS [cal mol ⁻¹ K ⁻¹]	ΔG [kcal mol ⁻¹]
$\text{HT}_6(1\text{Al}) \rightleftharpoons \text{T}_6^-(1\text{Al}) + \text{H}^+$	297.9	20.4	291.8	295.5	20.4	289.4
$\text{H}_2\text{T}_6(2\text{Al}) \rightleftharpoons \text{HT}_6^-(2\text{Al}) + \text{H}^+$	303.9	3.7	302.8	298.1	3.7	297.0
$\text{Al}(\text{OH})_3\cdot\text{HT}_6(1\text{Al}) \rightleftharpoons \text{Al}(\text{OH})_3\cdot\text{T}_6^-(1\text{Al}) + \text{H}^+$	309.6	13.1	305.7	303.4	13.1	299.5
$\text{Al}(\text{OOH})\cdot\text{HT}_6(1\text{Al}) \rightleftharpoons \text{Al}(\text{OOH})\cdot\text{T}_6^-(1\text{Al}) + \text{H}^+$	367.5	14.5	363.2	360.8	14.5	356.4
$\text{AlO}^+\cdot\text{HT}_6(2\text{Al}) \rightleftharpoons \text{AlO}^+\cdot\text{T}_6^-(2\text{Al}) + \text{H}^+$	347.7	27.3	339.6	342.6	27.3	334.5
$\text{Al}(\text{OH})_2^+\cdot\text{HT}_6(2\text{Al}) \rightleftharpoons \text{Al}(\text{OH})_2^+\cdot\text{T}_6^-(2\text{Al}) + \text{H}^+$	298.6	21.0	292.3	294.2	21.0	287.9
$\text{Al}(\text{OH})^{+2}\cdot\text{HT}_6(3\text{Al}) \rightleftharpoons \text{Al}(\text{OH})^{+2}\cdot\text{T}_6^-(3\text{Al}) + \text{H}^+$	311.2	31.1	301.9	305.8	31.1	296.6

[a] All frequency calculations were done at the B3LYP6-31G** level.

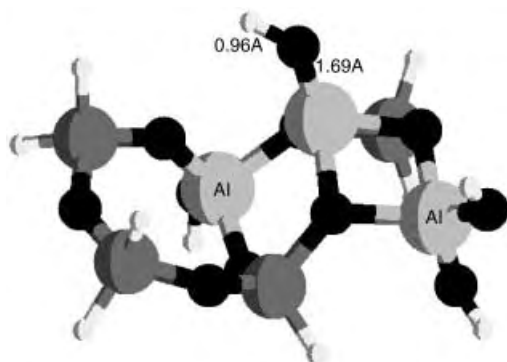


Figure 2. Optimized structure of the $\text{AlO}^+\cdot\text{HT}_6(2\text{Al})$, showing the formation of an EFAL hydroxy group (framework Al assigned).

involves considerably higher energy, compared to deprotonation of the isolated acid site. For the AlOOH and AlO^+ species, the intramolecular proton transfer forms a stable structure, as indicated by the highest deprotonation enthalpy among the calculated structures. This may imply that $\text{Al}=\text{O}$ bonds are not favored, but are protonated to form EFAL hydroxy groups.

From the calculations at the B3LYP6-311++G** level, the highest acidity was found for the $\text{Al}(\text{OH})_2^+\cdot\text{HT}_6(2\text{Al})$. If we take the ΔG (G is the Gibbs free energy) between the $\text{Al}(\text{OH})_2^+\cdot\text{HT}_6(2\text{Al})$ and the two EFAL free clusters, $\text{HT}_6(2\text{Al})$ and $\text{HT}_6(1\text{Al})$, to estimate the increase in acidity, we find that the EFAL has an effect on the $\text{p}K_a$ value by about -15.5 for $\text{HT}_6(2\text{Al})$ and -2.5 for $\text{HT}_6(1\text{Al})$ at 298.15 K. Nevertheless, this increase in acid strength is not due to a Brønsted/Lewis synergism, but to hydrogen bonding between the EFAL hydroxyls and the oxygen atoms of the formed AlO_4^- tetrahedral (Figure 3). Hence, anion stabilization through hydrogen bonding, instead of Brønsted/Lewis synergism in the acid form, should account for the enhanced activity of USY zeolites. Therefore, structural defects in USY, formed upon the release of framework aluminum atoms, might also contribute to the increased activity of these zeolites, compared with those dealuminated by $(\text{NH}_4)_2\text{SiF}_6$.

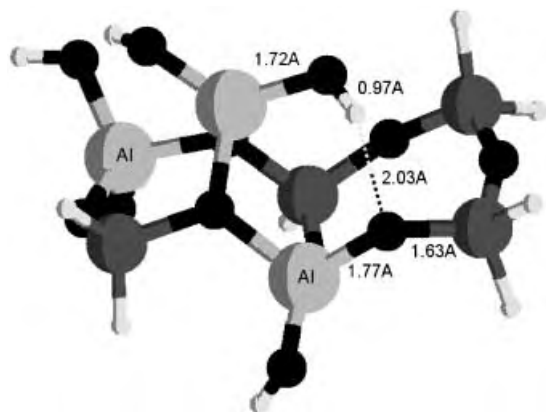


Figure 3. Structure of the conjugated base of $\text{Al}(\text{OH})_2\cdot\text{HT}_6(2\text{Al})$, showing the hydrogen bonding between the EFAL hydroxy group and the framework oxygen atom (framework Al assigned).

The defects generate SiOH nests, which could also contribute to anion stabilization through hydrogen bonding.

We also ran calculations with ammonia as base, as suggested by one of the referees. We have identified that there are many possibilities for the NH_3 molecule to interact with the EFAL, which constitutes a separate work. However, we found the same behavior of reducing the acid strength with respect to an isolated acid site. For the $\text{Al}(\text{OH})_2^+\cdot\text{HT}_6$ interacting with ammonia, we found, at least two possible reaction pathways: one leading to $\text{NH}_4^+\cdot\text{Al}(\text{OH})_2^+\cdot\text{T}_6$, in which the proton is transferred to ammonia; the other leading to the formation of a complex between NH_3 and $\text{Al}(\text{OH})_2^+\cdot\text{HT}_6$. This second structure was found to be more stable by $4.6 \text{ kcal mol}^{-1}$ at B3LYP6-31G** level, and involves an internal proton transfer to the EFAL OH group, which is hydrogen bonded with the NH_3 and the zeolite conjugated base. This may explain the better stabilization of this structure. Nevertheless, we stress that these are initial results and a careful investigation of the adsorption of NH_3 on EFAL· HT_6 is under way.

It was recently proposed^[13] that the presence of cationic species near the acid site stabilizes the conjugated base and increase zeolite acidity. Our calculations show that hydrogen bonding of the hydroxylated EFAL species with the conjugated base also contribute to an increase in acid strength. These concepts may change the direction of developing new structural modifications in zeolites to improve catalytic activity.

Supporting Information: Structure of the optimized minima.

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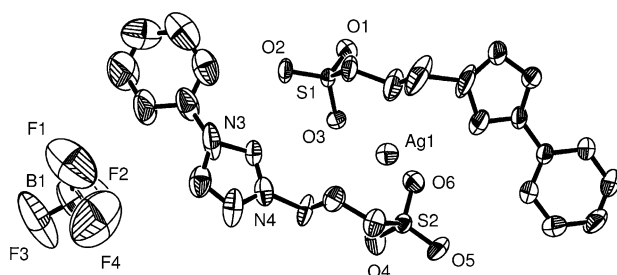


Figure 1. Molecular structure of **2c**-BF₄. Ellipsoids are shown at 30% probability levels. The unlabeled atoms are carbon atoms and hydrogen atoms have been omitted for clarity. Selected bond lengths [Å] and angles [°]: Ag(1)-O(3) 2.314(5), Ag(1)-O(6) 2.369(6), S(1)-O(1) 1.447(6), S(1)-O(2) 1.443(5), S(1)-O(3) 1.465(5), S(1)-O(3)-Ag(1) 137.06(3), O(3)-Ag(1)-O(6) 94.46(2), S(2)-O(6)-Ag(1) 118.02(3).

the sulfonates and the silver ion, Ag(1)-O(3) and Ag(1)-O(6), are 2.314 and 2.369 Å, respectively.

Melting points of the zwitterionic complexes were determined by differentiating scanning calorimetry (DSC). An ionic liquid is defined as the ionic compound that has a melting point lower than 100°C.^[8] From this point of view, zwitterionic complexes, **2a**-BF₄ and **2c**-BF₄ can be considered as ionic liquids.

Zwitterionic silver complexes were tested as transport carriers for the separation of isoprene from *n*-pentane through composite membranes. The composite membrane was prepared by casting an aqueous solution of a zwitterionic silver complex onto a polyester microporous membrane (47 mm, 0.1 µL, Whatman) by using a coater, and then dried under a vacuum.

SEM-WDS (WDS = wavelength-dispersive spectrometry) photographs in Figure 2 display the cross sections of the prepared composite membrane containing **2a**-BF₄ before and after transportation study. The white solid lines denote the distribution of silver across the membrane. WDS images clearly show that **2a**-BF₄ is immobilized in the porous structure of the polyester membrane and distributed mostly at the inlet and outlet of the pores.

A comparison of the WDS images before and after use of the membranes indicates that the initial distribution of **2a**-BF₄ remains almost unchanged during the separation process. It is hard to figure out the state of the carriers immobilized in the pores of the membrane. From the SEM images shown in the Supporting Information, the carriers seem to exist as a thin film on the inlet surface of the polyester membrane. However, in the pores of the membrane, the carriers are likely to be in a well-dispersed solid state, which might transform into a swollen gel film upon interaction with a feed mixture.

Figure 3 shows the selectivity of isoprene over *n*-pentane with time through composite membranes containing zwitterionic silver complexes as transport carriers. The selectivity was strongly dependent on the types of counterions of the zwitterionic silver complexes in the membranes. In contrast to polymer membranes that consist of polyvinyl alcohol and silver salts in which the counterion effect was negligible,^[19] the zwitterionic complex prepared from AgNO₃ showed much higher selectivity than those from AgBF₄ and AgClO₄.

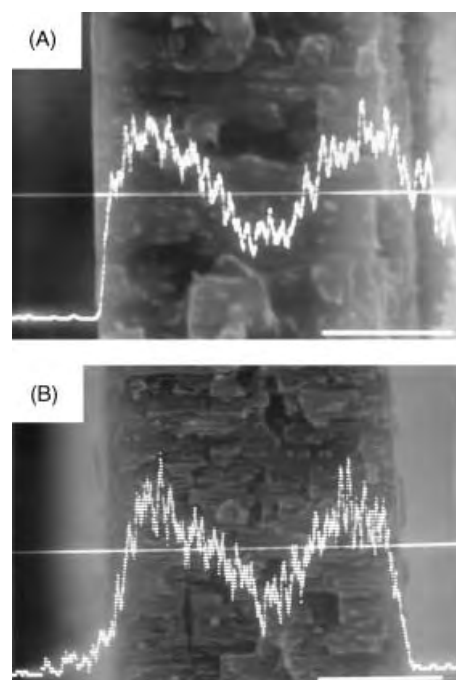


Figure 2. Cross section SEM-WDS photograph of the composite membrane containing **2a**-BF₄ A) before, and B) after 10 h of use. Bar = 10 µm

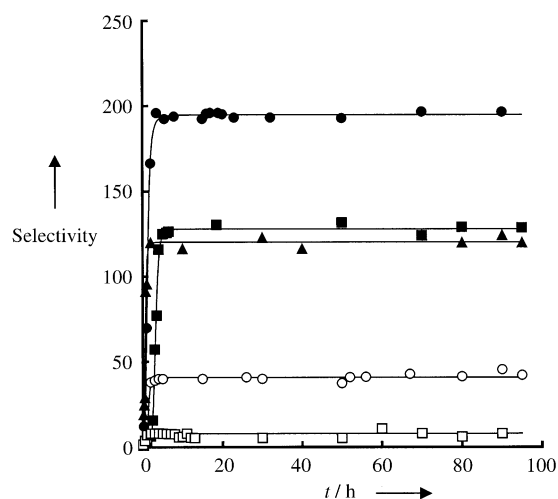


Figure 3. Change of selectivity of isoprene over *n*-pentane with time for various membranes containing zwitterionic silver complexes; ● **2a**-NO₃, ■ **2a**-ClO₄, ▲ **2a**-BF₄, ○ **2b**-BF₄, □ **2c**-BF₄.

Investigation of the anion effect on isoprene transport is in progress. The selectivity also varied significantly with the change of alkyl group on the imidazolium cations. Of the zwitterionic silver complexes that have the BF₄⁻ counterion, **2a**-BF₄ exhibits the highest selectivity and **2c**-BF₄ the lowest. The lower performance of phenyl-substituted imidazolium silver complex, **2c**-BF₄ can be largely attributed to the fact that the vacant site of silver ion is occupied by an additional sulfopropyl imidazolium ion, thereby blocking the coordination of isoprene to the silver ion to a certain extent. The

selectivities for isoprene over *n*-pentane increased rapidly with time up to about 2 h and then remained nearly constant throughout the experiments, thus demonstrating the stability of zwitterionic silver complexes towards reduction of the silver ions. In fact, the initial white color of the membrane was retained even after a long period of use. This result is a clear indication that the reduction of silver ions did not occur during the separation process. Such a high stability of silver ions in zwitterionic complexes is believed to stem from the strong interaction of silver ion with the sulfonate groups and a favorable electronic effect of the sulfopropyl imidazolium ions.

The permeation fluxes measured were 8×10^{-9} for **2a**-NO₃, 9×10^{-9} for **2a**-BF₄, 6×10^{-9} for **2a**-ClO₄, 9×10^{-9} for **2b**-BF₄, and 4×10^{-10} mol cm⁻² s⁻¹ for **2c**-BF₄. As expected from the reduced number of silver ions present in the membrane, **2c**-BF₄ gave the lowest flux, but other zwitterionic silver complexes exhibited similar permeation fluxes. The initial fluxes were maintained throughout the separation experiments.

The membranes containing zwitterionic silver complexes were also effective for the separation of mixtures of 1-pentene and *n*-pentane. When **2a**-NO₃ and **2a**-BF₄ were used as carriers, selectivities of 93 and 47 were obtained, respectively. The lower selectivities for 1-pentene compared with those for isoprene can be attributed to the fact that the binding ability of 1-pentene to silver ions is weaker than that of isoprene.

It should be mentioned here that this is the first attempt to use ionic liquid-based silver complexes as carriers for the facilitated transport membranes in separating unsaturated compounds from their corresponding alkanes. More importantly, the membranes containing zwitterionic silver complexes are easier to prepare, more stable, and more selective for separating isoprene from *n*-pentane compared with conventional ion-exchange membranes.

Efforts to improve the performance of the membranes by modifying the imidazolium moiety in the zwitterionic silver complexes are in progress as well as the detailed investigation of the effect of water on the performance and stability of the membranes.

Experimental Section

Compounds **1a**, **1b**, and **1c** were prepared by treating 1,3-propane sultone with the corresponding 1-alkyl or 1-phenylimidazole, similarly to the literature procedure employed for the preparation of 1-ethyl-3-sulfopropyl imidazolium compound.^[14] Isoprene and *n*-pentane were purchased from Aldrich and distilled prior to use. All other chemicals were obtained from Aldrich and used as received. Melting points were measured by using a DSC (TA Instruments).

2a-BF₄: A solution of AgBF₄ (11 mmol) in THF (30 mL) was treated with **1a** (10 mmol) at room temperature for 3 h. The solution was filtered, washed with THF to remove unconverted AgBF₄, and dried under a reduced pressure to give air stable white solid. Yield: 96%; mp 94.9°C; elemental analysis calcd (%): C 21.08, H 3.03, N 7.02; found: C 21.12, H 3.05, N 7.00; ¹H NMR (300 MHz, D₂O, 25°C): δ = 2.17 (m, 2H; CH₂), 2.78 (t, 2H; CH₂), 3.75 (3, 3H; CH₃), 4.22 (t, 2H; CH₂), 7.30 (s, 1H; CH), 7.38 (s, 1H; CH), 8.61 ppm (s, 1H; CH).

2a-NO₃: AgNO₃ (11 mmol) was treated with **1a** (10 mmol) in MeOH (30 mL) at room temperature for 3 h. The solution was

filtered to remove unconverted AgNO₃, and the solvent was removed under vacuum to give an air-stable white solid. Yield: 97%; mp: 119.9°C; elemental analysis calcd (%): C 22.47, H 3.23, N 11.23; found: C 22.63, H 3.21, N 11.20; ¹H NMR (300 MHz, D₂O, 25°C): δ = 2.17 (m, 2H; CH₂), 2.77 (t, 2H; CH₂), 3.74 (3, 3H; CH₃), 4.21 (t, 2H; CH₂), 7.29 (s, 1H; CH), 7.30 (s, 1H; CH), 8.61 ppm (s, 1H; CH).

Other zwitterionic silver complexes were prepared in a similar manner to that of **2a**-BF₄ or **2a**-NO₃.

2a-ClO₄: Yield: 98%; mp: 103.4°C; elemental analysis calcd (%): C 20.43, H 2.94, N 6.81; found: C 20.62, H 3.01, N 6.78; ¹H NMR (300 MHz, D₂O, 25°C): δ = 2.16 (m, 2H; CH₂), 2.77 (t, 2H; CH₂), 3.74 (3, 3H; CH₃), 4.21 (t, 2H; CH₂), 7.29 (s, 1H; CH), 7.36 (s, 1H; CH), 8.60 ppm (s, 1H; CH).

2b-BF₄: Yield: 97%; mp: 104.0°C; elemental analysis calcd (%): C 27.24, H 4.11, N 6.35; found: C 27.34, H 4.10, N 6.41; ¹H NMR (300 MHz, D₂O, 25°C): δ = 0.78 (t, 3H; CH₃), 1.18 (m, 2H; CH₂), 1.72 (m, 2H; CH₂), 2.19 (m, 2H; CH₂), 2.79 (t, 2H; CH₂), 4.07 (t, 2H; CH₂), 4.24 (t, 2H; CH₂), 7.39 (s, 1H; CH), 7.41 (s, 1H; CH), 8.69 ppm (s, 1H; CH).

2c-BF₄: Yield: 97%; mp: 77.4°C; elemental analysis calcd (%): C 39.63, H 3.88, N 7.70; found: C 39.80, H 3.92, N 7.68; ¹H NMR (300 MHz, D₂O, 25°C): δ = 2.63 (m, 2H; CH₂), 2.86 (t, 2H; CH₂), 4.35 (t, 2H; CH₂), 7.50 (s, 5H; C₆H₅), 7.60 (s, 1H; CH), 7.76 (s, 1H; CH), 9.17 ppm (s, 1H; CH).

Single crystals of **2c**-BF₄ suitable for X-ray diffraction studies were grown in methanol at -10°C.

Performance of membranes: Composite membranes were prepared by casting an aqueous solution of a zwitterionic silver complex (2.0 mmol in 0.5 mL H₂O) onto a polyester microporous membrane support (0.1 μ m, 47 mm, Whatman Industries Inc.) by using a coater. The coated membrane was dried in an oven at room temperature for 12 h under a stream of nitrogen, and then further dried in a vacuum oven at 40°C for 24 h. The separation of isoprene/*n*-pentane (50:50 wt. %) was performed at room temperature with the cast membranes in a module.^[20] Helium gas (10 mL min⁻¹) was used as a sweep gas and the flow rate of He was controlled by using a mass flow controller. A mixture of isoprene/*n*-pentane (10 mL, v/v = 50:50) was charged into a FEP tube attached to the lid of the module. The permeate was collected at -20°C in a trap filled with *n*-heptane and analyzed by gas chromatography (Younglin, Model 600D equipped with an FID and a unibead 2S 60/80 packed column).

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Keywords: facilitated transport · ionic liquids · membranes · silver · zwitterions

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Boranes

Conversion of Alkyltrifluoroborates into Alkyldichloroboranes with Tetrachlorosilane in Coordinating Solvents**

Byung Ju Kim and Donald S. Matteson*

Addition of tetrachlorosilane to organotrifluoroborates, RBF_3K (R = alkyl or aryl), in THF or acetonitrile at 20–25 °C results in immediate evolution of gaseous tetrafluorosilane and formation of the corresponding solvated organo-

[*] Dr. B. J. Kim, Prof. D. S. Matteson
Department of Chemistry, Box 644630
Washington State University
Pullman, WA 99164-4630 (USA)
Fax: (+1) 509-335-8867
E-mail: dmatteson@wsu.edu

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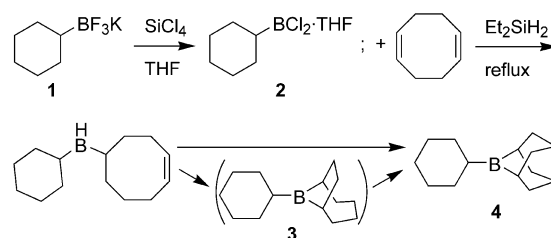
dichloroborane, RBCl_2 . This reaction completes a mild and efficient route from boronic esters to reactive alkyldichloroboranes, which are promising intermediates for asymmetric syntheses.

Previously known chemistry of RBF_3K includes the reaction of PhBF_3K with Me_3SiCl in acetonitrile to form PhBF_2 .^[1] Allylic RBF_3K derivatives react with aldehydes by defluorination with $\text{BF}_3\text{-OEt}_2$ to give the intermediates RBF_2 ,^[2] or by defluorination with $\text{Bu}_4\text{NI/H}_2\text{O}$.^[3] Alkylboronic esters of $\approx 99\%$ stereopurity are available by the reaction of boronic esters of chiral diols with LiCHCl_2 .^[4] Conversion of a stereopure (5-azido-1-phenylbutyl)boronic ester to $\text{N}_3(\text{CH}_2)_3\text{-CH(Ph)BF}_3\text{K}$ followed by treatment with Me_3SiCl or SiCl_4 has been shown to yield 2-phenylpyrrolidine without loss of stereopurity,^[5] but the nature of the alkyldihaloborane intermediates was not investigated. The chemistry of RBF_3^- salts has been reviewed in 2003.^[6] No previous instance of direct conversion of an RBF_2 to an RBCl_2 by means other than B-F/B-Cl exchange has been found in a search of the literature.^[7]

The barrier to F/Cl exchange is thermodynamic. Conversion of BF_3 and SiCl_4 into BCl_3 and SiF_4 [Eq (1)] is endothermic in the gas phase, $\Delta H^\circ_{298} = +74.014 \text{ kJ mol}^{-1}$ or $+6.168 \text{ kJ mol}^{-1} \text{ bond}^{-1}$; $\Delta G^\circ_{298} = +64.755 \text{ kJ mol}^{-1}$ or $+5.396 \text{ kJ mol}^{-1} \text{ bond}^{-1}$.^[8]



The ^{11}B NMR spectra of the products from treatment of potassium (cyclohexyl)trifluoroborate (CyBF_3K , **1**; Scheme 1), phenyltrifluoroborate (PhBF_3K), or (5-azidopentyl)trifluoroborate [$\text{N}_3(\text{CH}_2)_5\text{BF}_3\text{K}$] with SiCl_4 in THF or



Scheme 1. Conversion of potassium (cyclohexyl)trifluoroborate (**1**) into cyclohexyl-9-BBN (**4**).

CH_3CN (Table 1) correspond to solvated RBCl_2 and show only minor impurities, including all $\text{SiF}_n\text{Cl}_{(4-n)}$ ($n = 1\text{--}4$),^[9] in the ^{19}F NMR spectrum. In CH_2Cl_2 with a catalytic amount of [18]crown-6, the reaction stops at CyBF_2 or PhBF_2 , and in Et_2O an unidentified mixture was obtained. The products from Me_3SiCl in all solvents were RBF_2 . Commercial PhBCl_2 and PhBF_2 from $\text{PhBCl}_2\text{-NaBF}_4$ showed similar NMR spectra. The ^{11}B and ^{19}F chemical shifts of RBF_2 (Table 1) are consistent with tetracoordinate boron in THF but also tricoordinate boron in CH_3CN .^[10] The RBCl_2 derivatives are tetracoordinate in both solvents. The stronger solvation of RBCl_2 evidently favors its formation.

Tetrachlorosilane in acetonitrile is the preferred reagent for converting alkyltrifluoroborates and alkyl azides into secondary amines.^[5] It is now apparent that the relevant

Table 1: NMR spectroscopic data for RBF₃K, RBF₂, and RBCl₂ in various solvents.

Compound	Solvent	¹¹ B, δ	¹⁹ F, δ	Other
CyBF ₃ K ^[a]	CD ₃ CN	5.4	−145.2 ^[b]	J _{BF} = 63 Hz
CyBF ₂	THF/CDCl ₃	9.6	−149.5	
CyBF ₂	THF/CD ₃ CN	9.5	−149.8	
CyBF ₂	CH ₃ CN/CDCl ₃	28	−79.5	
CyBF ₂	CH ₃ CN/CD ₃ CN	33	−79.5	
CyBCl ₂	THF/CDCl ₃	15.7	none	
CyBCl ₂	CH ₃ CN/CD ₃ CN	5.7	none	
PhBF ₃ K	CD ₃ CN	4.0	−141	J _{BF} = 55 Hz
PhBF ₂	CDCl ₃	24.6	−92	
PhBF ₂	CD ₃ CN	11.6	−127.9	
PhBF ₂	THF/CD ₃ CN	7.2	−148.6	
PhBF ₂	THF/CDCl ₃	7.2	−149.8	
PhBCl ₂ ^[c]	CDCl ₃	55	none	
PhBCl ₂	CD ₃ CN	3.3	none	
PhBCl ₂	THF/CD ₃ CN	12.6	none	
N ₃ (CH ₂) ₅ BF ₃ K	CDCl ₃	6.1	−139.2	
N ₃ (CH ₂) ₅ BF ₂	THF/CDCl ₃	9.5	−145.8	
N ₃ (CH ₂) ₅ BCl ₂	THF/CDCl ₃	14.6	none	
N ₃ (CH ₂) ₅ BCl ₂	PhCH ₃ /CDCl ₃	7.5	none	

[a] Cy = cyclohexyl. [b] Also measured in D₂O, δ = −143 ppm.

[c] Commercial PhBCl₂ (Aldrich).

factors are that RBCl₂ reacts faster than RBF₂ with alkyl azides and that RBCl₂–CH₃CN dissociates more easily than RBCl₂–thf. The more weakly solvated primary RBCl₂ in diethyl ether react with alkyl azides at 25 °C.^[11]

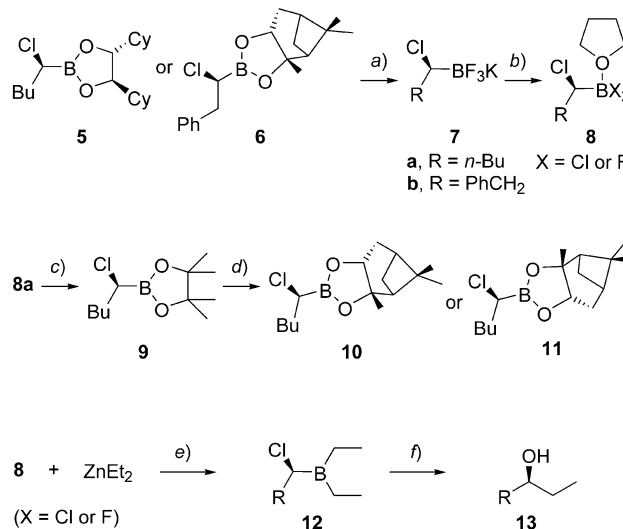
The higher reactivity of RBCl₂ compared to RBF₂ is significant in hydroboration chemistry. In noncoordinating solvents, the addition of an alkylsilane to RBCl₂ and an alkene results in rapid hydroboration, with clean formation of dialkylchloroborane if the molar ratio of reactants is 1:1:1.^[12] However, CyBF₂ (≈0.3 M in CH₂Cl₂) with Et₂SiH₂ does not hydroborate 1-hexene in 24 h at 20–25 °C. Hydroboration with sterically hindered RBCl₂ in the presence of diethyl ether has been reported.^[13] Accordingly, we briefly investigated hydroborations of alkenes with Et₂SiH₂ and CyBF₃K (**1**) in THF. Hydroboration is greatly retarded and no pure single product prior to trialkylborane can be produced.

Conversion of CyBCl₂ (**2**) and 1,5-cyclooctadiene to cyclohexyl-9-BBN (**4**) in refluxing THF requires ≈4 h, or only ≈1 h if an equimolar amount of 1-(dimethylamino)-naphthalene is added (Scheme 1). In accord with previous reports,^[14] the borabicyclo[4.2.1]nonane isomer **3** (¹¹B NMR: δ = 90.1 ppm) formed nearly as rapidly as the [3.3.1] isomer **4**, (¹¹B NMR: δ = 86.3 ppm), but rearranged into **4**. In contrast to the reaction in the absence of coordinating solvent,^[12] 1-hexene could not be converted to CyBClR, R = *n*-hexyl, without generating CyBR₂ (¹¹B NMR spectrum: δ = 85.3 ppm).

Reaction of CyBF₂ with BCl₃ in CH₂Cl₂ liberates gaseous BF₃ and free CyBCl₂, opening the way to the previously described stepwise control of hydroboration,^[12] but this chemistry is predictably restricted to RBCl₂ without alkoxy or other nucleophilic substituents and has not yet been explored.

Asymmetric (α-chloroalkyl)boronic esters can be converted into (α-chloroalkyl)dichloroboranes without measur-

able loss of stereopurity. [(*S*)-1-Chloropentyl]boronic ester **5**, prepared from the (butyl)boronic ester and LiCHCl₂,^[4,15] was converted into the trifluoroborate salt **7a**,^[5] then treated with SiCl₄ in THF to provide **8a** (Scheme 2). Treatment with methanol and pinacol yielded boronic ester **9** (95 % from **7**). Transesterification of separate portions with the enantiomeric (*S*)- and (*R*)-pinanediol esters produced **10** and **11** in high stereopurity, as shown by their ¹H NMR signals in the typically differentiated region,^[15] δ = 1.15–1.20 ppm (Figure 1, Scheme 2).



Scheme 2. Reactions of asymmetric alkyldihaloboranes. a) KHF₂.^[3] b) For X = Cl, SiCl₄ in THF; for X = F, Me₃SiCl/THF. c) 1. MeOH, 0 °C; 2. Pinacol, 25 °C, 1 h, 95 % from **7**. d) (*S*)-Pinanediol yields **10**, (*R*)-pinanediol **11**; filtered through silica. e) Et₂Zn. f) 1. MeOH, NaOMe; 2. H₂O₂, NaOH.

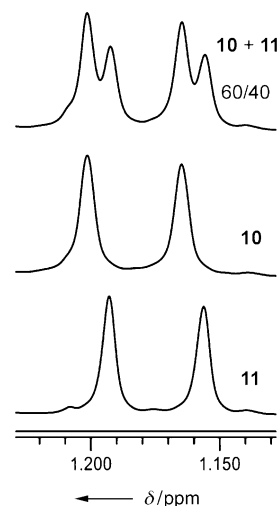


Figure 1. Differentiated ¹H NMR signals of (*S*)- and (*R*)-pinanediol (*S*)-1-(chloropentyl)boronates **10** and **11**.

An anticipated future synthetic application of asymmetric (α-chloroalkyl)dihaloboranes such as **8** is for joining two alkyl groups, especially combinations in which neither is available from a Grignard reagent. Connection to boron by hydro-

boration is not an option because (α -chloroalkyl)hydroboranes undergo rapid self-reduction in THF.^[16] but organometallic reagents should be useful. In view of the wide variety of organozinc reagents that have become available from the work of Knochel's group,^[17] diethylzinc was chosen for preliminary tests. All four (α -chloroalkyl)dihaloboranes **8** yielded optically active secondary alcohols **13** after treatment with excess diethylzinc followed by sodium methoxide and then alkaline hydrogen peroxide. The enantiomeric purities of **13** have not been verified, but rotations are in the expected range. Yields were satisfactory for **13a**, excellent for the less volatile **13b**. Intermediate **12** from **8a**, X = F, was verified by the ¹¹B NMR signal at δ = 87.5 ppm.

Experimental Section

13a: Trifluoroborate **7a** (1.57 g, 7.4 mmol) was stirred for 4 h with SiCl₄ (1.2 mL, 14.8 mmol) in THF (20 mL) at 20–25 °C under argon. Most of the excess SiCl₄ was removed by concentration under vacuum. The residue of **8a** (X = Cl) in THF (20 mL) at 0 °C was treated with ZnEt₂ (15 mL, 1 M in hexanes). After 12–16 h MeOH (5 mL) was added at 0 °C. When gas evolution ceased, the mixture was treated with NaOMe (2 g, 37 mmol) at 0 °C, then stirred for 4 h at 20–25 °C. After cooling to 0 °C, aqueous NaOH (5 mL, 3 M) and H₂O₂ (30%, 5 mL) were added. After 3 h, the mixture was worked up with ether and water and the residue was purified by flash chromatography (10% ether/pentane) and bulb to bulb distillation of **13a** (0.507 g, 59%); ¹H NMR (CDCl₃): δ = 3.52 (m, 1 H), 1.73 (bs, 1 H), 1.23–1.63 (m, 8 H), 0.94 (t, J = 7.2 Hz, 3 H), 0.91 (t, J = 6.9 Hz, 3 H); ¹³C NMR (CDCl₃): δ = 73.3, 36.6, 30.1, 27.8, 22.8, 14.1, 9.9 ppm; $[\alpha]_D^{22}$ = +7.9 (c = 0.03 in CHCl₃), $[\alpha]_{546}^{22}$ = +8.9 (c = 0.03 in CHCl₃); (lit., $[\alpha]_D$ = +5.83 (CHCl₃) (“95% ee”));^[18] $[\alpha]_D$ = +6.7 (neat), $[\alpha]_D$ = +8.0 (EtOH), $[\alpha]_D$ = +8.33 (Et₂O).^[19]

13b: Similar treatment of **7b** (partially epimerized, d.r. 86:14) yielded **13b** (94%); ¹H NMR (CDCl₃): δ = 7.18–7.34 (m, 5 H), 3.73 (m, 1 H), 2.82 (AB, dd, J = 13.5, 4.2 Hz, 1 H), 2.63 (AB, dd, J = 13.5, 8.4 Hz, 1 H), 1.64 (bs, 1 H), 1.52 (m, 2 H), 0.99 ppm (t, J = 7.8 Hz, 3 H); ¹³C NMR (CDCl₃): δ = 138.6, 129.4, 128.5, 126.3, 74.0, 43.5, 29.5, 10.0 ppm; $[\alpha]_D$ = +15.7 (ee < 72%, c = 0.054 in Et₂O); $[\alpha]_{546}$ = +20.0; no literature data available.

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Keywords: alkyl boranes · asymmetric synthesis · boron · hydroboration

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Cyclic Phosphanes

Efficient One-Pot Synthesis of Secondary Cyclic Phosphanes with Easy Regeneration of the Phosphorus-Donor Reagent Used**

Graziano Baccolini, Carla Boga, and Matteo Galeotti*

New syntheses of cyclic phosphanes are of considerable current interest, principally because they play a central role in

[*] Prof. Dr. G. Baccolini, Dr. C. Boga, Dr. M. Galeotti
Dipartimento di Chimica Organica
Università di Bologna
Viale Risorgimento, 4-40136 Bologna (Italy)
Fax: (+39) 051-209-3654
E-mail: baccolin@ms.fci.unibo.it

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coordination chemistry and homogeneous catalysis,^[1] but to date the most widely used procedures for obtaining secondary cyclic phosphanes give very low overall yields (3–5%).^[2–5]

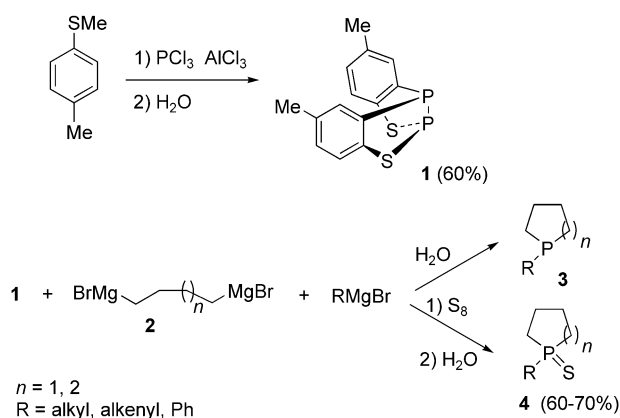
Secondary phosphanes are prepared by multistep procedures in which the final step is reduction of a phosphorus compound containing P–O, P–S, or P–Cl bonds with a wide variety of reagents and reaction conditions. However, whereas secondary acyclic phosphanes can be synthesized by several routes, only a few procedures for secondary five- (phospholanes) and six-membered cyclic phosphanes (phosphinanes) have been reported.^[2] For example, phospholane **5a** (see Scheme 3) was prepared^[3] by reaction of tetramethylenebis(magnesium bromide) $\text{BrMgC}_4\text{H}_8\text{MgBr}$ with dimethylphosphoramidous dichloride $(\text{Me})_2\text{NPCl}_2$ at -78°C to give the aminocyclophosphane $(\text{Me})_2\text{NPC}_4\text{H}_8$ in 8% yield. This aminocyclophosphane was treated with B_2H_6 and then kept in a sealable tube at 220°C . The tube was then sealed up and heated for 21 h at 210°C , and subsequent distillation gave a fraction containing the desired $\text{C}_4\text{H}_8\text{PH}$ (30%) and aminoborane impurities, which were separated by treatment with HCl. The overall yield of this multistep procedure was not higher than 3%. In another recent preparation, phospholane^[4] was obtained in approximately 5% yield by flash vacuum pyrolysis of butyldichlorophosphane at 600°C .

Phosphinane **5b** is obtained by similar multistep procedures^[5] or by flash pyrolysis.^[4] Other recent syntheses of these cyclic phosphanes use (trimethylsilyl)phosphane^[6] or an organolanthanide-catalyzed hydrophosphination/cyclization reaction,^[7] but the former reagent is very difficult and dangerous to prepare, and the latter procedure^[7] often gives a mixture of phospholane and phosphinane.

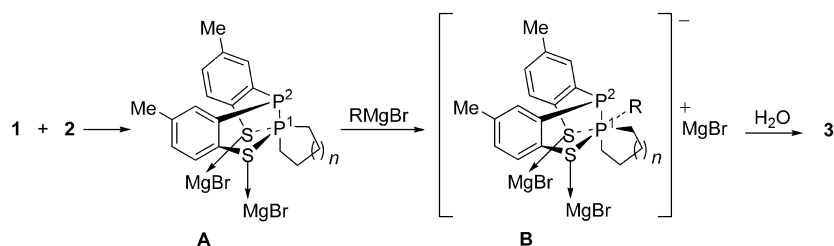
Herein we report a highly efficient and economical new method for one-pot preparation of **5a** and **5b** (70–80% yield) using an unusual phosphorus-donor reagent, namely, the benzothiadiphosphole **1** which, at the end of the process, can be easily regenerated by simple reaction of its end product **6** with PCl_3 .

We have reported^[8] that **1** is easily obtained by simple treatment of *p*-methylthioanisole with PCl_3 and AlCl_3 , and that it can be isolated by crystallization from the reaction mixture. Compound **1** is an air-stable solid that can be stored for several years without particular precautions, and it is also easy to handle. Subsequently, we found^[9] that **1** can be used as a phosphorus donor, and we recently reported^[10] that simultaneous or sequential addition of an equimolar mixture of a bis(Grignard reagent) **2** ($n=1, 2$; Scheme 1) and a Grignard reagent RMgBr ($\text{R}=\text{alkyl, phenyl, alkenyl}$) to an equimolar amount of **1** gave phosphanes **3** or, after addition of elemental sulfur, their sulfides **4** in good yield at room temperature.

The above results were explained by the intervention of hypervalent (penta- and hexacoordinate) phosphorus intermediates^[11] such as **A** and **B** (Scheme 2) in which the “dibenzo-butterfly” moiety of reagent **1**, as depicted in Scheme 2, might favor their formation. In pentacoordinate intermediate **A** coordination of the magnesium atom by a



Scheme 1. Preparation of **1** and synthesis of cyclic tertiary phosphanes **3** and their sulfides **4**.

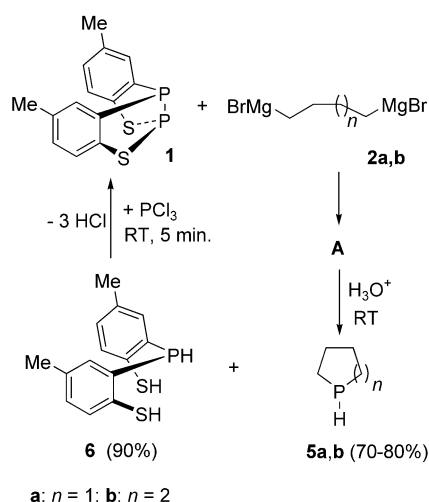


Scheme 2. Proposed reaction pathway for the formation of cyclic phosphanes **3**.

sulfur atom would activate P^1 toward further nucleophilic attack to give unstable hexacoordinate intermediate **B**. Treatment of **B** with water or sulfur gives phosphane **3** or its sulfide **4**, respectively.

To develop further applications of this reaction we then studied what happens when intermediate **A**, formed by reaction of **1** with one equivalent of **2**, is treated with water. Surprisingly, in this case we found that it is possible to obtain secondary cyclic phosphanes **5** in 70–80% yields (based on **2**). In addition, from the aqueous solution it is also possible to isolate, in very good yield (90% based on **1**), the new compound 4-methyl-2-[(5-methyl-2-sulfanylphenyl)phosphanyl]benzenethiol (**6**) which is the end product derived from **1** (Scheme 3).

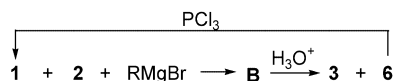
As depicted in Scheme 3, we first prepared intermediate **A** by reaction of equimolar amounts of **1** and a bis(Grignard reagent) **2** in THF. Partial evaporation of the solvent, treatment of the reaction mixture with aqueous acid followed by extraction with organic solvent (CH_2Cl_2 , diethyl ether) gave a mixture of secondary phosphanes **5** and residue **6**. These can be easily separated by treating the solution with aqueous NaOH; in this way the sodium salt of **6** dissolves in the aqueous solution, whereas the organic phase contains almost pure phosphanes **5** (70–80%), which can be purified by bulb-to-bulb distillation. Compounds **5a** and **5b** were characterized principally by ^1H , ^{31}P NMR, and IR spectroscopy and mass spectrometry, the data from which agree with the reported values.^[4,6a,b] Compound **6** can be recovered from the basic aqueous layer by acidification and extraction, and purified by distillation. It was stored under argon and



Scheme 3. Synthesis of secondary cyclic phosphanes **5a,b** and regeneration of the starting reagent **1** from end product **6**.

characterized by ^1H and ^{31}P NMR spectroscopy and HR-MS.^[12]

Simply treating a dry solution of **6** with an equimolar amount of PCl_3 regenerates **1** in sufficiently pure form that it can be reused without further purification (Scheme 3). Finally, we carried out the reaction shown in Scheme 1 to obtain tertiary phosphanes **3** using the same reaction conditions and separation procedure used to obtain compounds **5**, and we found that also in this case it was possible to isolate **6** (Scheme 4).



Scheme 4. Regeneration of **1** from **6**, obtained in the preparation of tertiary phosphanes **3**.

In conclusion, the syntheses of secondary and tertiary cyclic phosphanes reported herein can be carried out in a very simple, efficient, and low-cost procedure that gives higher yields than those previously reported. In addition, this synthesis is atom-economic^[13] and environmentally friendly, because by-product **6** is easily transformed quantitatively into starting reagent **1**, which can be recycled.

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Structure and Phase Behavior of a 2:1 Complex between Arene- and Fluoroarene-Based Conjugated Rigid Rods**

Stephen W. Watt, Chaoyang Dai, Andrew J. Scott, Jacquelyn M. Burke, Rhodri Ll. Thomas, Jonathan C. Collings, Christopher Viney, William Clegg, and Todd B. Marder*

Conjugated molecular materials are important^[1] owing to their role in organic devices such as organic light-emitting diodes (OLEDs) and thin-film transistors (TFTs).^[2] The performance of such devices depends not only on the intramolecular properties, but also on intermolecular interactions,^[3] both of which can be modified by introduction of fluorine substituents.^[4] Fluorinated oligomers have been utilized as electron-transporting layers in OLEDs.^[5] Recently, an oligothiophene with terminal perfluorophenyl groups was used in an *n*-type TFT and shown to exhibit a high mobility,

partially attributed to intermolecular arene–perfluoroarene stacking interactions.^[6] The arene–perfluoroarene interaction itself has been known for over 40 years,^[7] and has been shown to occur in numerous 1:1 complexes of arenes and fluoroarenes, including those of dissimilar shape,^[8] as well as in partially fluorinated conjugated molecules,^[9] in which it has been used to effect topological polymerizations.^[10]

Phenylene–ethynylenes such as 1,4-bis(phenylethynyl)benzene (**HHH**) have attracted attention, particularly with regard to their photophysical behavior.^[11,12] We have reported the crystal structure of a 1:1 complex of 1,4-bis(phenylethynyl)tetrafluorobenzene (**HFH**) and 1,4-bis(pentafluorophenylethynyl)benzene (**FHF**), which exhibits nematic liquid-crystalline (LC) phase behavior that is not present in either of the pure components.^[12] Herein we report the structure and preliminary LC phase behavior of a novel^[13] 2:1 arene–perfluoroarene complex formed between **HHH** and its perfluorinated analogue **FFF**.

Both components were synthesized by Sonogashira cross-coupling methodology.^[14] Crystals of the 2:1 complex (**2HHH**)**FFF** were obtained reproducibly from an equimolar mixture of **HHH** and **FFF** in toluene, whereby residual **FFF** crystallized separately. The single-crystal structures of the complex and of the two pure components were determined from X-ray diffraction data.^[15,16]

The complex (**2HHH**)**FFF** crystallizes in space group *Fddd*. The molecules pack in infinite stacks, in contrast to the respective herringbone and nonstacked layer arrangements found in crystals of pure **HHH** and **FFF**. In the complex, the molecules of **FFF** lie on crystallographic twofold rotation axes which pass through opposite C–C bonds of its central ring and are symmetrically sandwiched by two molecules of **HHH** (Figure 1). The molecules are essentially planar; the dihedral angles between the outer and central rings are 3.7 and 2.3° for **HHH**, and 2.0° for **FFF**, as opposed to 3.1 and 1.1° for pure **HHH** and **FFF**, respectively.

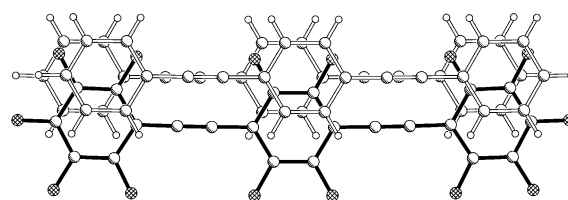


Figure 1. One **HHH**–**FFF**–**HHH** sandwich, which corresponds to twice the asymmetric unit of the crystal structure of (**2HHH**)**FFF** showing the overlap of the three molecules; filled bonds = **FFF**, hollow bonds = **HHH**.

The approximately parallel stacking of the molecules is not restricted to the (**2HHH**)**FFF** unit, but is propagated infinitely throughout the crystal structure; adjacent molecules of **HHH** are separated by 3.553 Å. Within each stack, adjacent molecules are approximately parallel, offset laterally from each other, and approximately equidistant; the mean stacking separation is 3.571 Å, typical of arene–perfluoroarene complexes. The stacks of molecules lie along directions parallel to the two *bc* diagonals of the orthorhombic unit cell,

[*] Dr. J. M. Burke, Dr. R. Ll. Thomas, Dr. J. C. Collings, Prof. T. B. Marder
Department of Chemistry
University of Durham
South Road, Durham, DH1 3LE (UK)
Fax: (+44) 191-384-4737
E-mail: todd.marder@durham.ac.uk

Dr. S. W. Watt, Prof. C. Viney^{††}
Department of Chemistry
School of Engineering and Physical Sciences
Heriot-Watt University, Edinburgh, EH14 4AS (UK)

Dr. A. J. Scott, Prof. W. Clegg
School of Natural Sciences (Chemistry)
University of Newcastle upon Tyne
Newcastle upon Tyne, NE1 7RU (UK)

Dr. C. Dai
Department of Chemistry
University of Waterloo
Waterloo, Ontario, N2L 3G1 (Canada)

[†] Current address:
Department of Chemistry, University of Cambridge
Lensfield Road, Cambridge CB1 1EW (UK)

[††] Current address:
University of California Merced, School of Engineering
P.O. Box 2039, Merced, CA 95344 (USA)

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almost but not quite orthogonal to one another (angle of 82.7°); the two types of stacks form layers in the *bc* plane, alternating with a displacement of *a*/4 relative to each other (Figure 2).

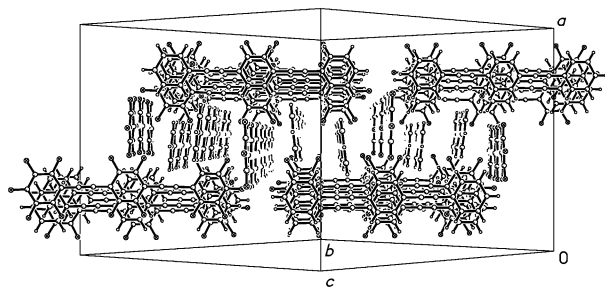


Figure 2. Packing of the triple units in the crystal structure of (2 HHH) FFF.

The principal interactions between stacks of molecules are of two types. Between stacks in a given layer, the closest contacts are F...F interactions between the fluorine atoms at the ends of the two molecules. The F...F separation of 2.528 Å compares to a sum of van der Waals (vdW) radii of 2.90 Å.^[17] Between stacks in adjacent layers, there are several H...F contacts, the shortest of which are 2.515 and 2.659 Å (sum of vdW radii = 2.55 Å).^[18] The near-perpendicular arrangement of stacks is proposed to arise from the unequal (2:1) ratio of H and F substituents in the stacks, which, in contrast to 1:1 complexes with parallel stacks, such as **HHFHFF**,^[12] precludes the establishment of a perfectly complementary network of stabilizing edge-to-edge H...F interactions.^[19]

Differential scanning calorimetry (DSC) and transmitted polarized optical microscopy were used to probe the mesomorphism of the 2:1 **HHH:FFF** system, which proved to be very complex. Thus, when a 2:1 mixture of **HHH** and **FFF** is cooled from the isotropic phase, surprisingly it appears to exhibit two different sequences of phase changes, with apparently no preference for either, over several samples and runs. In one pathway, the melt cools via a mesophase (ordered smectic phase S1) to a solid (X1) which, on reheating, exhibits similar thermal transitions to solution-grown cocrystals. However, in other runs, cooling leads to what appears to be a different mesophase (S2, also an ordered smectic mesophase, as judged by an optical texture different from that of S1), and then to a solid (X2), which does not have the same morphology as X1. On reheating, X2 does not behave like the solution-grown solid; instead, it first recrystallizes to a solid phase resembling X1. The DSC thermograms show subtle differences in the two cooling/heating cycles, although they also contain events which may be those of the individual components.

Solids X1 and X2 appear to be related paramorphotically to S1 and S2, respectively, and must therefore be linked to the smectic phase through particular kinetic pathways. That two different cooling pathways are found on exiting the isotropic state is highly curious, and consequently a more detailed account of the liquid-crystalline behavior of the **FFFHHH** system will be the subject of a future report, as will detailed computer-modeling studies on this system.

The 2:1 complex formed between 1,4-bis(phenylethynyl)-benzene and its perfluorinated analogue is unique in that it represents the first example of a non-1:1, hydrocarbon-perfluorocarbon:arene-perfluoroarene complex. The inter-stack geometry is also unusual and probably results from the lack of opportunity to maximize the stabilizing H...F edge-to-edge interactions which are generally observed in complementary 1:1 complexes. The complex phase behavior of this system suggests that alternative arrangements are close in energy.

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Single DNA Rotaxanes of a Transmembrane Pore Protein**

Jorge Sánchez-Quesada, Alan Saghatelian,
Stephen Cheley, Hagan Bayley, and M. Reza Ghadiri*Dedicated to Professor Julius Rebek, Jr.
on the occasion of his 60th birthday

Recent advances in stochastic DNA sensing technologies,^[1] especially those that exploit the transmembrane protein pore α -hemolysin (α -HL), have led to the realization that rapid single-molecule DNA sequencing may be feasible.^[2] As part of an ongoing study to better ascertain the nucleobase recognition capacity of α -HL and its potential utility in DNA sequencing, we report herein methods for capturing single-stranded DNA–poly(ethylene glycol) (DNA-PEG) hybrid molecules inside the α -HL pore. The DNA– α -HL rotaxanes were characterized at the single-species level by electrophysiological techniques and display a stable and reversible two-state switching capacity that depends on the applied potential, the orientation, and the method of threading and capture used.

Staphylococcus aureus α -HL forms large heptameric protein pores in lipid bilayers. Its crystal structure shows a mushroom-shaped assembly with a central channel approximately 10 nm long with a diameter of 1.5 nm at the narrowest point.^[3] Macrocyclic adapters have been shown to lodge inside and modify the conductance characteristics of the α -HL pore. This approach has been used to devise stochastic sensors for a range of small organic molecules.^[4] In addition, the pore structure of α -HL can accommodate PEG molecules, which can be used either to measure the pore size^[5] or in α -HL-based biosensors for protein recognition.^[6] Nucleic acids

[*] Dr. J. Sánchez-Quesada, Dr. A. Saghatelian, Prof. Dr. M. R. Ghadiri
Departments of Chemistry and Molecular Biology and
The Skaggs Institute for Chemical Biology
The Scripps Research Institute
10550 North Torrey Pines Road
La Jolla, CA 92037 (USA)
Fax: (+1) 858-784-2798
E-mail: ghadiri@scripps.edu
Prof. Dr. H. Bayley
Department of Chemistry
Chemistry Research Laboratory
University of Oxford
Mansfield Road
Oxford, OX1 3TA (UK)
Dr. S. Cheley
Department of Medical Biochemistry and Genetics
Texas A&M Health Science Center
440 Reynolds Medical Building, College Station
TX 77843-1114 (USA)

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can also be detected during transit transport through the α -HL pore.^[2,7] Homopolymers and block copolymers of DNA such as poly(A) and poly(C) can be distinguished by different reductions in the α -HL channel conductance caused by the presence of these polymers.^[2b] These elegant studies provide the basis for the designs and studies reported herein.

Rotaxanes are among the most commonly used classes of functional supramolecular structures. These assemblies have promising applications in nanotechnology as molecular switches in which the thread unit contains at least two dynamic binding sites for the macrocyclic component, each defined by a distinct signal.^[8] In the study described herein, we sought to form rotaxanes by threading single-stranded (ss) DNA-PEG copolymers of defined lengths through the α -HL transmembrane pore. The pore has a dual function in our design: its protein structure plays a role equivalent to that of the macrocyclic subunit of typical small-molecule rotaxanes, and perturbations of its native ion conductance provide the sensing signature for evaluating the functional characteristics of the rotaxanes.

The thread component of our rotaxane is an appropriately functionalized ss-DNA-PEG hybrid molecule designed to afford two different conductance states, depending on the portion of the strand (DNA or PEG) that is occupying the channel. DNA hybrid **1** was constructed as a continuous linear arrangement of the following four segments: The 3'-end consists of a 20-base region designed to bind to a specific oligonucleotide of complementary sequence. It is followed by a poly d(A) 40-mer. This segment is extended with 10 units of hexaethylene glycol phosphate and is capped with a biotinyl group at the 5'-terminus. This design includes DNA and PEG regions of the appropriate length for full threading of the α -HL pore by either of the two polymeric segments. The design also provides a mechanism to lock thread **1** at both ends after its insertion into the pore. Formation of a DNA duplex^[2a] at the 3'-end of the polymer and binding of

streptavidin to the 5'-biotin unit^[6,7b,d] create structures at the ends that are wider than the α -HL entrances and thus can be used to lock-in the rotaxane complex (Figure 1 a). Similarly, DNA hybrid **2** has a double-stranded 3'-DNA stem-loop structure^[2c] designed to allow control of the threading orientation and rotaxane formation (Figure 1 b). We exploited the anionic character of the ss-DNA-PEG chains to drive the threading process by applying a transmembrane potential. Once a rotaxane has been formed, the relative position of the thread inside the pore can be switched simply

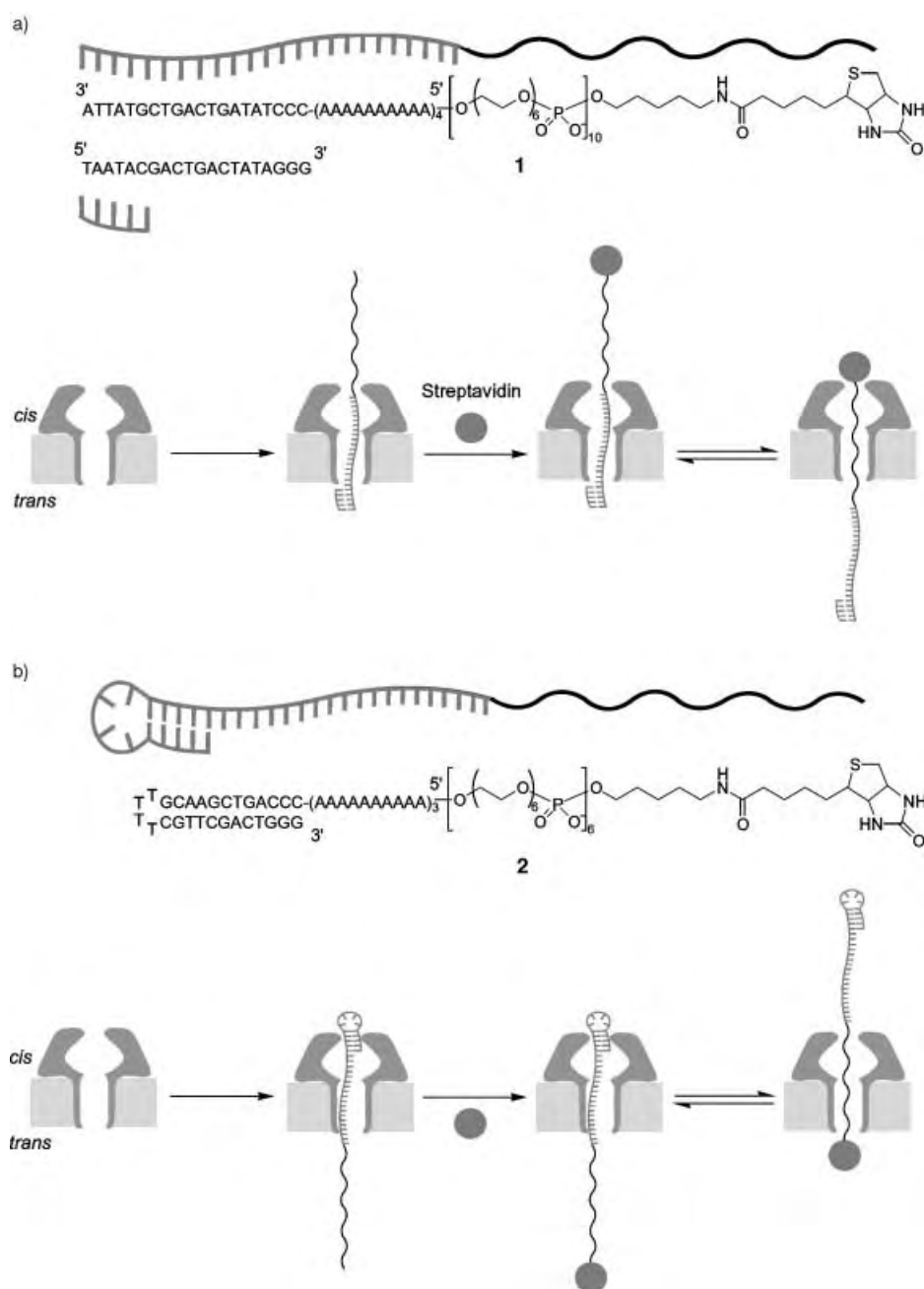


Figure 1. Schematic representation of the molecular components and processes employed for synthesis of transmembrane protein rotaxanes. a) System employed for rotaxane formation through capture by streptavidin on the *cis* side of the pore. b) Thread molecule with a DNA hairpin for rotaxane formation by capture with streptavidin on the *trans* side.

by adjusting the sign and magnitude of the applied transmembrane potential.

Initial experiments were performed with the uncapped ss-DNA-PEG thread **1**, which was added either to the *cis* or the *trans* side of the bilayer.^[9] At positive transmembrane holding potentials (negative when **1** is added in the *trans* chamber), blocking events were detected at two distinct current levels, which suggests that the PEG and DNA segments can be differentiated (Figure 2a,b). The events can be grouped in two classes according to which level is detected first. We have established (see below) that the higher blocking step (Level 2) results from the DNA segment traversing the pore, while the lower blocking level (Level 1) corresponds to the passage of the PEG portion of the strand. Events that display the higher blocking level first are more numerous than those that begin with the lower level, which indicates that the favored orientation for strand threading is with the 3'-DNA tail first.

Topoisomeric pseudorotaxanes of α -HL, which differ only in the orientation of the threading polymer **1**, were used to assign the two observed conductance states unequivocally. The conductance levels attributed to PEG and DNA could be established by sterically blocking the 3'- or 5'-terminus of **1** from entering the pore, either by DNA duplex formation at the 3'-tail or complexation of streptavidin to the 5'-biotin moiety. In experiments with the thread and streptavidin both present in the same chamber, the 5'-tail was blocked and thus only the DNA end was available to go through the α -HL pore. As expected, the current showed an initial high-level of block (passage of DNA), followed by a recovery of the channel conductance to a lower level which is assigned to the assembly with the PEG segment within the channel (Figure 2c,e). The conductance level was stable as long as the transmembrane voltage was applied, as we expect for a pseudorotaxane. This result indicates that the strength of the interaction between biotin and streptavidin as well as the steric bulk of the complex prohibit the strand from translocating through the channel pore to the other side of the bilayer.

Experiments performed under asymmetric salt conditions allowed the charge selectivity of the threaded channel to be determined.^[10] Free α -HL displays a slight anion selectivity ($P_{K^+}/P_{Cl^-} = 0.6$ in bilayers that separate solutions containing 300 and 500 mM KCl on the *cis* and *trans* sides, respectively). When the pore is threaded from the *trans* side by the streptavidin-biotin strand complex, the P_{K^+}/P_{Cl^-} value is 2.0, which suggests that the negatively charged phosphate groups in the PEG segment of the strand change the charge density inside the pore and transform α -HL into a somewhat cation-selective pore (Figure 3a).

Hybridization of thread **1** to its complementary 20-mer DNA sequence prevents the 3'-DNA tail from entering the α -HL lumen; only the 5'-tail (PEG segment) is free to enter the pore. When **1** and the complementary short DNA were both added to the *trans* side of the pore (Figure 2f), the blocking events observed under negative transmembrane potentials remained stable over time and the high blocking level associated with the presence of the DNA segment of the thread inside the α -HL pore was seen. High charge selectivity was observed ($P_{K^+}/P_{Cl^-} > 100$) when the same experiment was

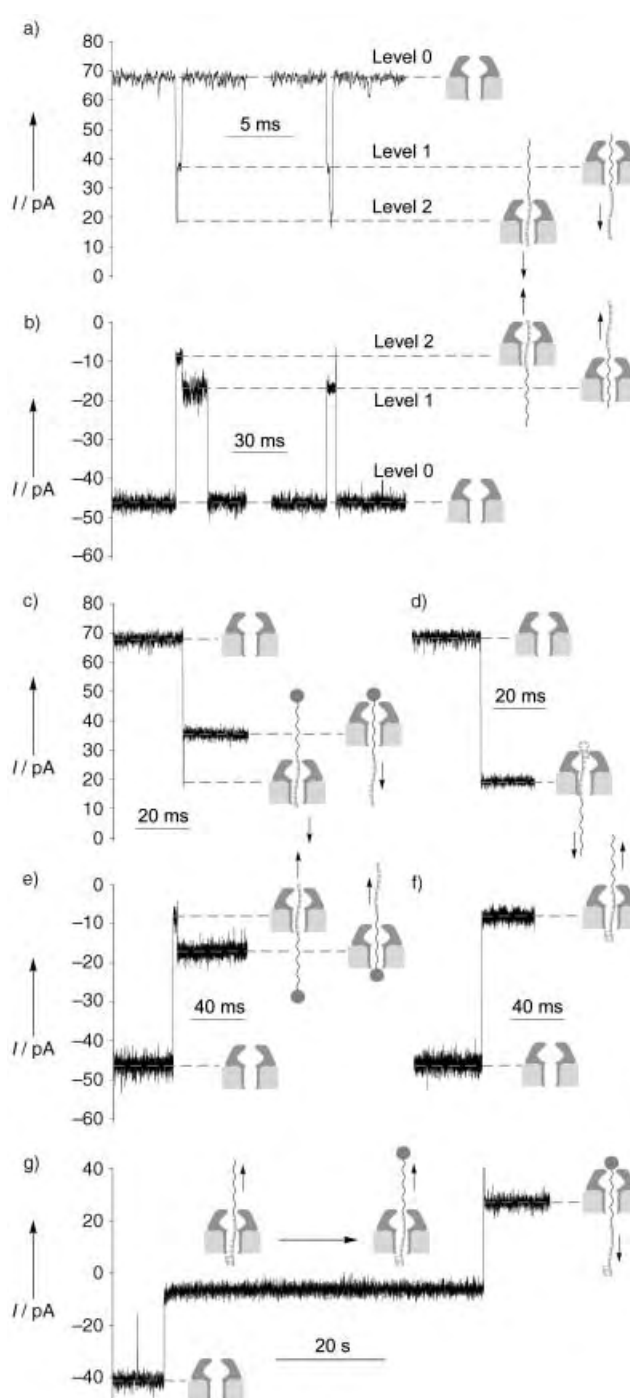


Figure 2. Blocking events caused by thread molecules. Typical events shown are from ion conductance traces recorded at +140 mV when the following thread molecules were added to the *cis* chamber: a) 1 μ M **1**, c) 1 μ M **1** in the presence of 10 μ M streptavidin, and d) 1 μ M hairpin thread **2**. Events were also observed at -140 mV with thread molecules added to the *trans* side: b) 1 μ M **1**, e) 1 μ M **1** and 10 μ M streptavidin, and f) 1 μ M **1** and 5 μ M complementary oligonucleotide strand. g) Rotaxane formation at -120/+120 mV transmembrane potential with 1 μ M **1** and 5 μ M complementary oligonucleotide strand on the *trans* side, as well as 10 μ M streptavidin on the *cis* side. Traces were recorded under symmetric conditions, KCl (500 mM) and morpholino-propanesulfonic acid (MOPS; 5 mM) at pH 7.5, bessel-filtered at 5 KHz, and sampled at 6 μ s (a) and 100 μ s (b-f), or bessel-filtered at 2 KHz and sampled at 200 μ s (g).

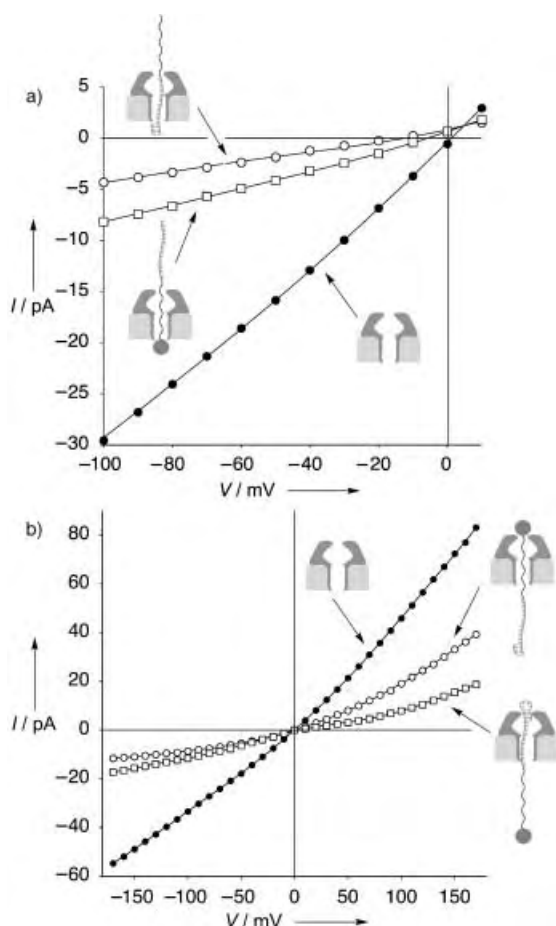


Figure 3. a) Current versus voltage relationships obtained under asymmetric conditions (300 and 500 mM KCl in the *cis* and *trans* chambers, respectively, both buffered with 5 mM MOPS to pH 7.5) for α -HL (●) and for α -HL threaded from the *trans* side with the streptavidin complex of **1** (□) or with **1** hybridized with the complementary oligonucleotide (○). b) Current versus voltage for α -HL (●), α -HL rotaxane with thread **1** (○), and α -HL rotaxane with strand **2** (□) in KCl (500 mM) and MOPS (5 mM) at pH 7.5.

performed under asymmetric conditions (Figure 3a). This selectivity can be accounted for by the presence of a high density of DNA phosphate groups inside the pore.

Formation of a single-molecule α -HL/DNA-PEG rotaxane is a two-step process. The first step involves formation of a pseudorotaxane, as described above. This process is driven by an applied transmembrane potential, which forces threading of the free tail of a polymer capped at one end (double-stranded DNA sequence or streptavidin) through the transmembrane pore and holds it stably in that configuration. The second step (locking) requires efficient capture of the protruding end of the thread on the other side of the bilayer. We found that the best approach for rotaxane formation was to employ a prehybridized strand on the *trans* side of the pore, with streptavidin present on the *cis* side (Figure 1a). We observed that, once the pseudorotaxane was captured (typically in less than a minute under the conditions employed), the α -HL conductance could no longer be returned to its

native conductance state, even when the sign of the applied transmembrane potential was changed (Figure 2g). Instead, the α -HL/DNA-PEG rotaxane displayed the conductance value corresponding to the presence of the PEG moiety in the pore at positive potentials, and the conductance level corresponding to the assembly with the DNA segment residing inside the pore at negative potentials. The rotaxane structure proved to be stable over the range of transmembrane potentials studied (−170 to +170 mV) and could be switched multiple times between the DNA and PEG states (Figure 2g).

The DNA-PEG polymer **2**, which has a double-stranded 3'-DNA stem-loop structure, was used to construct a corresponding rotaxane structure of opposite strand orientation to that described above (Figure 1b). The DNA stem-loop structure is known to prevent entry into the vestibule of the α -HL channel when the polymer is applied to the *cis* side of the pore.^[2c] Addition of **2** to the *cis* side of the α -HL pore resulted in the formation of a pseudorotaxane, as shown by the observed blocking of the pore when a high positive transmembrane potential was applied. The residual conductance corresponded to the blocking effect expected when DNA is inside the pore (Figure 2d). The α -HL native conductance could be fully recovered by switching back and forth between negative and positive transmembrane potentials (threading/dethreading). However, when streptavidin was added to the *trans* side of the pore, the thread was permanently trapped in the α -HL channel as a result of the formation of a rotaxane structure. This new rotaxane showed a conductance level at negative holding potentials typical of the channel when the PEG segment is lodged inside, which signifies the formation of a rotaxane of the opposite thread orientation to that described above for **1**. This second rotaxane structure was also shown to be stable over a wide range of applied transmembrane potentials (−170 to +170 mV; Figure 3b).

In summary, we have described a methodology for the controlled formation of individual rotaxanes of desired strand orientation with tunable conformational states. We expect this methodology to open up new possibilities for studying nucleic acids and other polymers at the single-molecule level. In addition, this study provides a direct method for trapping DNA strands of defined lengths and sequences inside the α -HL channel structure. Thereby, this method might be useful to studies aimed at establishing the scope and limitations of the nucleobase recognition capacity of α -HL and its utility in DNA sequencing. Our results also suggest that DNA- α -HL rotaxanes might provide a means for multiple-pass single-molecule DNA sequencing, which is likely to have significantly lower sequencing error rates than single-pass analyses.

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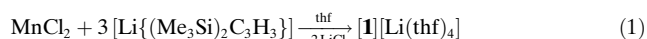
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A Manganese(II) Allyl Complex: Synthesis, Structure, and Magnetic Properties of $[\text{Li}(\text{thf})_4][\text{Mn}\{\eta^3\text{-(Me}_3\text{Si)}_2\text{C}_3\text{H}_3\}\{\eta^1\text{-(Me}_3\text{Si)}_2\text{C}_3\text{H}_3\}_2]^{**}$

Richard A. Layfield* and Simon M. Humphrey

The synthetic and structural organometallic chemistry of manganese(II) is atypical of organotransition metal chemistry due to the observation of essentially ionic C–Mn^{II} bonds, a property more commonly observed in organometallic compounds of the alkali and alkaline-earth metals.^[1] Hence Mn^{II} forms no isolable complexes with neutral π -acidic ligands such as carbon monoxide, olefins, or arenes, but does form an extensive series of cyclopentadienide (Cp[−]) complexes such as manganocene, [Cp₂Mn].^[2] Despite the ubiquitous nature of the cyclopentadienide ligand in organomanganese(II) chemistry no examples of π coordination to an Mn^{II} center by other anionic ligands of the “-enide” family are known: attempts at preparing “open manganocenes” that contain a bis-(η^5 -pentadienide)Mn arrangement, have only resulted in the formation of the unusual associated salts of general formula $[(\eta^5\text{-pentadienide})_2\text{Mn}^I]_2[\mu\text{-Mn}^{II}]$.^[3] Thus, we have sought to explore the capacity of the Mn^{II} ion for forming stable complexes with other π -bonded ligands of the -enide family.

Since π coordination of the sterically hindered allyl (or propenide) ligand $[1,3\text{-(Me}_3\text{Si)}_2\text{C}_3\text{H}_3]^-$ to a range of s-block metal cations has been observed, this ligand seemed the ideal candidate for use in the chemistry of the Mn^{II} ion.^[4] Furthermore, the stability and crystalline properties of the $[(\eta\text{-Cp})_3\text{M}]^-$ ions (M = Mn, Sn, Pb) suggested to us that the targeted synthesis of a tris(allyl)manganate anion would be prudent.^[5] Thus we now report the synthesis and structure of $[\text{Mn}\{\eta^3\text{-(Me}_3\text{Si)}_2\text{C}_3\text{H}_3\}\{\eta^1\text{-(Me}_3\text{Si)}_2\text{C}_3\text{H}_3\}_2][\text{Li}(\text{thf})_4]$ (**1**) [Li(thf)₄], which a subsequent X-ray crystallographic analysis revealed to contain both σ - and π -bonded $[(\text{Me}_3\text{Si)}_2\text{C}_3\text{H}_3]^-$ allyl ligands. The anion **1** is the first structurally authenticated example of π -allyl coordination to a manganese(II) center [Eq. (1)].



The reaction between $[\text{Li}\{(\text{Me}_3\text{Si)}_2\text{C}_3\text{H}_3\}]$ and MnCl₂ (3:1 equivalents) in thf solvent at −78°C produced a dark red solution. Removal of thf followed by the addition of pentane afforded, on storage of a concentrated solution at 5°C for

[*] Dr. R. A. Layfield, S. M. Humphrey
University of Cambridge, Department of Chemistry
Lensfield Road, Cambridge, CB2 1EW (UK)
Fax (+44) 122-333-6362
E-mail: ral34@cam.ac.uk

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

several days, very air-sensitive, red-brown crystals of **1** [Li(thf)₄]⁺ suitable for a single-crystal structure determination.^[6]

The structure of the [Mn{η³-(Me₃Si)₂C₃H₃}]{η¹-(Me₃Si)₂C₃H₃}[−] ion (**1**) contains an unsolvated Mn^{II} atom coordinated by one η³- (or π-) and two η¹-bonded [(Me₃Si)₂C₃H₃][−] ligands (Figure 1).

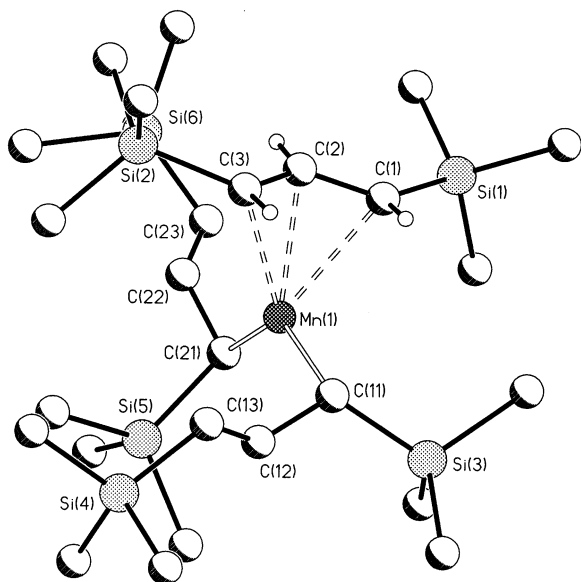


Figure 1. Molecular structure of the anion **1**. For clarity, only the η³-allyl hydrogens are shown. The [Li(thf)₄]⁺ cation has no remarkable structural features. Selected bond lengths [Å] and angles [°]: Mn(1)–C(1) 2.470(4), Mn(1)–C(2) 2.348(3), Mn(1)–C(3) 2.398(4), C(1)–C(2) 1.390(5), C(2)–C(3) 1.400(5), Mn(1)–C(11) 2.184(4), C(11)–C(12) 1.460(5), C(12)–C(13) 1.337(5), Mn(1)–C(21) 2.187(4), C(21)–C(22) 1.487(5), C(22)–C(23) 1.314(6).

The C(2)–Mn(1)–C(11), C(2)–Mn(1)–C(21) and C(11)–Mn(1)–C(21) bond angles of 117.54(15), 110.81(14) and 131.05(14)°, respectively, show that Mn(1) resides in a distorted tetrahedral coordination environment. The Mn(1)–C(11) and Mn(1)–C(21) η¹ bond lengths are essentially identical at 2.184(4) and 2.187(4) Å, respectively, and are similar to the average C–Mn^{II} bond length of 2.185(10) Å observed in the structure of the related ion-contacted η¹-pentadienide complex [K{3-Me-1,5-(Me₃Si)₂C₃H₄}Mn].^[7] The Mn(1)–C(1), Mn(1)–C(2), and Mn(1)–C(3) π bond lengths are 2.470(4), 2.348(3), and 2.398(4) Å, respectively, and indicate an appreciable degree of asymmetry in the Mn(1)–{η³-(Me₃Si)₂C₃H₃} interaction, which probably arises as a consequence of the sterically congested coordination environment, although the influence of crystal-packing forces cannot be completely discounted.^[8] Although no other examples of (η³-allyl)manganese(II) complexes are presently known to enable structural comparisons, it is worthy of note that the lengths of the Mn(1)–C π bonds observed in the structure of **1** are similar to those commonly observed in the structures of high-spin manganocenes and the [(η²-Cp)₃Mn][−] ion.^[2,5a] In contrast, however, the Mn(1)–C(η³-allyl) distances

in **1** are markedly longer than those of 2.10–2.28 Å observed in the structure of the Mn^I allyl complex [(η³-pentadienyl)–Mn^I(dmpe)₂]^[9] (dmpe is 1,2-bis(dimethylphosphanyl)ethane) and also the mean Mn–C(η⁵-Cp) distance of 2.157 Å observed in compounds of the type [Cp[′]Mn(CO)₃], in which Cp[′] is a substituted cyclopentadienyl derivative.^[10] All allyl hydrogen atoms in **1** were located in the peak-difference map and allowed to refine freely: the C(1)–C(2)–C(3)–H(3) and C(3)–C(2)–C(1)–H(1) torsional angles are identical at 18(3)° and show that the terminal allyl hydrogen atoms are tilted away from Mn(1), whereas the C(2)–H(2) bond is tilted 9(1)° toward Mn(1). The significance of the bending of the allyl hydrogen atoms of the [(Me₃Si)₂C₃H₃][−] ligand from the C(1)–C(2)–C(3) plane has been discussed for the structures of the related complexes [Ca{η³-(Me₃Si)₂C₃H₃}₂(thf)₂]^[4a] and [(η³-(Me₃Si)₂C₃H₃)Li(tmeda)] (tmeda is tetramethylethylenediamine),^[4b] which display similar distortions relative to the calculated structure of the free [(Me₃Si)₂C₃H₃][−] ion. Thus, in the structure of **1** H(1) and H(3) are oriented away from Mn(1) to reduce steric congestion and H(2) is oriented towards Mn(1) to improve the metal–ligand bonding. Theoretical investigations on the series of unsubstituted allyl–alkali-metal complexes [M(η³-C₃H₅)] (M = Li–Cs) suggest that bending of the allyl hydrogen atoms as in **1** is common for ionic metal allyls^[11] as it is for typical transition-metal allyls such as [Ni(η³-C₃H₅)].^[12]

Whereas allylmagnesium complexes usually contain η¹-bonded ligands,^[13] for other s-block metals η³-allyl bonding is normally observed;^[4] the observation of both σ- and π-allyl bonding in **1** is, therefore, unique for an ionic metal allyl (resulting in a formal valence electron count of 13), although the square-planar allyl metallates [(η³-C₃H₅)M(η¹-C₃H₅)₂][−] (M = Ni, Pd) are structurally related to **1**.^[14] It is possible that the lower hapticities of the η¹-allyl ligands allow a reduction in steric congestion in the coordination pocket that would otherwise arise from the voluminous Me₃Si substituents: on electrostatic grounds the 17 valence electron anion “[Mn{η³-(Me₃Si)₂C₃H₃}][−]” (compare with [Cp₂Mn]) would be preferred, although in the absence of other [Mn^{II}(allyl)_{*n*}] complexes with which to compare the structure of **1** such assertions must be tentative.

