Molecular Containers for Anions Based on Triply-Linked Bis(Cyclopeptides)

vom Fachbereich Chemie der Technischen Universität Kaiserslautern
zur Verleihung des akademischen Grades
„Doktor der Naturwissenschaften“ genehmigte Dissertation

D 386

Datum der wissenschaftlichen Aussrache: 28.08.2013

vorgelegt von
Eugenia Petkova Eugenieva-Ilieva
geboren in Sofia (Bulgarien)

Betreuer: Prof. Dr. Stefan Kubik
Kaiserslautern, Juni 2013

Die vorliegende Arbeit wurde unter der Leitung von Herrn Prof. Dr. S. Kubik im Fachbereich Chemie der Technischen Universität Kaiserslautern in dem Zeitraum von Januar 2007 bis Dezember 2010 angefertigt.

Betreuer:	Prof. Dr. S. Kubik
Dekan:	Prof. Dr. Ing. J. Hartung

Prüfungskommission

Vorsitzender	Prof. Dr. S. Ernst
1. Gutachter	Prof. Dr. S. Kubik
2. Gutachter	Prof. Dr. L. Gooßen

Tag der mündlichen Prüfung: 28.08.2013

Eidesstattliche Erklärung

Hiermit versichere ich, dass ich die vorliegende Arbeit eigenständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet, sowie Literaturzitate kenntlich gemacht habe.
Ich erkläre außerdem, dass diese Dissertation weder in gleicher noch in ähnlicher Form bereits in einem anderen Prüfungsverfahren vorgelegen hat.

Kaiserslautern, den _____ _____

(Eugenia Eugenieva-Ilieva)

Teile dieser Arbeit wurden bereits veröffentlicht:

Z. Rodriguez-Docampo, E. Eugenieva-Ilieva, C. Reyheller, A. M. Belenguer, S. Kubik, S. Otto, *Chem. Commun.* **2011**, *47*, 9798. <DOI: 10.1039/C1CC13451E>

Für meinen Schutzengel

Acknowledgements

I would like to express my deepest gratitude to my supervisor Professor, Ph.D. S. Kubik for guiding, helping and supporting me throughout the whole study. He was extreme patient with me and always available when needed, inspiring me with his knowledge, high morals and energy to pursue and find answers to existing problems. I am grateful to the Marie Curie Research Training Network on Dynamic Combinatorial Chemistry for funding this project (MRTN-CT-2006-035614).

My gratitude goes to all the people who helped me in accomplishing my work and inspired me with their ideas and professional attitude. Special thanks to former co-workers of the Kubik group Carsten Reyheller, Thomas Fiehn, Martin Krause, Sabine Stüwe and my colleagues, and friends Laura Gilberg, Astrid Schaly, Raquel Belda Vidal and Michael Zengerle. It was pleasure to work with you having fun in the laboratory and in spare time. I also appreciate the preparative contributions to this work by the students Yuan He and Christine Schur. I am grateful to our collaborators Professor S. Otto (University of Groningen, The Netherlands) and Z. Rodriguez-Docampo (Cambridge University, UK) and the people from the research training network for the inspiration, helpful ideas, and the great time spent together.

The staff of the departments of physical and analytical chemistry particularly Fabian Menges, Ruth Bergsträßer and Birgit Dusch I thank for measuring ESI-TOF MS spectra and elemental analyses as well as Christiane Müller for recording the NMR spectra. The members of the working groups of Prof. L. Gooßen and Prof. J. Hartung I thank for their kind cooperation and support.

I would like to thank Prof. A. Dobrev, Prof. O. Petrov, and Ass. Prof. M. Kandinska from Sofia University in Bulgaria who initiated interest in the field of Organic Chemistry and advised me to undertake the step of going to Kaiserslautern.

I also thank Prof. L. Gooßen for being the second referee of my thesis and Prof. S. Ernst for presiding the examination commission.

My heartiest gratitude to my closest friends who helped me and were always next to me sharing good and bad moments, pushing me, and inspiring me never to give up: Angel, Yordanka, Anton, Asja, Suzi, Geri and Dian.

Last but not least, my love and special thanks goes to my family Sarafka Lukanova, Petko Petkov, Svetlana Eugenieva, and Ivaylo Iliev and my lovely daughters Sophia and Elisara who are the ones most close to me and who never stopped believing in me. I would like to ask for forgiveness to all those who did not support or understand some of my actions and I forgive all those who involuntarily or voluntarily hurt me.

Finally I would like to dedicate this thesis to my late friend, Angel, who could not witness the end of this project and to my family who have always been with me or in my heart through the toughest times of this work. **Thank you!**

Table of contents

1. Molecular encapsulation of anions

Molecular encapsulation encompasses recognition of molecules within molecular structures that surround the guest from all sides. The complexes formed can be stabilized by the existence of multiple binding interactions between the host, i.e. the capsule, and the included guest. A recognition process is strong and selective when the host is electronically and geometrically complementary to the guest and adopts the optimal binding conformation prior to complex formation (preorganization).[1] Usually, rigid and highly symmetric structures like capsules and cages are particularly well suited to form stable complexes.

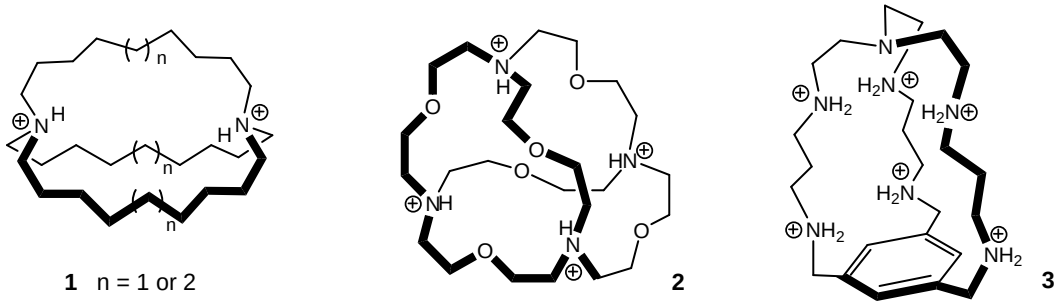
In the language of supramolecular chemistry, the words capsule and cage are often used as synonyms although some authors use the term capsule for receptors with a fully enclosed cavity that undergo rupture or deformation of bonds to exchange their guest(s). Cages on the other hand, do not change their structures upon guest exchange.[2] Guest molecules enclosed inside a cage-type receptor are prevented from contacts with other molecules and even unstable molecules therefore tend to be unreactive. A guest molecule thus has different properties when encapsulated as it would have when in solution.

Historically, cations and neutral molecules were initially captured within closed-frameworks. Encapsulation of anionic species received attention only later as it concerns binding of “more difficult” guests.[3] Compared to their isoelectronic cations, anions are better solvated, larger, have a lower charge density, and exhibit weaker electrostatic binding interactions with a receptor.[4] Moreover, anions display, different geometries, some are spherical, others trigonal planar, tetrahedral, octahedral, or have even more complex structures.[5] Finally, the effect of pH and solvent effects on the protonation state of anions must not be overlooked.[6] Anions such as acetate, for example, can lose their negative charge at low pH as an effect of protonation.

Anion binding is usually stronger in non-aqueous, aprotic, non-polar solvents.[7] However, the development of synthetic receptors for recognition of anions in highly competitive (aqueous) mixtures is significantly more relevant as it would allow the binding and sensing of anions in their natural environment. Potential target anions play crucial roles in biological systems (DNA, RNA, carboxypeptidase A),[8] medicine (chromates are carcinogenic, dysfunction of anion transport causes various diseases)[9] and environment (nitrate, sulfate, phosphate, arsenate and pertechnetate are dangerous pollutants).[10] For recent advances in anion coordination chemistry see the special issue of Chemical Society Reviews published in 2010.[11]

Since particularly strong binding can be expected by using cage-type receptors I aimed in my thesis at the development of new anion-binding molecular cages based on the anion-binding cyclopeptides developed in the Kubik group. In this introduction of my thesis previous attempts to synthesize anion-binding cages are summarized. These attempts involve synthesis of capsules

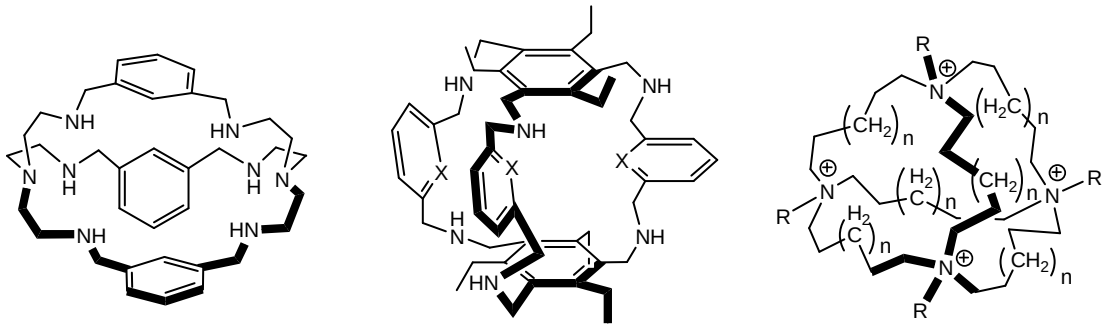
through covalent, coordinative, and non-covalent interactions. In addition, capsules can be synthesized under the influence of anions that act as kinetic or as thermodynamic templates.[2a, 12] The first receptors for anionic species, the bicyclic *katapinand* (**1**) of Simmons and Park[13] and the macrotricylic *cryptand* (**2**) of Lehn[14] have a cage-type architecture (Scheme 1). Both compounds **1** and **2** form inclusion complexes with chloride anions, but the complexes of the latter proved to be about three orders of magnitude more stabile when compared to **1** as a result of an extra strap. Important features of these hosts are good water solubility and controlled complex stability via change of the degree of host-protonation (pH dependency).[15]



Scheme 1 Amine functionalized receptors: katapinand **1**, cryptand **2** and azaphane **3**.

Aromatic units were utilized as spacers for the development of oligocyclic polyamines with increased inner space to include larger or polyatomic anions.[16] Hosts like azacyclophane **3,** for example, are conformationally restricted and endowed with additional binding sites that allow anion-π interactions.[17] Incorporation of three aromatic rings within the structure of octaazaphane **4** results in a relatively nonpolar cavity which offers enough room for the formation of a cascade complex:[18] two copper cations can be coordinated at the tren-binding sites and one carbonate[19] or one cyanide[20] ion can be centrally placed (Scheme 2). The selectivity of metal-free receptor **4** toward anions is highly pH dependent. The hexaprotonated host can co-encapsulate $F^-•H_2O$,[21] or entrap oxyanions (nitrate, perchlorate, sulfate, perrhenate, thiosulfate, chromate, and selenate with $K_a = 10^5 – 10^{7.5}$ M^{-1}),[22] while the tetraprotonated version proved to stabilize linear triatomic anions such as Cl_2H^-.[23]

The oxalate complex of $\mathbf{4}•H_6^{6+}$ possesses very high stability due to additional interactions between the host's aromatic π-systems and the C=O groups of the anion ($K_a = 3.1 \times 10^{10}$ M^{-1}).[24] Replacement of the tren-groups in **4** by two 1,3,5-tris-(aminomethyl)-2,4,6-triethylbenzene subunits afforded hexaamine **5a** with increased selectivity for tetrahedral anions in aqueous solution.[25] This receptor is highly selective for sulfate over dihydrogenphosphate under acidic conditions, while the situation reverses for **5b** as a result of additional hydrogen-bonding interactions between the $H_2PO_4^-$ and the pyridine nitrogen atoms. Incorporation of the pyridine spacers also allowed internal hydrogen bonds between the pyridine nitrogen atoms and the adjacent NH-groups, a preorganizational effect that also leads to enhanced binding of $H_2PO_4^-$.



4 **5a** X = CH; **5b** X = N **6** R = Me, n = 2 or 4

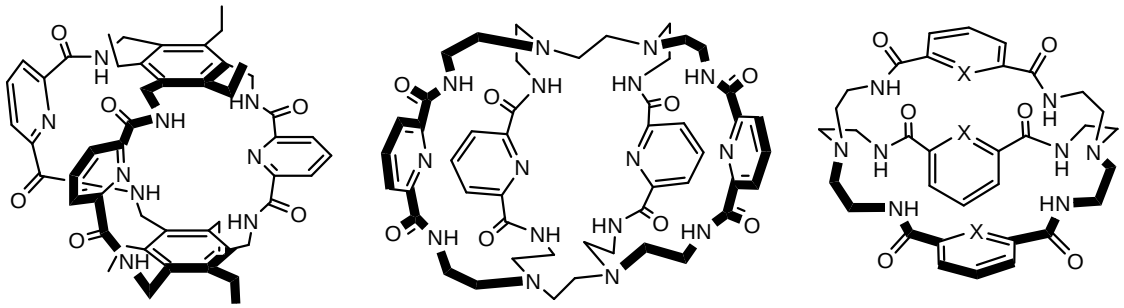
Scheme 2 Structures of azacyclophane receptor **4**, hexaamine cage **5** and the quaternary ammonium compound **6**.

In cases where anion binding needs to be independent of the pH or binding under neutral conditions is pursued, receptors containing quaternary ammonium units such as **6** can be used.[26] The effectiveness for anion binding of **6** is lower than that of receptors with protonated ammonium groups, as the quaternary nitrogen atoms are unable to form hydrogen bonds with an anion. However, Schmidtchen demonstrated that the encapsulation strength may be tuned by changing the length of the methylene chains between the ammonium centers (for **6**, n = 2: $Br^- \approx I^- > Cl^-$).[27] One of these cages (n = 4) was even found to act as a molecular catalyst in nucleophilic aromatic substitutions stabilizing the anionic transition state.[28] A major disadvantage of positively charged receptors is the competition of the receptor's counterions and the anionic substrate in complex formation. A remedy lies in the construction of electroneutral receptors.

The design of the latter usually involves introduction of hydrogen bond donor groups as binding units. These functionalities must be precisely positioned within the receptor framework so that their hydrogen atoms point towards the interior of the cavity and are located close to the bound anion. Other important features of such receptors are the balance between rigidity and flexiblility implemented in the host structure combined with a suitable cavity size, shape, and dimensionality. Major problems for neutral anion hosts to overcome are their generally low water solubility and their weak anion affinity in polar or even aqueous solutions.

Container molecules that rely on ion-dipole interactions to entrap an anion can be classified according to the type of functional group containing the hydrogen-bond donor, namely amide, urea, thiourea, pyrrole, hydroxyl etc. Use of amide groups as binding sites within receptors **7**, **8** and **9** was demonstrated by Anslyn and Bowman-James.[29] All of these receptors contain NH groups pointing toward the center of the cavity. However, not all of the available amide protons are usually able to interact with a bound anionic substrate: receptor **7** forms two bifurcated hydrogen bonds with a planar acetate;[29a] the complex of tricyclic cage **8** with linear FHF^- was stabilized by four hydrogen bonds;[29c] while only protonated tren-derivative **9a**•H_2^{2+} uses all eight

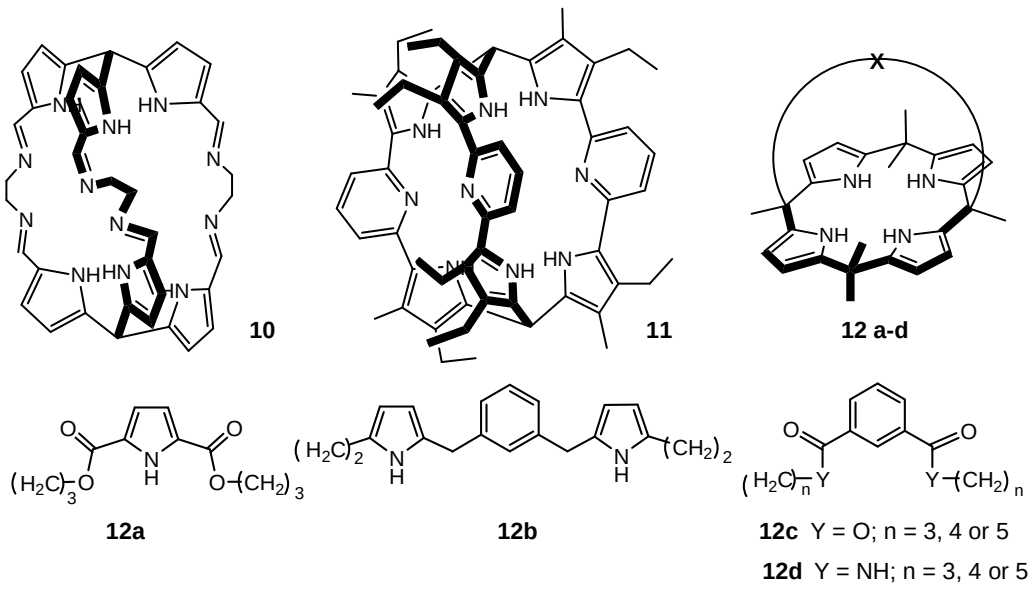
possible hydrogen bonds for sulfate encapsulation[30] (Scheme 3). Additional interactions such as fluoride-aryl CH protons (from *m*-xylyl) were found within **9b** to assist the binding process.



7 **8** **9a** X = N; **9b** X = CH

Scheme 3 Structures of hexaamide cyclophanes **7** and **9**, and the tricyclic octaamide **8**.

Calix[n]pyrroles or other pyrrole-derived subunits were also utilized to generate container molecules for anions.[31] Interesting examples are Beer's imine linked tripyrromethane dimer **10**[32] and the dipyrrylpyridine cryptand **11**,[33] although for both receptors no anion binding studies were performed (Scheme 4).



10 **11** **12 a-d**

12a **12b** **12c** Y = O; n = 3, 4 or 5

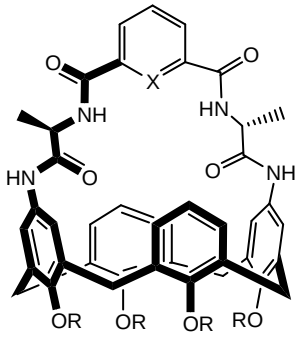
12d Y = NH; n = 3, 4 or 5

Scheme 4 Beer's tripyrromethane dimer **10**, Watanabe's cryptand **11**, and Lee's strapped calix[4]pyrroles **12**.

Strapped calix[4]pyrroles such as **12a** and **12b** in which conformational flexibility is restricted by the integration of a strap containing one or two pyrrole groups were developed by Lee and Sessler to bind halides.[34] Variation of the length of the strap in **12c** provided control over receptor properties thus leading to a nearly two orders of magnitude higher selectivity for fluoride over chloride (K_a = 3.9 x 10^6 M^{-1} of fluoride complex in DMSO).[35] Surprisingly, substitution of the

ester with an amide strap (**12d**) yielded no significant improvement of the binding strength in comparison to **12c**, although anion affinity is significantly higher than that of unstrapped calix[4]pyrroles.[36]

Ungaro et al. extensively investigated calix[n]arenes and their strapped derivatives as efficient building blocks for the design of cage-like architectures.[37] Such molecules possess a hydrophobic core and their selectivity toward substrates can be adjusted by increasing the number of arene units, or by incorporation of hydrophilic groups as straps or arms at the upper and/or lower rims. For example, amino acids or (fluorescent) peptides containing aromatic unit(s) have been attached to calix[4]arenes (**13**) to bind carboxylates but not carboxylic acids (Scheme 5).[38] Receptor **13** has higher affinity toward aromatic rather than aliphatic amino acid derived carboxylates and proved to be enantioselective for chiral α-amino carboxylates.[39] Generally, anion affinity is low but π-π stacking interactions between a phenyl group of the guest and the aromatic units of the host were found to improve complex stability.



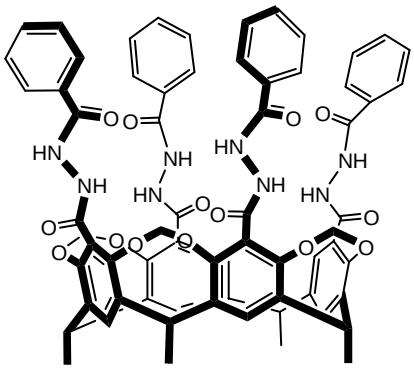
13 X = CH or N; R = $(CH_2)_2CH_3$

Scheme 5 Structure of C-strapped calix[4]arene **13**.