Although solution-phase isotopic perturbation experiments have proved to be useful in rationalizing the structures of [Li(η³-allyl)] fragments^[15] the paramagnetism of **1** [Li(thf)₄]⁺ has precluded similar attempts at corroborating the solid-state structure of the anion **1** by NMR spectroscopy. Indeed, the broad resonances and poor signal-to-noise ratio in the ¹H NMR spectrum of **1**[Li(thf)₄]⁺ only confirm the various chemical environments.

The magnetic properties of **1**[Li(thf)₄]⁺ were investigated by superconducting quantum interference device (SQUID) magnetometry in the temperature range 5–300 K and an applied field of 100 G (see Supporting Information). Under these conditions the complex behaves as a Curie paramagnet: the effective magnetic moment, μ_{eff}, at the low-temperature limit of the experiment is 2.92 BM with the value steadily rising with temperature to 4.03 BM at 300 K. Attempts to model this behavior on the basis of zero-field splitting were not successful,^[16] which is likely to indicate a low-spin/high-

spin equilibrium between the $S = 1/2$ and $S = 5/2$ states of Mn^{II} within **1** as the temperature is raised. Our conclusion is similar to those reached regarding the magnetic properties of the $[(\eta^2-Cp)_3Mn]^-$ ion and of “magnetically dilute” manganocenes.^[5a,17]

In summary, we report the first allyl complex of manganese(II). The structure of the formally 13-electron $[Mn\{\eta^3-(Me_3Si)_2C_3H_3\}\{\eta^1-(Me_3Si)_2C_3H_3\}_2]^-$ ion provides a unique example of a manganese(II) “-enide” and the observation of both η^1 - and η^3 -allyl coordination to an Mn^{II} center is without precedent for an ionic metal allyl.

Experimental Section

A freshly prepared solution of $[(Me_3Si)_2C_3H_3Li]$ (8.6 mmol) in thf (20 ml) was added dropwise to a stirred suspension of $MnCl_2$ (0.36 g, 2.9 mmol) in thf (5 ml) at $-78^\circ C$. The resulting red/brown mixture was slowly warmed to room temperature and stirred (2 h). The thf was evaporated, replaced with pentane (20 ml) and the solution filtered (Celite, porosity 3). The filtrate was reduced in volume to about 2–3 ml and stored at $+5^\circ C$ affording, after several days, red/brown plates of **1** [$[Li](thf)_4$] (1.10 g, 42%), mp $60-62^\circ C$. The extreme air-sensitivity of **1** [$[Li](thf)_4$] meant that accurate elemental analysis proved difficult to obtain: elemental analysis calcd (%) for $C_{43}H_{95}LiMnO_4Si_6$: C 56.97, H 10.56; found: C 54.95, H 10.31. ^1H NMR (C_6D_6 , 300.15 MHz, 298 K), due to the paramagnetism of the anion **1** most resonances were broadened to the extent that ^{J} coupling patterns were obliterated and have been assigned tentatively: $\delta = 0.06$ (s, 18H, Me_3Si), 0.21 (s, 18H, Me_3Si), 0.37 (s, 18H, Me_3Si), 1.67 (m, 16H, thf CH_2CH_2), 2.76 (br, 2H η^1 -allyl C-H), 3.61 (m, 16H thf CH_2OCH_2), 5.25, 5.97, 6.17, 6.45 ppm (br, m, η^3 -allyl and vinyl C-H). IR (Nujol mull, KBr plates): $\tilde{\nu} = 3033.1$ (w, sh, allyl C-H stretch) 1036.2 cm^{-1} (m, sh, thf C-O stretch).

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Keywords: allyl ligands · magnetic properties · manganese · structure elucidation

0.0473, 99.2% completeness to $\theta = 23.00^\circ$). The structure was solved by direct methods and refined by full-matrix least squares on F^2 : $R1 = 0.0608$ and $wR2 = 0.1657$. CCDC-231180 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

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Carbohydrate Synthesis

**Potent, Versatile, and Stable: Thiazolyl
Thioglycosides as Glycosyl Donors****

Alexei V. Demchenko, Papapida Pornsuriyasak,
Cristina De Meo, and Nelli N. Malysheva*

The majority of biologically important carbohydrates exist as polysaccharides or glycoconjugates in which monosaccharide

[*] Prof. Dr. A. V. Demchenko, P. Pornsuriyasak, Dr. C. De Meo,
Dr. N. N. Malysheva
Department of Chemistry and Biochemistry
University of Missouri–St. Louis
8001 Natural Bridge Road, St. Louis, MS 63121 (USA)
Fax: (+1) 314-516-5342
E-mail: demchenko@umsl.edu

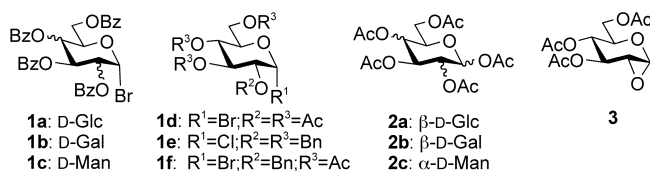
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Supporting information for this article is available on the WWW under <http://www.angewandte.org> or from the author.

units are joined by *O*-glycosidic bonds.^[1] The importance of chemical and/or enzymatic synthesis of complex oligosaccharides has come to the fore because of the low availability of these compounds from natural sources. As a result, many glycosyl donors have been developed and employed in oligosaccharide synthesis.^[2,3] Despite enormous progress, no general glycosylation method for oligosaccharide synthesis in solution or on a polymer support has yet emerged.^[4] Recently, we became interested in a class of glycosyl donors with the generic leaving group $\text{SCR}_1=\text{NR}_2$ (substituted thioimidoyl derivatives). We have already reported the synthesis of *S*-benzoxazolyl (SBox) glycosides and their evaluation in stereoselective 1,2-*cis* and 1,2-*trans* glycosylations.^[5,6] We demonstrated that the SBox glycosides provide high stereoselectivity and remarkably high yields. The lower stability of the SBox glycosides toward some extreme reaction conditions (triflic acid (TfOH) or NaH) in comparison to the corresponding *S*-alkyl/aryl glycosides prompted us to continue the search for suitable leaving groups of this class. Here, we describe the synthesis of novel glycosyl donors, the thiazolyl (Taz) thioglycosides, their application in stereoselective glycosylations, and an evaluation of their usefulness in convergent oligosaccharide synthesis.

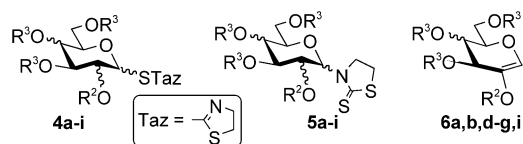
We reasoned that various synthetic precursors, such as anomeric halides (**1a–f**, Scheme 1), acetates (**2a–c**), or 1,2-anhydrosugars (**3**)



Scheme 1. Precursors for the synthesis of thiazolyl thioglycosides. **1a–c** and **2a–c** are derivatives of D-glucose, D-galactose, and D-mannose, as indicated. Bn = benzyl, Bz = benzoyl.

could serve as suitable precursors for the synthesis of thiazolyl thioglycosides (**4a–i**, Scheme 2). 2-Thiazoline-2-thiol (HSTaz) is an odorless solid, readily available from a variety of commercial sources; as a matter of fact, it is even cheaper than commonly used thiophenol. However, the ambiguous reactivity of HSTaz could lead to the formation of the *N*-linked product (**5a–i**). Also, the relatively high basicity of HSTaz ($\text{p}K_{\text{a}}=13.01$) and its conjugate base would promote undesired β elimination, which would result in glycal (1,2-dehydro derivative) formation (**6a,b,d–g,i**).

In many cases the side reactions could be significantly suppressed by varying the reaction conditions. To our delight, conversion of **2** or **3** allowed formation of the desired *S*-linked derivatives with complete stereoselectivity and in high yields.



Scheme 2. Thiazolyl thioglycosides synthesized. **a:** $\text{R}^2=\text{R}^3=\text{Bz}$ (β -D-Glc), **b:** $\text{R}^2=\text{R}^3=\text{Bz}$ (β -D-Gal), **c:** $\text{R}^2=\text{R}^3=\text{Bz}$ (α -D-Man), **d:** $\text{R}^2=\text{R}^3=\text{Ac}$ (β -D-Glc), **e:** $\text{R}^2=\text{R}^3=\text{Bn}$ (β -D-Glc), **f:** $\text{R}^2=\text{Bn}$, $\text{R}^3=\text{Ac}$ (β -D-Glc), **g:** $\text{R}^2=\text{R}^3=\text{Ac}$ (β -D-Gal), **h:** $\text{R}^2=\text{R}^3=\text{Ac}$ (α -D-Man), **i:** $\text{R}^2=\text{H}$, $\text{R}^3=\text{Ac}$ (β -D-Glc).

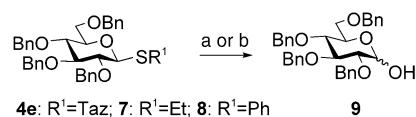
Similarly, derivatives of D-galactose (**4b,g**) and D-mannose (**4c,h**) were obtained. These results are summarized in Table 1.

Table 1: Synthesis of thiazolyl thioglycosides **4a–i**.

Precursor	Conditions ^[a]	Products	Yield [%]
1a	HSTaz, NaH, MeCN, RT	4a, 5a, 6a	49, 0, 41
1a	NaSTaz, [15]crown-5, MeCN, RT	4a, 5a, 6a	50, 0, 41
1a	KSTaz, [18]crown-6, MeCN, RT	4a, 5a, 6a	41, 0, 39
1a	NaSTaz, [15]crown-5, acetone, RT	4a, 5a, 6a	60, 0, 20
1b	KSTaz, [18]crown-6, MeCN, RT	4b, 5b, 6b	90, 0, 0
1c	KSTaz, [18]crown-6, MeCN, RT	4c, 5c, 6a	70, 0, 0
1d	NaSTaz, [15]crown-5, MeCN, RT	4d, 5d, 6d	53, 11, 13
1e	NaSTaz, [15]crown-5, acetone, RT	4e, 5e, 6e	55, 38, 0
1f	NaSTaz, [15]crown-5, MeCN, RT	4f, 5f, 6f	46, 28, 21
2a	HSTaz, $\text{BF}_3 \cdot \text{Et}_2\text{O}$, 3-Å MS, CH_2Cl_2 , 45 °C	4d, 5d, 6d	91, 0, 0
2b	HSTaz, $\text{BF}_3 \cdot \text{Et}_2\text{O}$, 3-Å MS, CH_2Cl_2 , 45 °C	4g, 5g, 6g	85, 0, 0
2c	HSTaz, $\text{BF}_3 \cdot \text{Et}_2\text{O}$, 3-Å MS, CH_2Cl_2 , 45 °C	4h, 5h, 6d	70, 0, 0
3	HSTaz, ZnCl ₂ , CH_2Cl_2 , RT	4i, 5i, 6i	78, 0, 0

[a] MS = molecular sieves.

Having synthesized a number of thiazolyl thioglycosides (**4a–i**), our attention turned to an evaluation of their stability toward hydrolysis in the presence of acidic thiophilic reagents (*N*-bromosuccinimide (NBS) or *N*-iodosuccinimide (NIS)/TfOH), common conditions for the thioglycoside hydrolysis.^[7,8] For these studies per-benzylated **4e** was compared with ethyl per-*O*-benzyl-1-thio- β -D-glycopyranoside (**7**, Scheme 3)^[9] and its 1-*S*-phenyl counterpart, **8**.^[10] Thus, **4e**, **7**,



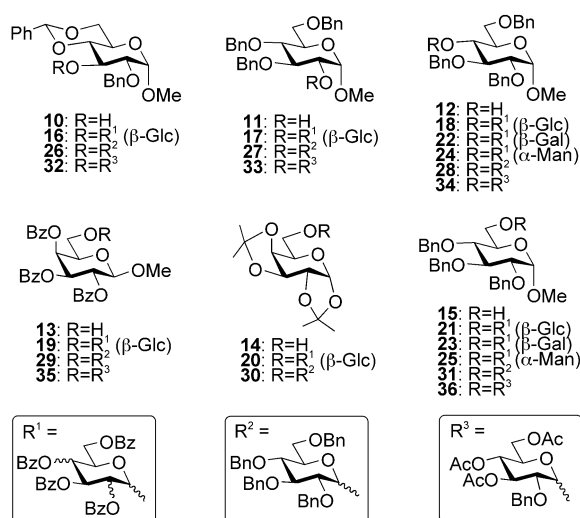
Scheme 3. Stability of STaz versus SEt/SPh toward acidic hydrolysis. Conditions: a) NIS/TfOH, aqueous dichloromethane (RT, 1 h); b) NBS, aqueous acetone (RT, 1 h).

or **8** was treated under standardized conditions to afford hemiacetal **9**. We were thrilled to find that **4e** appears to be much more stable than either **7** or **8**. For example, treatment of **4e** with NIS/TfOH in aqueous CH_2Cl_2 (conditions a) resulted in trace amounts of **9** (< 5%) in 1 h, while hydrolysis of either **7** or **8** was complete in less than 5 min.

Also, the thiazolylthio moiety was found to be stable toward common protective-group manipulations involving strong bases. For example, conversion of **4d** into **4e** by sequential deacetylation (NaOMe/MeOH) and benzylation (BnBr/NaOH in *N,N*-dimethylformamide) proceeded in high overall yield (83 %).

Having established the relative stability of the STaz glycosides, we turned our attention to an investigation of their glycosyl donor properties. It should be noted that, although **4d**^[11] and a number of thiazolyl thiofuranosides^[12] have been reported, to the best of our knowledge this class of compounds has never been glycosidated. We discovered that the STaz glycosides could be activated under a range of reaction conditions, for example, with MeOTf, a common activator for the glycosidation of thioglycosides, as well as AgOTf or Cu(OTf)₂. It should be highlighted that virtually no reaction takes place in the presence of NIS/catalytic TfOH, whereas the reaction is smoothly driven to completion in the presence of stoichiometric amounts of TfOH.

Per-benzoyl derivatives of glucose, galactose, and mannose (**4a–c**) were chosen to perform test reactions for the synthesis of 1,2-*trans*-linked disaccharides. Differently protected glycosyl acceptors **10–15**^[13–17] were selected for this purpose (Scheme 4). Disaccharides **16–25** were obtained in



Scheme 4. Glycosyl acceptors **10–15** and disaccharides **16–36** formed by use of thiazolyl thioglycosides.

the presence of each promoter; representative reactions are highlighted in Table 2. Complete stereoselectivity was achieved reliably with the assistance of a participating substituent at the C-2 position.^[18]

We also chose to demonstrate that 1,2-*cis*-glycosides could be obtained with the use of the STaz glycosides bearing a nonparticipating substituent at the C-2 position, for example, **4e** or **4f**. Although stereoselectivity is average in 1,2-dichloroethane (DCE), similar to that with the *S*-alkyl/aryl glycosides, it is apparent that these results can be significantly improved by varying common factors that influence the stereoselectivity at the anomeric center.^[4] Some of these studies are outlined in Table 3. Thus, the use of partially

Table 2: Synthesis of 1,2-*trans*-linked disaccharides **16–25**.

Donor	Acceptor	Promoter	Time	Product	Yield [%]
4a	10	AgOTf	16 h	16	91
4a	11	MeOTf	20 h	17	99
4a	12	NIS/TfOH	16 h	18	93
4a	13	AgOTf	2 h	19	90
4a	14	MeOTf	1 h	20	82
4a	15	Cu(OTf) ₂	4 days	21	99
4b	12	AgOTf	40 min	22	92
4b	15	AgOTf	30 min	23	84
4c	12	AgOTf	6 days	24	85
4c	15	AgOTf	6 days	25	87

Table 3: AgOTf-promoted synthesis of 1,2-*cis*-linked disaccharides **26–36**.

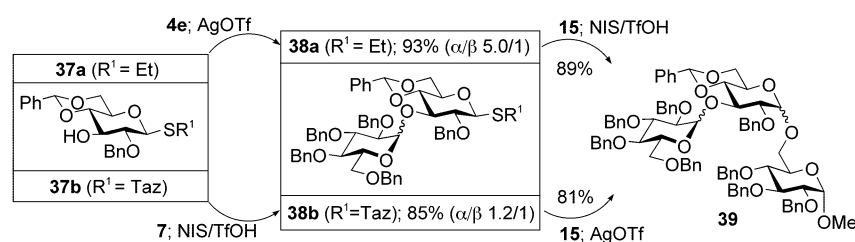
Donor	Acceptor	Solvent ^[a]	Product	Yield [%]	α/β ratio
4e	10	DCE	26	96	2.9/1
4e	10	T/D	26	85	7.7/1
4e	11	DCE	27	88	1.5/1
4e	11	T/D	27	97	4.5/1
4e	12	T/D	28	95	3.5/1
4e	13	T/D	29	82	4.1/1
4e	14	T/D	30	76	4.0/1
4e	15	T/D	31	85	2.7/1
4f	10	DCE	32	78	1.5/1
4f	11	DCE	33	88	9.3/1
4f	12	DCE	34	89	> 19/1
4f	13	DCE	35	74	4.8/1
4f	15	DCE	36	70	2.7/1

[a] T/D = toluene/dioxane, 1/1 (v/v).

acetylated glycosyl donors or a toluene/dioxane participating solvent mixture^[19] in the presence of Cu(OTf)₂ or AgOTf was found to be especially beneficial.

Additionally, we decided to evaluate the applicability of the STaz method to chemoselective glycosylation strategies for convergent oligosaccharide synthesis. We assumed that STaz glycosides could be activated over *O*-pentenyl glycosides (with MeOTf, AgOTf, or Cu(OTf)₂) or thioglycosides (with AgOTf or Cu(OTf)₂), as found for SBox glycosides.^[5,6] Conversely, it should be possible to activate other glycosyl donors over STaz glycosides, for example, bromides (with Koenigs–Knorr or Helferich conditions), trichloroacetimidates (with trimethylsilyl triflate), and even “stable” glycosyl donors such as *S*-alkyl/phenyl or *O*-pentenyl glycosides (with NIS/cat. TfOH).^[2,3] To prove this hypothesis we synthesized trisaccharide **39** through two conceptually different approaches: activation of the STaz moiety in **4e** over the SET moiety in **37a**^[20] with AgOTf and activation of the SET moiety in **7** over the STaz group in **37b** with NIS/cat. TfOH (Scheme 5). The obtained disaccharides **38a** and **38b** were coupled with **15** to provide **39**, which was isolated in 83 and 69 % overall yields over the two-step activation sequence STaz–SET–OMe and SET–STaz–OMe, respectively.

Based on the presented results, we conclude that the STaz glycosides can be successfully applied to the synthesis of both 1,2-*trans*- and 1,2-*cis*-glycosides. In addition, a fully orthogonal character of thiazolylthio and ethylthio moieties has



Scheme 5. Selective activation of STaz over SET and vice versa. Tf = trifluoromethanesulfonyl.

been discovered. These derivatives fulfill major requirements for the “ideal” glycosyl donor: accessibility, high stability toward protecting group manipulations, mild activation conditions, orthogonality toward other glycosyl donors, and good stereoselectivity. Many other synthetic strategies can be developed with these unique glycosyl donors, further evaluation of which is underway in our laboratory.

Experimental Section

Preparation of the STaz glycosides:

Method A from glycosyl halides: [15]Crown-5 (or [18]crown-6, 0.6 mmol) and NaSTaz (or KSTaz, 6.0 mmol) were added to a stirred solution of a glycosyl halide (**1a–f**, 3.0 mmol) in acetone (or MeCN, 4 mL) under argon. The reaction mixture was stirred for 1 h at RT. The mixture was then diluted with toluene (30 mL) and washed with 1 % aqueous NaOH (15 mL) and water (3 × 10 mL). The organic phase was separated, dried, and concentrated. The residue was purified by column chromatography on silica gel (ethyl acetate/hexane gradient elution) to afford the STaz glycoside (**4a–d**).

Method B from glycosyl acetates: A solution of a glycosyl acetate (**2a–c**, 0.128 mmol), HSTaz (0.256 mmol), and activated 3-Å molecular sieves (100 mg) in CH₂Cl₂ (1.0 mL) was stirred under argon for 30 min at RT. BF₃·Et₂O (0.256 mmol) was added dropwise and the reaction mixture was left for 45 min at RT. Additional portions of HSTaz (0.256 mmol) and BF₃·Et₂O (0.256 mmol) were added and the reaction mixture was left for 1 h under reflux conditions (45 °C). Upon completion, the STaz glycoside (**4d, g**, or **h**) was isolated and purified as described in Method A.

Method C from Brigl’s anhydride **3:** HSTaz (0.347 mmol) and ZnCl₂ (0.0087 mmol) were added to a stirred solution of **3** (0.174 mmol) in anhydrous CH₂Cl₂. The reaction mixture was stirred for 10 min at RT. Et₃N was then added dropwise until a neutral pH value was reached. The mixture was subjected to aqueous work-up and column chromatography to afford **4i**.

Method D from thioglycosides: The solution of **37a** (0.128 mmol) and 3-Å molecular sieves (70 mg) in CH₂Cl₂ (2 mL) was stirred under argon for 1 h. A freshly prepared solution of Br₂ in CH₂Cl₂ (1.2 mL, 1/165 (v/v)) was then added and the reaction mixture was left for 5 min at RT. The CH₂Cl₂ was then evaporated off under reduced pressure at RT. The crude residue was treated with NaSTaz (0.51 mmol) in dry MeCN (1 mL) under an argon atmosphere for 2 h at RT. The mixture was then diluted with toluene, the solid was filtered-off, and **37b** was isolated and purified as described in Method A.

Preparation of di- and trisaccharides:

Typical AgOTf-promoted glycosylation procedure: A mixture the glycosyl donor (0.11 mmol), glycosyl acceptor (0.10 mmol), and 3-Å molecular sieves (200 mg) in CH₂Cl₂ (2 mL) was stirred under argon for 1.5 h. Freshly conditioned AgOTf (0.22 mmol) was added and the reaction mixture was stirred for 1–2 h at RT, then diluted with CH₂Cl₂. The solid was filtered off and the residue was washed with

CH₂Cl₂. The combined filtrate (30 mL) was subjected to aqueous work-up and column chromatography.

All synthesized compounds have adequate ¹H and ¹³C NMR spectroscopy data and high-resolution MS data (see the Supporting Information).

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Keywords: carbohydrates · chemoselectivity · glycosides · glycosylation

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Template Synthesis

Mutual Stabilization between Imine Ligands and Copper(I) Ions in Aqueous Solution**

Jonathan R. Nitschke*

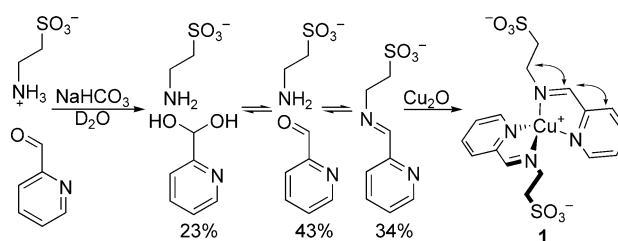
Dedicated to Professor T. Don Tilley
on the occasion of his 50th birthday

Imine donors are known to stabilize Cu^I ions in environments that would otherwise favor disproportionation to Cu^{II} and Cu⁰, such as aqueous solution. Herein, it is shown that the converse is likewise true, that Cu^I ions are capable of stabilizing and templating the formation of appropriate imine ligands from pyridine-2-carboxaldehyde and sulfonated amines in aqueous solution. This stabilization is mutual and cooperative, since neither Cu^I ions nor imines are ordinarily stable in water.

Selective exchange of the amine components of the imine ligands has also been demonstrated, thus representing an advance within the field of directed ligand/component assembly around metal centers.^[1–3] This branch of dynamic self-assembly^[4,5] is based upon ligand rearrangement reactions (changes in ligand conformation and supramolecular architecture induced by transmetalation,^[6] redox processes,^[7] or the presence of guest species^[8]).

The self-assembly reaction which generates these Cu^I–imine complexes proceeds readily in the presence of a dynamic combinatorial library of potential ligand components.^[5,9] The best ligand for the chelation of a Cu^I ion is formed exclusively, which results in the elimination of its components from the library. This causes a partial “collapse” of the library by eliminating all the other members that had integrated into these components.

The reaction of taurine (2-aminoethanesulfonic acid, 0.14 mmol) with pyridine-2-carboxaldehyde (0.14 mmol) and sodium bicarbonate (0.07 mmol) in deuterium oxide (0.5 mL) yields a product mixture whose ¹H NMR spectrum corresponds to the mixture of compounds shown in Scheme 1. The addition of copper(I) oxide (0.035 mmol) to this mixture under anaerobic conditions produces a dark red/brown solution as the copper(I) oxide dissolves over the course of several hours in an ultrasound bath. The ¹H and ¹³C NMR



Scheme 1. The synthesis of **1**. Double-headed arrows indicate observed NOE interactions.

spectra of this product demonstrate that it is diamagnetic and that the mixture of four components initially present has collapsed down to give a single product: the copper(I)–bis(dimine) **1**.

The quantitative formation of an imine ligand was verified by the observation of NOE difference peaks between the imine proton and the pyridine and methylene protons (Scheme 1). The diastereotopic methylene groups α to the imine nitrogen atom exhibit a single proton resonance in water or methanol at room temperature, which is indicative of rapid racemization around the Cu^I stereocenter. This signal decoalesces in [D₄]methanol at 233 K and gives rise to two broad resonances that reach a maximum separation of 121 Hz at 183 K. This process corresponds to a $\Delta G^\ddagger = 46 \text{ kJ mol}^{-1}$ at 233 K,^[10] a value slightly lower than those observed for Cu^I–bis(2-iminopyridine) complexes in chlorinated solvents.^[11] Electrostatic repulsion between the sulfonate groups may account for this lower barrier to racemization.

Complex **1** represents a novel example of imine self-assembly around a copper(I) template. The self-assembly of bidentate imine ligands having no negatively charged atom directly bound to the metal center is also noteworthy. With the exception of a tetracopper-grid structure synthesized in acetonitrile from 3,6-pyridazinedicarboxaldehyde and 1,3-diaminopropane,^[2] previous examples of metal-templated imine synthesis have involved the formation of a site with three or more metal-binding atoms and/or metal-bound anions to stabilize the resulting complex through chelate and electrostatic effects.^[3,12] The preference of the Cu^I center for a tetra-imine ligand may eliminate the need for additional stabilizing factors, so preventing the imines from hydrolyzing even in an aqueous environment.

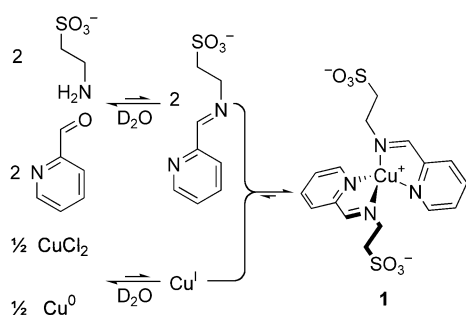
A solution of **1** reacts with atmospheric oxygen to give a pale green product, the NMR spectra of which show broad features, indicative of the presence of Cu^{II} ions. Complex **1** is stable to the presence of oxygen on a time scale of weeks in the solid state.

Complex **1** is stable in solution in the absence of oxygen. Its thermodynamic stability is demonstrated by the reaction shown in Scheme 2. The comproportionation of copper(II) chloride (0.03 mmol) and copper powder (0.03 mmol) in the presence of taurine (0.12 mmol), pyridine-2-carboxaldehyde (0.12 mmol), and sodium hydrogencarbonate (0.12 mmol) in deuterium oxide (1 mL) yields **1**, which is shown to be identical by NMR spectroscopic analysis to samples prepared from copper(I) oxide. The interaction of the products arising from the equilibria shown on the left of Scheme 2 gives rise to a new equilibrium, which lies entirely to the right under the

[*] Dr. J. R. Nitschke
Department of Organic Chemistry, University of Geneva
30 quai Ernest Ansermet, 1211 Genève 4 (Switzerland)
Fax: (+41) 223-793-215
E-mail: jonathan.nitschke@chiorg.unige.ch

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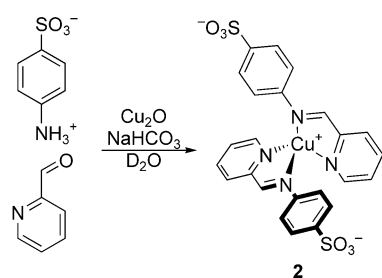
Supporting information for this article is available on the WWW under <http://www.angewandte.org> or from the author.



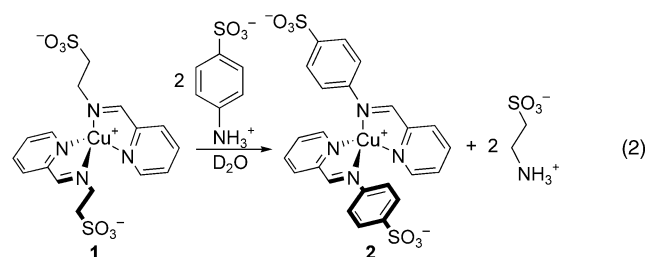
Scheme 2. The mutual stabilization of imine ligands and Cu^{I} ions during the generation of **1**.

experimental conditions employed, as evidenced by the quantitative formation of **1**. This equilibrium represents a mutual selection of the correct ligand by and for the metal in the correct oxidation state, that is, the cooperative stabilization of two species that are not stable in the absence of each other.

The generality of this reaction is demonstrated by the synthesis of **2**, prepared by using the same procedure as **1**, except that sulfanilic acid (4-aminobenzenesulfonic acid) was used in place of taurine. This synthesis is shown in Eq. (1).



The addition of sulfanilic acid (0.14 mmol) to a solution of **1** (0.07 mmol) in deuterium oxide (0.5 mL) also gives a near-quantitative yield of **2** [Eq. (2)]. This ligand/component

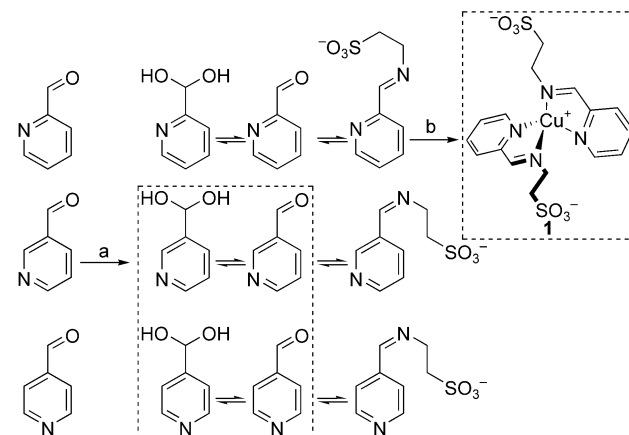


exchange occurs with a selectivity conservatively estimated as greater than 95%, based on ^1H NMR integration: only about 2% of the taurine initially incorporated into **1** is not released. The exchange occurs within seconds of the addition at room temperature.

The driving force behind this ligand/component exchange may be understood in terms of the difference in $\text{p}K_{\text{a}}$ values: the NH_3^+ group of taurine is substantially less acidic ($\text{p}K_{\text{a}} = 9.1$)^[13] than that of sulfanilic acid ($\text{p}K_{\text{a}} = 3.2$).^[14] This favors

the displacement of the protonated form of the weaker acid (taurine) from **1** and the incorporation of the deprotonated form of the stronger acid (sulfanilic acid) during the formation of **2**. The more extensively conjugated π system of the ligands in **2** should also stabilize it with respect to **1**, as well as providing lower-energy π^* orbitals that would have an enhanced ability to stabilize the electron-rich Cu^{I} center through back-donation.

In addition to the selection of amines, the system is capable of selecting pyridine-2-carboxaldehyde from a library of its isomers based on the ability to form a bidentate imine ligand (Scheme 3). The mixture of the three pyridinecarbox-



Scheme 3. The Cu^{I} -mediated selection of pyridine-2-carboxaldehyde from a library of its isomers. a) Taurine, sodium bicarbonate, deuterium oxide; b) copper(I) oxide. The compounds remaining after the addition of copper(I) oxide are outlined.

aldehydes and taurine (0.12 mmol of each) with sodium bicarbonate (0.06 mmol) in deuterium oxide (1 mL) gives a complex ^1H NMR spectrum, which shows resonances attributed to the aldehydes, their gem-diol hydrates, and their taurine-derived imines, all of which undergo dynamic interconversion. This situation corresponds to a dynamic combinatorial library of ten members (including free taurine). NMR spectroscopic analysis shows that addition of copper(I) oxide (0.03 mmol) to this mixture, followed by stirring overnight, gives a product mixture in which all of the components of the library derived from taurine and pyridine-2-carboxaldehyde (six out of ten initial members) have been removed by complexation. The product mixture has thus collapsed into **1** and the subset of aldehydes outlined in Scheme 3. This selection works equally well with a library containing sodium 2-formylbenzenesulfonate and 3,4-dihydroxybenzaldehyde as non-incorporated aldehydes in place of pyridine-3-carboxaldehyde and pyridine-4-carboxaldehyde, which suggests there is a certain generality.

The development of this aqueous Cu^{I} chemistry should thus enable an extension of the wealth of copper(I) self-assembly chemistry^[15] into the aqueous domain, where an interface with biology may be possible. Future work will focus on the investigation of Cu^{I} -templated self-assembly as a means to link copper centers to specific amino groups in biomolecules (such as peptides and nucleic acids). The use of

ligand component exchange to program the self-assembly of larger structures containing multiple copper centers will likewise be developed.

Experimental Section

All manipulations were carried out under argon or nitrogen atmospheres using degassed solvents.

Na⁺1⁻: Pyridine-2-carboxaldehyde (1.08 g, 10.1 mmol), taurine (1.27 g, 10.1 mmol), copper(I) oxide (0.362 g, 2.53 mmol), and sodium bicarbonate (0.425 g, 5.06 mmol) were added to a 100-mL Schlenk flask. The flask was sealed, a magnetic stirring-bar added, and the atmosphere was deoxygenated by three evacuation/argon fill cycles. Water (50 mL) was then added, which caused gas evolution and the development of a dark-red/brown color. Once gas evolution had ceased the flask was sealed and the reaction was allowed to stir for 8 h at 22°C. Volatiles were then removed under dynamic vacuum until approximately 5 mL of material remained, which was then triturated with methanol (10 mL) and *tert*-butanol (40 mL). A precipitated brown, microcrystalline solid was then allowed to settle, and the supernatant was removed with a cannula filter. The product (2.50 g, 96%) was then dried under dynamic vacuum for 4 h. ¹H NMR (400 MHz, 300 K, D₂O, referenced to *t*BuOH at 1.24 ppm as the internal standard; peak assignments are consistent with COSY and NOESY spectra): δ = 8.73 (s, 2H, imine), 8.51 (d, *J* = 5.8 Hz, 2H, 6-pyridyl), 8.07 (t, *J* = 10.2 Hz, 2H, 4-pyridyl), 7.87 (d, *J* = 10.2 Hz, 2H, 3-pyridyl), 7.64 (m, 2H, 5-pyridyl), 4.21 (t, *J* = 8.5 Hz, 4H, NCH₂CH₂S), 3.14 ppm (t, *J* = 8.5 Hz, 4H, NCH₂CH₂S); ¹³C NMR (75.48 MHz, 300 K, D₂O, referenced to *t*BuOH at 30.3 ppm as the internal standard): δ = 162.7, 150.4, 149.2, 138.3, 128.3, 127.0, 54.6, 51.2 ppm; ESI-MS: *m/z* 489.0 [1⁺], 213.3 [(metal-free imine ligand of 1)⁺]. Elemental analysis (%) calcd for C₁₆H₁₈CuN₃NaO₆S₂·2(CH₃OH): C 37.46, H 4.54, N 9.70; found: C 37.33, H 4.53, N 9.79.

Complex **2** may be synthesized in an identical fashion in 92% yield using sulfanilic acid in place of taurine. Characterization data for Na⁺2⁻: ¹H NMR (400 MHz, 300 K, D₂O, referenced to *t*BuOH at 1.24 ppm as the internal standard): δ = 8.92 (s, 2H, imine), 8.15 (d, *J* = 3.9 Hz, 2H, 6-pyridyl), 8.00 (t, *J* = 7.7 Hz, 2H, 4-pyridyl), 7.91 (d, *J* = 7.7 Hz, 2H, 3-pyridyl), 7.48 (m, 6H, 5-pyridyl, 2,6-sulfanil), 7.16 ppm (d, *J* = 7.3 Hz, 4H, 3,5-sulfanil); ¹³C NMR (75.48 MHz, 300 K, D₂O, referenced to *t*BuOH at 30.3 ppm as the internal standard): δ = 160.7, 150.7, 149.7, 149.0, 143.3, 139.3, 129.8, 127.9, 129.4, 123.0 ppm; ESI-MS: *m/z* 585.0 [2⁺], 261.0 [(metal-free imine ligand of 2)⁺]. Elemental analysis (%) calcd for C₂₄H₁₈CuN₄NaO₆S₂·CH₃OH: C 46.87, H 3.46, N 8.75; found: C 46.66, H 3.32, N 9.01.

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Intermolecular Dienyl Pauson–Khand Reaction**

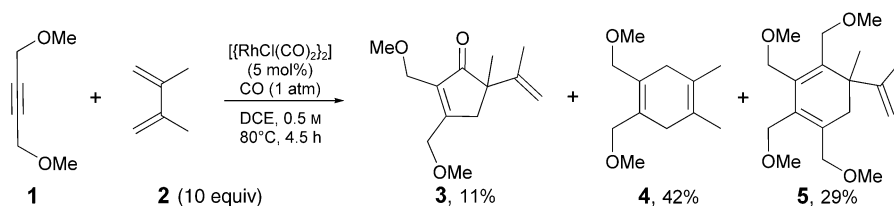
Paul A. Wender,* Nicole M. Deschamps, and
Travis J. Williams

The design and discovery of new reactions that produce molecular complexity and value with step economy are preeminent goals of organic synthesis.^[1] Our studies toward this objective have thus far resulted in new metal-catalyzed [4+4],^[2] [4+2],^[3] [5+2],^[4] and [6+2]^[5] cycloadditions and three-component [5+2+1],^[6] [4+2+1], and [2+2+1] reactions.^[7] In the course of our studies on the rhodium(I)-catalyzed intramolecular dienyl [2+2+1] Pauson–Khand reaction, we observed that when a diene is used in place of an alkene the reaction rate is significantly accelerated.^[7] The present study was aimed at determining whether this enhanced reactivity would enable the use of dienes in a catalyzed intermolecular [2+2+1] Pauson–Khand reaction.

The intermolecular Pauson–Khand reaction is a powerful complexity-increasing process for the construction of cyclopentenones, in which three carbon–carbon bonds and two stereocenters are produced in one operation. Although this reaction originally required stoichiometric amounts of cobalt,^[8] catalytic versions based on Co,^[9] Rh,^[10] Ir,^[11] and Ru^[12] have recently emerged. The catalyzed intermolecular reaction often requires a reactive alkene. For example, Narasaka and co-workers reported that attempts to conduct a Rh^I-catalyzed intermolecular Pauson–Khand reaction with simple alkenes (styrene or 1-phenyl-3-butene) and 1-phenylpro-

pyne (1 atm CO, dibutyl ether, 130 °C) were unsuccessful. Only more activated alkenes (norbornene and ethylene) gave [2+2+1] cycloaddition products, and then in only low to moderate yields.^[10a] We now report that dienes can be used in an intermolecular [2+2+1] process to produce alkenyl cyclopentenones, often in high yields and from commercially or readily available starting materials.

Our initial investigation of the intermolecular reaction of alkyne **1** and diene **2** was marginally encouraging; it provided the [2+2+1] cycloadduct **3** as a single regioisomer but in only 11% yield along with the [4+2] cycloadduct **4** and the [2+2+2] cycloadduct **5** (Scheme 1). Studies to control the selectivity of the reaction explored the effect of variations in temperature, solvent, CO pressure, diene equivalents, and concentration (Table 1). A decrease in temperature resulted



Scheme 1. Competing [2+2+1], [4+2], and [2+2+2] cycloadditions.

Table 1: Studies of the [2+2+1] cycloaddition reaction of **1**, **2**, and CO.^[a]

Entry	T [°C]	Solvent ^[b]	c [M] ^[c]	CO [atm]	Equiv. of 2	t [h]	Yield of 3 [%] ^[d]
1	80	DCE	0.5	1	10	6	(11) ^[e]
2	60	DCE	0.5	1	10	6	> 99 (98)
3	60	TCE	0.5	1	10	7	96
4	60	dioxane	0.5	1	10	23	65
5	60	toluene	0.5	1	10	23	85
6	60	neat	—	1	10	9	86
7	60	DCE	0.5	2	10	22	51
8	60	DCE	0.5	0.5	10	6	(54) ^[f]
9	60	DCE	0.5	1	2	6	> 99
10	60	DCE	0.5	1	1.1	7	54
11	60	DCE	1	1	2	3	85

[a] $[\{\text{RhCl}(\text{CO})_2\}_2]$: 5 mol %. [b] DCE = 1,2-dichloroethane; TCE = 1,1,2,2-tetrachloroethane. [c] Alkyne concentration. [d] Indicated yields were measured by GC; yields of the isolated product are denoted with parentheses. Unless otherwise specified, < 5% of **4** was observed by GC, and **5** was not observed. [e] Compounds **4** and **5** were obtained in 42% and 29% yield, respectively. [f] Compounds **4** and **5** were obtained in 14% and 9% yield, respectively.

in a remarkable increase in the selectivity of the reaction, which now provided **3** in nearly quantitative yield (Table 1, entry 2). Under similar conditions (in accord with prior work),^[10a] the use of norbornene in place of the diene gave the corresponding [2+2+1] adduct in only 4% yield (see Supporting Information). Other solvents studied afforded **3** in acceptable yields (Table 1, entries 3–5) but were not as efficient as DCE. Significantly, the reaction can be run successfully in the absence of solvent (Table 1, entry 6), thus avoiding atom-uneconomical solvent waste. Increasing the CO pressure to 2 atm resulted in a longer reaction and a decrease in yield, whereas lowering the CO pressure to 0.5 atm resulted in a shorter, less selective reaction (Table 1, entries 7 and 8). Decreasing the amount of diene to 2 equiv-

[*] Prof. P. A. Wender, N. M. Deschamps, T. J. Williams
Department of Chemistry, Stanford University
Stanford, CA 94305-5080 (USA)
Fax: (+1) 650-723-7259
E-mail: wenderp@stanford.edu

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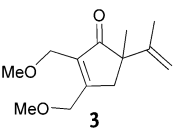
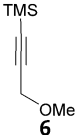
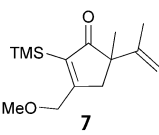
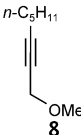
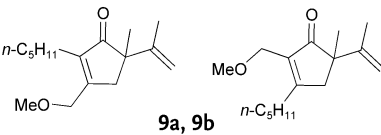
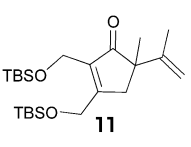
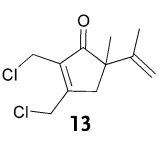
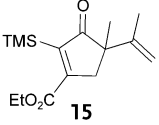
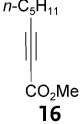
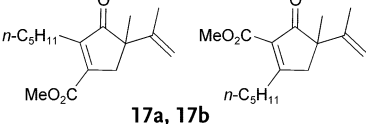
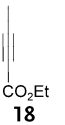
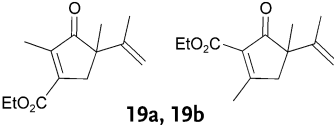
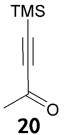
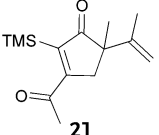
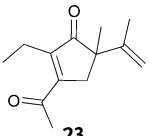
Supporting information for this article is available on the WWW under <http://www.angewandte.org> or from the author.

alents had no effect on the yield; however, only a 54% yield was observed when 1.1 equivalents of **2** were used (Table 1, entries 9 and 10). Finally, an increase in the concentration of the reaction resulted in a slight decrease in yield (Table 1, entry 11).

Table 2 summarizes the effect of alkyne structure on the reaction. Various functionalities are tolerated, and under optimal conditions several alkynes underwent the desired reaction to provide the corresponding [2+2+1] cycloadducts in excellent yields (>85%; Table 2, entries 1–4, 6). The reaction of the differentially substituted alkyne **6** proceeded in a completely regioselective fashion (Table 2, entry 2), whereas the reaction of the *n*-pentyl-substituted alkyne **8** gave a 1:1.6 mixture of regioisomeric products **9a** and **9b** (Table 2, entry 3). Replacement of the methyl ether groups with more synthetically versatile TBS ethers resulted in a slight decrease in yield (see Table 2, entries 1 and 4). The reaction of 1,4-dichlorobutene (**12**) afforded the cycloadduct **13** in only a modest 38% yield (Table 2, entry 5). Ethyl trimethylsilylpropynoate (**14**) proved to be an excellent substrate for this reaction, which afforded the cycloadduct **15** in 95% yield with complete regioselectivity. Methyl 2-octynoate (**16**) and methyl 2-butynoate (**18**) reacted to give the corresponding mixtures of regioisomers in moderate yields. Ynones, such as **20** and **22**, were less effective substrates under the current conditions. A striking difference was also noticed between the reactions of the silylated ynone **20** and ynoate **14** (Table 2, entries 6 and 9); in the latter reaction the yield of the product was more than twice that in the former. Although not a problem in most cases, the cycloadduct **23** exhibited product-inhibitory behavior, thus accounting for the longer reaction time required for its formation (see Supporting Information for more details).

Variation in the substitution of the diene was next examined. Other methyl-substituted dienes did not react as efficiently as 2,3-dimethyl-1,3-butadiene under the optimized conditions described above. Isoprene and (*E*)-3-methyl-1,3-pentadiene reacted at the less substituted double bond to give the cyclopentenones **25** and **27** in moderate yields (Table 3), the former arising through isomerization of the initially formed product. 1,3-Butadiene did not undergo reaction, whereas the reactions of piperylene (1,3-pentadiene) and 2-methyl-1,3-pentadiene gave complex mixtures of products under

Table 2: Intermolecular diene Pauson–Khand reaction of various alkynes with **2** and CO.^[a]

Entry	Alkyne	t [h]	Solvent ^[b]	Product(s)	Yield [%]
1		6	DCE		98 ^[c]
2		30	DCE		93
3		30	DCE		55, 35
4		6	DCE		87
5		5	1:1 DCE/TCE		38
6		24	1:1 DCE/TCE		95 ^[c]
7		28	DCE		31, 22
8		28	DCE		18, 39
9		32	TCE		39
10		72	TCE		63

[a] [$\{\text{RhCl}(\text{CO})_2\}_2$] (5 mol %), CO (1 atm), 60 °C, **2** (10 equiv). [b] Concentration of alkyne: 0.5 M. [c] Yield >99% by GC analysis. TBS = *tert*-butyldimethylsilyl, TMS = trimethylsilyl.

Table 3: Reactions of alkyl-substituted dienes.^[a]

Entry	Diene	Alkyne	t [h]	Solvent ^[b]	Product	Yield [%]
1			68	1:1 TCE/DCE		31
2			22	DCE		37

[a] $[\{\text{RhCl}(\text{CO})_2\}_2]$ (5 mol %), CO (1 atm), 60 °C, diene (10 equiv). [b] Concentration of alkyne: 0.5 M.

our previously optimized reaction conditions. Adjustment of the steric bulk of the diene, however, was well tolerated. Table 4 shows the results of some reactions of 2,3-dibenzyl-1,3-butadiene. Gratifyingly, these reactions were high yielding with only 2 equivalents of the diene.

Although the reaction of the free diol 2-butyne-1,4-diol led to no cycloadduct, the desired diol product can be readily

Table 4: Reactions of 2,3-dibenzyl-1,3-butadiene.^[a]

Entry	Alkyne	t [h]	Solvent ^[b]	Product	Yield [%]
1		9	DCE		81
2		24	DCE		98
3		6	DCE		75 ^[d]
4		24	1:1 DCE/TCE		99 ^[e]
5		27	TCE		61 ^[f]

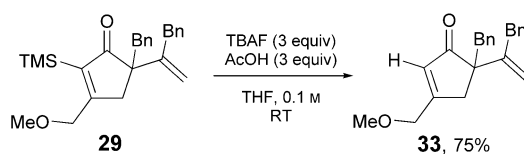
[a] $[\{\text{RhCl}(\text{CO})_2\}_2]$ (5 mol %), CO (1 atm), 60 °C, diene (10 equiv). [b] Concentration of alkyne: 0.5 M. [c] Structure verified by X-ray crystallographic analysis.^[13] [d] With 1.2 equivalents of the diene: 63 % yield. [e] With 1.2 equivalents of the diene: 82 % yield. [f] The [4+2] adduct was also obtained in 10 % yield.

obtained by desilylation of **11** (see Supporting Information). Moreover, although terminal alkynes did not undergo cyclization under the present conditions, the desilylation of silylated surrogates provides access to these cycloadducts (Scheme 2).

Since the process is biphasic, there is a possibility that if it were run on a larger scale complications could arise. As shown in Table 5, scale changes over two orders of magnitude did not significantly affect the outcome.

In summary, the first examples of a metal-catalyzed, intermolecular dieny Pauson–Khand reaction have been reported. For many alkynes and 2,3-disubstituted 1,3-dienes, the reaction leads to the

regioselective formation of alkenyl cyclopentenones under mild conditions and often in excellent yields from commercially or readily available starting materials. Further studies on this and other novel multicomponent processes are in progress.



Scheme 2. Desilylation of **29**. TBAF = tetrabutylammonium fluoride, RT = room temperature.

Table 5: Scalability of the reaction.

Entry	14 [mmol]	Yield of 15 [%] ^[a]	Yield of 34 [%] ^[a]
1	0.10	95	0
2	1.0	91	0
3	10.0	81	6

[a] Yield of isolated product.

Experimental Section

Full procedural details and characterization data are given in the Supporting Information.

Typical procedure: Ethyl 3-trimethylsilylpropionate (**14**; 180 μL , 170 mg, 1.00 mmol), 2,3-dimethyl-1,3-butadiene (1.13 mL, 822 mg, 10.0 mmol), 1,1,2,2-tetrachloroethane (distilled, 1.00 mL), and 1,2-dichloroethane (distilled, 1.00 mL), were mixed in an oven-dried 16 $\times$ 150-mm test tube with a stir bar. Chlorodicarbonylrhodium(I) dimer (19.4 mg, 50.0 μmol) was then added to the mixture. The test tube was capped with a septum and was evacuated and refilled with CO three times. The resulting solution was stirred at 60 °C for 24 h under a CO atmosphere and then purified by flash column chromatography on silica gel (eluant: 2 % diethyl ether in pentane). Product-containing

fractions were identified and concentrated by rotary evaporation to yield the alkenyl cyclopentenone **15** (254 mg, 91 %) as a yellow oil.

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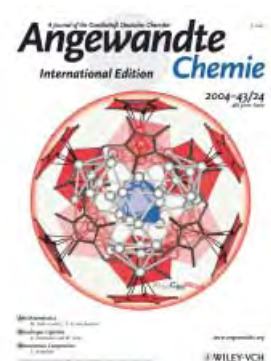
Keywords: cycloaddition · dienes · multicomponent reactions · Pauson–Khand reaction · rhodium

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- [13] CCDC-232055 (**28**) and CCDC-232056 (**29**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK; fax: (+44)1223-336-033; or deposit@ccdc.cam.ac.uk).

Cover Picture

Jean Vollet, Jens R. Hartig, and Hansgeorg Schnöckel*

A model Al_{50} cluster is part of the largest known molecular organometallic compound comprised of metal, carbon, and hydrogen. A central Al_8 unit (blue in the picture) is made up of 42 Al atoms (silver), 12 of which carry pentamethylcyclopentadienyl (Cp^*) groups. The 60 methyl groups of the Cp^* ligands (red pentagons) produce a surface topology reminiscent of a football. Further details on this $\text{Al}_{50}\text{Cp}_{12}^{12}$ cluster can be found in the Communication by H. Schnöckel et al. on page 3186 ff.



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The following Communication is available online (in Wiley InterScience). You can find it, as well as forthcoming Reviews, Highlights, and Essays, at www.angewandte.org, under Early View.

A. Gutnov,* B. Heller,* C. Fischer, H.-J. Drexler, A. Spannenberg,

B. Sundermann, C. Sundermann:

Cobalt(I)-Catalyzed Asymmetric [2+2+2] Cycloaddition of Alkynes and Nitriles: Synthesis of Enantiomerically Enriched Atropoisomers of 2-Arylpyridines

DOI: 10.1002/anie.200454164

Published online: May 26, 2004

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Crystallography: Conserving an Endangered Species.

H.-B. Bürgi and T. Weber

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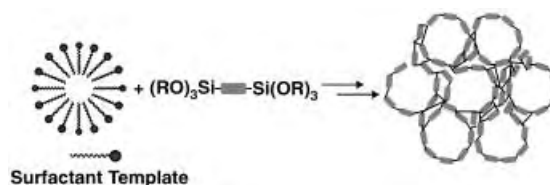
reviewed by B. Cornils

Highlights

Mesoporous Composites

G. Kickelbick* _____ 3102–3104

Hybrid Inorganic–Organic Mesoporous Materials



Another brick in the wall: Organic groups that contain several alkoxysilane substituents are applied in the synthesis of hybrid inorganic–organic mesoporous materials (see scheme). The resulting materials contain the organic moiety as an

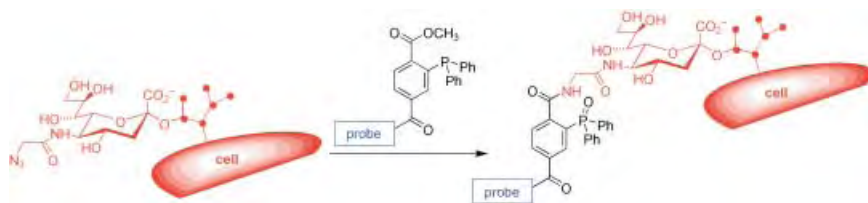
integral part of the wall. Depending on the functionality of the organic groups and on the properties of the functionalized porous material a plethora of potential applications can be envisioned.

Minireviews

Bioorganic Chemistry

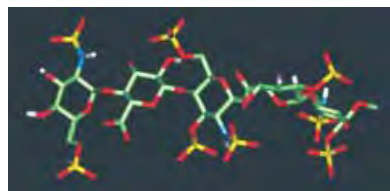
M. Köhn, R. Breinbauer* — 3106–3116

The Staudinger Ligation—A Gift to Chemical Biology



Bioconjugation made easy: The Staudinger ligation between azides and acyl-modified phosphanes is probably the mildest and most chemoselective ligation

reaction. It has found widespread application in chemical biology and can even be used in the complex environment of a living cell (see scheme).



Coagulation inhibition: Heparin has been used clinically as an antithrombotic agent since the 1940s. For decades researchers have been working on the development of alternatives with improved pharmacological properties and better-defined modes of action. The picture shows the synthetic pentasaccharide fondaparinux in the conformation it adopts when complexed with antithrombin III.

Reviews

Antithrombotics

M. Petitou,*
C. A. A. van Boeckel* — 3118–3133

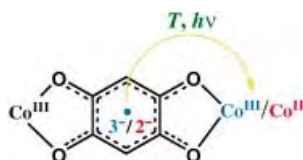
A Synthetic Antithrombin III Binding Pentasaccharide Is Now a Drug! What Comes Next?

Communications

Electron Transfer

C. Carbonera, A. Dei,* J.-F. Létard,*
C. Sangregorio, L. Sorace — 3136–3138

Thermally and Light-Induced Valence Tautomeric Transition in a Dinuclear Cobalt–Tetraoxolene Complex



Getting in tune: A material that undergoes valence tautomerism was obtained by tuning the electronic properties of a cobalt–polyoxolene complex (shown schematically) which contains a tri-positive metal ion acting as an ancillary counterpart. The tautomerism, which can be thermally or light induced, is quantitative upon irradiation at 617 nm.

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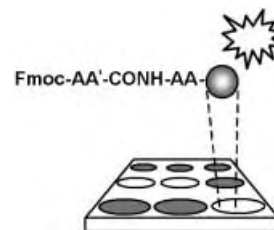
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Enzyme Screening

R. H. P. Doeze, B. A. Maltman, C. L. Egan,
R. V. Ulijn, S. L. Flitsch* — 3138–3141

Profiling Primary Protease Specificity by
Peptide Synthesis on a Solid Support

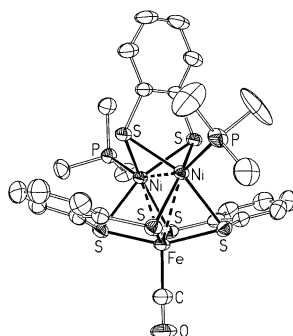
Reverse screening: A greatly simplified primary screening of protease specificity has been achieved by monitoring the fluorescence during the protease-catalyzed coupling of amino acids instead of peptide hydrolysis on a solid support (see picture, AA = amino acid). This approach paves the way for flexible, rapid, high-throughput identification and characterization of proteases without the need for expensively labeled peptide arrays.



Bioinorganic Chemistry

D. Sellmann, F. Lauderbach,* F. Geipel,
F. W. Heinemann, M. Moll — 3141–3144

A Trinuclear [NiFe] Cluster Exhibiting
Structural and Functional Key Features of
[NiFe] Hydrogenases



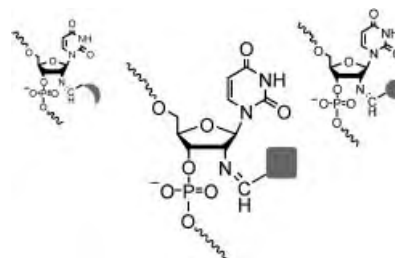
Charged but not changed: A trinuclear [NiFe] cluster combining the key structural features and reactivity of [NiFe] hydrogenases has been prepared (structure shown). Protons oxidize this cluster to its cation, which has the same structural parameters as the neutral form and therefore shows an important key feature of many oxidoreductases: structural rigidity during the electron-transfer processes.

Oligonucleotide Complexes

A. Bugaut, J.-J. Toulmé,
B. Rayner* — 3144–3147

Use of Dynamic Combinatorial Chemistry
for the Identification of Covalently
Appended Residues that Stabilize
Oligonucleotide Complexes

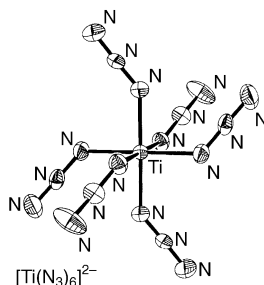
Amplification with kissing: Rapid identification of covalently appended residues that stabilize oligonucleotide complexes has been achieved by using a dynamic combinatorial approach that leads to amplification of the best ligand (see schematic representation). Both a DNA duplex and an RNA loop–loop (kissing) complex were investigated. Melting-temperature experiments confirmed that the amplified species stabilize the complexes.



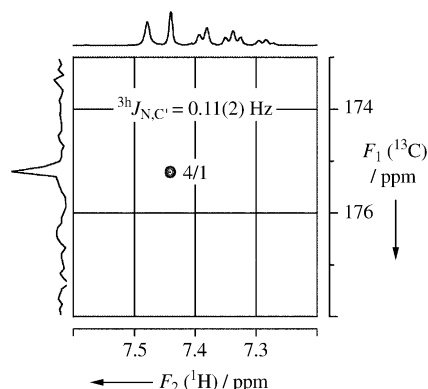
Azide Complexes

R. Haiges,* J. A. Boatz, S. Schneider,
T. Schroer, M. Yousufuddin,
K. O. Christe* — 3148–3152

The Binary Group 4 Azides
[Ti(N₃)₄], [P(C₆H₅)₄][Ti(N₃)₅], and
[P(C₆H₅)₄]₂[Ti(N₃)₆] and on Linear
Ti–N–NN Coordination



Out of line? Theory predicts that [Ti(N₃)₄] should exhibit unprecedented linear Ti–N–NN bond angles as a consequence of the α -N atoms of the azide groups acting as tridentate donors into the empty tetrahedral d⁰ orbitals of a (+IV) Group 4 metal atom. The title compounds were prepared and characterized (see structure). They do not possess linear Ti–N–NN angles because their coordination numbers exceed four.

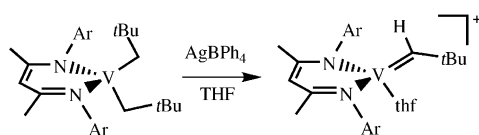


Differentiating helices: The direct observation of hydrogen bonds by $^3hJ_{N,C'}$ scalar couplings (see picture) is not only possible for α -helix and β -sheet peptides and proteins, but for 3_{10} -helical peptides as well. The method also provides information on terminal fraying of helices and allows the discrimination between α and 3_{10} helices.

Helical Structures

M. Bellanda, M. Rainaldi,
Q. B. Broxterman, B. Kaptein,
F. Formaggio, M. Crisma, S. Mammi,
C. Toniolo* 3152–3155

Direct Observation of Intramolecular
Hydrogen Bonds in Peptide 3_{10} Helices by
 $^3hJ_{N,C'}$ Scalar Couplings



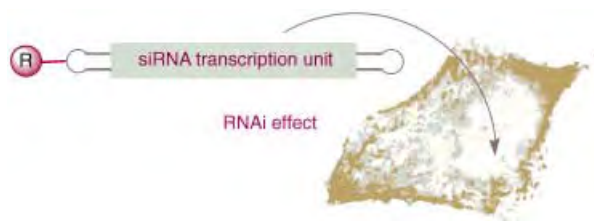
Kinetically stable, four-coordinate vanadium(IV) neopentylidene complexes such as $[(\text{Nacnac})\text{V}=\text{CHtBu}(\text{thf})](\text{BPh}_4)$ ($\text{Nacnac}^- = [\text{Ar}] \text{NC}(\text{Me})\text{CHC}(\text{Me})\text{N}[\text{Ar}]$, $\text{Ar} = 2,6\text{-(CHMe}_2)_2\text{C}_6\text{H}_3$), which was pre-

pared by oxidatively induced α -hydrogen abstraction from $[(\text{Nacnac})\text{V}(\text{CH}_2\text{tBu})_2]$ with AgBPh_4 in THF (see structure), contain the shortest $\text{V}=\text{C}$ bonds known to date.

Vanadium–Alkylidene Complexes

F. Basuli, U. J. Kilgore, X. Hu, K. Meyer,
M. Pink, J. C. Huffman,
D. J. Mindiola* 3156–3159

Cationic and Neutral Four-Coordinate
Alkylidene Complexes of Vanadium(IV)
Containing Short $\text{V}=\text{C}$ Bonds



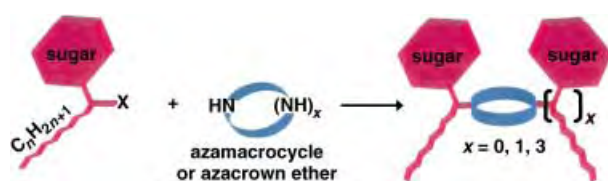
A dumbbell-shaped DNA, expected to be useful for gene therapy, was synthesized by using a novel method. The dumbbell-shaped vector (see picture) contains a

small interfering RNA (siRNA) coding sequence, and the vector was used to investigate the RNA interference (RNAi) effect inside cells.

DNA Synthesis

M. Taki, Y. Kato, M. Miyagishi, Y. Takagi,
K. Taira* 3160–3163

Small-Interfering-RNA Expression in Cells
Based on an Efficiently Constructed
Dumbbell-Shaped DNA



Each block carries out a designated task: Amphiphilic molecules are obtained by grafting one or several surfactant blocks with a bulky sugar headgroup onto a macrocyclic complexing block. This

modular approach allows control of the structure of the aggregates (see scheme) and formation of micelles insensitive to the presence of complexed metal ions.

Sugar-Based Surfactants

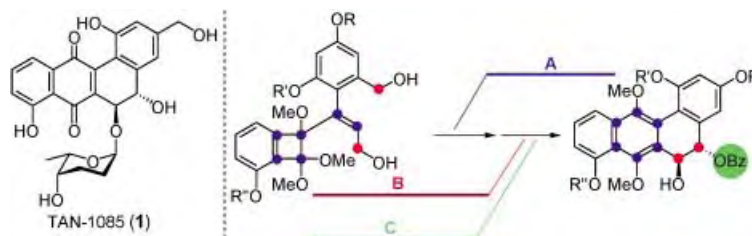
C. Larpent,* A. Laplace,
T. Zemb 3163–3167

Macrocyclic Sugar-Based Surfactants:
Block Molecules Combining Self-Aggregation and Complexation Properties

Natural Products Synthesis

K. Ohmori, K. Mori, Y. Ishikawa,
H. Tsuruta, S. Kuwahara, N. Harada,
K. Suzuki* 3167–3171

Concise Total Synthesis and Structure
Assignment of TAN-1085



Rapid access to the tetracyclic core of TAN-1085 (**1**) was possible by exploiting three efficient processes: a tandem electrocyclic reaction (A), a pinacol cyclization

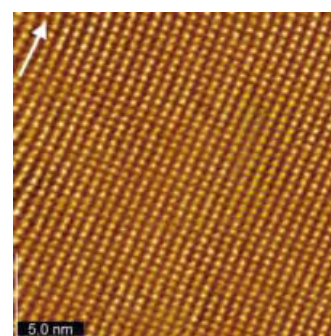
(B), and a regioselective monobenzylation of a 1,2-diol (C), which allowed the first total synthesis of **1**.

Mixed-Valence Structures

S. Takaishi,* H. Miyasaka, K.-i. Sugiura
M. Yamashita,* H. Matsuzaki, H. Kishida,
H. Okamoto, H. Tanaka, K. Marumoto,
H. Ito, S. Kuroda, T. Takami 3171–3175

Visualization of Local Valence Structures
in Quasi-One-Dimensional Halogen-
Bridged Complexes $[\text{Ni}_{1-x}\text{Pd}_x(\text{chxn})_2\text{Br}]_n\text{Br}_2$
by STM

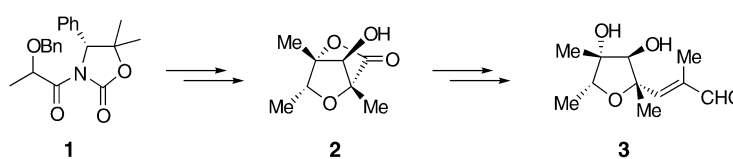
State secrets: Mott–Hubbard and charge-density-wave states have been observed in the quasi-1D bromine-bridged Ni and Pd complexes $[\text{M}(\text{chxn})_2\text{Br}]_n\text{Br}_2$, respectively, using STM (see image for the Ni complex, the arrow denotes the direction of the chain). The spin soliton was also visualized in real space in the Ni-Pd mixed-metal complexes for the first time. chxn: (1*R*,2*R*)-diaminocyclohexane.



Natural Products Synthesis

Y. Murata, T. Kamino, T. Aoki,
S. Hosokawa,
S. Kobayashi* 3175–3177

Highly Efficient Total Synthesis of
(+)-Citreoivral



Only eight steps were required for the total synthesis of (+)-citreoivral (**3**) from chiral imide **1** in 18% overall yield. The key steps included a highly *anti*-selective aldol reaction, the stereoselective iodolacton-

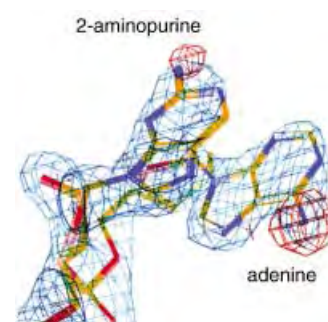
ization of a γ,δ -unsaturated β -hydroxy-imide, and an intramolecular $\text{S}_{\text{N}}2$ reaction of a tertiary alcohol to form intermediate **2**.

RNA Structures

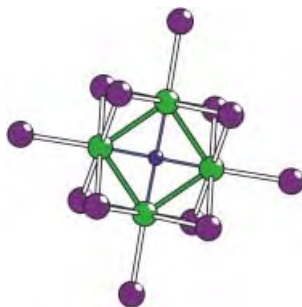
S. Shandrick, Q. Zhao, Q. Han, B. K. Ayida,
M. Takahashi, G. C. Winters,
K. B. Simonsen, D. Vourloumis,
T. Hermann* 3177–3182

Monitoring Molecular Recognition of the
Ribosomal Decoding Site

Adenine flips out: A combination of X-ray crystallography, 2-aminopurine fluorescence labeling, and the use of aminoglycosides as ligands is exploited to demonstrate conformational transitions in the RNA domain that ensures accurate protein synthesis (see picture). The triggering of a conformational change of an adenine unit in the RNA by ligand binding can be used as the basis of a screening method to discover antibiotics.



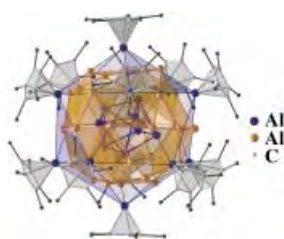
A round peg in a square hole: The unusual bonding situation within the cluster ion $[\{\text{Ti}_4\text{O}\}\text{I}_{12}]^{4-}$ (see picture) arises through the optimization of strong Ti–Ti and Ti–O σ bonding as well as to the absence of Ti–Ti multiple (π) bonding and the avoidance of Ti–I antibonding interactions.



Cluster Compounds

L. Jongen, A.-V. Mudring, A. Möller,
G. Meyer* _____ **3183–3185**

An Oxygen-Centered Titanium Square
Embedded in a Cuboctahedron of Iodine
in the Salt $\text{K}_4[\{\text{Ti}_4\text{O}\}\text{I}_{12}]$

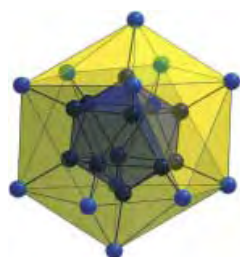


The largest organometallic cluster compound consisting of Al, C, and H only ($\text{Al}_{50}\text{Cp}_{12}^*$, $\text{Cp}^* = \text{C}_5\text{Me}_5$) is a fascinatingly simple structure of comparably high symmetry (see structure). The pentamethylcyclopentadienyl ligands form a carbon shell similar to the arrangement found in fullerene structures.

Cluster Compounds

J. Vollet, J. R. Hartig,
H. Schnöckel* _____ **3186–3189**

$\text{Al}_{50}\text{C}_{120}\text{H}_{180}$: A Pseudofullerene Shell of 60
Carbon Atoms and 60 Methyl Groups
Protecting a Cluster Core of 50 Aluminum
Atoms



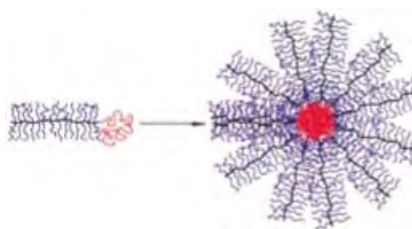
Shell suits: Two shells of Ga_{12} icosahedra characterize the arrangement of 24 gallium atoms in the title compound (see structure). The structure indicates connectivity possibilities of Ga_{12} units that are analogous to the topology of the α -boron modification.

Cluster Compounds

T. Duan, E. Baum, R. Burgert,
H. Schnöckel* _____ **3190–3192**

The Polyhedral Gallium Subhalide
 $[\text{Ga}_{24}\text{Br}_{22}]\cdot 10\text{THF}$: The First Step on the
Path to a New Modification of Gallium?

Giant rod–coil amphiphilic block copolymers were prepared by metallocene-catalyzed polymerization. The hydrophilic coiled block consists of polymethacrylic acid and the rod component of a hydrophobic cylindrical brush polymer. The high solubility of the stiff block results in the micelles being formed with the stiff block forming the solubilizing corona.



Rod–Coil Copolymers

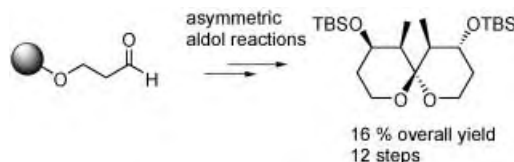
M. W. Neiser, S. Muth, U. Kolb,
J. R. Harris, J. Okuda,*
M. Schmidt* _____ **3192–3195**

Micelle Formation from Amphiphilic
“Cylindrical Brush”—Coil Block Copoly-
mers Prepared by Metallocene Catalysis

Asymmetric Synthesis

O. Barun, S. Sommer,
H. Waldmann* _____ 3195–3199

Asymmetric Solid-Phase Synthesis of
6,6-Spiroketals



Structurally simplified spiroketals derived from natural products often retain the biological activity of the parent compound. An asymmetric solid-phase synthesis with boron-mediated aldol reactions as key stereodifferentiating

transformations provides 6,6-spiroketal in just 12 steps in high yield and with high stereoselectivity (see scheme). The procedure is suitable for the creation of compound libraries for use in medicinal chemistry.



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Corrigendum

In the Communication “Direct Evidence for Extremely Facile 1,2- and 1,3-Group-Migrations in an FeSi₂ System” by H. Tobita et al. (*Angew. Chem.* **2004**, 116, 223–226; *Angew. Chem. Int. Ed.* **2004**, 43, 221–224, DOI 10.1002/anie.200352519),

In the Communication “Hydrogen-Bonded Helices in the Layered Aluminophosphate (C₂H₈N)₂[Al₂(HPO₄)(PO₄)]” by J. Yu, R. Xu, et al. (*Angew. Chem.* **2004**, 116, 2453–2456; *Angew. Chem. Int. Ed.* **2004**, 43, 2399–2402, DOI 10.1002/

the ¹H NMR data of complex **4** in the Experimental Section were given incorrectly. The correct data for **4** are given below: ¹H NMR (300 MHz, [D₆]benzene): δ = 0.69 (s, 3H, SiMesMe₂), 0.97 (s, 3H, SiMesMe₂), 1.03 (s, 3H, SiMesMe), 1.09

anie.200353118), on page 2400, left column, line 27, an oxygen atom was inadvertently mislabeled. The sentence should be: P(1) shares two O atoms with adjacent Al atoms and has one terminal P–OH group (P(1)–O(12) 1.563(4) Å)

(s, 9H, CMe₃), 1.46 (s, 15H, C₅Me₃), 2.15 (s, 3H, *p*-Me), 2.23 (s, 3H, *p*-Me), 2.45 (s, 6H, *o*-Me), 2.52 (s, 3H, *o*-Me), 2.64 (s, 3H, *o*-Me), 6.78 (s, 2H, *m*-H), 6.84 (s, 1H, *m*-H), 6.88 ppm (s, 1H, *m*-H).

and one terminal P=O group (P(1)=O(1) 1.470(3) Å), while P(2) and P(3) each share three oxygen atoms with adjacent Al atoms and have one terminal P=O group (P(2)=O(2) 1.493(3) Å, P(3)=O(5) 1.489(3) Å).

Hybrid Inorganic–Organic Mesoporous Materials

Guido Kickelbick*

Keywords:

mesoporous materials · organic–inorganic hybrid composites · self-assembly · silanes · sol–gel processes

Periodic mesoporous materials have gained a lot of interest in recent years, because of the possibility of tailoring the pore structure, framework composition, and morphologies over a wide range. Many potential applications arise from the promising properties of these high-surface-area materials, including separation technology (chromatography, membranes, etc.), catalysis, nanoelectronics, sensors, and spatially defined host materials for substances or reactions.^[1]

The synthesis of hexagonally ordered silica or aluminosilica mesoporous materials (the MCM-41 materials) using liquid-crystalline phases of ionic surfactants as templates was described for the first time in 1992 by Kresge et al.^[2,3] Soon it became clear that this new approach could be expanded towards the synthesis of other porous inorganic materials, such as various oxides,^[4–6] metal sulfides,^[7,8] phosphates,^[9] and metals.^[10,11] A variety of lyotropic liquid-crystalline surfactants and phase-separated block copolymers were used as templates for the assembly of the inorganic frameworks, which were formed as disordered, hexagonal, or cubic structures with a high control of the channel diameter between 2–100 nm.

Organic functionalization soon became a major topic of research because it offered a further possibility to tailor the chemical properties of the porous materials. The addition of organic

groups by post-synthetic grafting of RSi(OR')_3 , RSiCl_3 , or R_3SiCl onto the surface of the pores resulted in functional mesoporous inorganic–organic hybrid materials (Figure 1). This post-synthetic grafting process has been widely employed to anchor various organic groups onto the surface of the pores, including organometallic species, amino- and thiol groups, and epoxides. However, this method often led to a quite low loading, an inhomogeneous distribution of the functional groups, and a decrease of the pore volume. The alternative route of applying organosiloxanes directly during the synthesis by co-condensing them with tetraalkoxysilanes (Figure 1) led to a higher loading but above a limit of around 25% RSiO_3 groups the mesostructure collapsed. Functional groups, such as vinyl,^[12,13] 3-sulfanylpropyl,^[14,15] phenyl,^[16] aminopropyl,^[14,17] cyanoethyl,^[17] diphenylphosphanylpropyl,^[18] were incorporated by this method into the inorganic network. Recently, based on this approach, amphiphiles with a cleavable alkyl chain and a condensable head group were used as templates for the

synthesis for mesoporous silica materials.^[19] After the synthesis, the cleavage and removal of the alkyl chains left nanopores with a densely functionalized surface.

An expansion of the above mentioned concepts was the incorporation of bifunctional organosiloxane precursors, also referred to as bridged silsesquioxanes, of the general formula $[(\text{R}'\text{O})_3\text{Si}]_m\text{R}$ ($m \geq 2$) as the network-forming species (Figure 2). The resulting materials, called periodic mesoporous organosilicas (PMOs), contain the organic groups as an integral part of the inorganic-oxide framework and the inorganic and organic moieties are covalently linked to each other. Hence, the “chemistry of the void space” explored by the classical MCM-41-type materials was extended to the “chemistry of the walls”.^[20] This novel route offers materials with some important features that cannot be obtained by other approaches. First, the organic moieties are homogeneously dispersed within the channel wall and a maximum loading of up to 100% is obtained. Second, organic groups incorporated into the channel

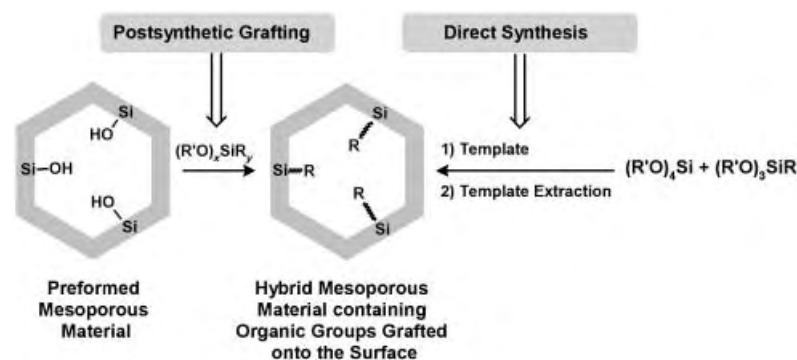


Figure 1. Synthesis of hybrid mesoporous materials containing functional groups that dangle into the channels.

[*] Dr. G. Kickelbick
Institute of Materials Chemistry
Vienna University of Technology
Getreidemarkt 9, 1060 Vienna (Austria)
Fax: (+43) 1-58801-15399
E-mail: guido.kickelbick@tuwien.ac.at

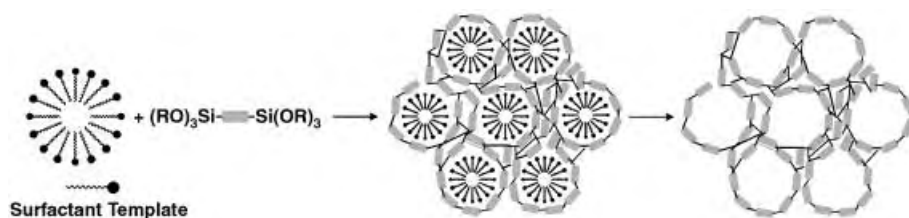


Figure 2. Synthesis of the periodic mesoporous organosilicas (PMOs).

walls do not block the pores. Integrating the “soft” organic groups should deliver mechanical properties different from those of the classical, purely inorganic mesoporous materials.^[21] And last but not least, changing the organic precursors can result in pore walls with different chemical and physical properties.^[22]

The advantages of the double-substituted molecules are that bonding is already established between the organic and inorganic species and thus the stoichiometry between silicon and carbon in the framework is assured. Examples of organosiloxanes that were successfully incorporated into the pore walls are bis(triethoxysilyl)methane,^[20] bis(triethoxysilyl)ethane,^[23,24] bis(triethoxysilyl)ethene,^[25] bis(triethoxysilyl)acetylene,^[22] 1,4-bis(triethoxysilyl)benzene and derivatives thereof,^[22,26–28] 2,5-bis(triethoxysilyl)thiophene,^[22] 1,4-bis(triethoxysilyl)ferrocene,^[22] and bis(triethoxysilyl)bithiophene^[22] (Figure 3). These organic precursors were either mixed with classical inorganic-oxide network builders, such as tetraethoxysilane (TEOS), or were used as single-source precursors. The nature of the organic group greatly influences the hydrolytic stability of the Si–C bond, whereas the simple precursors contain-

ing methylene, ethylene, or ethynylene groups show high stability in the synthesis, the use of more complicated groups results in a significant Si–C bond cleavage as well as a decrease in the degree of order in the material.^[22]

Generally, the resulting products are comparable to MCM-41 materials in that they have high Brunauer–Emmett–Teller (BET) surface areas of between 600 and 1700 m² g^{−1}, average pore diameters—depending on the surfactant and reaction conditions used—from 3 nm up to 10 nm, wall thicknesses of 0.7–3.3 nm, and narrow pore size distributions.