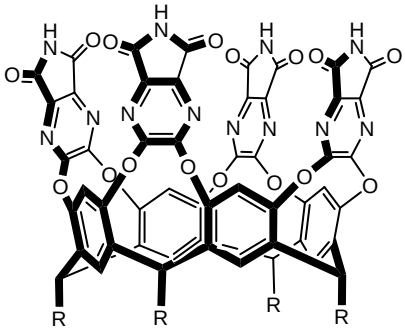
The potential of reversible reactions for the formation of molecular capsules capable to include anionic guests was also explored.[40] Strategies are based on hydrogen-bonding and also metal-ligand interactions to mediate self-assembly of a capsule from smaller components.[41] Self-assembly based on metal-ligand coordination will be discussed later in more detail (Section 2.3). Considering that aggregates stabilized by reversible interactions constantly exchange their building bocks in solution, the lability of such capsules is an important limitation. Despite this restriction, the use of self-assembly is a far more economical way for the construction of cage compounds than the methods of covalent chemistry. In order to obtain a capsular structure, the building blocks should recognize each other through non-covalent interactions or metal coordination and possess a certain degree of curvature. Suitable components proved to be calix[n]arenes,[42] resorcin[n]arenes,[43] pyridine containing bi-[44] or multi-dentate[45] ligands coordinated to transition metals or transition metal complexes.

Park and Paek[46] demonstrated that quantitative assembly into dimeric capsules can be induced by functionalization of the upper rim of resorcin[n]arene[47] molecules. A dimer formed from cavitand **14** not only encapsulates two acetate molecules in an antiparallel mode, orienting the carboxylic functions close to the eight hydrogen bonds, but is even able to extract polar anions into non-polar solvents (Scheme 6). Hydrogen bonds stabilize the cylindrical dimer of compound **15** and allow for numerous ion-dipole interactions with included anionic guests (TsO^-, $C_4F_9SO_3^-$, IO_4^-, ReO_4^-, PF_6^- , BF_4^-).[48] Solvent assisted space filling was detected in the complex of dimer $(\mathbf{15})_2$ in which two chloroform molecules interact with one bound chloride anion.

The group of Böhmer examined the behavior of a mixture of $(\mathbf{15})_2$ and a tetraurea-based calixarene dimeric capsule in the presence of NEt_4PF_6.[49] Separation (at about 10 Å) of the cation and the anion of this salt was observed upon encapsulation of each type of ion into the complementary capsule (PF_6^-•$(\mathbf{15})_2$ and Et_4N^+•tetraurea-containing calixarene capsule).[50]



14

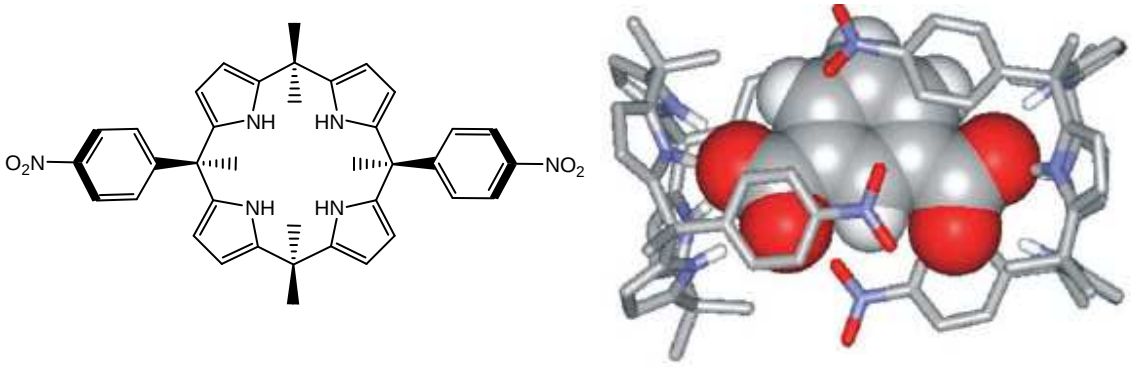


15 R = $C_{11}CH_{23}$

Scheme 6 Resorcin[4]arene **14** and cavitand **15** self-assemble into dimers.

Anion templation has been extensively used in supramolecular chemistry to produce interlocked structures like rotaxanes and catenanes, but also for the assembly of large polycyclic cages with included anions.[51]

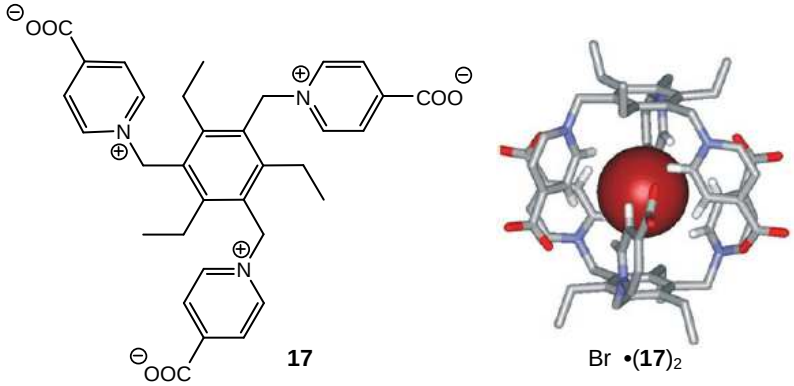
Encapsulation of dicarboxylates (1,3- and 1,4-benzenedicarboxylates) within a calix[4]pyrrole derived capsule $(\mathbf{16})_2$ was achieved by Kohnke and co-workers, with the introduced anion templating and accelerating formation of 1,3-$^-OOCC_6H_4COO^-$•$(\mathbf{16})_2$ (Figure 1).[52] The thermodynamic stability of the complex was found to be dependent on the nature of the counterion as well. Cations like Cs^+, and ball-shaped Me_4N^+, or n-Bu_4N^+ stabilize the anionic calixpyrrole complex by reduction of its conformational freedom.



16 1,3-$^{-}$OOCC$_6$H$_4$COO^{-} •(**16**)$_2$

Figure 1 Structure of calix[4]pyrrole monomer **16** and the X-ray crystal structure of the dianionic capsule 1,3-$^{-}$OOCC$_6$H$_4$COO^{-}•(**16**)$_2$.

In a recent publication, Steed and coworkers reported on the templating effect of bromide anions to induce the formation of a dimeric water-soluble capsule (Figure 2).[53] The self-assembled zwitterionic cage (**17**)$_2$ forms in D_2O and entraps one bromide anion. The structure is stabilized by hydrophobic and ion–ion intermolecular interactions, as well as by unusual aromatic CH$^{\cdots}$Br^{-} interactions. The resulting complex is so stable that it proved to be impossible to obtain the host in a guest-free form.



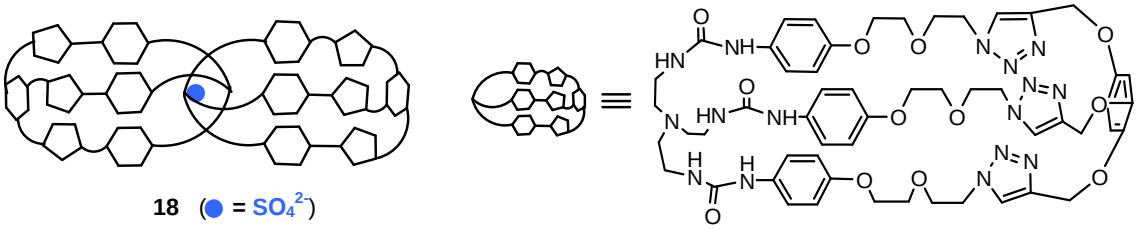
17 Br •(**17**)$_2$

Figure 2 Steed's zwitterionic building block **17** and the X-ray crystal structure of the bromide capsule Br $^{-}$•(**17**)$_2$.

Finally, use of anion templation in combination with one or more reversible reactions resulted in interesting receptor architectures such as bis(cyclopeptide) cages reported by Kubik et al. (Section 2.3.4)[54] and catenane-type cages described by Beer and coworkers.[55]

Beer and Li synthesized the first triply interlocked sulfate-binding catenane **18** with the anion permanently entrapped within the cavity (Figure 3).[55] Repeated attempts to remove sulfate by precipitation with $BaCl_2$ were unsuccessful, indicating an exceptionally strong complexation. Isolation of compound **18**, although in low yield (9 %), was also achieved in the absence of the

template, but even in this product a bound sulfate anion was found that was probably taken up during the purification process.



18 (● = SO_4^{2-})

Figure 3 Beer's triply interlocked sulfate-binding catenane **18**.

These examples of anion-binding cages demonstrate that guest binding benefits not only from direct interactions between the guest and the functional groups of the host, but also from non-covalent interactions within the host structure, from interactions with encapsulated solvent molecule(s), and attractive contacts with counterions.[47, 55-56] Either obtained from traditional covalent synthesis or produced using the methods of templating and self-assembly, container molecules ranging from nanometer to micrometer size with high affinities toward anions have been generated. Although some of these compounds could be applied for sensing, catalysis, nanotechnological or medicinal applications, very few systems if any are known whose affinities surpass those of biological anion receptor systems.

Based on this situation, interest arose in the Kubik group to prepare anion-binding capsules derived from known anion-binding cyclopeptides by using the principles of dynamic covalent chemistry under thermodynamic control.

2. Reversible chemistry for cage synthesis

Different from the traditional organic synthesis, where reactions should be irreversible to ensure formation of strong covalent bonds and highly selective for efficient production of a single product, dynamic covalent chemistry deals with the selective preparation of stable molecules from mixtures by using reversible covalent bond formation.[57] The use of different reversible interactions to bring organic molecules together has led to key discoveries in the fields of supramolecular chemistry,[58] chemistry of self-assemblies, and systems chemistry.[59]

Dynamic covalent chemistry proceeds under thermodynamic control where covalent bonds are being continuously broken, formed and reconstructed. It provides the possibility of "error checking" during the synthesis to eliminate thermodynamically unstable products.[60] The product distribution is controlled by a favorable change of the free energy during reaction progress (ΔG negative) and by the relative stabilities of the final products (Figure 4, **A** provides product **B** instead of **C** by the acid catalyzed reaction of glycerin, for example).[61] Stabilization of a desired product and hence product properties may be controlled by introduction of instruction motives into the starting materials or by manipulating the equilibrium (use of an excess of one of the building blocks or condensation of a product). Dynamic covalent chemistry is an elegant way to circumvent the difficulties occurring by the traditional organic synthesis where reactions are usually carried out under kinetic control. Being irreversible the latter proceeds preferentially via the most stable transition states (Figure 4, **A** gives product **C** rather than **B** at high temperatures) and if the states leading to different products are similar in energy, product mixtures result.[62] Hence, the isolation of the desired product is complicated and frequently renders low yields.

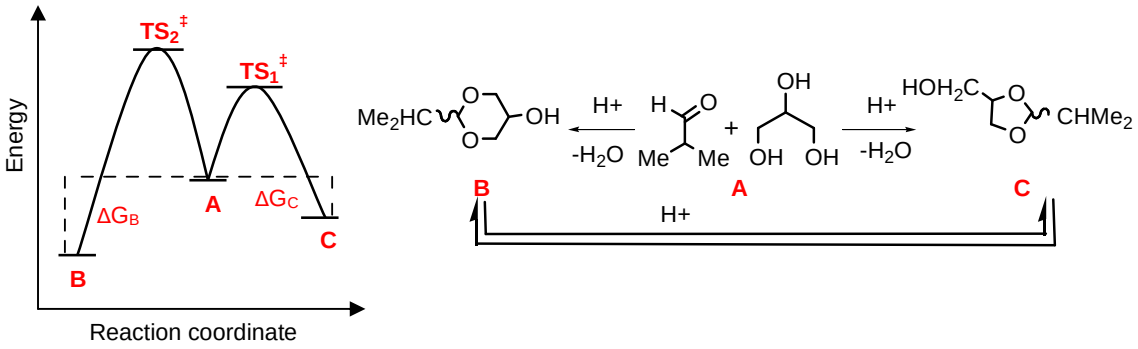


Figure 4 Energetic profile of the acid catalyzed reaction of glycerin with isobutyroaldehyde (**A**).[63] Product distribution under kinetic control ($TS_1^{‡}$) gives dioxolane (**C**) while performing the reaction under thermodynamic control ($TS_2^{‡}$) provides dioxane derivative (**B**).

The idea of my thesis was to take advantage of the reversibility offered by dynamic covalent chemistry to obtain covalently linked cages from an equilibrium mixture of all possible products. The stabilization and isolation of the targeted bis(cyclopeptides) should be promoted by

introduction of a template molecule to the reaction mixture. Dynamic combinatorial chemistry (DCC), a new field in dynamic covalent chemistry that emerged recently, combines formation of many different components in a combinatorial library with non-covalent molecular recognition leading ultimately to the identification of library members that interact with an added template most strongly. [60] DCC proved to be a useful strategy for the synthesis of macromolecules obtained through reversible covalent, non-covalent as well as coordinative interactions. In the following chapter, the principles of DCC will be shortly summarized.

2.1 Principles of DCC and basic requirements

The rapid development of DCC during the past decade resulted in the discovery of novel synthetic receptors,[57b, 64] catalysts[65] and sensors,[66] as well as in new supramolecular materials,[67] ligands for biomacromolecules,[68] and self-synthesizing molecules.[69] DCC combines rapid generation and screening of vast collections of molecules in one single process.[70] Diversity and complexity results from different combinations and continuous recombination of simple building blocks linked by reversible reactions. Such a pool of interconverting molecules under equilibrium, termed a dynamic combinatorial library (DCL), is an essential feature of DCC. In a DCL, the connections between individual fragments may be covalent or non-covalent. The composition of a DCL is dependent on the environment in that either the internal properties of the product(s) (self-selection) or the interaction with external entities (e. g. template molecule, physical stimuli, or phase change) may result in the generation of a given type of species (Figure 5).

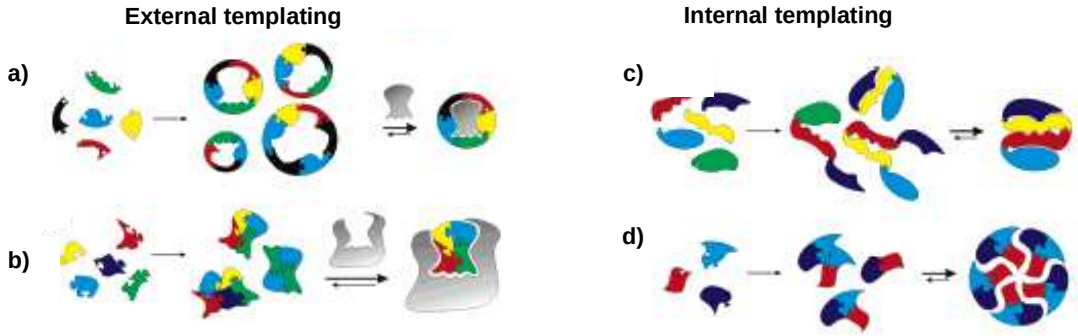


Figure 5 Schematic representations of the ways to control the product distribution in a dynamic library on the basis of non-covalent interactions. Selection operates in response to either external templating such as molding (a) or casting (b) or internal templating such as intramolecularly stabilized foldamers (c) or intermolecularly stabilized aggregates (d).

The addition of a template molecule, ranging from metal ion to protein, displaces the equilibrium by forming favorable non-covalent interactions with one or more library members.[71] Hence, a

particular constituent is additionally stabilized and brought to a lower energy state (Figure 6a). According to Le Châtelier's rule the library recognizes the new global energy minimum and adjusts its composition to reach it. In this way, an increased accumulation of the stabilized component (amplification) results, causing the less stable products to re-adjust and disappear ("proof of reading").[57a] This re-equilibration also serves purposes such as minimizing side reactions and achieving the desired stereochemistry. Amplification facilitates the identification of the selected species and after halting the exchange of building bocks the isolation of the target molecule directly from a "frozen" library becomes possible (Figure 6b).

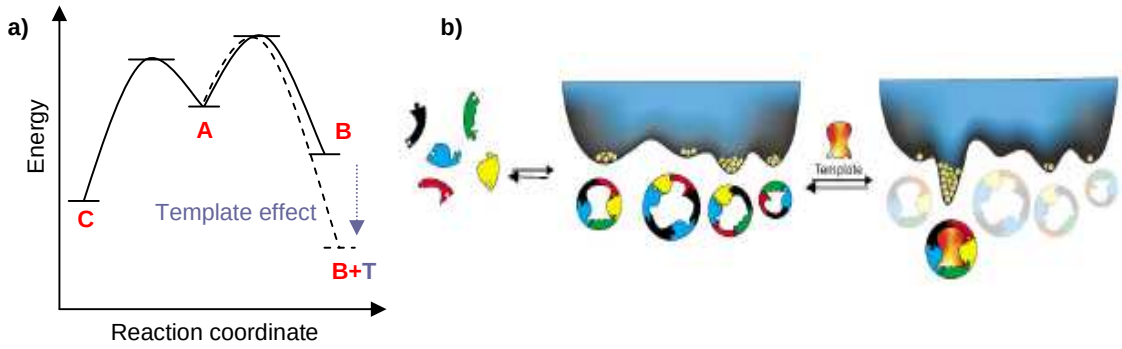
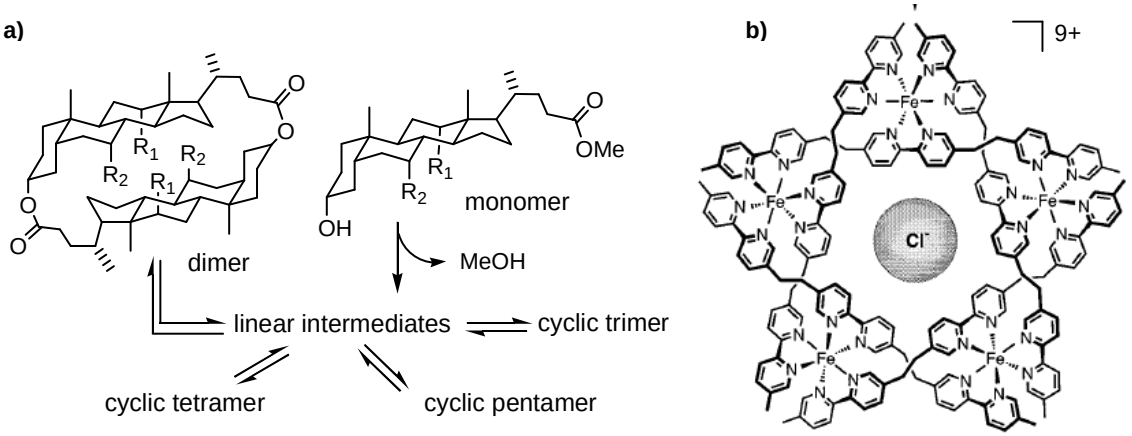


Figure 6 a) Change of the energetic profile of a reversible reaction upon addition of a template molecule (T) that selectively binds to one of the host species (B) from a mixture of starting materials (A) and other products (C); b) Introduction of a template to a DCL under thermodynamic control shifts the equilibrium to favor the formation of the energetically most stable product.

The relatively young area of DCC appeared clearly conceptualized as late as in the mid-1990s independently developed by the groups of Sanders and Lehn (Scheme 7). Sanders and his co-workers utilized reversible base-catalyzed transesterification processes combined with the presence of small alkali metal ions as external stimuli to dictate the product distribution of interconverting cyclic oligocholates, thus favoring the formation of larger polyester rings.[72] Lehn considered the notion of dynamic combinatorial coordination processes with the observation that the structure of an iron(II)-based circular helicate was directed by the nature of the counterion.[73] Since these first examples of DCC appeared in the literature, external templating is still the most extensively used means of directing a library's distribution. Huc and Lehn considered two possible routes for external templation of a system: *molding* (substrate-induced assembly) or *casting* (receptor-induced assembly).[74] Both DCC processes involve the identification of the optimal binding partners through spontaneous combination of building blocks in response to the addition of a suitable template.



Scheme 7 a) An equilibrium mixture of steroidal oligomers interconverting through transesterification reaction described by Sanders. Templating with metal ion, for example Li^+, leads to the formation of trimer (76 %), tetramer (16 %) and pentamer (7 %). b) Lehn's self-assembly of a pentanuclear circular helicate with a chloride anion bound in the center.

While *casting* concerns the identification of a molecular species that binds within a cavity or stabilizes a macromolecule,[75] *molding* is a process that addresses the selection of a host by a separately introduced guest (Figure 5b and a, respectively). *Molding* was utilized for the discovery of artificial linear, macrocyclic, or cage-like receptors as well as for the generation of functional interlocked structures.[76]

The molecular function that the identified host would express relies on the nature of the template applied. For example, Otto and Sanders reported on the first example of a catalyst obtained using DCC in their screening studies against a transition state analogue (TSA) for an acetal hydrolysis reaction and for a Diels-Alder reaction.[77] Specifically, among all amplified disulfide macrocycles only the larger cyclophane **19** binds TSA **20** and causes, respectively, a 2-fold and a 10-fold increase in the rate of the corresponding reactions (Figure 7a). Targeting organic cations, [2]-catenane receptor **21** composed of two interlocked hydrazone trimers was discovered and found to possess an impressive nanomolar affinity (100 nm) for the neurotransmitter acetylcholine (Figure 7b).[78] Alternatively, the response of the entire library was used by Buryak and Severin to report on the nature of the guest thus achieving the differentiation of structurally similar dipeptides or nucleotides.[79]

Powerful ways to control the selection processes in the dynamic combinatorial assembly such as the casting strategy**,** the self-selection of library members through internal templating (based on intramolecular or intermolecular non-covalent interactions, Figure 5c and d) or the displacement of library composition by external stimuli (irradiation, electric field or changes in the temperature and pH) will not be discussed here. These aspects have been recently explored by the groups of Balasubramanian**,**[75b] Li,[80] Gellman,[81] Lehn,[82] Guiseppone[83] and Fujita.[84]

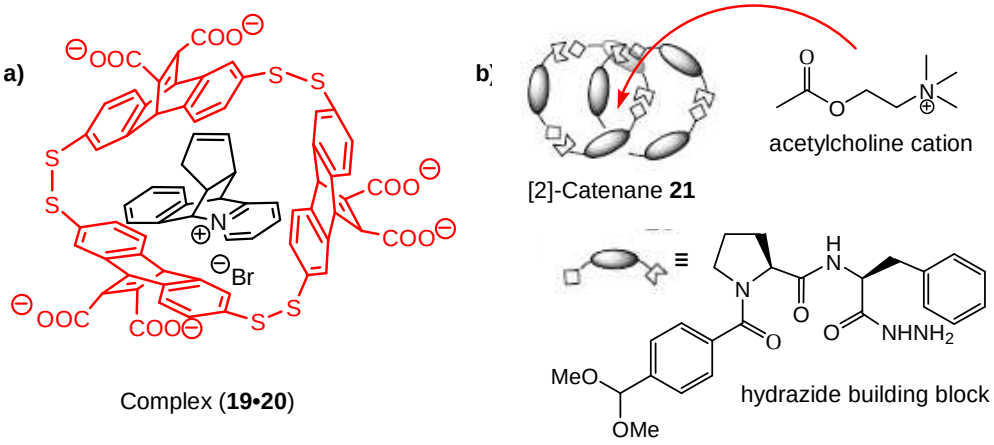


Figure 7 a) Cyclophane receptor **19** (red) acts as a receptor for TSA **20** (black) and as a catalyst for the Diels-Alder reaction; b) [2]-Catenane **21** binds acetylcholine where the two rings meet.

Some basic prerequisites have to be met to set up a combinatorial library:

• As DCC is not suited for the synthesis of small molecular weight compounds, the building blocks need to be carefully designed prior to do the experiment. Building blocks should ideally be relatively simple, inexpensive to synthesize, and unambiguous to analyze. Building blocks must be suitably functionalized such that they can react with one another and must contain a recognition element for interaction with a guest to allow templation. For DCLs constructed to generate receptors, the relative balance between rigidity and flexibility of the constituents' backbone should be considered. Another key parameter of a DCL is the concentration of building blocks as it influences the rates of the exchange reactions. Below a critical concentration, for example, essentially only small macrocycles are formed, because cyclization is faster than chain extension.

• Importantly, also the template concentration must be low enough to ensure that the amplification is selective for the best binder.[85] Use of limited template concentration, with a template to building block ratio of 1:10, usually yields satisfactory library response. At increased template concentration, a DCL may choose to generate two cyclic dimers instead of one cyclic tetramer (the better binder) as it profits twice the template-dimer binding energy versus only once the template-tetramer binding energy (Figure 8).[86] To verify that the library responds with amplification of the best receptor after addition of a template one must guarantee that the affinity of the best receptor is at least 20 fold higher than affinities of other potential library members.

• A reversible reaction capable to operate selectively on a reasonable time scale under mild conditions has to be selected. The exchange reaction needs to be fast enough, but slower than complexation/decomplexation kinetics of the template.[87] Tolerance to the functional groups required for template recognition is also necessary. A simple but fast change (so that no re-

equilibration occurs) in the conditions, such as pH, temperature, pressure, or electric field should allow efficient control over the exchange process in order to allow isolation of the selected product.

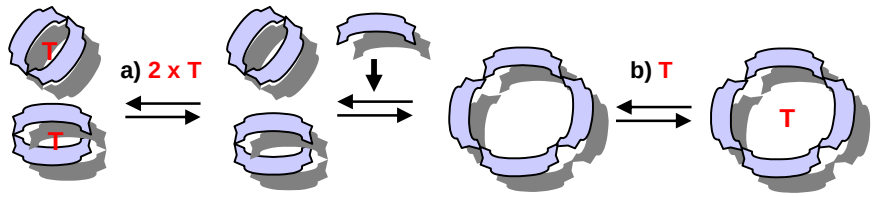
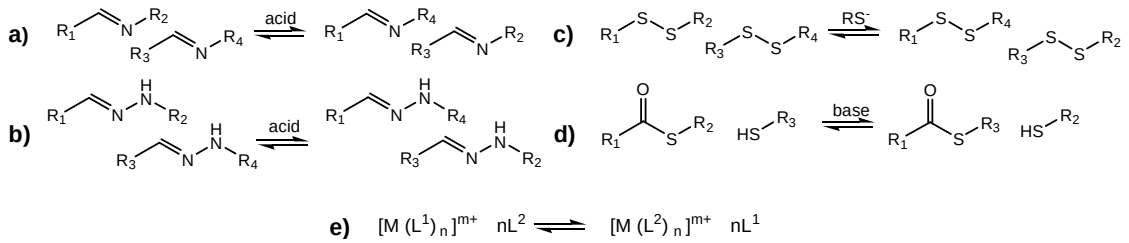


Figure 8 Effects of template concentration on library distribution. Guest excess may cause (a) amplification of a large number of smaller macrocycles, while low guest concentration ensures production of the best binder (b).

• A straightforward procedure for screening of the library composition and for identification and characterization of the expressed constituent(s) has to be available. Typically, highly sensitive analytical methods such as HPLC, LC-MS, or NMR techniques in the absence and presence of template ensure efficient analyses of dynamic systems composed of small or even relatively large libraries. [59c, 88]

Among all reversible interactions (covalent interactions,[74, 89] non-covalent interactions[90] and metal-ion coordination[91]) that tightly hold together the library constituents within a supramolecular assembly,[73e] the most important for DCC are imine exchange,[74, 92] hydrazone exchange,[93] disulfide exchange,[94] thioester exchange[81, 95] and metal-ligand exchange (Scheme 8).[91b, 96] However, new reversible chemistries available for the generation of DCLs are continuously being developed.[97]

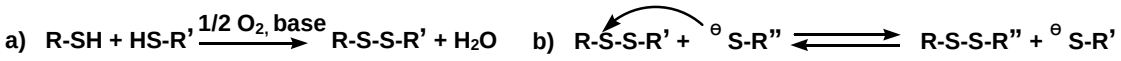


Scheme 8 Most important reversible reactions used in DCC: (a) transimination; (b) hydrazone exchange; (c) disulfide exchange; (d) transthioesterification; (e) metal-ligand exchange.

The construction of bis(cyclopeptide) cages in my thesis is based on thiol-disulfide interconversion and on metal-ligand exchange. Therefore, the characteristic features of both reactions will be discussed as well as some of their applications. In particular, use of these reactions for the synthesis of cage-like structures will be presented.

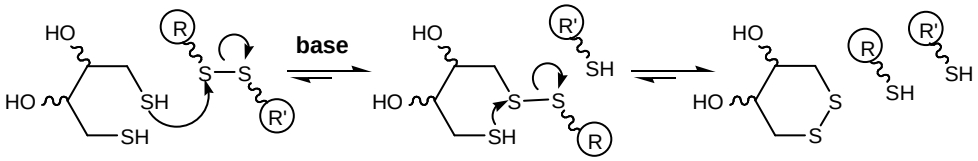
2.2 Disulfide exchange reaction

Although the reversibility of thiol-disulfide exchange is known and has been exploited for many years, the first use of disulfide exchange in the context of DCC was that of Hioki and Still involving the synthesis of an artificial peptide receptor.[94a] Since this breakthrough in 1998 disulfide exchange developed into one of the most popular and important DCC exchange reactions. Studies on the exchange mechanism revealed that the initial step consists in the irreversible air oxidation of thiols to disulfides in the presence of a small amount of base (pH 7-9).[98] Then, a catalytic amount of thiolate anions mediates the nucleophilic attack on the disulfide bond along the sulfur-sulfur axis (S_N2 - reaction). Scheme 9 illustrates the two steps of the disulfide interchange reaction. Exchange requires deprotonated thiol. Therefore, a simple change in the pH of the solution permits to switch on and off the dynamic process. Practically no exchange is detected under acidic conditions (pH ≤ 2.5).



Scheme 9 Mechanistic representation of the thiol-disulfide exchange reaction a) oxidation of thiols to disulfides under air and b) disulfide exchange in the presence of thiolate anions.

In theory, the same library mixture as that obtained from free thiols can be generated starting directly with corresponding "preoxidized" disulfides as building blocks.[99] To start exchange, a catalytic amount of reducing agent such as dithiothreitol (DTT),[94b] 1,4-butanedithiol (BDT),[100] or a free thiol such as 2-mercaptoethanol and a small amount of base must be added in this case to initiate exchange.[101] DTT, for instance, was shown to be a very selective reducing reagent for disulfide bonds as it efficiently oxidizes to a highly stable 6-membered cyclic disulfide that does not further participate in the interconversion of the library members (Scheme 10).[102]



Scheme 10 Reduction of a disulfide by dithiothreitol (DTT) proceeds through two subsequent S_N2 reactions.

The thiol-disulfide interconversion thus fulfills the above mentioned prerequisites. Firstly, the disulfides undergo rapid interchange with thiols at moderate to high pH (≥7), while the bond formed remains stable at low pH. Secondly, the thiol-disulfide reaction does not interfere in the recognition process and is highly chemoselective, efficiently proceeding in the presence of

various functional groups. The exchange can be carried out under mild conditions in organic solvents[103] as well as in the aqueous environment[104] and produces chemically stable products. Furthermore, no external catalyst is required for the exchange process to proceed. Last but not least, disulfide exchange is one of the few reversible covalent processes proven to be highly compatible with biomolecules.[94c, 105]

2.2.1 Some applications of disulfide exchange

An early application of disulfide exchange for the generation of a DCL in aqueous buffer employing a protein target (the plant lectin concanavalin A) has been described by Lehn and Ramström.[94c] The authors demonstrated the selection of a bis-mannose substrate **22** from a pool of four to six homo- or heterodimeric hexopyranosides and pentopyranosides containing linking units of different length (with 2 or 3 methylene groups) (Figure 9). Addition of immobilized Con A to the dynamic library caused a shift in the equilibrium in favor of homodimer **22**.

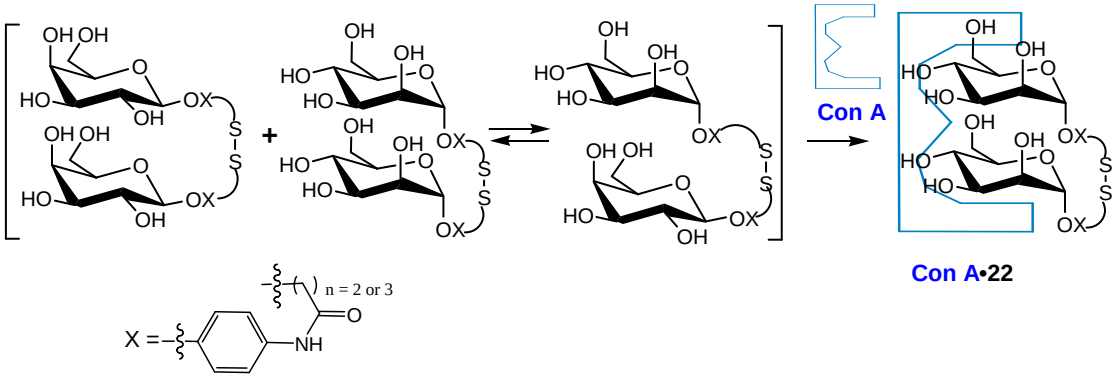
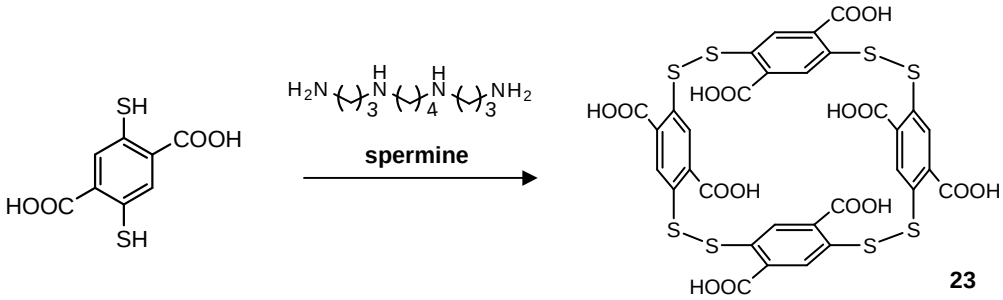


Figure 9 Selection of a bis-D-mannose disulfide by concanavalin A (Con A) from a mixture of disulfide-linked carbohydrate dimers reported by Lehn and Ramström.

Subsequently, disulfide exchange was used by Sanders, Otto, Kubik, Takata and others to generate macrocyclic[88d, 103, 106] or cage-type[107] receptors through DCC.

Examples are the report by Waters et al. on the discovery of small aromatic cage-like receptors for trimethyl lysine that bind to this substrate with comparable affinities and selectivities as the native HP1 chomodomain,[108] or the highly potent macrocyclic spermine receptor **23** developed by Otto.[109] This cyclophane, obtained from a relatively simple library, recognizes spermine, a polyamine that influences cell proliferation and apoptosis, with such a high affinity (K = 4.5 x 10^7 M^{-1} in TRIS buffer at pH 7.4) that it is able to remove this compound from one of its natural hosts, DNA (Scheme 11). Usually, interaction of DNA with spermine at low concentrations results in the formation of a left-handed DNA helix, but upon addition of **23** the DNA reverts back to its normal right-handed form due to complexation of spermine by **23**. Some years later Sanders and co-

workers isolated a linear receptor able to transport spermine from an aqueous source phase to an aqueous receiver phase separated by a bulk membrane.[110]



Scheme 11 Oxidation of thiol building blocks gives macrocyclic receptor **23** in more than 90 % yield after exposal to spermine.

Other publications described the utilization of disulfide-based DCC for the formation of interlocked structures such as catenanes[111] and (pseudo)rotaxanes.[99, 112] As an extension of their work on the ability of DCLs to generate [2]catenanes in water,[113] the Sanders group reported on a controllable template driven synthesis of two different donor-acceptor [2]catenanes **24** (D-A-A-D stacks) and **25** (D-A-D-D stacks) (Figure 10).[114] The yield of either catenane **24** or **25** was improved by up to 55 % by increasing the building block concentration, solvent polarity (water), and ionic strength ($NaNO_3$) of the reaction medium. The results indicate that hydrophobic interactions played the major role in the formation of the unusual D-A-D-D stacks in **25**.

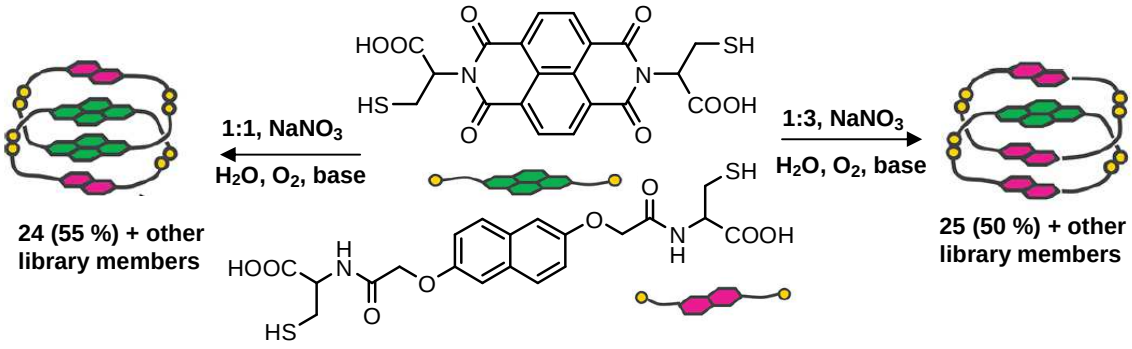
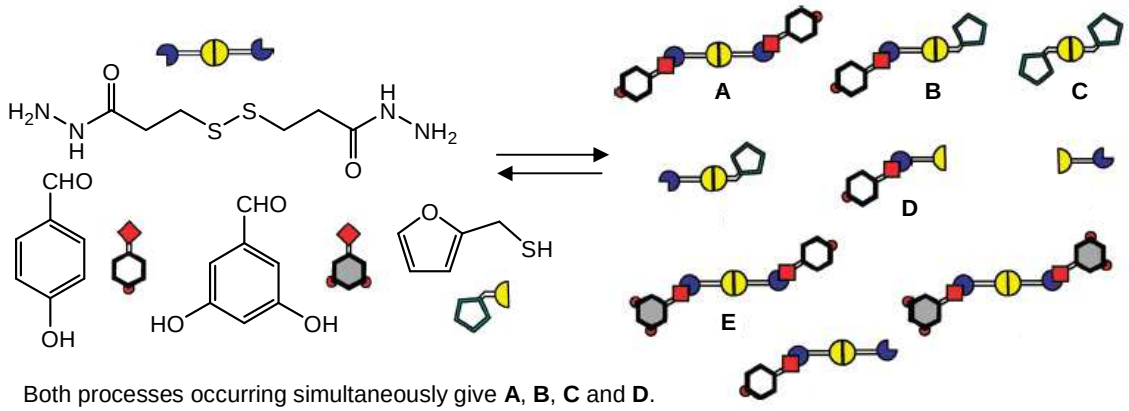


Figure 10 Increase of the building block concentration in a donor-acceptor disulfide DCL generates [2]catenanes **24** and **25** at different extents.

The first example of two covalent dynamic combinatorial chemistries operating simultaneously and responding orthogonally to pH changes in the system was reported by Otto and Rodriguez-Docampo.[115] Different product distributions were obtained depending on the order in which the disulfide and hydrazone exchange processes were activated by adjustment of the pH of the solution (from pH 8.5 to 2.5 and conversely) and when these processes were allowed to occur simultaneously (Figure 11). Such orthogonal exchange is a very promising tool for applications in

systems chemistry[116] since it may give rise to product distributions very different from the global thermodynamic minimum of a simple DCL. Other recently developed orthogonally[117] exchanging systems with disulfides include the combination of disulfide and thioester exchange,[118] and also the combined use of disulfide, hydrazone and thioester chemistry.[119]



Both processes occurring simultaneously give **A**, **B**, **C** and **D**.
Activation of disulfide then hydrazone exchange produces **B** and **D**.
Activation of hydrazone then disulfide exchange produces **B** and **E**.

Figure 11 Schematic representations of the building blocks (left) and all possible products (right) involved in the sequential and simultaneous activation of disulfide and hydrazone exchange reactions.

The group of Otto demonstrated that combining two individual reversible processes like disulfide exchange reaction and self-assembly can provide an exciting entry into the emerging field of systems chemistry.[59] A DCL made from aromatic building block **26** bearing a short peptide chain was found to spontaneously cyclize into hexamers or heptamers, which then self-assemble into thin micrometer-long fibres stabilized by β-sheet formation of the peptide chains (Figure 12).[120] Interestingly, shaking of the reaction solution caused preferential formation of fibres made up by cyclic hexamer **27** whereas stirring induced formation of the fibres composed of heptamer **28**. Autocatalytic growth of the fibres was attributed to breaking under the mechanical stress which released chain ends that templated the formation of new macrocycles. Different stabilities of the fibres explain why one requires stirring for disruption while the other breaks already upon shaking.