The chemical reactivity of the incorporated organic groups was demonstrated, for example, by the bromination of unsaturated spacers where by the structural integrity and order of the material was maintained.^[23,25] Furthermore, mesoporous aminosilica materials were obtained by the thermal ammonolysis of methylene or ethylene PMOs.^[29]

An interesting property was observed for the mesoporous silica materials that contain bridging methylene groups in the walls. This material showed a so-called metamorphosis upon heating under inert-gas atmosphere, that is, the bridging methylene groups

are transformed into terminal methyl groups and the mesoordered structure remains intact.^[20]

Structural order in the pore walls, on the molecular scale, was usually not observed, however, if aromatic systems were used as bridging groups the presence of arylsilica ordering arising from π – π stacking was observed leading to a kind of “double self-assembly” process involving simultaneous preorganization and co-assembly of both surfactant micellar and the organosilica precursor species.^[26,27,30] Hence, a hierarchically ordered mesoporous solid is produced with a molecular-scale pore-surface periodicity.

A further important step in the use of organosiloxane precursors was the change from bis(trialkoxysilane)-substituted organic groups, whose only function was to incorporate organic groups into the framework, to tri- or multi-alkoxysilane substituted units, which also act as cross-linkers in the network formation.^[31,32]

Recently, a novel ring-shaped precursor was introduced, that is, 1,3,5-tris[diethoxysilyl]cyclohexane [(EtO)₂SiCH₂]₃,^[33,34] in which each silicon atom has two organic substituents compared to one in all previous systems. This arrangement allows for a higher degree of organic functionalization in the walls. It forms networks through the six hydrolyzable groups in the ring and allows the preparation of mesoporous powders and oriented films. The resulting material contains mesoscale channels with a spacing of about 4.5 nm, mesopores with a diameter of approximately 2.2 nm, and an estimated wall thickness of 2.3 nm. Unlike other complex precursors, no Si–C bond cleavage was observed in the final material. The framework showed quite high thermal stability, and a thermal transformation of bridging CH₂ into terminal CH₃ groups was observed as in other methylenesilica systems. The thin films obtained by spin coating were calcined at 300 °C under a nitrogen atmosphere without cracking or loss of mesostructure and also the Si–C bonds remained intact. Hence, these materials are interesting low-dielectric-constant (*k*) materials for use in future integrated circuits and have a high thermal and a good mechanical stability. Films with a varying organic content were synthe-

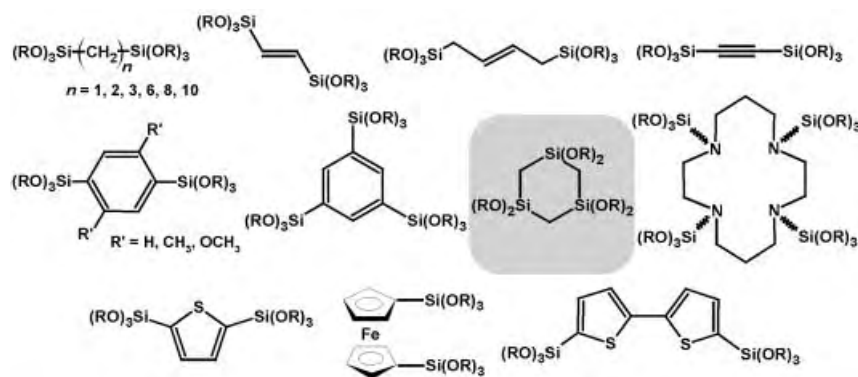


Figure 3. PMO building blocks.

sized from mixtures of tetramethoxysilane (TMOS) and the silsesquioxane ring. In these films a linear decrease of the k value with increasing organic content was observed. While the films made entirely of $[(\text{EtO})_2\text{SiCH}_2]_3$ had $k \approx 2.5$, this value could be decreased to 2 by thermal treatment up to 400°C. A unique feature of the described precursor is the possibility to functionalize one of the bridging methylene groups by a lithiation reaction followed by a nucleophilic substitution and the use of the functionalized ring thus formed for the formation of PMOs. The resulting materials contained functional groups, such as I and Br, which were still intact after synthesis and extraction. The chemical modification of these groups will allow a plethora of novel materials to be prepared. This novel precursor is a prototype of a new class of mesoporous hybrid materials and we can look forward to future developments in this class.

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The Staudinger Ligation—A Gift to Chemical Biology

Maja Köhn and Rolf Breinbauer*

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bioconjugates · bioorganic chemistry · chemical biology · peptides · Staudinger ligation

Although the reaction between an azide and a phosphane to form an aza-ylide was discovered by Hermann Staudinger more than 80 years ago and has found widespread application in organic synthesis, its potential as a highly chemoselective ligation method for the preparation of bioconjugates has been recognized only recently. As the two reaction partners are bioorthogonal to almost all functionalities that exist in biological systems and react at room temperature in an aqueous environment, the Staudinger ligation has even found application in the complex environment of living cells. Herein we describe the current state of knowledge on this reaction and its application both for the preparation of bioconjugates and as a ligation method in chemical biology.

1. Introduction

In their studies of biological systems molecular biologists and chemists are often faced with the need to link two molecular entities covalently, for example, to link a complex carbohydrate with a peptide or to attach a small molecular probe (such as a fluorescent dye, a radical probe, or an affinity tag) to a biopolymer. As biological systems are both rich in structural complexity and diverse in their functional reactivity, chemoselective ligation reactions have to be developed in which two mutually and uniquely reactive functional groups can be coupled, usually in an aqueous environment under physiological conditions. These uniquely reactive functional groups should be selective for one another and also tolerate a diverse array of other functionalities, thus rendering the use of protecting groups unnecessary and, in the ideal case, allowing its application in the complex environment of a living cell.

Although several bioconjugation techniques are available for the in vitro preparation of bioconjugates substituted with a limited number of functional groups,^[1] truly chemoselective

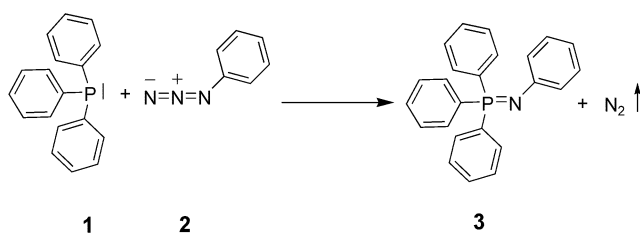
ligation reactions are rather limited. Most ligation reactions rely on the reaction of an electrophile with a nucleophile. As biological systems are rich in diverse electrophilic and nucleophilic sites, only a few functional groups are available that exhibit or-

thogonal reactivity to the functional groups present.^[2] Recently, two reactions were introduced in which the azide moiety serves as a reactive functional group, with the following three advantages: 1) the azide moiety is absent in almost all naturally occurring compounds (“bioorthogonal”); 2) despite their high intrinsic reactivity, azides undergo a selective ligation with a very limited number of reaction partners; 3) the azide group is small and can be introduced into biological samples without altering the molecular size significantly. Whereas the “click-chemistry” reaction of Sharpless and co-workers ([3+2] cycloaddition between an azide and a terminal alkyne) requires the presence of a copper catalyst,^[3] the Staudinger ligation introduced by Saxon and Bertozzi exploits the smooth reaction between an azide and a phosphane to form a phosphazide-ylide. This ylide can be trapped by an acyl group with formation of a stable amide bond.^[4] Herein we summarize recent applications of this chemoselective ligation method with two bioorthogonal groups.

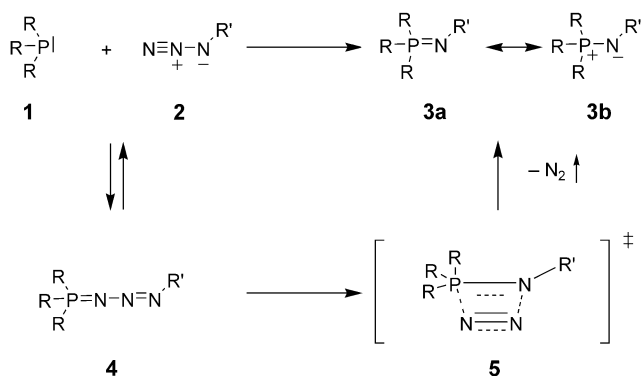
2. Staudinger Reaction

In 1919 Staudinger and Meyer reported that azides react smoothly with triaryl phosphanes to form iminophosphoranes (Scheme 1).^[5] This imination reaction proceeds under mild conditions, almost quantitatively, and without noticeable formation of any side products. Over the last century detailed mechanistic studies have revealed that the mechanism of this reaction involves several intermediates (Scheme 2).^[6] In a

[*] Dipl.-Chem. M. Köhn, Dr. R. Breinbauer
Department of Chemical Biology
Max-Planck-Institut für molekulare Physiologie
Otto-Hahn-Strasse 11, 44227 Dortmund (Germany)
and
Universität Dortmund, Fachbereich 3, Organische Chemie
44227 Dortmund (Germany)
Fax: (+49) 231-133-2499
E-mail: rolf-peter.breinbauer@mpi-dortmund.mpg.de



Scheme 1. Staudinger reaction between a phosphane and an azide to form an iminophosphorane.

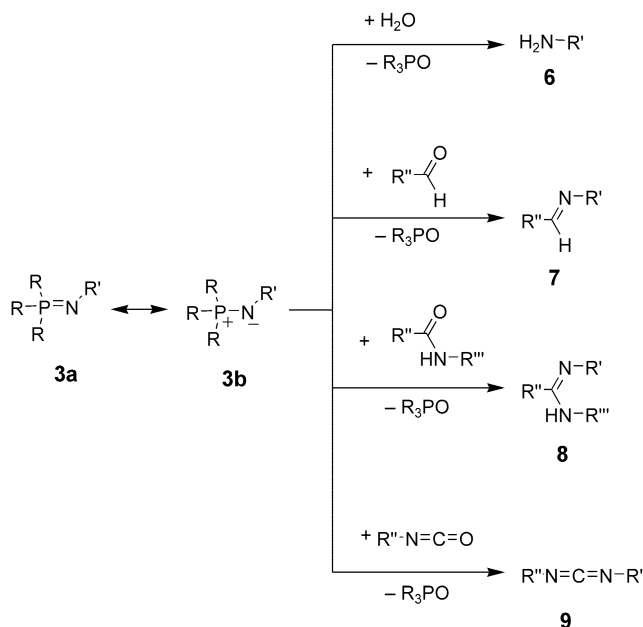


Scheme 2. Mechanism of the Staudinger reaction.

primary imination reaction **1** and **2** react to form a phosphazide **4**, which, as a rule, decomposes during the reaction with loss of nitrogen. It is interesting that the rate of the formation of **4** is controlled only by the inductive properties of the groups attached to the phosphorus atom and the azide, and that no significant steric influence is observed. Phosphazides **4** are stable at room temperature in organic solvents if substituents are present that delocalize the positive charge on the phosphorus atom and/or provide steric shielding of the phosphorus atom. The subsequent loss of dinitrogen is thought to proceed via the 4-membered-ring transition state **5** with retention of the original configuration at phosphorus, without the participation of either free radicals or nitrenes.

The iminophosphorane **3**, with its highly nucleophilic nitrogen atom, can react with almost any kind of electrophilic

reagent, thus resulting in many reactions of significant synthetic importance (Scheme 3).^[6,7] If the Staudinger reaction is carried out in an aqueous solvent, the readily formed iminophosphorane **3** is hydrolyzed rapidly to generate the primary amine **6** and phosphane(v) oxide. The so-called Staudinger reduction is a frequently used method for the



Scheme 3. The aza-ylide **3b** can be trapped by a plethora of electrophiles.

smooth reduction of azides to amines. Staudinger himself discovered that **3** can react with aldehydes or ketones to form imines.^[8] The synthetic value of the aza-Wittig reaction is reflected by the fact that it has found application in many total syntheses, for example, in syntheses of croomine, quinine, and dendrobine.^[7] Less reactive carbonyl electrophiles, such as amides or esters, also undergo reaction with **3**, especially if the electrophilic attack proceeds in an intramolecular fashion. To name one example, the reaction of 2-azidobenzoyl chloride with a suitable amide is an excellent method for the construction of quinazolines (Eguchi protocol).



Born in Schärding (Austria) in 1970, Rolf Breinbauer studied chemistry in Vienna and Heidelberg. After his doctoral studies with Prof. M. T. Reetz at the Max-Planck-Institut für Kohlenforschung in Mülheim an der Ruhr (Germany) he went as an Erwin Schrödinger Fellow to Prof. E. N. Jacobsen at Harvard University. Since 2000 he has led a research group at the Max-Planck-Institut für molekulare Physiologie and the Institute of Organic Chemistry of the Universität Dortmund (Germany). His research interests include combinatorial chemistry and chemical biology. (Foto: Jürgen Huhn)



Maja Köhn was born in 1975 in Kiel (Germany), where she also studied chemistry. For her diploma project she carried out research on the derivatization of carbohydrates under the supervision of Prof. T. K. Lindhorst. As a visiting scholar at the CSIC in Seville (Spain) she worked with Dr. J. M. Garcia Fernandez on the synthesis of glyco-clusters. Since 2001 she has been a graduate student in the Department of Chemical Biology (Prof. H. Waldmann) at the Max-Planck-Institut für molekulare Physiologie and the Universität Dortmund.

3. Staudinger Ligation

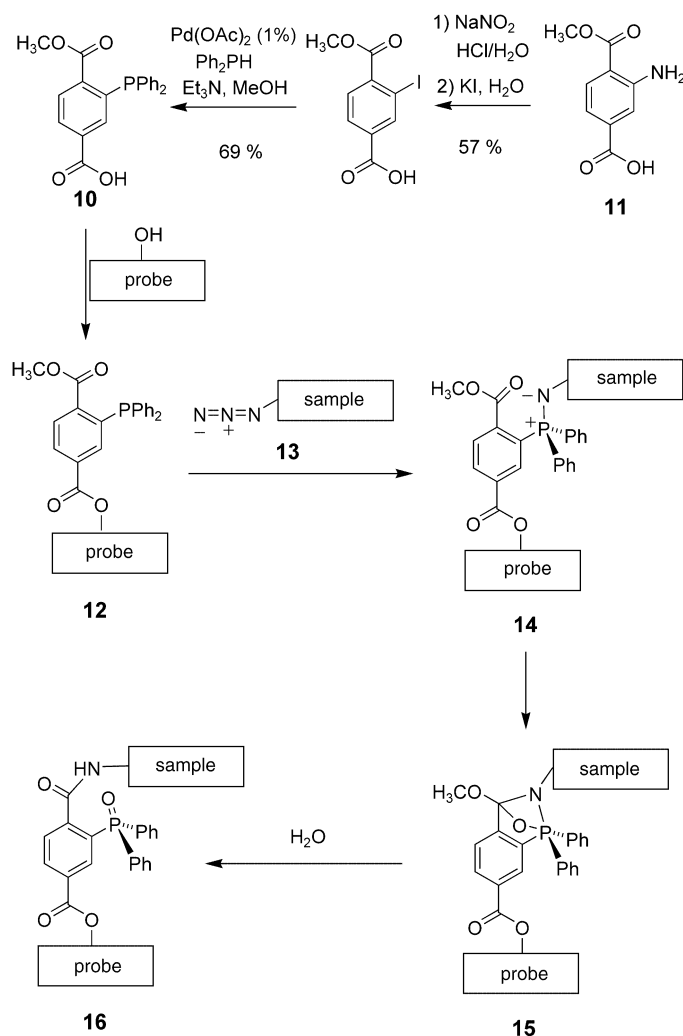
3.1. Nontraceless Staudinger Ligation

In their pioneering studies on the metabolic engineering of cell surfaces, Bertozzi and co-workers recognized the limitation of the hydrazone ligation method, in which conjugates are formed between a hydrazine probe and a ketone-modified sample.^[2] Their search for a milder reaction of two truly bioorthogonal functionalities led them to the Staudinger reaction. As described above, the product of the reaction of an azide with a phosphane, the aza-ylide **3**, undergoes spontaneous hydrolysis to the amine and phosphane oxide in an aqueous environment. Saxon and Bertozzi designed the ligand **10** based on the rationale that an appropriately located electrophilic trap, such as an ester moiety, within the structure of the phosphane, would capture the nucleophilic aza-ylide **14** by intramolecular cyclization (Scheme 4).^[4] This process would ultimately produce a stable amide bond before the competing aza-ylide hydrolysis could take place. The ligand **10**, which is not yet commercially

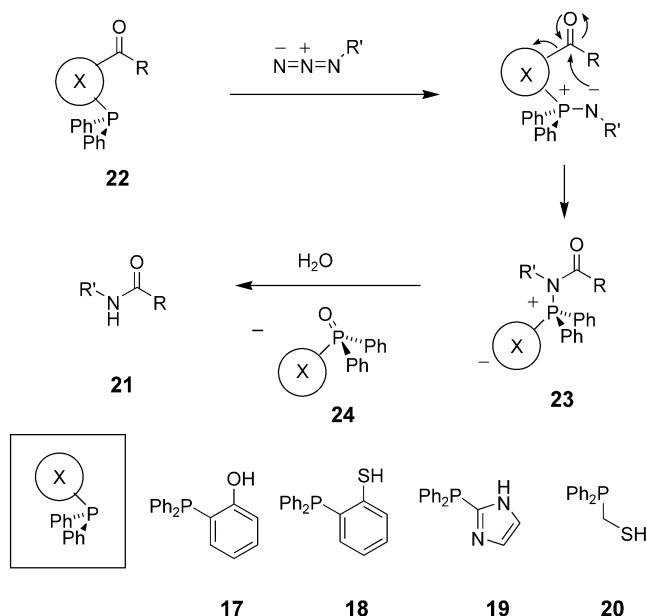
available, can be synthesized readily from the aminoterephthalic acid ester **11** by diazotization, followed by iodination and subsequent Pd-catalyzed phosphanylation.^[4,9] Standard esterification or amidation methods allow the attachment of the phosphane to the probe to form the conjugate **12**, which undergoes reaction in an aqueous solution with the azide **13**. Mechanistic studies by ³¹P NMR spectroscopy identified the aza-ylide **14** and the oxaphosphetane **15** as intermediates in the ligation reaction.^[10]

3.2. Traceless Staudinger Ligation

Although the reaction detailed above works well in a biological environment, a modification in which an amide bond is formed between the two coupling partners to give a product without a triaryl phosphane oxide moiety appears even more attractive. Shortly after their first report, Bertozzi and co-workers^[11] and—in a parallel effort—Raines and co-workers^[12] reported a traceless Staudinger ligation, in which the phosphane oxide moiety is cleaved during the hydrolysis step (Scheme 5).



Scheme 4. Preparation of the phosphane **10** and its application in the Staudinger ligation.



Scheme 5. Traceless Staudinger ligation.

In this reaction, the phosphanes **17**–**20** are first acylated and then treated with the azide. The nucleophilic nitrogen atom of the aza-ylide then attacks the carbonyl group to cleave the linkage with the phosphonium species. Hydrolysis of the rearranged product **23** produces the amide **21** and liberates the phosphane oxide **24**. Among the phosphanes tested, 2-diphenylphosphanylphenol (**17**; readily prepared by the Pd-catalyzed reaction of 2-iodophenol with diphenylphosphane)^[11] and diphenylphosphanylmethanethiol (**20**; now commercially available) exhibit the best reactivity profiles and have found widespread application, as will be detailed below. Because of its alkyl substituent, **20** is readily

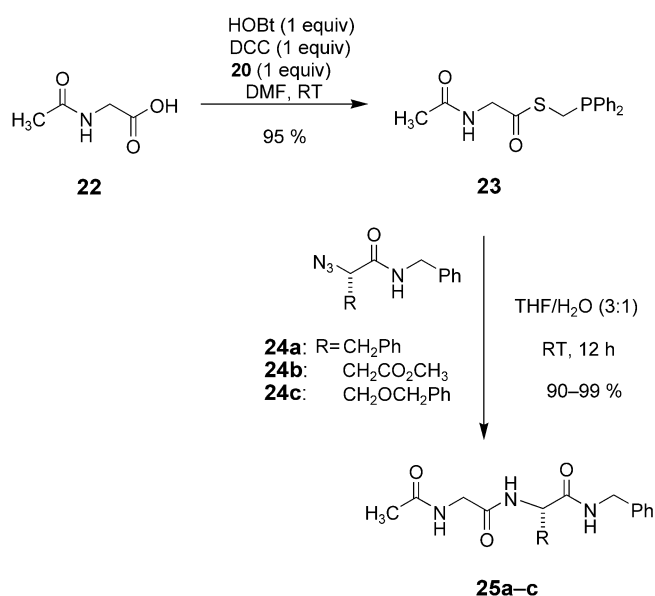
oxidized in air and is best handled as an adduct with BH_3 , from which it can be liberated by treatment with a powerful amine nucleophile, such as dabco (1,4-diazabicyclo[2.2.2]octane).

4. Applications

4.1. Peptide Ligation

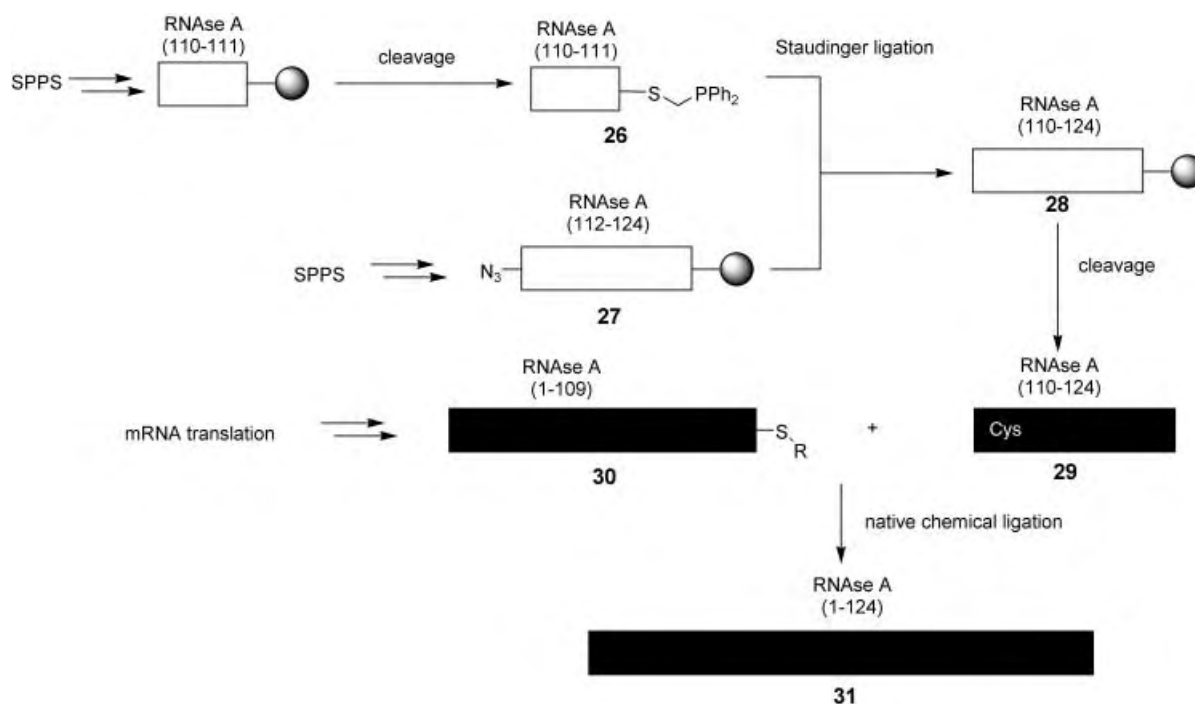
The total synthesis of proteins requires chemoselective ligation methods in which shorter peptide fragments (which can be synthesized by solid-phase peptide synthesis) are assembled to give the final protein.^[13] The most common ligation method, the native chemical ligation of Wieland et al. and Kent and co-workers, requires the presence of a Cys residue at the N terminus of the junction site.^[14] Raines and co-workers have proposed the Staudinger ligation as a peptide ligation method which does not depend on the side chain present.^[12b] In a proof-of-concept experiment they converted *N*-acetylglycine (**22**) into the thioester **23**, which reacted smoothly in aqueous THF with the protected azido amino acids **24a–c** to give the dipeptides **25a–c** in very good yields and without any detectable epimerization (Scheme 6). The required azido amino acids can be prepared quite simply from the corresponding amino acids by the diazo transfer procedure of Roberts and Wong.^[15,16]

In an impressive effort demonstrating the power of methods involving orthogonal chemical ligation, Raines and co-workers completed the total synthesis of ribonuclease A (**31**, RNase A, 124 amino acids) by linking three fragments (Scheme 7).^[17] RNase A(110–111) was synthesized as the C-terminal phosphanyl thioester FmocCys(Trt)Glu(OrBu)-



Scheme 6. Peptide formation through a traceless Staudinger ligation by Raines and co-workers.

SCH_2PPh_2 (**26**) by using a Kenner-type safety-catch linker. RNase A(112–124) was synthesized as the N-terminal azide $\text{N}_3\text{CH}_2\text{C}(\text{O})\text{Asn}(\text{Trt})\text{ProTyr}(\text{tBu})\text{ValProValHis}(\text{Trt})\text{PheAsp}(\text{OrBu})\text{AlaSer}(\text{tBu})\text{Val}$ (**27**) by a standard Fmoc strategy on a PEGA resin. The two peptide fragments were connected on the resin by a Staudinger ligation. After cleavage from the resin and deprotection of the acid-labile protecting groups, RNase A(110–124) (**29**) was isolated in 61% yield. The N-terminal Cys residue of this fragment allowed its attachment



Scheme 7. Total synthesis of RNase A with three different chemical ligation methods (Raines and co-workers).

by native chemical ligation to the RNase A(1–109) thioester **30** produced biosynthetically. The resulting protein **31** proved after folding to be fully functional RNase A.

This impressive result should not detract from the fact that the full scope and limitations of the Staudinger ligation in peptide chemistry have not yet been fully explored. Future efforts will be aimed at the ligation of more-complex peptide fragments, which should answer the question of whether or not side-chain protection is required.

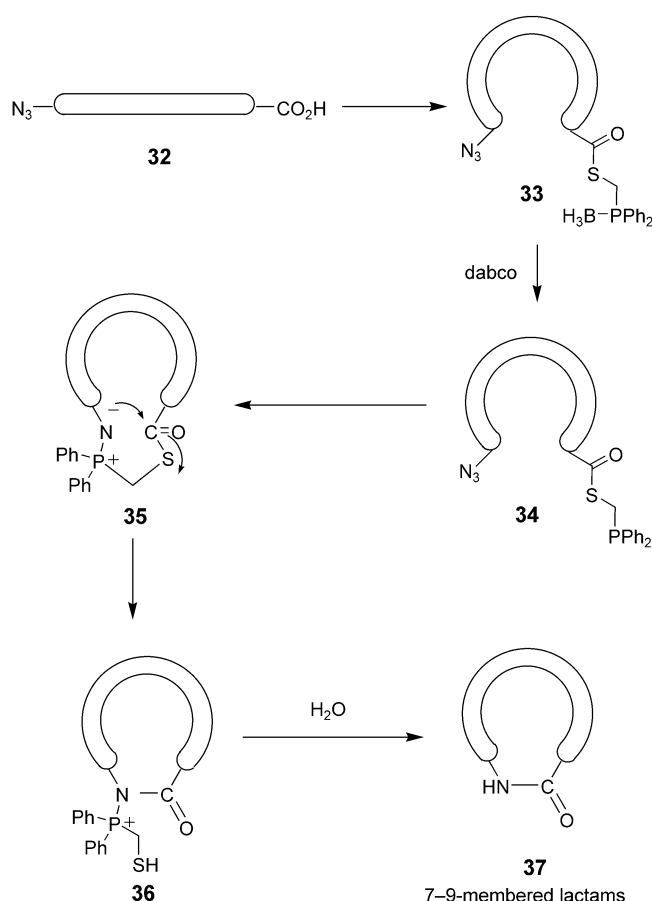
Liskamp and co-workers have begun to address this issue by studying the ligation of peptide fragments in which no glycine residue is present at the junction site.^[18] Starting from N-terminal α -azido peptides and C-terminal-peptide ortho-(diphenylphosphanyl)phenyl esters (readily prepared from the phosphane **17**) they prepared tetra- and pentapeptides in 6–36% yield under non-aqueous conditions. The coupling of an unprotected peptide containing a Lys residue not only delivered the desired product but also the product of non-specific aminolysis caused by nucleophilic attack of the ϵ -amino group of the Lys residue. Kinetic studies revealed that in all cases the formation of the aza-ylide intermediate proceeded very fast, thus suggesting that the slow formation of the ligation product might be a result of the increased steric congestion in the intermediates or the presence of amino acid side chains in the peptide fragments.

An interesting application of this amide-bond-forming reaction was disclosed by Maarseveen and co-workers, who present a solution to the problem of ring-closure to form medium-sized lactams.^[19] The reaction sequence is outlined in Scheme 8. The acyclic azido carboxylic acid **32** is converted into the borane-protected phosphanyl thioester **33**. Upon deprotection with dabco the phosphorus atom regains its ability to act as a nucleophile. Nucleophilic attack at the azide occurs with formation of the aza-ylide **35**, and electrophilic trapping of the ylide by the thioester then reduces the ring size by three atoms. In this reaction the enthalpy gain associated with amide formation compensates for the large increase in steric strain accompanying the formation of medium-sized rings. Hydrolysis removes the phosphane auxiliary and furnishes the 7–9-membered lactams **37** in yields that are typically higher than those of traditional lactamization methods.

4.2. Synthesis of Bioconjugates

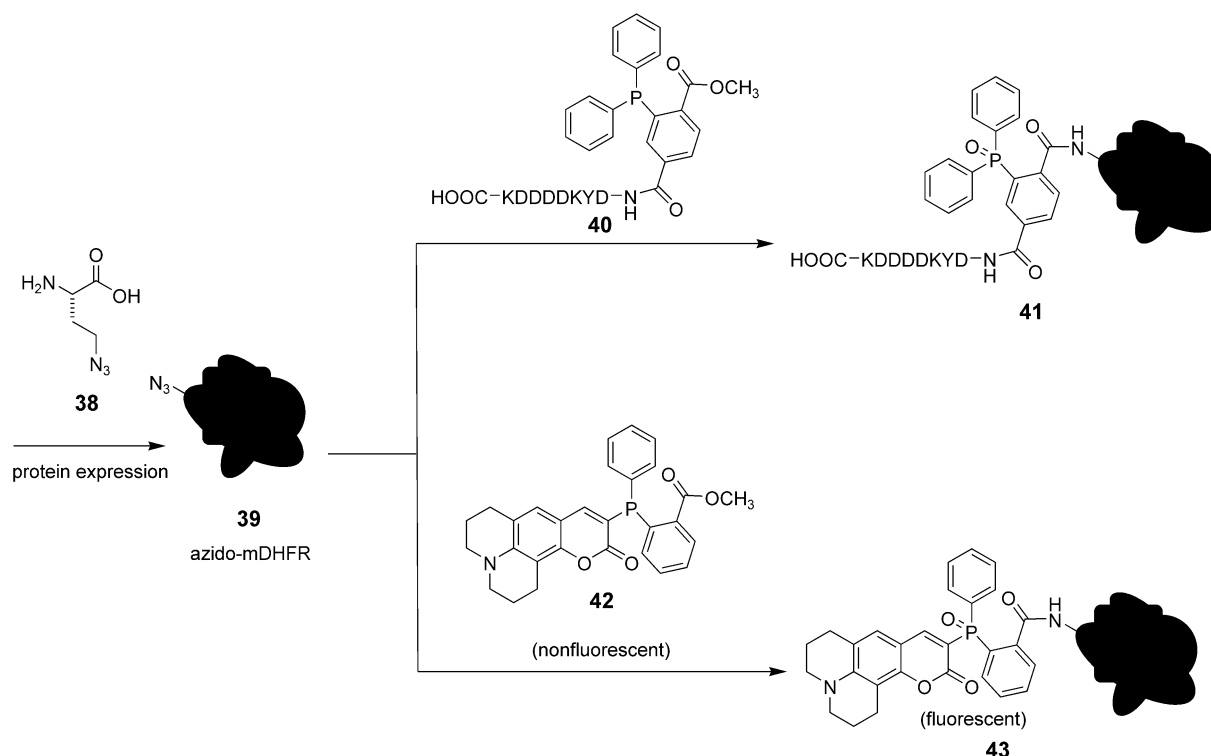
At the heart of chemical biology is the preparation of bioconjugates in which a reporter group (dye, spin label, affinity tag, recognition motif) is attached to a biological sample or biopolymer.^[1]

The research groups of Tirrell and Bertozzi have described a generally applicable strategy for the incorporation of azides into recombinant proteins for subsequent chemo-selective modification by the Staudinger ligation.^[20] Azido-homoalanine (**38**) is activated by the methionyl-tRNA synthetase (MetRS) of *Escherichia coli* and replaces methionine in proteins expressed in methionine-depleted bacterial cultures. In a proof-of-concept experiment murine dihydrofolate reductase (mDHFR) was expressed in an *E. coli*



Scheme 8. A general strategy for the formation of 7–9-membered lactams.

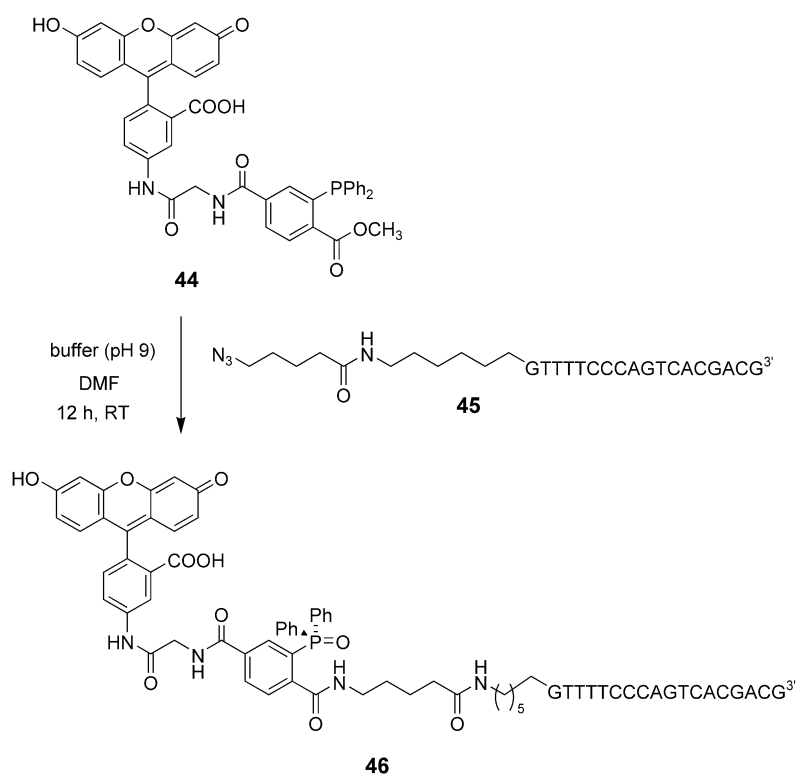
methionine auxotroph in a medium supplemented with azido-homoalanine (**38**). Amino acid analysis of the purified protein **39** indicated $95 \pm 2\%$ replacement of methionine. The purified protein was subjected to Staudinger ligation with two different probes (Scheme 9). Azido-mDHFR (**39**) was treated with the phosphane **40** (250 μM) containing the FLAG peptide (Asp-Tyr-Lys-Asp-Asp-Asp-Lys) in a buffer at 47°C for 6 h. The labeled protein **41** was characterized either by incubation with a FITC anti-FLAG antibody (FITC = fluorescein isothiocyanate) or by tryptic digestion and MALDI-TOF analysis. Although the Staudinger ligation took place at several sites, exhaustive modification of all eight possible sites was not observed. Reduction of the azide of the azido-homoalanine residue had probably already taken place to a certain extent. Interestingly and importantly, the labeling process also functions with a crude cell lysate. To avoid a two-step labeling procedure, which would impose limitations on the use of real biological samples, Bertozzi and co-workers designed the fluorogenic coumarin phosphane dye **42**, which itself is nonfluorescent but is activated by the Staudinger ligation with azides.^[21] The treatment of **42** with azido-mDHFR (**39**) resulted in the specific labeling of the azido protein. Unlike in the previous experiment with the FLAG conjugate, the labeled protein **43** could be observed directly, without the need for Western blotting, washing, or secondary labeling steps.



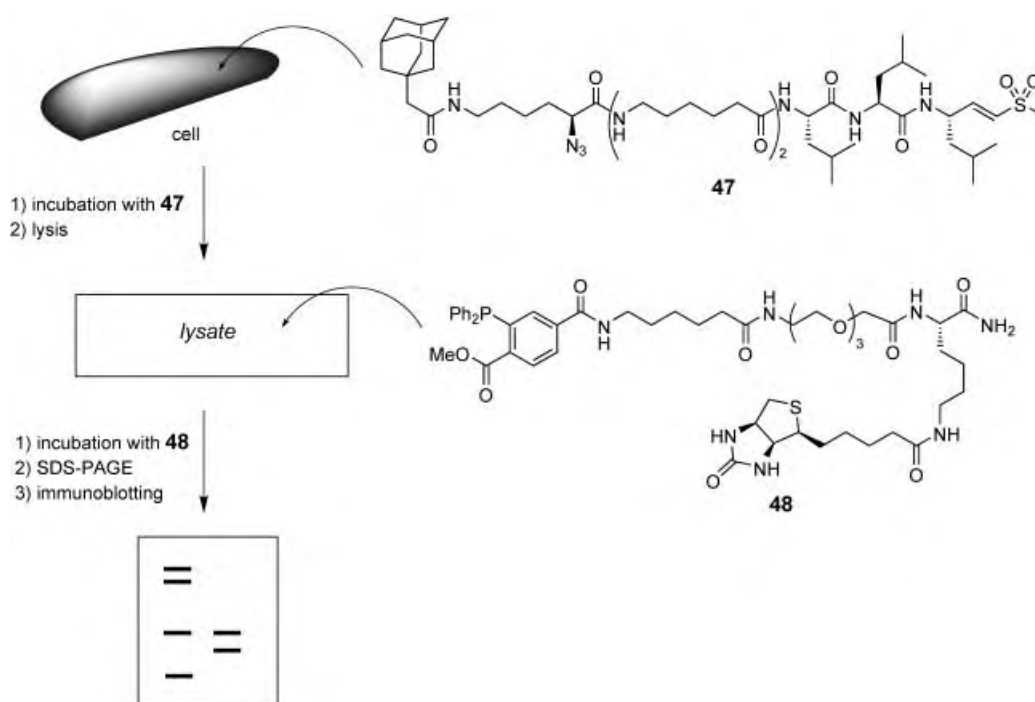
Scheme 9. Recombinant expression of azide-bearing proteins and their labeling with the Staudinger probes **40** and **42**.

Ju and co-workers have demonstrated the site-specific labeling of DNA by using the Staudinger ligation.^[22] An oligonucleotide **45** modified at its 5' end with an azido group underwent selective reaction with the fluorescein-modified phosphane (Fam) **44** under aqueous conditions to produce the Fam-labeled oligonucleotide **46** in approximately 90% yield (Scheme 10). The fluorescent oligonucleotide **46** was then used as a primer in a Sanger dideoxy sequencing reaction to produce fluorescent DNA extension fragments, which were analyzed with a fluorescence electrophoresis DNA sequencer.

Chemistry-based approaches in functional proteomics have been developed in which synthetic compounds that modify a selected subset of proteins covalently and irreversibly are used. Irreversible protease inhibitors have been used in the profiling of serine proteases and cysteine proteases.^[23] Overkleeft and co-workers designed the azide-containing inhibitor **47**, which inhibits all catalytically active β subunits of both the constitutive and the interferon- γ -inducible immunoproteasome.^[24] EL-4 cells were incubated with **47** overnight. After lysis the cell extract was incubated with the biotinylated phosphane probe **48** (Scheme 11). After separation by SDS-PAGE and immunoblotting the different subunits of the proteasome could be visualized. In this experiment *in vivo* labeling proved more effective than the corresponding procedure carried out *in vitro*.



Scheme 10. Preparation of a fluorescent-dye-labeled DNA oligonucleotide.



Scheme 11. Detection of active proteasomes by using an irreversible inhibitor probe, which is derivatized through a Staudinger ligation.

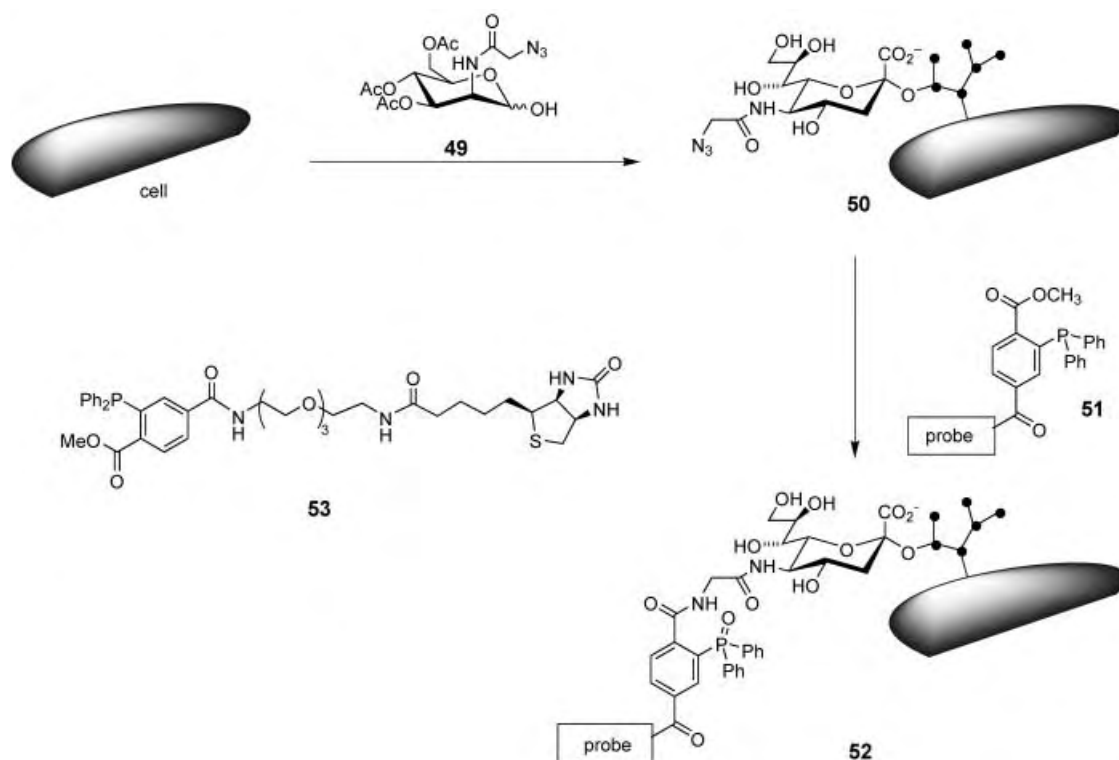
4.3. Metabolic Cell Engineering

The interactions that take place on the surface of a cell are of critical importance to the cell cycle and to the communication of cells within complex tissues, including cell–cell adhesion and virus–cell interactions. Carbohydrates have been recognized as central players in these recognition events. Although targeted disruption of glycosylation genes has provided significant information about the function and diversity of carbohydrates, this approach has several limitations, such as embryonic lethality and the upregulation of compensating pathways.^[9] Therefore, there is tremendous interest in chemical methods that modulate cell-surface molecules so that their function in the context of intercellular communication can be probed. The research group of Bertozzi has applied a technique in which non-natural biosynthetic precursors are converted into non-natural cell-surface polysaccharides with altered biological functions.^[4,9,10] Of particular interest for the application of the Staudinger reaction is the observation that mammalian cells incubated with peracetylated azidoacetylmannosamine (Ac₄ManNAz, **49**) take up this substrate and process it in their own sialic acid biosynthesis pathway to produce azidoacetylsialic acid (SiaNAz), which is incorporated instead of sialic acid into cell-surface glycoconjugates (Scheme 12). The cell-surface azide groups react with a phosphane–probe conjugate **51** (e.g. the biotinylated phosphane **53** or the FLAG phosphane **40**) in a Staudinger ligation. Thus, the probe is attached covalently to the surface glycoprotein, which makes the use of flow cytometry possible for quantitative measurements. In comparison experiments with their own cell-surface ketone–hydrazine reaction the Bertozzi research group could demonstrate that the cell-surface Staudinger reaction was superior

in several aspects: a) after cell metabolism to the sialic acid derivative, the Staudinger ligation led to a twofold increase in fluorescence relative to the ketone–hydrazine reaction; b) in contrast to a ketone, an azide has a unique reactivity in a cellular context as a result of its abiotic nature; c) the yield of the Staudinger ligation appears to be pH-independent over a wide range of pH values (5–8.5), whereas the hydrazine–ketone reaction is slow at pH 7.0 and has optimal reactivity at pH 6.5, an acidic environment that causes partial cell death. Control experiments revealed that neither azide reduction by endogenous monothiol (such as glutathione) nor the reduction of disulfides on the cell surface by the phosphane probe takes place. Thus, no side reactions that could obscure results occur.

By using known inhibitors of glycosylation the type of glycoprotein that hosts SiaNAz on the cell surface was identified. Incubation with tunicamycin (an inhibitor that blocks the *N*-linked glycosylation of proteins entirely) led to a steep decrease in cell-surface azide expression relative to that observed in control experiments, thus indicating that the metabolic product derived from Ac₄ManNAz (**49**) is resident within *N*-linked oligosaccharides. An incubation experiment with deoxymannojirimycin (a mannosidase I inhibitor that leads to truncated glycans which lack terminal residues) indicated that the cell-surface azides are present in the terminal sugars of *N*-linked glycans, such as sialic acid.

O-Linked glycosylation (at Ser or Thr residues) is the second major type of protein glycosylation. The predominant form of *O*-linked glycosylation is the mucin-type, which is characterized by an initial *N*-acetylgalactosamine (GalNAc) residue α -linked to the hydroxy groups of Thr or Ser side chains. Bertozzi and co-workers have reported a strategy for labeling such mucin-type *O*-linked glycoproteins with a

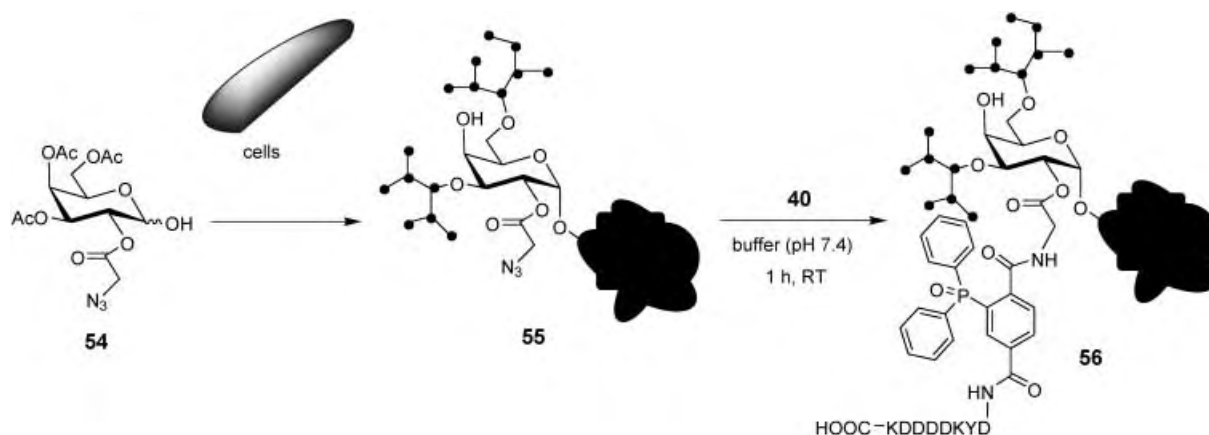


Scheme 12. Metabolic cell-surface engineering with azide-modified glycoproteins prepared biosynthetically.

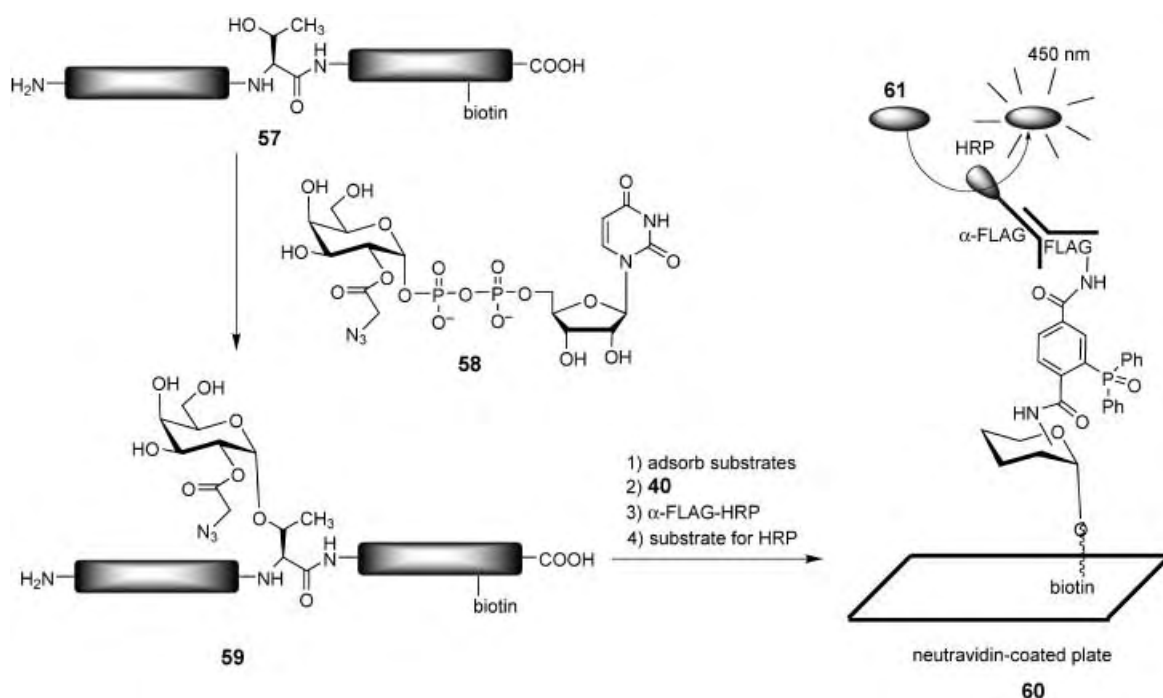
bioorthogonal azide tag. In this approach they exploit the one common feature of these glycoproteins: the conserved GalNAc residues.^[25] The strategy involves feeding CHO (Chinese hamster ovary) cells the peracetylated GalNAc analogue **54** (tetraacetyl-*N*-azidoacetyl-galactosamine, $\text{Ac}_4\text{-GalNAz}$), which is incorporated metabolically into mucin-type *O*-linked glycoproteins **55**, which then react with the probe **40** in a Staudinger ligation to produce labeled glycoproteins **56** (Scheme 13). This method is sensitive enough that glycoproteins expressed at endogenous levels in mammalian cells could be detected in complex cell lysates.

One challenge in glycobiology is the identification of the substrates and functions of the glycosyltransferases that build glycans within the secretory compartments. The polypeptide

N- α -acetyl-galactosaminyltransferases (ppGalNAcTs, a family with approximately 24 isoforms) play a critical role in the biosynthesis of mucin-type *O*-linked glycoproteins by attaching the initial GalNAc unit to Ser or Thr residues of the polypeptide scaffold. In this process UDP-*N*-acetyl-galactosamine (UDP-GalNAc; UDP = uridine diphosphate) serves as the glycosyl donor. Bertozzi and co-workers have described an “azido-ELISA” assay (ELISA = enzyme-linked immunosorbent assay) in which a biotinylated protein substrate **57** is glycosylated by ppGalNAcTs with UDP-GalNAz (**58**) as the glycosyl donor (Scheme 14).^[26] The reaction mixture is adsorbed onto a 96-well plate coated with neutravidin, to which the FLAG-phosphane **40** and an anti-FLAG antibody chimera with horse radish peroxidase (HRP) are added. The



Scheme 13. Metabolic approach to the labeling of mucin-type *O*-linked glycoproteins.



Scheme 14. "Azido-ELISA" allows profiling of the activity of glycosyltransferases.

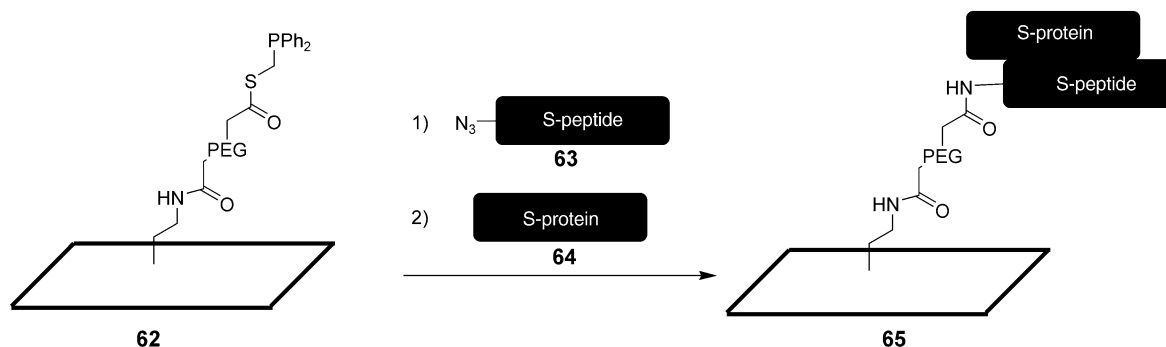
process is quantified based on measurements of the conversion of the HRP substrate **61** at 450 nm. This platform can be used to profile the substrate specificity of an entire enzyme family.

4.4. Preparation of Arrays

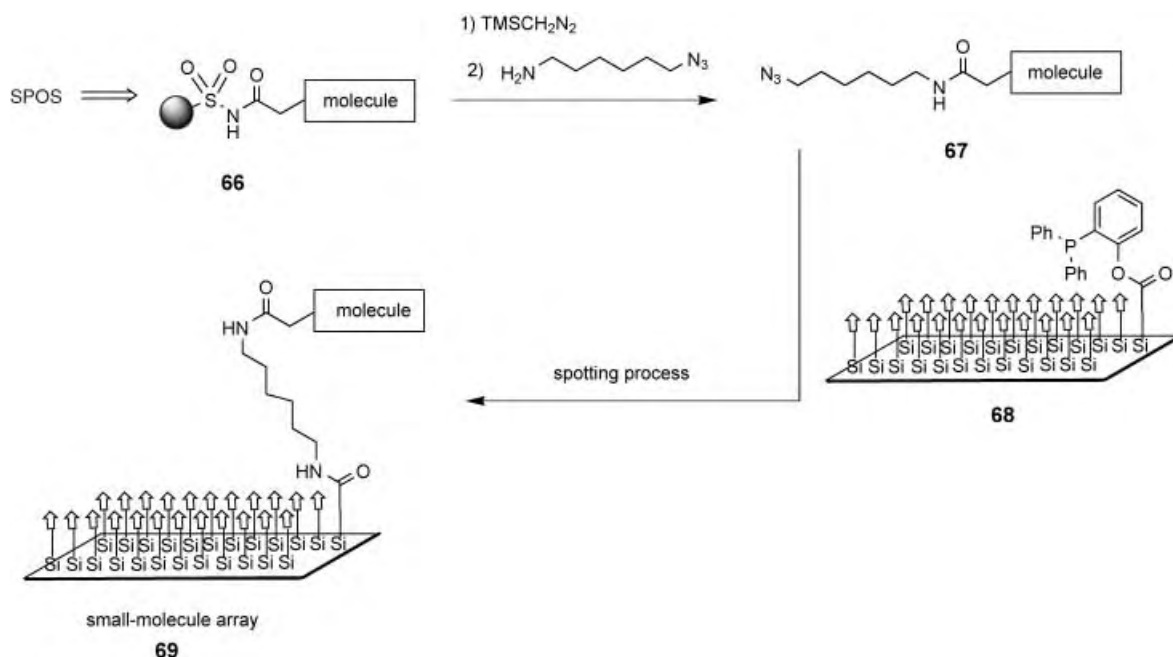
The unique chemoselectivity of the Staudinger ligation has stimulated its application as an immobilization technique for the preparation of protein and small-molecule arrays. Raines and co-workers modified an aminopropylsilane-functionalized glass slide with a bifunctional polyethyleneglycol (PEG) spacer and finally attached the phosphane **20**.^[27] The azide-functionalized N terminus of the S-peptide **63** (residues 1–15 of RNase A) is ligated covalently to the slide **62** derivatized with a phosphanyl thioester. Incubation with the S-protein **64** (residues 21–124 of RNase A) leads to the

formation of an adduct **65**, which can be assayed based on ribonucleolytic activity and immunostaining (Scheme 15). Thus, it was shown in a proof-of-concept experiment that the traceless Staudinger ligation can be exploited for the preparation of protein arrays.

In a collaborative effort, the research groups of Breinbauer, Niemeyer, and Waldmann used the phosphane **17** for the traceless immobilization of small molecules, which can be prepared by combinatorial solid-phase synthesis by using the Kenner safety-catch linker **66**.^[28] The azide functionalities are introduced upon cleavage from the solid phase. Staudinger ligation of the azide-modified compounds **67** with the phosphane-derivatized dendrimer coating on the slide **68** resulted in the covalent immobilization of the molecules in the sample. The small-molecule arrays **69** prepared in this way were screened for binding with fluorescence-labeled proteins (Scheme 16). The Staudinger ligation provides the advantage of chemoselectivity over many other ligation methods for the



Scheme 15. Site-specific immobilization of proteins through the Staudinger ligation.



Scheme 16. Preparation of a library of azide-terminated small molecules and their immobilization on phosphane-decorated glass slides for the preparation of small-molecule arrays. SPOS = solid-phase organic synthesis.

preparation of small-molecule arrays, thus tolerating a wider range of functional groups that might be necessary for efficient ligand–target binding.

5. Summary and Outlook

Although the Staudinger ligation is just three years old, many examples, as described above, give testimony to its high practical value in research in chemistry and biology. In particular, the phosphane reagent **10** introduced by Bertozzi and co-workers has proven its practicability not only in model experiments but also in actual biological studies. Although it can not be used in a traceless ligation, its stability against oxygen and superior functional-group tolerance makes it the reagent of choice for most applications. At present the Staudinger ligation is probably the mildest and most chemoselective ligation reaction. It should therefore be the first reaction considered for the preparation of bioconjugates or when chemistry has to be carried out in the complex environment of a living cell.

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Antithrombotics

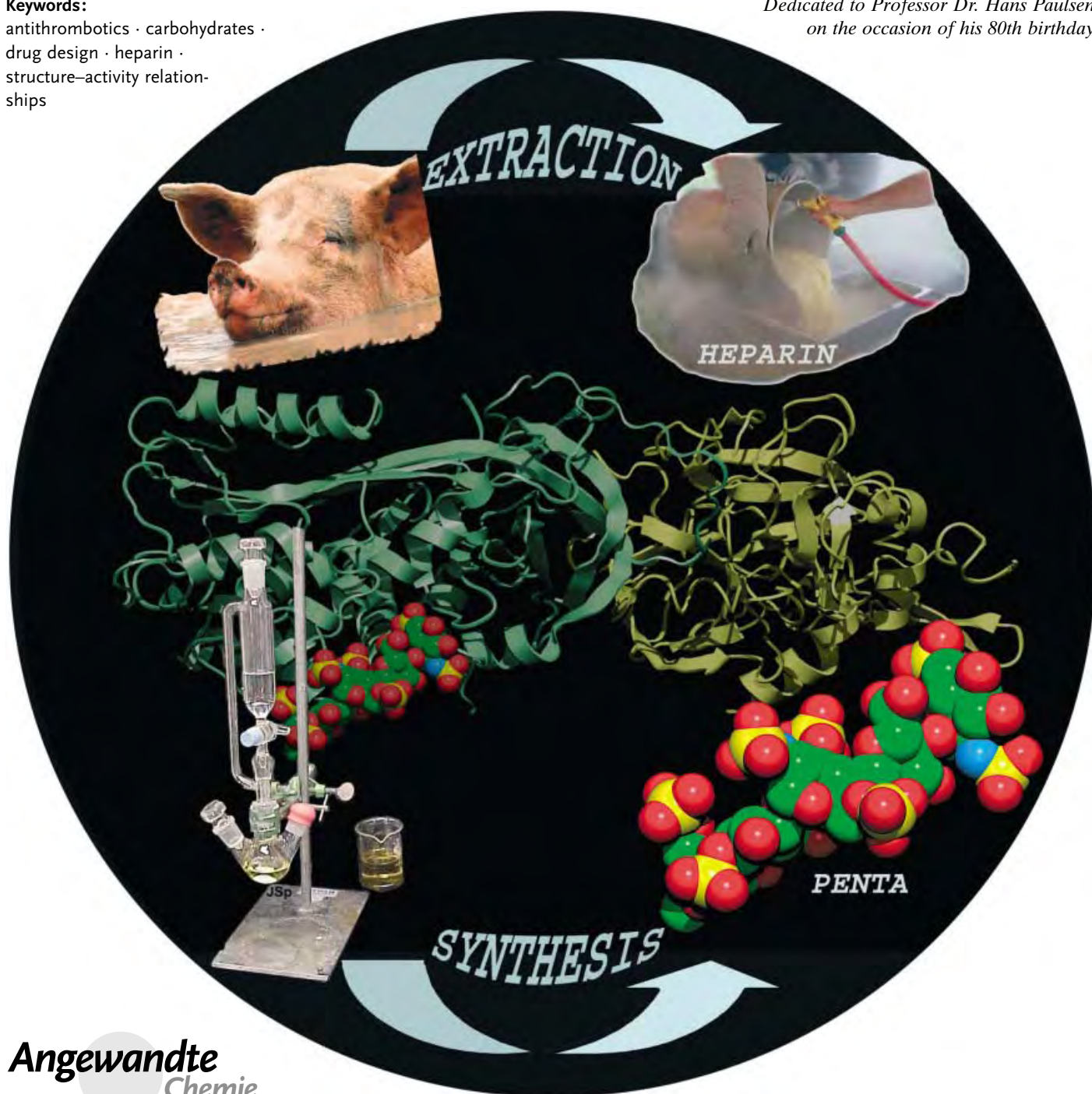
A Synthetic Antithrombin III Binding Pentasaccharide Is Now a Drug! What Comes Next?

Maurice Petitou* and Constant A. A. van Boeckel*

Keywords:

antithrombotics · carbohydrates ·
drug design · heparin ·
structure–activity relationships

*Dedicated to Professor Dr. Hans Paulsen
on the occasion of his 80th birthday*



Angewandte
Chemie

Heparin is a sulfated glycosaminoglycan isolated from animal organs that has been used clinically as an antithrombotic agent since the 1940s. In the early 1980s it was discovered that a unique pentasaccharide domain in some heparin chains activates antithrombin III (AT-III), a serine protease inhibitor that blocks thrombin and factor Xa in the coagulation cascade. Sanofi-Synthelabo and Organon developed a synthetic analogue of this pentasaccharide. The resulting anti-thrombotic drug arixtra, which went on the market in the USA and Europe in 2002, shows superior antithrombotic activity and brings about AT-III-mediated activity against factor Xa exclusively. Structure-based design has subsequently led to analogues with longer-lasting activity, such as idraparinux, as well as novel conjugates and long oligosaccharides with specific anti-Xa and antithrombin activities. The new drug candidates are more selective in their mode of action than heparin and less likely to induce thrombocytopenia.

1. Introduction

In 1993 we published a Review entitled “The Unique Antithrombin III Binding Domain of Heparin: A Lead to New Synthetic Antithrombotics”.^[1] In that article we outlined the requirement for endogenous antithrombin III (AT-III) if heparin is to accelerate the inhibition of two key proteases in the blood-coagulation cascade, factor Xa and thrombin. The inhibition requires the binding of a unique sequence-specific pentasaccharide domain of heparin to AT-III. The interaction of the pentasaccharide induces an activating conformational change in AT-III, which accelerates the inhibition of factor Xa but not of thrombin (Figure 1). Thrombin inhibition additionally requires AT-III and thrombin to bind to the same heparin chain in a ternary bridging complex. In the early 1980s, the efforts of several research groups^[2] led to the

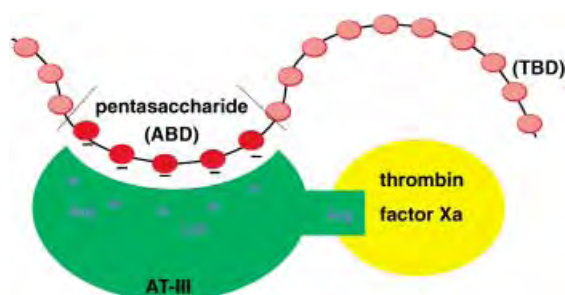


Figure 1. Heparin interacts with antithrombin III (AT-III) to bring about a conformational change in the serine protease inhibitor, thus allowing the loop with the reactive center to interact with coagulation enzymes, such as thrombin and factor Xa. To induce the conformational change in AT-III, a unique pentasaccharide domain—also called the antithrombin III binding domain (ABD)—should be present in the heparin chain. The pentasaccharide stimulates exclusively the AT-III-mediated inactivation of factor Xa (anti-Xa activity), whereas longer heparin fragments comprising both the pentasaccharide domain (ABD) and a thrombin-binding domain (TBD) are required for stimulating antithrombin activity.

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structural elucidation of the unique pentasaccharide domain through identification of the consensus sequence of hexa- and octasaccharides, which were isolated by heparin degradation and affinity chromatography on immobilized AT-III (Figure 2).^[3] A few years after the structural elucidation our research groups independently synthesized the first pentasaccharide analogue (**1** in Figure 3) of the active domain, which contains an *N*-sulfate group instead of an *N*-acetyl group in unit D.^[4,5] The synthetic pentasaccharide does indeed only promote the AT-III-mediated inhibition of factor Xa. Thus, a basis was established for a research and development program on a new class of carbohydrate-based antithrombotics: selective inhibitors of factor Xa.

At that time this new drug-discovery program was very controversial. For instance, more than 60 steps were required in the chemical synthesis of the first pentasaccharide, which was produced in extremely low yield and insufficient purity for use as a synthetic drug. As the pentasaccharide showed anti-Xa activity but no antithrombin activity, many people believed that it would be a poor antithrombotic. The half-life in humans was also questioned. Heparin (a high-molecular-weight polysaccharide) has a half-life of only 1 hour, and it was anticipated that the half-life of the small pentasaccharide would be even shorter because of faster renal clearance. Fortunately, a number of scientists were convinced not only that the pentasaccharide could be synthesized, but that through molecular modification and design, structurally

[*] Dr. M. Petitou
Département Cardiovasculaire/Thrombose
Sanofi-Synthelabo
195 route d'Espagne, 31036 Toulouse cedex (France)
Fax: (+33) 56-116-2286
E-mail: maurice.petitou@sanofi-synthelabo.com
Prof. Dr. C. A. A. van Boeckel
Medicinal Chemistry, N.V. Organon
P.O. Box 20, 5340 BH, Oss (The Netherlands)
Fax: (+31) 412-663-508
E-mail: stan.vanboeckel@organon.com

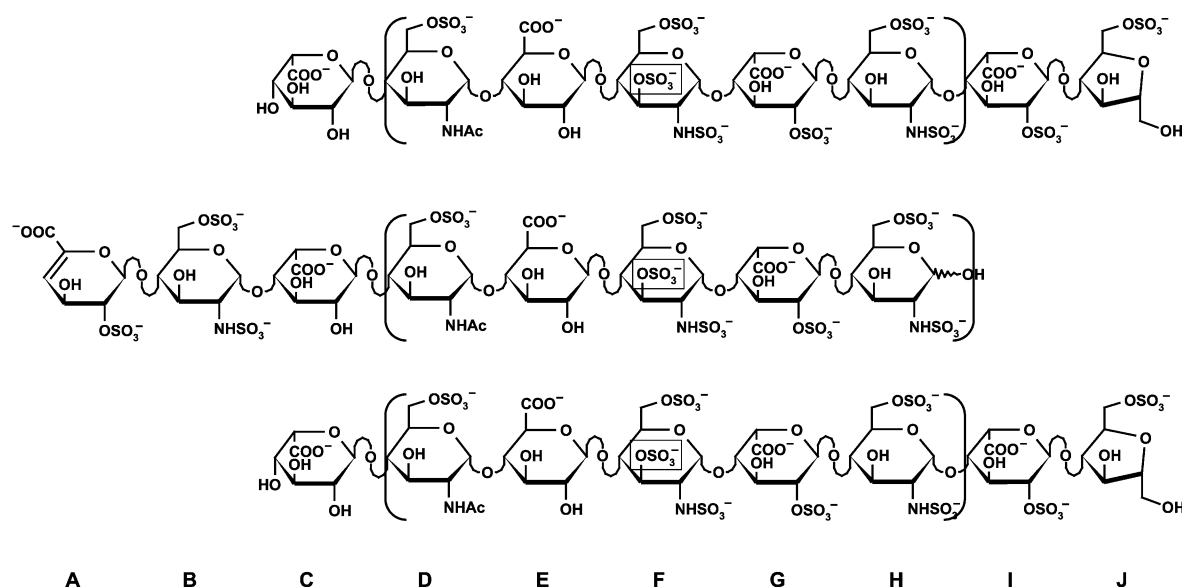


Figure 2. Typical fragments obtained through the degradation of heparin by heparinase or nitrous acid followed by affinity chromatography on immobilized AT-III. The three fragments contain a common pentasaccharide sequence “DEFGH” (within brackets) and a 3-O-sulfate group in the glucosamine unit F, which ensure high affinity for AT-III.

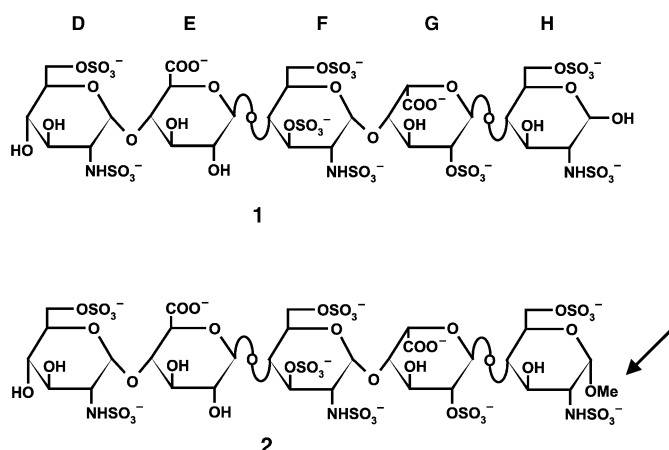


Figure 3. The first synthetic counterpart **1** of the AT-III-activating domain of heparin and its stabilized methyl glycoside **2**, the antithrombotic agent fondaparinux.

simplified analogues could be prepared, probably with tailor-made pharmacological properties. The chemists were quite optimistic, reasoning that the “active substance” of heparin itself, which only comprises a low percentage of the heparin carbohydrate content, might turn out to be a superior drug. In the early 1980s low-molecular-weight (LMW) heparins, obtained through heparin fragmentation, were also investigated in various clinical trials. Quite remarkably, the LMW heparins were found to be efficacious in the prevention of deep venous thrombosis and displayed a better therapeutic window than heparin, although they have a much higher anti-Xa/antithrombin ratio. A breakthrough for the project came in 1987 when the companies involved, Sanofi (now Sanofi-Synthelabo) and Organon, decided to continue their high-risk research and development activities in a joint venture. In that year the pentasaccharide **2** (Figure 3), which is closely related to the natural sequence and had been prepared by both companies (under the codes SR 90107 and Org 31540, respectively), was selected for further development.^[1,9] The analogue **2** with a methyl group at the anomeric center was



Constant A. A. van Boeckel was born in 1956 in Brunei (formerly British Borneo) and studied chemistry at the University of Leyden. He received a degree in chemistry in 1979 and his PhD in 1982 under the supervision of J. van Boom. He then went into the pharmaceutical industry (N.V. Organon, Oss). Currently he is head of Medicinal Chemistry at Organon, where his main area of interest is drug discovery. He is Adjunct Professor of Bioorganic Chemistry at the University of Leyden.



Maurice Petitou, born in 1949 in France, studied chemistry in Clermont-Ferrand and Orléans, where he received a doctorate in 1974. He worked for three years as a Fellow of the Ligue Nationale Française Contre le Cancer (LNFC), then from 1978 at the Institut Choay on the chemistry of heparin. He was appointed associate director of research of the Centre National de la Recherche Scientifique (CNRS) in 1988. Since 1989 he has been head of the Carbohydrate Chemistry Department, now part of the Cardiovascular Research Department of Sanofi-Synthelabo, Toulouse (France).

selected as a development candidate based on the observation that such derivatives are easier to synthesize and purify, because the reactive aldehyde group at the reducing end of the pentasaccharide is permanently protected.^[1] Indeed, in 1989 chemists provided more than 20 g of highly purified SR 90107/Org 31540 for toxicology studies and phase I clinical trials in healthy volunteers. In 2001, after a successful clinical development program in the 1990s, SR 90107/Org 31540 (**2**, fondaparinux) was registered in the USA and Europe as a new antithrombotic drug under the name arixtra.^[7] This drug is currently used for the prevention of venous thromboembolic events following knee- or hip-replacement surgery and after hip fractures. Further development is directed towards other prophylactic indications, as well as the treatment of deep venous thrombosis, pulmonary embolisms, and coronary artery diseases.

With regard to the pessimistic view of fifteen years ago we may say today that, even though it still comprises many steps, the multi-kg synthesis of the highly pure pentasaccharide **2** is performed successfully in industry. Its half-life in humans is approximately 17 h, which makes once-daily administration possible, instead of twice or three times daily as required for heparins. Moreover, in several clinical trials it was shown that the use of **2** in major orthopedic surgery led to a decrease in the risk of thrombosis of more than 50% relative to low-molecular-weight (LMW) heparin.^[7] In these studies 2.5 mg day⁻¹ of arixtra was applied, whereas the required dosage of the LMW heparin enoxaparin is 40 mg once daily or 30 mg twice daily.

In our previous Review we also described the synthesis of many analogues and detailed structure–activity relationships (see also Section 2).^[1] For example, a fully *O*-sulfated, *O*-methylated pentasaccharide (**3**, Figure 4) is not only much

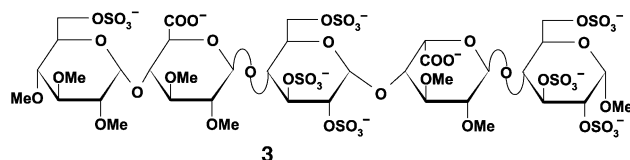


Figure 4. Idraparinux (**3**) is an analogue of the naturally occurring pentasaccharide, which because of its long half-life (that is, longer-lasting antithrombotic activity) needs only to be administered once a week. This compound is currently in the last phase of clinical development.

easier to synthesize than **2** but also displays higher activity and a longer duration of action (half-life in man approximately 120 h), thus allowing once-weekly administration.^[8,9] In a phase II dose-ranging study (PERSIST) it was shown that at a dose of 2.5 mg once weekly the pentasaccharide **3** (idraparinux) was at least as effective as existing treatments in the prevention of VTE (venous thromboembolic events). Idraparinux is now in late clinical development (phase III) for the prevention of thromboembolic events in patients with atrial fibrillation, and for the prevention and treatment of VTE (comparison with heparin plus vitamin K antagonists). Subsequently, our research groups shifted their attention towards pentasaccharide derivatives that also display antithrombin activity. Following a structure-based approach (see Section 3)

we designed pentasaccharide conjugates with tailor-made antithrombin activity (see Section 4). We also synthesized long heparin-like oligosaccharides (see Section 5) with superior antithrombin activity to that of heparin. Several of these compounds are in preclinical and early clinical development.

2. Structure–Activity Relationships of Synthetic Pentasaccharides

2.1. Structure–Activity Relationships of Heparin-Like Pentasaccharides

To study the role of the various negatively charged groups of the pentasaccharide many analogues were synthesized and tested for anti-Xa activity and binding to AT-III. The synthesis of the pentasaccharide **2** is summarized in Figure 5. An important feature of the synthesis is the

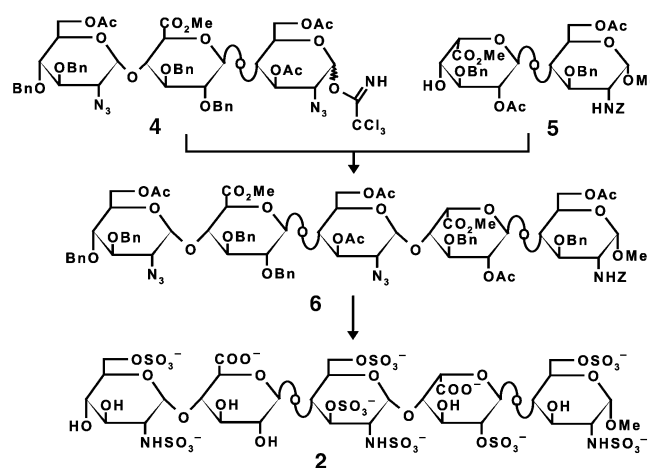


Figure 5. The antithrombotic agent fondaparinux (**2**) is synthesized through a block synthesis in about 55 steps from naturally occurring carbohydrates. The fully protected trisaccharide **4** is coupled with the disaccharide **5** to give the fully protected pentasaccharide **6**. In this approach, the acetyl (Ac) protecting groups mask the hydroxy groups to be sulfated, whereas benzyl ethers (Bn) block the “free” hydroxy groups, which are deprotected after hydrogenolysis.

application of an orthogonal-protection strategy^[1] in which hydroxy groups to be sulfated are esterified, whereas those corresponding to the free hydroxy groups in the product are masked as benzyl ethers. The fully protected pentasaccharide **6**, which is prepared through the coupling of the units **4** and **5**, is first saponified. The resulting free hydroxy groups are then *O*-sulfated. Subsequent hydrogenolysis of the benzyl and azide groups, followed by selective *N*-sulfation, delivers the desired pentasaccharide **2**.

Following this strategy it is relatively easy to prepare analogues lacking *O*-sulfate groups at defined positions,^[1] by exchanging an ester functionality for a benzyl ether at the desired position in the protected molecule. To investigate the role of the carboxylate group one can not rely on altering the protecting-group scheme, but one has to resort to new

carbohydrate building blocks. For example, to study the role of the carboxylate group of L-iduronic acid (unit G) an analogue was synthesized containing a D-xylopyranose moiety, which lacks the carboxylate group of iduronic acid. The role of the *N*-sulfate groups was established by testing analogues bearing a hydroxy or an *N*-acetyl group at the corresponding position.

In this context it is worth mentioning that the biological activity of a synthetic pentasaccharide analogue *N*-acetylated in unit D and thus representing the most widespread AT-III-binding domain of porcine heparin (see Figure 2)^[1] is only half as high as that of fondaparinux (**2**). In other words, arixtra displays stronger affinity towards AT-III and has a higher anti-Xa activity than the AT-III-binding pentasaccharide domain in standard heparin and the low-molecular-weight heparins. Finally, the role of the individual charged groups of the pentasaccharide **2** in the activation of AT-III was determined unambiguously as indicated schematically in Figure 6.

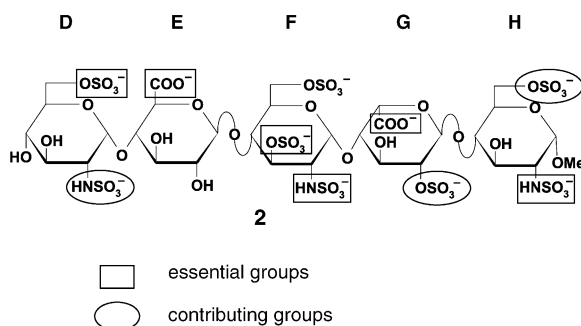


Figure 6. Structure–activity relationships of fondaparinux (**2**). Analogues were synthesized that lacked one particular sulfate or carboxylate group. In this way it was established that the groups highlighted in the boxes are absolutely essential for the activation of AT-III, whereas the groups in the circles only help to increase the biological activity.

The above-mentioned structure–activity relationships created a solid basis for the design of further analogues. Based on ¹H NMR spectroscopy and molecular modeling we postulated an interaction model with two AT-III–pentasaccharide binding sites.^[1] As shown in Figure 7, an extended AT-III binding region was determined to be complementary to the southern part of the pentasaccharide, whereas a second, smaller interaction domain was located near the north-eastern part. Interestingly, the results of this receptor mapping were confirmed 10 years later by X-ray crystal-structure analysis^[10] of AT-III cocrystallized with the analogue **8**^[8] (see Figure 8 for a schematic overview). The model described above inspired us to synthesize the analogue **7**, which contains an extra sulfate group at the 3-O position of unit H. This sulfate group was expected to display a favorable interaction with the proposed second binding site of AT-III (see Figure 7). Indeed, the pentasaccharide **7** binds more strongly to AT-III by one order of magnitude and exhibits enhanced anti-Xa activity.^[11] In fact the increased interaction with AT-III brought about by the extra sulfate group at 3-O of unit H is

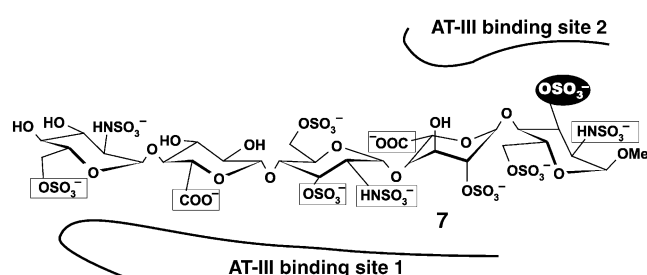


Figure 7. In the late 1980s, an interaction model was postulated in which two tentative AT-III binding sites were mapped around a model of the pentasaccharide **2**. This interaction model was obtained by reasoning that the essential negatively charged groups on the pentasaccharide (highlighted by the boxes) interact with complementary (positively charged) residues in the heparin-binding site of AT-III. This model inspired us to introduce an extra 3-O-sulfate group in unit H (in the black ellipse), which should enhance interaction with AT-III. Indeed, the binding of analogue **7** to AT-III was much stronger.