Another breakthrough in the field of disulfide DCC was achieved with the discovery that the selectivity of a solution-based molecular recognition can be transferred to solid phase DCLs.[121] In this context, immobilized templates were used during the equilibration process to produce a good binder for the cellular target of vancomycin[122] and a very efficient receptor for the adamantyl ammonium ion (1×10^7 M^{-1}).[123]

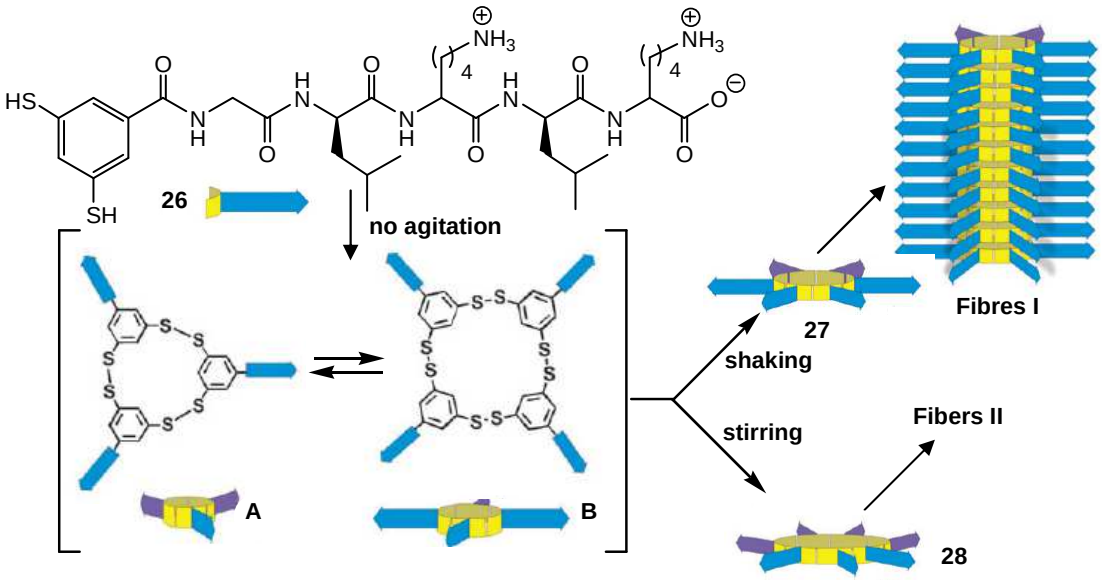


Figure 12 Peptide-functionalized dithiol building block **26** gave initially a mixture of macrocycles **A** and **B**. Shaking the DCL mixture produced hexamer **27** while stirring provided heptamer **28**, both aggregating into long fibres held together by non-covalent interactions between library members.

Rather than solid supported templates, Miller et al. have used a set of 150 supported cysteine-containing building blocks and an identical set of solution-phase monomers to target RNA binding.[68g] Theoretically, 11.325 library members could be formed both on the surface of the beads and free in solution after disulfide exchange was initiated. Upon addition of a fluorescence-labeled template (RNA), the Miller team succeeded in identification and isolation of several molecules with good affinity and significant selectivity for binding to RNA with a $(CUG)_n$ sequence, an agent responsible for myotonic dystrophy T1 (Figure 13).[124] Furthermore, the amplified compounds were shown to inhibit interaction between the $(CUG)_n$ repeat and the RNA-MBNL1 protein *in vitro*. The solid supported disulfide DCLs are the largest libraries constructed to date. When searching for strong ligand-receptor interactions it appears reasonable to work with libraries of similar or even larger size with more than 10^4 members.[88b, 125]

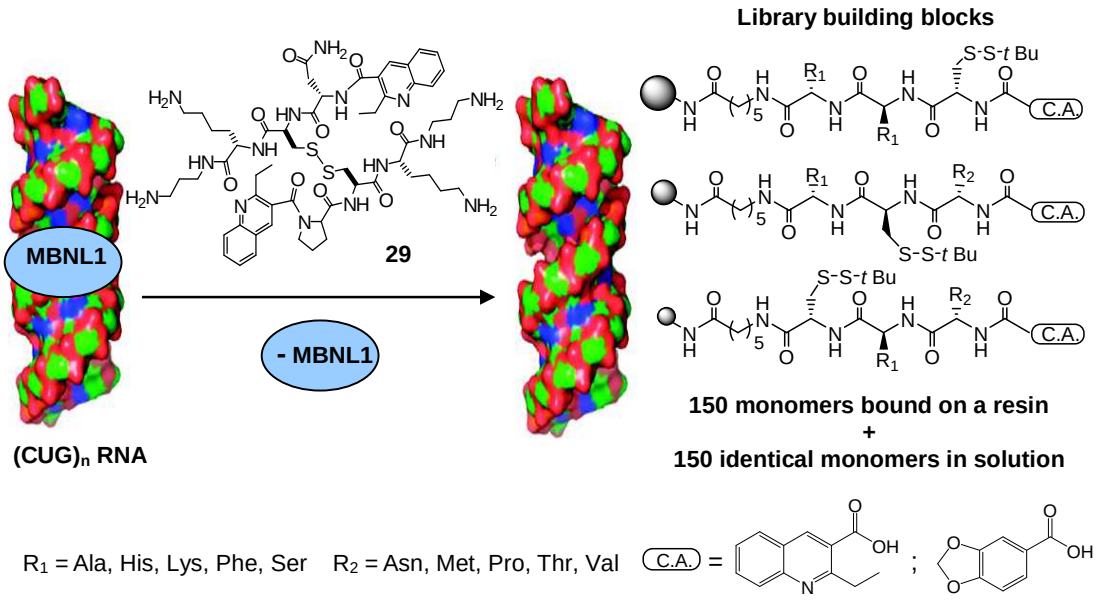
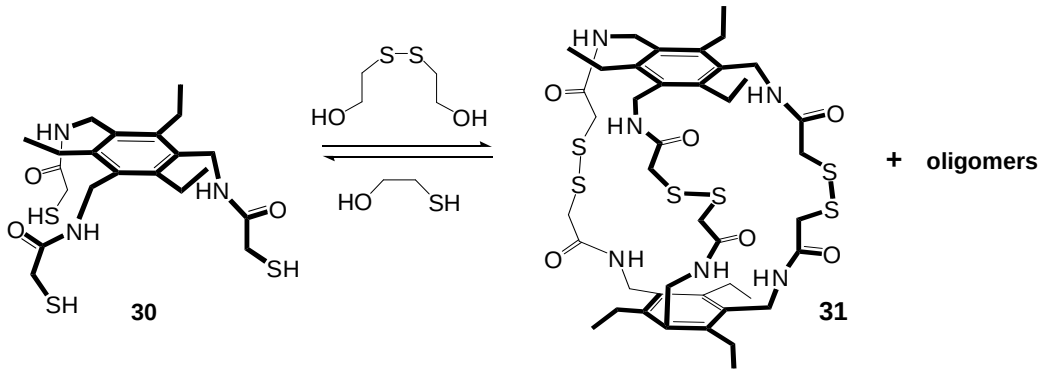


Figure 13 Discovery of lead compounds targeting myotonic dystrophy T1 through resin bound dynamic combinatorial selection. One of the amplified heterodisulfides **29** inhibits the $(CUG)_n$ repeat RNA-MBNL1 interaction. (MBNL1-RNA: binding protein acting as splicing agent).

2.2.2 Molecular capsules based on disulfides

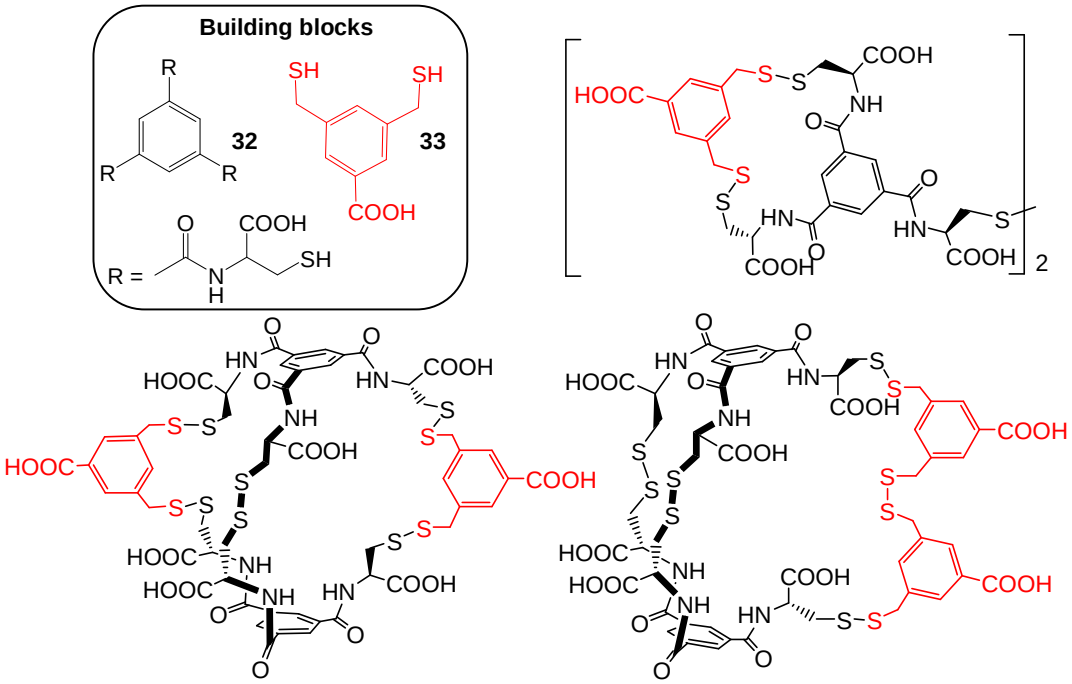
Although DCC made a great impact on the invention of new artificial receptors, its contribution to the field of molecular encapsulation is still in its infancy and waits for expansion. The synthesis of covalently assembled capsules through dynamic combinatorial chemistry was established by the groups of Fujita, Albrecht, Raymond, Rebek, Reinhoudt, Sanders, Stang, Steed, Yamaguchi, and others.[91c, 91e, 91g, 126] In this chapter, molecular capsules deriving from disulfide exchange reactions are mainly considered. Until now there are only few examples in the literature of capsules of this kind. It should be noted that some cage-type structures containing disulfide bonds have been described that were constructed under kinetic control.

In 1999, Tam-Chang et al. described the formation of a small macrobicyclic tris(disulfide) cage **31** (Scheme 12).[127] Equilibration of aromatic trithiol **30** in the presence of bis(2-hydroxyethyl) disulfide produced a mixture of the starting material, of dimer **31**, and of an oligomeric compound of unknown structure. Evaluation of the equilibrium mixture revealed that only 30% of it represented cage **31**. Addition of 2-mercaptoethanol to this library caused the cleavage of the cage and the re-accumulation of the starting material **30**. Although the effects of external templating during disulfide exchange were not investigated, one can speculate that addition of a suitable guest being able to template the formation of **31** could direct the library toward the production of a larger amount of the desired product.



Scheme 12 Equilibration of trithiol **30** to produce cage **31**.

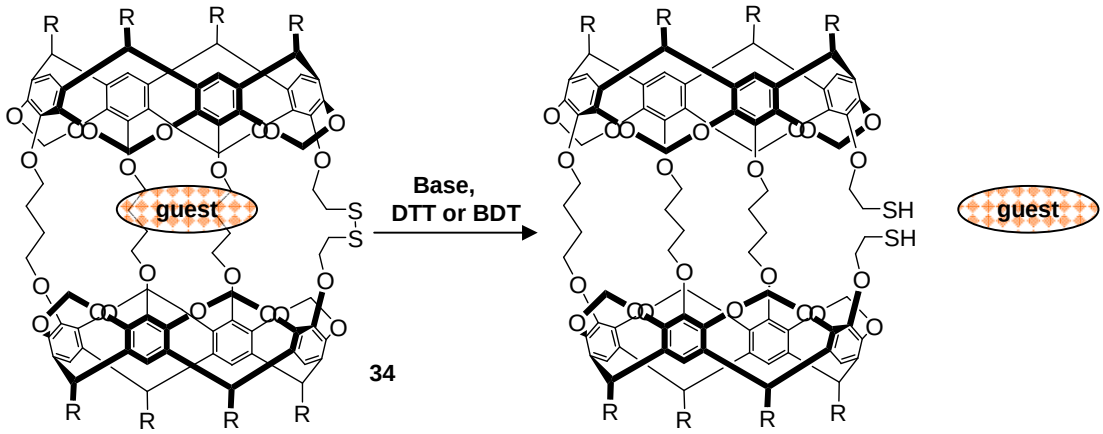
Synthesis of cage-like compounds using DCC and disulfide exchange was also pursued by West and Otto. A DCL composed of building block **32** containing three cysteine residues almost quantitatively dimerizes to give $(\mathbf{32})_2$.[2b] If oxidation of the trithiol **32** was conducted in the presence of dithiol **33** formation of mixed isomeric cages was observed with at least three different species having the composition $(\mathbf{32})_2(\mathbf{33})_2$ (Scheme 13). No guest encapsulation was observed.



Scheme 13 Representation of several isomeric macrobicyclic structures $(\mathbf{32})_2(\mathbf{33})_2$ generated from a DCL containing monomers **32** and **33**.

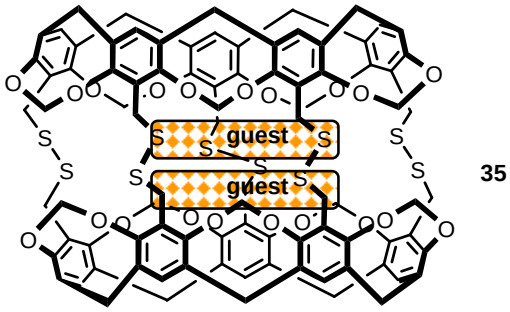
Introduction of a disulfide gate unit into the linker of a cage-type host represents a promising way to control the entry and exit of guest(s). In this context, the disulfide-dithiol reversibility was used by Houk and coworkers to introduce a redox-controlled gating mechanism into

hemicarceplexes.[128] When the gate is closed (disulfide) the resorcin[4]arene-based hemicarcerand **34** containing one disulfide linkage must be heated in the presence of an excess of guest (substituted benzenes) for complexation to take place. Complexation proceeds through thermally induced conformational change of the linker in the host structure. Exposing the hemicarceplex to reducing conditions by adding a small amount of base (1,8-diazabicyclo[5.4.0]undec-7-ene, DBU) and a dithiol (DTT or BDT) resulted in opening of the disulfide bond (Scheme 14). As a consequence, facile guest release became possible. Moreover, under appropriate conditions the gate could also be closed again.



Scheme 14 In the presence of reducing agent and catalytic amount of base the disulfide gate of hemicarcerceplex **34** opens to liberate the guest.

Sherman and co-workers reported the synthesis of a C_5-symmetric carcerand (**35**), in which the two halves of the capsule were held together by five $ArCH_2$-S-S-CH_2Ar linkages (Scheme 15).[129] Although obtained under kinetic control, this carcerand is a promising candidate to study thermodynamic template effects acting on its formation by subjecting the corresponding thiol precursor to equilibrium conditions.



Scheme 15 Sherman's carcerand **35** contains five disulfide linkages. Guest = DMF or DMA.

Work in Kubik group demonstrated that the use of thiol-disulfide DCC for structural optimization of the spacer between two covalently linked cyclopeptide rings renders efficient neutral receptors for

recognition of inorganic anions in aqueous media.[130] More details about cyclopeptide-based receptors are provided in Section 2.3.4.

Another example of the use of multiple disulfide bonds to bridge two macrocyclic building blocks involves the cyclodextrin dimers described by Kraus and co-workers. In a first paper, the researchers reported on the efficient synthesis of duplex cyclodextrin **38** composed of two α-cyclodextrin macrocycles bridged by two disulfide bonds in transannular ($C6^{I}$ and $C6^{IV}$) positions.[131] In principle, air oxidation of the starting thiol derivative **36** could result in the formation of products including a cyclodextrin with an intramolecular disulfide bond as well as various cyclic or linear dimers, oligomers, and higher polymeric products. However, under thermodynamic control exclusive formation of two C_2-symmetrical products was observed with the dimer **38** significantly dominating over **37** containing the intramolecular disulfide bond (Figure 14).

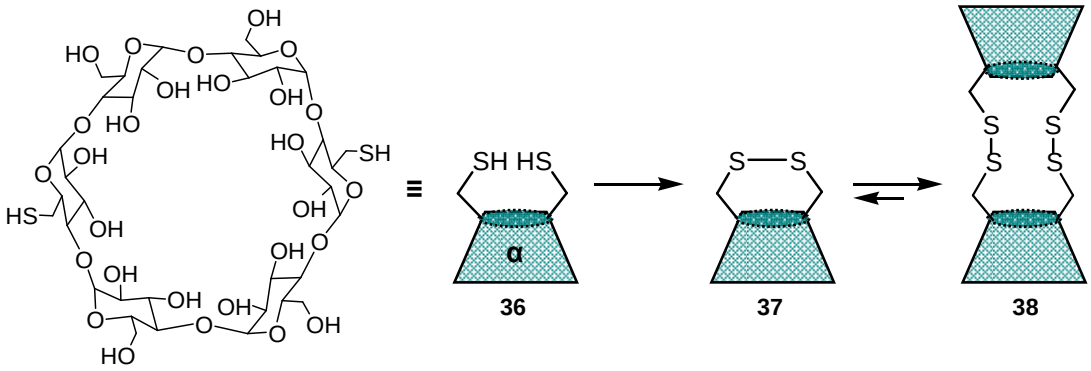


Figure 14 Thermodynamically driven template-free formation of α-cyclodextrin duplex **38** (87%) and intramolecular disulfide **37** from an aqueous solution of derivative **36**.

The duplex **38** was shown to form inclusion complexes with α,ω-alkanediols and 1-alkanols possessing aliphatic hydrocarbon chains longer than 10 carbon atoms. Isothermal titration calorimetric (ITC) measurements with different α,ω-alkanediol guests demonstrated that dimer **38** exhibits about 2 orders of magnitude higher binding affinity than a related α-cyclodextrin dimer containing only one disulfide bridge. The impressive stability constant of K_a = 8.6 x 10^9 M^{-1} determined for the 1,14-tetradecanediol complex of **38** in aqueous solution was attributed to the improved preorganization of **38** for complex formation. Performing the synthesis of **38** in the presence of appropriate templates caused a significant improvement in the yield. Among all α,ω-bifunctional derivatives, 1,16-hexadecanedioic acid proved to be the best template giving the corresponding inclusion complex of **38** in a yield of 92 %. This complex turned out to be so stable that the substrate could barely be removed from the cavity.

The efficiency of the template-free dimerization approach was applied by the same group to engineer a related α-cyclodextrin duplex **39** containing three symmetrically placed disulfide bonds ($C6^{I}$, $C6^{III}$ and $C6^{V}$) (Figure 15).[132] The increased number of the linking units caused improvement of stability but no significant enhancement in affinity of **39** toward α,ω-alkanediols

(C_{11} - C_{13}). Thus, the binding constants obtained for the inclusion complexes of dimer **39** were comparable to those of the doubly-bridged duplex **38**. In the crystal lattice of **39**, the α-cyclodextrin duplexes were found to be tightly stacked forming channels partially filled with water molecules or guest species. Such tubular structures exemplify that disulfide exchange is a promising reaction for the covalent synthesis of nanotubes and may lead to applications of DCC for the development of ion channels and novel delivery devices.

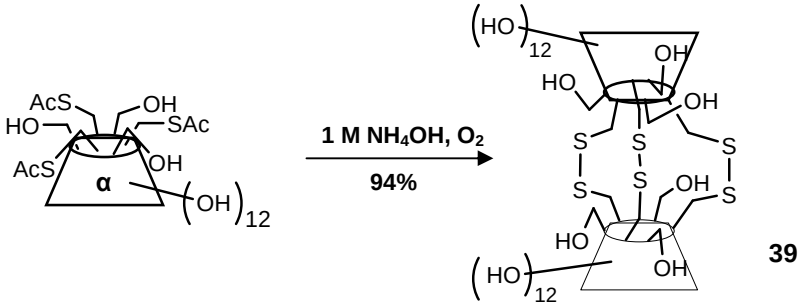


Figure 15 One-pot synthesis of duplex **39**.

In this context, Chechik et al. showed that spontaneous aggregation of perthiolated β-cyclodextrin **40** in aqueous solution followed by air oxidation leads to formation of nano-sized, hollow capsules (63% yield) able to entrap Reichardt's dye (Figure 16).[133] Thermodynamic templating of **40** with Reichardt's dye in water resulted in its entrapment inside the polymeric cyclodextrin-derived structure so that dye removal by dialysis proved to be impossible. Although unable to enter the cyclodextrin cavity, the dye molecule forms very stable complexes with the polymer so that its release could only be mediated by reduction of the disulfide bonds with mercaptoethanol.

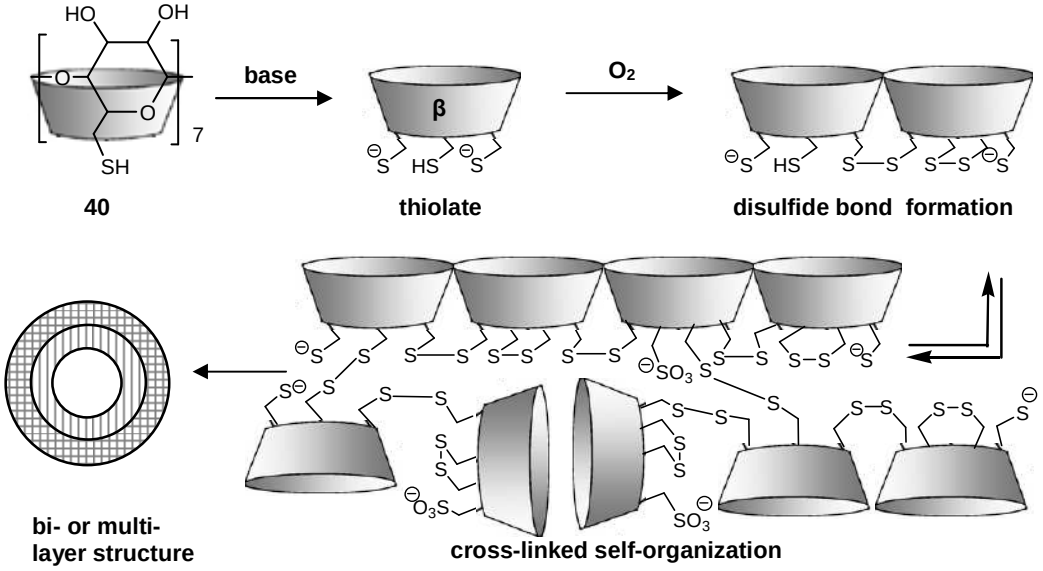


Figure 16 Deprotonation of **40** followed by air oxidation leads to the formation of water-soluble capsules, cross-linked through inter- and intramolecular disulfide bonds.

This short overview of disulfide based molecular capsules demonstrates that these compounds could possess exceptional properties. Developing also cage-type anion receptors based on bis(cyclopeptides) may therefore be of high interest.

2.3 Coordination-driven self-assembly[134]

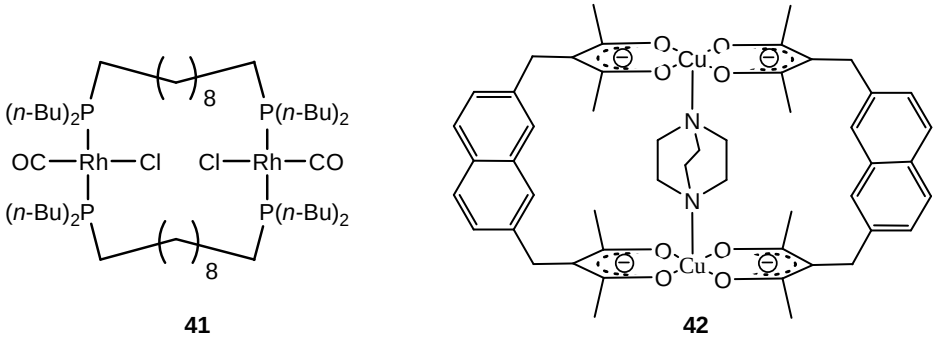
In nature, self-assembly is responsible for protein folding, quaternary structure of nucleic acids, storage and transcription of genetic information, arrangement of the capsid protein shells of viruses etc.[135] The synthetic use of self-assembly processes in supramolecular chemistry resulted in various innovative architectures such as rosettes,[136] racks, ladders and grids,[137] tweezers,[138] helices,[139] molecular containers,[134a, 140] rotaxanes and catenanes,[141] polygons and polyhedra,[142] metallodendrimers[143] and others. Self-assembly (SA) is a thermodynamically driven process of spontaneous organization of building blocks through reversible non-covalent or coordinative interactions into discrete and usually highly symmetric supramolecular structures.[144] Akin to dynamic covalent chemistry, reversibility is crucial to SA and likewise a change of the reaction parameters can shift the balance from one aggregate to another one possessing significantly different properties (Chapter 2.1, Figure 5d).[145]

Depending on the interactions involved between the constituents, SAs are divided into the following main types: (a) those that employ H-bonding motifs,[146] (b) those that use other types of non-covalent interactions such as ion-ion, ion-dipole, π-π stacking, cation-π, van-der-Waals, or hydrophobic contacts[40c, 147] and (c) those that utilize metal-ligand interactions.[145c, 148] A desired SA can be synthesized according to one of the three available main methods: symmetry-interaction approach (SIA),[137b, 149] weak-link approach (WLA),[150] and directional-bonding approach (DBA). Although all processes proceed under thermodynamic control, the latter has been extensively used in the past decade for the synthesis of multi-metallic architectures.

The DBA was chosen in this thesis to construct a trigonal-prismatic cage via self-assembly. In the following section, main features of metal-directed self-assembly with regard to DBA and some key aspects affecting the outcome of a metal-ligand exchange reaction are summarized.

2.3.1 Metal-ligand coordination

The invention of macrocyclic metalla-complex **41** described by Pryde, Shaw and Weeks is considered to represent the origin of the field of metal-coordinated self-assemblies (Scheme 16). Almost ten years later Maverick and Klavetter reported on the first self-assembled cyclic metal-receptor **42**, which preferentially forms inclusion complexes with DABCO (diazabicyclo-[2,2,2]octane) instead with other available nitrogen-containing guests.[151] Since then, a vast majority of architectures have been constructed.



41 **42**

Scheme 16 Dinuclear rhodium(I) complex **41** of Weeks and a copper-chelated macrocyclic receptor **42** reported by Maverick.

Invented by Verkade,[152] DBA, also termed molecular library model, takes advantage of the ability of metal complexes containing both weakly coordinated and blocking ligands to exchange the former with rigid multitopic ligand(s) during SA (Figure 17).[145d, 152-153] Properly protected metal ions are usually operative to control the number of coordinating sites and the directionality of the organic ligands. The presence of blocking ligands favors cage formation with respect to alternative open species. In some cases, when the metal precursor contains weakly coordinating anions to allow for exchange with the incoming organic ligands, these anions may act as templates for the assembly of the optimal receptor from the equilibrium mixture.[42, 154] Of particular interest for DBA are transition-metal-based systems, as they afford predictable geometries and contain multiple binding sites due to higher coordination numbers. Once the most stable species is formed it is unlikely to undergo further structural changes.[155]

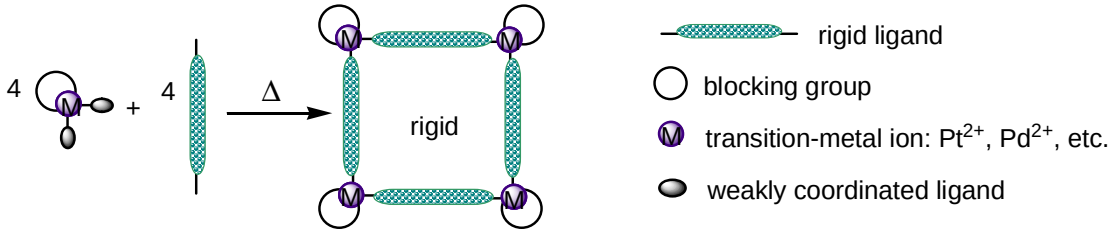


Figure 17 Schematic representation of the directional-bonding approach (DBA) towards formation of metal-coordinated SA. Charges and counterions are omitted for clarity.

Crucial for DBA is the metal-ligand exchange reaction, which is also the central reaction for the construction of metal-coordinated cages. It consists in substitution of one or more ligands coordinatively interacting with a central metal atom from a given complex by different ligands (Scheme 17). The mechanism of the reaction varies, depending on both the metal ions and the coordinated ligands. In a classical example, the anticancer agent *cisplatin* (*cis*-diamminodichlorido platinum(II)) exchanges its ligands via an associative process, where the

incoming ligand coordinates first as a fifth ligand, after which one of the original ligands dissociates. Octahedral Ru(II) complexes, on the other hand, tend to lose a ligand before a new ligand associates.[156]

$$[M(L^1)_n]^{m+} + nL^2 \rightleftharpoons [M(L^2)_n]^{m+} + nL^1$$

Scheme 17 Metal-ligand exchange reaction (M = metal atom, L^1 and L^2 = multidentate ligands).

Metal-ligand SAs contain relatively strong bonds between the metal centers and the ligands characterized with bond energies ranging between strong covalent bonds (ca. 60 - 120 kcal / mol) and weaker electrostatic interactions (π-π-stacking and hydrogen bonding ca. 0.5 - 10 kcal / mol). However, these connections are to some extend kinetically labile to allow for thermodynamic control of the self-assembling process in favor of discrete, well-ordered aggregates over oligomers or polymeric species. Thus, the synthetic efforts to obtain metal-ligand-directed SAs are relatively low, as synthesis of such assemblies starts from simple building blocks and uses one-pot procedures to obtain stable products in high yields.

The most commonly utilized organic ligands include nitrogen-containing heteroaryls,[148a, 157] followed by catecholates,[134a] hydroxamates, phosphorus-based ligands, and more rarely, cyano-substituted or other types of ligands.[158] Frequently employed metal building block units for the construction of positively charged matallocycles contain tetrahedral copper(I), octahedral iron(II), cobalt(II), nickel(II), and square planar palladium(II) or platinum(II) ions,[159] while coordination to zinc(II), ruthenium(II), molybdenum(II or IV), rhodium(I or II), rhenium(I), and iridium(I) ions was explored to produce predominantly neutral complexes.

The ultimate form of a self-assembled entity is determined by the symmetry, the number of binding sites, and the orientation of the Lewis-basic sites in the ligand. The design of polycyclic (polyhedra) and three-dimensional architectures requires building blocks of which at least one has three binding sites.[160] Dominant formation of a specific aggregate, out of a set of ordered and disordered supramolecules within a SA, can be achieved by control of the self-organization (SO) process, [148d, 161] by the use of highly symmetric building blocks, and/or by reducing the number of the involved subunits, for example by mixing only one metal-acceptor with one metal-donor. Most of the requirements discussed in Section 2.1 are relevant for cage SAs as well.

In particular, the outcome of metal-organonitrile or metal-pyridine coordination-based SAs leading to cage-type assemblies depends on a wide variety of factors as demonstrated by the groups of Fujita, Stang, Dalcanale and Hong. Generally, these factors can be divided into structural parameters of the donating ligand, geometrical constraints concerning the acceptor subunit, and external parameters like temperature, solvent, and the presence of a template.[91d, 162] Important is that a multidentate organic ligand is properly preorganized in order to serve as building block for the construction of complexes with enclosed cavities (see also Sections 2.3.2 and 2.3.3).

Changes in the acceptor subunit involving metal precursor, coordination angle and geometry, size of chelating ligand, and counterions may completely switch off the SA process. As the stability of a product relies on the strength of the metal-ligand interaction,[42] in the absence of other effects, the tendency of nitrogen-containing subunits correlates with the relative strength of the respective N-M dative bonds (M = Pt^{2+} > Pd^{2+} > Ni^{2+}).[159, 163] [164] A prerequisite for cage SA is the *cis*-coordination geometry of an acceptor component as possible, for example, in square-planar complexes. The major driving force to direct the SA to a cage is a coordination angle between the "capping" ligands (L-M-L) at the metal center. A final problem to be faced is the characterization of the resulting products or product mixtures. X-ray crystal structure determination turned out to be the most useful method for characterization if products can be isolated and crystallized, although there is always the problem of structural differences in solution and in the solid state.

With respect to the main objective of this thesis, the following section will focus on cage SAs based on metal-pyridine and metal-organonitrile coordination. Their properties and ability to bind or encapsulate mono- and poly-atomic species will be discussed. Indefinite aggregates or systems that were not designed to act as receptors such as micelles, liquid crystals, monolayers, and solid state assemblies will not be considered.

2.3.2 Molecular capsules for anions based on metal-pyridine coordination

Coordination of a ligand to a metal center is one of the strongest possible interactions that can be used to target capsules assembled in aqueous solution. Thus, a large number of cage-type hosts of diverse size, shape and dimensionality for the entrapment of anions have been synthesized that usually possess high symmetry.[2a, 165] A major portion of these receptors have been generated employing platinum(II) or palladium(II)-pyridine interactions as demonstrated by Stang, Fujita, Shionoya, and others.[145c, 145d, 145g, 148g, 153b]

An early example of the use of "induced-fit" molecular recognition[62, 166] in water was reported by Fujita. Trigonal prism **43** possessing D_{3h} symmetry was assembled from tris-(4-pyridylmethyl)benzene and ethylenediamine capped palladium ions in the presence of neutral or anionic guests containing both bulky hydrophobic groups and a carboxylate group (Figure 18).[167] The highest yield of cage **43** (> 90%) was obtained with 1-adamantanecarboxylic acid or 2-phenylpropionic acid as templates of which one molecule can be entrapped within the cage.

A system similar to molecular gyroscopes[168] was constructed by Shionoya et al. to bind guests like BF_4^- anions by coulombic interactions.[169] The 2 nm large, ball-shaped cage **44** forms spontaneously and quantitatively upon heating of a rigid bidentate norbornene ligand with palladium(II) ions in polar solvents. It was found that the two BF_4^- ions occupying the hollow space can be exchanged through the four large portals with guest molecules like 1,1'-ferrocene

bis(sulfonate) possessing similar distance between the anionic centers as the two included BF_4^- ions.

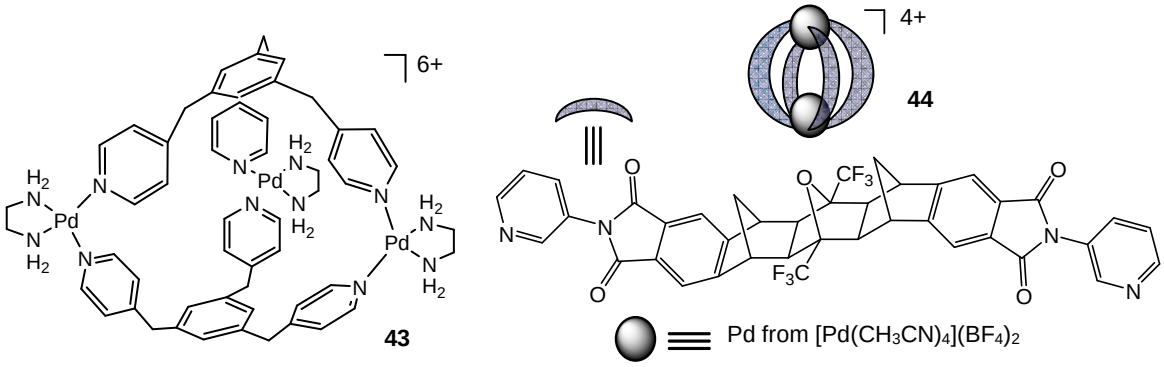


Figure 18 Schematic representations of Fujita's trigonal prism **43** and Shionoya's ball-shaped cage **44**.

Targeting inclusion of hydrophilic anions, the group of Custelcean synthesized a tetrahedral cage $[Ni_4(\mathbf{45})_6]^{6+}$ that strongly interacts with a sulfate anion. Sulfate affinity is as strong as that of the sulfate binding protein (SBP)[170] in aqueous solution (K_{app} $[Ni_4(\mathbf{45})_6]^{6+}$ = 6 x 10^6 M^{-1} versus K_a (SBP) = 8.3 x 10^6 M^{-1}) (Figure 19).[171] Binding is due to hydrogen bond formation between the NH moieties of the urea groups and the included sulfate anion as well as Coulomb attractions. Earlier, the same group reported on tris(2-aminoethyl)amine based receptors that saturated the coordination number of sulfate through encapsulation inducing the formation of a 1D silver-organic framework (MOF).[172]

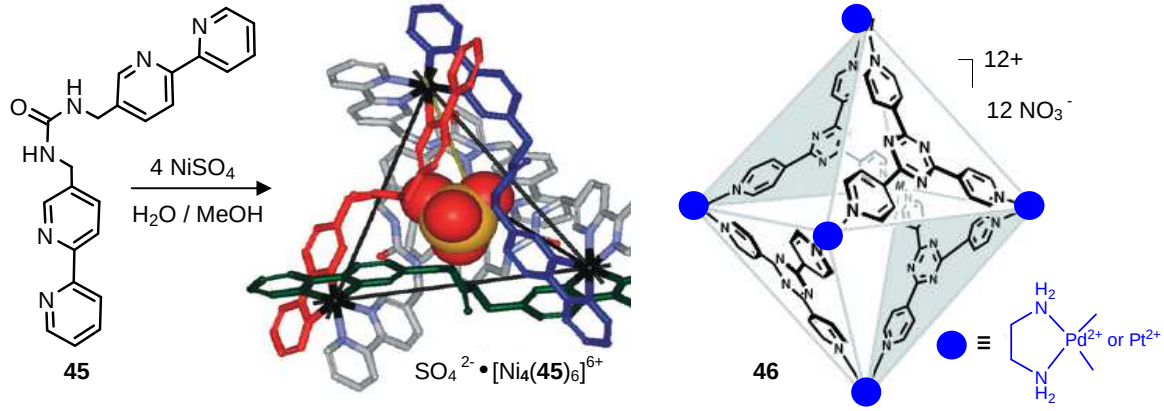


Figure 19 Urea functionalized ligand **45** with the X-ray crystal structure of encapsulated SO_4^{2-}•$[Ni_4(\mathbf{45})_6]^{6+}$. Octahedral cage **46** and its building blocks.

Other researchers, for example Raymond, Nitschke, Saalfrank, Ward, Albrecht etc., demonstrated that cage systems of the M_4L_6 type possess not only interesting inclusion properties but can also

be used in a number of applications including catalysis.[149h, 173] To extend the structural space accessible by the principles of the self-assembly, ligands have also been used that can reside on the faces of polyhedral cages while the metal centers constitute the vertices (molecular paneling).[145c] Thus, many nanometer-sized cages of different shapes (octahedral, cubic, cuboctahedral etc.) possessing large inner spaces were generated. Such aggregates, like the octahedral cage **46**, not only recognize and encapsulate more than one substrate, but also often exhibit interesting functions. They can, for example, act as storage devices, stabilize reactive molecules and intermediates, serve as reaction chambers to mediate regio- or stereoselective reactions, interact with biologically relevant compounds, or act as catalysts.

The group of Fujita used a template assisted combinatorial approach to generate in one step a receptor for trichloroacetate.[126e] Addition of this substrate to a library comprising tripyridyl and dipyridyl ligands gave a single product, namely the palladium(II)-linked capsule **47** (Figure 20). Also the addition of neutral CBr_4 as guest selected predominantly this host.

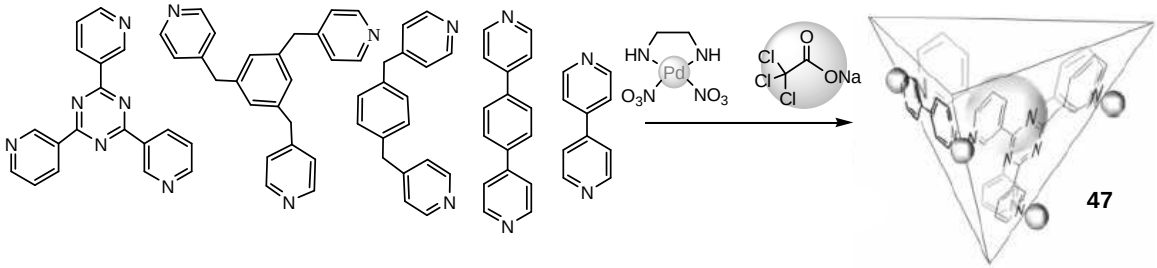


Figure 20 Assembly of trichloro-acetate receptor **47** from a library of dipyridyl and tripyridyl ligands used as building blocks.