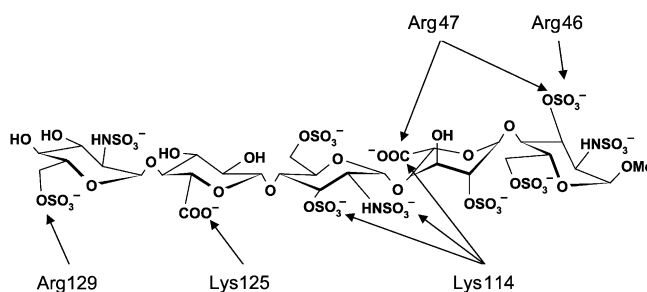


Figure 8. In the late 1990s the X-ray crystal-structure analysis of AT-III in a complex with a pentasaccharide confirmed the presence of two distinct binding sites with AT-III, as postulated earlier (see Figure 7). The extra 3-O-sulfate group at unit H interacts with Arg46 and Arg47, thus explaining the stronger binding of this class of analogues with AT-III.

unusual, as additional sulfate groups at other positions have a detrimental effect on the activity. The crystal structure mentioned above shows that the extra 3-O-sulfate group interacts with positively charged amino acids (Arg46 and Arg47) of AT-III (Figure 8). The type of charge also appears to be crucial for the biological activity,^[1] as the sulfate group can not be exchanged for a phosphate or a carboxylate group without affecting the biological activity.

2.2. Methylated and Other Structurally Simplified Analogues

As the synthesis of heparin analogues in AT-III binding is lengthy and cumbersome, we turned our attention to analogues that are easier to synthesize but still biologically active. We reasoned that an analogue bearing *O*-sulfate instead of *N*-sulfate groups and *O*-methyl ethers instead of hydroxy groups would be much easier to synthesize.^[1] Thus, as no orthogonal protecting groups are required and no amino or azido sugars have to be prepared, deprotection and sulfation procedures are shortened. A nice example of this strategy is the full modification as described above of the derivative **7** to give **8**,

which hardly affects the biological activity at all (Figure 9).^[8] Many derivatives, which we termed “non-glycosaminoglycan” analogues, were prepared in this series. The synthesis of derivatives with a “pseudo”-alternating sequence, such as the

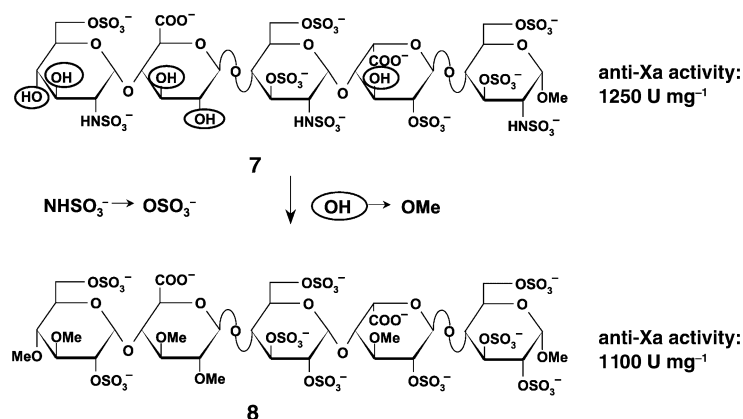


Figure 9. Simplified analogues were designed and synthesized by replacing *N*-sulfate groups with *O*-sulfate groups and protecting all hydroxy groups permanently as methyl ethers. These analogues (e.g. **8**) had similar activity to that of their natural counterparts.

pentasaccharide **3** (idaraparinix), proved to be particularly promising. As the two disaccharide fragments of **3** within brackets in Figure 10 have the same substitution pattern they

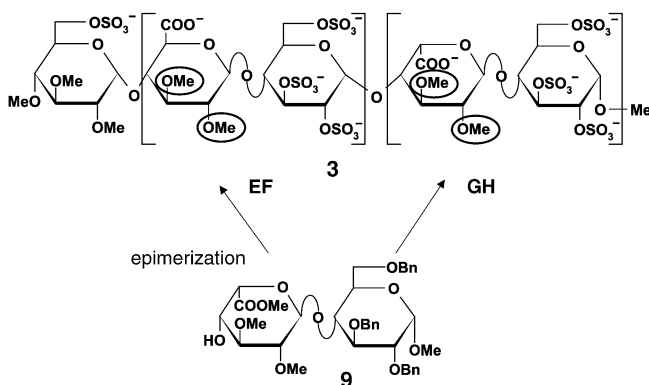


Figure 10. Simplified pentasaccharides with a “pseudo”-alternating sequence could be prepared from a single disaccharide building block (e.g. synthesis of **3** from **9**). The *L*-iduronic acid moiety of the GH building block **9** undergoes epimerization to *D*-glucuronic acid upon treatment with a base to deliver a suitable EF building block.

can be derived from a single disaccharide precursor **9**, which in turn is prepared from glucose.^[8,12] With this synthetic strategy the total synthesis comprises only approximately 25 steps.

As mentioned above, the pentasaccharide **3** ($K_D \approx 1$ nM) interacts more strongly with AT-III than the pentasaccharide **2** ($K_D = 50$ nM), and **3** also exhibits superior anti-Xa activity (1600 versus 700 U mg^{−1}).^[1,8,13] This increased activity can be partly attributed to the presence of the 3-*O*-sulfate group in unit H. However, this can not be the only explanation, as two

other important functionalities, the 2-*N*- and 2-*O*-sulfate groups in units D and G, respectively, are absent in **3**. Additional contributing factors to the observed higher activity are the presence of methyl ethers (which are thought to interact with complementary lipophilic groups at the protein surface) and the exclusive existence of iduronic acid in the favorable skew-boat conformation (see Section 2.3).

Some other modifications that we performed are depicted in Figure 11. For example, we replaced unit D, which contains an essential 6-*O*-sulfate group, with a flexible *O*-sulfoglycol-2-*O*-methyl entity,^[14] whereby 50% of the biological activity was conserved (step a). In our previous Review we showed that the replacement of *D*-glucuronic acid (unit E) or *L*-iduronic acid (unit G) with flexible “open” pyranose analogues (that is, 2-*O*-methylglyceric acid moieties) led to virtually complete loss (step b) or 85% loss (step c) of the biological activity, respectively.^[1] Another example of chemical modification was the exchange of the interglycosidic oxygen atom (between units D and E) for a carbon atom,^[15] which led to only a slight decrease in activity.

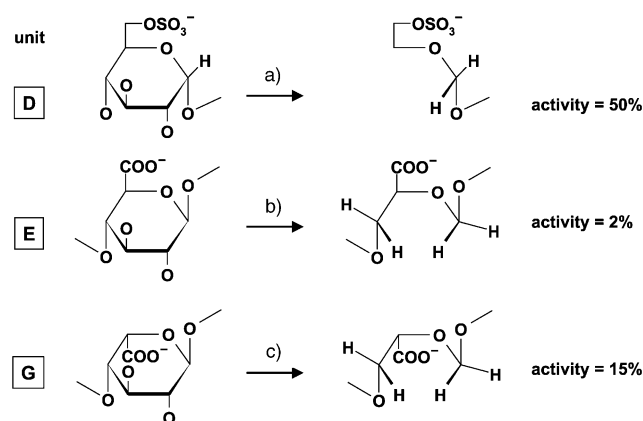


Figure 11. The influence of the rigidity of the carbohydrate units in the pentasaccharide was studied. It appears that if some carbohydrate units (e.g. units D and G) are replaced with more flexible mimetics significant anti-Xa activity remains (50 and 15%, respectively), but for others the rigidity is essential. For example, only 2% of the anti-Xa activity remains if the glucuronic acid unit E in the pentasaccharide is replaced with a flexible mimetic.

2.3. The Implications of Flexible *L*-Iduronic Acid

In our earlier Review we reported on the conformational analysis of the pentasaccharide and the peculiar conformational behavior of the iduronic acid moiety (unit G) in particular.^[1] It was observed that *L*-iduronic acid in heparin-like molecules can adopt three different conformations: ¹C₄, ⁴C₁, and ²S₀ (Figure 12). ¹H NMR spectroscopic analysis and molecular modeling showed that in the biologically active pentasaccharides the iduronic acid moieties exist in an equilibrium between the ¹C₄ and ²S₀ conformations in which

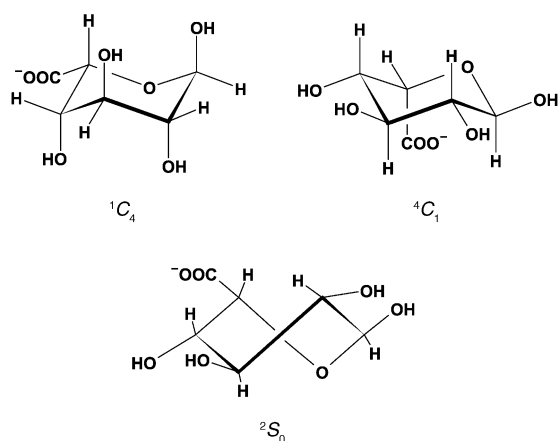


Figure 12. In theory, L-idopyranuronic acid may occur in three different conformations: $^1C_4$, $^4C_1$, and $^2S_0$, but in heparin-like molecules only the $^1C_4$ and $^2S_0$ conformations are observed.

the latter conformation prevails. In agreement with these findings it was later found that the biological activity decreases when L-iduronic acid in the pentasaccharide **2** is replaced by 3-deoxy-L-iduronic acid, the conformation of which is shifted towards $^1C_4$ as a result of the elimination of 1,3-diaxial interactions.^[16] The three-dimensional representation of the pentasaccharide **2** in Figure 13 shows L-iduronic

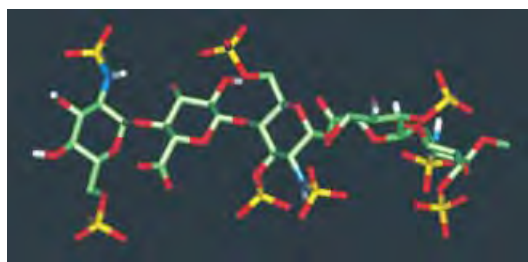


Figure 13. Three-dimensional structure of the pentasaccharide **2** (fondaparinux) with the L-iduronic acid moiety in the $^2S_0$ conformation, as observed in the crystal structure of the complex with antithrombin III.

acid in the preferred $^2S_0$ conformation. As the $^2S_0$ conformation is occupied exclusively in analogues such as **3**, it was tempting to conclude that this is the active conformation. However, under different conditions (e.g. if the pentasaccharide is bound to AT-III) the preferred conformation of L-iduronic acid may be different from that in solution. For example, experiments at high salt concentrations indicated a shift in the equilibrium from the $^2S_0$ towards the $^1C_4$ conformation. Nevertheless, considerable evidence suggests that the $^2S_0$ form is the active conformation of L-iduronic acid when the pentasaccharide is bound to AT-III. Thus, a pentasaccharide with a rigidified L-iduronic acid moiety fixed in the $^1C_4$ conformation (or $^4C_1$ conformation) was found to be biologically inactive,^[17] whereas an equivalent derivative with L-iduronic acid locked in the $^2S_0$ conformation had similar potency to that of the parent compound (see Figure 14).^[18,19] Convincing evidence was also provided by a high-resolution crystal structure of the AT-III complex with

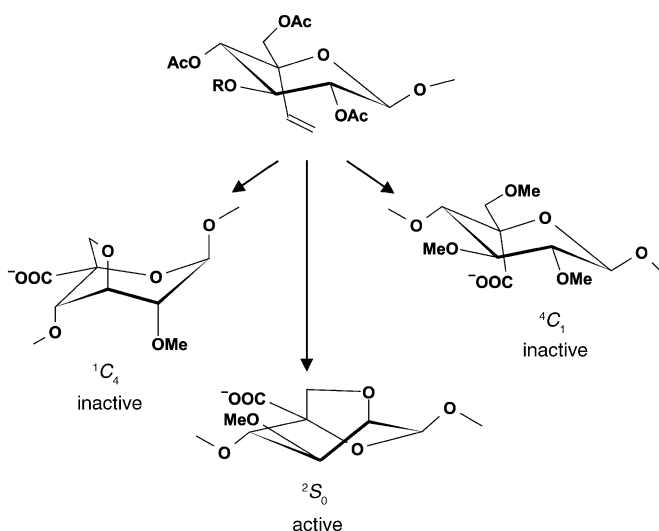


Figure 14. Synthetic analogues were prepared in which the L-iduronic acid moiety is fixed in the $^1C_4$, $^2S_0$, or $^4C_1$ conformation. Only the pentasaccharide with the L-iduronic acid equivalent fixed in the $^2S_0$ conformation is biologically active.

the potent pentasaccharide analogue **8**, for which the electron-density map corresponded to iduronic acid in the $^2S_0$ conformation.^[10]

3. Activation of AT-III towards Various Serine Proteases

Having gained experience in the synthesis of complex pentasaccharides we felt confident to attempt the total synthesis of more-complex molecules that can catalyze the AT-III-mediated inhibition of other serine proteases, particularly thrombin, which is considered by many pharmacologists as a key target for antithrombotic drugs. Before we describe our chemical approach we need to recall the structural rules that govern antithrombin activation towards various serine proteases.

3.1. Inhibition of Factor Xa

In Section 1 it was shown that the unique pentasaccharide domain of heparin induces a conformational transition in AT-III, to bring the loop with the reactive center into an active conformation capable of inhibiting coagulation proteases such as factor Xa (Figure 1). Based on the crystal structure of native AT-III (Figure 15a) and related serine protease inhibitors we proposed the following mechanism for AT-III activation:^[20] The interaction of the pentasaccharide with AT-III leads to an elongation of helix D with concomitant exposure of the loop with the reactive center and closure of the A sheet, as was confirmed when the crystal structure of the complex was elucidated (Figure 15b). The catalytic activity of the pentasaccharide can be explained by the reverse event: After complex formation with the protease

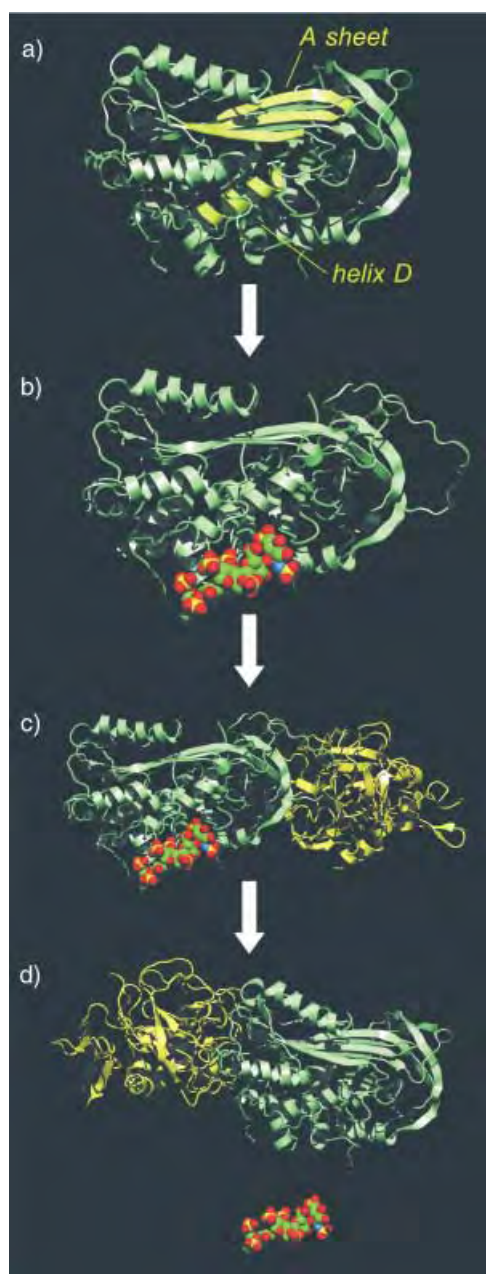


Figure 15. The crystal structures of AT-III (a), the AT-III/pentasaccharide complex (b), factor Xa (c; factor Xa in yellow), and a protease inhibitor/protease complex (d) provide precise structural information as to how the pentasaccharide catalyzes the AT-III-mediated inhibition of factor Xa (see text).

(Figure 15 c), the cleaved loop reinserts into the A sheet, and helix D unwinds to release the pentasaccharide (Figure 15 d).

As the trisaccharide DEF is composed of three monosaccharides barely represented in heparin, it was tempting to speculate that this trisaccharide is the part of the unique pentasaccharide sequence that is critically involved in the recognition of AT-III. We could confirm first by fluorescence spectrometry,^[21] then by fast-flow kinetic studies,^[13] that DEF is indeed able to trigger the conformational change described above (Figure 16). We therefore proposed the following mechanism for the activation of AT-III by the pentasaccha-

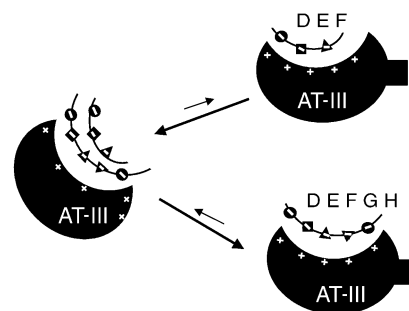


Figure 16. The DEF part of the pentasaccharide binds first to anti-thrombin III and induces the conformational change that creates the binding site for GH. The complex formed with DEF is far less stable than the complex formed with the pentasaccharide DEFGH.

ride DEFGH: The segment DEF binds first, inducing a conformational change in the protein, which is subsequently locked by the segment GH, whereby the L-iduronic acid (unit G) adopts the ${}^2S_0$ conformation.

3.2. Inhibition of Thrombin

In the case of thrombin, this conformational change is not sufficient to neutralize the enzyme. The unique pentasaccharide domain must be present, but heparin should also act as a negatively charged template to which both AT-III and thrombin bind to form a so-called ternary complex (for a schematic representation, see Figure 1).^[22] The most likely order of events is that AT-III first interacts with the unique pentasaccharide domain of heparin, then thrombin binds to a remote domain of heparin and becomes suitably oriented. The interaction between thrombin and the latter heparin domain is less specific in nature and weaker by about three orders of magnitude than the interaction between AT-III and the pentasaccharide. Investigations of isolated heparin fragments revealed that the heparin fragment should be approximately 18 saccharides long to show inhibitory activity against thrombin.^[23]

In 1995 we described a useful model of the ternary AT-III/heparin/thrombin complex based on the crystal structure of AT-III and thrombin.^[24] Structure–activity relationships of the pentasaccharide–AT-III interaction, as well as the crystal structure of AT-III and information on its heparin-binding amino acid residues, were used to construct a model of the AT-III/pentasaccharide complex. The asymmetric distribution of complementary interaction points suggested a unique orientation of the pentasaccharide, in which its nonreducing carbohydrate unit (unit D) points in the direction of the loop of AT-III with the reactive center. Five years later this orientation was confirmed by crystal-structure analysis, although the position of the pentasaccharide is shifted by a few Ångströms relative to the model. Subsequently, a model of the ternary complex was designed by using the crystal structure of thrombin (in which it is possible to recognize its heparin binding site), the above-mentioned AT-III/pentasaccharide complex, and a molecular model of heparin. The model of the ternary complex (Figure 17) clearly shows that

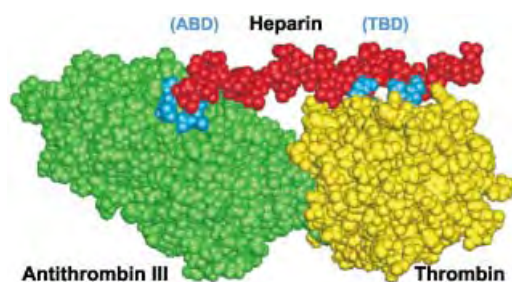


Figure 17. Molecular model of the ternary AT-III/heparin/thrombin complex, which clearly shows that heparin forms a bridge between the proteins. Six to eight sulfated monosaccharide units in the bridge region do not interact with the proteins.

the thrombin-binding domain of heparin should be located near the nonreducing terminus (unit D) of the pentasaccharide. Another important finding was that heparin forms a bridge between the two positively charged regions of the proteins, but that this bridge, which is approximately eight sugars in length, does not interact with positively charged protein residues. This model inspired us to design and synthesize novel tailor-made glycoconjugates that show AT-III-mediated inhibition of factor Xa and thrombin.^[24,25] Two approaches were used to this end: In the first we connected a pentasaccharide with an AT-III-binding domain (ABD) to a thrombin-binding domain (TBD) through a flexible non-carbohydrate spacer (see Section 4); in the second compounds composed entirely of oligosaccharides were synthesized (see Section 5), in an attempt to also definitively prove the mechanism of activation of AT-III by heparin.

4. Pentasaccharide Conjugates with Antithrombin Activity

4.1. Design and Synthesis of Conjugates with Tailor-Made Anti-Xa/Antithrombin Activity

The model of the ternary AT-III/heparin/thrombin complex (Figure 17) serves as the basis for the working hypothesis that is schematically depicted in Figure 18.^[24] Thus, synthetic molecules with mixed anti-Xa/antithrombin activity can be obtained by coupling a synthetic pentasaccharide—the AT-III-binding domain (ABD)—at its nonreducing end with a negatively charged thrombin-binding domain (TBD) through a molecular spacer. Furthermore, we hypothesized that the molecular spacer could be neutral,^[24] which would allow the preparation of heparin mimetics that were less negatively charged and thus more selective in their mode of action because they would undergo less nonspecific binding to other positively charged proteins. As it was proposed in the literature that the TBD of heparin interacts nonspecifically with thrombin, we anticipated that a simple sulfated oligosaccharide could be used as the TBD. Taking into account our model and the underlying phenomena, the conjugate **13** was synthesized (Figure 19b).^[25] The conjugate **13** comprises a non-glycosaminoglycan pentasaccharide as the ABD, a molecular spacer of about 50 atoms in length, and a

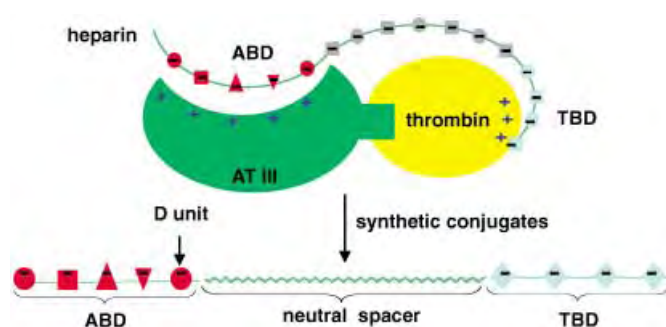


Figure 18. By using the model of the ternary complex in Figure 17 new synthetic conjugates were designed that should display AT-III-mediated antithrombin activity. Accordingly, the conjugates contain a specific pentasaccharide as the AT-III-binding domain (ABD), which is linked at its nonreducing end (unit D) to a neutral spacer of sufficient length, which in turn is connected to a negatively charged thrombin-binding domain (TBD).

persulfated maltotriose moiety as the TBD. The key synthetic steps are summarized in Figure 19a. Thus, following a slightly modified procedure to introduce a 13-azidotetraethylene glycol moiety at the reducing end of the pentasaccharide, the fully protected ABD **10** was synthesized by a similar route to that used for the related non-glycosaminoglycan pentasaccharides. After hydrogenolysis of the benzyl and azide groups, followed by saponification of the remaining acetyl esters, the resulting 13-aminotetraethylene glycol moiety was extended with a tetraethylene glycol spacer containing an acetyl-protected thiol. The ABD moiety **11** was then obtained by *O*-sulfation of the free hydroxy groups. The functionalized *O*-sulfated maltotriose **12** (the TBD), which bears a reactive amine group, was synthesized in several steps from maltotriose. Finally, the ABD and TBD units were linked in a one-step procedure by using the commercially available bifunctional linker sulfo-SIAB to furnish **13** in good yield after size-exclusion chromatography.^[25] The conjugate **13** did indeed display substantial antithrombin activity (140 U mg⁻¹; heparin: 160 U mg⁻¹). The modular composition of these conjugates comprising ABD, spacer, and TBD components allowed a straightforward approach in the design of new tailor-made antithrombotics. For example, we anticipated that alterations in the ABD would influence the anti-Xa activity and half-life in circulation (Figure 20). Changes in the spacer (length and rigidity) and in the charge density of the TBD were expected to affect the antithrombin activity. We were delighted to observe that the structure–activity relationships of these conjugates do indeed follow the anticipated profiles.

In one series of conjugates we conserved the ABD and the TBD, while varying the spacer length between the two units (Figure 21).^[25] As expected, there is a clear relationship between spacer length and antithrombin activity: if the spacer is too short, no ternary complex can be formed. Figure 22 shows a series of conjugates in which only the charge density of the TBD is varied.^[25–27] It is clear that antithrombin activity is proportional to negative charge density on the TBD. The type of negatively charged group on the TBD seems to be less relevant. Phosphate groups are more effective than sulfate

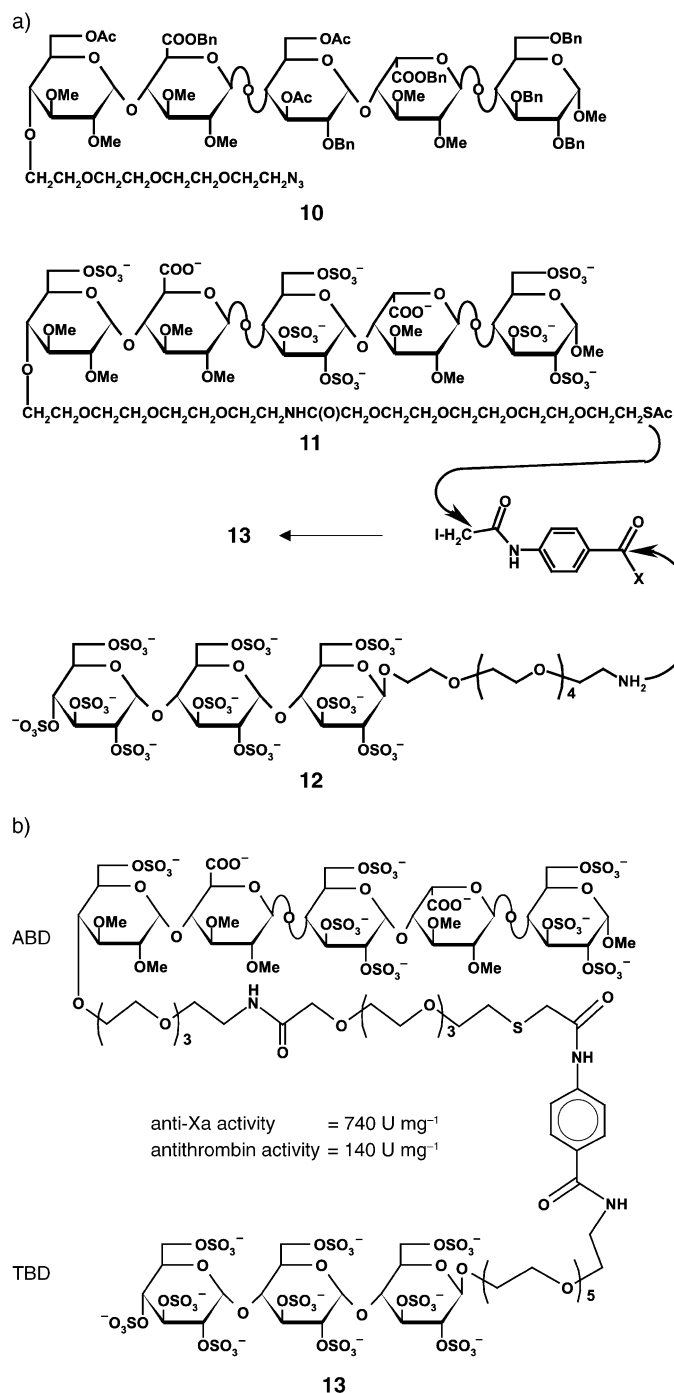


Figure 19. a) Conjugates designed for AT-III-mediated antithrombin activity could be prepared from pentasaccharides such as **10**, which contain a small molecular spacer with a reactive amine group attached to unit D. By coupling the amine group to a bifunctional linker, the length of the spacer can be increased as desired. The resulting intermediate **11**, which contains a masked thiol group, can be connected in turn to the thrombin-binding domain **12** (by using a bifunctional reagent) to deliver the conjugate **13**. b) Conjugate **13**, comprising an AT-III-binding domain (ABD) and a sulfated trisaccharide as the thrombin-binding domain (TBD) connected through a flexible spacer, shows substantial antithrombin activity.

groups, as their charge density is higher. This phenomenon demonstrates that the TBD–thrombin interaction is less specific in nature than the ABD–AT-III interaction. For

Structure – Activity Relationships of the Conjugates



Possibilities	Predicted consequences
change ABD:	effect on half-life and Xa inhibition
change TBD:	effect on thrombin inhibition
change spacer:	effect on thrombin inhibition

Figure 20. Following a modular approach the ABD, TBD, and spacer of the conjugates can be modified to create novel antithrombotics with tailor-made profiles.

CHANGE SPACER Role of the Spacer Length

TBD	Spacer length number of atoms	Antithrombin activity [U mg ⁻¹]
10 SO ₃ ⁻	18	1
10 SO ₃ ⁻	32	15
10 SO ₃ ⁻	46	20
10 SO ₃ ⁻	59	120

Figure 21. Structure–activity–relationships of the conjugates: the antithrombin activity increases substantially on elongation of the molecular spacer between the ABD and the TBD.

CHANGE TBD Role of the Charged Groups

TBD	Negatively charged groups	Antithrombin activity [U mg ⁻¹]
dermatan-like	4 SO ₃ ⁻	2
dermatan-like	5 SO ₃ ⁻	10
heparin-like	6 SO ₃ ⁻	10
cellobiose	7 SO ₃ ⁻	10
cellobiose	7 SO ₃ ²⁻	26
maltotriose	10 SO ₃ ⁻	65
maltotriose	10 SO ₃ ²⁻	167
maltopentaose	16 SO ₃ ⁻	330

Figure 22. Structure–activity–relationships of the conjugates: the antithrombin activity of conjugates is proportional to the charge density on the TBD.

example, sulfates can not be replaced by phosphates in the ABD (see Section 2.1). By adjusting the ABD part, a conjugate with unprecedented anticoagulant character could also be prepared. Thus, when a relatively low-affinity pentasaccharide (ABD: $K_D \approx 1000$ nM) was linked to a persulfated maltopentaose (TBD) through a long spacer of

56 atoms, the first heparin mimetic was produced for which the ratio anti-Xa/antithrombin activity was significantly lower (anti-Xa: 40 U mg⁻¹, antithrombin: 280 U mg⁻¹) than for heparin itself (anti-Xa: 160 U mg⁻¹, antithrombin: 160 U mg⁻¹).

The conjugates described have a flexible molecular spacer between their ABD and TBD. However, on formation of the ternary complex the adoption of the proper orientation by the flexible spacer should be accompanied by an unfavorable loss of entropy. Consequently, we anticipated that a rigid linker with the appropriate configuration would enhance the formation of the ternary complex and thus lead to increased antithrombin activity (Figure 23). As a neutral rigid spacer we used an octasaccharide made up of glucopyranose units with alternating α - and β -1,4-glycoside linkages,^[28] which closely resembles the backbone structure of heparin. This rigid conjugate is a hexadecasaccharide (**15** in Figure 23) and has superior antithrombin activity (1200 U mg⁻¹) to the conjugate **14** (40 U mg⁻¹) and to heparin (160 U mg⁻¹). The hexadecasaccharide belongs to a family of synthetic long heparin-like fragments that show superb antithrombin activity (see Section 5).^[28]

5. Synthetic Long Fragments

The conjugate concept offered the advantage of simplified chemistry compared to the synthesis of an oligosaccharide comprising about 20 units. Nevertheless, a further necessary step was the synthesis of such longer oligosaccharides, to be able to assess the extent to which the chemical structure, particularly that of the neutral spacer, influenced thrombin inhibition. This work also offered the opportunity to definitively answer several questions about the molecular structures involved in the inhibition of serine proteases by heparin-activated AT-III: 1) What is the minimum length of an

oligosaccharide for thrombin inhibition to be observed in the presence of AT-III? 2) What is the position of the TBD relative to the ABD? 3) What is the influence of the chemical structure on the inhibition of thrombin (and other coagulation factors)? 4) Is it possible to design heparin mimetics that are more potent than heparin but devoid of its side effects?

5.1. Thrombin Inhibitory Activity Requires At Least a 15-mer

Our first goal was to assess experimentally the minimum size of an oligosaccharide able to mimic heparin with respect to thrombin inhibition. In Section 3 we showed that the inhibition of thrombin requires the formation of a ternary heparin/AT-III/thrombin complex in which heparin plays the role of a template that binds both to AT-III through an ABD and to thrombin through a TBD. In most heparin molecules the ABD is at each end connected to a potential TBD (Figure 24, top), but because of the precise docking of AT-III on heparin (which results in an asymmetrically oriented complex) only the domain located close to the inhibitory loop of AT-III is actually useful for thrombin inhibition. As we had not taken into account whether the useful TBD was located at the reducing end or at the nonreducing end of the ABD, we reasoned that a molecule to which AT-III could bind at any hexasaccharide sequence (a continuum of ABDs) should be able to inhibit thrombin as well as factor Xa as soon as the size of the fragment is long enough to accommodate both AT-III and thrombin.^[29] In fact, an ABD may attract thrombin electrostatically and thus also serve as a TBD.

The hexasaccharide **16** (Figure 24), which closely resembles the high-affinity pentasaccharide **3**, was identified as a potential candidate for testing this hypothesis^[29,30] and synthesized from a single disaccharide **17**. Although the affinity of **16** for AT-III was much lower than that of **3** (0.35 μ M versus 0.0019 μ M), we felt it would be sufficient for

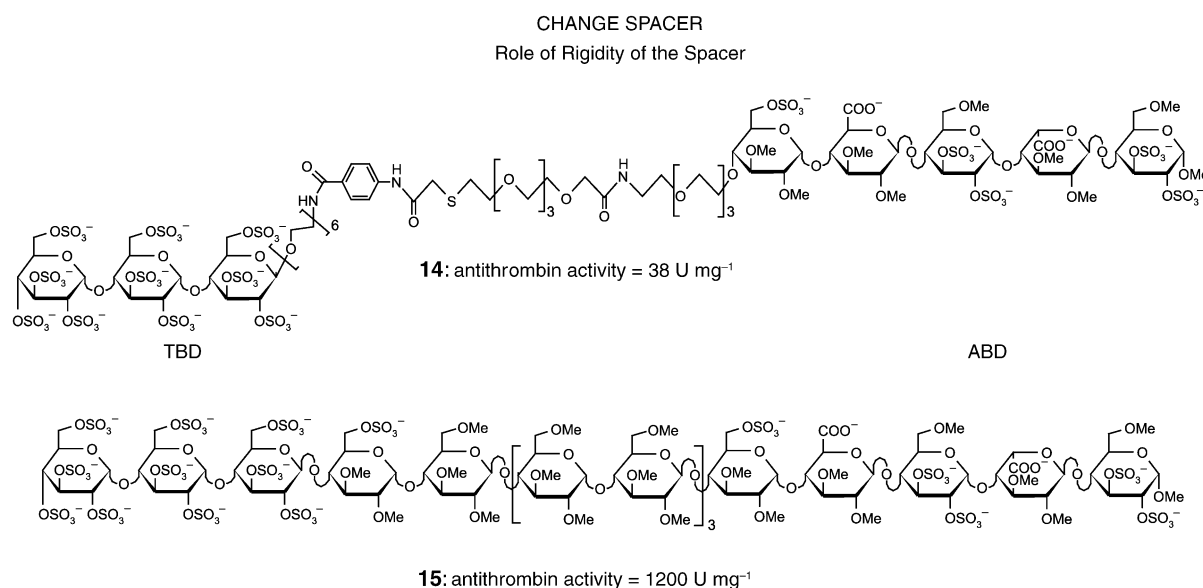


Figure 23. Structure–activity relationships of the conjugates: the antithrombin activity increases enormously if the flexible molecular spacer is replaced by a rigid carbohydrate spacer. Moreover, the antithrombin activity of the rigidified conjugate is superior to that of heparin.

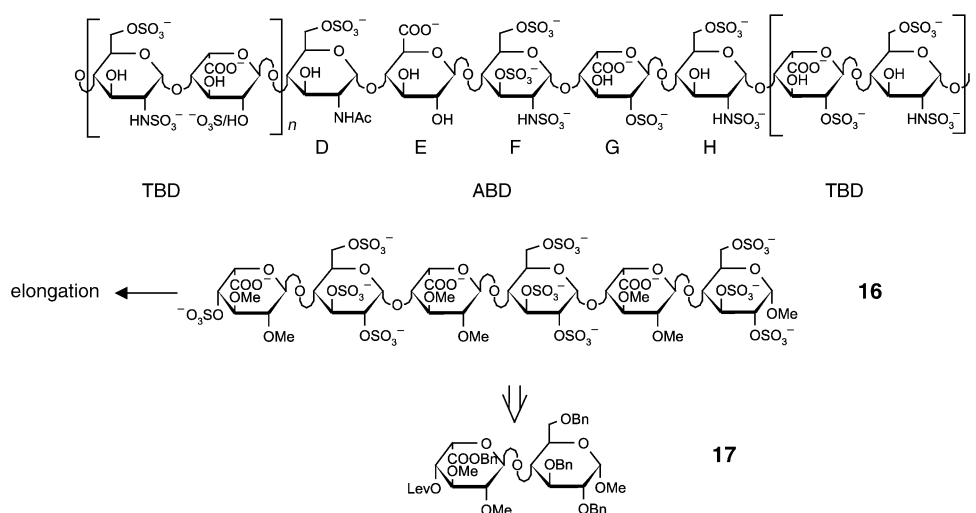


Figure 24. The hexasaccharide **16** binds to AT-III although it has an alternating sequence. Longer fragments containing the same repeating disaccharide unit have been used to study the minimum length required for antithrombin activity by heparin-like molecules (see Figure 25). For long fragments of this type it should be irrelevant whether the TBD is connected at the reducing end or the nonreducing end of the ABD (see upper part), because every hexasaccharide sequence of the oligomer is capable of activating AT-III. All fragments were synthesized from the suitably protected disaccharide building block **17**.

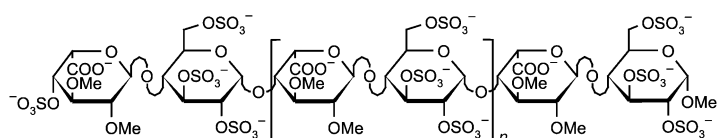
determining the minimum size of an oligosaccharide able to catalyze thrombin inhibition. Therefore, larger oligosaccharides were synthesized through the “polymerization” of the disaccharide **17**, which can be converted readily into an imideate (glycosyl donor) after acetolysis. As shown in Figure 25, all the oligosaccharides obtained catalyzed the inhibition of factor Xa in the presence of AT-III ($1.5\text{--}2\text{ U mmol}^{-1}$), whereas only the 16-mer and larger compounds displayed thrombin-inhibitory activity. The increasing activity with increasing chain length was reflected in the IC_{50} values, which decreased from $130\text{ }\mu\text{g mL}^{-1}$ for the 16-mer to $6.7\text{ }\mu\text{g mL}^{-1}$ for the 20-mer.^[29–31]

5.2. Relative Location of the Thrombin-Binding and AT-III-Binding Domains

The molecular model of the ternary heparin/AT-III/thrombin complex suggested that the productive TBD is located at the nonreducing end of the ABD (Figure 17). The corresponding synthetic conjugates with an ABD connected through a flexible spacer to a TBD were shown to inhibit thrombin (see Section 4). We wished, however, to obtain experimental evidence on the relative position of these two domains by comparing the inhibitory properties of heparin mimetics with similar charge densities and charge distributions to those of heparin, and with a specific ABD and TBD in the two possible arrangements.

The 17-mer **18** and the 18-mer **19** (Figure 26) in the non-glycosaminoglycan series (see Section 2.2) were selected as targets,^[32] for relative ease of preparation. We selected the high-affinity pentasaccharide **3** as the ABD and, to further simplify the synthesis, replaced 2-*O*-sulfonato- α -L-iduronic acid in the TBD with 2,6-di-*O*-sulfonato- β -D-glucose. This simplification was based on the consideration that the binding of thrombin to heparin mainly results from the electrostatic attraction between the anion-binding exosite II of the protein and the anionic polysaccharide. In this way we hoped to conserve the charge density (number of charges per saccharide unit) of heparin. Molecular modeling showed that in spite of the difference in the stereochemistry of the D-glucose monomer to that of L-ido, the mimetics are very similar to heparin in terms of their global shape and the localization of negative charges. This phenomenon appears to be virtually independent of the conformation of the L-idose ring (${}^1C_4$ or ${}^2S_0$).

Our strategy for the synthesis of the 17-mer **18** and the 18-mer **19** relied on the preparation of the fully



Oligosaccharide	Size	Anti-Xa activity [U mg ⁻¹]	Antithrombin activity IC ₅₀ [ng mL ⁻¹]
heparin	—	170	3
<i>n</i> = 3	10-mer	325	>10 000
<i>n</i> = 4	12-mer	405	>10 000
<i>n</i> = 5	14-mer	360	>10 000
<i>n</i> = 6	16-mer	310	130
<i>n</i> = 7	18-mer	360	23
<i>n</i> = 8	20-mer	290	6.7

Figure 25. Long heparin-like fragments with an alternating sequence, in which any hexasaccharide moiety is capable of activating AT-III. All fragments display anti-Xa activity, but AT-III-mediated antithrombin activity is only observed if the polysaccharide contains at least six monosaccharide units. (IC₅₀ is the concentration at which thrombin activity is diminished to 50%).

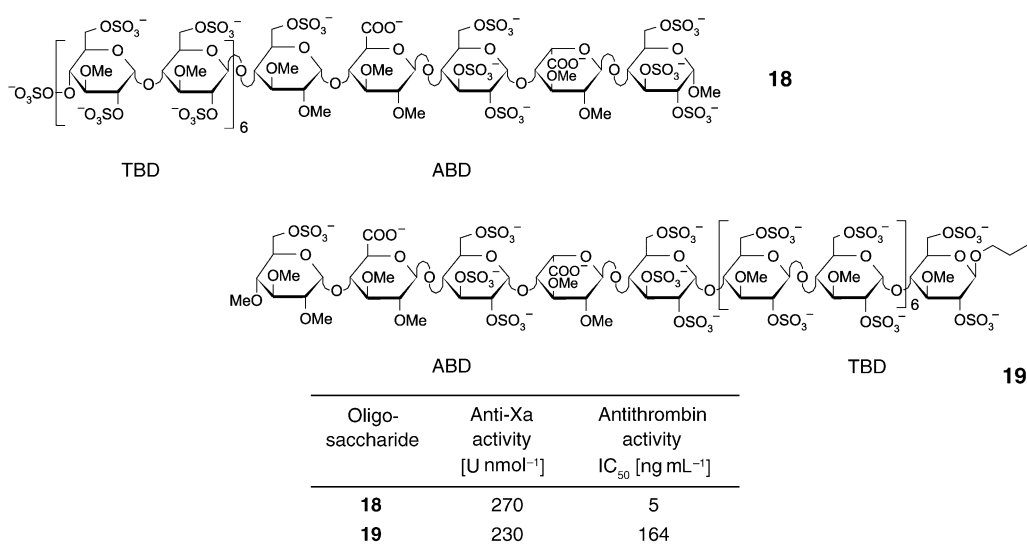


Figure 26. Fragments **18** and **19** were synthesized to show unambiguously that the TBD in heparin should be connected to the nonreducing end of the ABD (pentasaccharide), as had been indicated by earlier modeling studies and results obtained with the conjugates.

protected precursors **20** and **21**, in which benzyl ethers and acetyl esters protect the positions to be sulfated, and the carboxylate groups of glucuronic and iduronic acids (units E and G) are masked as benzyl esters (Figure 27). After deprotection and sulfation the final compounds were obtained in three high-yielding steps. In this way tedious and costly purification steps at this late stage of the synthesis were avoided. Investigations into the biological properties of these compounds clearly showed that, whereas both catalyze the inhibition of factor Xa, only **18** can catalyze thrombin inhibition.^[32] This may be viewed as convincing evidence that the TBD must be located at the nonreducing end of the ABD in a heparin molecule for it to inhibit thrombin.

5.3. Influence of Size and Charge on Serine Protease Inhibition

Having established that AT-III-mediated thrombin inhibition requires a charged molecule that is at least as long as a pentadecasaccharide and has an ABD located at the reducing end, we synthesized several oligosaccharides to assess the influence of the size and charge of the TBD on the potency of thrombin inhibition.^[33] In the oligosaccharide **22** the TBD is two monosaccharides shorter than in **18**, whereas in **23** it is two monosaccharides longer. As expected, the size of a molecule affects its ability to inhibit thrombin, but not its anti-Xa activity (Figure 28). In the oligosaccharides **24**, **25**, and **26**, the TBD is made up of α -linked glucose units, each with a

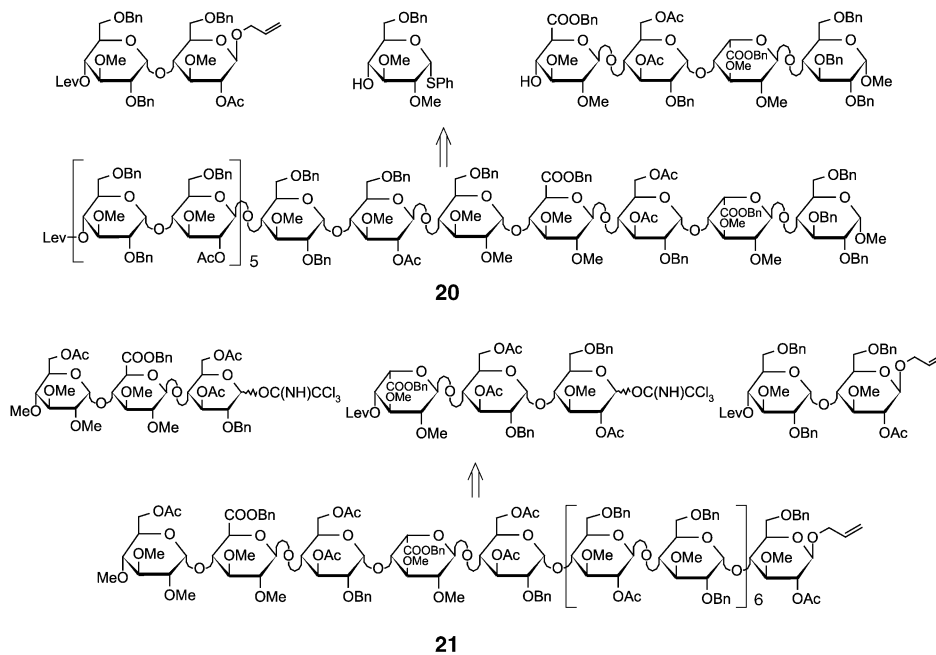
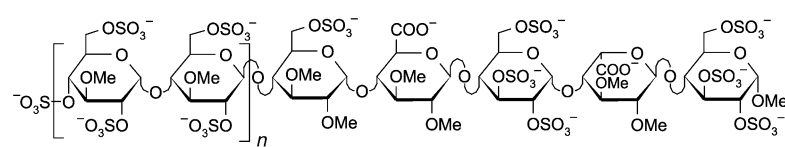


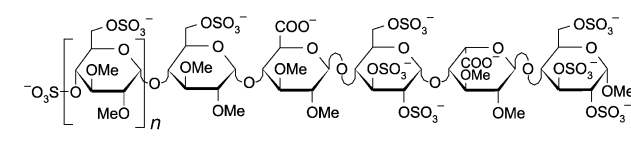
Figure 27. The synthesis of the long heparin-like fragments **18** and **19** required the preparation of the fully protected counterparts **20** and **21**, respectively. Each fragment could be prepared from three building blocks, as is outlined retrosynthetically.



Oligo-saccharide	<i>n</i>	Anti-Xa activity [U nmol ⁻¹]	Antithrombin activity IC ₅₀ [ng mL ⁻¹]
22	5	370	41
18	6	270	5
23	7	290	2

Figure 28. For long heparin-like molecules the thrombin-inhibitory activity is—as expected—related to the size of the molecule, whereas the anti-Xa activity remains similar.

single sulfate group at position 6 (Figure 29). The thrombin-inhibitory activity of these compounds was lower than that of the more heavily charged compounds **18**, **22**, and **23** (Figure 28).



Oligo-saccharide	<i>n</i>	Antithrombin activity IC ₅₀ [ng mL ⁻¹]
24	11	490
25	13	360
26	15	88

Figure 29. Heparin-like molecules in which the TBD consists of α -linked 6-O-sulfated glucose moieties also show antithrombin activity, but to a lesser extent than long fragments with a more heavily charged TBD.

Thus, by using these series of synthetic compounds we could confirm experimentally the well-known rules governing the biological properties of heparin, namely, that a strong correlation exists between thrombin inhibition and molecular size and charge. The same structural parameters also govern the interaction of heparin (and, more generally, of polyanions) with positively charged biological components. The question then arose as to whether chemical synthesis could be exploited for the modification of active, heparin-like structures to make them more favorable for drug development.

5.4. Influence of the Spacer between the ABD and the TBD on Thrombin Inhibition: Synthesis of Long Fragments with Improved Biological Profiles

During the clinical development of the pentasaccharide **2** the long half-life of this compound (17 h in young healthy subjects) came as a welcome surprise, because the reference compounds in the field at that time, heparin and LMW heparin, both had relatively short half-lives in humans. The reason for this difference between fondaparinux and the heparins is that the long, charged molecules that constitute any heparin preparation interact with blood and blood-vessel components, and are therefore rapidly eliminated, whereas the small pentasaccharide is entirely bound to AT-III and therefore protected from elimination. Besides the problem of half-life, related to the interaction with the cells that line the vessel wall, the interaction of heparin with the protein PF4, which is released by platelets, plays a critical role in the development of the most feared side effect of heparin therapy: heparin-induced thrombocytopenia (HIT). The interaction of heparin with PF4 leads to an immunological reaction caused by the immunogenic PF4-heparin complex.^[34] The studies performed so far have shown that the pentasaccharide does not interact with PF4 and does not trigger HIT. It is known also that LMW heparins are less prone to interact with PF4 than standard heparin. We wondered if we could modify the structure of the thrombin-inhibitory oligosaccharides described above so that they behave like the pentasaccharide **2** (which does not interact with PF4) rather than like heparin and LMW heparin. This could be an insurmountable challenge, if one considers that for the formation of a complex with PF4 the optimal size of an oligosaccharide is a 16-mer.^[35] However, two key observations led us to believe there might be a solution: 1) a heparin oligosaccharide must be at least the size of a 8-mer to bind significantly to PF4;^[36] 2) in the ternary heparin/AT-III/thrombin complex the central units are not involved in the interaction with the proteins (see Figure 17). Thus, an oligosaccharide in which a charged ABD and TBD are separated by a neutral domain might be able to catalyze thrombin inhibition while avoiding undesired interactions, particularly with PF4. To this end we prepared a series of compounds, such as **27** and **28**, in which various TBDs and an ABD are separated by a neutral fully methylated domain (Figure 30).^[28,37] We found that the size of the thrombin-binding site can even be as small as a mono- or disaccharide fragment, and again we confirmed that the interaction with thrombin also depends on charge density. The experience gained during the synthesis of the conjugates was very useful in the design of the TBD. The various domains of the structure can be modified to influence the pharmacodynamic and pharmacokinetic profile of the compounds specifically. After structure–activity-relationship studies it appeared that the 16-mer **28** (SanOrg 123781) is suitable for pharmaceutical development.^[38]

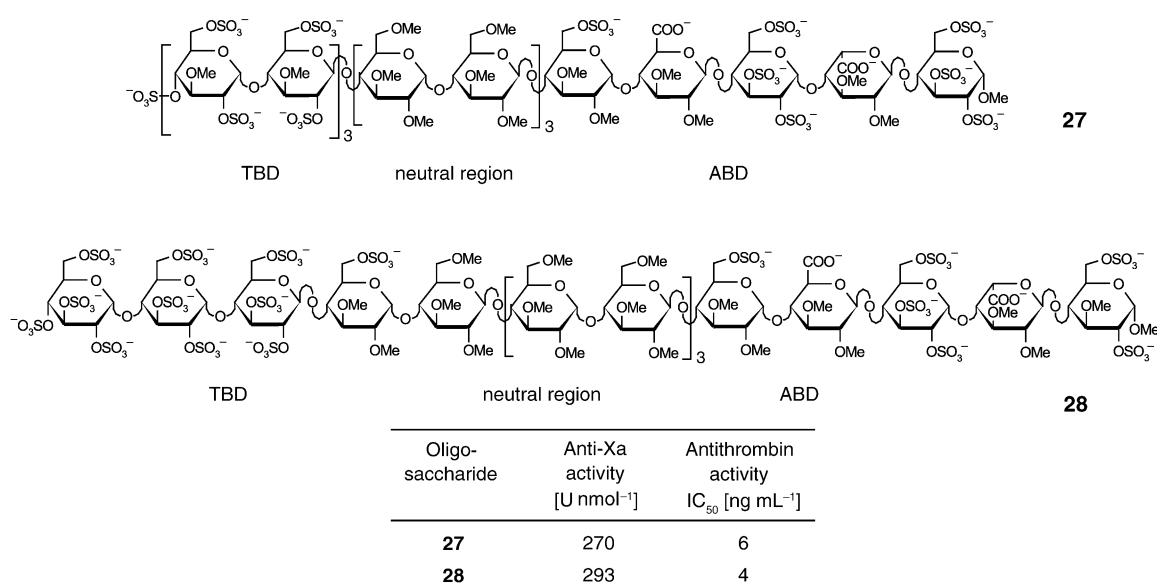


Figure 30. Long heparin-like fragments such as **27** and **28** (see also **15** in Figure 23) contain neutral spacer moieties, so that these molecules have a lower charge density than heparin. The low charge density in these designer molecules is very important for avoiding interaction with the protein PF4 released from platelets, which in a complex with heparin can be immunogenic and cause heparin-induced thrombocytopenia. In fact the relatively low charged molecules **27** and **28** show no interaction with PF4, but, because of their design, display superior antithrombin activity to that of heparin.

6. Conclusions and Prospects

In the early 1980s heparin, the main drug in the field of venous thrombosis, was submitted to chemical or enzymatic fragmentation, and the resulting LMW heparins became the market leaders in antithrombotics, with current world sales of about two billion dollars. At the same time the AT-III-binding site of heparin was identified, and, following this breakthrough, programs in carbohydrate chemistry were initiated with the ambitious objective of obtaining synthetic drug substances with the excellent antithrombotic properties of the complex polysaccharides of animal origin. After years of risky research and development programs, oligosaccharide synthesis entered the field of medicinal chemistry when the first drug issued from these programs proved its efficiency in patients. It must be underlined that the risky program included not only the multistep synthesis on a laboratory scale of the most complex oligosaccharides prepared to date, but also the scale-up of such syntheses.

Fondaparinux, the first synthetic antithrombotic pentasaccharide, is now available throughout the world as arixtra. Other substances with different and promising pharmacological profiles are under clinical investigation. Among the most important characteristics of all these new agents are their molecular purity and their precisely defined mechanism of action. A shortage of the raw materials of animal origin is a threat to the market for heparin antithrombotics. However, innovation in chemistry allows us to synthesize from glucose, the most abundant organic chemical on earth, powerful antithrombotic agents that can replace the extracted polysaccharides.

The chemical methods developed during the last 20 years, first by us and more recently also by others, allow the synthesis of all oligosaccharides of the heparin/heparan sulfate family.^[39] Considering the emerging physiological importance of this family of natural polysaccharides, it is highly probable that based on this chemistry other drugs, which are useful in the treatment of other diseases, will be developed. Furthermore, we have demonstrated that through organic synthesis and rational design heparin-like compounds can be prepared, whereby not only the AT-III-mediated anti-Xa and antithrombin activity can be tuned precisely but also the half-life in circulation, and nonspecific interactions with other basic proteins can be diminished.

The research and development activities summarized in this Review were performed by various teams at Sanofi-Synthelabo and Organon spanning a period of about fifteen years. We are very grateful to all the individuals in these teams, who were very supportive and enthusiastic and who made the project a great success.

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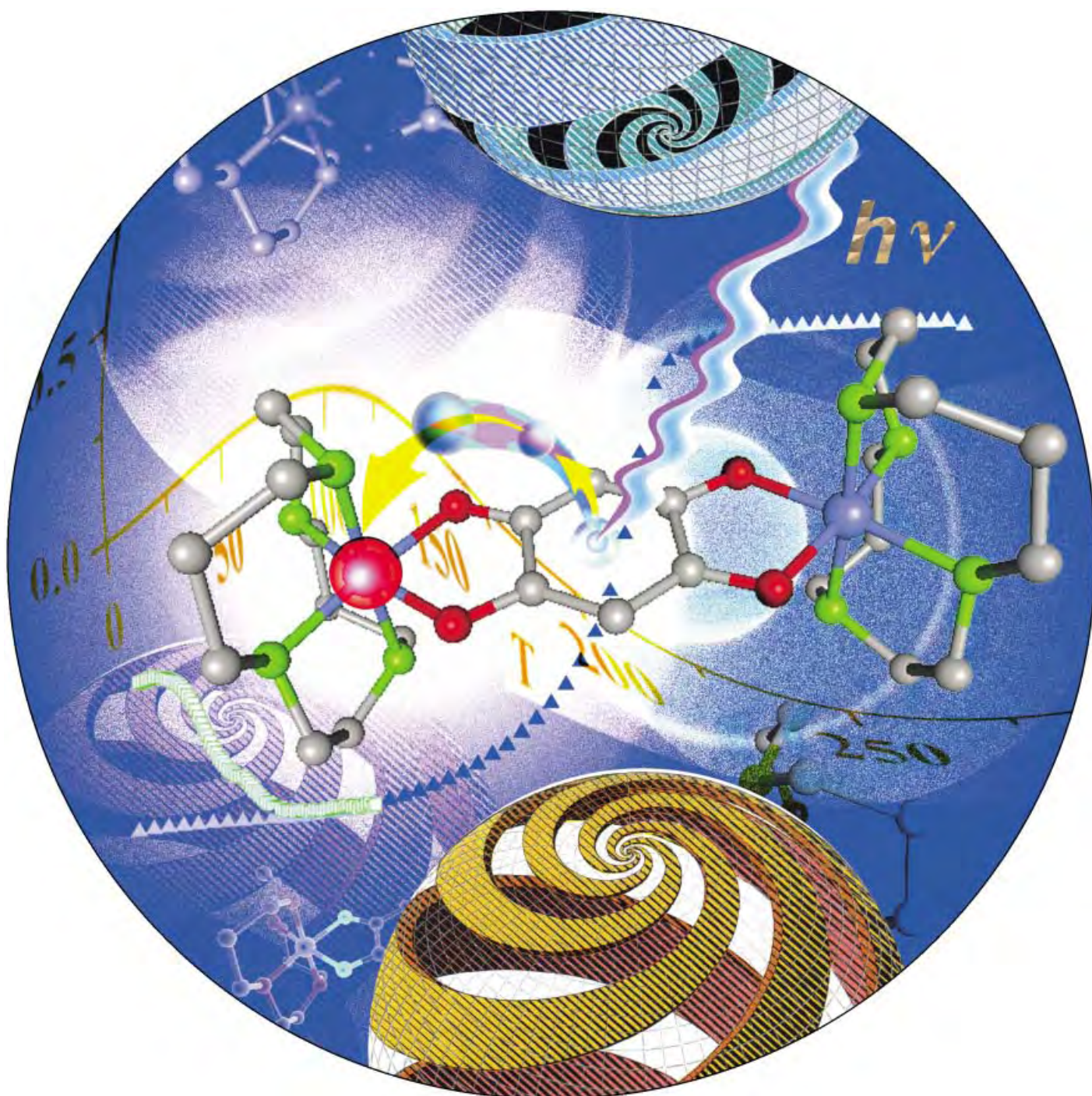
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Communications



A photoswitchable system that undergoes entropy-driven valence tautomerism has been obtained by successfully tuning the electronic properties of a dinuclear cobalt-polyoxolene complex by molecular techniques. For more information see the Communication by A. Dei, J.-F. Létard, and co-workers on the following pages.

Electron Transfer

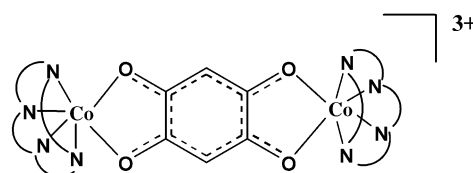
Thermally and Light-Induced Valence Tautomeric Transition in a Dinuclear Cobalt–Tetraoxolene Complex**

Chiara Carbonera, Andrea Dei,* Jean-François Létard,* Claudio Sangregorio, and Lorenzo Sorace

The design of optically switchable molecules is one of the main challenges in modern materials chemistry because such compounds have potential applications in the development of new electronic devices. Particular attention has been devoted to molecules exhibiting electronic bistability, that is, the property to exist in two different electronic states depending on the external parameters.^[1] Among the different classes of bistable molecules investigated to date, there is no doubt that those showing electronic states with different magnetic properties are the most attractive. These include spin-cross-over metal complexes and cobalt–dioxolene complexes that undergo valence tautomerism.^[2,3] Photoinduced spin-state interconversion in spin-cross-over iron(II) complexes has been described.^[4] Transient interconversions were also observed at room temperature for valence tautomeric complexes,^[5] but recently it was found that a low-spin cobalt(III) complex of the general formula [Is-Co^{III}(N–N)(cat)(sq)] (N–N is a diimine ligand, cat and sq represent the catecholato and semiquinonato forms of 3,5-di-*tert*-butylcatechol) undergoes transition to the high-spin isomer [hs-Co^{II}(N–N)(sq)₂] after illumination at low temperature.^[6,7] We have recently observed that the same phenomenon occurs for the 1:1 cobalt–macrocyclic dioxolene adduct [Co^{III}(cth)(phencat)](PF₆)·H₂O (cth = (*dl*)-5,7,7,12,14,14-hexamethyl-1,4,8,11-tetraazacyclotetradecane, phencat = catecholato form of 9,10-phenanthrenequinone).^[8] However, although several transition-metal complexes exhibit spin crossover or valence tautomerism, only the aforementioned metal complexes afford photoswitchable derivatives. It seems reasonable to believe that this is related to the large changes in the acceptor–donor bond lengths (0.15–0.20 Å) associated with the transitions. This is partic-

ularly disappointing because the number of molecular systems of potential interest is strongly limited.

With the aim of preparing a new family of cobalt–dioxolene systems that undergo valence tautomerism, we have synthesized [[Co(cth)]₂(dhbq)](PF₆)₂ (**1**, dhbq = deprotonated 2,5-dihydroxy-1,4-benzoquinone). This complex—as the previously reported Mn, Cr, Fe, and Ni analogues^[9]—is postulated to contain two six-coordinate high-spin cobalt(II) ions bridged by the dinegative tetraoxolene ligand. One-electron oxidation of this compound with a stoichiometric amount of AgPF₆ in acetone affords [[Co(cth)]₂(dhbq)](PF₆)₃ (**2**, Scheme 1). This complex can be reasonably formulated as



Scheme 1. Schematic view of the cationic part of complex **2**.

containing 1) two cobalt(II) ions bridged by the mononegative tetraoxolene ligand if a ligand-centered oxidation takes place, 2) a mixed-valence cobalt(III)–cobalt(II) couple bridged by the dinegative tetraoxolene if a metal-centered oxidation occurs, or 3) two cobalt(III) ions bridged by the trinegative radical tetraoxolene. In the last case, the oxidation involves both the metal ions and reduction of the ligand occurs as well, as found for the iron analogue.^[9]

The electronic (see below) and infrared spectra (see the Experimental Section) of **2** at room temperature strongly support the formulation of the compound as a mixed-valence Co^{II}–Co^{III} derivative (hypothesis 2). The temperature-dependent magnetic susceptibility measurements additionally show the existence of a transition between two different species (Figure 1). The $\chi_M T$ product of **2** at low temperature is close to 0.45 cm³ K mol^{−1}, in agreement with a species with a doublet ground state, while the limiting value reached in the

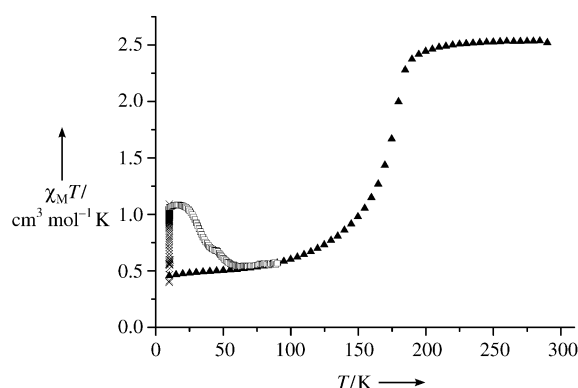


Figure 1. Plot of the $\chi_M T$ product as a function of temperature T for complex **2** measured before (▲) and after (□) irradiation at 647 nm. The crosses (x) indicate the increase of the $\chi_M T$ product with time upon irradiation of the sample. The bump at 45 K on the irradiation curve is a result of a small amount of oxygen.

[*] Prof. A. Dei, Dr. C. Sangregorio, Dr. L. Sorace
INSTM Research Unit and LAMM Dipartimento di Chimica
Università di Firenze
Via della Lastruccia 3
50019 Sesto Fiorentino, Firenze (Italy)
Fax: (+39) 055-4573-372
E-mail: andrea.dei@unifi.it
Dr. C. Carbonera, Prof. Dr. J.-F. Létard
Groupe des Sciences Moléculaires
Institut de Chimie de la Matière Condensée de Bordeaux
UPR CNRS No 9048, Université Bordeaux 1
87 Av. du Doc. A. Schweitzer, 33608 Pessac (France)
Fax: (+33) 540002649
E-mail: letard@icmcb-bordeaux1.fr

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high-temperature region ($2.53 \text{ cm}^3 \text{ K mol}^{-1}$) is consistent with the presence of a $S = 3/2$ paramagnetic species.

Therefore, both hypotheses, a spin-crossover transition involving the cobalt(II) ion and a valence tautomeric interconversion involving the $[\text{ls-Co}^{\text{III}}-(\text{dhbq}^{3-})-\text{ls-Co}^{\text{III}}]$ and the $[\text{hs-Co}^{\text{II}}-(\text{dhbq}^{2-})-\text{ls-Co}^{\text{III}}]$ species, are valid. However, the EPR spectrum recorded at 4 K is unambiguous. The strong signal at $g = 2.00$ (Figure 2) proves the existence of a radical

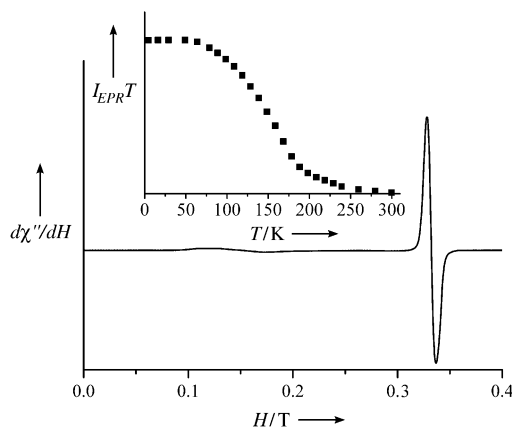


Figure 2. The X-band (9.24 GHz) EPR spectrum of **2** recorded at 4 K. Inset: Plot of the temperature dependence of the product of the intensity of the radical signal, calculated as the area under the curve, with temperature ($I_{\text{EPR}}T$).

ligand and rules out a $[\text{ls-Co}^{\text{II}}-(\text{dhbq}^{2-})-\text{ls-Co}^{\text{III}}] \rightleftharpoons [\text{hs-Co}^{\text{II}}-(\text{dhbq}^{2-})-\text{ls-Co}^{\text{III}}]$ spin-crossover equilibrium. A weak signal at $g = 4.3$ indicates the presence of a small amount (less than 10 %) of cobalt(II) ions,^[10] which can account for the deviation of the $\chi_{\text{M}}T$ product from the expected value of $0.375 \text{ cm}^3 \text{ K mol}^{-1}$. The progressive disappearance of the radical species above 100 K is evidenced by the investigation of the temperature dependence of the signal at $g = 2.00$ (inset of Figure 2). Indeed if the amount of radical were unchanged at different temperatures, the value of $I_{\text{EPR}}T$, which is proportional to the $\chi_{\text{M}}T$ value of the radical moiety, should remain constant. The temperature-induced transition of **2** can be then reasonably attributed to a valence tautomeric interconversion involving an intramolecular one-electron transfer between the tetraoxolene ligand and the low-spin cobalt(III) acceptor with a spontaneous change in spin state from $\text{ls-Co}^{\text{III}}$ ($S = 0$) to hs-Co^{II} ($S = 3/2$) and the concomitant transformation of the ligand from a paramagnetic ($S = 1/2$) to a diamagnetic ($S = 0$) species. This transition, which occurs around 175 K (Figure 1), can be classified as gradual, in analogy with the definition currently used for spin-crossover complexes;^[2] no thermal hysteresis was observed.

As a further step we analyzed the effect of light on the magnetic properties of **2**. At 10 K, the sample was initially in the $[\text{ls-Co}^{\text{III}}-(\text{dhbq}^{3-})-\text{ls-Co}^{\text{III}}]$ state. Upon irradiation with light (647.1–676.4 nm),^[11] the magnetic moment increased immediately, and the limiting value of $\chi_{\text{M}}T$ measured after a long period of irradiation was almost $1.2 \text{ cm}^3 \text{ K mol}^{-1}$. The light was then switched off and the temperature was increased at 0.3 K min^{-1} . The $\chi_{\text{M}}T$ value decreased smoothly and around

60 K the signal recovered the initial value obtained before irradiation. This result shows that the light-induced phenomenon is perfectly reversible. Experiments to increase the level of photoexcitation by increasing the irradiation time or power (up to 30 mW cm^{-2}) or changing the wavelength (488.0–514.5 nm, 752.5–799.3 nm) were unsuccessful.

The highest photoinduced rate of population corresponds to 43 % if we consider that the $[\text{ls-Co}^{\text{III}}-(\text{dhbq}^{3-})-\text{ls-Co}^{\text{III}}] \rightarrow [\text{hs-Co}^{\text{II}}-(\text{dhbq}^{2-})-\text{ls-Co}^{\text{III}}]$ process occurs. There are many different explanations for the magnitude of the $\chi_{\text{M}}T$ product under irradiation with light. One of them concerns the strong opacity of the sample (dark purple), which might prevent the penetration of light in the bulk phase; this has been reported for some iron(II) spin-crossover complexes.^[7] In this context, the properties of **2** were investigated with a home-built set-up that allows collection of the reflectivity spectra between 400 and 850 nm at a given temperature as well as monitoring of the temperature dependence of the signal at a selected wavelength ($\pm 5 \text{ nm}$). Figure 3 displays two selected absorp-

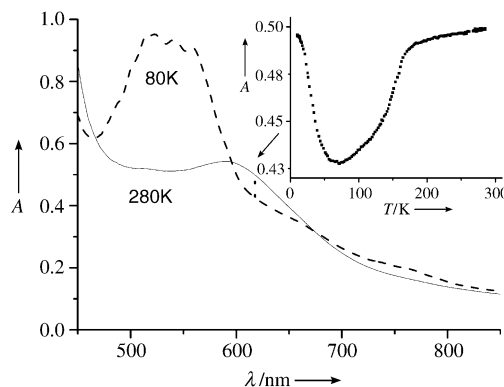


Figure 3. Diffusive absorption spectra of **2** recorded at room temperature and at 80 K. Inset: Plot of the reflectivity at $617 \pm 5 \text{ nm}$ as a function of temperature (see text for details). The absorbance A is given in arbitrary units.

tion spectra collected at room temperature and at 80 K. This confirms there is a clear modification of the absorption spectra accompanying the thermally induced valence tautomeric interconversion. The inset of Figure 3 shows the change of the reflectivity signal as a function of temperature at $617 \pm 5 \text{ nm}$. The gradual thermally induced valence tautomeric process is clearly observed and the shape of the curve perfectly follows the magnetic data shown in Figure 1. Most remarkably, an almost quantitative photoinduced interconversion occurs at low temperature at the surface. The magnitude of the reflectivity signal recorded at 10 K is in fact close to that recorded at room temperature.

In conclusion, we have prepared a new dinuclear $[\{\text{Co}(\text{cth})\}_2(\text{dhbq})](\text{PF}_6)_3$ complex that undergoes a tautomeric transition. A gradual thermal transition occurs at around 175 K and quantitative photoconversion was observed at the surface by using a reflectivity technique. In the bulk sample, the level of photoexcitation, as a result of the strong opacity of the sample, is estimated at around 40 %. Further

work is now in progress to fully characterize the relaxation kinetics as well as the thermodynamic parameters.

In addition to the novelty of the system undergoing valence-tautomeric transition, these results offer an interesting perspective. Indeed, the role of the tripositive metal ion in the above-discussed compound is magnetically ancillary, since its function is that of tuning the redox potential of the bridging ligand. However, the same effect can be, in principle, obtained by substituting the cobalt(II) metal ion with another tripositive paramagnetic metal ion, thus offering the possibility of obtaining valence-tautomeric complexes with different electronic ground states.

Experimental Section

1: A solution of $[\text{Co}(\text{cth})\text{Cl}_2]$ (2 mmol) in methanol (40 mL) was mixed with an alkaline solution of 2,5-di-hydroxy-1,4-benzoquinone (1 mmol) in methanol (30 mL) under argon. The resulting solution was heated at reflux and then mixed with an aqueous solution of KPF_6 . Brown crystals of **1** precipitated.

2: A solution of **1** (0.5 mmol) in acetone (50 mL) was mixed with a solution of AgNO_3 (0.5 mmol) in acetone/water (1/1, 30 mL). The resulting suspension was stirred at room temperature for 0.5 h, the silver metal was removed by filtration, and an aqueous solution of KPF_6 was added. Brown-violet microcrystals of **2** unsuitable for X-ray data collection were obtained. Elemental analysis calcd (%) for $\text{C}_{38}\text{Co}_2\text{H}_{74}\text{N}_8\text{O}_4\text{P}_3\text{F}_{18}$: C 36.23, H 5.92, N 8.89; found: C 35.84, H 5.99, N 8.65.

The IR spectra of **1** and **2** were very similar to those of the previously reported Mn and Ni analogues.^[9] The magnetic susceptibility measurements were carried out on a solid sample with a MPMS-55 Quantum Design SQUID magnetometer operating at $H = 2$ T in the 2–300 K temperature range. Irradiation was achieved with a Krypton laser connected through an optical fiber to the SQUID magnetometer. The power on the sample was adjusted to 5 mW cm^{-2} . Bulk attenuation of light intensity was limited as much as possible by preparation of a thin layer of the compound. The weight of the thin layer was determined by comparing the thermal valence tautomeric transition curve with the curve recorded for a heavier sample of the same compound.^[11] The reflectivity spectra were recorded on a solid sample with a home-made set-up with a CVI spectrometer. The EPR spectra of polycrystalline powder samples were recorded on a Bruker E5000 spectrometer equipped with an ^4He continuous flow cryostat for varying the temperature.

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Enzyme Screening

Profiling Primary Protease Specificity by Peptide Synthesis on a Solid Support**

Ron H. P. Doeze, Beatrice A. Maltman, Claire L. Egan, Rein V. Ulijn, and Sabine L. Flitsch*

An estimated 2–3 % of the mammalian proteome consists of proteases: enzymes that hydrolyze proteins and peptides.^[1] Many of these enzymes are involved in essential physiological functions such as immunological defense and cell differentiation. Several proteases are involved in disease states, such as HIV, Alzheimer's disease, hepatitis C, Candida infections, and pancreatitis.^[2] In addition, proteases have found applications as (industrial) biocatalysts in synthetic organic chemistry as a result of their ability to selectively hydrolyze (or synthesize) peptide bonds with high specificity

[*] R. H. P. Doeze, Dr. B. A. Maltman, C. L. Egan, Prof. S. L. Flitsch
School of Chemistry
The University of Edinburgh
King's Buildings, West Mains Road, Edinburgh, EH9 3JJ (UK)
Fax: (+44) 131-650-4737
E-mail: slf01@staffmail.ed.ac.uk

Dr. R. V. Ulijn
Manchester Materials Science Centre
University of Manchester/UMIST
Grosvenor Street, Manchester, M1 7HS (UK)

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

under mild conditions.^[3] Given the large number of proteases and their importance in both synthetic chemistry and biomedical science, high-throughput screening methods for the measurement of protease specificity are required.

Key to the characterization of this class of enzymes is the definition of substrate specificity, that is, the selectivity for amino acids flanking the scissile peptide bond. The number of potential substrates for proteases can be large. There are 400 possible dipeptide sequences if only the “primary” amino acids directly adjacent to the scissile bond are considered. This number increases to 160 000 when secondary sites are considered. Several methods have been developed to determine the specificity of proteases. Large peptide libraries are used where the library members are attached on solid supports such as polymer beads^[4] or microarrays.^[5] For example, Meldal and co-workers have developed screening methods for large combinatorial peptide (or peptide mimic) libraries by using quenched fluorogenic labels that become fluorescent upon peptide hydrolysis. Both the synthesis and the biological screening of these libraries could be carried out directly on polyacrylamide/poly(ethylene glycol) (PEGA copolymer) polymers that are compatible with enzyme activity.^[4] Recently, the research groups of Ellman and Yao independently developed successful peptide chips where the members of a peptide library were directly arrayed onto glass slides. This method is so far restricted to the analysis of the specificity for amino acids positioned on the carboxylic side of the cleaved peptide bond.^[5a,b]

Here we describe a simple new method for the profiling of the primary specificities of proteases. The primary specificity describes the protease preference for the amino acids on either side of the amide bond (P_1 and P'_1).^[6] The assay is based on our recently reported discovery that the peptide hydrolysis equilibrium can be reversed toward peptide synthesis on PEGA polymer beads.^[7] Instead of studying the cleavage of peptides, the reverse reaction, that is, the protease-catalyzed coupling of amino acids is monitored (Figure 1).

Biocompatible polymer materials are required for the successful analysis of enzymatic reactions on polymer sup-

ports. PEGA₁₉₀₀ has been identified as a suitable biologically inert material where all reactive sites on the polymer are accessible to small enzymes. Literature reports have shown that proteases are catalytically active within PEGA₁₉₀₀ beads.^[4,6]

To assess the validity of our approach, the selectivity of the protease thermolysin from *Bacillus thermoproteolyticus* Rokko in the hydrolysis and synthesis directions was compared by analytical HPLC analysis. Attachment of two amino acids (Asp and Phe) and two Fmoc-protected dipeptides (Fmoc-Asp-Phe and Fmoc-Phe-Asp) through the Wang linker to PEGA₁₉₀₀ resin provided solid-supported substrates for the synthesis and hydrolysis reactions, respectively. Analysis of the products (Fmoc-Phe-Asp and Fmoc-Asp-Phe after acidic cleavage of the Wang linker or of Fmoc-Asp and Fmoc-Phe, respectively) was performed by LC-MS. Figure 2 shows that thermolysin-catalyzed synthesis and

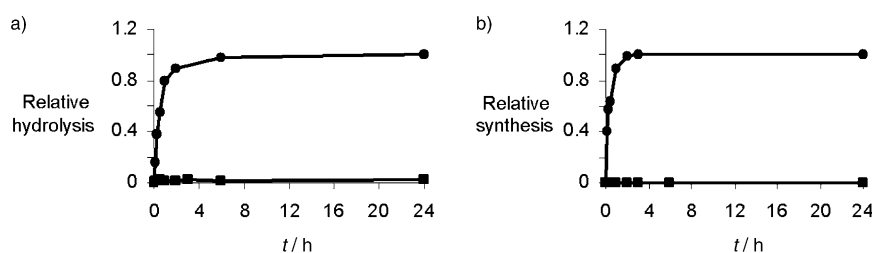


Figure 2. Thermolysin-catalyzed hydrolysis and synthesis of Fmoc-Asp-Phe-Wang-PEGA₁₉₀₀ and Fmoc-Phe-Asp-Wang-PEGA₁₉₀₀. a) Hydrolysis of Fmoc-Asp-Phe (circles) is favored over hydrolysis of Fmoc-Phe-Asp (squares). b) In the presence of excess Fmoc-Asp or Fmoc-Phe, synthesis of Fmoc-Asp-Phe (circles) is favored over synthesis of Fmoc-Phe-Asp (squares). Analyses were performed by LC-MS.

hydrolysis follow the same selectivity, with a marked preference for the Fmoc-Asp-Phe over the Fmoc-Phe-Asp sequence. The rates of the reactions appear similar because the kinetics are dominated by the rate-limiting diffusion of enzyme into the beads, as shown previously.^[8]

For comparison of two different proteases, commercially available bovine pancreatic α -chymotrypsin and thermolysin from *B. thermoproteolyticus* Rokko, which display opposite but complementary specificities for certain P_1/P'_1 amino acid combinations were selected. While α -chymotrypsin is selective for aromatic amino acids in the P_1 position, it is rather unspecific for the P'_1 residue. By contrast, thermolysin exhibits a preference for large hydrophobic residues in the P'_1 position and is nonspecific for the P_1 residue. Hence, one would expect hydrolysis/synthesis of the amide bond in Asp-Phe to be catalyzed preferentially by thermolysin and that of Phe-Asp by α -chymotrypsin.^[9]

To test whether this specificity could be measured with the present method of peptide synthesis, PEGA-immobilized Phe and Asp were exposed to Fmoc-Asp and Fmoc-Phe in the presence of either of the two enzymes. After overnight incubation, the relative fluorescence intensities were measured; these directly indicated the level of protease-catalyzed peptide synthesis. Figure 3 shows that the relative fluorescence intensities observed indeed corresponded to the reported specificities for both enzymes. These experiments

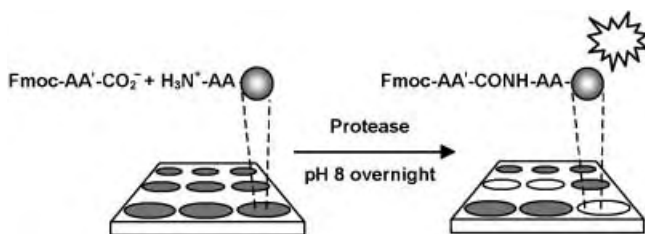


Figure 1. Protease specificity assay on a microtiter plate. Each well contains 1 of the 20 different P'_1 amino acids (AA) directly linked to PEGA-NH₂ through the carboxylic acid terminus. The P_1 amino acid AA' carries a fluorescent 9-fluorenylmethoxycarbonyl (Fmoc) protecting group and is added in a buffer solution containing the protease of interest. After incubation of the beads for 16 h at RT, they are washed and the Fmoc fluorescence is read by using a plate reader.

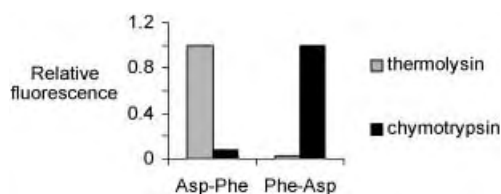


Figure 3. Comparison of substrate selectivity of two complementary proteases. Reactions were performed as outlined in Figure 1 with either Asp or Phe in positions P_1 or P'_1 . "Relative fluorescence" is a measure of transfer of the respective P_1 amino acid by thermolysin (gray) or chymotrypsin (black). The experiment shows that thermolysin favors formation of Fmoc-Asp-Phe-PEGA while chymotrypsin favors formation of Fmoc-Phe-Asp-PEGA.

suggest that solid-phase screening through peptide synthesis can give the same specificity as observed in peptide hydrolysis. Indeed, protease specificity is expected to be identical in either the hydrolysis or synthesis directions because the same transition states are involved.

The assay was then applied in a 96-well microtiter plate format by using thermolysin as a model system. Thermolysin specificity against three P_1 amino acids carrying side chains with different physical properties was monitored: phenylalanine (aromatic side chain), aspartic acid (negatively charged), and glycine (smallest amino acid). The fluorescence intensities were measured, compared directly (Figure 4, top images), normalized, and converted into a gray-scale (bottom image) with white representing the highest fluorescence intensity and black representing no signal above the background level.

The reported specificity for large hydrophobic amino acids, specifically leucine (L), isoleucine (I), methionine (M), and phenylalanine (F) is indeed observed for the three P_1 amino acids studied here. Alanine (A), glycine (G), and tyrosine (Y) were recognized to a lesser extent as they are smaller or less hydrophobic than the preferred substrates. Tryptophan (W) appears to be too bulky to fit into the active site and is not accepted by thermolysin.^[10]

Figure 4 also shows that the nature of P_1 significantly affects the selectivity for P'_1 in thermolysin. It appears that a large hydrophobic phenyl group in the P_1 position restricts P'_1 to large hydrophobic amino acids (mainly Ile, Leu, and to a lesser extent Val, Met, and Phe), while for smaller Gly residues the substrate specificity is more relaxed and a wider range of substrates are accepted. This highlights the importance of screening all combinations of P_1 and P'_1 rather than both sites independently, to fully characterize substrate specificity.

In summary, we have presented a new approach to screening for protease specificity. Primary screening of protease specificity is greatly simplified by taking advantage of the reversal of peptide hydrolysis (that is, peptide synthesis) on a solid support. The choice of label for each added amino acid is flexible; indeed, different fluorescent labels could be used for different amino acids and thus might aid simultaneous detection and analysis. The methodology has been demonstrated for a microtiter plate system but should be also applicable to the array format. Specificity for sites away from the primary positions can also be monitored by

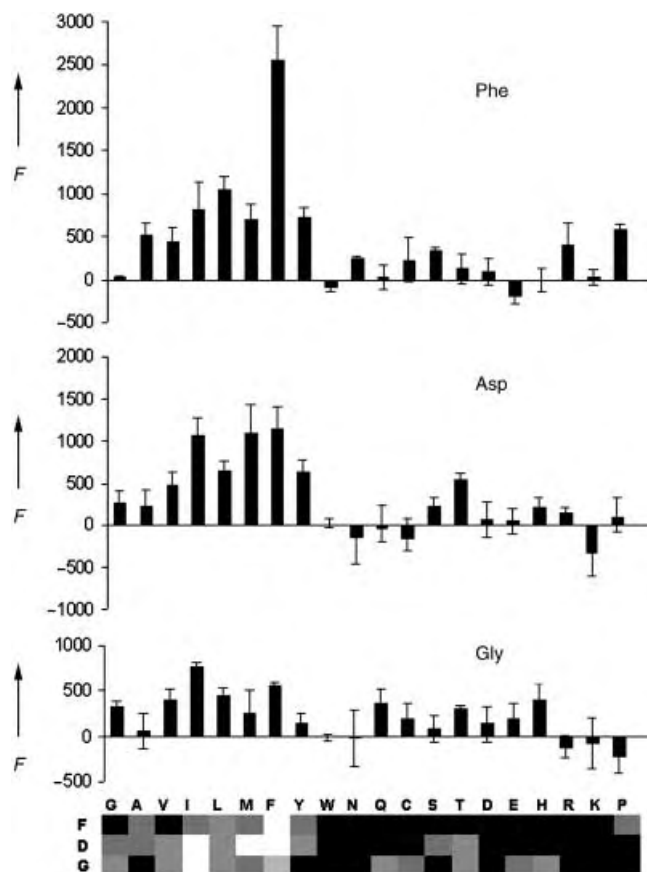


Figure 4. Primary thermolysin specificity as determined by reverse peptide hydrolysis on a solid support. Three different P_1 amino acids were supplied as Fmoc derivatives in separate experiments and all of the natural P'_1 amino acids were directly linked to PEGA₁₉₀₀. The histograms show the average fluorescence response obtained over three measurements. (The large error bars on the data are mainly as a result of inaccuracies resulting from dispensing bead suspensions in microtiter plates.) The bottom image shows the relative fluorescence normalized against the P'_1 amino acid which gave rise to the highest intensity. Lighter shades indicate increased fluorescence observed.

incubation with di-, tri-, and polypeptides instead of Fmoc-protected amino acids. Work on such an extension of the methodology is currently in progress. We believe that the present approach paves the way for flexible rapid high-throughput identification and characterization of proteases without the need for expensively labeled peptide arrays.

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Bioinorganic Chemistry

A Trinuclear [NiFe] Cluster Exhibiting Structural and Functional Key Features of [NiFe] Hydrogenases**

Dieter Sellmann, Frank Lauderbach,* Franz Geipel, Frank W. Heinemann, and Matthias Moll

Hydrogenases are enzymes that control formation and consumption of protons and electrons by catalyzing of the reversible H_2 oxidation [Eq. (1)]. Therefore, they play a central role in the natural hydrogen and energy metabolism.^[1]



[*] Prof. Dr. D. Sellmann,* Dipl.-Chem. F. Lauderbach, Dr. F. Geipel, Dr. F. W. Heinemann, Dr. M. Moll
Institut für Anorganische Chemie
Universität Erlangen-Nürnberg
Egerlandstrasse 1, 91058 Erlangen (Germany)
Fax: (+49) 9131-852-7367
E-mail: lauder@anorganik.chemie.uni-erlangen.de

[†] deceased

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Hydrogenases are essential for many microorganisms which receive energy by reduction of CO_2 , carbonates, sulfates, or nitrates.^[2] These enzymes are of great biotechnological interest^[3] owing to their capability to produce molecular hydrogen.^[4] [NiFe] hydrogenases are the most abundant of the four types, the [NiFe], [NiFeSe], Fe-only, and metal-free hydrogenases.^[3,5] Figure 1 depicts the active center of hydrogenase from *Desulfovibrio gigas*.

Whereas all hydrogenases perform the H_2 production/consumption reversibly, the [NiFe] hydrogenases generally have the greatest reaction rates for H_2 consumption and the "Fe-only" hydrogenases for H_2 production.^[7] Not all [NiFe] hydrogenases obey to this rule. Some of them are known to be more active in catalyzing H_2 production than H_2 oxidation.^[8]

The mechanism of the redox reaction is still poorly understood. [NiFe] hydrogenases exist in numerous redox states designated Ni-A, Ni-B, Ni-SU, Ni-SI, Ni-C, and Ni-R that can be labeled by their EPR spectra and individual $\nu(CO)$ frequencies.^[3,9] The relationship of these redox states to the metal oxidation states is still unknown. Furthermore, it is not known where the H_2 molecule is activated and turned over and why two different metals are needed in [NiFe] hydrogenases. To shed light on these questions, several di- and oligonuclear compounds have been synthesized by various research groups.^[10] Despite this research the number of compounds containing sulfur bridged Ni and Fe centers remains very small.^[11]

We recently reported the complex $[(S_2^2-Ni-\mu(S_3^2))Fe(CO)(PMe_3)_2]$ ($S_2^{2-} = 1,2$ -benzenedithiolate(2-), $S_3^{2-} = \text{bis}(2\text{-mercaptophenyl)sulfide}(2-)$).^[12] This complex is currently the closest approach to the active site of [NiFe] hydrogenases since nickel and iron are bridged by two thiolate donors and a carbonyl ligand is linked to the iron center. Nevertheless, it can only serve as a structural model compound for the active site as it does not show hydrogenase activity. This is also the case in the other structural model compounds for [NiFe] hydrogenases reported to date.

Herein, we report the synthesis of $[(S_2^2)\{Ni(PMe_3)_2\}Fe(CO)(S_2^2)]$ (**1**), the first trinuclear [NiFe] cluster that shows structural (iron and nickel centers in sulfur dominated coordination spheres, iron bound CO) as well as functional properties of the active site of [NiFe] hydrogenases and, therefore, serves as the first structural and functional model. The trinuclear [NiFe] cluster **1** resulted from the reaction of $[Fe(CO)_2(S_3^2)]_2$ with labile $[Ni(PMe_3)_2(S_2^2)]$ according to Equation (2) in 19% yield. Monitoring the $\nu(CO)$ frequency by IR spectroscopy showed that in addition to **1** several other CO-containing complexes formed as byproducts on which we recently reported.^[12]

The trinuclear cluster **1** consists of a square-pyramidal $[Fe(CO)(S_2^2)_2]$ fragment which is connected by thiolate bridges to two $[Ni(PMe_3)]$ fragments that are linked through

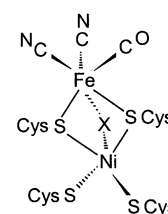
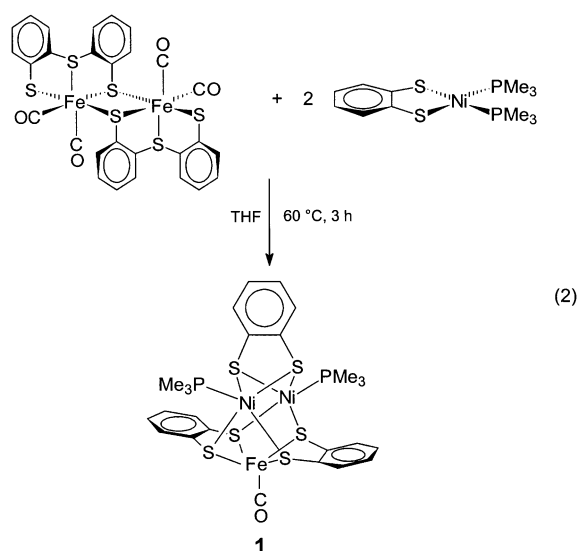


Figure 1. Schematic structure of the active center from *D. Gigas* hydrogenase in the oxidized form ("X" represents a bridging group, probably OH^- or O^{2-}).^[6]



thiolates. With an average Ni–Fe separation of 259.6(1) pm and a Ni–Ni separation of 240.5(1) pm all metal atom positions are very close to each other so that there are not only M–S–M bridging but also metal–metal interactions. The Fe–S bond (av. 221.9(1) pm, see Figure 3) in the {Fe(CO)(‘S₂’)} unit and the Ni–S bond lengths (av. 230.4(1) pm) in the {μ-(‘S₂’){Ni(PMe₃)₂}} fragment are shorter than the Ni–S bonds in between these units (av. 237.0(1) pm). Although [Ni(‘S₂’)₂][Ni(PPh₃)₂(‘S₂’)], a complex very similar to **1** in which the Fe–CO fragment is replaced by an Ni atom, was reported a few years ago,^[13] no information is available on its redox properties.

As hydrogenases catalyze the reversible interconversion of protons and electrons into molecular dihydrogen and consequently show redox activity electrochemical studies of model hydrogenase compounds are essential. Figure 2 shows the cyclic voltammogram of **1**. The three quasireversible redox waves at –1071 mV, –95 mV, and 844 mV are assigned the redox couples **1**^{0/–}, **1**^{0/+}, and **1**^{+2/+}, respectively. The redox potential of the redox wave at –95 mV (**1**^{0/+}) lies within a potential range observed for several typical [NiFe] hydro-

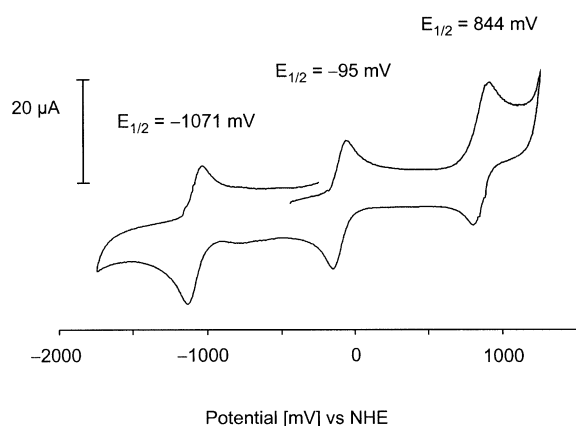
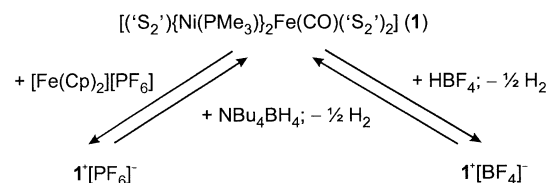


Figure 2. Cyclic voltammogram of **1** in CH₂Cl₂. NHE = normal hydrogen electrode.

genase redox reactions.^[3,14] This encouraged us to treat **1** with HBF₄ and to monitor this reaction by IR spectroscopy, the ν(CO) vibration serving as a probe for the cluster electron density. In CH₂Cl₂ the corresponding band at 1916 cm^{–1} is shifted to 1976 cm^{–1} upon addition of HBF₄. After subsequent treatment with NBu₄BH₄ the original band reappeared at 1916 cm^{–1}. When solutions of **1** in CH₂Cl₂ were combined with one equivalent of ferrocenium salt [Cp₂Fe][PF₆] (a one-electron oxidant (Cp = C₅H₅)) the ν(CO) band was shifted again to 1976 cm^{–1}. These findings confirmed that the shift of 60 cm^{–1} corresponds to single-electron-transfer steps. The corresponding EPR spectra of the cationic compounds prepared with HBF₄ and ferrocenium salts exhibit identical singlets at room temperature (*g* = 2.092). The glass-spectra consist of a broad singlet at almost the same position (*g* = 2.087) and a rhombic spectrum of low intensity (*g*₁ = 2.181, *g*₂ = 2.054, *g*₃ = 2.025), which could not be related unambiguously to the molecular structure of the paramagnetic complex cation.

Scheme 1 depicts the redox reactions of **1** with oxidants and reductants. In an NMR tube a solution of **1** in CD₂Cl₂ was



Scheme 1. Redox activity of **1** with HBF₄ and [Cp₂Fe][PF₆]. The resulting ion **1**⁺ was then reduced with NBu₄BH₄.

treated with HBF₄. Monitoring the reaction by ¹H NMR spectroscopy showed signals of a diamagnetic species at δ = 7.28, 6.71, 6.57, 6.22, and 2.09 ppm before the addition of HBF₄. After the addition, owing to the paramagnetism of **1**⁺, these signals vanished and a sharp signal at δ = 4.6 ppm appeared which was assigned to free dihydrogen. After addition of NBu₄BH₄ the signals of the diamagnetic **1** reappeared and as a result of the oxidation of borohydride to dihydrogen and diborane the singlet arising from free dihydrogen gains in intensity. When oxidized by [Cp₂Fe][PF₆], the ion **1**⁺ proved to be stable in solution over several weeks, whereas oxidation through HBF₄ led to a decrease of the ν(CO) frequency of about 25 % after 24 h. Thus, the species resulting from oxidation with the ferrocenium salt could be crystallized and an X-ray structure determination could be performed (Figure 3).^[15]

It is anticipated that many oxidoreductases undergo no or only marginal structural changes while redox reactions occur. The structural rigidity means a low reorganization barrier and therefore favors rapid electron transfer.^[16] This criterion would fit to the redox pair **1**/**1**⁺ because no significant changes in bond lengths or angles take place. So the average Fe–Ni separation before oxidation is 259.6(1) pm and afterwards 258.2(2) pm, the Ni–Ni bond length in **1** changes from 240.5(1) pm to 243.1(2) pm, and the average Fe–S separation from 221.9(1) pm to 220.5(6) pm after oxidation. Even the

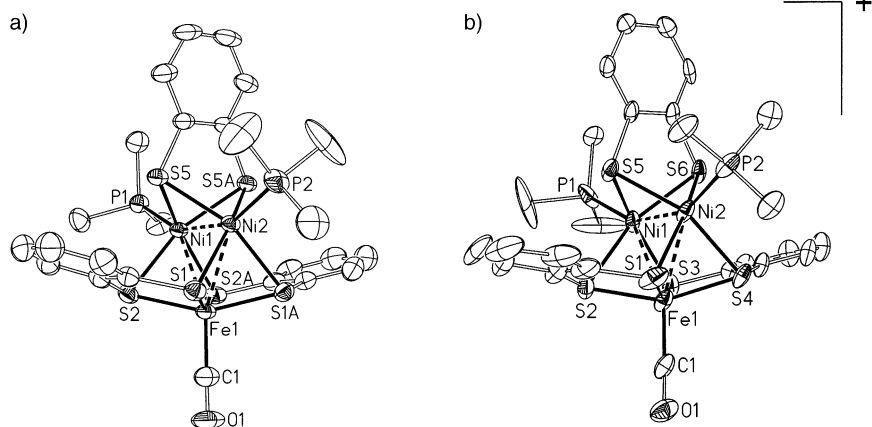


Figure 3. Molecular structures of **1** (a) and **1⁺** (b; H atoms omitted). Selected interatomic distances [pm] and angles [°] of **1**: Fe1–S1 222.0(1), Fe1–S2 221.8(1), Fe1–C1 173.8(5), Fe1...Ni1 262.3(1), Fe1...Ni2 256.9(1), Ni1...Ni2 240.5(1), Ni1–S2 236.1(1), Ni2–S1 237.9(1), Ni1–P1 221.3(2), Ni2–P2 219.3(2), Ni1–S5 230.4(1), Ni2–S5 232.4(1); S1–Fe1–S2 90.00(4), S1–Fe1–S1A 84.01(5), S1–Fe1–S2A 154.24(5), Ni2–Fe1–Ni1 55.18(3), Ni2–Ni1–Fe1 61.27(3), Ni1–Ni2–Fe1 63.54(3). Selected interatomic distances [pm] and angles [°] of **1⁺**: Fe1–S1 220.4(2), Fe1–S2 219.7(2), Fe1–C1 174.4(8), Fe1...Ni1 258.8(2), Fe1...Ni2 257.7(2), Ni1...Ni2 243.1(2), Ni1–S2 232.8(2), Ni2–S1 232.3(2), Ni1–P1 220.8(2), Ni2–P2 221.7(2), Ni1–S5 229.3(2), Ni2–S5 229.0(2); S1–Fe1–S2 91.37(8), S1–Fe1–S4 83.33(8), S1–Fe1–S3 154.17(9), Ni2–Fe1–Ni1 56.14(3), Ni2–Ni1–Fe1 61.70(4), Ni1–Ni2–Fe1 62.15(4).

Ni–S bond lengths of the Ni–S–Ni bridges change only marginally from 230.4(1) pm to 229.6(2) pm after oxidation. The average Ni–S bond of the Ni–S–Fe bridges shortens a little after oxidation from 237.0(1) pm to 233.1(9) pm. The Ni–Fe–Ni angle increases from 55.18(3)° in the neutral compound **1** to 56.14(3)° in the ion **1⁺**. In summary cluster **1** combines structural properties of the [NiFe] hydrogenases active site, hydrogenase reactivity, and structural rigidity during electron transfer processes.

Experimental Section

All manipulations were carried out in absolute solvents under exclusion of air. $[\text{Fe}(\text{CO})_2(\text{S}_2\text{C}_6\text{H}_5)_2]$ and $[\text{Ni}(\text{PMe}_3)_2(\text{S}_2\text{C}_6\text{H}_5)_2]$ were prepared by literature methods.^[12,17] IR spectroscopy: Perkin Elmer 983, 1620 FT-IR and 16PC FT-IR; NMR spectroscopy: Lambda LA 400; EPR: Bruker ESP 300E spectrometer; mass spectrometry: JEOL MSTATION 700; elemental analysis: Carlo Erba EA 1106 or 1108.

1: A THF suspension (40 mL) of $[\text{Fe}(\text{CO})_2(\text{S}_2\text{C}_6\text{H}_5)_2]$ (535 mg, 0.74 mmol) and $[\text{Ni}(\text{PMe}_3)_2(\text{S}_2\text{C}_6\text{H}_5)_2]$ (523 mg, 1.49 mmol) was heated to 60°C for 3 h. MeOH (80 mL) was added to the resulting dark red to black solution. Black-grey microcrystals precipitated and were removed from the mother liquor after 48 h and washed with MeOH (20 mL). Yield: 215 mg (19%). ¹H NMR (399.7 MHz, $[\text{D}_8]\text{THF}$): δ = 7.28 (m, 4H, C_6H_4), 6.71 (m, 4H, C_6H_4), 6.57 (m, 2H, C_6H_4), 6.22 (m, 2H, C_6H_4), 2.09 ppm (s, 18H, PC_3H_9); ¹³C{¹H} NMR (100.4 MHz, CD_2Cl_2): δ = 207.3 (CO), 146.9, 130.9, 130.3, 125.1, 123.3, (C_6H_4), 18.3 ppm (PC_3H_9); ³¹P{¹H} NMR (161.7 MHz, CD_2Cl_2): δ = 214.7 ppm (br, PC_3H_9); IR (KBr): $\tilde{\nu}$ = 1910 cm^{-1} (CO); IR (CH_2Cl_2): $\tilde{\nu}$ = 1916 cm^{-1} (CO); MS (FD, $[\text{D}_8]\text{THF}$): m/z : = 774 ($[\text{Ni}_2\text{Fe}(\text{CO})(\text{PMe}_3)_2(\text{S}_2\text{C}_6\text{H}_5)_3]^+$); elemental analysis calcd (%) for $\text{C}_{25}\text{H}_{30}\text{FeNi}_2\text{OP}_3\text{S}_6$: C 38.79, H 3.91, S 24.85; found: C 38.58, H 3.79, S 24.86.

1⁺[PF₆][−]: $[\text{Fe}(\text{Cp})_2][\text{PF}_6]$ (14 mg, 0.04 mmol) was added to a CH_2Cl_2 solution (5 mL) of **1** (34 mg, 0.04 mmol). Evaporation of solvent yielded a brown powder. IR (KBr): $\tilde{\nu}$ = 1951 cm^{-1} (CO); IR (CH_2Cl_2): $\tilde{\nu}$ = 1976 cm^{-1} (CO); EPR (9.43 GHz, CH_2Cl_2): 300 K:

$\langle g \rangle$ = 2.092 (s); 135 K: g = 2.181, 2.087, 2.054, 2.025; MS (FD, CH_2Cl_2): m/z : = 774 ($[\text{Ni}_2\text{Fe}(\text{CO})(\text{PMe}_3)_2(\text{S}_2\text{C}_6\text{H}_5)_3]^+$); single crystals suitable for X-ray structure determination were obtained by slow diffusion of *n*-pentane in a saturated CH_2Cl_2 solution of **1⁺**[PF₆][−] at 10°C; some of the crystals were crushed and dried in vacuum; elemental analysis calcd (%) for $\text{C}_{25}\text{H}_{30}\text{FeNi}_2\text{OP}_3\text{S}_6\text{F}_6$: C 32.67, H 3.29, S 20.93; found: C 32.93, H 3.27, S 20.83.

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- [15] X-ray structure analysis of **1**·2THF: Black prisms of **1** were obtained from a THF solution. A suitable single crystal was embedded in protective perfluoropolyether oil. Data were collected at 210 K on a Siemens P4 diffractometer using MoK α radiation ($\lambda = 71.073$ pm), graphite monochromator, and the ω -scan technique. Lorentz, polarization, and absorption corrections (Psi-scans, $T_{\min} = 0.090$, $T_{\max} = 0.139$) were applied. C₃₃H₄₆FeNi₂O₃P₂S₆, crystal size 0.62 × 0.56 × 0.24 mm, monoclinic space group *Cm* (no. 8), $a = 1044.7(2)$, $b = 1872.0(4)$, $c = 1016.2(2)$ pm, $\beta = 97.80(2)^\circ$, $V = 1.9690(7)$ nm³, $Z = 2$, $\rho_{\text{calcd}} = 1.549$ g cm⁻³, $\mu = 1.742$ mm⁻¹, 4666 measured reflections ($4.0 < 2\theta < 54.0^\circ$), 4431 unique reflections, 4223 observed reflections [$I > 2\sigma(I)$], 272 parameters, $wR_2 = 0.0796$ (all data), $R_1 = 0.0301$ [$I > 2\sigma(I)$]. The structure was solved by direct methods and refined by full-matrix least-squares procedures on F^2 using SHELXTL NT 6.12. All non-hydrogen atoms have been refined anisotropically. The complex molecule is situated on a crystallographic mirror plane resulting in *Cm* symmetry. The compound crystallizes with two molecules THF per formula unit. The solvent molecules are disordered, two alternative sites could be refined giving site occupancy factors of 0.5 each. All hydrogen atoms were geometrically positioned. X-ray structure analysis of **1**·PF₆·1.33CH₂Cl₂: Black plates of **1**·PF₆·1.33CH₂Cl₂ were obtained from a CH₂Cl₂/*n*-pentane mixture. A suitable single crystal was embedded in protective perfluoro-polyether oil. Data were collected at 100 K on a Bruker-Nonius KappaCCD diffractometer using MoK α radiation ($\lambda = 71.073$ pm) and a graphite monochromator. Images were taken using ϕ - and ω -rotations with a rotation angle of 1.1° and an irradiation time of 55 s per frame. Lorentz, polarization, and semiempirical absorption corrections (SADABS using multiple scans, $T_{\min} = 0.798$, $T_{\max} = 1.000$) were applied. C_{26.33}H_{32.67}Cl_{2.67}F₆FeNi₂OP₃S₆, crystal size 0.40 × 0.30 × 0.06 mm, monoclinic space group *C2/c* (no. 15), $a = 5543.2(3)$, $b = 1048.8(2)$, $c = 2051.7(2)$ pm, $\beta = 104.056(6)^\circ$, $V = 11.571(2)$ nm³, $Z = 12$, $\rho_{\text{calcd}} = 1.778$ g cm⁻³, $\mu = 2.025$ mm⁻¹, 49275 measured reflections ($7.0 < 2\theta < 52.8^\circ$), 11049 unique reflections, 6677 observed reflections [$I > 2\sigma(I)$], 890 parameters, $wR_2 = 0.1433$ (all data), $R_1 = 0.0656$ [$I > 2\sigma(I)$]. The structure was solved by direct methods and refined by full-matrix least-squares procedures on F^2 using SHELXTL NT 6.12. All non-hydrogen atoms have been refined anisotropically. The asymmetric unit contains 1.5 molecules of **1**⁺. Half a molecule is situated on crystallographic twofold axis resulting in strong disorder. No hydrogen atoms have been included for this strongly disordered cation. Disorder is also observed for one of the PF₆⁻ counterions and the CH₂Cl₂ solvate molecules. A number of crystals has been investigated, all of them exhibiting the same type of disorder. Hydrogen atoms for the non-disordered cation and the solvate molecules were geometrically positioned. CCDC 224468 (**1**) and CCDC 224469 (**1**⁺) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).
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Use of Dynamic Combinatorial Chemistry for the Identification of Covalently Appended Residues that Stabilize Oligonucleotide Complexes**

Anthony Bugaut, Jean-Jacques Toulmé, and Bernard Rayner*

Selective molecular recognition between synthetic oligonucleotide ligands and nucleic acid targets plays an important role in molecular biology, biotechnology, and molecular medicine. Whereas natural DNA or RNA oligonucleotides, either rationally designed or identified through *in vitro* selection, usually generate lead compounds, the improvement of their properties (affinity, nuclease resistance, membrane permeability) requires introduction of chemical modifications. Nevertheless, the design of a chemical modification is not a trivial task, particularly for binders to structured RNA targets.^[1] Lead optimization may benefit from combinatorial methods that allow the generation and screening of large populations of modified oligonucleotides.^[2] Herein, we report a novel methodology, which employs dynamic combinatorial chemistry (DCC), for the identification of covalently appended small molecules that stabilize nucleic acid complexes.

DCC is an emerging field which offers an alternative approach to traditional combinatorial chemistry (CC).^[3] Whereas CC involves the use of irreversible reactions to generate a static library of related compounds, DCC involves the use of reversible reactions to generate an equilibrating mixture of molecules, that is, a dynamic combinatorial library (DCL). The composition of a DCL is able to respond to molecular-recognition events resulting from the addition of a target of interest. The preferential binding of one member of the DCL to the target induces a shift in the equilibrium towards the formation of that particular compound. Thus, whereas in CC library synthesis and screening are two separate processes that are performed sequentially, DCC offers *in situ* screening of the combinatorial library simply by comparing its composition in the absence or presence of the target.

For our purpose, an oligonucleotide ligand bearing a reactive amino group will be allowed to react reversibly with a set of aldehydes in an aqueous media. According to the DCC

[*] A. Bugaut, Dr. J.-J. Toulmé, Prof. B. Rayner
INSERM U386
Université Victor Segalen Bordeaux 2
146, rue Léo Saignat, 33076 Bordeaux Cedex (France)
Fax: (+33) 5-5757-1014
E-mail: bernard.rayner@bordeaux.inserm.fr
Dr. J.-J. Toulmé
Institut Européen de Chimie et Biologie (IECB)
2, rue Robert Escarpit, 33607 Pessac Cedex (France)

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

principle, the resulting mixture of imines at equilibrium will be responsive to its environment.^[4] Addition of a nucleic acid target that develops interactions with imine products should promote the formation of the strongest binders. The reactive amino group will be introduced in the oligonucleotide as a 2'-amino-2'-deoxynucleotide. The pK_a value, determined to be 6.2, and the higher nucleophilicity of this primary amine compared to those present in nucleobases will allow the reaction to take place at near physiological conditions and specifically at this position.^[5] Subsequently, the mixture of interconverting imine species can be selectively reduced by sodium cyanoborohydride (NaBH_3CN) in the form of chemically stable secondary amines, which will facilitate isolation and analysis of the final mixture.^[6]

We first used a model system for an initial proof-of-principle study. This system was formed by a self-complementary hexadeoxyribonucleotide **1** bearing a 2'-amino-2'-deoxyuridine (U^*) at its 3'-terminus (Scheme 1).^[7] We assumed that hybridization between two self-complementary strands would drive the reversible reaction with a set of aldehydes towards the preferential formation of the conjugated oligonucleotide corresponding to the most stable duplex. Self-complementary oligonucleotide **1** was allowed to react with a set of three aldehydes **2a–c** in the presence of NaBH_3CN (Scheme 1).^[8] Aliquots of the reaction mixture were periodically withdrawn and analyzed by reversed-phase HPLC.^[9] Proportions (as estimated from relative peak areas) of resulting conjugates **3a–c** were compared to those of the corresponding conjugates obtained in a control experiment involving reaction of non-self-complementary hexadeoxyribonucleotide, 5'-TTTCGU*, with the same set of aldehydes

under the same conditions.^[10] A plot, as a function of time, of the percentage amplification in the proportion of each conjugated self-complementary product over the corresponding non-self-complementary one is shown in Figure 1.^[11]

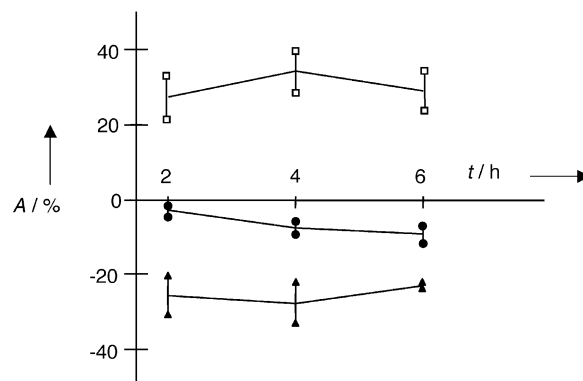
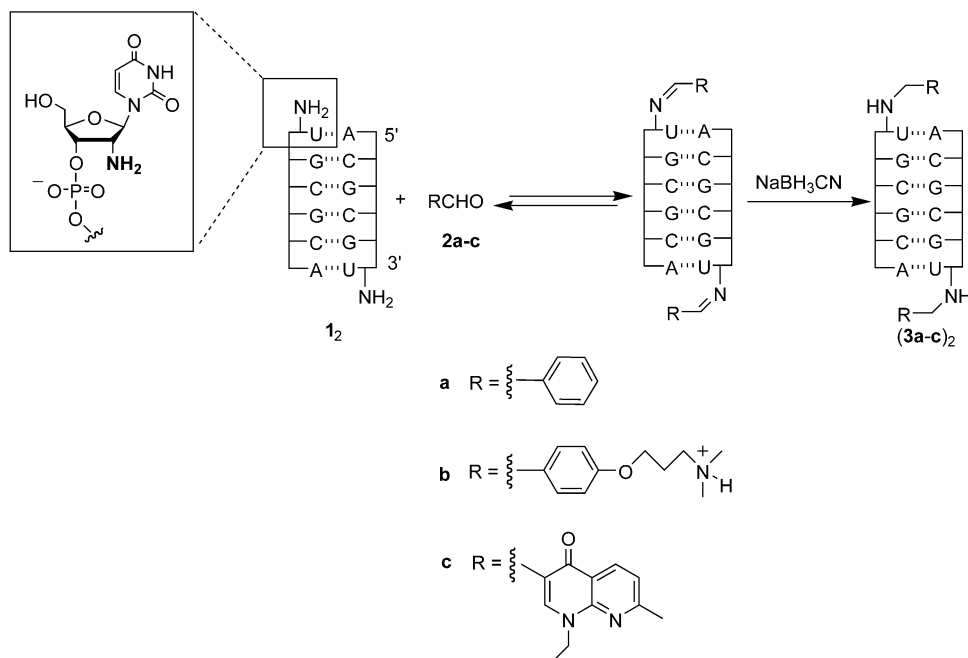


Figure 1. Time course for the DCC experiment showing the relative amplification (A) of **3a** (▲), **3b** (●), and **3c** (□) over the corresponding controls. **1** (0.25 mM) or 5'-TTTCGU* (0.25 mM) was incubated with **2a** (7 mM), **2b** (9 mM), and **2c** (0.8 mM) at room temperature (ca. 23 °C) in 20 mM phosphate buffer (pH 6) containing 100 mM NaCl and 5 mM NaBH_3CN . The reactions were complete after 6 h. Data shown correspond to duplicate experiments.

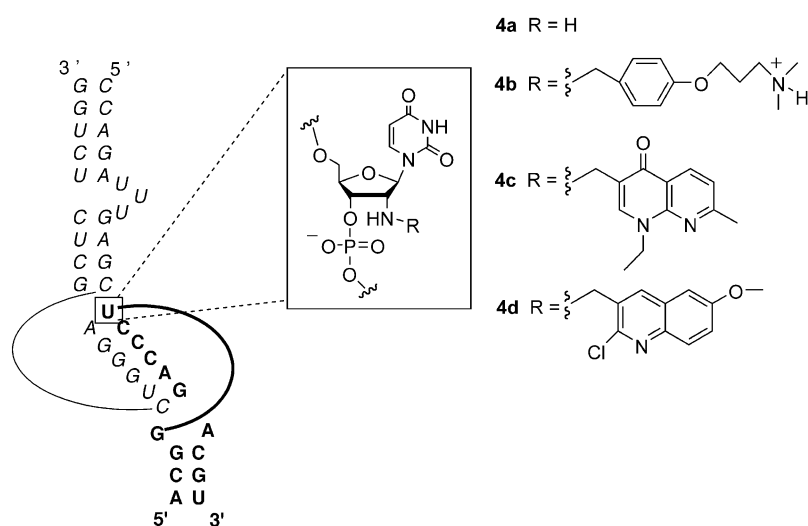
Apparent invariance of the percentage amplifications during the time-course experiment is consistent with a rapid formation of interconverting imines followed by their rate-limiting reduction. When reductive amination was performed on the reaction mixture with the self-complementary oligo-



Scheme 1. Selection by DCC of 3'-appended residues that stabilize a DNA duplex. Self-complementary oligonucleotide **1** bearing a 2'-amino-2'-deoxyuridine at its 3'-terminus is allowed to reversibly react with a set of aldehydes (benzaldehyde (**2a**), 4-[3-(dimethylamino)propoxy]benzaldehyde hydrochloride (**2b**), and nalidixic aldehyde (**2c**)) in an aqueous medium. This reaction provides a dynamic mixture of conjugated duplexes whose proportions are dictated by their relative thermodynamic stabilities. Subsequently, imines are reduced by NaBH_3CN to chemically stable secondary amines, thus allowing HPLC analysis of the composition of the mixture.

nucleotide, a significant enrichment in the nalidixic conjugated product **3c** was obtained relative to the reaction involving the non-self-complementary oligonucleotide. This occurred at the expense of the other products **3a** and **3b**.

Next, to confirm that the relative amplification values are related to the thermodynamic stabilities of the three conjugated duplexes, each self-complementary product was subjected to UV-monitored melting experiments. The results (Table 1) show a direct correlation between the melting-temperature (T_m) values obtained and the ranking order of amplification effects observed in the DCC experiment. However, the dynamic amplification occurs at equilibrium and reflects the stabilizing effects of the imino-connected residues, whereas the thermodynamic stabilities were measured with reduced



Scheme 2. Structures of appended R groups (**b–d**) that were screened by DCC for stabilizing a kissing complex formed between the MiniTAR (italic letters) target and the aptamer (**4a**, bold letters) incorporating a 2'-amino-2'-deoxyuridine.

Table 1: Melting temperatures (T_m) for duplexes^[a] and relative amplifications observed in the course of the DCC experiment.

Duplexes	T_m [°C]	ΔT_m [°C]	Amplification ^[b]
1₂	30.0		
(3a)₂	30.3 (31.3) ^[c]	+0.3 (+1.3) ^[c]	–28%
(3b)₂	34.2	+4.2	–8%
(3c)₂	40.3 (47.3) ^[c]	+10.3 (+17.3) ^[c]	+34%

[a] UV-monitored (260 nm) melting experiments were performed with 0.25 optical density units of each oligomer in 20 mM phosphate buffer (0.2 mL; pH 6) containing 100 mM NaCl. T_m values are given with a maximum standard deviation of $\pm 0.5^\circ\text{C}$. [b] Values are those obtained after a four-hour reaction. [c] T_m values of the amido-linked derivatives.

conjugated duplexes. It may be argued that the amino linker present in the reduced compounds **3a–c** is more flexible than the imino link operating during selection. Consequently, we synthesized more rigid amido-linked analogues of products **3a** and **3c**.^[9] These derivatives can be viewed as kinetically stable analogues of the transient imine compounds. We then performed UV-monitored melting experiments with these derivatives (Table 1). The results are consistent with those obtained with the amino-linked products: the amido-linked benzyl residue does not significantly improve the thermodynamic stability of the duplex, whereas the nalidixic residue increases the T_m value of duplex by 17.3°C .^[12]

Studies with this regular double-stranded model allowed us to successfully demonstrate that DCC can be used to identify covalently appended residues that increase the thermodynamic stability of a duplex. We next used this approach in the context of a tertiary-structured RNA complex formed by a loop–loop interaction between an RNA hairpin aptamer, identified by systematic evolution of ligands by exponential enrichment (SELEX) in our laboratory, and its target, the TAR RNA hairpin element of HIV-1.^[13] A loop–loop complex, also known as a kissing complex, was

attractive because it combines the fundamental base-pairing complementarity of nucleic acids with the added specificity of a constrained three-dimensional structure. For our experiment, we used a 14-nucleotide version of the aptamer (**4a**, Scheme 2) in which the ribonucleotide U in the loop was replaced by a U*, a set of three aldehydes (**2b**, **2c**, and 2-chloro-6-methoxy-3-quinolinecarboxaldehyde, **2d**), and a 27-nucleotide form of TAR (MiniTAR). Two reaction mixtures containing **2b–d** and the aptamer ligand **4a** were prepared, one in the absence and one in the presence of MiniTAR (one molar equivalent relative to **4a**).^[8] Reaction mixtures were incubated at 20°C in an aqueous buffer at pH 6 for 16 h and then analyzed by reversed-phase HPLC.^[9] We observed that the presence of MiniTAR promotes the formation of **4c** (+20%) at the expense of the other products (Figure 2).^[10] This amplification was correlated with a 2.6°C increase in the T_m value of the kissing complex **4c**/MiniTAR relative to that of **4a**/MiniTAR, while a slight destabilization was found with the conjugates **4b** and **4d**.^[14] To verify that the amplification effect was a consequence of loop–loop interaction and was not caused by any nonspecific effect, a mutated target, MiniTAR 3A, was also used as a control. This mutated target, in which the three central G residues in the loop are replaced by three A residues, prevents the formation of the kissing complex. As shown in Figure 2, when the reaction was carried out in presence of MiniTAR 3A, no significant change in the product distribution was observed, which demonstrates that the variations observed were caused by formation of a kissing complex between the conjugated ligands and MiniTAR.

The question arises as to why the nalidixic group was selected for stabilizing both complexes. Although the use of small libraries increases the probability of selecting the same aldehyde, the mode of interaction of the nalidixic group in stabilizing a DNA duplex or a RNA kissing complex is probably different. Structural evidence was provided that oxolonic acid (a quinolone related to nalidixic acid) disrupts the terminal T:A base-pair and stacks on the contiguous G:C

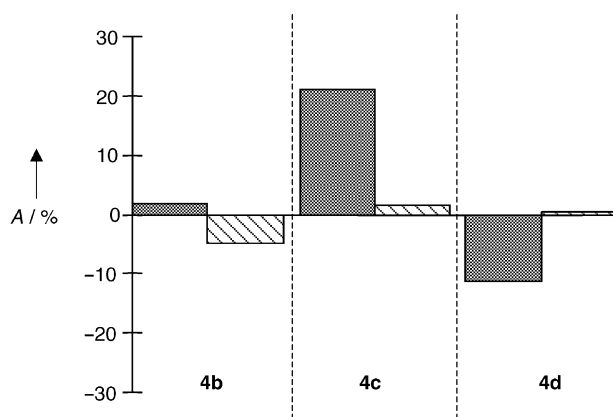


Figure 2. Relative amplifications of conjugated aptamers **4b–d**, after 16 h of incubation in the presence of 0.1 mM MiniTAR (gray) or 0.1 mM MiniTAR 3A (lined), over corresponding controls that were incubated in the absence of target. **4** (0.1 mM) was incubated at 20°C with **2b** (1.2 mM), **2c** (0.25 mM), and **2d** (0.35 mM) in 20 mM sodium cacodylate (pH 6) containing 140 mM KCl, 20 mM NaCl, 3 mM MgCl₂, 5% dimethylsulfoxide, and 5 mM NaBH₃CN. Results are averages of duplicate experiments.

base-pair when appended on termini of DNA duplexes.^[15] Such a stabilizing but base-disrupting effect appears unlikely within the highly constrained structure of a kissing complex.^[16]

In conclusion, we have described the first successful use of DCC for the rapid identification of a non nucleic acid residue appended to an oligonucleotide ligand that stabilizes the complex formed with its nucleic acid target. This was achieved both with a DNA duplex and with a tertiary-structured RNA–RNA complex. Therefore, DCC represents a powerful methodology for optimization of nucleic acid ligands. Additive effects of several appended groups would lead to a high-affinity and nuclease-resistant nucleic acid ligand. We are currently investigating the possibility of increasing the size of the set of aldehydes as well as the number of 2'-amino-2'-deoxynucleotides present in the ligand.

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- Experimental details are given in the Supporting Information.
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The Binary Group 4 Azides $[\text{Ti}(\text{N}_3)_4]$, $[\text{P}(\text{C}_6\text{H}_5)_4][\text{Ti}(\text{N}_3)_5]$, and $[\text{P}(\text{C}_6\text{H}_5)_4]_2[\text{Ti}(\text{N}_3)_6]$ and on Linear Ti–N–NN Coordination**

Ralf Haiges,* Jerry A. Boatz, Stefan Schneider, Thorsten Schroer, Muhammed Yousufuddin, and Karl O. Christe*

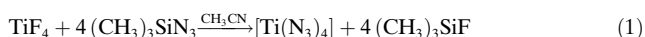
Whereas numerous partially azide-substituted titanium compounds had been reported,^[1–7] no binary Group 4 azides were known. In a recent theoretical study, the Group 4 metal tetrazides $[\text{M}(\text{N}_3)_4]$ ($\text{M} = \text{Ti}, \text{Zr}, \text{Hf}, \text{Th}$) were predicted^[8] to be vibrationally stable, exhibiting tetrahedral structures with unique linear $\text{M}–\text{N}–\text{NN}$ bond angles (see Figure 1). All the



Figure 1. Predicted tetrahedral structure of free gaseous $[\text{Ti}(\text{N}_3)_4]$ with linear $\text{Ti}–\text{N}–\text{NN}$ bonds.

characterized covalent binary azide species possess bent $\text{M}–\text{N}–\text{NN}$ angles.^[9] Herein, we report the synthesis, isolation, and characterization of the first binary Group 4 azide species $[\text{Ti}(\text{N}_3)_4]$, $[\text{Ti}(\text{N}_3)_5]^-$, and $[\text{Ti}(\text{N}_3)_6]^{2-}$, and provide explanations for the observed and predicted $\text{Ti}–\text{N}–\text{NN}$ bond angles.

The reaction of TiF_4 with $(\text{CH}_3)_3\text{SiN}_3$ in acetonitrile solution at room temperature results within minutes in complete fluoride-azide exchange and yields a clear orange solution of $[\text{Ti}(\text{N}_3)_4]$ according to Equation (1).



Removal of the volatile products $(\text{CH}_3)_3\text{SiF}$ and excess $(\text{CH}_3)_3\text{SiN}_3$ at ambient temperature results in the isolation of $[\text{Ti}(\text{N}_3)_4]$ as an amorphous orange solid. All attempts to obtain single crystals by recrystallization or sublimation were unsuccessful.

As expected for a highly endothermic, covalent polyazide species, $[\text{Ti}(\text{N}_3)_4]$ is very shock sensitive and can explode violently when touched with a metal spatula or when exposed to a rapid change in temperature (e.g. freezing with liquid nitrogen). Its identity was established by the observed material balance, and vibrational and NMR spectroscopy. The presence of covalent azido ligands^[10–15] is confirmed by the observed ^{14}N NMR shifts of $\delta = -134$ ppm (N_β , $\Delta\nu_{1/2} = 26$ Hz), -195 ppm (N_γ , $\Delta\nu_{1/2} = 39$ Hz) and -255 ppm (N_α , extremely broad) in DMSO solution at 25°C .

The observed Raman and IR spectra of solid $[\text{Ti}(\text{N}_3)_4]$ are shown in Figure 2, and the frequencies and intensities are listed in the Experimental Section. The experimental vibra-

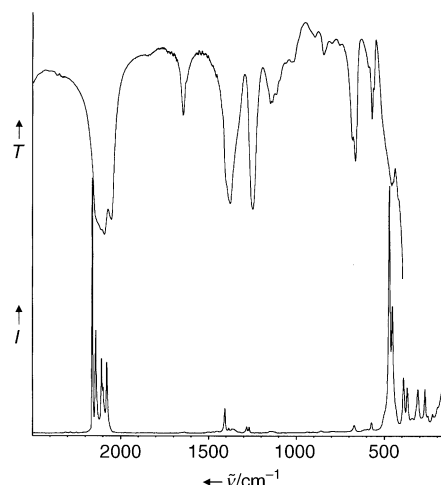


Figure 2. IR (top) and Raman (bottom) spectra of solid $[\text{Ti}(\text{N}_3)_4]$.

tional spectra deviate significantly from those calculated for free $[\text{Ti}(\text{N}_3)_4]$ at the B3LYP level of theory,^[8,16] and resemble those of higher-coordinated compounds with bent $\text{M}–\text{N}–\text{NN}$ bonds. Therefore, the predicted^[8] tetrahedral structure with linear $\text{Ti}–\text{N}–\text{NN}$ bonds could not be confirmed. The discrepancy between the calculated and observed spectra arises from solid-state effects. The Ti atoms in $[\text{Ti}(\text{N}_3)_4]$ are coordinatively unsaturated seeking higher coordination numbers by the formation of nitrogen bridges, and crystal-structure data will be required for a reliable determination of the precise arrangement of the azido ligands in solid $[\text{Ti}(\text{N}_3)_4]$. The growing of single crystals for such a study will be difficult because the compound is hard to recrystallize and does not sublime without decomposition. The structure determination of free monomeric $[\text{Ti}(\text{N}_3)_4]$ and proof for its predicted tetrahedral structure with linear $\text{Ti}–\text{N}–\text{NN}$ bonds will be even more difficult.

[*] Dr. R. Haiges, Dr. S. Schneider, Dr. T. Schroer, M. Yousufuddin, Prof. Dr. K. O. Christe
Loker Research Institute
University of Southern California
Los Angeles, CA 90089-1661 (USA)
Fax: (+1) 213-740-6679
E-mail: haiges@usc.edu
kchriste@usc.edu

Dr. J. A. Boatz, Prof. Dr. K. O. Christe
ERC, Inc. and Space and Missile Propulsion Division
Air Force Research Laboratory (AFRL/PRSP)
10 East Saturn Boulevard, Bldg 8451, Edwards Air Force Base, CA 93524 (USA)

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The reaction of $[\text{Ti}(\text{N}_3)_4]$ with one equivalent of ionic azides leads to the formation of the $[\text{Ti}(\text{N}_3)_5]^-$ ion according to Equation (2).



The $[\text{PPh}_4][\text{Ti}(\text{N}_3)_5]$ salt was isolated as an orange solid. It is less sensitive than $[\text{Ti}(\text{N}_3)_4]$ and does not explode upon freezing with liquid nitrogen. It was characterized by its ^{14}N NMR spectrum, and by infrared and Raman spectroscopy. The observed Raman and IR spectra of $[\text{PPh}_4][\text{Ti}(\text{N}_3)_5]$ are shown in Figure 3. The frequencies and intensities are listed in

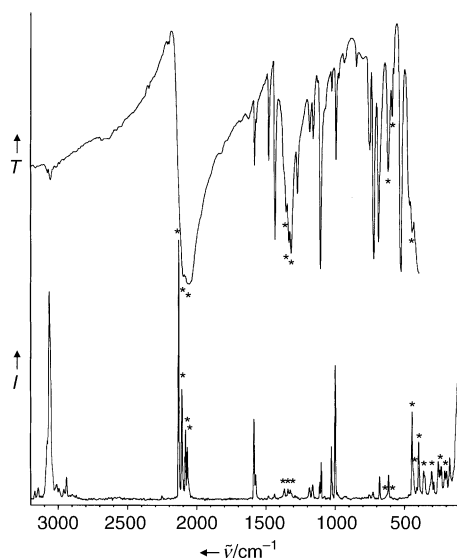
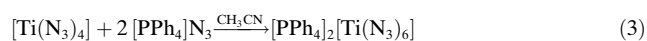


Figure 3. IR (top) and Raman (bottom) spectra of $[\text{PPh}_4][\text{Ti}(\text{N}_3)_5]$. The bands belonging to the $[\text{Ti}(\text{N}_3)_5]^-$ ion are marked with asterisks.

the Experimental Section. Three well-resolved ^{14}N NMR resonances were found the spectrum run in DMSO at 25 °C. It shows a sharp signal at $\delta = -133$ ppm ($\Delta\nu_{1/2} = 28$ Hz) for the N_β atoms, a medium-sharp resonance at $\delta = -194$ ppm ($\Delta\nu_{1/2} = 40$ Hz) for the N_γ atoms, and a very broad signal at $\delta = -263$ ppm ($\Delta\nu_{1/2} = 160$ Hz) for the N_α atoms, in accord with our expectations for covalently bound azido groups and quadrupole relaxation effects. The calculated structure for the free gaseous anion is that of a trigonal bipyramid, and its calculated frequencies are listed in the Theoretical Methods section.

By reaction of $[\text{Ti}(\text{N}_3)_4]$ with two equivalents of ionic azide, the $[\text{Ti}(\text{N}_3)_6]^{2-}$ ion is formed [Eq. (3)].



$[\text{PPh}_4]_2[\text{Ti}(\text{N}_3)_6]$ was isolated as an orange solid and is stable at room temperature. Because of the presence of two large counterions, it is much less sensitive and explosive than $[\text{Ti}(\text{N}_3)_4]$. It can even be heated to its melting point at 191 °C without any signs of decomposition. The compound was characterized by its crystal structure,^[17] and vibrational and ^{14}N NMR spectroscopy. Single crystals were obtained from a solution in CH_3CN .

$[\text{PPh}_4]_2[\text{Ti}(\text{N}_3)_6]$ crystallizes in the triclinic space group $P\bar{1}$ and is the first structurally characterized binary titanium azide. Figure 4 depicts the structure of the $[\text{Ti}(\text{N}_3)_6]^{2-}$ ion that is only slightly distorted from perfect S_6 symmetry. The

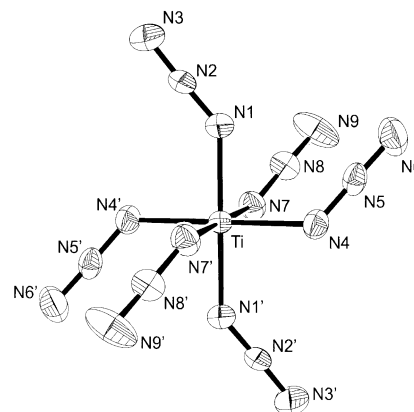


Figure 4. ORTEP drawing of the dianion of the crystal structure of $[\text{PPh}_4]_2[\text{Ti}(\text{N}_3)_6]$. Thermal ellipsoids are set at 50% probability. Selected bond lengths [Å] and angles [°]: Ti–N1 2.002(2), Ti–N4 2.019(2), Ti–N7 2.048(2), N1–N2 1.173(2), N2–N3 1.138(2), N4–N5 1.200(2), N5–N6 1.146(3), N7–N8 1.197(2), N8–N9 1.140(3); N1–N2–N3 177.5(2), N4–N5–N6 177.6(2), N7–N8–N9 176.9(2), N1–Ti–N4 91.68(9), N1–Ti–N7 90.18(7), N4–Ti–N7 89.73(9), Ti–N1–N2 140.64(14), Ti–N4–N5 126.01(13), Ti–N7–N8 129.67(14).

structure consists of an asymmetric TiN_9 unit with three azido groups covalently bonded to give a trigonal pyramidal coordination environment around the titanium center. The remaining three azide groups are generated by symmetry (symmetry operation $-x+2, -y+1, -z$). The average Ti–N distance of 2.02 Å is in good agreement with the one reported for $[(\text{C}_5\text{H}_5)_2\text{Ti}(\text{N}_3)_2]$ (2.03(1) Å).^[6] In the $[\text{PPh}_4^+]_2[\text{Ti}(\text{N}_3)_6]^{2-}$ salt, the anions are well separated by twice as many large counterions, and the closest Ti···N contacts between neighboring anions are 7.1 Å.

Further support for the presence of the $[\text{Ti}(\text{N}_3)_6]^{2-}$ ion is provided by the NMR spectrum. In analogy with $[\text{Te}(\text{N}_3)_4]$ and $[\text{Ti}(\text{N}_3)_5]^-$, the ^{14}N NMR spectra in DMSO show at $\delta = -134$ ppm (N_β , $\Delta\nu_{1/2} = 30$ Hz), -199 ppm (N_γ , $\Delta\nu_{1/2} = 35$ Hz) and -264 ppm (N_α , $\Delta\nu_{1/2} = 165$ Hz) resonances characteristic for covalent azides.

The observed Raman and IR spectra of $[\text{PPh}_4]_2[\text{Ti}(\text{N}_3)_6]$ are shown in Figure 5, and the observed frequencies and intensities are listed in the experimental section. Assignments of the observed spectra were made by comparison with those calculated at the B3LYP/SBKJC + (d) level of theory^[16] and are given in the Experimental Section.

Although the occurrence of linear Ti–N–NN bond angles could not be confirmed experimentally, we have confirmed by independent computations the correctness of the previous predictions^[8] for free gaseous $[\text{Ti}(\text{N}_3)_4]$. Furthermore, it was shown, both computationally and experimentally by our crystal structure determination, that in higher coordinated binary titanium azide species, such as $[\text{Ti}(\text{N}_3)_6]^{2-}$, and in the main-group tetra-azides $\text{M}(\text{N}_3)_4$ (M = Si, Ge, Sn) the M–N–NN bond angles are normal and strongly bent. It was also

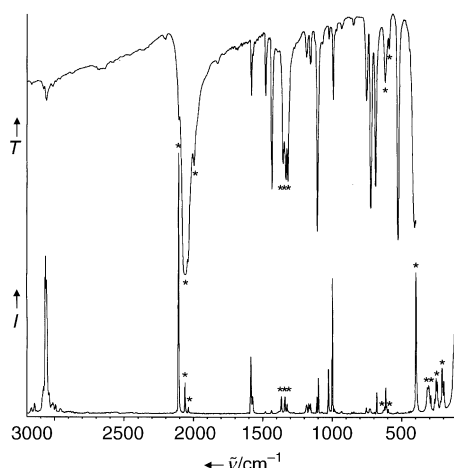


Figure 5. IR (top) and Raman (bottom) spectra of $[\text{PPh}_4]_2[\text{Ti}(\text{N}_3)_6]$. The bands belonging to the $[\text{Ti}(\text{N}_3)_6]^{2-}$ ion are marked with asterisks.

shown that the occurrence of linear M–N–NN bond angles should not be limited to Group 4 tetra-azides, but might also occur in compounds, such as $[\text{Fe}(\text{N}_3)_2]$.^[18] Normal coordinate analyses were carried out for the binary Ti-azides of this study and showed that the modes owing to the azido ligands were highly characteristic, while those of the $[\text{TiN}_n]$ skeletal modes were strongly mixed and, therefore, are not reported herein. However, the $[\text{TiN}_n]$ stretching-mode clusters clearly exhibit the frequency decreases expected for increasing polarities of the Ti–N bonds with increasing negative formal charges. Thus, the range of the $[\text{TiN}_n]$ stretching modes decreases from 472–371 cm^{-1} in $[\text{Ti}(\text{N}_3)_4]$ to 448–355 cm^{-1} in $[\text{Ti}(\text{N}_3)_5]^-$ and 398–307 cm^{-1} in $[\text{Ti}(\text{N}_3)_6]^{2-}$.

In the previous theoretical paper on the linearity of the M–N–NN bond angles for M = Ti, Zr, and Hf, the linearity of the M–N–NN angles was explained by M–N–N–N conjugation.^[8] In our opinion, the linearity of the M–N–NN angles is due to a nearly ideal overlap between the three valence electron pairs on the α -nitrogen atoms of the azide ligands and the lobes of the d orbitals on the central metal atom. Because the d orbitals of the (+IV) central metal atoms are unoccupied, the three valence electron pairs of the α -nitrogen atoms can donate electron density equally into three lobes of the empty d orbitals resulting in the α -nitrogen acting as a tridentate donor ligand. The symmetry of the central axes of these d orbitals is tetrahedral. Therefore, this tridentate type of overlap is possible only for the tetracoordinate azide complexes of Group 4 transition metals with d^0 configurations. For other coordination numbers or electron configurations, the α -nitrogen atoms act usually as monodentate donors with only one pair donating and two sterically active free valence electron pairs, thus resulting in strongly bent M–N–NN bond angles. An excellent example for the validity of our explanation is the known crystal structure of $[\text{Zr}(\text{BH}_4)_4]$ (see Figure 6) involving a (+IV) Group 4 d^0 transition-metal central atom and for $\eta^3\text{-BH}_4$ ligands.^[19] Another example for a special geometry, dictated by the empty d^0 orbitals of a (+IV) Group 4 transition metal is $[\text{Ti}(\text{O}_2\text{ClO}_2)_4]$ in which the perchlorato groups act as bidentate ligands.^[20]

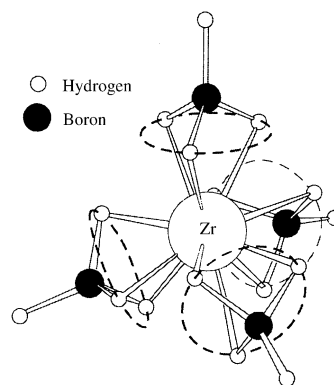


Figure 6. Crystal structure of $[\text{Zr}(\text{BH}_4)_4]$ demonstrating the perfect geometrical overlap of a tridentate donor into the empty d^0 orbitals of a (+IV) Group 4 transition-metal atom.^[19]

Experimental Section

Caution! Covalent azides are potentially hazardous and can decompose explosively under various conditions! They should be handled only on a scale of less than 2 mmol with appropriate safety precautions (safety shields, safety glasses, face shields, leather gloves, protective clothing, such as leather suits, and ear plugs). Teflon containers should be used, whenever possible, to avoid hazardous fragmentation. Pure $[\text{Ti}(\text{N}_3)_4]$ has to be cooled or heated carefully and slowly. A sample at ambient temperature must not be cooled directly with liquid nitrogen. Doing so can result in violent explosions. Handle the materials, whenever possible, in solution to avoid detonation propagation. The use of large inert counterions as spacers and the formation of anions which increases the partial negative charges on the terminal N_γ atoms and thereby reduce the $\text{N}_\beta\text{--N}_\gamma$ triple bond character, facilitate the manipulation of these materials. **Ignoring safety precautions can lead to serious injuries.**

All reactions were carried out in Teflon-FEP (FEP = fluoroethylene-propylene copolymer) ampules that were closed by stainless steel valves. Volatile materials were handled in a Pyrex glass vacuum line. All Teflon reaction vessels were passivated with ClF_3 prior to use. Nonvolatile materials were handled in the dry argon atmosphere of a glove box.

Raman spectra were recorded at -80°C in the range 4000–80 cm^{-1} on a Bruker Equinox 55 FT-RA spectrophotometer using a Nd-YAG laser at 1064 nm with power levels of less than 200 mW. Pyrex melting point tubes that were baked out at 300°C for 48 h at 10 Torr vacuum or Teflon-FEP tubes with stainless steel valves that were passivated with ClF_3 were used as sample containers. Infrared spectra were recorded in the range 4000–400 cm^{-1} on a Midac, M Series, FT-IR spectrometer using KBr or AgCl pellets. The pellets were prepared inside the glove-box using an Econo press (Barnes Engineering Co.).

^{14}N NMR spectra were recorded unlocked at 36.13 MHz on a Bruker AMX 500 spectrometer using solutions of the compounds in DMSO in sealed standard glass tubes. Neat CH_3NO_2 (0.00 ppm) was used as the external reference.

The starting materials TiF_4 and $[\text{P}(\text{C}_6\text{H}_5)_4]\text{I}$ (both from Aldrich) were used without further purification. $(\text{CH}_3)_3\text{SiN}_3$ (Aldrich) was purified by fractional condensation prior to use. Solvents were dried by standard methods and freshly distilled prior to use. $[\text{P}(\text{C}_6\text{H}_5)_4]\text{N}_3$ was prepared from $[\text{P}(\text{C}_6\text{H}_5)_4]\text{I}$ and AgN_3 .

$[\text{Ti}(\text{N}_3)_4]$: A sample of TiF_4 (0.96 mmol) was loaded into a Teflon-FEP ampule, followed by the addition of CH_3CN (3 mL) and $(\text{CH}_3)_3\text{SiN}_3$ (4.32 mmol) in vacuo at -196°C . The mixture was allowed to warm to room temperature. Within minutes, the mixture turned yellow, and the color intensified while the reaction proceeded.

After 1 hour, all volatile material was pumped off, leaving behind an orange solid (0.20 g, weight calculated for 0.96 mmol $[\text{Ti}(\text{N}_3)_4] = 0.21$ g). The obtained orange solid was characterized by vibrational and NMR spectroscopy. IR (AgCl): $\tilde{\nu} = 2141(\text{vs})$, 2093(vs), 2051(vs) ($\nu_{\text{as}}\text{N}_3$), 1376(s, br), 1253(s) ($\nu_{\text{s}}\text{N}_3$), 684(s), 668(s), 665(s), 575(m), 561(m) (δN_3), 458(w) cm^{-1} (νTiN_n). Raman (50 mW, -80°C): $\tilde{\nu} = 2160(10.0)$, 2141(4.0), 2108(2.9), 2100(1.8), 2079(2.8) ($\nu_{\text{as}}\text{N}_3$), 1407(1.0), 1385(0.2), 1366(0.2), 1283(0.3), 1269(0.3) ($\nu_{\text{s}}\text{N}_3$), 671(0.3), 574(0.4) (δN_3), 472(9.6), 454(4.9), 391(2.2), 371(1.8) (all νTiN_n), 310(1.7), 270 (1.7), 227 (0.8), 168 (2.1), 135(2.0) cm^{-1} . For NMR spectroscopy data see text.

$[\text{PPh}_4][\text{Ti}(\text{N}_3)_5]$. A solution of $[\text{Ti}(\text{N}_3)_4]$ (0.5 mmol) in CH_3CN (3 mL) was added to a mixture of $[\text{PPh}_4]\text{N}_3$ (0.5 mmol) in CH_3CN (2 mL) at -64°C . The reaction mixture was warmed to ambient temperature where it formed a clear orange solution. After 2 h, all volatiles were slowly removed in a dynamic vacuum at 20°C , leaving behind an orange solid (0.450 g, weight calculated for 0.5 mmol $[\text{PPh}_4][\text{Ti}(\text{N}_3)_5] = 0.443$ g). IR of $[\text{Ti}(\text{N}_3)_5]^-$ (KBr): $\tilde{\nu} = 2100(\text{vs})$, 2070(vs), 2058(vs) ($\nu_{\text{as}}\text{N}_3$), 1373(m), 1356(s), 1320(s) ($\nu_{\text{s}}\text{N}_3$), 621(m), 595(w), 591(w), 580(vw) (δN_3), 448(w), 440(vw) cm^{-1} (both νTiN_n). Raman of $[\text{Ti}(\text{N}_3)_5]^-$ (50 mW, -80°C): $\tilde{\nu} = 2133(10.0)$, 2110(4.4), 2083(2.9), 2070(2.2) ($\nu_{\text{as}}\text{N}_3$), 1367(0.3), 1342(0.3), 1325(0.2), 1314(0.1) ($\nu_{\text{s}}\text{N}_3$), 623(0.2), 608(0.1), 597(0.1) (δN_3), 445(3.4), 438(1.0), 412(0.6), 398(2.2), 363(0.9), 355(0.7) (all νTiN_n), 306(0.9), 245(1.3), 210(0.9), 174(1.6) cm^{-1} . For NMR spectroscopy data see text.

$[\text{PPh}_4][\text{Ti}(\text{N}_3)_6]$. A solution of $[\text{Ti}(\text{N}_3)_4]$ (0.5 mmol) in CH_3CN (3 mL) was added to a mixture of $[\text{PPh}_4]\text{N}_3$ (1.0 mmol) in CH_3CN (2 mL) at -64°C . The reaction mixture was warmed to ambient temperature and an orange precipitate was formed. After 8 h, all volatiles were removed in a dynamic vacuum at 20°C , leaving behind an orange solid (0.768 g, weight calculated for 0.5 mmol $[\text{PPh}_4][\text{Ti}(\text{N}_3)_6] = 0.778$ g). Single crystals were grown from a solution in CH_3CN by slow evaporation in a dynamic vacuum. IR of $[\text{Ti}(\text{N}_3)_6]^{2-}$ (KBr): $\tilde{\nu} = 2109(\text{m})$, 2061(vs), 2041(vs) ($\nu_{\text{as}}\text{N}_3$), 1356(s), 1322(s) ($\nu_{\text{s}}\text{N}_3$), 622(m), 615(m), 595(w) cm^{-1} (δN_3). Raman of $[\text{Ti}(\text{N}_3)_6]^{2-}$ (50 mW, -90°C): $\tilde{\nu} = 2110(10.0)$, 2063(1.2), 2038(0.3) ($\nu_{\text{as}}\text{N}_3$), 1366(0.7), 1341(0.7), 1325(0.4) ($\nu_{\text{s}}\text{N}_3$), 631(0.2), 622(0.3), 608(0.3) (δN_3), 398(5.4), 316(1.1), 307(1.1) (νTiN_n), 246(1.3), 210 (1.8), 103(0.5), 95(0.5) cm^{-1} . For NMR spectroscopy data see text.

Theoretical Methods: The molecular structures and harmonic vibrational frequencies were calculated at the DFT level using the B3LYP hybrid functional,^[16a] which included the VWN5 correlation functional.^[16b] The SBKJC^[16c] effective core potential and the corresponding valence-only basis set was used for titanium. The all-electron 6-31G basis set,^[16d] augmented with a d polarization function^[16e] and a diffuse s + p shell^[16f] and denoted as 6-31 + G(d), was used for nitrogen. All calculations were performed using the GAMESS^[16g] quantum chemistry program. Unscaled calculated frequencies [cm^{-1}] and (infrared, kmol^{-1}) and [Raman, $\text{\AA}^4\text{amu}^{-1}$] intensities for $[\text{Ti}(\text{N}_3)_4]$: 2293 (0.0) [794], 2251 (5737) [1022], 1497 (0.0) [4.5], 1475 (2005) [48], 573 (110) [0.3], 571 (0.0) [0.3], 569 (0.0) [0.0], 516 (933) [67], 373 (0.0) [103], 192 (17) [7.3], 167 (0.0) [14.0], 14.8 (0.0) [50], 14.0 (0.0) [71]. $[\text{Ti}(\text{N}_3)_5]^-$ (pseudo-trigonal bipyramid of C_1 symmetry): 2248 (72) [751], 2212 (3406) [42], 2206 (1441) [160], 2202 (1836) [77], 2200 (142) [278], 1445 (48) [28], 1433 (390) [4.3], 1415 (180) [16], 1404 (174) [17], 1402 (384) [6.9], 620 (50) [0.3], 618 (18) [0.8], 615 (57) [0.7], 602 (13) [1.2], 601 (12) [0.1], 599 (10) [0.7], 595 (12) [0.8], 594 (1.0) [0.4], 584 (19) [0.9], 577 (43) [0.4], 487 (325) [1.2], 475 (347) [0.7], 456 (534) [1.8], 401 (1.2) [77], 322 (0.3) [4.2], 263 (2.6) [8.6], 252 (5.3) [7.3], 231 (3.9) [7.2], 228 (0.8) [19], 200 (0.7) [8.1], 131 (2.8) [4.8], 103 (1.1) [11], 94 (0.2) [12], 91 (0.5) [7.1], 66 (0.1) [1.9], 54 (1.1) [4.6], 40 (0.1) [18], 36 (0.1) [22], 33 (0.6) [18], 30 (0.2) [19], 24 (1.1) [2.4], 19 (0.7) [8.1]. $[\text{Ti}(\text{N}_3)_6]^{2-}$: 2234 (1.4) [798], 2186 (3723) [9.5], 2185 (2778) [90], 2180 (2776) [74], 2168 (25) [180], 2167 (468) [134], 1438 (3.6) [70], 1429 (161) [18], 1428 (19) [46], 1425 (182) [23], 1424 (186) [24], 1424 (165) [17], 626 (4.1) [5.9], 622 (34) [1.2], 621 (39) [2.8], 620 (59) [3.9], 620 (0.0) [2.9], 616 (3.1) [3.9], 608 (5.2) [0.2], 605

(8.8) [0.0], 604 (3.9) [0.1], 599 (9.4) [0.1], 599 (9.9) [0.0], 598 (0.6) [0.4], 409 (675) [0.4], 404 (540) [0.9], 404 (601) [1.2], 364 (1.2) [78], 284 (0.2) [11], 280 (0.1) [8.4], 264 (3.0) [7.0], 261 (5.4) [0.2], 250 (3.5) [6.1], 235 (3.6) [8.7], 209 (0.1) [19], 205 (0.2) [7.4], 151 (1.2) [25], 150 (0.2) [15], 142 (0.4) [25], 78 (1.9) [2.2], 63 (0.1) [11], 62 (2.7) [0.4], 44 (2.2) [20], 40 (0.5) [28], 39 (0.0) [14], 33 (1.0) [28], 28 (2.0) [2.7], 23 (0.1) [13], 21 (1.2) [4.1], 16 (0.1) [4.5], 15 (1.2) [6.8].

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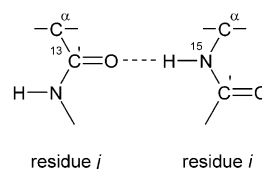
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Direct Observation of Intramolecular Hydrogen Bonds in Peptide 3_{10} Helices by $^3J_{N,C'}$ Scalar Couplings

Massimo Bellanda, Mario Rainaldi,
Quirinus B. Broxterman, Bernard Kaptein,
Fernando Formaggio, Marco Crisma, Stefano Mammi,
and Claudio Toniolo*

In a recent series of papers, Grzesiek and co-workers reported the observation of cross hydrogen-bond ^{15}N – $^{13}\text{C}'$ scalar couplings ($^3J_{N,C'}$) in proteins characterized by α -helix and β -sheet conformations.^[1] This coupling establishes an electron-mediated connection between the amide nitrogen and carbonyl carbon nuclei of two amino acid residues involved in an intramolecular $\text{C}=\text{O}\cdots\text{H}-\text{N}$ hydrogen bond (see schematic representation). The discovery of this spin–spin cou-



pling phenomenon between nuclei across hydrogen bonds offers an important new tool to unequivocally determine the donor and the acceptor (the latter is particularly difficult to identify) of a hydrogen bond in isotopically enriched biomolecules. Other groups have recently published significant theoretical and experimental papers on this methodology by examining α -helix and β -sheet proteins.^[2] Direct evidence for a central α -helical region in the peptaibol chrysospermin C in micelles was obtained by using this approach.^[3]

The principal limitation for a successful detection of these very weak correlations stems from the low sensitivity of the related NMR spectroscopic experiments. Detection of the weak $^3J_{N,C'}$ couplings relies on the use of long de- and rephasing intervals. For large proteins, the sensitivity of this long-range H(N)CO experiment rapidly decreases because of ^{15}N transverse-relaxation losses during the extended magnetization-transfer periods. Although the absolute size of the $^3J_{N,C'}$ couplings is small, it is possible to trace out nearly

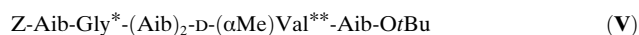
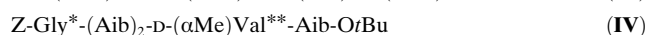
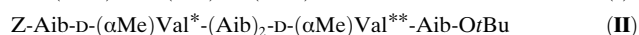
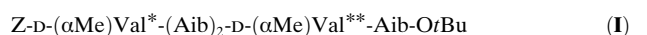
[*] Dr. M. Bellanda, Dr. M. Rainaldi, Prof. F. Formaggio, Dr. M. Crisma, Prof. S. Mammi, Prof. C. Toniolo
Department of Chemistry, University of Padova
and CNR ICB, Padova Unit
via Marzolo 1, 35131 Padova (Italy)
Fax: (+39) 049-827-5139
E-mail: claudio.toniolo@unipd.it

Dr. Q. B. Broxterman, Dr. B. Kaptein
DSM Pharma Chemicals,
Advanced Synthesis and Catalysis
P.O. Box 18, 6160 MD, Geleen (The Netherlands)

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complete hydrogen-bond networks in small, nondeuterated proteins (≤ 10 kDa) by using the long-range H(N)CO method. An improvement in sensitivity can be obtained by quenching the scalar-coupling-mediated relaxation by using an adiabatic or a composite pulse decoupling on the aliphatic carbon atoms^[4] or, for larger proteins, by the TROSY approach^[1b,5] and deuteration. In the small protein ubiquitin, $^3J_{\text{N,C}}$ coupling constants down to 0.1 Hz have been detected.^[1a] More recently, Jaravine et al.^[1d] showed the applicability of this method to a marginally stable α -helical peptide and demonstrated that the size of the individual $^3J_{\text{N,C}}$ coupling constants can be used as a measure of the population of the helical conformation.

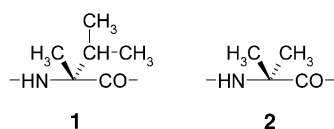
Hydrogen-bond correlations in ubiquitin have been observed for almost all hydrogen bonds in the α helix as well as in the various β sheets, but none of the hydrogen bonds in 3_{10} -helices seen in the crystal and NMR spectroscopically derived structures of ubiquitin give rise to observable correlations. Apparently, the failure to detect $^3J_{\text{N,C}}$ couplings in the 3_{10} -helices of ubiquitin stems from an unfavorable geometry for the hydrogen bonds.^[2g] In the present work, we measured the very small $^3J_{\text{N,C}}$ couplings in 3_{10} -helices for the first time. To this end, we synthesized the series of short peptides **I–V**, which contain, in addition to Gly, the



(Z = benzyloxycarbonyl; ^{*} = ¹³C' = O-labeled residue;

^{**} = ¹⁵N–H-labeled residue)

C ^{α} -tetrasubstituted α -amino acid residues D-(α Me)Val (**1**; C ^{α} -methyl-D-valine) and Aib (**2**; α -aminoisobutyric acid).



These penta- and hexapeptides were designed to fold in stable (left-handed) 3_{10} helices, by taking advantage of the predominant presence of D-(α Me)Val and Aib residues,^[6] and to incorporate selectively ¹³C- and ¹⁵N-labeled residues at appropriate positions suitable for monitoring helical C=O $\cdots$ H–N hydrogen bonds. More specifically, the limited main-chain length (< 8 residues) of the peptides and the 80–100% occurrence of C ^{α} -tetrasubstituted residues in their sequences are known to favor strongly the onset of a 3_{10} helix over an α helix.^[6] In any case, hexapeptide **III**, with its two consecutive ¹³C' = O-labeled residues, was tailored to distinguish between these two types of helical structures. The incorporation of a flexible Gly residue at the N-terminus (pentapeptide **IV**) or near the N-terminus (hexapeptide **V**) was expected to provide information on fraying of the helical end.

The X-ray diffraction structure of the terminally protected pentapeptide **I** represented unambiguous experimental proof

of the conformational preference of the above peptides for the 3_{10} helix in the crystal state (Figure 1). The molecules are indeed found in a regular, left-handed, 3_{10} -helical structure (from residues 1 to 4). The C-terminal Aib residue is helical

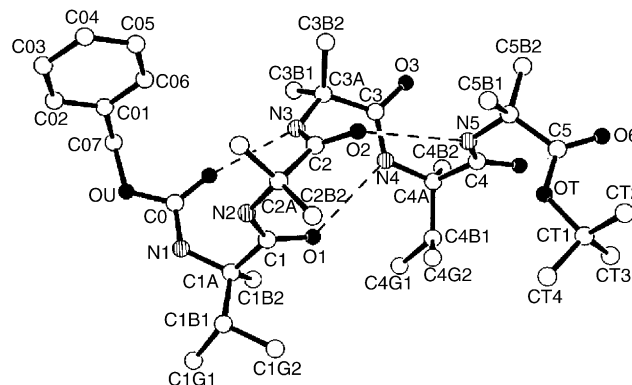


Figure 1. X-ray diffraction structure of the terminally protected, left-handed, 3_{10} -helical pentapeptide **I** with atom numbering. The three C=O $\cdots$ H–N intramolecular hydrogen bonds are represented by dashed lines.

too, but the signs of the ϕ, ψ torsion angles are opposite to those exhibited by the preceding residues (a common observation for 3_{10} -helical peptide esters).^[6a] The average ϕ, ψ torsion angles for the four left-handed helical residues are $+54.9^\circ, +28.7^\circ$, which are close to the values expected for a 3_{10} helix.^[7] Three consecutive C=O $\cdots$ H–N intramolecular hydrogen bonds of the $i \leftarrow i + 3$ type stabilize the helical structure (N3–H3 $\cdots$ O0=C0, N4–H4 $\cdots$ O1=C1 and N5–H5 $\cdots$ O2=C2). The corresponding N $\cdots$ O distances are 3.183(3), 3.003(3), and 3.107(3) Å, and the N–H $\cdots$ O angles are $158.5^\circ, 170.6^\circ$, and 167.3° , respectively.

The FTIR absorption spectra in the N–H stretching region of all five peptides in solution (CDCl₃; $c = 1$ mM), in which self-association is absent (not shown), are similar and characterized by two bands. As an example, Figure 2 illustrates the spectrum of pentapeptide **I**. The weak absorption at ~ 3430 cm^{–1} is assigned to free, solvated NH groups and the stronger absorption at ~ 3345 cm^{–1} to hydrogen-bonded NH groups.^[8a,b] These curves are typical of helical peptides. In addition, the ratios of the integrated molar extinction coefficients for the hydrogen-bonded and free NH groups of the five peptides fall nicely on the curve of these ratios as a function of the number of peptide units in 3_{10} -helical peptides, as recently reported and discussed by Pispisa et al.^[8c] These FTIR absorption results provided firm and independent evidence that the 3_{10} -helical structure preferred in the crystalline state by the (α Me)Val- and Aib-rich peptides is also largely populated in noncompeting solvents.