2.3.3 Molecular capsules for anions based on metal-organonitrile coordination

Bis- or oligo(nitrile) ligands have only rarely been employed for the assembly of metal-organic architectures because their interaction with metals is weaker than that between metals and pyridine ligands.[174] Initially, cyano-bidentate ligands and Pd or Pt(dppp)OTf_2 (dppp = 1,3-di(phenylphosphino)propane, OTf = $CF_3SO_3^-$) complexes were utilized for the construction of homo-,[175] heteronuclear,[174b, 176] and chiral[177] molecular squares, which were found to encapsulate solvent and/or water molecules in the hollow space. Since then, much attention has been devoted to study self-assembled supramolecular capsules that possess a defined nanocavity.[2a, 12e, 12f, 140a] It is expected that the shape and dimension of the cavity will control selectivity of binding or even direct reactions between two co-encapsulated guest molecules.

Custelcean, Moyer, and Hay utilized the strong hydrogen-binding ability of sulfate anions to template the formation of tren-containing capsules.[172] The aromatic unit of a tren(trisurea) ligand was functionalized in *meta* position with cyano groups and the resulting product **48a** was equilibrated with Ag_2SO_4 to obtain a three-dimensional metal-organic framework.[172] An X-Ray

crystallographic analysis confirmed the existence of cage-like structures in the solid state, each of which contains a single sulfate anion between two ligands bound via 12 hydrogen bonds.[178] Changing from cyanyl to pyridyl chelating groups yielded monomer **48b** that turned out to permanently entrap the guest upon cage-self-assembly induced with metal sulfate salts (Zn^{2+}, Cd^{2+}, Co^{2+} and Mg^{2+}) (Figure 21).[172, 178] Coordination polymer (**48a**)$_2$ is extremely selective for sulfate anions, a feature that could be useful for the clean-up of nuclear waste.

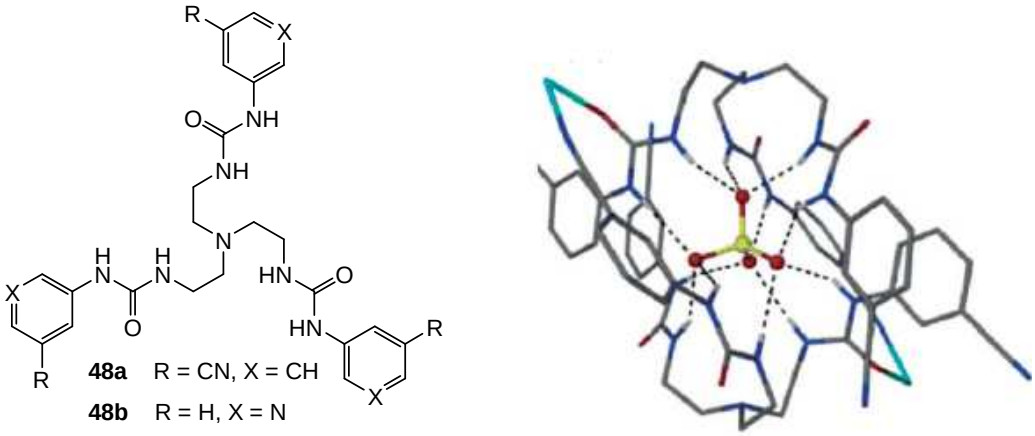


Figure 21 Structure of a tren-based monomer **48**, and crystal structure of (**48a**)$_2$ with a sulfate anion encapsulated and bound via 12 hydrogen bonds.

Many supramolecular capsules are composed of functionalized C_{4V} symmetric cavitands due to their conformational rigidity and the ease of synthetic accessibility.[12f, 146f, 146g, 147c, 148b, 148e, 179] Functional groups have been introduced both on the exterior and in some cases also in the interior of the cavitand molecules.[165b]

Incorporation of suitable donor groups at the upper rim of resorcinarene-based cavitands allowed the group of Dalcanale to extensively study the potential of these compounds for the construction of dimeric capsules. Besides some doubly-[180] and triply-[42] linked metalla-cages created as reference structures, the majority of these cavitand-based assemblies involved tetrasubstituted resorcinarenes.[179a, 181] In their first communication, Jacopozzi and Dalcanale achieved quantitative self-assembly of palladium(II)- and platinum(II)-containing cages from a 1:2 ratio of tetracyano cavitand **49** and square-planar complexes of M(dppp)(OTf)$_2$ (M = Pd^{2+} (**50a**) or Pt^{2+} (**50b**)) at room temperature (Figure 22).[174c] Cages **51a-d** formed immediately as the only product in various solvents and turned out to be extremely stable.[182] Surprisingly, one triflate ($CF_3SO_3^-$) anion was found to be encapsulated within the tetragonal prismatic cavity of dimers of **51**. The guest could only escape after dissociation of the cage induced by the addition of a competing ligand (*e.g.* NEt_3). Substitution of all nitrile groups in tetracyano cavitand **51a** with 4-benzonitriles gave rise to a cage with larger portals that allows release of the included triflate and fast exchange with external anions.[183]

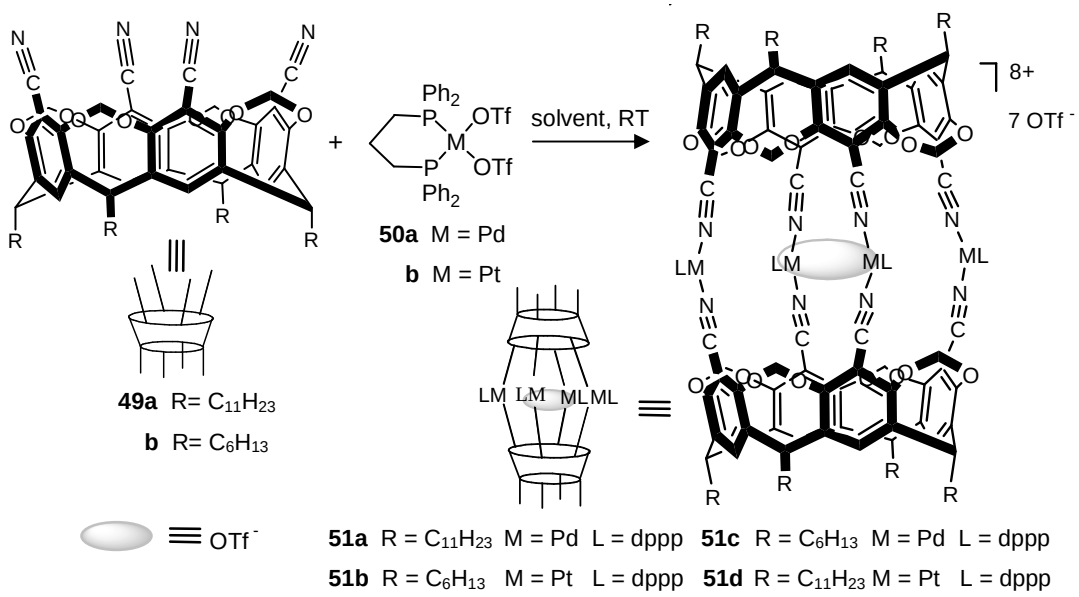


Figure 22 Synthesis of cavitand-based hosts **51** described by Dalcanale.

In a subsequent paper, analogous bis-tetracyano cavitands were synthesized and their ability to self-assemble while changing a) the Ph_2P-M-PPh_2 angle within the chelating ligand, b) the weakly coordinating counterions, and c) the preorganization of the tetradentate cavitand was systematically evaluated.[42] It was found that SA is favorable for methylene rather than for ethylene-bridged cavitands, with the best acceptor being the platinum(II) complex with dppp blocking groups. When comparing the selectivity of methylene-bridged cavitands **51a** and **51d** towards inclusion of monovalent anions, the size and the shape of the cavity as well as the free energy of guest desolvation were shown to be crucial for the encapsulation pattern ((BF_4^-•solvent) > $CF_3SO_3^-$ >> PF_6^-).

Formation of hetero-cavitand cages based on metal-ligand interactions was also reported.[148c, 148e, 148f, 179a, 182, 184] Dalcanale, Reinhoudt, and co-workers introduced alkyl chains of various lengths at the lower rim of cavitand monomers to achieve SA of hetero-cavitand cages first in solution and subsequently on gold surfaces. In chloroform solution, reaction between cavitand **52** and Pd-cage **51a** afforded Pd-homocage **53** and Pd-heterocage **54**, while a mixture of Pt-cage **51b** and cavitand **52** gave negligible ligand exchange as expected from the kinetic inertness of the underlying metal-ligand interaction (Figure 23). In further experiments, hetero-cages of type **54** (R' = C_6H_{13}) adsorbed on a gold surface were found to be very stable decomposing only upon addition of triethylamine.[185] This feature makes the immobilized cages promising candidates for the preparation of nanoscale electronic devices. Further studies on cavitand-based hetero- and heteronuclear cages have been reported by Kobayashi et al.,[186] and Dalcanale and co-workers.[187]

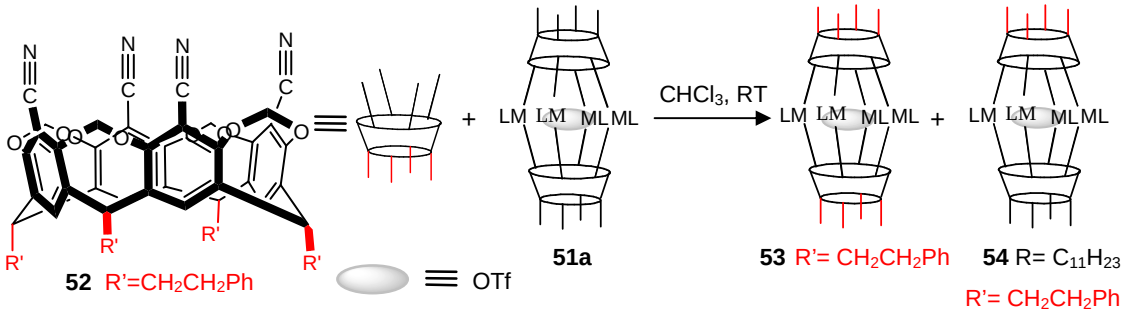


Figure 23 Reaction between cavitand **52** and triflate complex of **51a** gave homo and hetero complexes **53** and **54** (M = Pd^{2+}, L = dppp), respectively as a result of ligand exchange. The counterions ($7OTf^-$) and the positive charges (8+) of the complexes are omitted for clarity.

Also other transition metal centers (*e.g.* iron(II), cobalt(II), rhodium(II), and molybdenum(II)) were utilized together with organonitrile building blocks to obtain SAs with cage structures. A tetrahedral cage was, for example, obtained by the group of Huttnert through the combination of six-coordinated iron tridentate units and fumaronitriles.[194] Experiments revealed the encapsulation of a tetrafluoroborate anion in the cavity of the isolated product **55** (Figure 24). It is assumed that not the rigidity of the nitrile containing subunits but the templating effect of the BF_4^- anions is responsible for the organization of altogether 15 components.

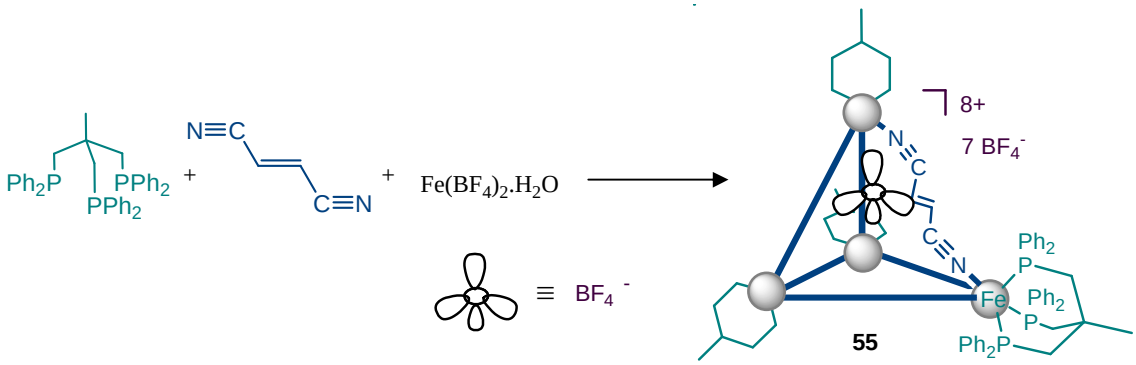


Figure 24 Self-assembly of Fe^{2+}-fumaronitrile tetrahedron **55** described by Huttnert.

To generalize, the principles outlined in Section 2.3 may alter the behavior and functions of metal-organic SAs but are not the only factors one must take into account when setting up an assembly. Nucleation by guests, solvation effects, entropic effects emerging from the presence of solvent molecules in the cage itself or adsorption on a solid phase are further aspects that make the challenge to design self-assembly processes even greater.

2.3.4 Former synthetic strategies toward bis(cyclopeptide) receptors

The usefulness of dynamic chemistry to prepare anion receptors from properly functionalized cyclopeptides has already been demonstrated by the group of Kubik. This methodology was employed because synthesis of such cage compounds is not trivial and is presumably very difficult if possible by using standard synthetic strategies involving the stepwise formation of bonds between appropriate precursors. Specifically, singly- and doubly-linked bis(cyclopeptides) have already been prepared by using disulfide exchange.[188] Even if not isolated as an analytically pure compound, formation of a bis(cyclopeptide) connected via three bridging units has also been achieved by means of olefin metathesis under thermodynamic control.[189]

Earlier work on such receptors revealed the effects that subtle structural changes within the peptide backbone have on host selectivity and anion coordination ability. In the parent cyclopeptide **56**, composed of alternating L-proline and 6-aminopicolinic acid subunits, the tertiary amide groups adopt the *cis*-conformation and all hydrogen atoms from the NH groups are oriented on the same side of the molecule (Figure 25). This overall cyclopeptide conformation turned out to be essential for anion recognition.[190] In aqueous solution, two of the peptide rings self-assemble in the presence of inorganic anions. The anion, located in the center of a hydrophilic cavity between the two cyclopeptide rings, is isolated from the bulk solvent and forms six hydrogen bonds with the amide NH hydrogen atoms.[191] The presence of the anion in the cavity causes deshielding of cyclopeptide protons located in close proximity, namely amide NH and proline H(α) protons, whose signals experience a downfield shift in a ^{1}H-NMR spectrum. Usually, signals of NH protons are not observed when the spectra are recorded in protic solvents and the downfield shift of the proline H(α) signals is therefore diagnostic for anion complexation. Stability of these sandwich-like complexes in aqueous solvent mixtures is not only due to the direct cyclopeptide anion interactions. In addition, hydrophobic interactions between the proline rings of the two cyclopeptide units also contribute to complex stability.[130b, 191] In DMSO, where these hydrophobic interactions are absent, only complexes with a 1:1 stoichiometry are observed.

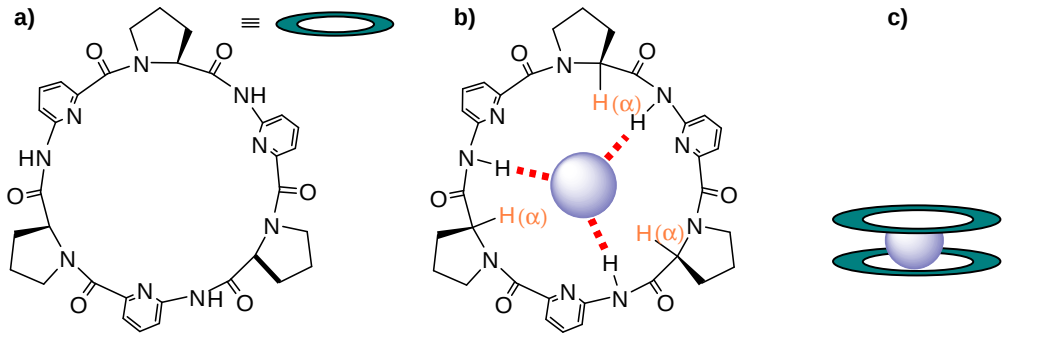


Figure 25 (a) Schematic representation of cyclopeptide **56**, (b) binding motif between **56** and an anion (in blue) and (c) 2:1 sandwich-type complex. Hydrogen bond formation between the guest and amide protons is marked in red and the proline H(α) protons in direct proximity of the anion are shown in orange.

Replacement of the 6-aminopicolinic acid with 3-aminobenzoic acid in compound **56** resulted in cyclopeptides with all amide groups adopting the *trans*-conformation.[192] Although these peptides were also shown to interact with anions, they do not bind anionic substrates in aqueous solution showing how important the correct overall conformation of **56** is for the binding properties.

Also, substituents in the γ-position of the proline rings and the configuration of this stereogenic center have a pronounced effect on binding properties. Host **57a**, which contains 4*R*-hydroxy proline units instead of proline subunits, had the advantage of improved water solubility (Figure 26).[193] This cyclopeptide is, however, unable to form sandwich-type complexes in aqueous solution with anions, presumably because of the higher polarity of the proline rings, which reduced the effectiveness of hydrophobic interactions. Cyclopeptide **57a** only forms 1:1 complexes with anions such as sulfate or halide which are moderately stable (log K_a = 1-2 in 80 % D_2O/CD_3OD).[193] The all 4*S*-configured analog **57b** is even less suitable for anion coordination, as the three proline amides adopt the *trans*-conformation in solution.

In search for more efficient anion binders, the group of Kubik also investigated the effects on anion affinity of the introduction of a linker between two units of cyclopeptide **56**. In this context, a family of singly-bridged bis(cyclopeptides) **59** (Figure 26) was developed from cyclopeptide **58**, some of which were designed by computational methods.[193-194] It was shown that introduction of appropriate linkers that do not prevent the cyclopeptide subunits from approaching each other allows formation of anion complexes with a 1:1 stoichiometry. These bis(cyclopeptides) possess an improved anion affinity with respect to $(\mathbf{56})_2$ because of the reduced entropic cost of complex formation. In addition, a systematic study on the influence of linker structure on anion affinity of bis(cyclopeptide) **59** was performed. The best receptor identified in this study contains a linker

with seven carbon atoms (log K_a (so_4) = 6 for **59a** in both 50 % D_2O/CD_3OD and 33 % D_2O/CD_3CN). Bis(cyclopeptide) **59b** containing a fluorescent linker was shown to sense the presence of sulfate in aqueous solvent mixtures by a change of fluorescence in the presence of an excess of competing anions such as chloride.[195]

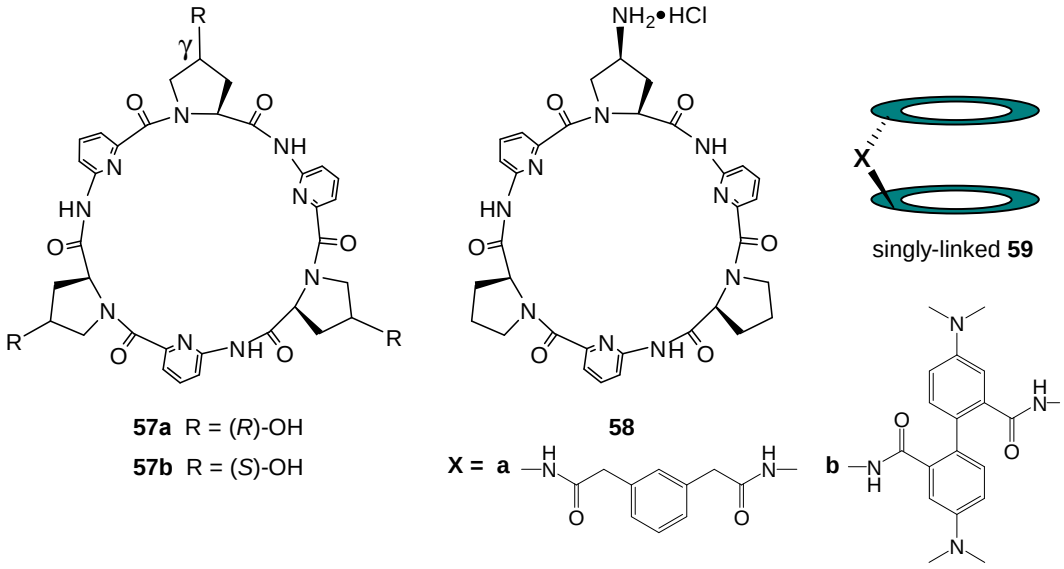
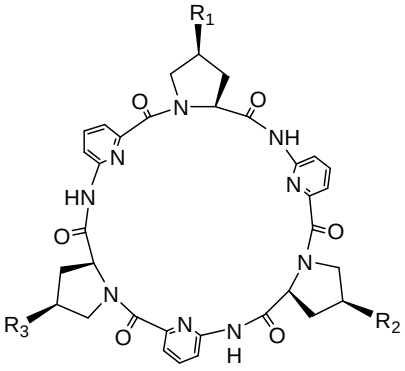


Figure 26 Structures of hydroxylated cyclopeptides **57** and (4*S*)-amino substituted precursor **58**. The latter was converted into various singly-linked receptors **59**.