For the detection of the J coupling across the hydrogen bond between the donor ¹⁵N and the acceptor carbonyl ¹³C, a 2D version of the pulse scheme proposed by Cordier and Grzesiek^[1a] was used, in which the chemical shift evolution of the ¹⁵N nucleus was not recorded. The sensitivity required to detect very small coupling constants was achieved for several reasons. First, the peptides examined have a strong tendency to fold in stable 3_{10} helices. More importantly, as they are very

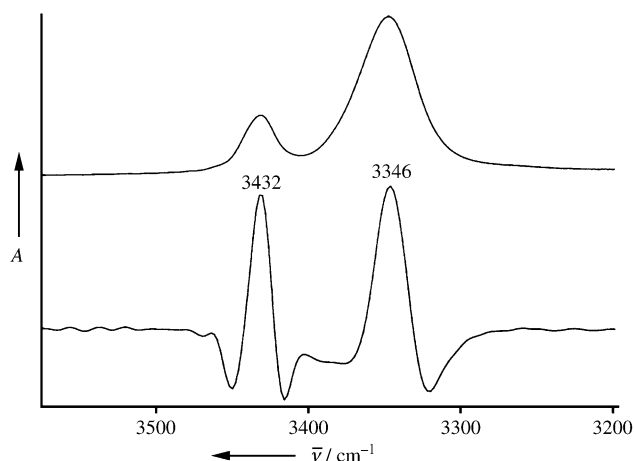


Figure 2. FTIR absorption and inverted second derivative spectra (3550–3200 cm^{-1} region) of the terminally protected pentapeptide I in CDCl_3 ($c = 1 \text{ mM}$).

soluble in MeOH, concentrations in the order of 10 mM were easily obtained. This was a key factor, because the chance to observe a cross-peak increases very slowly with the number of scans, making the use of high concentrations often the only possibility to detect small couplings. Furthermore, ^{15}N transverse-relaxation losses decrease owing to the very small size of the peptides and the scalar-coupling-mediated relaxation is negligible because the aliphatic carbon atoms are present at natural abundance. Considering a detection limit of three times the standard deviation of the noise obtained in the different experiments, couplings down to 0.03–0.04 Hz could be detected. The portions of the spectra showing the correlations of interest are shown in Figure 3.

The size of the long-range coupling was determined from the measured intensity ratio for the correlation $^{15}\text{N}_i, ^{13}\text{C}'_j$ in the long-range experiment (I_{lr}) relative to the $^{15}\text{N}_i, ^{13}\text{C}'_{i-1}$ correlation in the reference experiment (I_{ref}). The implicit equation for $I_{\text{lr}}/I_{\text{ref}}$ described by Cordier and Grzesiek^[1a] was solved by numerical inversion. This equation requires a knowledge of the $^1J_{\text{N}(\text{C}')_{i-1}}$ values, which cannot be determined easily with high accuracy with our labeling scheme. Therefore, the equation was solved with $^1J_{\text{N}(\text{C}')_{i-1}} = 15.6 \text{ Hz}$, a value in the middle of the range reported for the 3_{10} -helical segment in ubiquitin (15.1–16.1 Hz).^[9] A different choice would not produce very different results. Even considering the full range of couplings measured in ubiquitin (13.5–17.2 Hz), the calculated $^3hJ_{\text{N},\text{C}'}$ values would differ less than 0.02 Hz from those reported in Figure 3. The uncertainty in $^1J_{\text{N}(\text{C}')_{i-1}}$ is the main source of error in $^3hJ_{\text{N},\text{C}'}$. When three standard deviations of the noise are used as the statistical error for the experimental intensities, the error propagation on the simplified formula proposed in reference [1a] leads to much smaller errors in $^3hJ_{\text{N},\text{C}'}$. The role of the $^2J_{\text{N}(\text{C}')}$ passive coupling in the experimental error was considered to be negligible because the carbonyl group of the residue containing the ^{15}N amide is not isotopically enriched, and the values of this constant are small anyway.

With the exception of peptide IV, a signal indicating the presence of a hydrogen bond typical of the 3_{10} helix between

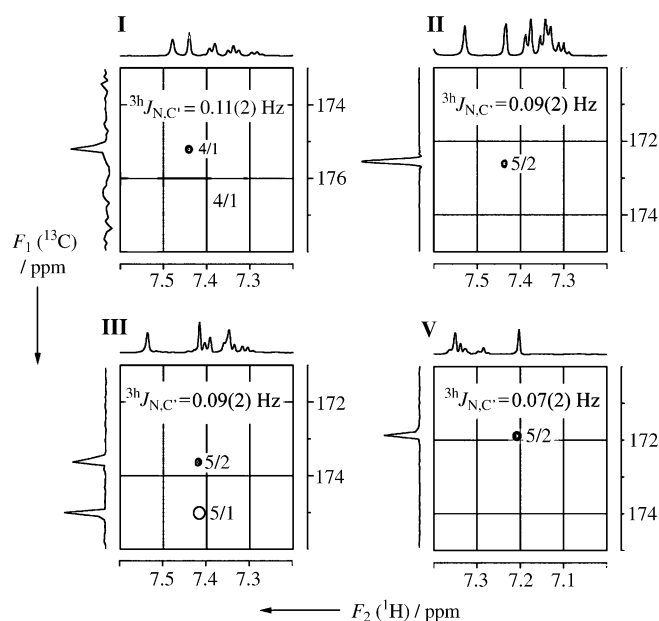


Figure 3. Portions of the long-range 2D H(N)CO spectra of the terminally protected peptides I, II, III, and V in CD_3OH ($c \sim 10 \text{ mM}$). Peaks are marked by the residue number of the donor N–H group followed by the residue number of the acceptor carbonyl group. In each spectrum, the value of $^3hJ_{\text{N},\text{C}'}$ is also indicated. The error in these values was estimated to be lower than 0.02 Hz. For peptide III, in which two ^{13}C -labeled (αMe)Val residues are present, resonances of the two N-terminal amino acids were assigned by combination of a ROESY experiment and an HMBC experiment (not shown). The area of the spectrum where the $(i+4)^{15}\text{N}$ – $(i)^{13}\text{C}'$ scalar coupling was expected, is indicated by an empty circle.

labeled C=O and N–H groups was always observed. The measured $^3hJ_{\text{N},\text{C}'}$ values (Figure 3) are significantly lower than those recorded for α -helical peptides, as expected from the less-than-optimal hydrogen-bond geometry in 3_{10} helices.

For peptide IV, which contains a Gly residue at the N-terminus, it was not possible to observe any signal above the detection limit, probably because of the high mobility of this protein residue. When an Aib residue was added at the N-terminus (peptide V), the peak corresponding to the hydrogen bond between Gly² and (αMe)Val⁵ was clearly observed, although the slightly lower value of $^3hJ_{\text{N},\text{C}'}$ might indicate that some fraying is still present. Finally, in peptide III, designed to allow direct detection of the hydrogen bond for both the 3_{10} - and the α -helical structures, only the signal indicating the presence of the 3_{10} helix was seen, unequivocally demonstrating the propensity of these short peptides for such a type of helical folding. From our data, the presence of a small population of α -helical peptides cannot be excluded. Based on the fact that the detection limit for this particular experiment is 0.03 Hz and that the average value found for the $^3hJ_{\text{N},\text{C}'}$ constants in α -helical hydrogen bonds of the fully folded ubiquitin is $0.38 \pm 0.12 \text{ Hz}$,^[1a] an α -helix population of less than about 10 % would not be observable.

In summary, we have determined the correlation between the ^{13}C -labeled (i) carbonyl group and the hydrogen-bonded proton of the ^{15}N -labeled $(i+3)$ NH group for five short 3_{10} -helical peptides. The magnetization transfer is achieved by

$^3J_{\text{N,C}}$ scalar coupling through the hydrogen bond in an H(N)CO-type experiment. Interestingly, in MeOH, a solvent compatible with peptide helical structures, this phenomenon is clearly seen with peptides **I**, **II**, **III** and **V**, but not with peptide **IV**, most probably because the labeled carbonyl residue (Gly) in the latter is quite flexible and located at the beginning of the main chain (N-terminal fraying). Of the five peptides, only pentapeptide **IV** does not bear a conformationally restricted C $^{\alpha}$ -tetrasubstituted α -amino acid at the N-terminus. This study is the first successful application of this novel and important NMR spectroscopic methodology to 3_{10} -helical peptides. It is also noteworthy that in peptide **III** the ^{15}N – ^{13}C scalar coupling between the (*i*) (α Me)Val carbonyl carbon atom and the (*i* + 4) (α Me)Val amide nitrogen atom is not seen, allowing us to conclude that the α -helical structure is essentially absent in this hexapeptide. This finding demonstrates the power of this technique to discriminate between the two most relevant helical structures (α vs. 3_{10}) in peptides and proteins. However, our results indicate that detection of intramolecular C=O...H–N hydrogen bonds in 3_{10} helices by $^3J_{\text{N,C}}$ scalar couplings is feasible only when peptide segments are conformationally rigidified.

Experimental Section

Preparation of unlabeled and ^{15}N –H- or ^{13}C –O-labeled, enantiopure C $^{\alpha}$ -methyl-D-valines was performed by DSM Pharma Chemicals.^[10] Terminally protected peptides were synthesized step by step in solution, beginning from the C-terminus. These sterically demanding peptide bonds were formed in moderate to good yields either by the *N*-ethyl, *N'*-[(3-(dimethylamino)propyl]carbodiimide/7-aza-1-hydroxy-1,2,3-benzotriazole^[11a] or by the acyl fluoride^[11b] C-activation method. For details of the synthesis and characterization of the five final peptides and their synthetic intermediates, see Supporting Information.

IR absorption: The solution IR absorption spectra were recorded on a Perkin-Elmer model 1720 X FTIR spectrometer. Spectrograde CDCl_3 (99.8%) was purchased from Aldrich.

NMR: The H(N)CO type experiments were carried out at 298 K, in CD_3OH on a Bruker Avance 600 instrument. Each spectrum results from $80(\text{t1}) \times 1024(\text{t2})$ complex data points and was recorded with 480 scans. The reference spectrum was acquired with the same parameters in an interleaved way, and the total measuring time for each pair of spectra was 56 h.

X-Ray diffraction: The data were collected on a Philips PW1100 four-circle diffractometer. Single crystals suitable for X-ray diffraction analysis were grown from ethyl acetate/petroleum ether by vapor diffusion.^[12]

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Keywords: helical structures · IR spectroscopy · NMR spectroscopy · peptides · X-ray diffraction

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- [12] Crystal-structure determination of **I** ($\text{C}_{36}\text{H}_{59}\text{N}_5\text{O}_8$): crystal dimensions $0.5 \times 0.2 \times 0.2 \text{ mm}^3$; orthorhombic, $P2_12_12_1$; $a = 11.710(3) \text{ \AA}$, $b = 16.319(4) \text{ \AA}$, $c = 21.269(5) \text{ \AA}$; $V = 4064.4(17) \text{ \AA}^3$; $\rho_{\text{calc}} = 1.127 \text{ Mg m}^{-3}$; $2\theta_{\text{max}} = 120^\circ$; $\text{Cu K}\alpha$ radiation ($\lambda = 1.54178 \text{ \AA}$), θ - 2θ scan mode, $T = 293 \text{ K}$; 3750 collected reflections, 3702 of which were independent; h, k, l limits: $-1 \leq h \leq 13$, $0 \leq k \leq 18$, $0 \leq l \leq 23$. Intensities were corrected for Lorentz and polarization effects, not for absorption ($\mu = 0.647 \text{ cm}^{-1}$). The structure was solved by direct methods by using the SIR 2002 program,^[13a] and anisotropically refined by full-matrix block least-squares on F^2 by application of the SHELXL 97^[13b] program. Data/restraints/parameters: 3702/1/430. Hydrogen atoms were calculated at idealized positions and refined as riding. $R_1 = 0.047$ [on $F \geq 4\sigma(F)$]; $wR_2 = 0.148$ (on F^2 , all data). Max./min. residual electron density peaks: $+0.512/-0.229 \text{ e \AA}^{-3}$. CCDC 230-250 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).
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Vanadium–Alkylidene Complexes

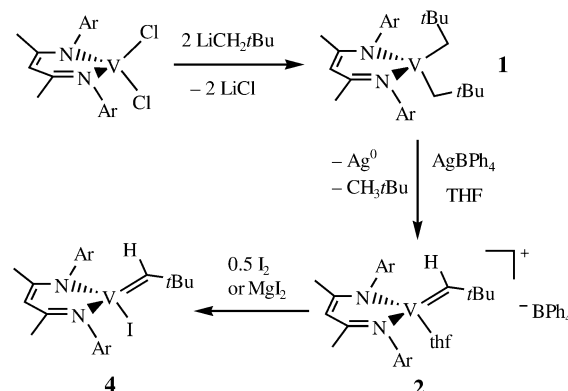
Cationic and Neutral Four-Coordinate Alkylidene Complexes of Vanadium(IV) Containing Short V=C Bonds**

Falguni Basuli, Uriah J. Kilgore, Xile Hu, Karsten Meyer, Maren Pink, John C. Huffman, and Daniel J. Mindiola*

High-oxidation state transition-metal alkylidenes have received considerable attention in the past few years since these systems are capable of catalyzing important processes such as olefin metathesis, alkyne polymerization, and Wittig-type reactions.^[1,2] Although transition-metal alkylidene complexes of the nucleophilic “Schrock-type” have been extensively studied for the 4d and 5d metals of Group 5, the chemistry for 3d vanadium systems has been far less explored. The first example of a vanadium(III)–alkylidene complex, [CpV(CH*t*Bu)(dmpe)] (dmpe = bis(dimethylphosphanyl)ethane), was reported by Hessen, Teuben, and co-workers and involved α -hydrogen abstraction of the bis(alkyl) precursor.^[3] In contrast to the heavier d^0 alkylidene congeners, which are prepared by α -hydrogen abstraction, the synthesis of the first vanadium(V)–alkylidene complex [CpV(NAr)(CHPh)(PMe₃)] (Ar = 2,6-(CHMe₂)₂C₆H₃) involved a two-electron oxidation of the precursor [CpV(NAr)(PMe₃)₂] with the Wittig reagent Ph₃P=CHPh by alkylidene transfer.^[4] Since there are only a handful of vanadium–alkylidene complexes known,^[3–6] we report the synthesis of the first cationic and neutral four-coordinate vanadium(IV)–neopentylidene complexes [(Nacnac)V=CH*t*Bu(thf)](BPh₄) and [(Nacnac)V=CH*t*Bu(I)] (Nacnac[−] = [Ar]NC(Me)CHC(Me)N[Ar], Ar = 2,6-(CHMe₂)₂C₆H₃), and subsequent thermolysis of each system. These d^1 paramagnetic species reported herein are kinetically stable, and contain the shortest V=C bonds reported so far.

Our recent strategy to prepare the first four-coordinate titanium–neopentylidene complex by an oxidatively induced α -hydrogen abstraction reaction^[7] motivated us to pursue other 3d transition metals containing this reactive motif.

Unlike titanium, vanadium displays more diverse redox chemistry, hence oxidation states ranging from +2 to +5 can occur. Using the precursor [(Nacnac)VCl₂]^[8] developed by Budzelaar and co-workers and two equivalents of LiCH₂*t*Bu,^[19] we prepared the corresponding bis(neopentyl) complex [(Nacnac)V(CH₂*t*Bu)₂] (**1**) in 80 % yield as dark brown needles (Scheme 1). Attempts to obtain crystals suitable for a



Scheme 1. Synthesis of the vanadium(IV)–alkylidene complexes **2** and **4**.

single-crystal X-ray structure determination were unsuccessful, but elemental analysis, magnetic susceptibility measurements (μ_{eff} = 2.96 μ_{B}), and reactivity (vide infra) are in agreement with the formation of **1**.^[9] Complex **1** is a close analogue of the *n*Bu derivative prepared by Budzelaar and co-workers, differing only by the lack of β -hydrogen atoms.^[8] Cyclic voltammograms of **1** (TBAH, THF; TBAH = tetra-*n*-butylammonium hexafluorophosphate) revealed reversible oxidation and reduction waves at −0.35 and −2.68 V, respectively, which correspond to the V^{III}/V^{IV} and V^{III}/V^{II} redox couple (referenced versus [FeCp₂]/[FeCp₂]⁺).^[9] Chemical oxidation of **1** with AgBPh₄ leads to formation of a Ag⁰ mirror concurrent with the vanadium(IV)–alkylidene complex [(Nacnac)V=CH*t*Bu(thf)](BPh₄) (**2**) in 74 % yield (Scheme 1). Complex **2** is stable in the solid state, but its solutions (Et₂O, C₆H₆, THF) gradually decompose over several hours at room temperature.

Dark brown single crystals of **2** were grown from THF solutions of the complex layered with Et₂O at −35 °C. The structure of **2** (Figure 1) reveals a cationic and four-coordinate vanadium center with pseudotetrahedral geometry, a very short V=C bond length (V(1)–C(33) 1.795(3) Å), and an obtuse V(1)–C(33)–C(34) angle of 159.8(4)°. The highly distorted neopentylidene ligand suggests that there is a significant α -agostic CH interaction with the metal center. Since **2** is a d^1 complex, α -elimination is inhibited, and the complex distorts to form an unusually short V=C bond. The vanadium center deviates from the N(2)–N(6)–C(33) mean plane by 0.592(6) Å, and as consequence of the cationic nature of the metal center, a coordinated molecule of THF occupies the fourth site. EPR (g_{iso} = 1.982, A_{iso} = 88 × 10^{−4} cm^{−1}, ⁵¹V, I = 7/2, 99.6 %, Figure 2) and magnetic susceptibility measurements (SQUID, μ_{eff} = 1.85 μ_{B} ; Evans’,

[*] Dr. F. Basuli, U. J. Kilgore, M. Pink, Dr. J. C. Huffman, Prof. Dr. D. J. Mindiola
Department of Chemistry and Molecular Structure Center
Indiana University, Bloomington, IN 47405 (USA)
Fax: (+1) 812-855-8300
E-mail: mindiola@indiana.edu

X. Hu, Prof. Dr. K. Meyer
Department of Chemistry and Biochemistry, Mail Code 0358
University of California, San Diego
9500 Gilman Drive, La Jolla, CA 92093-0358 (USA)

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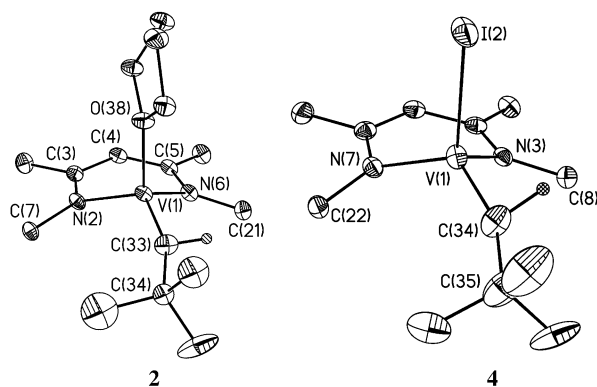


Figure 1. Molecular structure of complexes [(Nacnac)V=CH*t*Bu(thf)][−] (BPh₄)[−] (**2**) and [(Nacnac)V=CH*t*Bu(I)] (**4**) with thermal ellipsoids at the 50% probability level. Hydrogen atoms with the exception of the alkylidene carbon atoms (C(33), **2**; C(34), **4**), and aryl groups on the nitrogen atoms with the exception of the *ipso*-carbon atoms (C(7), C(21), C(8), C(22)) are omitted for clarity. The [BPh₄][−] ion and two disordered THF solvent molecules are omitted for clarity in the structure of **2**. Selected bond lengths [Å] and angles [°]: for **2**: V(1)–C(33) 1.795(3), V(1)–N(2) 1.942(3), V(1)–N(6) 1.964(3), V(1)–O(38) 2.000(2); V(1)–C(33)–C(34) 159.8(4), O(38)–V(1)–C(33) 101.87(13), C(33)–V(1)–N(6) 112.82(15), C(33)–V(1)–N(2) 122.03(15), N(6)–V(1)–N(2) 96.12(11). For **4**: V(1)–C(34) 1.787(3), V(1)–N(3) 1.989(2), V(1)–N(7) 1.935(2), V(1)–I(2) 2.6478(6); V(1)–C(34)–C(35) 158.7(3), I(2)–V(1)–C(34) 110.65(12), C(34)–V(1)–N(3) 107.08(12), C(34)–V(1)–N(7) 123.41(15), N(7)–V(1)–N(3) 94.93(9).

$\mu_{\text{eff}} = 1.87 \mu_{\text{B}}$) are in accordance with a d¹ paramagnetic species and unambiguously rule out the possibility that oxidation of a V–C bond in **1** occurred instead to give the V^{III} cation [(Nacnac)V(CH*t*Bu)(thf)]⁺. Similar species have been prepared by protonation reactions of the corresponding bis(alkyl) systems.^[11] The role of the anion in the oxidation of **1** to form **2** is key in the oxidatively induced α -hydrogen abstraction process inasmuch as oxidation of **1** with AgOTf oxidizes the vanadium–carbon bond instead of the vanadium center. Hence, the V^{III} complex [(Nacnac)V(CH*t*Bu)(OTf)] (**3**) is formed in good yields (78%) when **1** is treated with AgOTf. Elemental analysis, solution magnetic susceptibility data, and single-crystal X-ray diffraction studies are consistent with the connectivity proposed for complex **3**.^[9] Complex **2** can undergo smooth anion exchange with 0.5 equivalents of I₂ (47% yield) or excess MgI₂ (69% yield) to produce the neutral, four-coordinate vanadium–alkylidene complex [(Nacnac)V=CH*t*Bu(I)] (**4**) (Scheme 1). In the reaction of **2** with I₂, we also detect, by ¹¹B NMR spectroscopy, the formation of BPh₃ as a side product. This observation suggests that I₂ might be also oxidizing the B–C(phenyl) bond of the BPh₄[−] counter anion to afford **4**. Magnetic susceptibility measurements (Evans', $\mu_{\text{eff}} = 1.94 \mu_{\text{B}}$), single-crystal X-ray diffraction study, and solution EPR ($g_{\text{iso}} = 1.976$, $A_{\text{iso}} = 64 \times 10^{-4} \text{ cm}^{-1}$, ⁵¹V, $I = 7/2$, 99.6%, Figure 2) spectra are consistent with the d¹ vanadium(IV) complex **4** containing a terminal alkylidene functionality. Cyclic voltammograms of **4** (TBAH, THF) revealed irreversible oxidation and reduction waves at −0.18 and −2.28 V, respectively (referenced versus [FeCp₂]/[FeCp₂]⁺).^[9] This suggests that on the cyclic voltammetry time scale the putative vanadium(III) and vanadium(V) systems of

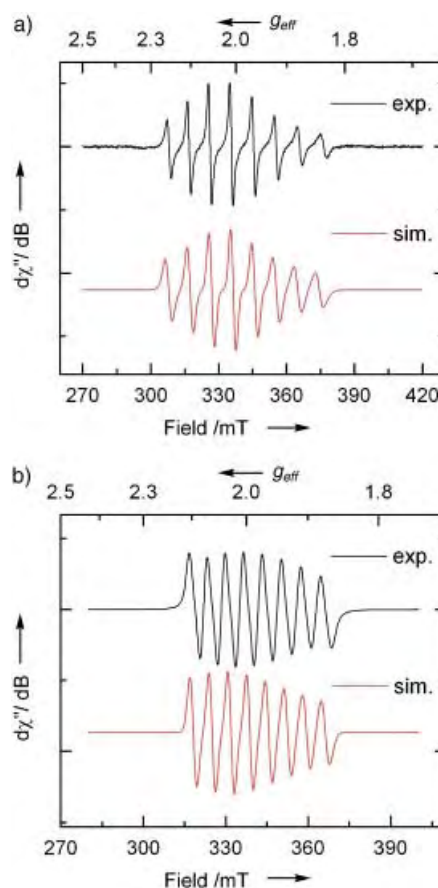
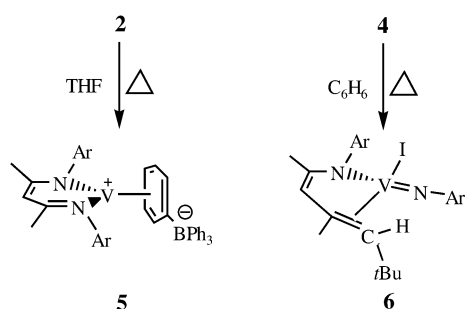


Figure 2. X-band EPR spectrum of **2** (a) and **4** (b) recorded at room temperature in toluene. Experimental spectra (exp.) are above the simulated spectra (sim.).

4, generated by one-electron reduction and one-electron oxidation, respectively, appear to be unstable. In addition, chemical reduction and oxidation of complex **4** resulted in decomposition products. The molecular structure of **4** is depicted in Figure 1 and reveals the shortest V=C_α{alkylidene} bond reported so far (V(1)–C(34) 1.787(3) Å) as well as an obtuse V–C_α–C_β angle (V(1)–C(34)–C(35), 158.7(3)°).^[12] Complexes **2** and **4** are isostructural, where the bound THF molecule and cationic charge in **2** are replaced with I[−] to generate the neutral complex **4**.

Owing to the instability of the previously reported four-coordinate alkylidene complex [(Nacnac)Ti=CH*t*Bu(OTf)],^[7] it was presaged that complexes **2** and **4** would also be prone to “Wittig-like” reactivity involving the metal–alkylidene functionality. Accordingly, when complex **2** is heated at 60 °C for 6 h in THF, extrusion of neopentane is observed (by ¹H NMR spectroscopy and GC MS analysis) concomitant with the formation of the zwitterion vanadium(II) product [(Nacnac)V(η⁶-C₆H₅BPh₃)] (**5**) (77% yield, Scheme 2). THF is dehydrogenated during the thermolysis inasmuch as [D₂]neopentane is observed when the reaction is carried out in [D₈]THF. Complex **5** is an orange, paramagnetic species, and the X-band EPR solution spectra in toluene, as well as solution and solid-state magnetic susceptibility measurements are in accordance with the molecule having a quartet ground



Scheme 2. Thermolysis of complexes **2** and **4** to prepare **5** and **6**, respectively.

state spin configuration (SQUID, $\mu_{\text{eff}} = 3.93 \mu_{\text{B}}$; Evans', $\mu_{\text{eff}} = 3.68 \mu_{\text{B}}$).^[9] The structure determined for **5** by using suitable single crystals also substantiates the proposed connectivity and illustrates clearly a low-valent, two-coordinate (Nacnac)-V^{II} scaffold coordinating to one of the phenyl groups attached to the borate (Figure 3).^[13] In contrast to the transformation

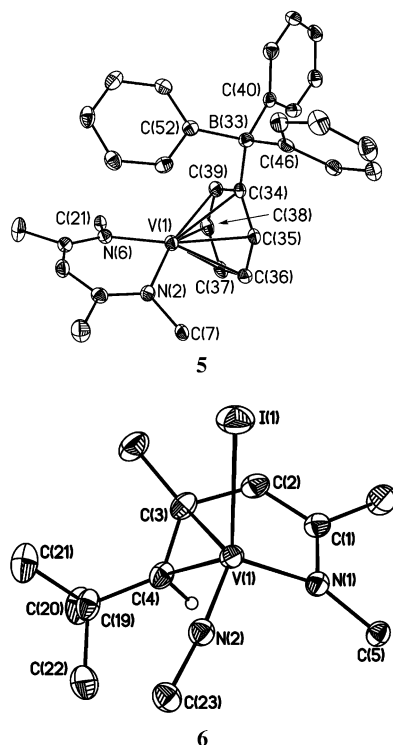


Figure 3. Molecular structure of complexes $[(\text{Nacnac})\text{V}(\eta^6\text{-C}_6\text{H}_6\text{BPh}_4)]$ (**5**) and $[(\text{Ar})\text{NC}(\text{Me})\text{CHC}(\text{Me})\text{CH}t\text{Bu})\text{V}=\text{NAr}(\text{I})]$ (**6**) with thermal ellipsoids at the 50% probability level. Hydrogen atoms with the exception of the olefinic carbon atom (C(4), **6**), and aryl groups on the nitrogen atoms with the exception of the *ipso*-carbon atoms (C(7), C(21), C(5), C(5)) are omitted for clarity. Selected bond lengths [Å] and angles [°]: For **5**: V(1)–C(34) 2.445(2), V(1)–C(35) 2.363(2), V(1)–C(36) 2.340(2), V(1)–C(37) 2.322(2), V(1)–C(38) 2.353(2), V(1)–C(39) 2.383(2), V(1)–N(6) 2.066(8), V(1)–N(2) 2.082(8); N(6)–V(1)–N(2) 90.89(7). For **6**: V(1)–N(1) 1.985(7), V(1)–N(2) 1.687(8), V(1)–I(1) 2.6589(4), V(1)–C(4) 2.219(2), V(1)–C(3) 2.387(2), C(4)–C(3) 1.404(3); C(3)–C(4)–C(19) 127.2(9), I(1)–V(1)–C(4) 125.89(6), N(2)–V(1)–N(1) 112.69(8), C(4)–V(1)–N(2) 100.42(8), C(4)–V(1)–N(1) 89.76(8).

of **2** to **5**, when a solution of **4** in benzene is thermolyzed for 6 h at 60 °C, “Wittig-like” reactivity is observed, which is evidenced by the formation of the vanadium(IV)–imide complex $[(\text{Ar})\text{NC}(\text{Me})\text{CHC}(\text{Me})\text{CH}t\text{Bu})\text{V}=\text{NAr}(\text{I})]$ (**6**) (57% yield; Scheme 2). Solution EPR and magnetic susceptibility measurements are consistent with a d^1 system.^[9] In addition, the molecular structure of **6** was obtained from single-crystal X-ray diffraction studies and disclosed a low-coordinate vanadium(IV) center supported by an imide, and an anilide with a η^2 -coordinated diene pendant arm.^[14] The low-coordinate environment at the metal center promotes η^2 coordination of the ene motif, which is now part of the former Nacnac[−] ligand (Figure 3). Similar transformations have been reported by our group for four-coordinate $[(\text{Nacnac})\text{Ti}^{\text{IV}}]$ complexes with reactive alkylidene or phosphinidene functionalities.^[7,15] Concentration-dependent experiments on the titanium–alkylidene system (by ¹H NMR spectroscopy) determined the cross-metathesis reaction to be first-order in titanium, hence we suspect the same to be valid for the thermolytic transformation of **4** to **6**.

In summary, the cationic and neutral four-coordinate complexes (**2** and **4**, respectively) derived from the one-electron oxidation of the bis(alkyl)vanadium(III) precursor **1** represent a new class of alkylidene systems in the context of the organometallic chemistry of vanadium. Further applications of these unique d^1 metal radicals of vanadium containing metal–ligand multiple bonds to reactions such as group- or atom-transfer radical polymerization (ATRP),^[16] and olefin metathesis will be described in due course.

Experimental Section

Synthetic and characterization details for compounds **1–6** (including single-crystal X-ray data for **2–6**)^[17] are described in the Supporting Information.

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Keywords: alkylidenes · carbenes · N ligands · oxidation · vanadium

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- [9] See Supporting Information for complete experimental, spectral, and crystallographic details.
- [10] Crystallographic details for **2·2 THF**: A dark crystal of approximate dimensions $0.30 \times 0.30 \times 0.06 \text{ mm}^3$ was selected and mounted on a glass fiber. A total of 88763 reflections ($-54 \leq h \leq 52$, $-20 \leq k \leq 20$, $-24 \leq l \leq 24$) was collected at $T = 136(2) \text{ K}$ in the range of 2.27 to 27.46° , of which 8308 were observed ($R_{\text{int}} = 0.0957$); $\text{MoK}\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$). A direct-methods solution was calculated which provided most non-hydrogen atoms from the E-map. All non-hydrogen atoms were refined with anisotropic displacement parameters. The residual peak and hole electron densities were 1.692 and $-0.604 \text{ e \AA}^{-3}$, respectively. The absorption coefficient was 0.213 mm^{-1} . The least-squares refinement converged normally with residuals of $R(F) = 0.0733$, $wR(F^2) = 0.2286$, and a GOF = 0.934 ($I > 2\sigma(I)$). $\text{C}_{70}\text{H}_{95}\text{BN}_2\text{O}_3\text{V}$, space group $C2/c$, monoclinic, $a = 42.3319(19)$, $b = 15.8991(7)$, $c = 19.1013(9) \text{ \AA}$, $\beta = 110.2890(10)^\circ$, $V = 12058.3(9) \text{ \AA}^3$, $Z = 8$, $\rho_{\text{calcd}} = 1.183 \text{ mg m}^{-3}$, $F(000) = 4648$.^[17]
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- [13] Crystallographic details for **5·2 Et₂O**: A crystal of approximate dimensions $0.25 \times 0.25 \times 0.08 \text{ mm}^3$ was selected and mounted on a glass fiber. A total of 43991 reflections ($-15 \leq h \leq 15$, $-19 \leq k \leq 19$, $-27 \leq l \leq 34$) was collected at $T = 111(2) \text{ K}$ in the range of 2.07 to 27.53° , of which 5447 were observed ($R_{\text{int}} = 0.1258$); $\text{MoK}\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$). A direct-methods solution was calculated which provided most non-hydrogen atoms from the E-map. Full-matrix least-squares/difference Fourier cycles were performed which located the remaining non-hydrogen atoms. A disordered diethyl ether solvent molecule was located in the cell at an inversion center. All non-hydrogen atoms were refined with anisotropic displacement parameters. The residual peak and hole electron densities were 0.267 and $-0.374 \text{ e \AA}^{-3}$, respectively. The absorption coefficient was 0.260 mm^{-1} . The least-squares refinement converged normally with residuals of $R(F) = 0.0461$, $wR(F^2) = 0.0839$ and a GOF = 0.835 ($I > 2\sigma(I)$). $\text{C}_{55}\text{H}_{66}\text{N}_2\text{O}_{0.5}\text{V}$, space group $P2(1)/n$, monoclinic, $a = 11.5823(11)$, $b = 15.0376(15)$, $c = 26.496(2) \text{ \AA}$, $\beta = 101.522(3)^\circ$, $V = 4521.8(7) \text{ \AA}^3$, $Z = 4$, $\rho_{\text{calcd}} = 1.212 \text{ mg m}^{-3}$.^[17]
- [14] Crystallographic details for **6**: A crystal of approximate dimensions $0.30 \times 0.30 \times 0.24 \text{ mm}^3$ was selected and mounted on a glass fiber. A total of 21477 reflections ($-12 \leq h \leq 12$, $-13 \leq k \leq 15$, $-23 \leq l \leq 23$) was collected at $T = 130(2) \text{ K}$ in the range of 2.03 to 30.02° , of which 7739 were observed ($R_{\text{int}} = 0.0320$); $\text{MoK}\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$). A direct-methods solution was calculated which provided most non-hydrogen atoms from the E-map. Full-matrix least-squares/difference Fourier cycles were performed which located the remaining non-hydrogen atoms. All non-hydrogen atoms were refined with anisotropic displacement parameters. The residual peak and hole electron densities were 1.105 and $-0.614 \text{ e \AA}^{-3}$, respectively. The absorption coefficient was 1.295 mm^{-1} . The least-squares refinement converged normally with residuals of $R(F) = 0.0341$, $wR(F^2) = 0.0869$ and a GOF = 1.020 ($I > 2\sigma(I)$). $\text{C}_{34}\text{H}_{51}\text{IN}_2\text{V}$, space group $P\bar{1}$, triclinic, $a = 9.0935(4)$, $b = 11.0457(5)$, $c = 17.0793(8) \text{ \AA}$, $\alpha = 102.4790(10)^\circ$, $\beta = 92.1090(10)^\circ$, $\gamma = 98.7240(10)^\circ$, $V = 1651.30(13) \text{ \AA}^3$, $Z = 2$, $\rho_{\text{calcd}} = 1.339 \text{ mg m}^{-3}$.^[17]
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DNA Synthesis

Small-Interfering-RNA Expression in Cells Based on an Efficiently Constructed Dumbbell-Shaped DNA**

Masumi Taki, Yoshio Kato, Makoto Miyagishi, Yasuomi Takagi, and Kazunari Taira*

Gene therapy is a promising approach for the treatment of human diseases from both acquired and genetic disorders.^[1] The need for improved, effective gene-transfer vector systems has been a roadblock to the successful pharmaceutical application of gene therapy, though both viral and nonviral vectors are currently under evaluation. Viral-vector systems are the most efficient tools for gene delivery into mammalian cells, achieving excellent delivery and expression efficiencies (usually > 90%). However, many disadvantages of a viral system, such as cost, immunogenicity, unpredictable integration, and difficulties in manufacturing, have shifted the focus of interest towards nonviral vectors.^[2,3] Among nonviral-vector systems, direct injection of naked plasmid DNA into cells is the simplest nonviral-gene-delivery method, and so far one of the most successful methods.^[3] However, although this method is the simplest form of gene delivery with plasmid DNA, it still has several disadvantages in its application for gene therapy. For example, these plasmid DNAs usually have extra genes that encode therapeutically unnecessary proteins. Moreover, these extra genes might contain immunostimulatory sequences, such as CpG motifs that can induce immunotoxicity during gene therapy.^[4,5] To overcome these problems, a minimal-size gene-transfer vector was invented.^[4,6] As shown in Figure 1, this new vector is a circular, covalently closed, dumbbell-shaped DNA molecule^[6] that includes only a transcription unit (a promoter and a gene of interest). The dumbbell vector is much smaller (by $\approx 90\%$) than recombi-

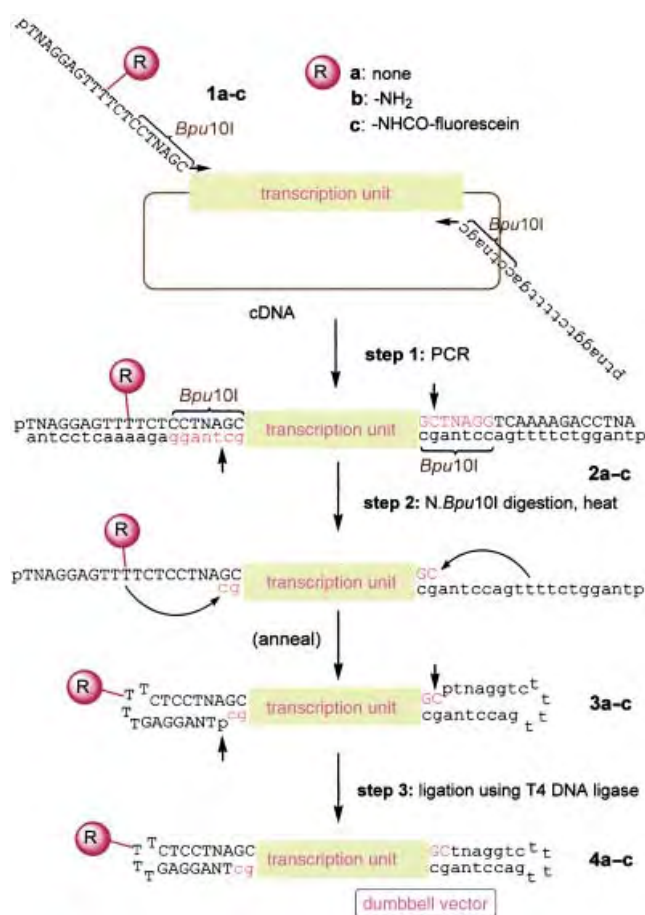


Figure 1. General scheme for the direct and efficient synthesis of dumbbell-shaped DNA by in vitro PCR. a) Plain DNA, b) N-functionalized DNA, c) fluorescein-labeled DNA. For the detailed chemical structures of the oligonucleotides, see the Supporting Information. N.Bpu10I is a site- and strand-specific endonuclease artificially engineered from Bpu10I that cleaves only one strand of the DNA within its recognition sequence on double-stranded DNA substrates; the capital letter (N) means *nicking enzyme*.

nant plasmids routinely used in transfection experiments, thus avoiding the expression of nontherapeutic genes. This vector can also be used to minimize CpG sequences, depending on the gene-transfer application. Another advantage is that circular dumbbell DNAs provide increased stability toward exonucleolytic degradation in a biological environment because of the absence of free termini.^[6-8] Dumbbell-shaped DNA is usually generated by a ligation reaction between one transcription unit backbone and two hairpin DNAs.^[4,6] However, it is extremely difficult to obtain sufficient dumbbell-DNA molecules through a conventional cloning method. (For a conventional cloning method, see the Supporting Information.)

To obtain the large amount of dumbbell-shaped DNA needed, we report herein a new strategy for gene construction in vitro by intramolecular ligation to form the hairpin-loop structure. We used this methodology to construct a small-interfering-RNA (siRNA) expression^[9] dumbbell-shaped vector, in which sense and antisense sequences against a target gene were placed under the control of a U6

[*] Dr. M. Taki, Y. Kato, Dr. M. Miyagishi, Prof. Dr. K. Taira
Gene Function Research Center
National Institute of Advanced Industrial Science and
Technology (AIST)
Central 4, 1-1-1 Higashi, Tsukuba Science City 305-8562 (Japan)
Fax: (+81) 29-861-3019
E-mail: taira@chembio.t.u-tokyo.ac.jp

Y. Kato, Dr. M. Miyagishi, Prof. Dr. K. Taira
Department of Chemistry and Biotechnology
School of Engineering, The University of Tokyo
Hongo, Tokyo 113-8656 (Japan)

Y. Takagi
iGENE Therapeutics, Inc.
Central 4, 1-1-1 Higashi, Tsukuba Science City 305-8562 (Japan)

Dr. M. Taki
Department of Bioscience and Biotechnology
Faculty of Engineering, Okayama University
3-1-1 Tsushima-naka, Okayama 700-8530 (Japan)

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promoter.^[10] The expression of siRNA is expected to trigger the sequence-specific target-gene silencing, called RNA interference (RNAi).^[9] Herein we demonstrate the novel synthesis, the enhanced stability, and, most importantly, the greater efficacy of a dumbbell-shaped siRNA expression cassette over a linear-shaped DNA. The three key steps for the construction of the dumbbell DNA are shown in Figure 1:

Step 1: A gene of interest is amplified by polymerase chain reaction (PCR) with a primer pair that can be chemically modified by various substituents.

Step 2: The amplified DNA is cleaved at two site-specific positions to yield two single-stranded DNA species at both ends of the double-stranded gene of interest. The nicking enzyme (*N.Bpu10I*) cleaves only one DNA strand within its quasi-symmetric recognition sequence on its double-stranded DNA substrate, resulting in an intramolecular annealing to form hairpin ends.

Step 3: The product is then treated with T4 DNA ligase, to form circularized dumbbell-shaped DNA. We emphasize that the yield of cyclization was found to be over 90% because the cyclization involves a simple intramolecular ligation reaction (see below), in contrast to the conventional intermolecular reactions among three DNA molecules.

Dumbbell-shaped DNAs are known to be protected against exonuclease degradation because of their hairpin oligodeoxynucleotide structures at the ends.^[6,8] To confirm an effective ligation with our strategy, the intermediate linear PCR product (**2c**) and the dumbbell-shaped vector (**4c**) were independently treated with exonuclease III and analyzed by an 8% polyacrylamide gel electrophoresis (PAGE). As shown in Figure 2, DNA with free termini (**2c**, lane 2) was totally

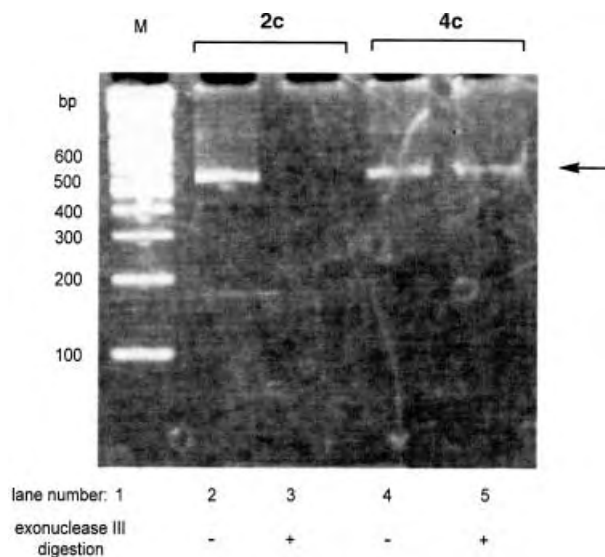


Figure 2. DNA stability toward exonuclease digestion. The same amount of DNA was subjected to 8% PAGE. The gel was stained with ethidium bromide, and the bands were visualized by using a transilluminator at $\lambda_{\text{ex}} = 302$ nm. The labels **2c** and **4c** correspond to those in Figure 1. The bands of approximately 600 bp correspond to fluorescein-labeled DNA.

degraded after incubation for 1 hour with the nuclease (lane 3), whereas the dumbbell DNA (**4c**, lane 4) clearly resisted nucleolytic degradation under the same conditions (lane 5). Next, we compared the conversion yield of our novel dumbbell-cloning method with that of the conventional dumbbell-cloning method.^[4,6] The ratio of band intensities before and after the nuclease digestion reflects the yield of conversion from the linear PCR vector to the dumbbell-shaped vector. As shown in Figure 3, the conversion yield of

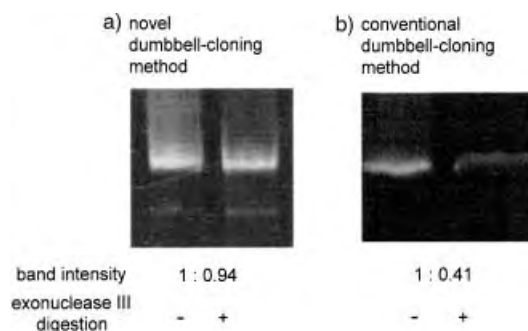


Figure 3. Conversion yields from linear vectors to dumbbell vectors. The same amount of DNA was subjected to 8% PAGE. The gel was then stained with ethidium bromide, and the bands were visualized by using a transilluminator at $\lambda_{\text{ex}} = 302$ nm.

our dumbbell-cloning method was, under all conditions (concentrations), usually over 90% because of the intramolecular reaction, whereas with the conventional dumbbell-cloning method it was around 40% under our optimized conditions because of the intermolecular nature among three independent molecules (two loops and a gene of interest). Our intramolecular ligation method avoids the use of excess amounts of wasteful capping oligonucleotides (loops). Thus, we can routinely obtain large amounts of dumbbell vectors in a single batch reaction.

When a primary amino group is chemically introduced at a predetermined position of an oligonucleotide, it can be functionalized with succinimidyl ester derivatives; the chemical reaction of the succinimidyl esters is limited only to the primary amino group.^[11] As shown in Figure 4, the dumbbell vector (**4b**) with a primary amino group only in the hairpin-loop (lane 1) was successfully converted into a fluorescent dumbbell DNA (yielding **4c**) by reaction with the succinimide ester of fluorescein (lane 2). In contrast, dumbbell DNA (**4a**) without the primary amino group (lane 3) did not react at all with the succinimide ester under the same reaction conditions (lane 4). This means that various substituents can be site-specifically introduced only at the noncoding hairpin-loop region of the dumbbell DNA. Fluorescent dumbbell DNA (**4c**) was also made from the fluorescent primer- (**1c**) mediated direct synthesis by PCR (data not shown).

Using plasmid, linear-, and dumbbell-shaped siRNA expression vectors, the inhibitory efficacies were tested against a target mRNA in cells. These different vectors were individually transfected into HeLa S3 cells that stably expressed EGFP protein. When vectors expressing siRNAs targeted against EGFP were used, a specific and effective

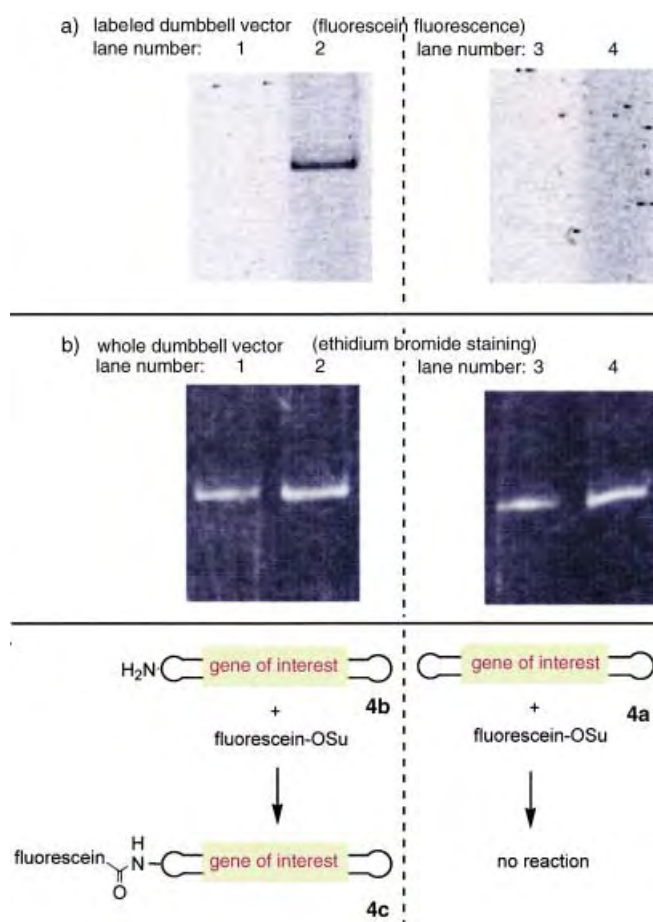


Figure 4. Loop-specific modification of dumbbell vectors by using the succinimide ester of fluorescein. The same amount of DNA was subjected to 8% PAGE. The bands were first visualized by using Fluor-Imager 595 (Molecular Dynamics) at $\lambda_{\text{ex}} = 488$ nm (a). The gel was then stained with ethidium bromide, and the bands were visualized by using a transilluminator at $\lambda_{\text{ex}} = 302$ nm (b). Lanes 1 and 2: dumbbell DNA with a primary amino group in the hairpin loop; lanes 3 and 4: dumbbell DNA with no primary amino group. Lanes 2 and 4 contain samples of the reaction mixture with the succinimide ester of fluorescein. Lanes 1 and 3 contain samples before the reaction.

down-regulation of the target was detected (Figures 5a,d, and e). We found that the best and most reproducible inhibition (more than 70%) was obtained with the dumbbell-shaped cassette (Figure 5d). We also found that the dumbbell-shaped siRNA expression vector was more effective than the linear-shaped PCR product after incubation for 48 h. It is known that RNA interference (RNAi) is one of the best methods for gene suppression.^[9,10,12–14] The RNAi effect triggered by the dumbbell-shaped DNA appears to be higher and to last longer than that triggered by the linear-shaped PCR products, as the effect by the latter PCR products decreased after 48 hours in HeLa S3/EGFP. This decrease suggests that the half-life of dumbbell-shaped DNA inside cells is longer than that of linear DNA. This suggestion can be supported by the fact that dumbbell DNA is stable toward exonuclease III digestion, whereas linear DNA is totally degraded within 1 hour (Figure 2). In contrast, mock transfection had no effect on the expression of EGFP (Figure 5b).

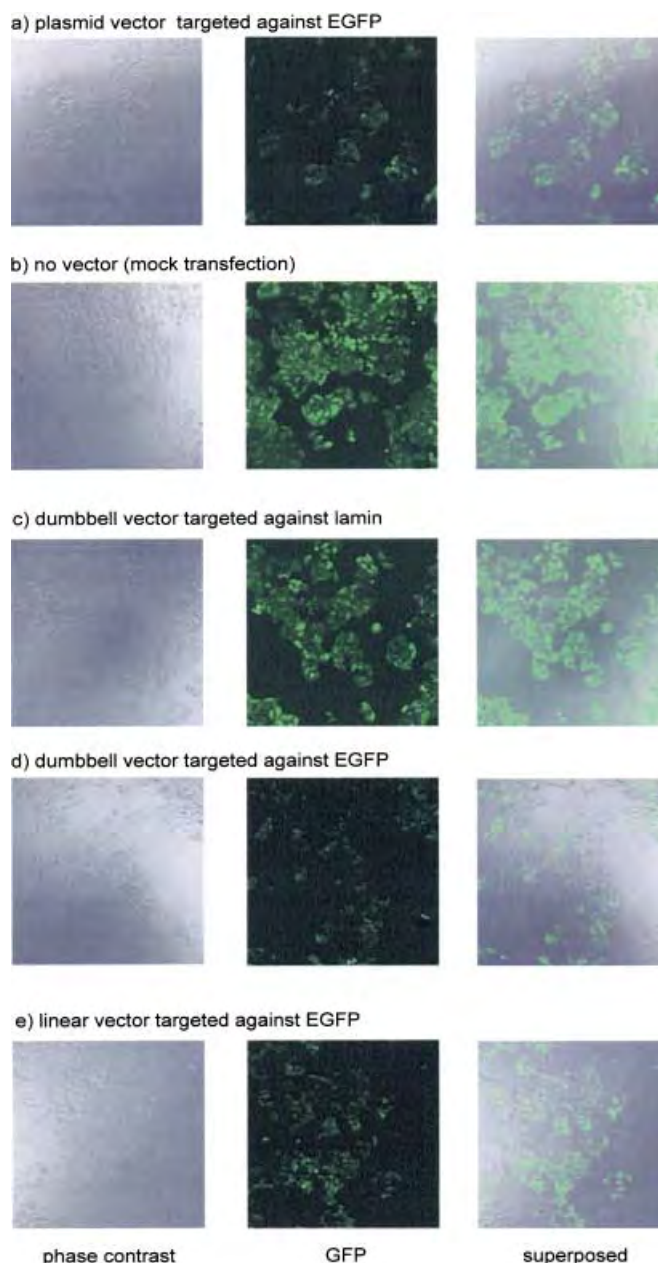


Figure 5. Inhibition of EGFP expression by using various vectors transfected in HeLa S3/EGFP cells.

Similarly, the control dumbbell-shaped U6 expression cassette expressing siRNA targeted against lamin had no effect on the expression of EGFP (Figure 5c), although the lamin level was reduced when using this vector against lamin (see the Supporting Information), which demonstrates the high level of specificity of the vector.

In conclusion, we have established a novel and convenient method for creating dumbbell DNA molecules in excellent yield. The size of the dumbbell DNA obtained by this method cannot be obtained by standard solid-phase cyclization procedures.^[15] Dumbbell-shaped DNA was found to be more stable than linear DNA both in vitro and in vivo. Recently, our group as well as Castanotto et al. reported a convenient, PCR-based approach for the rapid synthesis of

U6 promoter-based siRNA gene constructs without carrying out any subsequent cloning, and their products are now commercially available.^[14,16] By combining the PCR methodology and the present dumbbell-making technology, we can obtain long-term siRNA expressions without carrying out any time-consuming vector construction. This PCR-mediated minimal siRNA vector-construction system will open up new possibilities for biologically safe and stable gene therapy. Specific gene targeting might also be possible by the attachment of various moieties, such as functional peptides and proteins, through or in other primary amino groups on the hairpin-loop at one or both ends.

Experimental Section^[7]

PCR amplification of U6 promoter-driven siRNA coding linear DNA targeted against lamin: 1 μ M of each synthetic DNA primer was mixed with an siRNA expression plasmid against lamin (pU6i-lamin), and the PCR reaction was carried out with Ex Taq DNA polymerase (TaKaRa). The sequences of the sense and antisense *universal* primers for the PCR were 5'-pTTA GGA GTT X_nTC TCC TAA GCG TTT TCC CAG TCA CGA CGT TG-3' and 5'-pTTA GGT CTT TTG ACC TAA GCG AGC GGA TAA CAA TTT CAC ACA GG-3', respectively. Both primers contain the N.Bpu10I recognition sequence (underlined), and the 5'-ends were phosphorylated. The PCR product was isolated by using the Wizard^(R) SV Gel and PCR Clean-Up System (Promega). The sense primer contains unmodified (X₁; dT), amino-modified (X₂, amino-modified dT), or fluorescein-modified (X₃, fluorescein-modified dT) deoxythymidine. The HPLC-purified *universal* primers were purchased from Hokkaido System Science Co., Ltd. (Sapporo, Japan).

PCR amplification of U6 promoter-driven siRNA coding linear DNA targeted against EGFP: 1 μ M of each synthetic DNA primer was mixed with an siRNA expression plasmid against EGFP (pU6i-EGFP), and then the *first* PCR reaction was carried out with Ex Taq DNA polymerase (TaKaRa). The sequence of the sense and antisense primers for the *first* PCR were 5'-GTT TTC CCA GTC ACG ACG TTG AAG GTC GGG CAG GAA GAG-3' and 5'-GAG CGG ATA ACA ATT TCA CAC AGG AAA AAG GCT ACG TCC AGG AG-3', respectively. The *first* PCR product was isolated by 8% polyacrylamide gel electrophoresis (PAGE). The isolated PCR fragment was used as a template DNA in a *second* PCR reaction under the same conditions, using the above *universal* primers. The *second* PCR product was isolated by using the Wizard^(R) SV Gel and PCR Clean-Up System (Promega).

Conversion of the siRNA-expressing linear DNA to Dumbbell-shaped DNA: 18.8 μ g of each linear PCR product was mixed with 18.8 U of N.Bpu10I (MBI Fermentas, Hanover, MD), and the reaction mixture was incubated at 37°C overnight. The mixture was heated for 1 min at 95°C, and then brought from 95°C to room temperature over 1 h. During this step, the terminal hairpin-loop structures were formed. For intramolecular ligation at the hairpin termini, T4 DNA ligase (16000 U) and ligation buffer (TaKaRa) was added directly to the reaction mixture and incubated at 16°C for 3 h. **Note:** The above conversion processes are part of a one-pot reaction and were performed in one microtube. The ligated product was purified by extraction with phenol/chloroform and precipitated with ethanol.

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Sugar-Based Surfactants

Macrocyclic Sugar-Based Surfactants: Block Molecules Combining Self-Aggregation and Complexation Properties**

Chantal Larpent, Annabelle Laplace, and Thomas Zemb*

There is a rise in interest in the rational design of new surfactants that possess various chemical or biological functionalities and self-assemble into tunable and predictable

[*] Prof. C. Larpent, Dr. A. Laplace
Université de Versailles-St Quentin en Y.
SIRCOB UMR-CNRS 8086, LRC CEA DSM 95-1
45 Avenue des Etats-Unis, 78035 Versailles (France)
Fax: (+33) 1-3925-4452
E-mail: larpent@chimie.uvsq.fr
Dr. A. Laplace, Prof. T. Zemb
CEA-Saclay, Service de Chimie Moléculaire
91191 Gif/Yvette (France)

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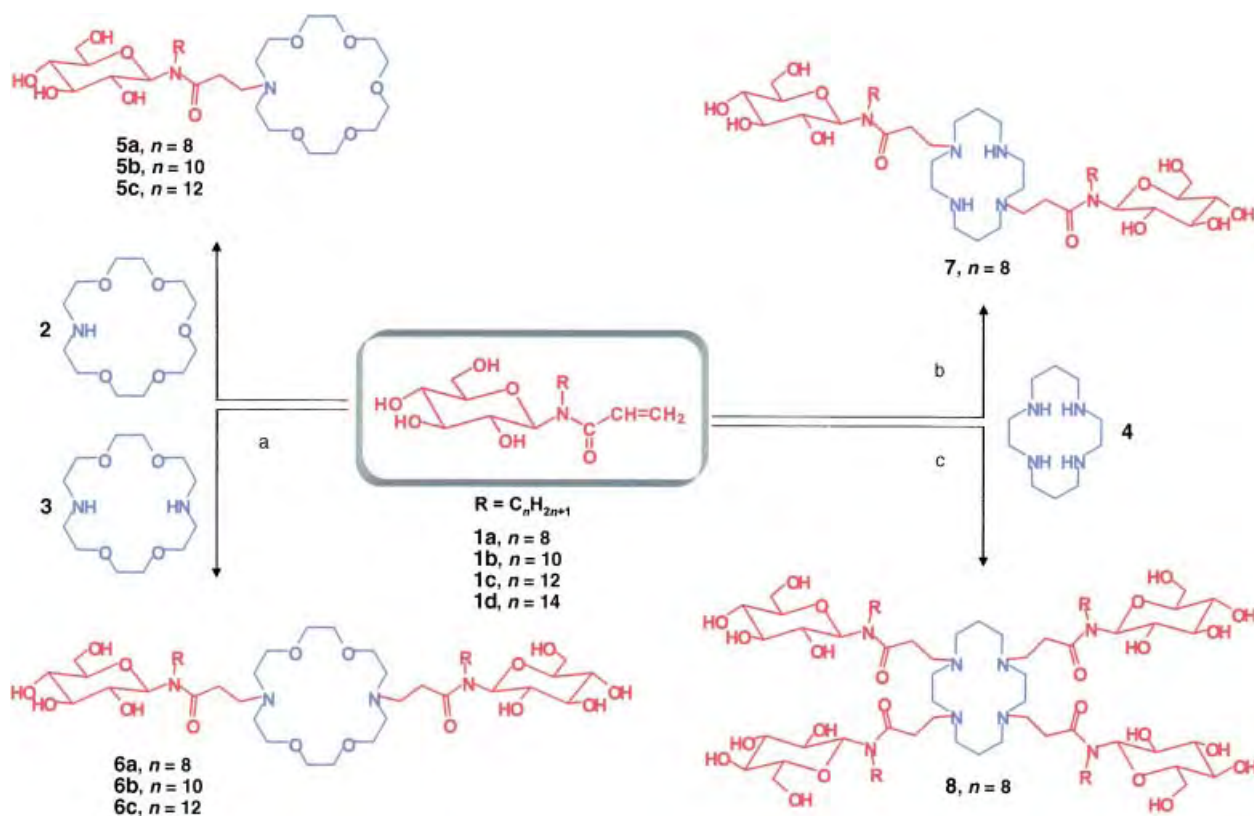
aggregate structures. In the past few years new amphiphiles (many of which are chiral and derived from peptides or carbohydrates) have been proved to be promising scaffolds for nanometer-sized structures and mimics of natural materials.^[1,2] On the other hand, it is now well known that micelles and other supramolecular assemblies can enhance the rates of chemical reactions as well as the regio- and/or stereoselectivity, especially when functional surfactants are used.^[3,4] Chelating surfactants and amphiphilic metal complexes are attracting considerable attention as catalysts for several types of reactions,^[3–5] including enantioselective ones,^[3,6] as well as in separation technology.^[1,7] The main difficulty in dealing with surfactants that have recognition properties is in the profound change associated with the binding of the guest. The complexation often completely changes the solubility and aggregation behavior of metal-binding surfactants in an unpredictable way.^[5,8,9] We have previously observed that micelles of a peculiar gemini sugar-based surfactant with a macrocyclic spacer were surprisingly not altered by the binding of cations.^[10] In this paper we show that this is general for a whole family of novel amphiphiles consisting of one or more sugar-based surfactant units covalently linked to a macrocyclic residue; these amphiphiles combine independent predictable self-aggregation behavior and tunable complexation properties. The bulky sugar-based surfactant units impose the curvature and, hence, the morphology of the aggregates irrespective of the macrocycle. On the other hand, the sugar headgroups provide a dominating water-solubiliza-

tion and thereby minimize the effect of ion association on the micellization.

A series of novel amphiphilic macrocycles is readily prepared by a one-step procedure, depicted in Scheme 1, from surfactant units which include a reactive group that permits the covalent linkage to various macrocyclic residues. *N*-alkylglucosylacrylamides **1a–d**, with octyl to tetradecyl tails, are a family of reactive nonionic sugar-based surfactants which exhibit properties very close to conventional alkylglucosides.^[11] Moreover, they contain an acrylamide group that acts as a Michael acceptor and undergoes nucleophilic addition of azamacrocycles in aqueous medium (Scheme 1).

Two types of macrocycles, with different cation binding selectivities,^[12] have been used: aza[18]crown-6 (compounds **2** and **3**) and cyclam (compound **4**). Crown derivatives **5** and **6**, with one or two surfactant units, are prepared from **2** and **3**, respectively. Amphiphilic crowns with octyl to dodecyl chains are soluble in water.^[13,14] Soluble cyclam derivatives **7** and **8**, with two or four surfactant blocks, are obtained from **4** and the octyl precursor **1a**.^[14,15]

Herein, we focus on the self-aggregation and complexation properties of cyclam/**1a** derivatives (**7**, **8**) and aza[18]-crown-6/**1c** derivatives (**5c**, **6c**). As expected from the molecular structures, the cation-binding capacity and selectivity of the macrocycles are retained after the linkage of one or several surfactant moieties. As illustrated in Figure 1, electrospray mass spectra show that amphiphilic crowns **5c** and **6c** form complexes with alkali cations with a marked



Scheme 1. Synthesis of sugar-based macrocyclic surfactants. a) $1/2 = 4:1$, $1/3 = 5:1$, MeOH/H₂O (3:2)/NMe₄OH (0.04 M), 25 °C, 7 days; yields: 65 (**5a**), 80 (**5b**), 86 (**5c**), 60 (**6a**), 70 (**6b**), 77% (**6c**). b) $1a/4 = 5:1$, H₂O/NaOH (0.1 M), 25 °C, 15 h; 90% yield. c) $1a/4 = 10:1$, MeOH/H₂O (2:1)/NaOH (0.06 M), 40 °C, 2 weeks, 70% yield. For experimental details and structural characterization, see the Supporting Information.

selectivity for potassium and sodium cations, like their parent crowns **2** and **3**.^[12,14,16] Cyclam **4** is a well-known ligand that exhibits a very high affinity for cupric ions;^[12] its derivatives **7** and **8** with two and four surfactant units, respectively, also give stable complexes with Cu^{II} ions, with characteristic absorptions at 640 and 670 nm, close to the absorptions of other N-substituted cyclam–copper complexes.^[17] The stability constants of the complexes, although not determined precisely, are high ($\log K > 6$), with quantitative complexation even at low copper concentrations (10^{-4} M).^[14]

The characteristic properties of any surfactant are 1) a well-defined critical micelle concentration (CMC) and 2) self-aggregation in the form of small stable micelles.^[18] Cage molecules with two or four surfactant moieties behave like oligomeric or so-called gemini surfactants^[19] and have low CMC values compared with their precursors: the CMC values of **7**, **8**, and **6c** are 0.14, 0.6, and 0.01 mM, respectively, to be compared with 12 and 0.14 mM for **1a** and **1c**.^[14] On the other hand, **5c** with only one surfactant block has a CMC value of

Table 1: Microstructural characteristics of the micelles.

Cmpd.	N_{Ag} ^[a]	N_s ^[b]	β ^[c]	Aggregate shape and size
8				
free ^[d]	8	32	< 0.01	prolate micelles ^[e]
Cu complex ^[f]	9	36	< 0.01	17/55 Å
7				
free ^[d]	17	34	< 0.1	prolate micelles ^[e]
Cu complex ^[f]	20	40	< 0.1	16/60 Å
1a	38	38		prolate micelles 17/55 Å
5c				
free ^[d,g]	66	66	< 0.1	prolate micelles ^[e]
Na complex ^[h]	60	60	< 0.1	25/78 Å
6c				
free ^[d,g]	35	70	< 0.01	prolate micelles ^[e]
Na complex ^[h]	33	66	< 0.15	25/76 Å
1c	> 800	> 800		cylinders 21/500 Å

[a] N_{Ag} = Aggregation number (average number of molecules per micelle) $\pm 10\%$. [b] N_s = average number of sugar-based surfactant units per micelle. [c] β = Dissociation ratio of counterions, defined by $\beta = Z_{eff}/Z_{str}$ where the effective charge Z_{eff} is the charge at the origin of electrostatic repulsions between micelles and Z_{str} is the structural charge. [d] At pH 7.5. [e] Similar size and shape, average dimensions R/L (see Figure 2). [f] In the presence of one equivalent of Cu(NO₃)₂. [g] The pH value was adjusted by addition of HNO₃. Similar aggregates are formed at pH 7.5 and pH 5.^[14] [h] In the presence of one equivalent of NaNO₃.

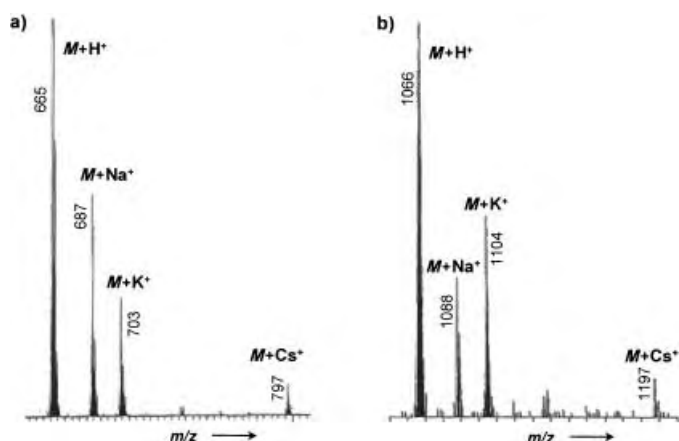


Figure 1. Electrospray mass spectra for a) **5c** and b) **6c** in the presence of one equivalent of alkali cations. [cage] = [NaBr] = [KNO₃] = [CsCl] = 10^{-2} M in CH₃CN/H₂O (80:20).

0.2 mM, a value of the same order of magnitude as that of its precursor **1c**. Micellar microstructures have been determined by combined small-angle X-ray and neutron scattering on the absolute scale.^[10,20,21] The microstructural characteristics of aggregates formed by the surfactant cage molecules, their complexes, and the starting surfactants are given in Table 1. All of the macrocyclic surfactants studied self-aggregate in water into small prolate micelles with a lipophilic core consisting of hydrocarbon chains and a hydrophilic shell containing the cages and the hydrated sugar moieties

(Figure 2).^[22] The complexation does not affect the self-aggregation: in every case, the aggregates formed by the free cages and their complexes with Cu(NO₃)₂ or NaNO₃ are

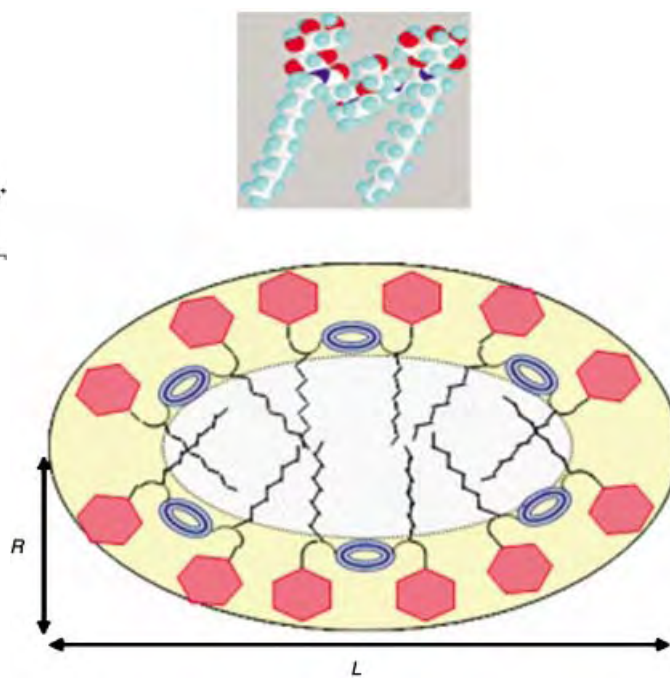


Figure 2. Schematic representation of prolate micelles (cross-section, bottom) and scaled CPK molecular model of **6c** (top).

similar in shape and size.^[23] A tight association of the counteranions within the sugar-rich shell may account for a screening of the positive charges of the complexes and, hence, for the resulting low effective charge of the micelles.^[24]

Moreover, as illustrated by the SANS and SAXS spectra shown in Figure 3, molecules of a given family form similar

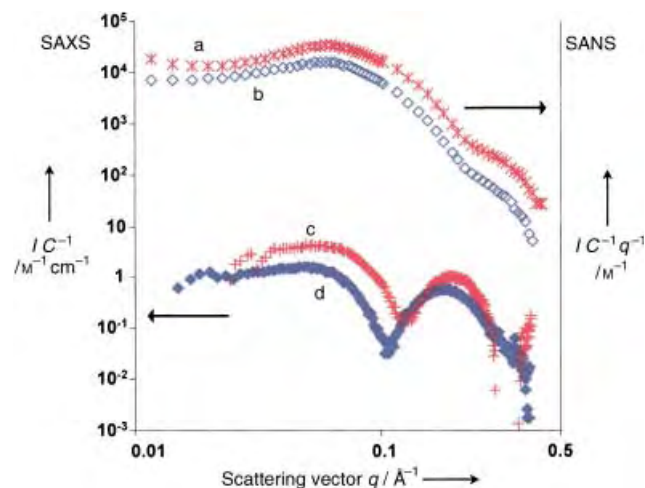


Figure 3. SANS spectra of globular micelles of a) **6c** and b) **5c**. SAXS spectra of globular micelles of c) **6c** and d) **5c**. [**5c**] = 9.4×10^{-2} M, [**6c**] = 3.1×10^{-2} M, D₂O, pH 5. Similar spectra are obtained at pH 7.5.^[14]

aggregates. The aggregation numbers of crowns **5c** and **6c** are about 30–35 and 60–65, respectively: the number of dodecyl sugar-surfactant blocks per micelle, about 60–70, is roughly constant regardless of the substitution pattern of the cage. Similar trends are observed for cyclam derivatives: **7** and **8** form comparable aggregates that contain about 35–40 surfactant blocks. The number of surfactant units per micelle increases as the length of the aliphatic chain increases, as for conventional surfactants.^[18] The self-aggregation process is governed by geometrical constraints, and the surfactant packing parameter, p , may be used to compare Gibbs energies of the different possible self-assemblies. This parameter p , defined as $p = V/al$ where V is the hydrophobic volume of the chains, l is the chain length, and a is the cross-sectional headgroup area, is a useful tool to predict the aggregate morphology.^[25] Since the macrocycle moieties lie in the polar shell,^[22] V/l is set by the surfactant unit **1**. Accordingly, amphiphiles of the same family (that is, a given macrocycle and a given surfactant unit) self-assemble into identical aggregates whatever the number of surfactant residues per cage.^[26] Remarkably, the cyclam derivatives **7** and **8** and their precursor **1a** form micelles comparable in size and shape and which contain the same number of surfactant blocks per micelle (≈ 35 –40). In this case, a medium-size cage tethered with a medium-tail surfactant, **a** is set by the bulky sugar headgroups, and the self-aggregation is entirely governed by the precursor chosen.^[26] On the other hand, the linkage of azacrowns to the dodecyl surfactant **1c** converts long cylinders (with high aggregation number) into small ellipsoi-

dal micelles for macrocyclic derivatives **5c** and **6c**. A contribution of the large macrocycle to the polar headgroup and the resulting increase of the area per molecule account for the reduction of the size of the micelles.^[25,26]

In conclusion, this strategy based on surfactant units is a straightforward and versatile method which gives access to a series of glycosylated macrocyclic amphiphiles with complexation and self-aggregation properties controlled independently by the nature of the cage and the nature of the surfactant unit. This first example of full control of self-aggregation of functional surfactants by assembling building blocks, where each block carries out its designed task, is hoped to be general, provided that surfactant units with bulky nonionic polar headgroups are used. The potential of these new chiral amphiphilic macrocycles for use in enantioselective metal-catalyzed reactions in aqueous micellar media is currently under investigation.

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- [15] Compound **8** is obtained in a water-methanol medium. Compound **7** precipitates when the reaction is performed in water. The solubility of **7** and **8** in water is about 0.02–0.03 M. Di- and tetrasubstituted cyclam derivatives with longer chains are poorly soluble in water. Alternatively, mono- and disubstituted cyclam derivatives can be prepared selectively in organic media as described in: H. Fensterbank, J. Zhu, D. Riou, C. Larpent, *J. Chem. Soc. Perkin Trans. 1* **1999**, 811–815; H. Fensterbank, P. Berthault, C. Larpent, *Eur. J. Org. Chem.* **2003**, 3985–3990.
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- [23] The nature of the associated anion seems to have little effect on micellization since we found that metallomicelles of **8** with CuF₂,^[10] Cu(CF₃SO₃)₂, and Cu(CH₃CO₂)₂ are quite similar in size, shape, and low effective charge.
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Natural Products Synthesis

Concise Total Synthesis and Structure Assignment
of TAN-1085**

Ken Ohmori, Keiji Mori, Yuji Ishikawa,
Hideyuki Tsuruta, Shunsuke Kuwahara,
Nobuyuki Harada, and Keisuke Suzuki*

TAN-1085 (**1**), an antibiotic produced by *Streptomyces* sp. S-11106,^[1] has attracted considerable synthetic interest, not only for its important biological activities, angiogenesis and aromatase inhibition, but also for its unique structural features, that is, a curved tetracyclic chromophore with a vicinal diol, one of which is glycosylated with a rhodnose unit.^[1,2] The relative and absolute stereochemistries of this compound, however, remained to be assigned.

Herein we report the first total synthesis of **1** and its 5,6-bis-epimer, thereby finally establishing the remaining stereochemical questions. The synthesis features three key transformations: 1) a tandem electrocyclic reaction for the facile construction of the aglycon precursor, 2) its stereoselective conversion into a *trans*-diol by SmI₂-mediated pinacol cyclization, and 3) discrimination of the resulting C5/C6 hydroxy groups by in situ benzylation. Furthermore, the stereochemical assignment of **1** was effected by exploiting the CD exciton chirality method.^[3]

Scheme 1 outlines our retrosynthetic analysis, predicated upon the construction of tetracyclic aglycon **I** from biaryl

[*] Dr. K. Ohmori, K. Mori, Y. Ishikawa, Dr. H. Tsuruta, Prof. Dr. K. Suzuki
Department of Chemistry
Tokyo Institute of Technology, CREST-JST Agency
2-12-1 O-okayama, Meguro-ku, Tokyo 152-8551 (Japan)
Fax: (+81) 3-5734-2788
E-mail: ksuzuki@chem.titech.ac.jp
S. Kuwahara, Prof. Dr. N. Harada
Institute of Multidisciplinary Research for Advanced Materials
Tohoku University
2-1-1 Katahira, Aoba-ku, Sendai 980-8577 (Japan)

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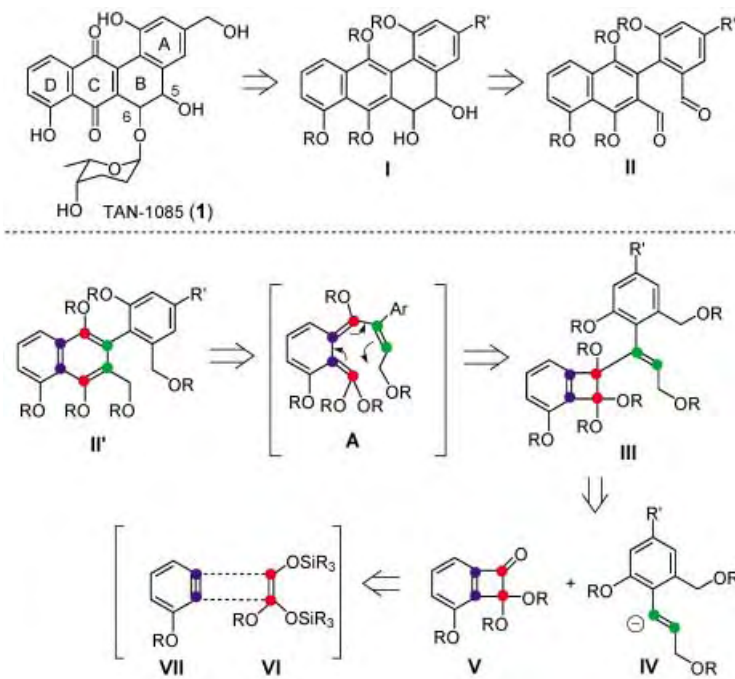


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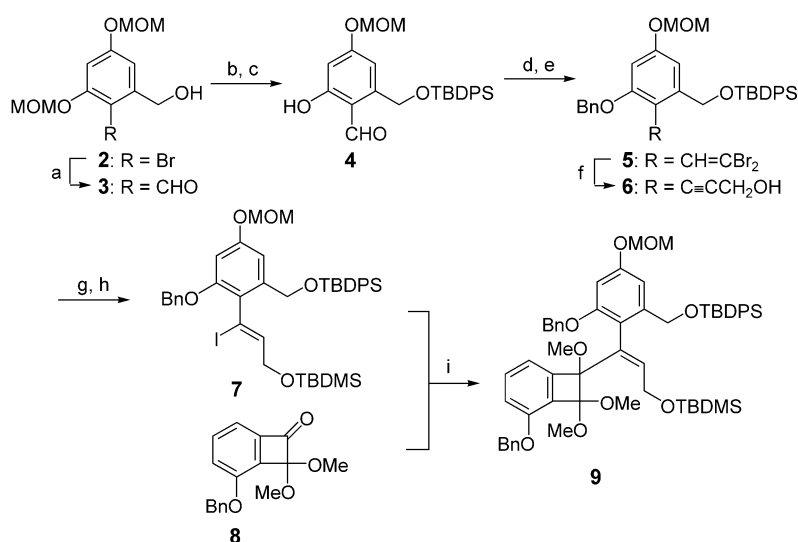
dialdehyde **II** by pinacol cyclization.^[4] We hoped that the sterically encumbered biaryl structure in **II** (or the precursor **II'**) could be accessible by the ring enlargement of benzocyclobutene **III** by way of tandem thermal electrocyclic reactions via **A**.^[5,6] The key intermediate **III** could be obtained by assembly of styryl anion **IV** and the selectively protected benzocyclobutenedione **V**, which in turn is readily available from silyl enol ether **VI** and benzyne **VII**.^[7]

Scheme 2 illustrates the preparation of **9** for the key electrocyclizations. Known bromide **2**^[8] was converted into aldehyde **3** by successive treatment with MeLi and *n*BuLi^[9] followed by DMF. After silylation, the MOM group proximal to the carbonyl function was selectively removed, giving phenol **4** in good yield. Benzoylation of **4** was followed by dibromoolefination^[10] to give **5** in 85 % yield, which was successively treated with *n*BuLi and paraformaldehyde to afford propargyl alcohol **6** in 91 % yield. Hydroalumination of **6** with Red-Al followed by quenching with iodine gave, after silylation, vinyl iodide **7**. Union of benzocyclobutenone **8**^[7] and the vinyl lithium species generated from **7** followed by treatment with methyl triflate in situ gave **9**, ready for the key tandem electrocyclic reaction.