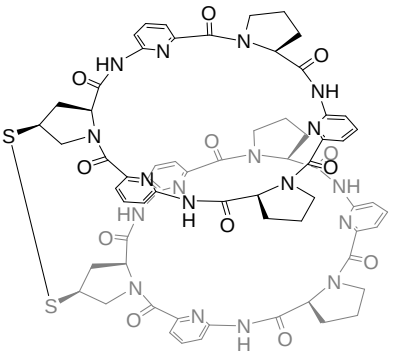
In another approach, dynamic combinatorial chemistry was used to optimize the linker between the subunits of the anion-binding bis(cyclopeptides).[130a] Either cyclopeptide thiol **60** with one 4*S*-configured thioproline unit or disulfide **62** were used as building blocks and mixed with different dithiols as potential linkers (Scheme 18). The hosts **63a-c** were identified as excellent anion receptors of which **63c** exhibits a one order of magnitude higher sulfate and iodide affinity than the computationally designed receptor **59a** (log K_a (so_4) = 7 for **63c** in 33 % D_2O/CD_3CN).[130b] Interestingly, receptor **63d** containing 3,5-dimercaptobenzoic acid as linker is able to coordinate sulfate in aqueous borate buffer (25 mM, pH 9) with an appreciable affinity log K_a (so_4) of 4.[188b] Similarly to **59a**, reinforced molecular recognition contributes to the high anion affinity of these receptors.[196]

Relying on the same synthetic strategy, also doubly-linked bis(cyclopeptides) **64** have been prepared from cyclopeptide dithiol **61** (Scheme 18). These receptors bind anions within their cavity in a similar 1:1 manner as the singly-linked analogs **63**. In contrast to the singly-linked bis(cyclopeptides) the nature of the spacer has a pronounced effect on anion binding of these compounds (Table 1, **63b** and **63c** vs. **64c** and **64d**).[64n, 188b] Incorporation of the second linker between the cyclopeptide subunits reduces the number of possible receptor conformations,

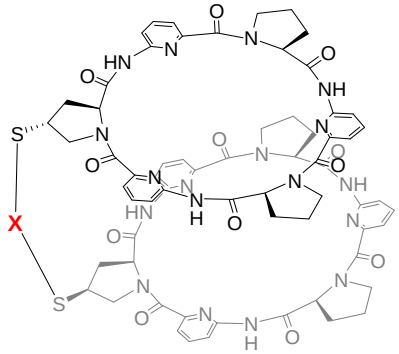
improving preorganization and thus producing better receptors towards oxyanions and halides. Such rigidification of the macrobicyclic structure of the bis(cyclopeptides) also enhances selectivity. Affinity of these receptors approaches the nanomolar range for tetrahedral anions such as sulfate and selenate. The highest stability constant was obtained for receptors **64b** containing two spacers derived from 1,2-ethanedithiol, which seem to allow for conformational adjustments within the system during complex formation (log K_a (SO_4) ≥ 8.67 for **64b** in 33 % D_2O/CD_3CN).[64n] Sulfate affinity of **64b** is ca. 12-times higher than that of receptors containing 1,3-benzenedithiol as linker (**64c**). Noteworthy is also the discriminative behavior of receptors **64b** and **64c** towards anions of similar sizes, charges, and geometries: **64b** binds sulfate 4 times stronger than selenate while selectivity of **64c** is even greater with a 10-times higher affinity for sulfate than for selenate. Thus, receptor **64b** is currently the most potent neutral sulfate host operating in aqueous mixtures and the second generation of bis(cyclopeptide)-derived receptors are the strongest anion binders of all synthetic receptors developed through DCC.



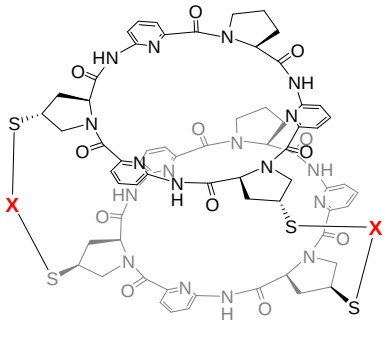
60 R_1 = SH, R_2 = R_3 = H
61 R_1 = R_2 = SH, R_3 = H



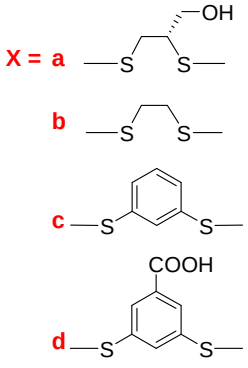
62



singly-linked **63**



doubly-linked **64**



linker

Scheme 18 Structures of singly- and doubly-linked bis(cyclopeptides) **63** and **64** that form 1:1 complexes with anions, and their precursors **60** (or disulfide **62**) and **61**, respectively.

	log K_a			
anion	**63b**	**64b**	**63c**	**64c**
sulfate	6.78	8.67	6.83	7.59
selenate	-	8.04	-	6.60
iodide	4.89	6.04	4.75	5.08

Table 1 ITC determined association constants for receptors **63b-c** and **64b-c** with Na_2SO_4, Na_2SeO_4 and KI in 33 % acetonitrile/water mixtures at 298 K. Binding studies for receptors **63b** and **63c** with Na_2SeO_4 were not performed.

How the overall degree of flexibility affects the binding properties of a bis(cyclopeptide) has also been demonstrated for a triply-linked cage (**65**) prepared by using the irreversible azide-alkyne 1,3-cycloaddition together with sulfate templation (Figure 27).[54] The click cage **65** captured sulfate between its two cyclopeptide rings with stability constants up to 10^5 - 10^6 M^{-1} in 1:1 H_2O/CH_3OH mixtures. Comparison of the sulfate affinity of triply-linked bis(cyclopeptide) **65** with the singly-linked analog **66** showed that binding affinity increased only by one order of magnitude upon introduction of the two additional linking units, however.[189] This result was attributed to the unfavorable endothermicity observed during sulfate binding of **65**. In contrast, sulfate binding of **66** is exothermic. Possible reasons for the enthalpically unfavorable sulfate binding of **65** are strained conformations of the linkers in the complex and high energetic costs of desolvation of the receptor during complex formation.

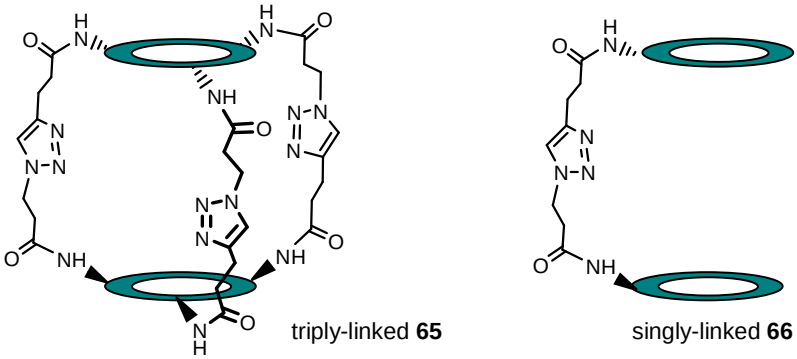


Figure 27 Triply-linked bis(cyclopeptide) **65** and its singly-linked analog **66** prepared by click chemistry.

Because of the fact that the thermodynamic templation delivered the most efficient anion receptors up to date (Sections 2.2.1-2.2.2 and Sections 2.3.2-2.3.3), I decided to use this strategy for the generation of novel triply-linked molecular cages based on analogous bis(cyclopeptides).

3. Objectives

The cyclic hexapeptide **56** containing alternating L-proline and 6-aminopicolinic acid groups that was developed in the Kubik group binds inorganic anions such as halides and sulfate in competitive aqueous solvent mixtures (Figure 28). Complex formation involves incorporation of the anions in the cavity between two cyclopeptide rings. By introducing a linker between the cyclopeptide rings, complex stoichiometry changed from 2:1 to 1:1 together with a significant improvement of anion affinity. Complex structure of the corresponding bis(cyclopeptides) is overall very similar to that of the parent cyclopeptide incorporating the anion between the two cyclopeptide rings (Figure 28, **I**).[190, 193] Increasing the number of linkers from one to two (host **II**) resulted in a further enhancement of affinity and selectivity for sulfate in comparison to host **I** in acetonitrile/water mixtures.[54, 130, 191, 194a, 195], [197] The nanomolar sulfate affinity exhibited by receptor **II** with the linkers **X** deriving from 1,2-ethanedithiol was ascribed to the optimal balance of spacer rigidity and flexibility.[64n]

While introduction of one or two linkers afforded receptors with sufficiently large entrance portals to allow fast binding and release of the anion it is to be expected that introduction of three linkers would give rise to carcerand-type receptors (Figure 28, structure **III**). Incorporation of a third linker between the cyclopeptide rings therefore represents the next consequent step in these studies that aims mainly at improving selectivity rather than affinity toward a substrate of interest.

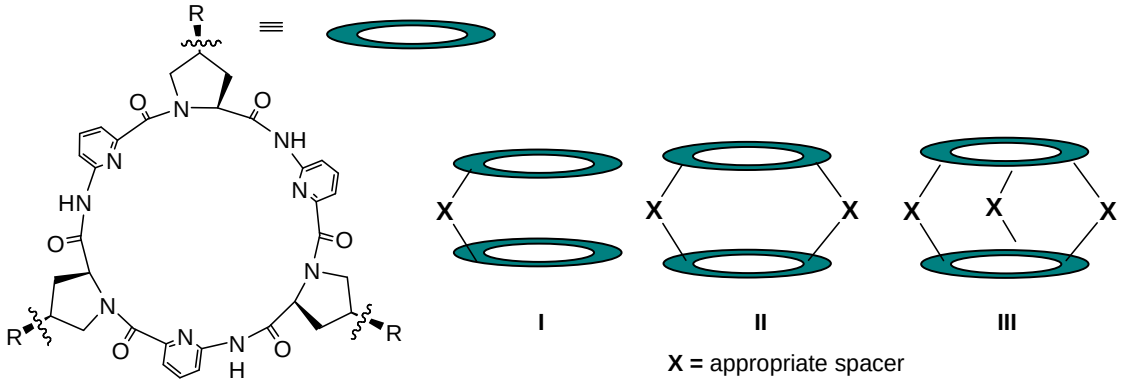
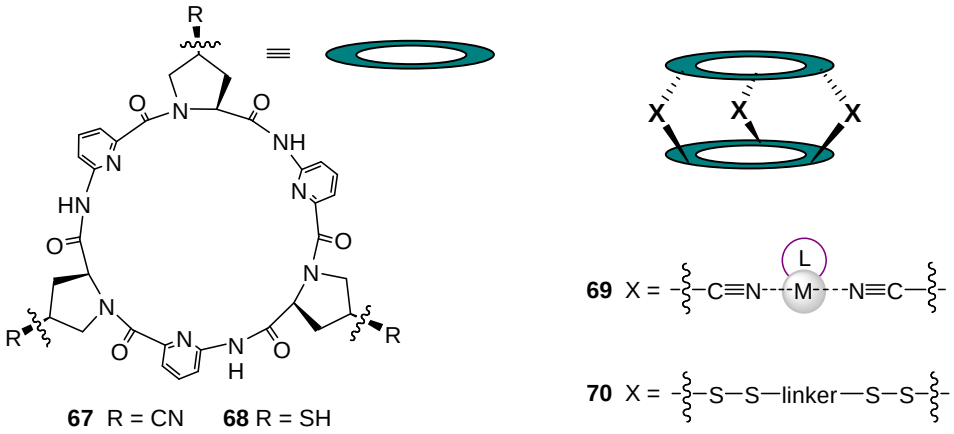


Figure 28 Structure of a cyclopeptide composed of alternating 6-amnopicolinic acid and L-proline subunits. General representations of singly-, doubly- and triply-linked bis(cyclopeptide) receptors are shown in cartoons **I**, **II**, and **III**.

With this goal in mind, my thesis involved the development of new molecular capsules derived from bis(cyclopeptides) containing three linkers between the cyclopeptide rings. Such complex architectures are most probably difficult to access by classical synthesis under kinetic control. Therefore, a synthetic approach was chosen that relies on dynamic chemistry under the template

effects of anions. Similar approaches have already afforded bis(cyclopeptides) of type **I** and **II**.[54, 130a, 198] The central idea was to prepare structurally related container molecules of type **III** for anion encapsulation by means of two different reversible reactions. Metal-ligand exchange reactions should be used for the construction of triply-linked coordination cage **69** starting from the cyclopeptide **67** (Figure 29). To this end, the new building block **67** bearing a cyano group in γ-position of each proline ring had to be synthesized. The potential of this cyclic precursor to self-assemble should be studied in the presence of appropriate metal complexes (ML). Resulting coordination cages should be studied with respect to anion encapsulation.



67 R = CN **68** R = SH

Figure 29 Structures of triply-linked metalla-cage **69** (M = metal, L = blocking ligand) and covalent capsule **70** (linker = aromatic or aliphatic unit) and their corresponding precursors **67** and **68**.

In addition, the preparation of a covalently constructed capsule was targeted. This cage **70** should be assembled by means of disulfide exchange using cyclopeptide trithiol **68** as building block, suitable dithiols as linkers, and inorganic anions as templates. In this context, identification of appropriate reaction conditions is necessary as well as identification of the best linkers. Subsequently, the products will be evaluated with respect to their anion binding properties. An interesting question in this context is whether covalently assembled capsules of type **III** are able to permanently entrap inorganic anions inside the cavity. If this is the case, a further objective would be to find appropriate conditions for guest release. If anion binding of capsules **III** turns out to be reversible, quantitative information about anion affinity and kinetics of complex formation should be obtained. Moreover, experiments will be carried to synthesize **III** in the presence of different dithiols as linkers to use the concept of dynamic combinatorial chemistry for receptor optimization.

4. Results and discussion

4.1 Triply-linked bis(cyclopeptide)-derived coordination cage

4.1.1 Building blocks for the coordination cage 69

The envisioned coordination cage **69** represents a trigonal prism, which is one of the simplest three-dimensional (3D) assemblies (Figure 30). Retrosynthetically, its assembly requires five building blocks, two appropriately functionalized cyclopeptide subunits (**67**) and three chelating subunits with easily exchangeable ligands such as Pd(dppp)OTf_2 (**50a**). The choice of the starting compounds was determined both by the straightforward accessibility of the Pd-ligands and by the experience in our group regarding the synthesis of substituted cyclohexapeptides. The goal was the preparation of cyclic peptide **67**, functionalized with three nitrile groups, to allow interaction with the chelating ligand. Cyclopeptide **67** consists of three identical dipeptide building blocks **71** containing 4*S*-configured cyanoproline subunits. The (*4S*)-configuration at the proline rings is necessary to allow for the arrangement of two cyclopeptide rings in a bis(cyclopeptide) required for anion complexation. Metal-ligand exchange reaction between **67** and *cis*-protected palladium(II) precursor **50a** mixed in a proper ratio should give coordinative cage **69** (Figure 30). In the course of self-assembly the palladium complex **50a** should liberate two triflate anions per complex and the palladium centers should coordinate to the nitrile groups. The triflate ions could act as templates shifting the equilibrium towards formation of the cage. Alternatively, other template anions could be added externally.

In analogy to the known preparation of similar mono-, di-, and trisubstituted cyclopeptides reported by the Kubik group, a related synthetic strategy was chosen for the preparation of **67**. It started with the construction of the dipeptide building block **71**. The reaction involves coupling of 6-aminopicolinic acid with a protected 4-cyanoproline derivative. Next, chain elongation proceeds between the aliphatic amine from the proline unit and the carboxylic acid group from another peptide to obtain the linear tetra- and hexapeptide. Finally, a cyclization reaction under high dilution affords the cyclic product. The reaction pathway for the synthesis of **67** is shown in Scheme 19.

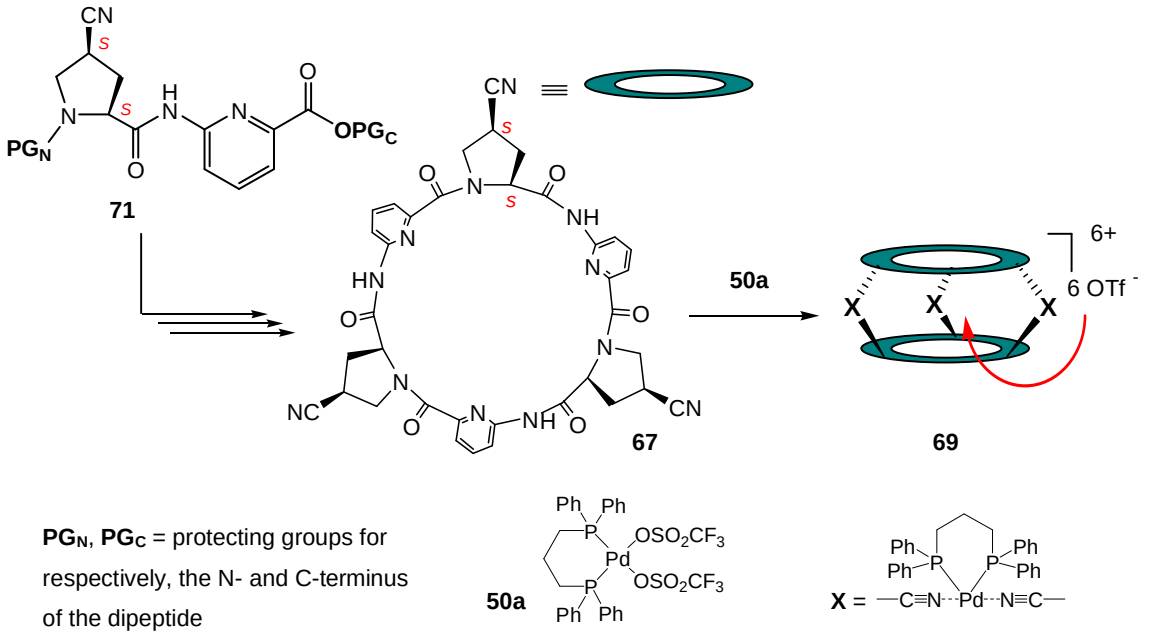
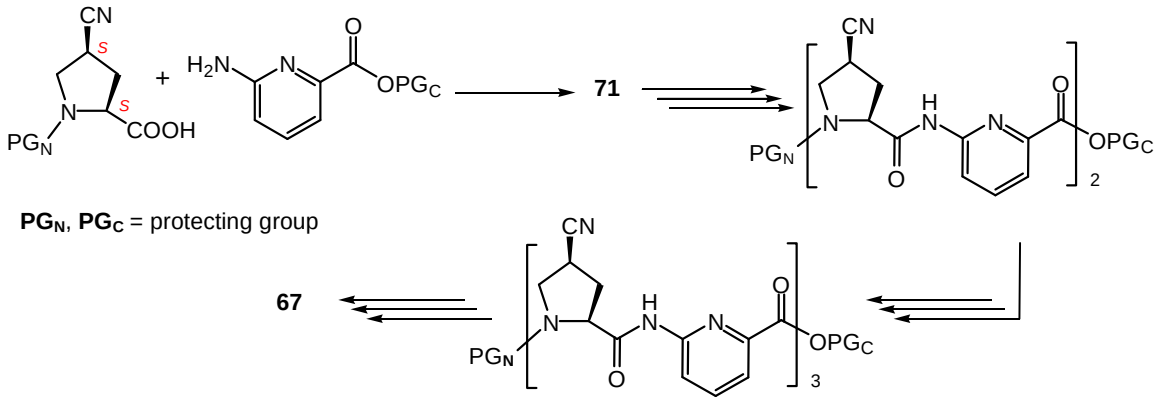


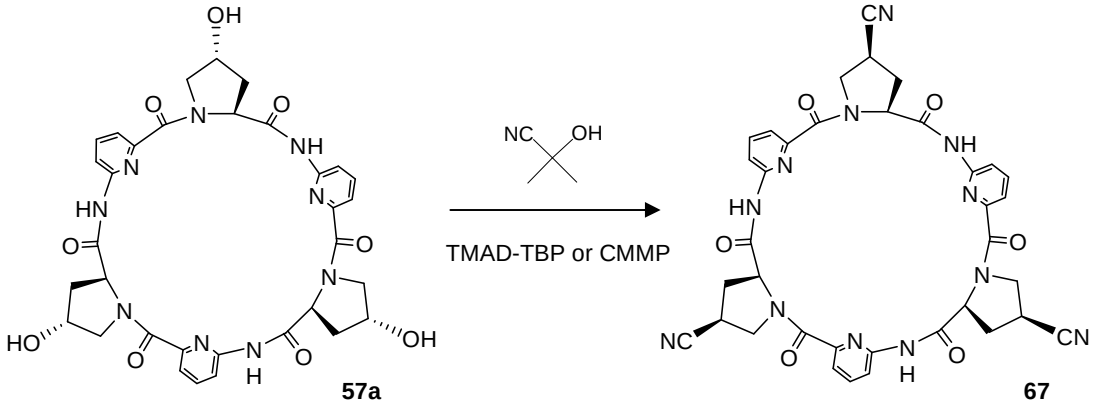
Figure 30 Suitably protected (*4S*)-4-cyanoproline-containing dipeptide **71** gives cyclopeptide **67** after chain elongation and cyclization. Then, the precursor **67** should self-assemble in the presence of *cis*-protected paladium(II) complex **50a**.