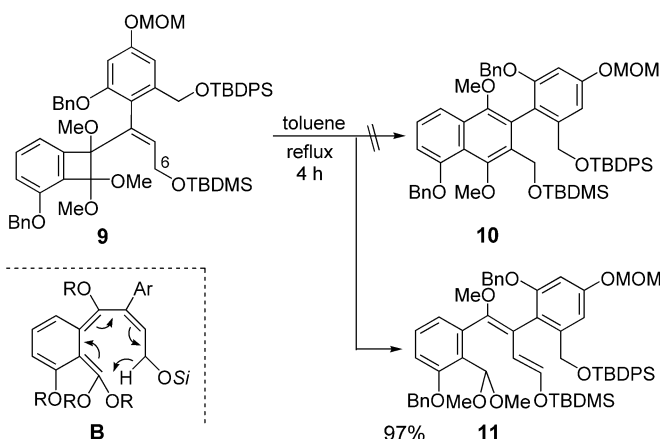
The planned reactions of **9**, however, were uniformly unsuccessful under various conditions owing to the prevalence of another pericyclic process, the 1,7-hydrogen shift shown in **B** (Scheme 3). For example, although the starting material **9** was completely consumed upon refluxing in toluene for 4 h, only silyl ether **11** was obtained.



Scheme 1. Retrosynthetic analysis of **1**.

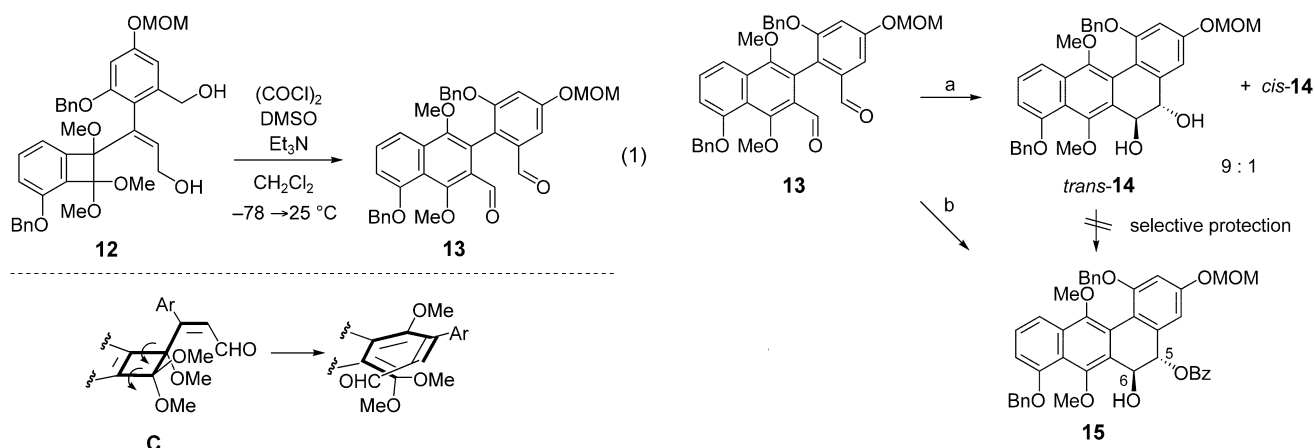


Scheme 2. Preparation of **9**. a) MeLi (1.1 equiv), THF, -78°C , 10 min; then *n*BuLi (1.2 equiv), DMF, -78°C , 5 min (97 %); b) TBDPSCl, imidazole, DMF, $0^{\circ}\text{C} \rightarrow \text{RT}$, 78 %; c) Montmorillonite K-10, benzene, room temperature, 11 h, 40°C , 50 min; d) BnBr, NaH, DMF, $0^{\circ}\text{C} \rightarrow \text{RT}$, 10 h, 75 % (two steps); e) CBr_4 , PPh₃, CH_2Cl_2 , 0°C , 20 min, 85 %; f) *n*BuLi (2.0 equiv), THF; then $(\text{HCHO})_n$, $-78^{\circ}\text{C} \rightarrow \text{RT}$, 91 %; g) Red-Al, Et_2O , $-78^{\circ}\text{C} \rightarrow -10^{\circ}\text{C}$, 50 min; then EtOAc, -10°C , 15 min; then I_2 , THF, $-78^{\circ}\text{C} \rightarrow 0^{\circ}\text{C}$, 1 h; h) TBDPSCl, imidazole, DMF, $0^{\circ}\text{C} \rightarrow \text{RT}$, 1.5 h, 93 %; i) **7** (1.0 equiv), *t*BuLi (2.1 equiv), Et_2O , -78°C , 20 min; then **8** (1.5 equiv), THF, -78°C , 25 min; then MeOTf (3.0 equiv), $-78^{\circ}\text{C} \rightarrow 0^{\circ}\text{C}$, 1.5 h, 85 %. DMF = *N,N*-dimethylformamide, TBDPS = *tert*-butyldiphenylsilyl, TBDMS = *tert*-butyldimethylsilyl, Red-Al = $\text{NaAlH}_2(\text{OCH}_2\text{CH}_2\text{OCH}_3)_2$, Tf = trifluoromethanesulfonyl.



Scheme 3. Attempts at thermal electrocyclic reactions.

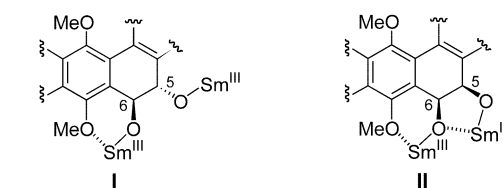
After considerable experimentation, we discovered a highly efficient solution to this problem [Eq. (1)]. Simply by raising the oxidation level of C6 in **9**, the 1,7-shift was completely suppressed, and the desired 6π process was remarkably facilitated. Thus, after removal of the two silyl groups in **9**, the resulting diol **12** was subjected to Swern oxidation. Importantly, when the Swern oxidation mixture was warmed to 25°C and kept standing for 8 h, the ring opening of **C** and subsequent 6π -closure occurred, thereby directly giving the biaryl dialdehyde **13**, the key intermediate in our synthetic plan. This remarkable rate enhancement of the pericyclic processes could be rationalized by the cooper-



ative effect of the electron-donating ($2 \times \text{OMe}$) and the electron-withdrawing (enal) substituents of the four-membered ring toward the required conrotation.^[11]

The biaryl dialdehyde **13** was subjected to a pinacol cyclization to the tetracyclic structure (Scheme 4). Treatment of **13** with SmI_2 ^[12] gave the corresponding diols, *trans*-**14** and *cis*-**14** (9:1), which were separable by column chromatography.^[13,14] For the regioselective introduction of the sugar moiety, discrimination of the C5/C6 hydroxy groups in *trans*-**14** was required, a challenging task owing to the local C_2 symmetry. All attempts at the monofunctionalization of diol **14** were unfruitful; no regioselectivity and/or double reaction at the C5/C6 diols occurred.

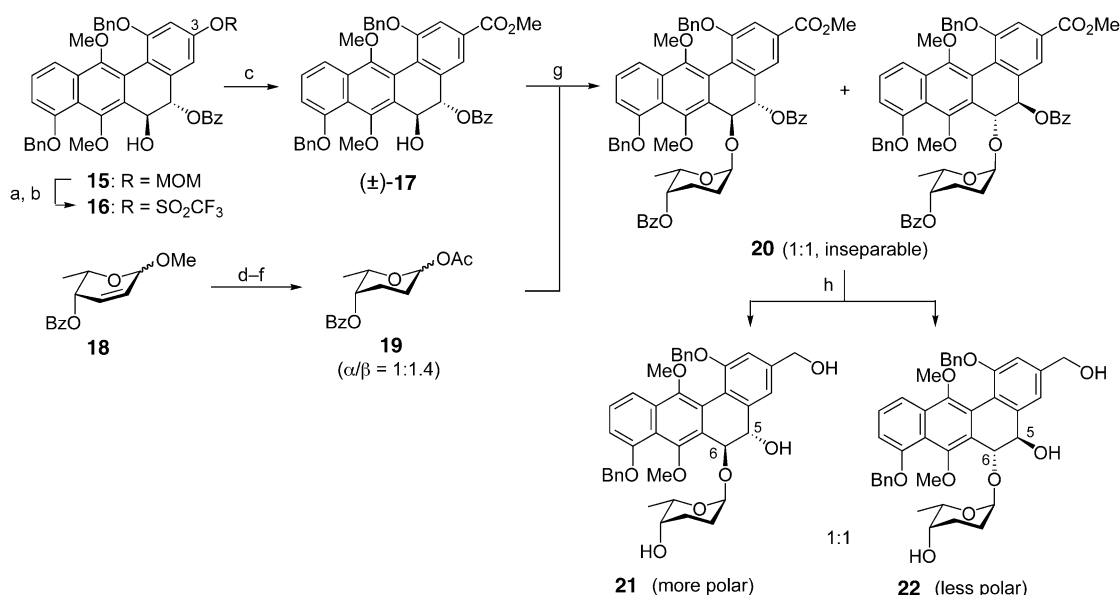
Gratifyingly, a felicitous solution to this issue was offered by direct quenching of the pinacol cyclization (see above). Thus, after treatment of **13** with SmI_2 as above, benzoyl chloride (1.5 equiv) was directly added to give C5-benzoate *trans*-**15** in 86% yield. Neither the C6 benzoate nor the



Scheme 4. Pinacol cyclization and discrimination of the two hydroxy groups. a) SmI_2 , THF, 0 °C, 2 min, 96%, *trans*/*cis* = 9:1; b) SmI_2 , THF, 0 °C, 2 min; then BzCl (1.5 equiv), 86%.

dibenzoate was detected, which could be rationalized by the difference in the nucleophilicity of two oxygen functions: The C6 samarium alkoxide is chelated as in **I**, and is therefore less reactive than the C5 alkoxide.^[15]

Construction of the aglycon portion was completed in three steps to give methyl ester **17**, primed for introduction of the sugar moiety (Scheme 5). Tetracyclic **17** was glycosylated by reaction with L-rhodosyl acetate **19**^[16,17] in the presence



Scheme 5. Construction of the aglycon **17** and its glycosylation with L-rhodoside **19**. a) H_2SO_4 (aq., 0.5 M), DME, 60 °C, 12 h, 91%; b) PhNTf_2 , K_2CO_3 , acetone, 0 °C, 3 h, 98%; c) CO (3 atm), $\text{Pd}(\text{OAc})_2$ (30 mol%), dppp (30 mol%), Et_3N , MeOH, DMF, 65 °C, 30 h, 91%; d) H_2 , Pd/C, MeOH, room temperature, 30 min; e) HCl (2 M), AcOH, THF (1:1:1), room temperature, 7 h, 80% (two steps); f) Ac_2O , pyridine, room temperature, 30 min, 94%, $\alpha/\beta = 1:1.4$; g) $\text{BF}_3 \cdot \text{OEt}_2$ (1.1 equiv), **19** (2.0 equiv, $\alpha/\beta = 1:1.4$), molecular sieves (4 Å), CH_2Cl_2 , 95%, 1:1 mixture of diastereomers; h) DIBAL, CH_2Cl_2 , $-78 \rightarrow -20$ °C, 1 h, 98%. dppp = 1,3-bis(diphenylphosphanyl)propane, DIBAL = diisobutylaluminum hydride.

of $\text{BF}_3 \cdot \text{OEt}_2$ to give the α glycoside **20** as an inseparable mixture of diastereomers (1:1). These isomers were the diastereomers arising from the racemic nature of the aglycon relative to the L-rhodoside. The anomeric stereochemistries of the diastereomers were both shown to be α by ^1H NMR spectroscopic analysis.^[18] Without separation, methyl ester **20** was treated with DIBAL, which led to the simultaneous removal of the two benzoyl groups to give diastereomeric products, which were separable by column chromatography ($\text{CHCl}_3/\text{EtOAc}$ = 1:30); **21** (R_f = 0.10), **22** (R_f = 0.17).

In spite of considerable efforts, we were frustrated by the inability to directly assign the stereochemistry of these diastereomers, and decided to rely on indirect methods. Thus, the sugar was detached from the more-polar isomer **21** to obtain the “resolved” aglycon **23** (with unknown absolute stereochemistry at this stage), which was converted into benzoate **24**^[19] for measuring the CD exciton chirality effects (Figure 1a).^[3]

The CD spectrum of alcohol **23** showed strong and complicated Cotton effects because of the markedly twisted π -electron system in the dihydrobenz[*a*]anthracene skeleton (blue line, Figure 1b). The CD spectrum of benzoate **24** also showed intense and complicated Cotton effects similar to

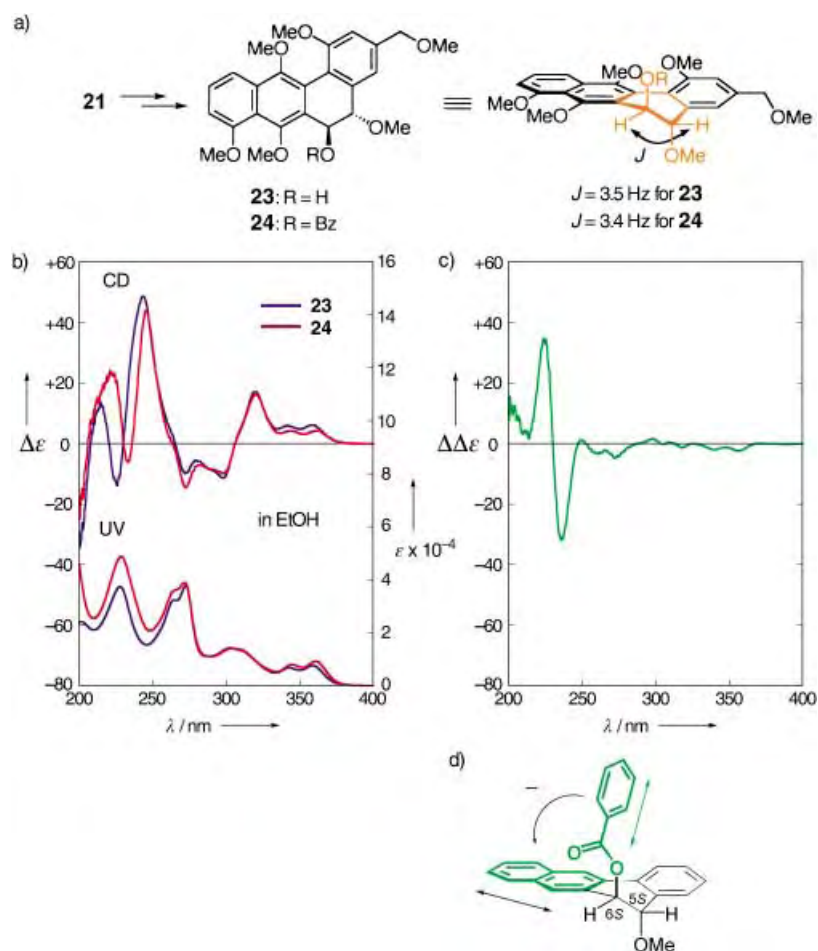
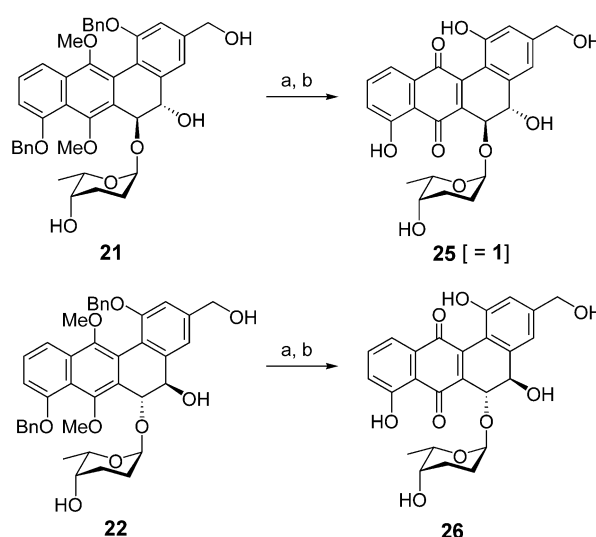


Figure 1. Application of the exciton chirality method. a) Conformations of tetracycle **23** and **24**, b) CD and UV spectra of **23** and **24**, c) difference CD curve between **24** and **23**: $\Delta\Delta\epsilon = \Delta\epsilon(\mathbf{24}) - \Delta\epsilon(\mathbf{23})$, d) Anticlockwise relationship between the long axis of the naphthalene and the benzoate chromophores.



Scheme 6. Construction of **1**. a) CAN, H_2O , CH_3CN , 0°C , 20 min, b) H_2 , Pd/C, MeOH (53% for **25** from **21**, 56% for **26** from **22**). CAN = ceric ammonium nitrate.

those of **23** (red line). Therefore, to detect the genuine exciton CD Cotton effects due to the interaction between the long axes of naphthalene and benzoate chromophores, the difference CD curve was calculated: $\Delta\Delta\epsilon = \Delta\epsilon(\mathbf{24}) - \Delta\epsilon(\mathbf{23})$. As shown in Figure 1c, the difference CD curve shows a typical pattern of exciton split CD, $\Delta\Delta\epsilon = -32$ at 236 nm (the first Cotton effect) and $\Delta\Delta\epsilon = +35$ at 224 nm (the second Cotton effect), $A = \Delta\Delta\epsilon(1\text{st}) - \Delta\Delta\epsilon(2\text{nd}) = -67$; the negative sign of the *A* value proved the counterclockwise relationship between these chromophores (Figure 1d). The *5S,6S* configuration was thus unambiguously assigned.^[20]

With the structures secured, diastereomeric triols **21** and **22** were converted into the final products by oxidation with CAN followed by deprotection (Scheme 6). Of the diastereomers **25** (from **21**) and **26** (from **22**), the former coincided with the authentic specimen of **1**.^[21]

In conclusion, the first synthesis of TAN-1085 was completed through the concise and efficient construction of the aglycon. The stereochemistry of natural product was concluded to be that of **25**.

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Keywords: antibiotics · electrocyclic reactions · glycosylation · structure elucidation · total synthesis

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- [20] This conclusion was further confirmed by the ¹H NMR anisotropy method (see Supporting Information).
- [21] All physical data of synthetic **25** (¹H and ¹³C NMR, IR, [α]_D) were identical to those of an authentic specimen of **1**.

Mixed-Valence Structures

Visualization of Local Valence Structures in Quasi-One-Dimensional Halogen-Bridged Complexes $[\text{Ni}_{1-x}\text{Pd}_x(\text{chxn})_2\text{Br}] \text{Br}_2$ by STM**

Shinya Takaishi, Hitoshi Miyasaka, Ken-ichi Sugiura, Masahiro Yamashita,* Hiroyuki Matsuzaki, Hideo Kishida, Hiroshi Okamoto, Hisaaki Tanaka, Kazuhiro Marumoto, Hiroshi Ito, Shin-ichi Kuroda, and Tomohide Takami*

One-dimensional (1D) electron systems have been attracting much attention because many characteristic physical properties have been observed, such as the spin-density wave (SDW)

[*] Dr. S. Takaishi, Dr. H. Miyasaka, Prof. K.-i. Sugiura, Prof. M. Yamashita
Department of Chemistry, Graduate School of Science
Tokyo Metropolitan University and CREST (JST)
1-1 Minamiosawa, Hachioji, Tokyo 192-0397 (Japan)
Fax: (+81) 426-77-2525
E-mail: takaishi@comp.metro-u.ac.jp
yamashita@comp.metro-u.ac.jp

H. Matsuzaki, Dr. H. Kishida, Prof. H. Okamoto
Department of Advanced Material Science
Graduate School of Frontier Science
The University of Tokyo, Kashiwa, Chiba 277-8561 (Japan)
Dr. H. Tanaka, Dr. K. Marumoto, Prof. H. Ito, Prof. S. Kuroda
Department of Applied Physics, Graduate School of Engineering
Nagoya University, Furocho, Chikusa-ku
Nagoya, Aichi 464-8603 (Japan)

T. Takami
Visionarts Research Inc.
5-3-22 Minami-aoyama, Minato-ku, Tokyo 107-0062 (Japan)

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and charge-density wave (CDW) in organic conductors,^[1] soliton, polaron, and bipolaron states in π -conjugated polymers,^[2] and slow relaxation of magnetization in several ferro- or ferrimagnetic compounds.^[3] Quasi-1D halogen-bridged complexes are also attractive materials because of their marked physical properties such as intense and dichroic intervalence charge-transfer bands,^[4] overtone progression of resonance Raman spectra,^[5] luminescence spectra with large Stokes shifts,^[6] large third-order nonlinear optical properties, and midgap absorptions attributable to solitons and polarons,^[7] as well as providing 1D model compounds of high critical temperature (T_c) copper oxide superconductors.^[8]

Theoretically, these MX chains are considered as Peierls–Hubbard systems where the electron–phonon interaction (S), the electron transfer (T), and the intra- and intersite Coulomb repulsion energies (U and V , respectively) compete or cooperate with each other.^[9] The Pt and Pd compounds form CDW or mixed-valence states represented as $-X\cdots M^{2+}\cdots X-M^{4+}-X\cdots$ as a result of the electron–phonon interaction, where the bridging halogen atoms are displaced from the midpoints between the neighboring two metal ions.^[10] Accordingly, the half-filled metallic band splits into the occupied valence band and unoccupied conduction band with finite Peierls energy gaps. Therefore, these compounds belong to class II of the Robin–Day classification for mixed-valence complexes.^[11] On the other hand, the Ni complexes form Mott–Hubbard states expressed as $-X-Ni^{3+}-X-Ni^{3+}-X-$ as a result of the strong electron correlation (U), where the bridging halogen atoms are located at the midpoints between two neighboring Ni ions.^[12] Therefore, these Ni complexes belong to class III of the Robin–Day classification.^[11] Very strong antiferromagnetic interactions among the spins located on the $Ni^{III}d_{z^2}$ orbitals through the bridging halogen ions are observed.^[13] These Ni complexes have also been of recent interest in applied science because the largest third-order, nonlinear optical susceptibility of 10^{-4} esu has been observed in $[Ni(chxn)_2Br]Br_2$ (chxn: (1*R*,2*R*)-diaminocyclohexane).^[14]

Recently, the mixed-metal complexes $[Ni_{1-x}Pd_x(chxn)_2Br]Br_2$ ($0 \leq x \leq 1$) were synthesized.^[15] The valence structures of these complexes are interesting because competition between the Ni^{3+} – Ni^{3+} Mott–Hubbard and Pd^{2+} – Pd^{4+} CDW states can be expected in single crystals. The electronic states of these complexes have been studied by infrared spectroscopy,^[16] magnetic susceptibility,^[17] and X-ray diffuse scattering.^[18] According to these studies, the valence structure of the complexes with $x < 0.8$ form Mott–Hubbard states, whereas the complexes with $x > 0.8$ show CDW states. However, no information on local structures was obtained. In addition, it is difficult to clarify the valence structures, so an X-ray crystal structure analysis cannot be applied to such mixed-metal compounds. A scanning tunneling microscope (STM) is a powerful tool for studying local electronic structures in such compounds. Day described the usefulness of the method for halogen-bridged complexes in 1985.^[19] Herein, we report the first visualized local valence structures in Ni–Pd mixed-metal complexes obtained by STM.

Figure 1 a shows an STM image of $[Ni(chxn)_2Br]Br_2$ in the range 200×200 Å. Bright spots in the image are observed

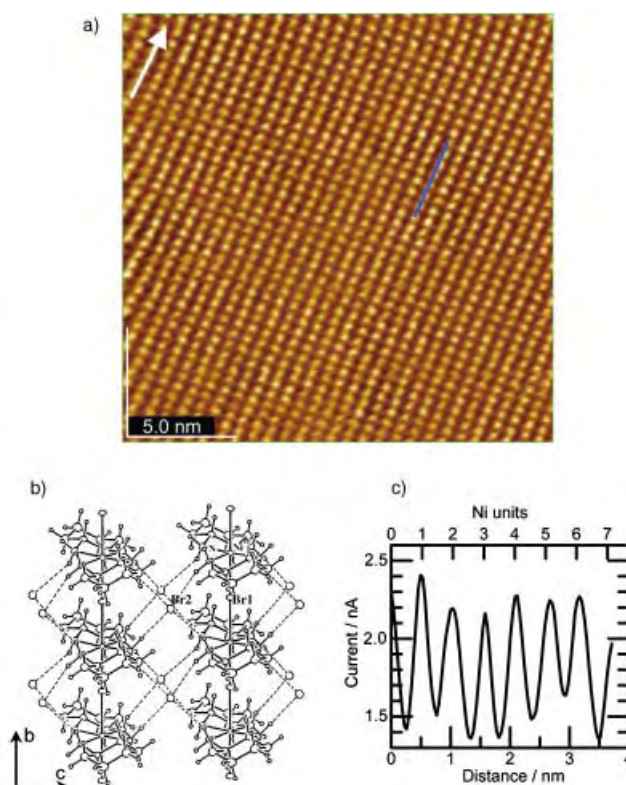


Figure 1. a) STM image of $[Ni(chxn)_2Br]Br_2$ on the bc plane (200×200 Å). The image was acquired with a sample bias of $V_s = +1.3$ V. The white arrow shows the 1D chain direction. b) Crystal structure of $[Ni(chxn)_2Br]Br_2$. c) Tunnel current profile of $[Ni(chxn)_2Br]Br_2$ on the blue line in Figure 1 a.

every 5×7 Å. The crystal structure of $[Ni(chxn)_2Br]Br_2$ is shown in Figure 1 b. The Ni···Ni distances along the b (1D chain) and c axes are 5.16 and 7.12 Å, respectively,^[12] and hence these spots reflect the periodicity of $[Ni(chxn)_2]$ units in the bc plane. To investigate the valence structure in detail we plotted a current profile along the 1D chain (on the blue line) in Figure 1 c. The maximum of the current is observed every Ni unit, which indicates that all the Ni centers are equivalent and in the Mott–Hubbard Ni^{3+} – Ni^{3+} state in $[Ni(chxn)_2Br]Br_2$. This finding is consistent with the result obtained by single-crystal X-ray analysis.

An STM image in the range 200×200 Å and the crystal structure of $[Pd(chxn)_2Br]Br_2$ are shown in Figure 2 a and b, respectively. Bright spots are observed every 10×7 Å. The Pd···Pd distances along the b (1D chain) and c axes are 5.29 and 7.07 Å, respectively,^[10] and thus these spots in the image reflect the twofold periodicity of the valence structure that result from the CDW structure of $[Pd(chxn)_2Br]Br_2$. The phase of the CDW is almost aligned in the bc plane, which is consistent with the X-ray diffuse scattering study.^[18] Therefore, correctly speaking, $[Pd(chxn)_2Br]Br_2$ should be formulated as $[Pd^{II}(chxn)_2][Pd^{IV}(chxn)_2Br_2]Br_4$.

Schematic band structures of $[Ni(chxn)_2Br]Br_2$ and $[Pd(chxn)_2Br]Br_2$ are shown in Figure 3. According to X-ray photoelectron spectra (XPS) and Auger spectra, the d_{z^2} band of Ni splits into occupied lower-Hubbard (LH) and unoccu-

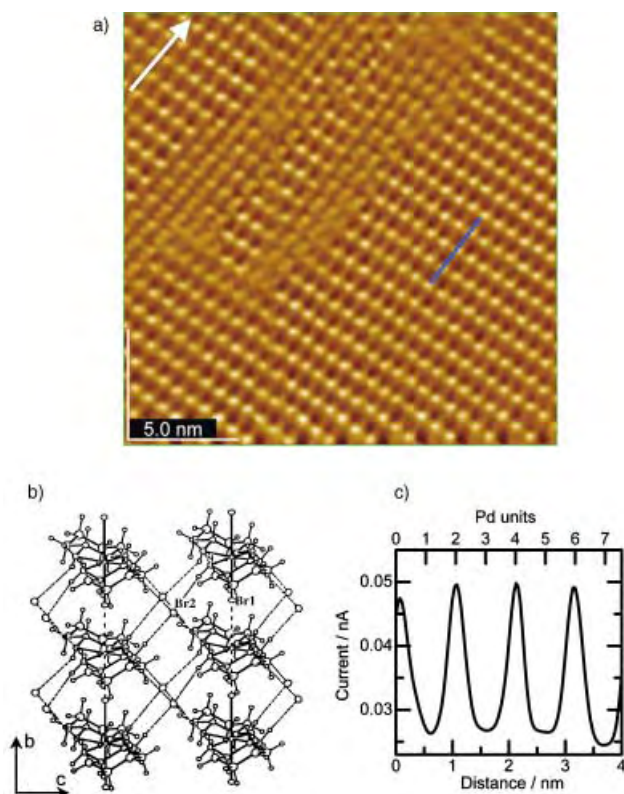


Figure 2. a) STM image of $[\text{Pd}(\text{chxn})_2\text{Br}]\text{Br}_2$ on the bc plane ($200 \times 200 \text{ \AA}$). The sample bias was $V_s = +1.0 \text{ V}$. The 1D chain direction is shown by a white arrow. b) Crystal structure of $[\text{Pd}(\text{chxn})_2\text{Br}]\text{Br}_2$. c) Current profile of $[\text{Pd}(\text{chxn})_2\text{Br}]\text{Br}_2$ on the blue line in Figure 2a.

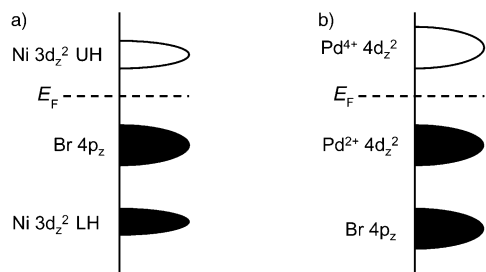


Figure 3. Schematic band structures of a) $[\text{Ni}(\text{chxn})_2\text{Br}]\text{Br}_2$ and b) $[\text{Pd}(\text{chxn})_2\text{Br}]\text{Br}_2$.

pied upper-Hubbard (UH) bands as a consequence of the strong electron correlation and, as a result, the valence and conduction bands of $[\text{Ni}(\text{chxn})_2\text{Br}]\text{Br}_2$ are the p_z band of bridging Br atoms and the UH band composed of the d_{z^2} orbital of Ni, respectively.^[20] In $[\text{Pd}(\text{chxn})_2\text{Br}]\text{Br}_2$, on the other hand, the valence and conduction bands are the d_{z^2} bands of Pd^{2+} and Pd^{4+} , respectively. As the STM measurements were performed with positive sample bias, the tunnel current is observed from the Fermi energy (E_F) of a tip to a conduction band of the sample. Therefore, in $[\text{Ni}(\text{chxn})_2\text{Br}]\text{Br}_2$, the tunnel current from E_F to the UH d_{z^2} band of Ni^{3+} is observed, whereas that from E_F to the d_{z^2} band of Pd^{4+} is observed in $[\text{Pd}(\text{chxn})_2\text{Br}]\text{Br}_2$, which affords the

twofold periodicity along the 1D chain observed in the STM image.

Next, we made STM measurements on the mixed-metal complexes $[\text{Ni}_{1-x}\text{Pd}_x(\text{chxn})_2\text{Br}]\text{Br}_2$ to determine their local valence structure. Figure 4 shows the STM images of the

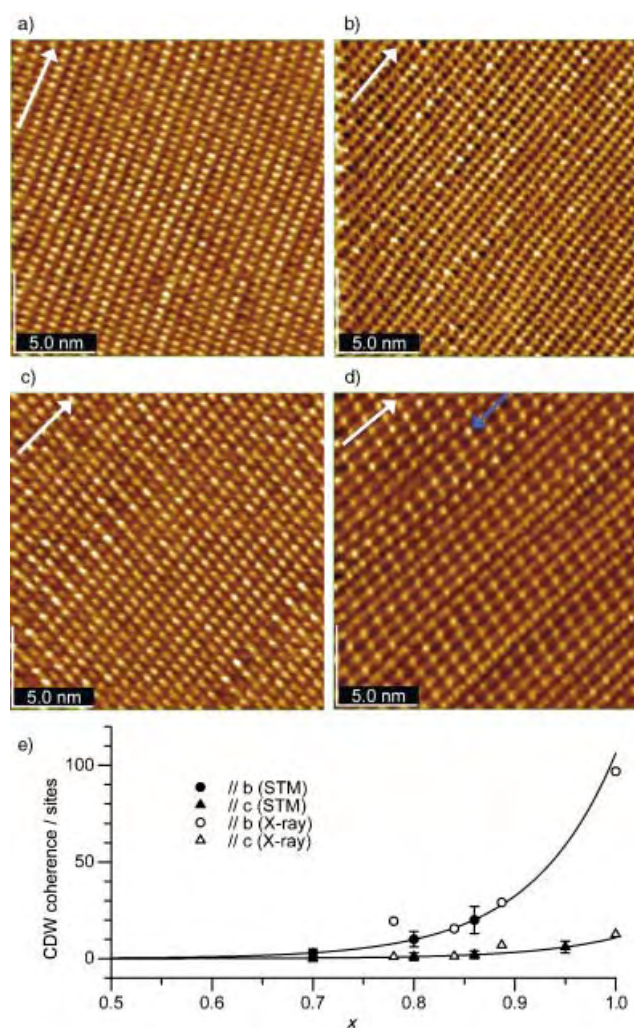


Figure 4. STM images of $[\text{Ni}_{1-x}\text{Pd}_x(\text{chxn})_2\text{Br}]\text{Br}_2$ with a) $x = 0.70$, b) $x = 0.80$, c) $x = 0.86$, and d) $x = 0.95$ on the bc plane ($200 \times 200 \text{ \AA}$). The sample bias was $V_s = +1.0 \text{ V}$. The 1D chain directions are shown as white arrows; the blue arrow shows the 1D chain on which the spin soliton is observed. e) CDW coherence as a function of x . The filled circle and triangle represent CDW coherence along the chain and c axis, respectively, determined by STM measurement. The empty circle and triangle represent those determined by X-ray diffuse scattering measurements reported by Wakabayashi et al. The solid lines are guides to the eye.

complex with a) $x = 0.70$, b) $x = 0.80$, c) $x = 0.86$, and d) $x = 0.95$ in an area of $200 \times 200 \text{ \AA}$. In the $x = 0.70$ complex (Figure 4a), an image similar to that of $[\text{Ni}(\text{chxn})_2\text{Br}]\text{Br}_2$ is observed, with almost no twofold periodicity. This result shows that the $x = 0.70$ complex forms almost in the Mott–Hubbard state, where the oxidation state of the Pd center is $3+$. In the $x = 0.80$ complex (Figure 4b), on the other hand, twofold periodicity is observed in several areas, which is

attributable to the CDW state. This CDW state has coherence over approximately 10 metal sites along the *b* axis, but almost no coherence along the *c* axis. In the $x=0.86$ complex (Figure 4c), the CDW coherence is clearly more propagated than that of the $x=0.80$ complex. In the $x=0.86$ complex, CDW coherence spreads over approximately 20 metal sites along the *b* axis, and 2 or 3 metal sites along the *c* axis. Figure 4d shows the STM image of the $x=0.95$ complex. A large part of this image has a twofold periodicity, that is, a CDW state. Along the *b* axis, a large part of the chains with a CDW state conserve the twofold periodicity within the measured length (ca. 40 metal sites), which shows that the CDW coherence along the chain is longer than 20 nm. Along the *c* axis, on the other hand, CDW coherence spreads over approximately 6 or 7 sites. We plotted the CDW coherent length as a function of x in Figure 4e together with the data evaluated by X-ray diffuse scattering measurements reported by Wakabayashi et al.^[18] The CDW coherence determined by STM measurements is in good agreement with the X-ray diffuse scattering results. It was revealed that CDW coherence is generated slightly from about $x=0.70$ and increasingly propagated with increasing value of x .

In addition, the phase of the CDW on the chain shown as a blue arrow in Figure 4d is reversed within the chain. This reverse can be recognized as the generation of a spin soliton. A schematic chain structure with the spin soliton in a CDW complex is shown together with *trans*-polyacetylene (*trans*-PA) in Figure 5. It is well established that the spin soliton in

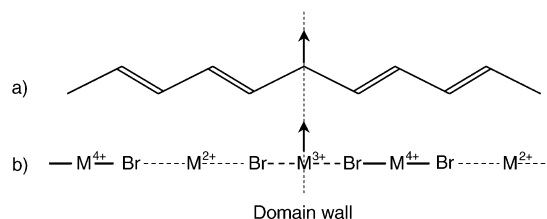


Figure 5. Schematic chain structure of the spin soliton in a) *trans*-polyacetylene and b) halogen-bridged CDW complexes.

trans-PA is made by a domain wall originating from the mismatch of the phase of alternating bonds.^[2] On the other hand, it has been revealed that spin solitons in the CDW complexes are made by mismatch of the CDW phase, and M^{3+} sites act as domain walls.^[13,21] This domain wall of the spin soliton observed in Figure 4d spreads over approximately 10 metal sites, which is consistent with theoretical prediction.^[22] Although the mechanism of the spin soliton in the complex is the same as that in *trans*-PA, the most marked difference between them is the activation energy for soliton motion. This energy has been evaluated as about 80 meV by light-induced ESR studies,^[21] which is much larger than that of *trans*-PA (2 meV).^[23] This finding shows that soliton motion in $[\text{Pd}(\text{chxn})_2\text{Br}]_2$ is much slower than that of *trans*-PA. As a result, the spin soliton has a lifetime of the order of a minute, so it can be observed by STM, which operates on the millisecond-order timescale. Although spin solitons are well known in π -conjugated polymers,^[2,23] this is the first time the spin soliton has been visualized in real space.

In summary, we have visualized the Mott–Hubbard and CDW states in quasi-1D halogen-bridged Ni and Pd complexes, respectively. In addition, we succeeded in visualizing the propagation of CDW coherence and the spin soliton in real space in the mixed-metal complexes $[\text{Ni}_{1-x}\text{Pd}_x(\text{chxn})_2\text{Br}]_2$ for the first time.

Experimental Section

Single crystals of $[\text{Ni}_{1-x}\text{Pd}_x(\text{chxn})_2\text{Br}]_2$ ($x=0, 0.70, 0.80, 0.86, 0.95$, and 1.0) were synthesized according to previous studies.^[16] The elemental ratio of Ni and Pd was determined by ICP emission measurements with a SEIKO SPS 7000 plasma spectrometer. STM measurements were performed at room temperature and ambient pressure. Single crystals of $[\text{Ni}_{1-x}\text{Pd}_x(\text{chxn})_2\text{Br}]_2$ were cut and mounted on a sample stage with carbon paste so that the surface of the *bc* plane could be observed. All STM images were acquired in the constant height mode using a JEOL JSPM-5200 microscope. A positive sample bias voltage (V_s) was used.

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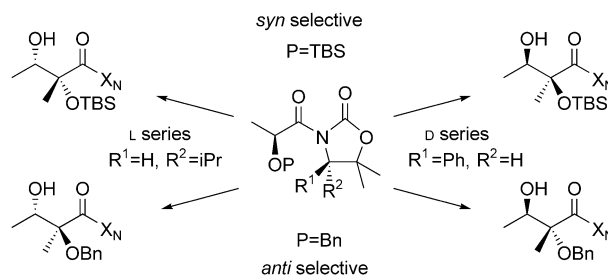
Keywords: bridging ligands · coordination modes · mixed-valent compounds · scanning probe microscopy · valence structures

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biological activities, these compounds have attracted a great deal of attention from a synthetic point of view. Indeed, total syntheses of citreoviral and citreoviridin have already been reported by several groups.^[5]

We recently reported a general methodology for the stereoselective construction of 1,2-diols including a stereogenic quaternary carbon center by a titanium-mediated aldol reaction of lactate-bearing chiral oxazolidin-2-one.^[6] Control of all the stereogenic centers is possible by the correct choice of chiral oxazolidin-2-one and the protecting group for the hydroxy function of the lactate unit (benzyl or TBS) (Scheme 1). In the tetrahydrofuran moiety of citreoviral and



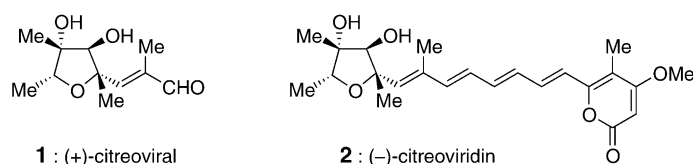
Scheme 1. Stereoselective construction of 1,2-diols that include a stereogenic quaternary carbon center.

Natural Products Synthesis

Highly Efficient Total Synthesis of (+)-Citreoviral

Yoshihisa Murata, Tomoyuki Kamino, Toshiaki Aoki, Seiji Hosokawa, and Susumu Kobayashi*

(–)-Citreoviridin (**2**) was isolated by Hirata and co-workers from *Penicillium citreoviride*^[1] as a potent inhibitor of the



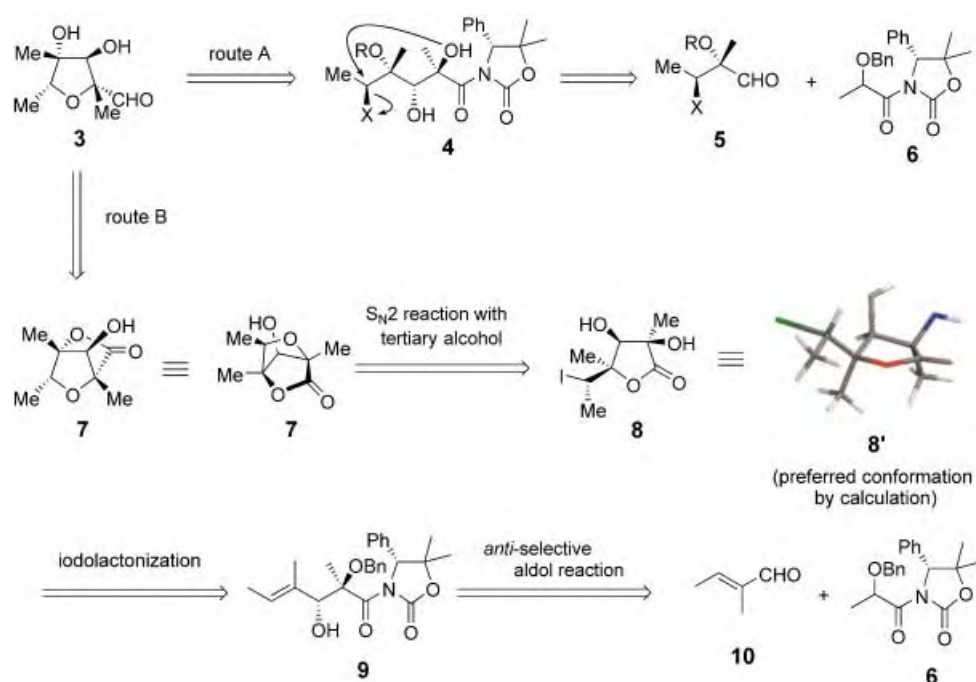
mitochondrial ATPase and oxidative phosphorylation^[2] and was recently reported to exhibit anti-HIV activity.^[3] Structurally related (+)-citreoviral (**1**) was also isolated from the same microorganism.^[4] These compounds have a highly substituted and oxygenated tetrahydrofuran moiety as a common subunit. Owing to the structural complexity as well as significant

citreoviridin, we can recognize several 1,2-diol units and we expected that our methodology could be effectively utilized for the synthesis of citreoviral and related compounds. Since the transformation of citreoviral into citreoviridin has already been established, we focused on the synthesis of citreoviral.

The tetrahydrofuran moiety of citreoviral includes two sets of adjacent secondary and tertiary alcohol groups. Our retrosynthetic analysis for the synthesis of **1** is shown in Scheme 2. Although the structure of **1** presents many opportunities for the use of our 1,2-diol-forming reaction, we decided to employ the strategy starting from an *anti* aldol product, as the stereoselectivity of *anti* aldol reactions is generally higher (>50:1) than that of *syn* aldol reactions (≈10:1). Our first analysis of the oxygenated furan **3**, the core structure of **1**, is shown as route A in which **4** would be synthesized by coupling of **5** and **6**. Although our *anti* aldol reaction should be effective in this route, there are two problems: 1) stereoselective construction of aldehyde **5** and 2) possible intramolecular S_N2 reaction of the tertiary alcohol group at the carbon center bearing the halide function. To avoid the long sequence required for the synthesis of **5** and the potentially problematic (albeit unprecedented) S_N2 reaction, we chose route B via the bicyclic intermediate **7** (Scheme 2). This route also involves an intramolecular S_N2 reaction with the tertiary alcohol function, but the iodide **8** has the preferred conformation **8**^[7] in which the tertiary alcohol group interacts strongly with the C–I⁺ bond; thus the desired S_N2 reaction should proceed smoothly. The iodide **8** can be obtained by stereospecific iodolactonization of **9**. There have been a number of precedents for the iodolactonization of γ,δ-unsaturated β-hydroxycarboxylic acids,^[8] and theoretical calculations of the transition states were investigated in

[*] Y. Murata, T. Kamino, T. Aoki, S. Hosokawa, Prof. S. Kobayashi
Faculty of Pharmaceutical Sciences
Tokyo University of Science
2641 Yamazaki, Nodashi, Chiba 278-8510 (Japan)
Fax: (+81) 4-7121-3671
E-mail: kobayash@rs.noda.tus.ac.jp

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Scheme 2. Retrosynthetic analysis.

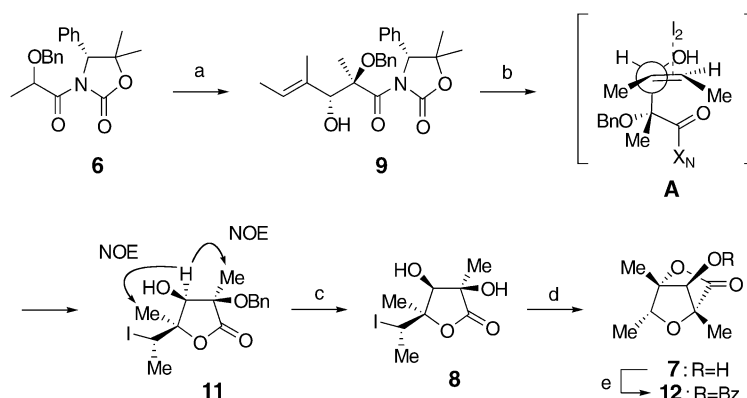
detail by Houk et al.^[9] Imide **9** would be obtained by our *anti*-selective aldol reaction between tiglic aldehyde (**10**) and lactate derivative **6**.

The actual synthesis of the (+)-citreoal core **3** is shown in Scheme 3. According to the established protocol, **6** was treated with LDA followed by $\text{Ti}(\text{OiPr})_3\text{Cl}$; the reaction of the resulting titanium enolate with **10** afforded the *anti* aldol **9** in 71 % yield. The stereoselectivity of this reaction is excellent (*anti*/*syn* > 99:1), and we could not detect the formation of any other isomers. Iodolactonization of the resulting aldol adduct **9** was next attempted. Unsaturated imide **9**, which includes a chiral auxiliary, was treated directly with I_2 and NaHCO_3 in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (4:1) at room temperature. As expected, iodolactonization proceeded smoothly to afford

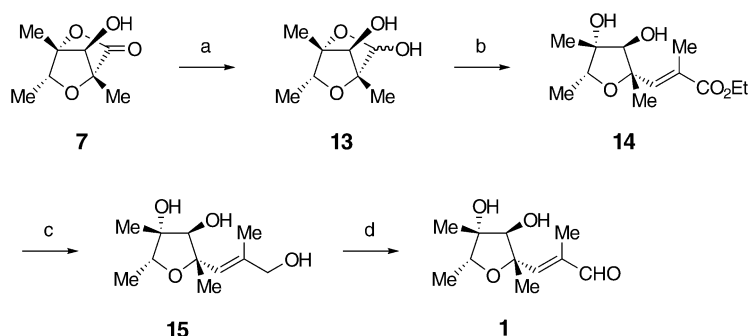
iodolactone **11** in 92 % yield as a single isomer. The employment of an imide^[10] as the substrate for iodolactonization accompanied by removal of the chiral auxiliary as well as the high selectivity were quite important from a practical point of view. The stereochemistry of γ -lactone **11** was confirmed by NOE studies, as shown in Scheme 3. The stereochemical course of the present iodolactonization could be explained by considering the “inside alkoxy effect”,^[9] as depicted in the transition state **A**.

The benzyl group in γ -lactone **11** was cleanly removed by exposure to BCl_3 in CH_2Cl_2 to obtain the free tertiary alcohol **8** in 79 % yield (Scheme 3). Next, we attempted the critical intramolecular $\text{S}_{\text{N}}2$ reaction of tertiary alcohol **8**. After a number of attempts with various bases and additives, we finally obtained the desired bicyclic lactone **7** in 92 % yield by treatment of **8** with AgNO_3 in DMF. The structure of **7** was confirmed unambiguously by conversion into the known benzoate **12**.^[5c] The spectral data of **12** were in good agreement with reported data. Thus, the tetrahydrofuran moiety of **1** was constructed in four steps in a completely stereoselective manner from chiral imide **6**.

The bicyclic lactone **7** was transformed into **1** by employing a sequence of reactions similar to those reported by Overman and co-workers^[5c] (Scheme 4). Reduction of **7** with DIBAL at -78°C in CH_2Cl_2 generated the lactol **13** in 94 % yield. Wittig reaction of **13** with (carboethoxyethylidene)triphenylphosphorane in refluxing benzene yielded a mixture of α,β -unsaturated esters (*E/Z* = 6:1), from which the desired isomer (*E*)-**14** was isolated in 82 % yield by chromatographic separation. The α,β -unsaturated δ,ϵ -dihydroxyester **14** was treated directly with DIBAL to afford triol **15** in 82 % yield. Finally, selective oxidation of allylic alcohol **15** with BaMnO_4 completed the total synthesis of (+)-citreoal (**1**). The data for synthetic **1** were identical in all respects with reported



Scheme 3. Reagents and conditions: a) LDA, $\text{Ti}(\text{OiPr})_3\text{Cl}$; then **10**, THF, -78°C $\rightarrow$ -40°C , 71 %; b) I_2 , NaHCO_3 , $\text{MeCN}/\text{H}_2\text{O}$ (4:1), room temperature, 92 %; c) BCl_3 , CH_2Cl_2 , -78°C $\rightarrow$ 0°C , 79 %; d) AgNO_3 , DMF, room temperature, 92 %; e) BzCl , NEt_3 , DMAP, CH_2Cl_2 , room temperature, 79 %. LDA = lithium diisopropylamide, DMF = *N,N*-dimethylformamide, DMAP = 4-dimethylaminopyridine.



Scheme 4. Reagents and conditions: a) DIBAL, CH_2Cl_2 , -78°C , 94%; b) (carboethoxyethylidene)triphenylphosphorane, benzene, reflux, 82%; c) DIBAL, CH_2Cl_2 , 0°C , 82%; d) BaMnO_4 , benzene, reflux, 58%. DIBAL = diisobutylaluminum hydride.

data. The eight-step sequence from **6** gave enantiomerically pure **1** in 18% overall yield.

In conclusion, we have completed a highly efficient total synthesis of (+)-citroviral through a route involving an *anti*-selective aldol reaction, the iodolactonization of an unsaturated imide, and the intramolecular $\text{S}_{\text{N}}2$ reaction of a tertiary alcohol. This short and highly stereoselective route can be applied to a variety of highly oxygenated bioactive compounds.

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Keywords: alcohols · aldol reaction · lactones · natural products · total synthesis

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RNA Structures

Monitoring Molecular Recognition of the
Ribosomal Decoding Site**

*Sarah Shandrick, Qiang Zhao, Qing Han,
Benjamin K. Ayida, Masayuki Takahashi,
Geoffrey C. Winters, Klaus B. Simonsen,
Dionisios Vourloumis, and Thomas Hermann**

Fidelity during protein synthesis is maintained by the ribosomal decoding site which monitors base pairing between the mRNA codon and the tRNA anticodon.^[1] Binding of cognate tRNA induces conformational changes in ribosomal RNA (rRNA) at the decoding site that translate into a signal for the progression of translation. An internal loop containing adenine residues A1492 and A1493 in 16S rRNA of the 30S (small) ribosomal subunit constitutes a major component of the bacterial decoding site (Figure 1a). Contacts of the

[*] S. Shandrick, Dr. Q. Zhao, Dr. Q. Han, Dr. T. Hermann
Department of Structural Chemistry
Anadys Pharmaceuticals, Inc.
9050 Camino Santa Fe, San Diego, CA 92121 (USA)
Fax: (+1) 858-527-1539
E-mail: thermann@anadyspharma.com

Dr. B. K. Ayida, M. Takahashi, G. C. Winters, Dr. K. B. Simonsen,
Dr. D. Vourloumis
Department of Medicinal Chemistry
Anadys Pharmaceuticals, Inc.

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

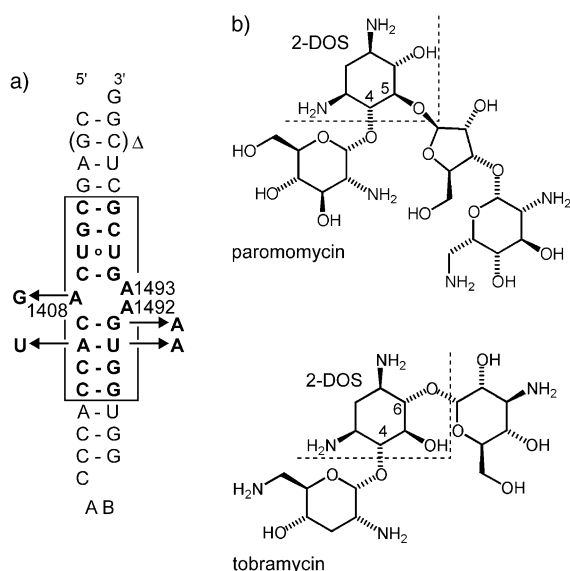


Figure 1. a) Bipartite oligonucleotide constructs AB and ABA containing the ribosomal decoding site used in this study. The box indicates the sequence that is identical to the bacterial decoding site. In the ABA RNA, one G–C base pair (in parentheses) is deleted in the upper flanking stem. Base changes in the eukaryotic sequence are indicated by arrows. The individual single-stranded oligonucleotides are referred to as A and B in the text. b) Aminoglycoside antibiotics paromomycin, a 4,5-disubstituted 2-deoxystreptamine, and tobramycin, a 4,6-disubstituted 2-deoxystreptamine (2-DOS = 2-deoxystreptamine).

conformationally flexible residues A1492 and A1493 across the shallow groove of the codon–anticodon hybrid have been proposed to be involved in sensing the base-pairing geometry between mRNA and tRNA, and discriminating against non-Watson–Crick alignments.^[2] Aminoglycoside antibiotics increase the error rate of translation by binding specifically to the bacterial decoding-site RNA (Figure 1),^[3] and locking the residues A1492 and A1493 in a conformation predisposed for interaction with the mRNA–tRNA hybrid. Pre-organization of part of the decoding site by bound aminoglycosides reduces the energetic cost of a ribosomal domain closure that is required for discrimination between noncognate and cognate tRNAs.^[4] Insights into the molecular recognition of the decoding site by antibiotic ligands and the mechanics of translational fidelity are emerging from three-dimensional (3D) structures of aminoglycosides in complex with decoding-site oligonucleotides^[5–7] and whole 30S ribosomal subunits.^[8]

To explore specific aminoglycoside interactions with the bacterial decoding site as a model of RNA-targeting,^[9] and as first step for the discovery of novel antibiotics, we have studied model oligonucleotides that retain the conformational flexibility of the decoding site in solution and that allow capture of distinct conformational states in RNA crystals. Fragments of rRNA containing the decoding site accurately represent the binding of natural aminoglycosides to the whole ribosome.^[10–12] To facilitate direct comparison of solution binding experiments and crystallographic structure investigations, we focused on an RNA construct that could be used as a faithful model of the ribosomal decoding site, including its aminoglycoside binding properties, both in solution and

crystallization studies. The bipartite oligonucleotides AB and ABA were chosen as optimal constructs, comprising the bacterial decoding-site sequence and flanking auxiliary base pairs that were added to enhance the crystallizability (Figure 1a). Both RNAs behaved identically in the fluorescence experiments described herein. In contrast to monopartite tethered decoding-site oligonucleotides used in binding studies by others,^[7,12,13] the AB RNAs do not contain an artificial loop structure that may interfere with ligand binding or crystallization.^[14]

We crystallized the AB oligonucleotide, and determined its 3D structure by X-ray diffraction (Table 1). While a range

Table 1: Crystal data and refinement statistics.

	AB native ^[a]	ABA-2AP1492 ^[a]
resolution range [Å]	8.0–1.7	8.0–2.2
highest resolution shell [Å]	1.76–1.70	2.28–2.20
space group	$P2_12_12_1$	$P2_1$
unit cell: a, b, c [Å]	32.04, 32.95, 92.69	31.64, 87.32, 32.79
unit cell: β [°]	90	93.27
unique reflections (fold redundancy)	11,015 (14.9)	8,903 (8.6)
completeness [%]	97.0 (81.6)	99.1 (96.1)
I/σ	32.7 (6.1)	15.3 (3.3)
R_{merge} ^[b]	0.044 (0.164)	0.09 (0.311)
R factor ^[c]	0.209 (0.337)	0.205 (0.299)
R_{free} ^[d]	0.238 (0.447)	0.266 (0.328)
RNA atoms	809	1396
solvent molecules	130	215
r.m.s.d. from ideality: bonds [Å]	0.023	0.004
r.m.s.d. from ideality: angles [°]	1.90	0.93
average B-factors [Å ²]: RNA	24.9	23.9
average B-factors [Å ²]: solvent	35.8	31.8

[a] Numbers in parentheses are statistics for data of the highest resolution shell, except for fold redundancy. [b] $R_{\text{merge}} = \sum_{hkl} \sum_i |I_i - \langle I \rangle| / \sum_{hkl} \sum_i I_i$ for all data with $I/\sigma > -3$. [c] R factor = $\sum_{hkl} ||F_{\text{obs}}| - k|F_{\text{calc}}|| / \sum_{hkl} |F_{\text{obs}}|$. [d] R_{free} was calculated from 10% of the data.

of crystallization conditions resulted in well-diffracting crystals of AB and ABA, hanging-drop vapor diffusion eventually allowed the growth of RNA crystals that captured two conformational states of A1492 within the decoding site (Figure 2). Electron density was found consistent with 50% partial occupancy of the A1492 base inside the RNA loop and 50% in a flipped-out conformation. In the inside conformation, the base of A1492 is fully stacked on G1491 and forms a *cis*-Watson–Crick pair^[15] with residue A1408 of the opposite strand (Figure 2b). The bases of A1492 and A1493 are partially stacked in the flipped-out conformation, which closely resembles the RNA structures in complexes of aminoglycosides bound to the decoding site.^[5,6,8] We were able to soak the aminoglycoside paromomycin into crystals of AB containing the flipped-out conformation of A1492 and observe electron density consistent with the aminoglycoside bound at the decoding-site loop.^[26]

The absence of crystal-packing contacts with A1492 in the flipped-out conformation in conjunction with the random distribution between the in- and out-states suggests that the two conformations are energetically similar. By variation of

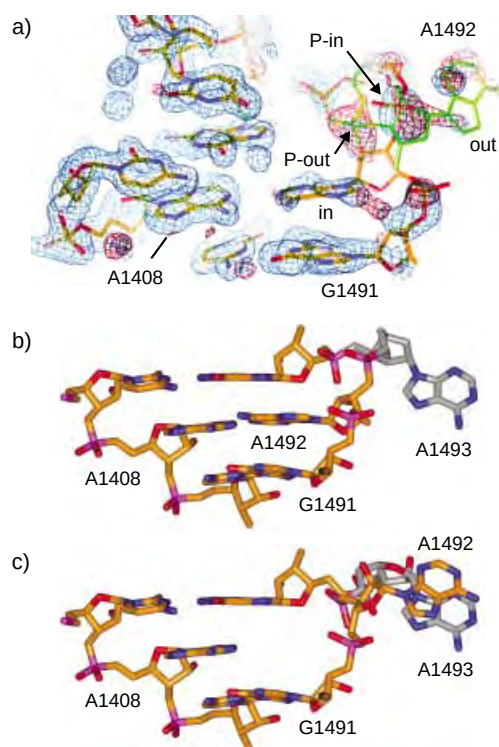


Figure 2. 3D structure of the decoding site in AB RNA as determined by X-ray diffraction of a crystal that captured a 1:1 random distribution of A1492 oriented inside and outside the RNA loop. a) Electron-density maps (blue: $2F_o - F_c$ contoured at 1.3σ ; red: $F_o - F_c$ at 3σ) around the decoding site, calculated using an AB model in which A1492 was oriented inside the RNA loop. For comparison, the flipped-out conformation of the A1492 residue is shown in green sticks (not included in map calculation). Positions of the phosphate groups (P) for the two conformations (in/out) are indicated (for density maps around a model with A1492 flipped out, see Supporting Information S1). b) Inside conformation of A1492 with the base stacking on G1491 in the interior of the decoding-site loop and forming a *cis*-Watson-Crick pair with residue A1408 of the opposite strand. c) Flipped-out conformation of A1492 with the base stacking partially on A1493 (gray sticks).

the conditions for crystallization of the AB and AB Δ RNAs, occupancy of either state could be driven to completeness. In contrast to the position of A1492, and independent from the crystallization conditions, the conformation of A1493 is locked in a flipped-out state by contacts in the shallow groove of a neighboring RNA helix (see Supporting Information).

To investigate the conformational states of the flexible residues A1492 and A1493 in solution, we replaced in AB Δ the adenines at either of these positions by the fluorescent 2-aminopurine (2-AP) base. This adenine analogue is a sensitive probe of structural environment since fluorescence of 2-AP is quenched when it is stacked with other bases, but increases significantly upon unstacking and exposure to solvent.^[16,17] Alternative conformations of residues in the decoding site in the crystal structures of the AB RNAs are thus expected to give rise to distinct fluorescence intensities corresponding to the stacking state of the 2AP label. Indeed, the observed fluorescence signal of the AB Δ RNA constructs, 2AP-labeled at either position 1492 or 1493, agreed well with the distinct

conformations of residues A1492 and A1493 in the crystal structures of AB and AB Δ (Figure 3a). The AB Δ RNA carrying 2AP at the 1492 position showed low fluorescence intensity, indicating that residue 2AP is stacked inside the

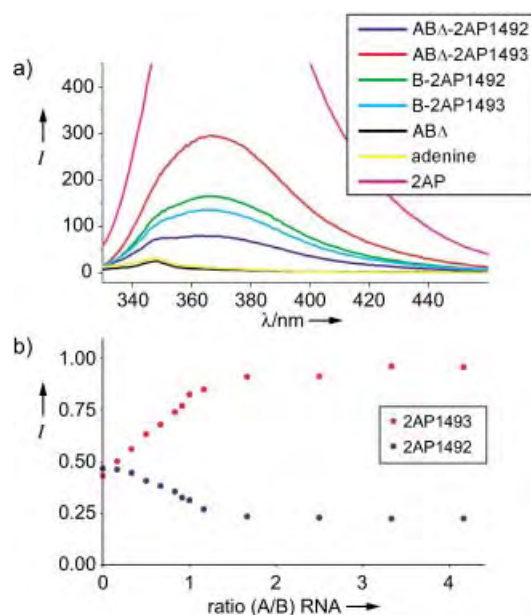


Figure 3. a) Fluorescence emission spectra of 2AP-labeled RNAs and controls at equivalent concentrations. AB Δ -2AP1492 = AB Δ RNA with 2AP at position 1492, AB Δ -2AP1493 = AB Δ RNA with 2AP at position 1493, B-2AP1492 = B oligonucleotide with 2AP1492, B-2AP1493 = B oligonucleotide with 2AP1493; Controls: AB Δ = unlabeled RNA construct; adenine and 2AP = monomeric bases. b) Normalized fluorescence signal at 370 nm during titration of single-stranded oligonucleotide A to single-stranded B that has the 2AP label at residue 1492 and 1493, respectively. (Experimentally determined oligonucleotide concentrations resulted in slight underestimation of the A/B ratio, presumably owing to the use of empirical extinction coefficients in RNA-concentration calculations.)

decoding-site loop. In contrast, the 2AP1493-labeled construct gave rise to high fluorescence, in line with a substantial solvent exposure of the 1493 residue. Fluorescence of the 2AP-labeled single-stranded B oligonucleotides (that is, in the absence of the A strand) was of an intermediate intensity, nearly independent from the position of the fluorescent base and perhaps reflecting little-defined conformations of the single-stranded RNA. The differences in fluorescence between the 2AP-labeled single-stranded B and double-stranded AB Δ RNAs were exploited to monitor structure formation in the decoding-site loop upon hybridization of the A and B oligonucleotides (Figure 3b). Titration of strand A to a constant amount of 2AP1493-labeled B resulted in a steady increase of fluorescence up to 1:1 stoichiometry of the oligonucleotides, after which the signal leveled off. The equimolar oligonucleotide ratio was marked by a clear inflection of the fluorescence signal, indicating that all the B strand was hybridized into AB Δ in which the flipped-out conformation of 2AP1493 resulted in high fluorescence. The precisely converse effect was observed when strand A was titrated to 2AP1492-labeled B oligonucleotide. These data

further suggest that the flipped-out conformation of residue A1493 in the crystal structure of the decoding-site construct AB is not an artifact of crystal packing, but rather reflects the capture of a conformational state that is preferred in solution.

To examine if replacement of the adenine base at position 1492 disturbs the conformational states of the decoding-site loop, we determined the 3D structure of 2AP-labeled ABA RNA by X-ray crystallography. We were especially interested in the ABA-2AP1492 construct, as we assumed that the inside conformation of residue 1492 might be affected by the exchange of adenine to 2AP, while the preferentially flipped-out residue 1493 would be less sensitive to base identity. The structure of ABA-2AP1492 in crystals grown under similar conditions as described above for AB, had the 2AP1492 residue in alternating inside and flipped-out conformations, arranged in a symmetry-related fashion (Figure 4 and Table 1). The residue A1493 is pointing outside

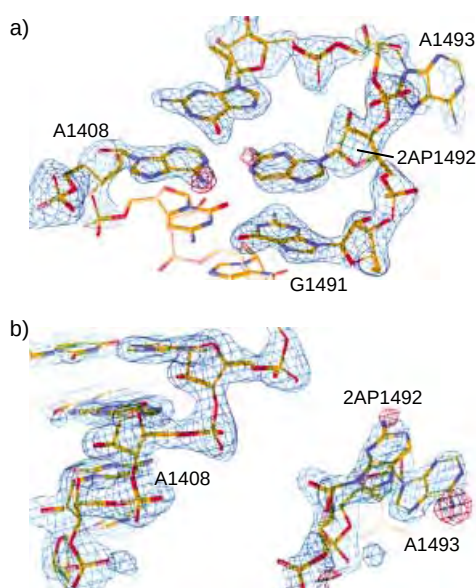


Figure 4. 3D structure of the decoding site in fluorescence-labeled ABA-2AP1492 RNA as determined by X-ray diffraction of a crystal that captured a symmetry-related 1:1 distribution of A1492 oriented inside and outside the RNA loop. a) Electron-density maps (blue: $2F_o - F_c$ contoured at 1.3σ ; red: $F_o - F_c$ at 2.8σ) around the decoding-site loop with 2AP1492 oriented inside the RNA. The exocyclic 2-amino group of 2AP1492 and 6-amino group of A1493 are omitted from the model used for density calculation, giving rise to $F_o - F_c$ density that unambiguously revealed the identity of the adenine and 2AP bases. b) Electron density maps around the decoding-site loop with 2AP1492 in the flipped-out state.

in all ABA-2AP1492 RNA molecules and involved in similar packing contacts as those observed in the AB RNA structure. In the inside-conformation of 2AP1492, the fluorescent base stacks on G1491, whereas in the flipped-out state, it is stacked on A1493 (see Supporting Information).

The conformation of the decoding-site loop in both states of the ABA-2AP1492 RNA closely resembles the structure of the unlabeled AB RNA, with exception of the 2AP1492–A1408 base pair in the inside conformation. Compared to the canonical *cis*-Watson–Crick geometry of the A1492–A1408

pair in the crystal structure of AB, the 2AP1492 residue in ABA-2AP1492 is rotated slightly towards the shallow groove to accommodate the 2-amino group. The 2AP1492–A1408 pair is stabilized by two hydrogen bonds, (A1408)N6–H...N1(2AP1492) and (2AP1492)N2–H...N1(A1408). The similarity of the native and 2AP-labeled constructs, both with regard to 3D structure as well as the geometry and conformational states of residues 1492 and 1493, led us to conclude that the modified RNAs might be used as sufficiently faithful representatives of the decoding-site in fluorescence measurements of aminoglycoside binding.

We reasoned that the fluorescence intensity changes associated with conformational rearrangements of residue 1492 in the 2AP-labeled decoding-site RNA might be exploited to monitor the interaction of ligands with the internal loop (Figure 5a). The aminoglycoside paromomycin was used as a representative ligand (Figure 1b), since structural investigations had shown earlier that upon binding this natural product induces the residues A1492 and A1493 to flip out of the decoding-site loop.^[5,6,8] We performed titrations of paromomycin to a variety of 2AP-labeled decoding-site constructs (Figure 5b and Supporting Information). As a control, paromomycin was also added to 2AP monomer, 2AP-labeled single-strand B oligonucleotide (not shown), and double-labeled (1492 and 1493) ABA RNA, none of which showed significant changes of fluorescence. In contrast, titration of the aminoglycoside to 2AP1492-labeled ABA RNA lead to a dramatic increase of fluorescence, corresponding to an EC_{50} value (EC_{50} is the concentration required for 50% response) of $0.75 \pm 0.05 \mu\text{M}$ for the paromomycin dose response. Fluorescence of 2AP1493-labeled ABA construct was quenched by paromomycin at an EC_{50} value that was identical within the experimental error ($0.95 \pm 0.20 \mu\text{M}$). The EC_{50} values calculated from both experiments are in good agreement with the binding affinity of paromomycin for decoding-site RNA ($K_d \approx 10^{-6} \text{M}$).^[11,12] The congruence of the fluorescence changes triggered by paromomycin addition to either the 2AP1492- or 2AP1493-labeled construct indicates that the same conformational event is monitored in both cases, and that the 2AP label does not alter the interaction of the aminoglycoside with the RNA target. We suggest that binding of paromomycin to the decoding site displaces residue 1492 from the internal loop, associated with unstacking of the base and increase of 2AP1492 fluorescence. As demonstrated by the structural studies on the decoding site, the flipping-out of A1492 forces the bases of residues 1492 and 1493 to partially stack onto each other, which leads to quenching of the 2AP1493 fluorescence. That fluorescence of 2AP1492-labeled RNA increases above the level of 2AP1493-labeled construct in the paromomycin-bound state (Figure 5b), although residues 1492 and 1493 are mutually stacked, might be attributed to the asymmetric overlap of the bases (see Supporting Information) and their distinct solvation, both of which influence the quantum yield of the fluorophore.^[17]

The preferentially flipped-out conformation of residue 1493 renders this position ideally suited for fluorescence labeling since base modifications are unlikely to interfere with either the structural integrity of the decoding site or with

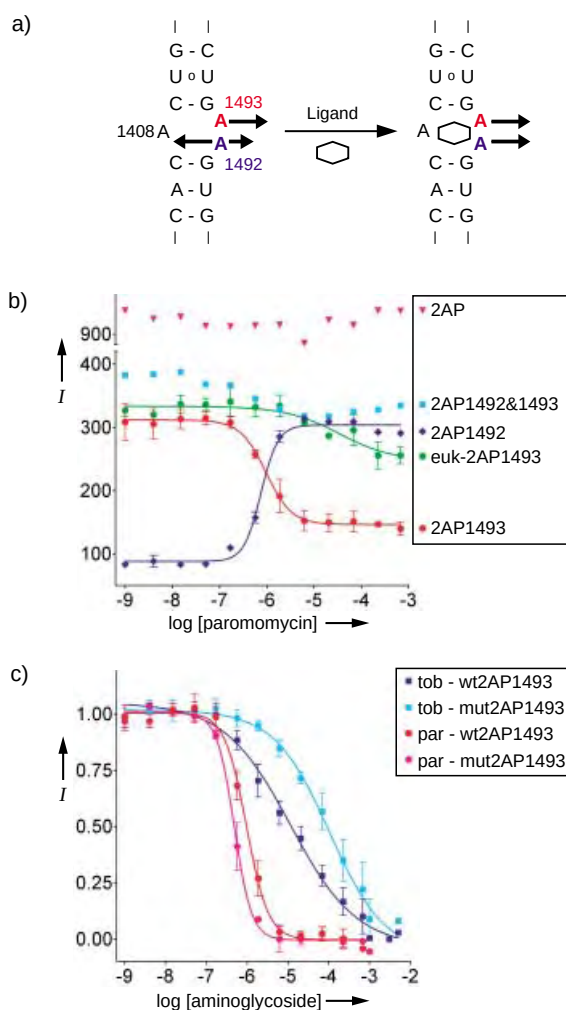


Figure 5. a) Model illustrating the conformational changes triggered by aminoglycoside ligand binding to the AB decoding-site RNA. See text for a discussion. b) Titrations of 2AP-labeled RNA constructs and 2AP monomer with paromomycin (see text for details). The fluorescence signal at 370 nm is plotted against log of aminoglycoside concentration (error bars correspond to 2σ calculated for the average of two experiments). c) Titrations of 2AP-labeled wild-type decoding site (wt2AP1493) and U1406A mutant construct (mut2AP1493) with paromomycin (par) and tobramycin (tob). Normalized fluorescence signals are plotted.

ligand binding. The concept of indirect fluorescence quenching by ligand-induced displacement of A1492 and stacking on residue 1493 can therefore be extended to other labels which have fluorescent characteristics that make them suitable for the testing of UV-active decoding-site ligands. Fluorescent pteridine base analogues, which have higher quantum yields along with emission maxima shifted to longer wavelengths,^[18] may replace 2AP as the preferred label to prepare decoding-site constructs for ligand binding studies.^[27]

The fluorescence-labeled RNA constructs described herein provide powerful probes to detect the specific binding of ligands to the ribosomal decoding-site and to discriminate against nonspecific RNA binders (see also Supporting Information). Importantly, 2AP-labeled RNA models of the eukaryotic decoding site can be used to investigate the target

selectivity of ligand binding. Titration of paromomycin to a 2AP-labeled RNA construct that carried the eukaryotic decoding-site sequence (Figure 1a) exhibited significantly lower affinity of the aminoglycoside for the eukaryotic compared to the bacterial target (euk-2AP1493, Figure 5b), substantiating the organism-specificity of these antibiotics that is exploited in therapy.^[12] Moreover, 2AP-labeled bacterial decoding-site constructs are useful tools with which to investigate the molecular discrimination associated with target mutations in aminoglycoside-resistant bacteria. As an example, we have tested aminoglycoside interaction with bacterial decoding-site RNA that carried the U1406A mutation, which is known to confer resistance selectively to 4,6-disubstituted 2-DOS (Figure 1b).^[19] The binding of paromomycin, a 4,5-disubstituted 2-DOS, is little affected by the mutation, whereas the affinity of tobramycin, which belongs to the 4,6-disubstituted 2-DOS class (Figure 1b), is noticeably reduced (Figure 5c). The EC_{50} values calculated for tobramycin binding to the wild type and U1406A mutant RNA (wt: $9.5 \pm 2.0 \mu\text{M}$; mut: $110 \pm 8.5 \mu\text{M}$) are in agreement with the minimum inhibitory concentration (MIC) shift for tobramycin in corresponding wild type and mutant bacteria reported by Pfister and co-workers.^[20] Crystal-structure analyses by Vicens and Westhof have shown that the U1406A substitution causes steric clash between the exocyclic amino group of A1406 and the sugar ring linked at the 6-position of 2-DOS.^[6] Molecular discrimination resulting from the U1406A target mutation is well reproduced by the 2AP-labeled decoding-site RNAs.

In summary, we have developed a set of fluorescence-labeled RNA constructs to investigate molecular recognition of the ribosomal decoding site, the target of many aminoglycoside antibiotics. Incorporation of fluorescent 2AP has been used before to probe conformational changes of RNA in solution,^[16,21] and while this manuscript was in preparation, Pilch and co-workers reported a similar concept for detecting conformational changes in ribosomal RNA by 2AP replacement of A1492.^[13] In the study described herein, we have combined structural data from X-ray crystallography and fluorescence measurements in a unique fashion to explore the conformational states of the decoding site. We exploited the indirect quenching of fluorescence-labeled residue 1493 by a conformational change in A1492, which is in turn induced by specific ligand binding to the decoding site. The approach disclosed herein provides a basis for affinity screening of the ribosomal target against novel RNA binders as highly selective lead candidates for the discovery of novel antibiotics. Consequently, our group has extensively used the fluorescence binding assay for the decoding-site target to establish structure–affinity relationships for designed aminoglycoside mimetics.^[22]

Experimental Section

Gel-purified and desalted oligonucleotides (Figure 1, constructs AB and ABA) were purchased from Dharmacon Research (Lafayette, CO). Double-stranded RNA was obtained by heating complementary oligonucleotides in buffer at 75 °C for 1 min and snap cooling on ice. Cacodylate buffer (30 mM, pH 6.8) was used to prepare RNA for

crystallization, and cacodylate or phosphate buffer (30 mM, pH 6.8) for fluorescence experiments.

Fluorescence measurements were performed on an RF-5301PC spectrofluorometer (Shimadzu, Columbia, MD) at 25 °C. Emission spectra were recorded at 1 μ M RNA concentration in 1-cm path-length quartz cells, irradiating at 310 nm. EC₅₀ values were calculated with the ORIGIN software (MicroCal, Northampton, MA) by fitting a dose response curve to the fluorescence intensity plotted against the log of ligand concentration.

RNA was crystallized by the hanging-drop vapor-diffusing method using sparse matrix screens (Hampton Research, Aliso Viejo, CA). Crystals of decoding-site constructs (0.3 mM RNA) grew within 2 weeks from 1.95 to 2.05 M ammonium sulfate, 50 mM Na cacodylate (pH 6.8), and 0.7 to 1.2 mM Mg acetate. For X-ray diffraction, crystals were transferred to glycerol (15% v/v) as a cryoprotectant and frozen. Data sets were collected with an R-AXIS IV imaging plate detector using Cu α radiation (λ = 1.54178 Å) of an RU-H3R rotating anode generator (Rigaku/MSO, The Woodlands, TX). Data were processed with DENZO/SCALEPACK.^[23] Structures were solved by molecular replacement using canonical A-form RNAs as the initial model with AMORE,^[24] and refined with CNS.^[25] For crystal data and refinement statistics see Table 1. Coordinates and structure factors have been deposited in the Protein Data Bank (accession codes 1T0E and 1T0D for native decoding site and 2-AP labeled RNA, respectively).

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Keywords: antibiotics · fluorescent probes · molecular recognition · RNA structures · structure elucidation

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Cluster Compounds

An Oxygen-Centered Titanium Square Embedded in a Cuboctahedron of Iodine in the Salt $K_4[\{Ti_4O\}I_{12}]^{**}$

Liesbet Jongen, Anja-Verena Mudring, Angela Möller, and Gerd Meyer*

The di- and triiodides of titanium, TiI_2 and TiI_3 , are excellent and chemically simple examples with which to study the formation of metal–metal bonds. According to single-crystal data TiI_3 undergoes a reversible one-dimensional Peierls transition at about 300 K.^[1] The low-temperature structure corresponds to that of ZrI_3 ,^[2] with Ti–Ti bond lengths of 309.6(2) pm at 293 K. TiI_2 exhibits the same anionic structure, that is, the hexagonal closest-packing of iodine ions. At high temperatures, half of all the octahedral interstices are occupied by Ti^{2+} ions just as in the CdI_2 type of structure. The TiI_2 structure undergoes a two-dimensional Peierls distortion upon cooling, at about 240 K, with a large hysteresis of about 110 K, according to magnetic data.^[1] Thereby triangular clusters $\{Ti_3\}^{6+}$ are formed with single Ti–Ti bonds of 300 pm. These triangles are also known from $Ti_7Cl_{16} \triangleq Ti[\{Ti_3\}Cl_8]_2$,^[3] KTi_4Cl_{11} ,^[4] and $Na_2[\{Ti_3\}Cl_8]$ ^[5] with bond lengths of 295.4, 295.5, and 299.6 pm, respectively. To some extent, the formation of the carbon-stabilized octahedral cluster in $\{Ti_6C\}Cl_{14}$ ^[6] can be interpreted as a three-dimensional Peierls distortion. In this case the Ti–Ti bonds are between 300 and 310 pm.

We have now found a titanium square with a central oxygen atom, $\{Ti_4O\}$, in the salt $K_4[\{Ti_4O\}I_{12}]$. This salt was first obtained in the pursuit of crystal growth of the complex salt $KTiI_3$. We are now able to produce pure samples of $K_4[\{Ti_4O\}I_{12}]$ when potassium iodide, titanium(IV) iodide, titanium, and titanium dioxide are allowed to react in the appropriate proportions in a sealed tantalum container at 798 K.^[7] $K_4[\{Ti_4O\}I_{12}]$ is obtained as good-quality black crystals with metallic luster. The crystal structure has a tetragonal body-centered symmetry, space group $I4/m$. No structural changes are observed between 300 and 10 K, according to X-ray powder diffractometry and single-crystal structure determinations at 293 and 170 K.^[8]

Four out of six faces of the cube of an iodine cuboctahedron with shortest I–I separations of 396 to 407 pm are centered by titanium atoms. This square of titanium atoms is itself centered by an oxygen atom, so that an isolated ion $[\{Ti_4O\}I_{12}]^{4-}$ is created (Figure 1). These anions are arranged

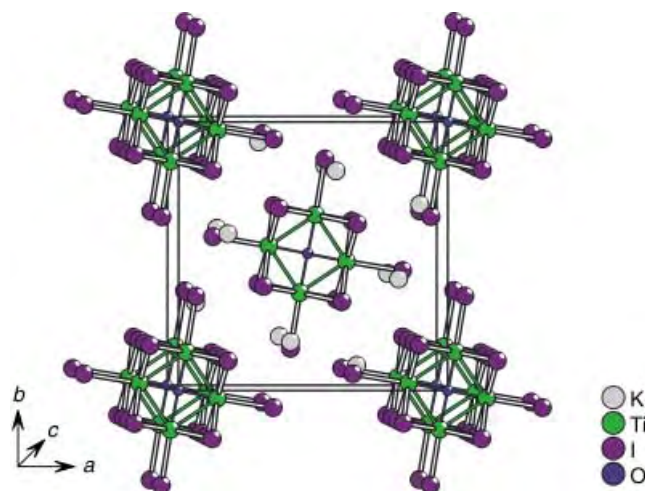


Figure 1. Crystal structure of $K_4[\{Ti_4O\}I_{12}]$.

in a tetragonal body-centered fashion and held together by potassium cations with K^+I^- separations ranging from 347.0(1) to 404.5(4) pm (Figure 1).

The Ti–Ti separations within the $\{Ti_4O\}$ square are unusually short, only 286.0(2) pm at 293 K. This situation also accounts for short Ti–O separations of 202.5(1) pm, a distance that is well known from Ti_2O_3 .^[10] With an oxidation state of +2.5 for titanium, the short Ti–Ti separation may either be the result of strong Ti–Ti bonding (1.5 electrons can be allocated to each of the four Ti–Ti bonds in the square) or of strong $Ti^{2.5+}-O^{2-}$ bonding. Naturally a quasi one-dimensional Peierls distortion of the square to a rectangle would be expected if such a simple picture should hold some truth (see below for a better theoretical description). Ti–I separations are within a narrow range between 282 and 287 pm, averaging to 284 pm. This mean distance is in good agreement with the 286 pm found in $CsTi_{4.3}I_{11}$.^[4] All Ti–O–Ti, O–Ti–I and I–Ti–I angles are 90 and 180°, or close to these ideal values deviating by 2° at most.

Quantum mechanical calculations for solid $K_4[\{Ti_4O\}I_{12}]$ (relativistic density functional theory and extended Hückel calculations) and for the isolated cluster ion $[\{Ti_4O\}I_{12}]^{4-}$ (extended Hückel calculations) were performed.^[11] Independent of the method used, a consistent picture of the electronic structure results.

The band structure obtained from DFT-LMTO studies (Figure 2, left) is characterized by comparatively flat bands indicative of very well localized electronic levels. The narrow bands around the Fermi level suggest an insulating behavior. The density of states can be divided into several distinctive regions. A projection of the density of states which allows for the partitioning of the total density of states into contributions of certain atoms or atomic orbitals (Figure 2, right) shows—from lower to higher energies—mainly oxygen p character,

[*] Dr. L. Jongen, Dr. A.-V. Mudring, Priv.-Doz. Dr. A. Möller, Prof. Dr. G. Meyer
Institut für Anorganische Chemie
Universität zu Köln
Greinstrasse 6, 50939 Köln (Germany)
Fax: (+49) 221-470-5083
E-mail: gerd.meyer@uni-koeln.de

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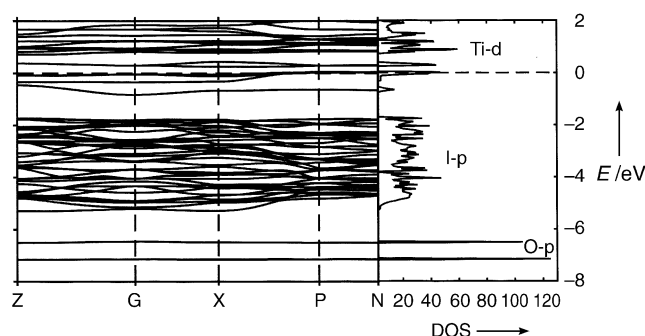


Figure 2. Band structure (left) and density of states (DOS, right) for $K_4[{\text{Ti}_4\text{O}}\text{I}_{12}]$ obtained from LMTO studies.

followed by a broad region stemming predominantly from iodine p orbitals. At even higher energies the titanium d levels follow which are only partially occupied. Fatband analysis of the valence bands of $K_4[{\text{Ti}_4\text{O}}\text{I}_{12}]$ shows that the valence levels are made up predominantly of titanium d_{z^2} and d_{xy} states (to comply with the local point-group symmetry, angular-dependent parts of the wave functions are given with respect to the local cluster coordinate system). A graphical interpretation of the crystal overlap Hamiltonian population (COHP) reveals that these levels are responsible for strong Ti–Ti bonding interactions (Figure 3a). These interactions lie right below the Fermi level and are locally of the same magnitude as titanium–iodine interactions (Figure 3b).

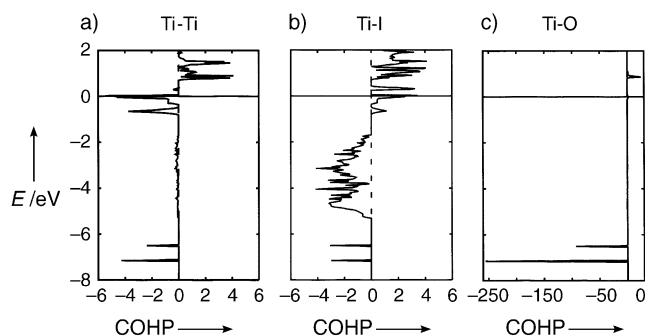


Figure 3. Crystal overlap Hamiltonian population (COHP) analysis of the data from LMTO studies for the a) Ti–Ti, b) Ti–I, and c) Ti–O interactions in $K_4[{\text{Ti}_4\text{O}}\text{I}_{12}]$.

For a more localized analysis of the bonding interactions, extended Hückel calculations were carried out for a distinct $[{\text{Ti}_4\text{O}}\text{I}_{12}]^{4-}$ cluster. The resulting molecular levels correlate directly with the states in the extended solid.

A closer inspection of the frontier orbitals of this cluster (Figure 4) reveals that three Ti–Ti σ -bonding HOMOs of b_{2g} and e_u symmetry exist. Additionally, the e_u orbital set has an oxygen p-orbital contribution.

Directly above the Fermi level a slightly bonding Ti–Ti level of a_{1g} symmetry is found (c.f. Figure 3a and Figure 4b). Population with electrons up to this energy, however, becomes unfavorable as the resulting weak Ti–Ti bonding is overcompensated by a strongly antibonding Ti–I interaction (see Figure 3b). Thus, the electron count of six electrons per

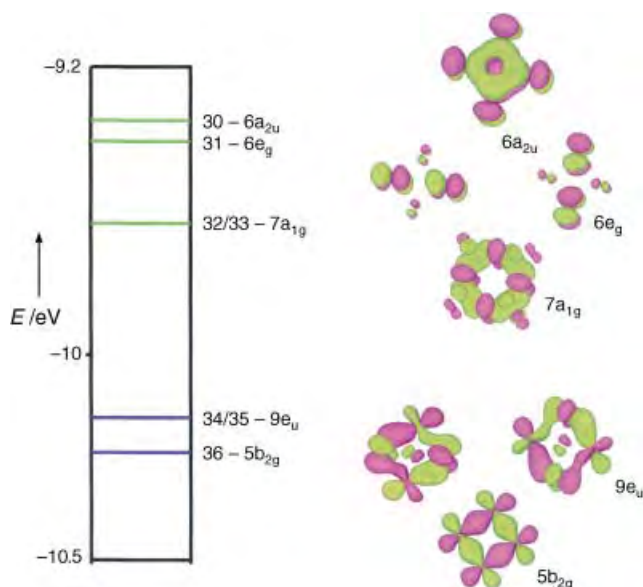


Figure 4. Energy level diagram for the valence orbitals of $[{\text{Ti}_4\text{O}}\text{I}_{12}]^{4-}$ (left) and corresponding orbitals (right) obtained from extended Hückel calculations.

$[{\text{Ti}_4\text{O}}]$ unit is necessary for the optimization of the bonding in this compound. Other interstitial atoms, such as carbon or nitrogen, would lead to less stable radical compounds. Furthermore, a comparison of the total energies obtained from theoretical calculations (extended Hückel) show that the total energy of the oxygen compound is about 10 % higher than that of a hypothetical oxygen-free compound. Note also the highly bonding COHP values of the Ti–O bond (Figure 3c). As a result, in $K_4[{\text{Ti}_4\text{O}}\text{I}_{12}]$ the shortest Ti–Ti distance ever observed is found owing to the strong stabilization by the oxygen atom. Without the interstitial atom, energetically higher cluster orbitals which no longer have σ symmetry with respect to the Ti–Ti bond would have to be occupied. To avoid this situation, incorporation of the oxygen atom in accord with the general tendency—well known for complex compounds and solids—that transition metals (especially the early ones) of the first series, in strong contrast to their heavier homologues try to avoid multiple metal-metal bonds.

First magnetic measurements^[12] reveal a small temperature independent paramagnetic (TIP) behavior for $K_4[{\text{Ti}_4\text{O}}\text{I}_{12}]$, in perfect accord with the calculated band structure. Furthermore, X-band ESR measurements between 2 and 300 K do not show a bulk signal. Below 20 K a very small signal with a g value of approximately 1.91 is observed which can be assigned to a Ti^{III} (d^1) impurity. The ESR results confirm the presence of oxygen in this cluster compound, since carbon and nitrogen should give a bulk signal (e.g. $g \approx 2.0$) owing to the odd total number of electrons and the resulting paramagnetic behavior.

In conclusion, with $K_4[{\text{Ti}_4\text{O}}\text{I}_{12}]$ we have a salt that contains an anion consisting essentially of a $[{\text{Ti}_4\text{O}}]^{8+}$ cluster embedded in a cuboctahedron of iodide ions. This $[{\text{Ti}_4\text{O}}\text{I}_{12}]^{4-}$ ion is an excellent example of an optimized bonding situation where strong Ti–Ti and Ti–O σ bonding as

well as the absence of Ti–Ti multiple (π) bonding and, furthermore, the avoidance of unfavorable Ti–I antibonding interactions stabilize the cluster anion.

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- [7] Synthesis: KI (Aldrich, 99%) was dried under vacuum at 473 K. TiI_4 was prepared from titanium (chips, Chempur, 99.6%) and iodine (Riedel de Haën, 99.8%), according to the procedure described in ref. [9]. TiO_2 was used as obtained (Kronos Titan). All storage and manipulation of all starting materials and products were handled under dry box conditions (MBraun, Garching). The reaction was carried out in a sealed tantalum container, jacketed with a silica tube under argon at a temperature of 798 K. The compound formed as black shiny crystals of good quality. X-ray powder diffraction measurements at room temperature revealed the product to consist of only one phase, $\text{K}_4[\text{Ti}_4\text{O}]\text{I}_{12}$. No phase transition was observed by X-ray powder diffraction in the temperature range between 293 K and 10 K. Indexed X-ray powder diffractograms of $\text{K}_4[\text{Ti}_4\text{O}]\text{I}_{12}$ were measured at room temperature on a transmission powder diffraction system, Guinier Image Plate Camera G670 (Huber, Rimsting), using monochromatic $\text{MoK}\alpha$ radiation (cell parameters at 298 K refined from powder data: $a = 1373.0(3)$ pm, $c = 807.1(2)$ pm). Low temperature X-ray powder diffractograms were measured on a Guinier diffractometer G645 (Huber, Rimsting, Germany), using monochromatic $\text{CuK}\alpha$ radiation, (cell parameters at 150 K refined from powder data: $a = 1369.1(4)$ pm, $c = 808.8(3)$ pm).
- [8] Crystal data and structure refinement: A suitable single crystal ($0.2 \times 0.2 \times 0.1$ mm) was mounted in a glass capillary. Intensity data were collected on an IPDS diffractometer (Stoe, Darmstadt) at 170 K and 293 K. 170 K: Tetragonal, space group $I4/m$; $a = 1364.5(1)$ pm, $c = 801.51(7)$ pm, $V = 1.4923(2)$ nm³; $Z = 2$; $\rho_{\text{calc}} = 4.199$ g cm⁻³; $2.11 < \theta < 29.97^\circ$; IPDS II, $\text{MoK}\alpha$ radiation ($\lambda = 71.073$ pm); $T = 170(2)$ K; $F(000) = 1616$; $\mu = 13.152$ mm⁻¹; 13 964 reflections were measured, of which 1169 are unique, 1104 are considered as observed. $R1 = 0.0196$ and $wR2 = 0.0426$ for $[I_0 > 2\sigma(I_0)]$. 293 K: Tetragonal, space group $I4/m$; $a = 1373.9(2)$ pm, $c = 810.1(2)$ pm, $V = 1.5293(5)$ nm³; $Z = 2$; $\rho_{\text{calc}} = 4.097$ g cm⁻³; $2.92 < \theta < 25.98^\circ$; IPDS I, $\text{MoK}\alpha$ radiation ($\lambda = 71.073$ pm); $T = 293(2)$ K; $F(000) = 1616$; $\mu = 13.658$ mm⁻¹; 6080 reflections were measured, of which 804 are unique, 536 are considered as observed. $R1 = 0.0261$ and $wR2 = 0.0385$ for $[I_0 > 2\sigma(I_0)]$. The data were processed with the program systems SHELX-97 [G. M. Sheldrick, SHELX-97, Universität Göttingen, **1997**]. Scattering factors were taken from the International Tables for Crystallography, Volume C [A. J. C. Wilson, *International Tables for Crystallography*, Vol. C, Kluwer Dordrecht, the Netherlands, **1995**]. Numerical absorption correction after crystal shape optimization was performed using the programs XRED and XSHAPE [Stoe, XRED 1.01 and XSHAPE 1.01, Darmstadt, **1996**]. Further details on the crystal structure investigations may be obtained from the Fachinformationszen-
- trum Karlsruhe, 76344 Eggenstein-Leopoldshafen, Germany (fax: (+49) 7247-808-666; e-mail: crysdata@fiz-karlsruhe.de), on quoting the depository numbers CSD-413504 and CSD-413505.
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- [10] W. R. Robinson, *J. Solid State Chem.* **1974**, *9*, 255.
- [11] Theoretical calculations: Semi-empirical extended Hückel calculations (EH) of the whole solid $\text{K}_4[\text{Ti}_4\text{O}]\text{I}_{12}$ were performed with the program package CAESAR [J. Ren, W. Liang, M.-H. Whangbo, CAESAR, PrimeColor Software Inc., Raleigh, NC, USA, **1998**] with the following parameters: H_{ij} [eV] (ζ_1 : O: 2s –32.29999 (2.275), 2p –14.8 (2.275), I: 5s –18.0 (2.679), 5p –12.7 (2.322) K: 4s –4.34 (0.874), 4p –2.73 (0.874); Ti: 4s –8.97 (1.075), 4p –5.44 (0.675) for Ti-3d a double zeta-function was used H_{ij} [eV], ζ_1 , coefficient 1, ζ_2 , coefficient 2: –10.81, 4.55, 0.4206, 1.4, 0.7839. The program package CACAO [C. Mealli, D. M. Proserpio, A. Ienco, CACAO, A package of programs for Molecular Orbital Analysis, Instituto per lo Studio della Stereo-chimica ed Energetic dei Composti di Coordinazione, Florence (Italy), D. M. Proserpio, *J. Chem. Educ.* **1990**, *67*, 399–402] was used for extended Hückel calculations of the electronic structure and interpretation of bonding for an isolated $[\text{Ti}_4\text{O}]\text{I}_{12}^{4-}$ cluster. Mulliken overlap populations [R. S. Mulliken, *J. Chem. Phys.* **1955**, *23*, 1397] are given for selected interactions. For graphical display of the cluster valence orbitals Hyperchem [HyperChem, Hypercube Inc., Gainesville, Florida, USA, **2001**] was used. Density functional studies were performed with the Stuttgart LMT0 program [R. W. Tank, O. Jepsen, A. Burckhardt, O. K. Andersen, *TB-LMTO-ASA Program*, Vers. 4.7, Max-Planck-Institut für Festkörperforschung, Stuttgart, Germany, **1998**] based on the linear-muffin-tin approximation using the local density functional LDA as the exchange-correlation functional and the atomic sphere approximation ASA [H. L. Shriver, *The LMTO Method*, Springer, Berlin, **1984**; O. Jepsen, M. Snob, O. K. Andersen, *Linearized Band-Structure Methods in Electronic Band-Structure and its Applications*, Springer Lecture Note, Springer, Berlin, **1987**; O. K. Anderson, O. Jepsen, *Phys. Rev. Lett.* **1984**, *53*, 2571]. Interstitial spheres are introduced to achieve space filling. The ASA radii as well as the positions and radii of additional empty spheres are calculated automatically. Reciprocal-space integrations are carried out using the tetrahedron method [O. K. Andersen, O. Jepsen, *Solid State Commun.* **1971**, *9*, 1763; P. Blöchl, O. Jepsen, O. K. Andersen, *Phys. Rev. B* **1994**, *34*, 16223] The basis set of short ranged, atom-centered TB-LMTOs contained for O 3s, 2p, 3d, for I 6 s, 5p, 5d, and 4f, for K 4s, 4p, 3d, and 4f and for Ti 4s, 4p, and 3d. For O the 3s and 3d, for I the 6s, 5d, and 4f, for K the 4p, 3d, and 4f and for Ti 4s, 4p, and 3d orbitals were treated with the downfolding technique [W. R. L. Lambrecht, O. K. Andersen, *Phys. Rev. B* **1986**, *34*, 2439; O. Jepsen, O. K. Andersen, *Z. Phys. B* **1995**, *97*, 35; G. Krier, O. Jepsen, O. K. Andersen, Max-Planck-Institut für Festkörperforschung, Stuttgart, Germany, unpublished] The crystal orbital Hamiltonian population (COHP) method is used for bond analysis [R. Dronskowski, P. Blöchl, *J. Phys. Chem.* **1993**, *97*, 8617]. Note that the values are negative for bonding and positive for antibonding interactions, the reverse of the signs used in crystal orbital overlap population (COOP; [S. Wijeyesekera, R. Hoffmann, *Organometallics* **1984**, *3*, 949]) diagrams in the semi-empirical Hückel treatment. This discrepancy emerges because, to obtain the COOP the DOS gets multiplied by the overlap population while for weighting the DOS in case of the COHP the corresponding element of the Hamiltonian is used.
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Cluster Compounds

Al₅₀C₁₂₀H₁₈₀: A Pseudofullerene Shell of 60 Carbon Atoms and 60 Methyl Groups Protecting a Cluster Core of 50 Aluminum Atoms**

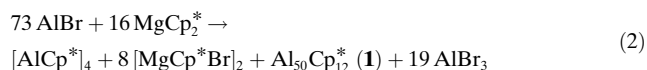
Jean Vollet, Jens R. Hartig, and Hansgeorg Schnöckel*

The controlled disproportionation of metastable Al^I halide solutions has been developed into a fruitful concept for the generation of metalloid Al cluster compounds as intermediates on the way to bulk phases of Al metal during the last years [Eq. (1), X = Cl, Br, I].^[1]



To prevent the thermodynamically favored formation of the metal, the halide ligands are substituted—simultaneously to the disproportionation reaction—by bulky ligands. The majority of the metalloid Al clusters prepared in our research group to date are protected by N(SiMe₃)₂ ligands.^[1] Here we introduce a large metalloid Al cluster containing only Al, C, and H atoms. The first hint of an unusual Al_nCp_m^{*} (*n* > *m*, Cp^{*} = pentamethylcyclopentadienyl) compound came from the observation of some very high-field ²⁷Al NMR signals in the spectra of some AlCp^{*}-containing samples, similar to that found in the substituted cluster SiAl₁₄Cp₆^{*}.^[2] The second indication of the formation of Al-rich Al_nCp_m^{*} species came recently from MALDI experiments, where Al₈Cp₄^{*} species were found to be either present or generated in the mass spectrometer when solid unpurified [AlCp^{*}]₄ was used in the experiments.^[3]

The preparation of the Al₅₀Cp₁₂^{*} cluster **1** described below follows, in principle, standard methods.^[4] Performing the synthesis at −78 °C would lead to an exchange of the halogen functionality and lead to Cp^{*} and [AlCp^{*}]₄ as the only products.^[4] However, the reaction between AlX and MgCp₂^{*} is allowed to proceed at conditions where disproportionation (that is, formation of an Al_n cluster) should occur more rapidly than the substitution of the halide atoms by the Cp^{*} ligands [see Eq. (2), which represents a proposed stoichiometry of the substitution and disproportionation reactions in toluene/THF at −30 °C].



[*] Dipl.-Chem. J. Vollet, Dipl.-Chem. J. R. Hartig, Prof. Dr. H. Schnöckel
Institut für Anorganische Chemie
Universität Karlsruhe (TH)
Engesserstrasse 15, Geb. 30.45, 76128 Karlsruhe (Germany)
Fax: (+49) 721-608-4854
E-mail: hansgeorg.schnoekel@chemie.uni-karlsruhe.de

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

chometry of the substitution and disproportionation reactions in toluene/THF at −30 °C].

A metastable solution of AlBr in a mixture of THF and toluene, obtained by trapping of the high-temperature AlBr molecule,^[4] reacts at −78 °C with decamethylmagnesiumocene (MgCp₂^{*}) dissolved in *n*-heptane. The reaction mixture is warmed to −30 °C and kept at this temperature for about 24 h. After reaching room temperature, the solution, which contains the Al-rich cluster compound, the Grignard species (MgCp^{*}Br·THF)₂^[5] (an analogue of (MgCp^{*}Cl·Et₂O)₂^[6]), and (AlCp^{*})₄ as well as the as yet uncharacterized Al^{III} compound, has a red color. The by-products can be gradually separated by using several concentration and cooling cycles (down to −25 °C). Finally, the Al₅₀ cluster compound **1** is separated from the [AlCp^{*}]₄ by removal of the toluene from the deep-red solution and subsequent extraction with pentane. Crystals of **1** suitable for X-ray crystallographic analysis were grown in [D₆]benzene.

The crystal structure analysis of **1**^[7] shows an Al₈ moiety with a distorted square-based anti-prism geometry (similar to a recently published Ga₈R₈^{2−} ion)^[8] at the center of the neutral cluster compound (Figure 1a). This Al₈ center is

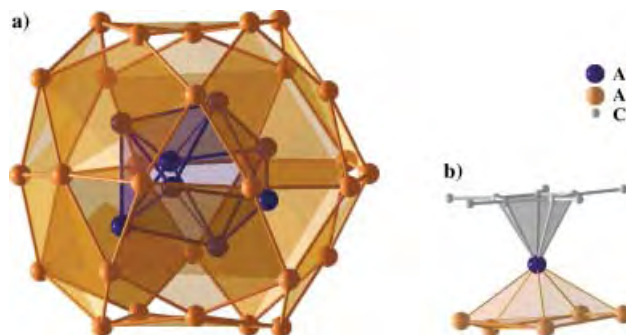


Figure 1. a) Ball and stick model of the Al₈ core (blue) and the surrounding icosidodecahedral Al₃₀ shell (orange); b) the “mixed sandwich” coordination of each of the outer 12 Al atoms through η⁵-C₅(CH₃)₅ and η⁵-Al₅.

surrounded by 30 Al atoms that form an icosidodecahedron with 12 pentagonal and 20 trigonal faces. Every pentagonal face is capped by an AlCp^{*} unit whose 12 Al atoms form a very regular icosahedron (Al–Al distances: found 570.2 pm, calcd 579.4 ± 9.4 pm). Each of these 12 Al atoms is coordinated by 10 atoms (5 Al and 5 C) in the form of a “mixed sandwich” (Figure 1b).

The average Al–Al bond lengths in **1** (Figure 2a) increase from the Al₈ unit, over the Al₃₀ unit, to the distances between the AlCp^{*} and the Al atoms of the pentagonal planes (this is also the weakest bond, Table 1). The Al–C and the C–C bond lengths are similar to those in (AlCp^{*})₄^[9] (Figure 2b). Density functional theory (DFT) calculations afford geometrical data which are in good agreement with the experimental values (Table 2).

The 60 CH₃ groups of the 12 C₅(CH₃)₅ ligands form a surface topology reminiscent of a “distorted fullerene”. The Archimedean polyhedron of fullerene, a truncated icosahedron, possesses 12 pentagonal and 20 hexagonal faces with

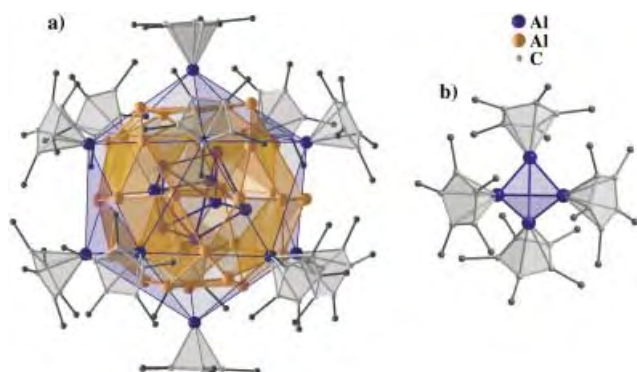


Figure 2. a) Representation of the whole $\text{Al}_{50}\text{Cp}^*_{12}$ molecule **1**. The icosahedral arrangement of the 12 ligands bearing Al atoms is shown in blue; b) representation of the Al_4Cp^*_4 molecule. H atoms are omitted for clarity.

Table 1: A comparison of the calculated (in brackets) and experimentally determined bond lengths of **1** and Al_4Cp^*_4 at 150 K (in pm).

	1			Al_4Cp^*_4		
	av	min/max	calcd	av	min/max	calcd
Al–Al	277.0	257.8/287.7	(285.0)	275.8	274.8/277.3	(282.1)
Al–C _{Cp}	232.0	228.8/236.2	(237.4)	234.3	230.8/238.8	(239.7)
C _{Cp} –C _{Cp}	142.1	140.3/143.4	(143.9)	141.9	140.1/143.1	(143.8)
C _{Cp} –C _{Me}	149.7	148.0/151.1	(150.4)	150.0	148.7/150.8	(150.5)
C _{Me} –H	(98.0)		(111.3)	(98.0)		(111.3)
C _{Pcenter} ^[a] –Al	198.1	197.2/198.3		200.8	198.8/201.9	
diameter ^[b]	1489.6		(1502.6)	739.0		

[a] C_{Pcenter} is the geometrical center of the Cp ring. [b] The diameter is twice the average distance between the molecular center and the C_{Pcenter} (the distance between the separate molecules of **1** in the crystal is significantly greater (about 2 nm)).

Table 2: Distances between Al atoms located in the same (for example, Al_8 shell) or different (for example, Al_8 – Al_{30}) shells of the cluster core of **1**. The distances between the closest methyl C atoms of adjacent ligands are also included (in pm).

	Experimental		Calculated
	av	min/max	
Al_8 shell	266.4	257.8/279.7	(271.2)
Al_8 – Al_{30}	280.9	260.3/300.3	(293.7)
Al_{30} shell	276.5	268.2/282.0	(280.5)
Al_{30} – Al_{12}	286.7	276.0/297.2	(294.8)
C _{Me} –C _{Me}	385.9	313.3/477.5	

60 vertices and 90 edges. In **1** the 20 hexagonal faces are divided into 80 trigonal faces which, together with the 12 pentagonal faces, form a snub dodecahedron^[10] with 60 vertices and 150 edges (Figure 3a). The remaining 60 carbon atoms of the C_5 units are located within each of the 12 pentagonal faces.

The topology of the 60 methyl groups in **1** thus closely resembles that of the 60 C atoms in fullerene (C_{60}), even though the carbon–carbon interactions in both molecules are completely different. The C–sp² centers in C_{60} are connected by covalent bonds, whereas **1** has 60 hydrogen-saturated sp³ centers and 60 sp²-type C atoms in the Cp rings. The average distances between nearest methyl groups of neigh-

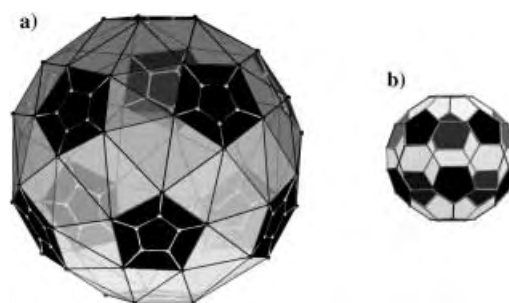


Figure 3. a) Polyhedral representation of the carbon shell of the $\text{Al}_{50}\text{Cp}^*_{12}$ molecule **1** (consisting of 60 methyl groups and 12 carbon pentagons); b) polyhedral representation of the fullerene C_{60} in the same length scale.

boring Cp* ligands (386 pm) are approximately twice the van der Waals radius of a single methyl group (195 pm). The entire $\text{Al}_{50}\text{Cp}^*_{12}$ molecule has a volume (calculated with single point calculations based on the experimental geometry) which is about five times larger than that of a C_{60} molecule (Figure 3b). The fivefold symmetry of the outer shell, which is also reflected in the second Al_{42} shell (Figure 2a), is merely broken by the Al_8 unit in the center. The Al–Al bond lengths of the Al_8 moiety are shorter than those in the Al_8 framework of $(\text{SiAl}_8)(\text{AlCp}^*)_6$ ^[2] (with an Al–Al bond length of 289.6 pm). Such a compression of the cluster core by the highly symmetrical outer shell has been found for Ga_4 and Ga_{13} units in the center of the Ga_{16} ^[11] and Ga_{51} ^[12] clusters, respectively. This neutral Al_{50} cluster **1** is very different from the anionic metalloid Al clusters Al_{77} ^[13] and Al_{69} ^[14] for which the topology essentially reflects the coordination motives of the solid metal and the Al–Al bond lengths decrease with coordination number from the center (278 pm, coordination number 12) to the outer sphere (268 pm, coordination number 4).

The calculated ²⁷Al NMR shifts of $\text{Al}_{38}[\text{Al}_{12}\text{Cp}^*_{12}]$ **1** are $\delta = -269.8$, -372.9 , and 108.5 ppm for the Al_8 , Al_{30} , and Al_{12} moieties, respectively. The difficult observation of the usually very broad ²⁷Al NMR shifts in solution have resulted in us having detected only one sharp signal at $\delta = -272$ ppm ($\omega_{1/2} = 584$ Hz), which we attribute to that of the Al atoms in the central Al_8 shell.

To determine the relative energy of **1** compared to metal atoms and bulk metal we performed model calculations, in which not only **1** but also Al_{38} clusters^[15] played an important role. The results are visualized in Figure 4: The capping of the Al_{38} cluster with 12 AlCp^* molecules leads to a stabilization and allows the isolation of **1** from solution. Thermochemical measurements should lead to a verification of the theoretical results.

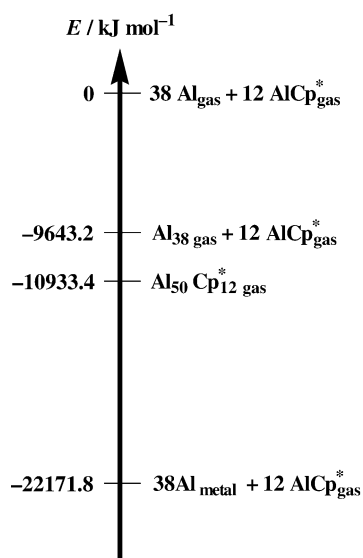


Figure 4. Calculated energy diagram of a model reaction starting from 38 Al atoms and 12 AlCp* molecules illustrate that **1** (gaseous) can be regarded as an intermediate in the formation of bulk metal starting from aluminum in the gas phase.

Why is this new Al₅₀ cluster and in particular its structure remarkable? The Al₅₀ cluster is the largest organometallic molecule characterized so far which contains only metal, carbon, and hydrogen atoms. Furthermore the 12 homoleptic bonds between the metal atom center and the 60 carbon atoms are singular (compare with SiAl₁₄Cp*₆^[2] and Ni₆Cp₆^[16]). This fascinating bonding situation in the Al₅₀ framework as well as in the C₁₂₀ shell could only be determined because suitable crystals of **1** could be grown. Their measurement carried out with X-ray diffraction methods gave the most precise structural information on regular nanostructures.^[17]

Besides the uniqueness of **1** in the fields of metalloid clusters and main group organometal systems, one surprisingly finds many structural motifs of it in different areas of the natural sciences. The fivefold symmetry elements of **1** show similarities to:

- fullerene-like species,^[18]
- Müller's highly symmetrical MoO-based clusters with pentagonal building blocks up to Keplerates,^[19]
- intermetallic compounds (as investigated by, for example, Corbett^[20] and Lidin^[21]),
- pure metal-carbon molecules, that is, metallocarbohedrenes such as Ti₈C₁₂ (which could only be detected mass spectroscopically),^[22]
- spherical viruses, for example, the Sindbis virus.^[23]

The individual Al₅₀ clusters, which represent “fullerene-like C₁₂₀H₁₈₀ molecules with a nanoscaled endohedral-like Al₅₀ core”, form a distorted cubic centered lattice (α -W type) in the crystal. Each cluster of **1** is surrounded by eight nearest neighbors (1807.4 pm), and additionally is coordinated to six other clusters (1921.7 pm, 2149.3 pm) in a distorted octahedral geometry. The near-spherical form and metal-rich center means that the Al₅₀ cluster species **1** can be regarded as large metal atoms which are separated from each other through a

nonconducting shell of carbon and hydrogen atoms only. Weak interactions between the individual clusters again resemble those of the fullerenes, which in both cases lead to solubility in nonpolar organic solvents. In particular, this property—which is exceptional for metalloid cluster compounds—promises to open up a wide field of physical measurements on single, structurally distinct metalloid Al₅₀C₁₂₀H₁₈₀ molecules and on molecular layers of these well-defined clusters.

Besides so many potential relationships and promising perspectives in various fields of chemistry and physics, this Al₅₀C₁₂₀H₁₈₀ compound may mark a new era of organometallic chemistry. A few examples demonstrate the rapid development seen in organoaluminum chemistry: trimethylaluminum (3c-2e bonds in Al₂(CH₃)₆), Al₂R₄^[24] (R = CH(SiMe₃)₂, as the first molecular compound with Al–Al bonds), Al₁₂R₁₂^{2–[25]} (R = *i*Bu), and [(AlCp*)₄]^[26] (as the first Al^I-organic compounds). Since the latter compound has been cited as a “milestone” in modern organometallic chemistry,^[27] **1** should belong to a “postmodern” organometallic chemistry, where ever larger cores consisting of “naked” metal atoms are enclosed by a shell of carbon and hydrogen atoms only. Perhaps one day, for example, Li_x(CH₃)_y ($x > y$) compounds such as the Al₅₀R₁₂ species may be obtained, and, in analogy to the alkaline suboxides (for example, Cs₁₁O₃^[28]) will open up a new field in solid-state chemistry and material science.

Experimental Section

General: All manipulations were carried out under nitrogen gas with solvents which were pre-dried by using standard procedures. The ligand MgCp*₂ was synthesized as described in the literature.^[29]

1 was synthesized in a flame-dried flask with 1 equivalent of the solution of Al^IBr (4 mmol) in a mixture of THF and toluene (1:3) and 1.25 equivalents (5 mmol) MgCp*₂ dissolved in *n*-heptane (20 mL). The reactants were combined at –78 °C and warmed to –30 °C under magnetic stirring. The reaction mixture was kept at this temperature for about 24 h. After the solution had reached room temperature it had a red color. The by-products [AlCp*]₄ and Mg₂Br₂Cp*₂·C₄H₈O could be gradually separated by using several concentration and cooling cycles (down to –25 °C). Finally, the Al₅₀ cluster compound **1** was separated from the [AlCp*]₄ by removal of the toluene from the deep-red solution and subsequent extraction with pentane. Black crystals of **1** (39 mg, 24.6%) suitable for X-ray crystallographic analysis were grown in [D₆]benzene. This synthesis could be reproduced repeatedly to provide ample amounts of **1** for physical measurements. ¹H NMR (250 MHz, C₆D₆): δ = 1.97 ppm (s, 180H; CH₃); ¹³C NMR (250 MHz, C₆D₆): δ = 10.7 (CH₃), 111.0 ppm (C₅); ²⁷Al NMR (78 MHz, C₆D₆): δ = –272 ppm (Al₈, $\omega_{1/2}$ = 584 Hz).

In solving the structure of **1**, the atoms Al1 and Al2 in the inner shell had to be split over two positions and were included in the refinement with occupation factors of 82 and 18%. The quantum-chemical calculations (DFT) relied on the Turbomole program package.^[30a] The BP method with the SVP basis set was used for all elements. NMR shifts were obtained using the MPSHIFT module. The molecular volumes were calculated using the Gaussian98 system at the SCF level and with 3-21G*-basis sets.^[30b] Single-point calculations based on experimental geometries were performed by applying the “ICPM solvation model”.^[30c]

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The Polyhedral Gallium Subhalide [Ga₂₄Br₂₂]·10 THF: The First Step on the Path to a New Modification of Gallium?*

Taike Duan, Elke Baum, Ralf Burgert, and
Hansgeorg Schnöckel*

To date, [Ga₈I₈]·6PEt₃ is the only structurally characterized gallium(i) subhalide.^[1] In this compound the gallium atoms form an eight-membered planar ring with two center–two electron (2c–2e) bonds. Although we have recently synthesized a series of metalloid gallium clusters from GaX-solution (X = Cl, Br, I),^[2,3] no further examples of Gallium subhalides have been reported.^[4] However, we have shown that the polyhedral aluminum subhalide, [Al₂₂X₂₀]·12 THF (**1**; X = Cl, Br) can be prepared from metastable AlCl or AlBr solution.^[5,6] The topology of the 22 aluminium atoms in **1** is unexpected: 10 aluminium atoms are directly bonded by 2c–2e bonds to a central Al₁₂ icosahedron such that two aluminum atoms at the apexes of the Al₁₂ icosahedron are “naked”. An equivalent framework has not even been reported for boron, although there are conjoined B₁₂ icosahedra in α-boron. Based on this discovery and in collaboration with other research groups, we found that the formation of **1**, as an intermediate from the disproportionation of AlX to aluminum metal and aluminum trihalide, could be plausibly interpreted as a step in the formation of new modification of aluminum with an analogous structure to α-boron.^[6] This conclusion has been further supported by theoretical studies by Häussermann et al.^[7] Furthermore, they found that for gallium a hypothetical modification with an α-boron type structure is energetically only 5 kJ mol^{−1} higher than α-gallium (for aluminum it would be 22 kJ mol^{−1}). With an expansion of the volume by about 20%, the gallium atoms of α-gallium would rearrange and approach an α-boron type framework.^[7] Motivated by these studies, we directed our investigations towards preparing the first polyhedral gallium subhalide, particularly a gallium subhalide with the same structure as the [Al₂₂X₂₀]·12 THF clusters. The existence of such a cluster with an icosahedral Ga₁₂ core was further suggested by the recently reported partially substituted Ga₂₂ gallium subhalide cluster [Ga₂₂Br₁₀R₁₀].^[8] Herein we report the novel compound [Ga₂₄Br₂₂]·10 THF (**2**), which may be the precursor of this kind of Ga₂₂-core compound.

A dark-brown to black solution of GaBr in a mixture of THF and toluene (1:20), made by cocondensation of the gaseous components (GaBr, THF, toluene),^[2,3] is slowly warmed from −78 °C to room temperature over several days. After the separation of precipitated metallic gallium and the stepwise concentration of the solution under vacuum, **2** crystallizes in the form of small orange square plates, the crystallization continues for several weeks. The crystal growth is interrupted every few days by the removal by filtration of precipitated gallium. Most of the crystals are very small (<0.1 mm), mechanically and chemically highly labile, and even a reaction is observed in the anhydrous perfluoropolyether oil at room temperature during the preparation of the crystals for X-ray structural analysis. The synthesis of **2** is repeatable although yields are low (2% to 16%), and a suitable crystal for X-ray structural analysis can seldom be selected. The X-ray structural analysis^[9] indicates **2** is an inversion-symmetric compound with a central, slightly distorted Ga₁₂ icosahedron to which the other 12 Ga atoms are joined with 2c–2e bonds (Figure 1). The Ga–Ga bond lengths

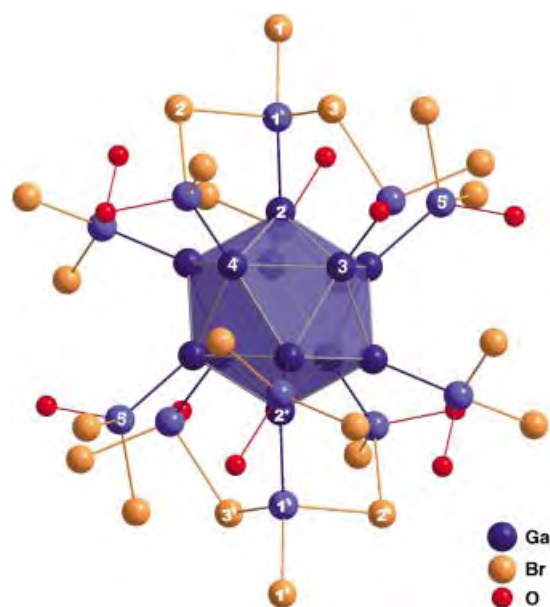


Figure 1. Molecular structure of [Ga₂₄Br₂₂]·10 THF (**2**), C and H atoms of THF are omitted for clarity.

in the central Ga₁₂ icosahedron range from 255 pm to 267 pm. The bonds from the Ga₁₂ icosahedron to terminal 12 Ga atoms (Ga₂–Ga₁) are shorter at about 240.0 ± 0.4 pm, this is expected since they are also bonded to Br atoms and have a higher oxidation state leading to shorter covalent radius. Compared to the central Ga₁₂ icosahedron, the external Ga₁₂ icosahedron is strongly distorted owing to the varied substitution of the outer gallium atoms: Two opposing apical gallium atoms (Ga₁/Ga₁) are bonded to three Br atoms, one exclusively, and the other two shared with neighboring gallium atoms. All the other external gallium atoms (such as Ga₅/Ga₅) bond with two bromine atoms and one oxygen atom from the THF molecule. The Ga–O bond length is on average 199 ± 2 pm, which is in good agreement with DFT calcula-

[*] M.Sc. T. Duan, Dr. E. Baum, Dipl.-Chem. R. Burgert, Prof. Dr. H. Schnöckel
Institut für Anorganische Chemie
Universität Karlsruhe (TH)
Engesserstrasse 15, Geb. 30.45, 76128 Karlsruhe (Germany)
Fax: (+49) 721-608-4854
E-mail: hansgeorg.schnoekel@chemie.uni-karlsruhe.de

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tions.^[10] The arrangement of $[\text{Ga}_{24}\text{Br}_{22}]\cdot 10\text{THF}$ clusters in the crystal corresponds to a distorted body-centered topology similar to the α -tungsten structure.

The Ga–Ga bond lengths in the central Ga_{12} icosahedron ($\text{Ga}_2\text{--Ga}_3$, $\text{Ga}_3\text{--Ga}_4$) of **2** (261 ± 6 pm) are in the range seen in other compounds containing a Ga_{12} icosahedron: $[\text{Ga}_{12}(\text{fluorenyl})_{10}]^{2-}$ (**3**; 264 ± 6 pm),^[11] $[\text{Ga}_{22}\text{Br}_2[\text{N}(\text{SiMe}_3)_2]_{10}\text{Br}_{10}]^{2-}$ (**4**; 259 ± 2 pm),^[8] and $[\text{Ga}_{22}\text{Br}[\text{N}(\text{SiMe}_3)_2]_{10}\text{Br}_{10}]^{3-}$ (**5**; 270 ± 12 pm).^[8]

The topology of the Ga_{24} framework of **2** is the same of the Ga_{22} clusters **4** and **5**, which implies that the subhalide cluster **2** may react further with the GaBr_3 present in the solution to give Ga_2Br_4 and the hypothetical $[\text{Ga}_{22}\text{Br}_{20}]\cdot 10\text{THF}$ cluster analogous to **1** and may thus be the precursor of the target Ga_{22} -subhalide cluster as well as **4** and **5**. Comparison of **1** and **2** shows that the 22 Al atoms or 24 Ga atoms are surrounded by a cage formed by 32 non-metal atoms (**1**: 20 Br atoms and 12 O atoms from THF, **2**: 22 Br atoms and 10 O atoms from THF). Single-point self-consistent field (SCF) calculations based on the experimentally determined geometries were performed to calculate the volume^[12] of the shells of the same electron density formed by the 32 non-metal atoms in **1** and **2**. They indicate that, although **2** has more metal atoms, the volume occupied by the 24 Ga atoms is almost 3% smaller than that of the 22 Al atoms in **1**. The result is consistent with the difference in the atomic radius of gallium and aluminum calculated from the $[\text{R}_2\text{M--MR}_2]$ compounds ($\text{R} = \text{CH}(\text{SiMe}_3)_2$; $\text{M} = \text{Ga}$ 254.1(1) pm and $\text{M} = \text{Al}$: 266.0(1) pm),^[13] which shows the gallium atom radius is 4.6% smaller. Although **1** and **2** have the same sum of Br and O atoms, they arrange in quite different ways: In **1**, 20 Br atoms adopt the geometry of a highly distorted pentagonal dodecahedron with the 12 O atoms of THF molecule in the centers of the pentagonal faces; in **2** the 22 Br atoms and 10 O atoms form three twisted eight-membered rings and two twisted four-membered rings.

To test the hypothesis that **2**, just like **1**, is an intermediate in the decomposition of the monohalide to an α -boron type modification of gallium or aluminum, respectively, quantum chemical calculations were carried out^[10] to compare the energy of **2** to GaBr and GaBr_3/Ga . In combination with thermodynamic data from the literature^[10,14] we get an energy level diagram that describes the disproportionation of GaBr and THF to α -gallium and $\text{GaBr}_3\cdot 2\text{THF}$ (Figure 2). The diagram shows that, GaBr disproportionates firstly to compound **2**, then to an α -boron type modification of gallium, and finally to the thermodynamically stable α -gallium and GaBr_3 . The postulated formation of the α -boron type modification of gallium also seems to be plausible under the experimental conditions: at low temperature (from -78°C to ca. 0°C) and under vacuum GaBr oligomers of large volume are formed as intermediates. By volume reduction of the primary species cluster **2** is formed by association and disproportionation of the oligomers in the solution, then the α -boron type gallium and finally the thermodynamically stable α -gallium is formed.

Together with the results of the different metalloid gallium clusters, especially the recently published Ga_{22} cluster,^[8] the results presented herein clearly illustrate that the connectivity diversity in the seven gallium modifica-

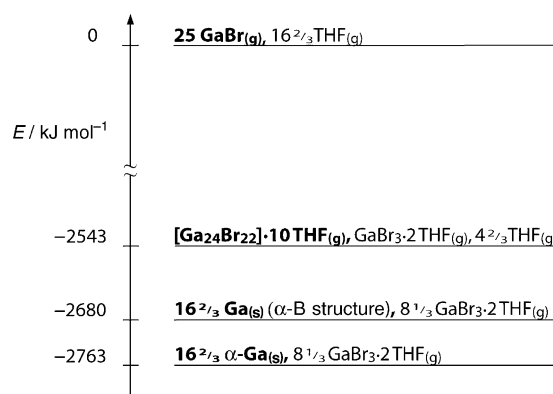


Figure 2. Energy level diagram for the disproportionation of molecular GaBr to α -gallium and GaBr_3 via the intermediate $[\text{Ga}_{24}\text{Br}_{22}]\cdot 10\text{THF}$ (**2**) and the hypothetical α -boron type gallium modification, based on DFT calculation and literature values.^[10,14]

tions,^[3,8,15] is also reflected in the of structural variety of the metalloid gallium clusters and the connectivities of their gallium frameworks. From the structure of **2** and the energy level diagram it seems plausible that a new modification of elemental gallium with an α -boron type structure may be experimentally realizable.

Experimental Section

GaBr (48 mmol) was cocondensed with toluene (100 mL) and THF (5 mL) according to the literature method.^[5] A portion (25 mL) of the ca. 0.41M black or dark-brown GaBr solution was warmed from -78°C to room temperature under vacuum over several days. The elemental gallium precipitate was separated and the dark filtrate was concentrated in stages and kept at room temperature under vacuum. Repeated concentration and filtration steps generated crystals of **2** from the filtrate in several weeks in the form of yellow orange square plates. The yields of **2**, owing to its extreme sensitivity to experimental conditions, range between 2% and 16%. Cluster **2** is mechanically and chemically highly labile, it decomposes even in solution as well as during the mounting in anhydrous perfluoropolyether oil for X-ray diffraction at room temperature. Mounting a crystal in cooled anhydrous perfluoropolyether oil makes X-ray diffraction at low temperature possible and gives suitable data for the structural analysis.

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Rod–Coil Copolymers**Micelle Formation from Amphiphilic “Cylindrical Brush”—Coil Block Copolymers Prepared by Metallocene Catalysis****

*Michael W. Neiser, Sandra Muth, Ute Kolb,
J. Robin Harris, Jun Okuda,* and Manfred Schmidt**

Although the synthetic access to rod–coil block copolymers has been known for a long time,^[1] the recent discovery of their self-organization into complex supramolecular assemblies has initiated a number of publications on the synthesis and properties of novel rod–coil block copolymers.^[2]

So far, most of the block copolymers prepared exhibit rather short rigid blocks consisting of either liquid-crystalline segments or helices. Herein we present the first report of micelle formation from novel giant rod–coil amphiphiles, which consist of a stiff cylindrical brush connected to a linear poly(methacrylic acid) chain.

[*] Dr. M. W. Neiser, S. Muth, Dr. U. Kolb, Prof. Dr. M. Schmidt
Institut für Physikalische Chemie
Johannes Gutenberg Universität
Jakob-Welder-Weg 11, 55128 Mainz (Germany)
Fax: (+49) 6131-392-2970
E-mail: mschmidt@mail.uni-mainz.de

Prof. Dr. J. Okuda
Institut für Anorganische Chemie
RWTH Aachen
Prof.-Pirlet-Strasse 1, 52056 Aachen (Germany)
Fax: (+49) 241-809-2644
E-mail: jun.okuda@ac.rwth-aachen.de

J. R. Harris
Institut für Zoologie
Johannes Gutenberg-Universität
55099 Mainz (Germany)

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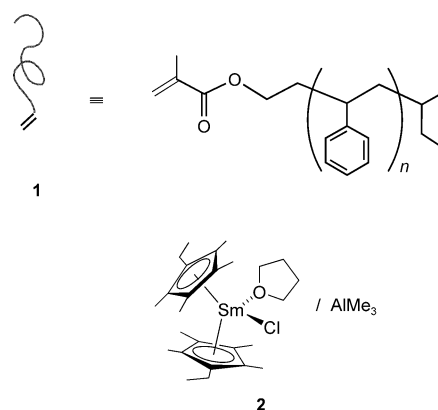
Supporting information for this article is available on the WWW under <http://www.angewandte.org> or from the author.

Regular comblike polymers with an extremely high grafting density of the side chains adopt the shape of cylindrical brushes, if the main chain is much longer than the side chains. The observed stiffening of the normally flexible main chain originates from the steric repulsion of the densely grafted side chains. Cylindrical brushes may be prepared by the polymerization of macromonomers,^[3] by the polymerization of the side chains from macroinitiators,^[4] or by grafting side chains onto functionalized linear chains.^[5] Each of these procedures has specific advantages and disadvantages as described elsewhere.^[6] Regularly branched side chains (“dendrons”) have been also successfully utilized for the preparation of cylindrical macromolecules.^[7]

So far, the polymerization of macromonomers to much higher degrees of polymerization for the main chain than the side chain could only be achieved by free-radical polymerization of highly concentrated macromonomer solutions.^[3] All other attempts to achieve this goal by other methods, particularly by living or controlled polymerizations such as anionic,^[8] radical,^[9] ring opening,^[10] or metallocene-catalyzed^[11] polymerization failed in so far that the degree of polymerization of the main chain hardly exceeded that of the side chain. The resulting structures could be described as spherical or star like.

Recently, we reported the synthesis of extremely high-molar-mass polymacromonomers by metallocene-catalyzed polymerization.^[6] The polydispersities of the samples were relatively broad (typically $1.8 \leq \bar{M}_w/\bar{M}_n \leq 2$; $\bar{M}_w$ and $\bar{M}_n$ are the mass-average and number-average molar masses, respectively) and the degree of polymerization was not controllable. Accordingly, we wondered whether living chain ends persisted during the course of the reaction. The synthesis of cylindrical-brush-coil block copolymers would prove that the metallocene-initiated polymerization of macromonomers is at least partly controlled and proceeds by a living-polymerization mechanism.

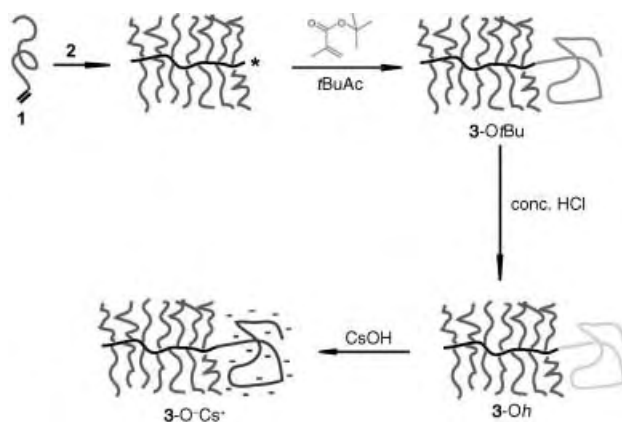
The synthesis is shown in Scheme 1. Briefly, a concentrated solution of methacryloyl end-functionalized polystyrene macromonomer **1** (PS-MM; $\overline{DP}_n = 18.3$, $\overline{DP}_w/\overline{DP}_n = 1.05$; $\overline{DP}_w$ and $\overline{DP}_n$ are the mass-average and number-average degrees of polymerization; Scheme 2) was polymerized by the



Scheme 2. Chemical structure of the macromonomer **1** and of the samarocene catalyst **2**.

organosamarium(III) catalyst **2** in THF for 2 h as described previously.^[6] After a small aliquot (500 μ L) had been taken from the reaction mixture, *tert*-butyl methacrylate (*t*BuMA) was added. After two more hours the polymerization was terminated by ethanol and the polymer **3-O*t*Bu** was precipitated. The sample was investigated by NMR spectroscopy, gel-permeation chromatography (GPC), light scattering, atomic force microscopy (AFM), and transmission electron microscopy (TEM). Details of the synthesis and characterization procedures are given in the Supporting Information.

The AFM image of the copolymer **3-O*t*Bu** is shown in Figure 1. Bright spots can be seen at the end of the cylindrical brush chains, which most probably originate from the collapsed *p*-*t*BuMA coil block (arrow 1). Furthermore, homopolymer brushes can be seen (arrow 2), which clearly did not initiate a linear coil block. Additional evidence for the formation of block copolymers is given by NMR spectroscopy, which shows an increase in the ratio of the aliphatic to aromatic protons from 0.8:1 to 0.87:1. From these numbers the block-length ratio is calculated: $\overline{DP}_n(\text{PS-MM}):\overline{DP}_n(\text{p-}t\text{BuMA}) = 2.0$. The degree of polymerization $\overline{DP}_w$ of the



Scheme 1. Synthesis of a cylindrical brush-coil block copolymer **3-O[−] Cs⁺**.

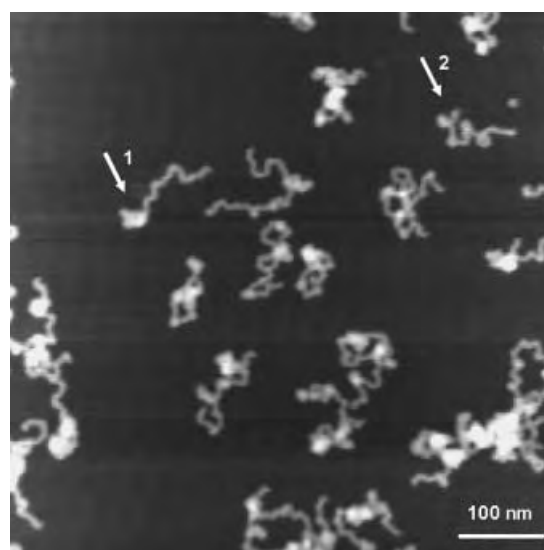


Figure 1. AFM picture of the cylindrical brush-coil copolymer **3-O*t*Bu**.

cylindrical brush block is estimated to be about 890 (see below and Supporting Information). If an unreacted macro-monomer fraction of 20% and $\overline{DP}_w/\overline{DP}_n \approx 2.1$ are taken into account, the minimum degree of polymerization of the *t*BuMA block $\overline{DP}_n^{\min}(t\text{BuMA})$ is estimated to be about 265 (see Supporting Information for details). $\overline{DP}_n^{\min}(t\text{BuMA})$ represents a lower limit because it is assumed that all PS-brush chains are living and able to initiate a *p-t*BuMA block. Unfortunately, there is no simple way to determine the fraction of cylindrical brush homopolymer in the reaction mixture, because the total molar-mass fraction of *p-t*BuMA in the copolymer amounts to 4% only. Static light scattering on the copolymer brush in toluene yields $\bar{M}_w = 2.0 \times 10^6 \text{ g mol}^{-1}$ and a gyration radius $\langle R_g^2 \rangle^{1/2} = 53 \text{ nm}$, which represent mostly the characteristics of the cylindrical brush block, because the mass fraction and contrast (an increment in the refractive index) of *p-t*BuMA in toluene are negligible. This value compares well to that estimated by GPC and to the results obtained from light scattering on a similar homopolymer brush (see Supporting Information).

To unambiguously demonstrate that a block copolymer was synthesized, the *p-t*BuMA block was hydrolyzed to the polymethacrylic acid **3-OH** by HCl in dioxane and neutralized with CsOH to give **3-O⁻Cs⁺** (Scheme 1). The resulting amphiphilic block copolymer **3-O⁻Cs⁺** in THF forms giant spherical micelles as shown in Figure 2. The increased

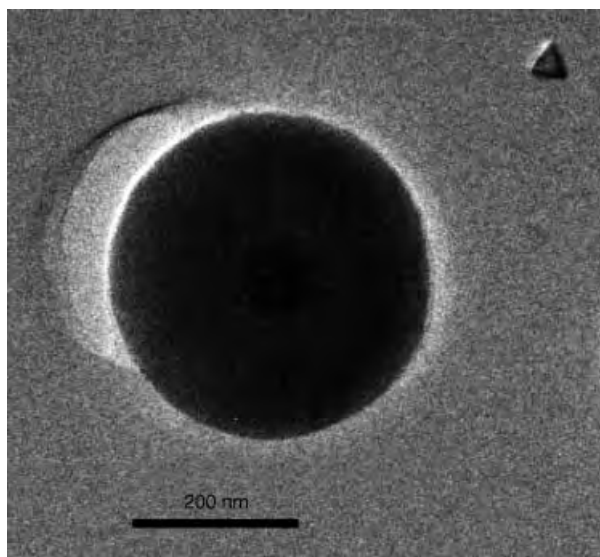


Figure 2. TEM picture of a giant cylindrical brush-coil block copolymer **3-O⁻Cs⁺**.

contrast at the core of the micelle can be ascribed to an increased concentration of cesium counterions. Additional pictures are presented in the Supporting Information, which show that the size distribution of the micelles is unusually broad. The micelle formation for such samples is quite complex and has not been investigated in detail. In particular, the formation of “twin” micelles and aging of the micellar solutions over weeks leading to an enhanced contrast of the

core provide insight into the peculiarities of the structures presented.

We have reported herein on the synthesis of a novel high molar-mass amphiphilic block copolymer, which consists of a coiled and highly soluble stiff cylindrical brush block. The block copolymers from core-shell micelles have a hydrophilic flexible core and a hydrophobic shell comprising the stiff block. In analogy to the known low-molar-mass rod-coil block copolymers, an interesting variety of micellar structures may be expected as a function of the composition of the block copolymers, which will be investigated in the future.

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libraries requires the availability of efficient and practical methods for the multistep solid-phase synthesis (typically more than 10 linear steps) of frameworks of biologically promising natural products. The efficiency and selectivity of these methods must be comparable to those of competing solution-phase techniques. To date this goal has been attained in only a few cases.^[1,4]

Herein we report a stereoselective solid-phase synthesis of 6,6-spiroketal^[5] with stereoselective aldol reactions of boron enolates as key stereodifferentiating transformations. The synthesis proceeds in 12 linear steps and provides access to the desired spiroketals in preparatively viable overall yields and with very high stereoselectivity.

6,6-Spiroketal are the prevalent underlying structural element in a wide range of important natural products with differing biological activity, such as the spongistatins **1** and okadaic acid (**2**; Scheme 1).^[6,7] Importantly, structurally simplified spiroketals derived from natural products retain their biological activity^[7] (see Scheme 1), so that the underlying 6,6-spiroketal structure is suitable as a starting point for the development of natural product derived compound libraries. The usefulness of spiroketals in combinatorial

Asymmetric Synthesis

Asymmetric Solid-Phase Synthesis of 6,6-Spiroketal^{**}

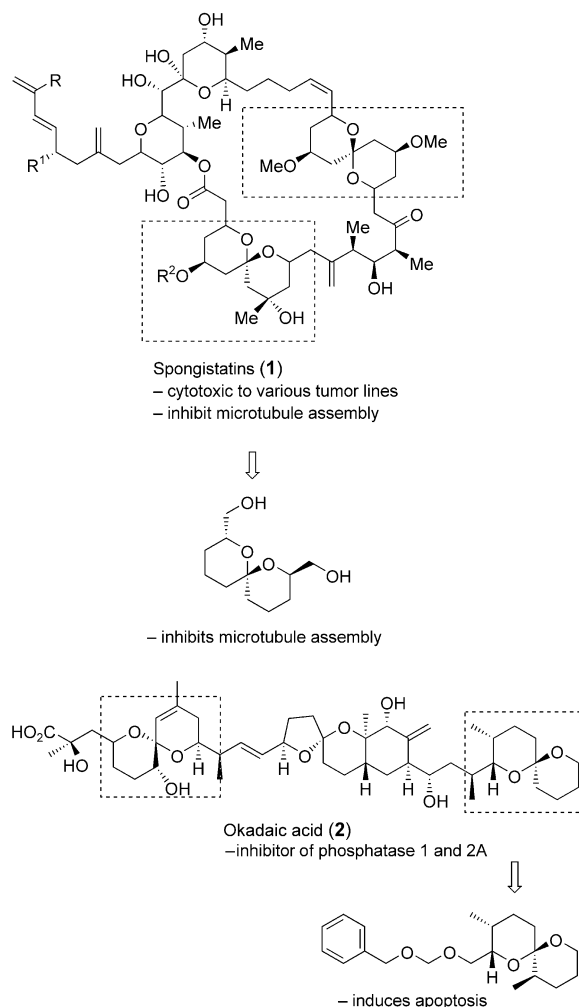
Okram Barun, Stefan Sommer, and Herbert Waldmann*

The solid-phase synthesis of compound libraries with a predetermined profile of properties has evolved as a key enabling technique in postgenomic chemical biology and medicinal chemistry. The underlying chemical structures of the compound libraries should have significance in nature. Ideally they should be biologically validated^[1] and/or feature so-called privileged structures, that is, structures that enable the library members to bind to several different proteins.^[2]

The precondition of biological validation is fulfilled by biologically active natural products, which can be regarded as ligands selected by evolution for structurally conserved yet genetically mobile protein domains.^[1] As this insight suggests, natural product derived compound libraries are promising starting points for research in chemical biology and lead development in medicinal chemistry.^[1,3] The synthesis of such

[*] Dr. O. Barun, Dipl.-Chem. S. Sommer, Prof. Dr. H. Waldmann
Max-Planck-Institut für molekulare Physiologie
Abteilung Chemische Biologie
Otto-Hahn-Strasse 11, 44227 Dortmund (Germany)
and
Universität Dortmund, Fachbereich 3, Organische Chemie
Fax: (+49) 231-133-2499
E-mail: herbert.waldmann@mpi-dortmund.mpg.de

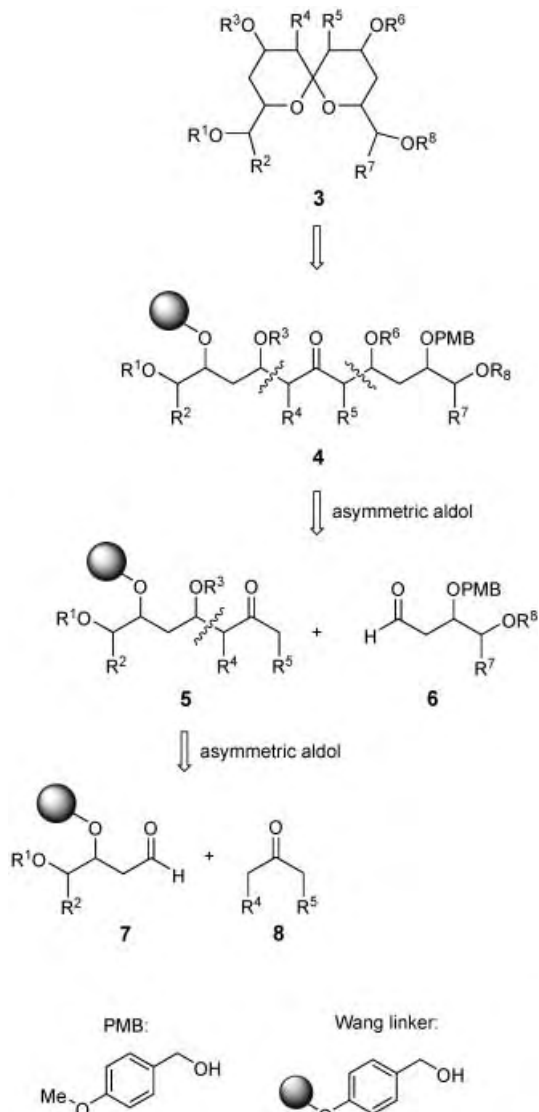
[**] This research was supported by the Max-Planck-Gesellschaft and the Fonds der Chemischen Industrie. O.B. was supported by a research fellowship from the Alexander von Humboldt Foundation and S.S. by a Kekulé Fellowship from the Fonds der Chemischen Industrie.



Scheme 1. Biologically active natural products with spiroketal substructures and structurally simplified biologically active analogues.

chemistry and a method for their synthesis on solid support have already been reported by Ley and co-workers.^[8]

In planning the synthesis we chose compounds of the general structure **3** as targets (Scheme 2), which were traced



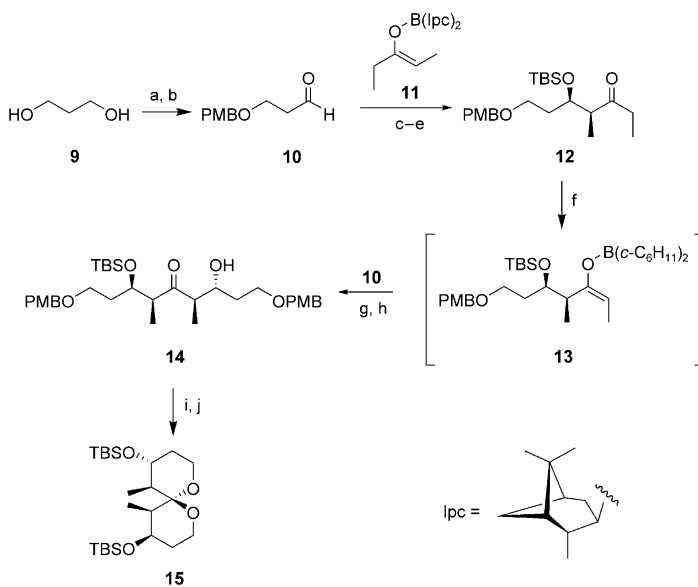
Scheme 2. Retrosynthetic analysis of the 6,6-spiroketal structures.

back in a retrosynthetic sense to aldol adducts **4**, **5**, and **7**. We planned to attach one of the hydroxy groups required for acetal formation to the solid support. The other alcohol group was to be protected by a functional group of the same chemical type as the anchor. Thus, final release from the polymeric carrier would be accompanied by cleavage of the protecting group, and under appropriate conditions by spontaneous ketalization to give the desired spiroketals **3** in a single step. We anticipated that these conditions would be fulfilled by the *p*-methoxybenzyl (PMB) ether protecting group and the corresponding Wang linker (Scheme 2).

Immobilized aldol intermediates **4** and **7** might be formed by means of asymmetric boron-mediated aldol reactions with

both polymer-bound and soluble chiral boron enolates. A few examples of stereoselective aldol reactions with soluble^[9] and polymer-bound^[10] chiral enolates have been described. However, the use of polymer-bound chiral boron enolates in aldol reactions has not yet been explored.

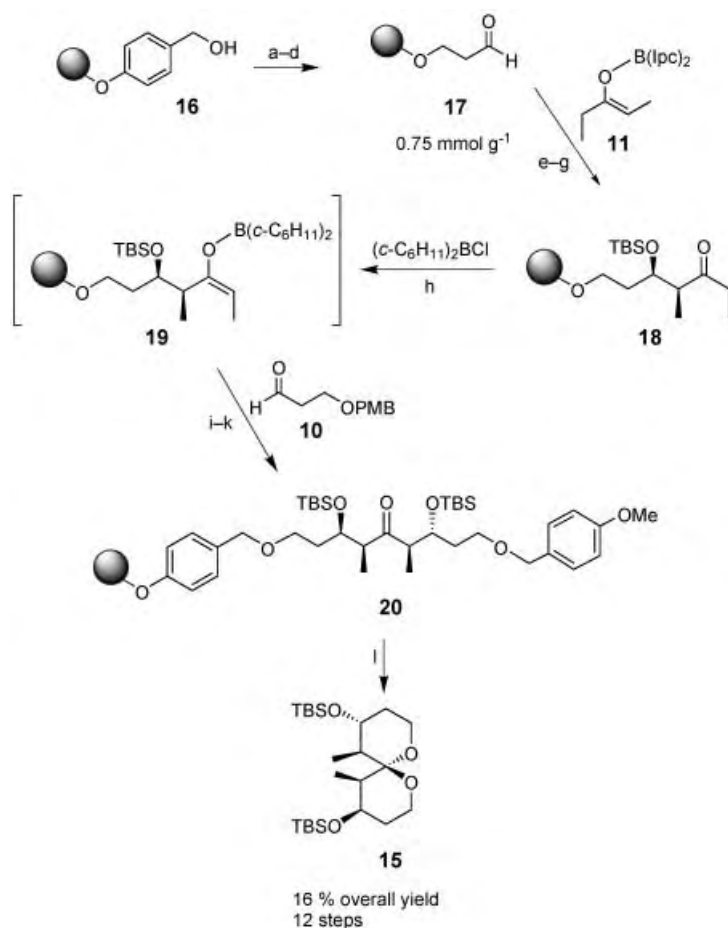
The feasibility of the strategy was investigated first in solution with the *Z* diisopinocampheyl borinate **11**^[11] and the *E* enolate **13**,^[12] which led to the formation of the corresponding *syn* and *anti* aldol adducts, respectively (Scheme 3).^[13] The absolute configuration of the aldol



Scheme 3. Solution-phase synthesis of the spiroketal **15**: a) NaH (1.5 equiv), PMBCl (1.1 equiv), DMF, 0°C→RT, 24 h, 63%; b) (COCl)₂ (1.5 equiv), DMSO (2.5 equiv), Et₃N (4 equiv), CH₂Cl₂, -78→0°C, 2 h, 93%; c) 3-pentanone (1 equiv), (–)-Ipc-BOTf (1.2 equiv), DIEA (1.5 equiv), CH₂Cl₂, -78→–30°C, 20 h; d) 30% aqueous H₂O₂/MeOH/buffer (pH 7) (1.5:5:1), 0°C→RT, 2 h, 69%; e) TBSCl (1.3 equiv), imidazole (2.1 equiv), DMF, room temperature, 24 h, 91%; f) (c-C₆H₁₁)₂BCl (1.3 equiv), Et₃N (1.5 equiv), Et₂O, 0°C, 4 h; g) **10** (1.4 equiv), Et₂O, -78→–30°C, 24 h; h) 30% aqueous H₂O₂/MeOH/buffer (pH 7) (1.5:5:1), 0°C→RT, 2 h, 89%; i) TBSCl (1.3 equiv), imidazole (2.5 equiv), DMF, room temperature, 24 h, 92%; j) DDQ (2.8 equiv), CH₂Cl₂/buffer (pH 7), 0°C→RT, 3 h, 88%. DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, DIEA = *N,N*-diisopropylethylamine, DMF = *N,N*-dimethylformamide, DMSO = dimethyl sulfoxide, TBS = *tert*-butyldimethylsilyl, Tf = trifluoromethanesulfonyl.

adduct **14** was assigned based on the assumption that the stereochemical course of the reaction is analogous to that observed in related cases.^[14] The simultaneous oxidative cleavage of both PMB protecting groups proceeded smoothly and was followed—as hoped—by the spontaneous formation of the spiroketal **15**, which was obtained as a single isomer. The configuration of compound **15** was ascertained after deprotection by means of NOE experiments and by comparison of spectroscopic data with those of known compounds.

After the development of this reaction sequence in solution we investigated whether it could be transferred to the solid phase (Scheme 4). The Merrifield resin equipped with the Wang linker (**16**; loading 1.2 mmol g⁻¹) was activated



Scheme 4. Solid-phase synthesis of the spiroketal **15**: a) CCl_3CN (8 equiv), DBU (3 mol %), CH_2Cl_2 , 0°C , 40 min; b) $\text{TBSO}(\text{CH}_2)_3\text{OH}$ (5 equiv), $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (3 mol %), cyclohexane, CH_2Cl_2 , room temperature, 15 min; c) TBAF (8 equiv), THF, room temperature, 14 h; d) IBX (8 equiv), DMSO, room temperature, 36 h; e) 3-pentanone (6 equiv), $(-)\text{-Ipc}_2\text{BOTf}$ (6.1 equiv), DIEA (7 equiv), CH_2Cl_2 , $-78 \rightarrow 0^\circ\text{C}$, 20 h; filter and wash (two cycles); f) 30 % aqueous $\text{H}_2\text{O}_2/\text{MeOH}/\text{DMF}/\text{buffer}$ (pH 7) (1.5:4:4:1), 0°C , 8 h; g) TBSCl (10 equiv), DMAP (1 mol %), imidazole (10 equiv), $\text{DMF}/\text{CH}_2\text{Cl}_2$ (1:1), room temperature, 24 h (two cycles); h) $(\text{C}_6\text{H}_{11})_2\text{BCl}$ (8 equiv), Et_3N (9 equiv), Et_2O , 0°C , 24 h; filter and wash (two cycles); i) **10** (10 equiv), Et_2O , $-78 \rightarrow 20^\circ\text{C}$, 26 h (2 cycles); j) 30 % aqueous $\text{H}_2\text{O}_2/\text{MeOH}/\text{DMF}/\text{buffer}$ (pH 7) (1.5:4:4:1), 0°C , 8 h; k) TBSCl (10 equiv), DMAP (1 mol %), imidazole (10 equiv), $\text{DMF}/\text{CH}_2\text{Cl}_2$ (1:1), room temperature, 24 h (two cycles); l) DDQ (10 equiv), $\text{CH}_2\text{Cl}_2/\text{buffer}$ (pH 7) (20:1), $0^\circ\text{C} \rightarrow \text{RT}$, 6 h. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, DMAP = 4-dimethylaminopyridine.

as the corresponding trichloroacetimidate,^[15] which was then subjected to nucleophilic displacement by mono-TBS-protected 1,3-propanediol. This two-step sequence was conveniently monitored by FT-IR spectroscopy. After cleavage of the TBS group the primary alcohol was oxidized to the corresponding aldehyde **17** by 2-iodoxybenzoic acid (IBX). At this stage resin loading was determined to be 0.75 mmol g^{-1} .^[16] The polymer-bound aldehyde **17** was then treated at -78°C with the preformed *Z* enolate **11** in dichloromethane, and the reaction mixture was allowed to warm to 0°C . This procedure was repeated once. After oxidative workup to cleave the B–O bond the secondary

alcohol was protected as its TBS ether to yield the immobilized aldol product **18** (monitored by FT-IR spectroscopy).

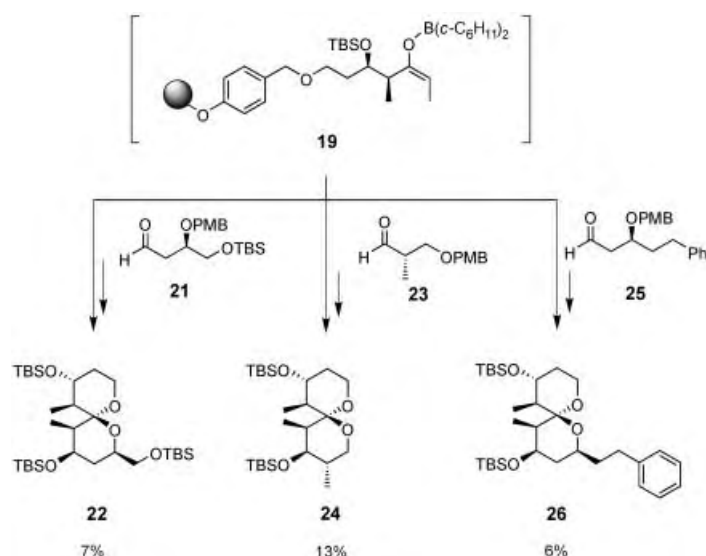
To form the polymer-bound chiral boron enolate required for the crucial second aldol reaction, the ketone resin **18** was swollen in diethyl ether and then a solution of chlorodicyclohexylborane and triethylamine in diethyl ether was added to the resin at 0°C . After 6 h the resin was washed and the addition of the enolate formation in solution (see above and reference [12]) the *E* dicyclohexylboron enolate **19** was formed on the solid support. The boron enolate resin **19** was then treated at -78°C with the aldehyde **10**, and after oxidative workup as described above the free secondary alcohol (strong absorption at 3504 cm^{-1} and 1714 cm^{-1} in the IR spectrum) was protected as the TBS ether **20**.

The treatment of the intermediate **20** with DDQ in a mixture of CH_2Cl_2 and an aqueous buffer (pH 7) resulted in the simultaneous cleavage of the PMB ether, release from the Wang resin, and spiroketalization. After purification by filtration through a short column of silica gel, the spiroketal **15** was obtained as a single stereoisomer, as determined by HPLC as well as ^1H and ^{13}C NMR spectroscopy.^[13] NOE investigations and comparison of spectroscopic data and the specific rotation revealed that the product was identical to the spiroketal **15** synthesized in solution as described above.

The spiroketal **15** was obtained in this 12-step solid-phase synthesis in an overall yield of 16 %, which corresponds to an average yield of 86 % per step. This compares very favorably with the overall yield of 27 % recorded for the 10-step synthesis in solution described above. The fact that the spiroketal obtained from the solid-phase synthesis had the same configuration as that obtained from the solution-phase synthesis is evidence that both aldol reactions on the polymeric support proceed by full analogy with the corresponding asymmetric transformations in solution. Furthermore, the degree of stereodifferentiation is very similar in both cases.

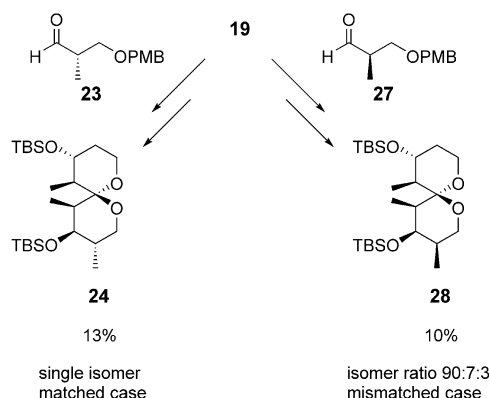
To demonstrate that the reaction sequence shown in Scheme 4 is amenable to the synthesis of compound libraries, three different functionalized spiroketals were synthesized. Thus, the chiral aldehydes **21**, **23**, and **25** were prepared^[17] and subjected to aldol reactions with the boron enolate **19** (Scheme 5). After workup and release from the solid support the spiroketals **22**, **24**, and **26** were obtained as single isomers in overall yields of 7, 13, and 6 %, respectively.

To gain further insight into the stereoselectivity of the asymmetric boron-mediated aldol reactions on the solid support, we investigated whether the principle of double diastereodifferentiation also operates in this case. To this end, the aldehyde **27** was prepared and treated with **19** under the aldol reaction conditions. The spiroketal **28** was obtained in 10 % overall yield as an inseparable mixture with two other



Scheme 5. Solid-phase synthesis of the spiroketals **22**, **24**, and **26**; for reaction conditions, see Scheme 4.

isomers in a ratio of 90:7:3 (Scheme 6; determined by GC–MS). Thus, in both aldol reactions of the chiral enolate **19** with the enantiomeric aldehydes **23** and **27** the *anti* adduct is



Scheme 6. Double diastereodifferentiation in the aldol reaction on the solid support.

formed as the major product. In accordance with related findings^[18] the combination of **19** with **23** represents the matched case and the combination of **19** with **27** the mismatched case.

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Keywords: asymmetric synthesis · chemical biology · natural products · solid-phase synthesis · spiro compounds

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- 44.4, 43.4, 35.5, 29.6, 26.1, 26.1, 18.4, 18.3, 14.0, 9.6, -3.9, -4.2, -4.4, -4.4 ppm; GC-MS (m/z , %): 444 (M^+ , 8), 387 ($[M-57]^+$, 15); HRMS (EI, 70 eV) calcd for $C_{23}H_{48}O_4Si_2$: 444.3091, found: 404.3099.
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