Scheme 19 General strategy for the preparation of cyclopeptide **67**.

Synthesis of **67** requires incorporation of a nitrile group at C(γ) atom of the proline ring. Introduction of substituents into functionalized cyclopeptides may take place as the final step after cyclization[130a] or at the beginning of the synthesis either at the stage of dipeptide[188a] or the proline derivative.[189, 199] Several cyclopeptides have been obtained along the first route. For example, derivatives of **66** containing one aminoproline or one thioproline subunit instead of an unsubstituted proline ring were prepared from a cyclopeptide containing a tosylated

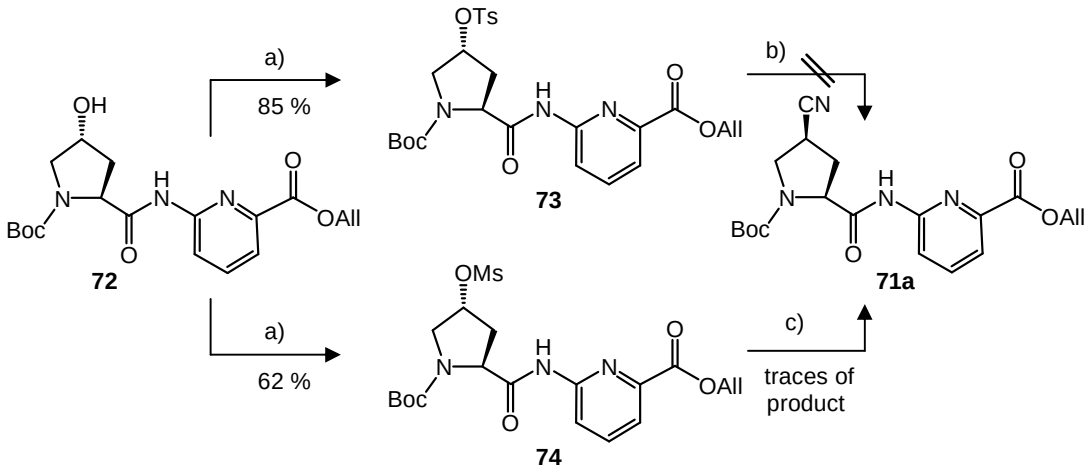
hydroxyproline subunit.[200] Following a similar route, **67** could be obtained from the known cyclic hexapeptide **57a** by converting the hydroxyl groups on the hydroxyproline units stereoselectively into nitrile groups by means of a Mitsunobu reaction (Scheme 20). This reaction requires the use of acetone cyanohydrin and the presence of azodicarboxamide and tributylphosphine (TMAD-TBP) or (cyanomethylene)trimethyl-phosphorane (CMMP) as mediator.[201] However, a major disadvantage concerns the fact that transformations of cyclopeptides are usually associated with formation of difficult to separate by-products and low yields especially in cases like here where more than one functional group has to be converted. Therefore this strategy was not pursued.



57a **67**

Scheme 20 Hexapeptide **57a** can potentially be transformed into **67** by using a Mitsunobu reaction and acetone cyanohydrin.

Previous work in the Kubik group demonstrated that substituents can be introduced into the proline subunits already at the stage of the dipeptide if these substituents are stable under the conditions required for the cleavage of protecting groups and peptide coupling conditions. Applying this strategy to the objectives of my project, preliminary experiments were conducted to prepare (4*S*)-4-cyanoproline-containing dipeptide **71a** by transformation of the tosylate (Ts) or mesylate (Ms) containing precursors **73** and **74**. Compounds **73** and **74** were synthesized from a dipeptide containing a hydroxyproline unit following well-known procedures (Scheme 21).[188, 199] Substitution of the leaving group by a nitrile group was tested by using different cyanide salts (Table 2). When tosylated dipeptide **73** was reacted with KCN or tetra-*n*-butylammonium cyanide (TBACN) in different amounts, no product formation was detected. The outcome of the reaction was not affected when the solvent was changed from DMSO to DMF. In contrast, heating the mesylated dipeptide **74** with an excess of KCN generated traces of product **71a**, accompanied with numerous unidentified impurities as detected by thin-layer chromatography (TLC) and MALDI-TOF MS. Although this approach is also not suitable for the synthesis of the desired product it indicated that the mesyl group might be better suited for the preparation of 4-cyanoproline derivatives than the tosylate group.



Scheme 21 Attempts towards synthesis of **71a** by using the tosylated or mesylated dipeptides **73** and **74**, respectively. Reaction conditions a) TsCl or MsCl, pyridine, CH_2Cl_2; b) see Table 2; c) 1.5 equiv. KCN, DMSO, 55 °C, 20 h.

reagent	equiv.	solvent	temperature / °c	stirring time / h
KCN	1.0	*dry* DMSO	55	20
	1.5			
TBACN	1.5	*dry* DMF		
	2.0			
	10.0			

Table 2 Reaction conditions tested to transform tosylate **73** into nitrile **71a**.

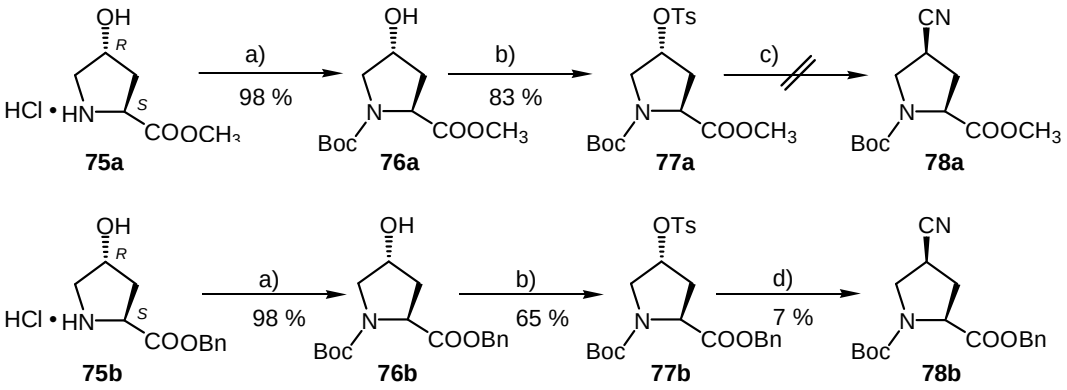
Further experiments in this context revealed that a better synthetic route toward dipeptide **71** involves introduction of the nitrile group at the step of the proline derivative. This route eventually afforded the central intermediate **71** in excellent yield. Aspects of this strategy to be considered are the type of leaving group that should be replaced with the nitrile group and the types of protecting groups. The commercially available L-hydroxyproline **75** should serve as a starting point (Scheme 23). Previous approaches reported in the literature for the synthesis of 4-cyanoproline derivatives are summarized in Scheme 22 and Table 3. Best yields of the desired product were obtained when tosyl esters were heated with NaCN in DMSO at 60 °C (Table 3, entries 5 and 2). On the other hand, transformation of mesylates gave lower but still useful yields and required shorter reaction times when TBACN in DMF was used (entry 3 *vs*. 4). The utilization of dry solvents was generally preferable. The main product was always that resulting from inversion of the configuration at C(γ) atom. However, one should take into account that there is a risk of side reactions, for example, elimination to the corresponding olefin as well as formation of a mixture of epimers at C(γ) and C(α) positions in particular at higher reaction temperatures (Scheme 22).

Scheme 22 Proline derivatives (see Table 3) and possible side products resulting from the introduction of a nitrile group at the C(γ)-position of the proline ring.

entry	R	R_1	R_2	reaction conditions	yield of (*4S*)-configured product / %
1[202]	Boc	$C(Ph)_2OMe$	OTs	KCN, 18-crown-6, DMSO, N_2, 80 °C, 24 h	31
2[203]	Boc	COOBn	OTs	NaCN, DMSO, 80 °C, 4 h	**58**
3[204]	Boc	COOEt	OMs	TBACN, *dry* DMF, 55 °C, 20 h	**52**
4[204]	Boc	COOEt	OMs	NaCN, *dry* DMF, 55 °C, 55 h	52
5[205]	Bn	COOEt	OTs	NaCN, *dry* DMSO, 60 °C, 4 h	**90**
6[205]	Bn	CH_2OTBS	OMs	NaCN, TBACN, *dry* DMSO, N_2, 70 °C, 24 h	38
7[206]	Cbz	COOMe	OTs	KCN, dibenzo 18-crown-6, CH_3CN, reflux, 44 h	33

Table 3 Proline derivatives and reagents used in the literature so far for the selective introduction of a nitrile group into the C(γ)-position of the proline ring.

The success of the synthesis under conditions shown in entries 2, 3, and 5 in Table 3 motivated me to initially test tosylates **77a** and **77b** for the preparation of 4-cyanoproline derivatives. These starting materials both contain Boc-groups for the protection of the nitrogen atom but differ in the protecting group on the carboxylate terminus. Compounds **77a** and **77b** were obtained as shown in Scheme 23 following described procedures.[188a, 207] A selection of tested conditions for the conversion of **77a** to the corresponding nitrile is summarized in Table 4. Progress of the substitution reaction was followed by TLC and MALDI-TOF MS. Neither treatment of **77a** with different amounts of NaCN /dry DMSO nor TBACN /dry DMF generated product **78a**. Employing the benzyl ester in combination with a tosyl group at the 4-position of hydroxyproline **75b** yielded 7 % of nitrile **78b** when the reaction was performed with 1.9 equiv. of NaCN in dry DMF at 90 °C for 72 h. An advantage of the benzyl esters is that, while the methyl (or ethyl) esters of Boc-protected proline derivatives are oils, many of the corresponding benzyl esters are crystalline compounds. It remained unclear, why the yield of 58 % for **78b** that is reported in the literature was not achieved.[203]



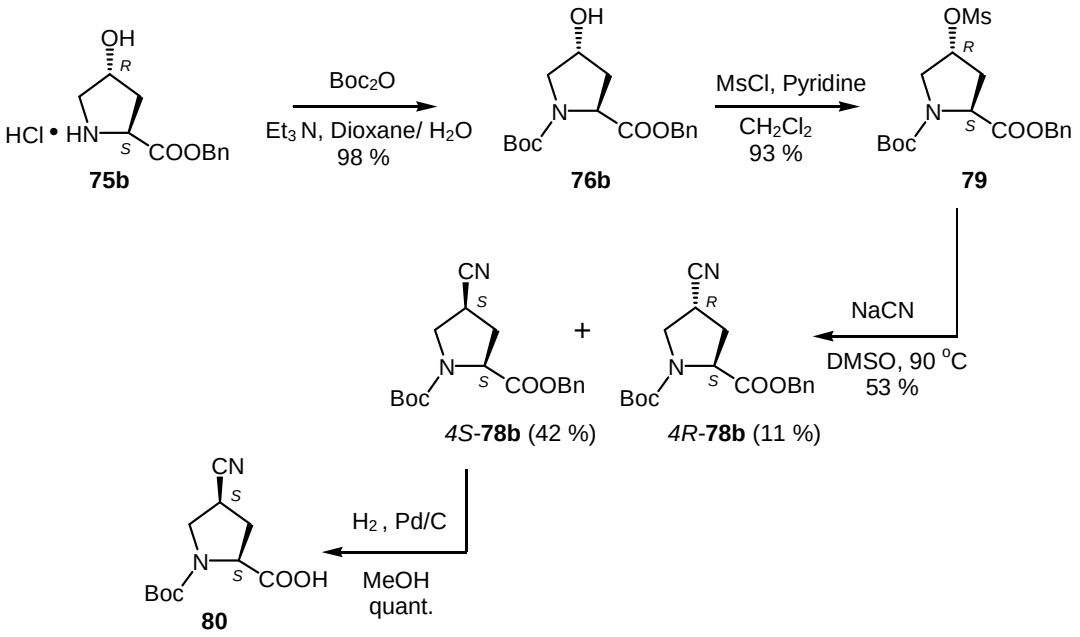
Scheme 23 Substrates and reagents used for the preparation of **78** from L-hydroxyproline **75**. Reaction conditions a) $(Boc)_2O$, Et_3N, 1,4-dioxane/ H_2O; b) TsCl, pyridine, CH_2Cl_2; c) see Table 4; d) 1.9 equiv. NaCN, dry DMF, 90 °C, 72 h.

reagent / equiv.		solvent	temperature / °c	stirring time / h
NaCN	1.9	*dry* DMSO	55	20
	10.0			
TBACN	1.5	*dry* DMF		
	1.9			
	10.0			

Table 4 Selection of tested reaction conditions for conversion of **77a** to cyanoproline **78a**.

Subsequently, Boc- and Bn-protected mesyl ester **79** was prepared and tested as starting material for the nitriles (Scheme 24). Mesyl esters were used because my experiments with dipeptide **71** indicated that they should be better suited than tosylates for this transformation. Conditions tested to convert **79** into the corresponding nitrile **78b** are summarized in Table 5.

Yields vary from 17 to 43 % depending on the cyanide salt used. The literature procedure involving the use of TBACN and heating at 55 °C proved to give the product **78b** in only 17 % yield much lower than the reported 52 %. If 1.5 equiv of NaCN or KCN were used approximately the same yields were obtained independently of the salt (33 % and 34 %, respectively). Use of 18-crown-6 together with 1.5 equiv. of KCN in dry DMSO increased the yield to 40 %. Nevertheless, the high cost of the crown ether additive renders this reaction less attractive. Similar yields of 42 – 43 % were reached by using 1.9 equiv. of NaCN in dry DMF or DMSO at 90 °C. Work-up of the product proved to be difficult when DMF was used and the reaction was therefore best performed in DMSO. Indeed, the results shown in Table 5 indicate that DMSO is generally the better solvent giving superior yields. The isolated yield of nitrile is also strongly dependent on the reaction time with best outcomes resulting after 3 days of stirring.



Scheme 24 Synthesis of **78b** and its subsequent benzyl deprotection.

reagent / equiv. / temperature	solvent	stirring time / h	inert atmosphere (N_2)	yield of main product **78b** / %
NaCN, 1.5, 90 °C	*dry* DMF	24	+	28
	DMSO	72	-	33
NaCN, 1.9, 90 °C	*dry* DMF	24	+	24
	DMF *	**72**	**-**	**43**
	DMSO	40	**-**	32
	DMSO *	**72**	**-**	**42**
NaCN, 2.0, 90 °C	*dry* DMF	24	+	38
KCN, 1.5, 80 °C	DMSO	36	**-**	29
	DMSO	72	**-**	34
	DMSO+*CE* **	**72**	**-**	**40**
KCN, 1.9, 90 °C	DMSO	65	**-**	23
TBACN, 1.9, 55 °C	*dry* DMF	24	**-**	17

Table 5 Tested reaction conditions for the conversion of mesylate **79** into nitrile **78b**. For reactions conducted under N_2, dry and degassed solvents were used (* *solvent was dried only over molecular sieves (4 Å); CE ** - addition of 18-crown-6 (1.5 equiv.)* [202, 208]

Synthesis of **80** involves a four step reaction pathway overall (Scheme 24). First, the (4*R*)-configured L-hydroxyproline **75b** was Boc-protected at the nitrogen atom. O-Activation of the hydroxyl group by treatment with MsCl resulted in the formation of mesylate **79** in 93 % yield after

chromatographic purification. Then, treatment with NaCN under the conditions mentioned above yielded *4S*-**78b** as the main product. In addition, the formation of a minor species was observed with a significantly different retention time in the TLC and HPLC analysis of the reaction mixture (Chart 1).[206] Both compounds were readily separable by flash chromatography. Some amount of the starting material (10 %) could also be recovered as described in the literature.[204] The major product *4S*-**78b** could additionally be purified by crystallization from *n*-pentane at 4 °C. This compound, isolated in yield of 42 %, represented the desired product *4S*-**78b** according to ^{1}H- and NOESY-NMR spectroscopy. The minor product obtained in a yield of 11 % is an isomer of **78b** according to MS. Based on the ^{1}H- and NOESY-NMR spectra, this compound was structurally assigned to represent the *4R*-epimer of **78b**, presumably formed as a result of the high temperatures used for the reaction.

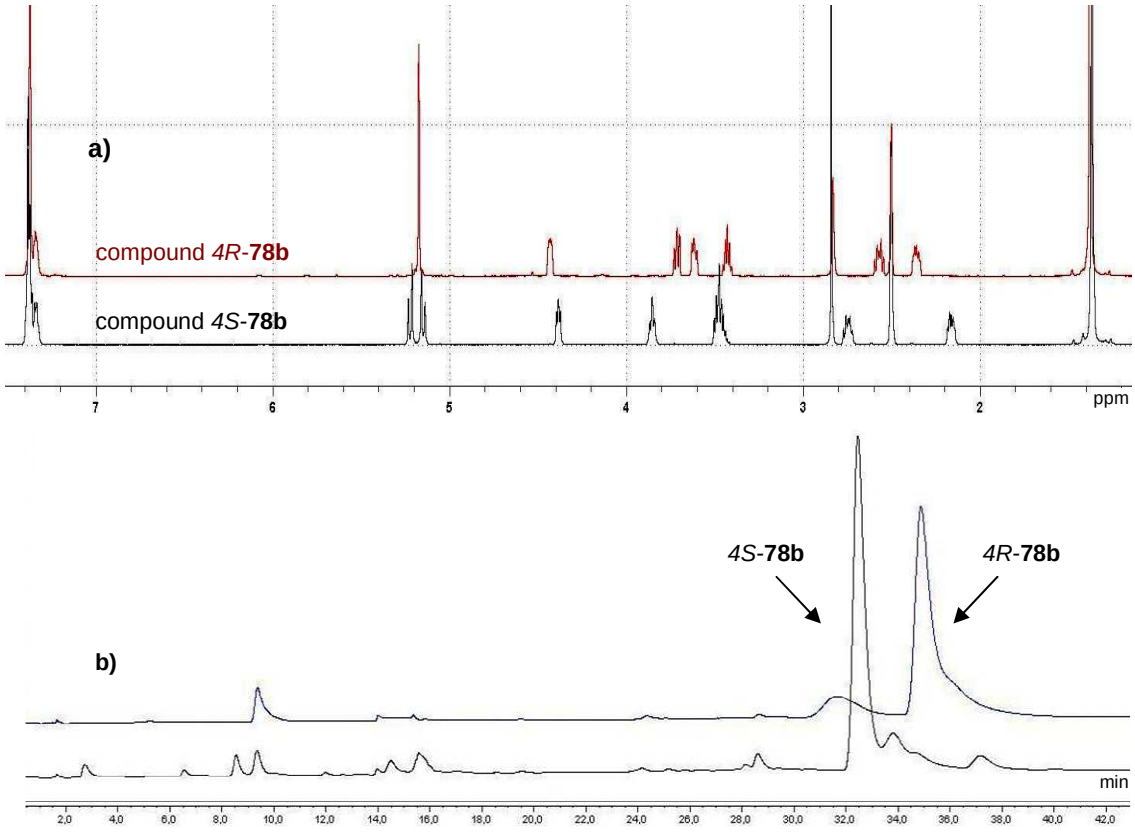
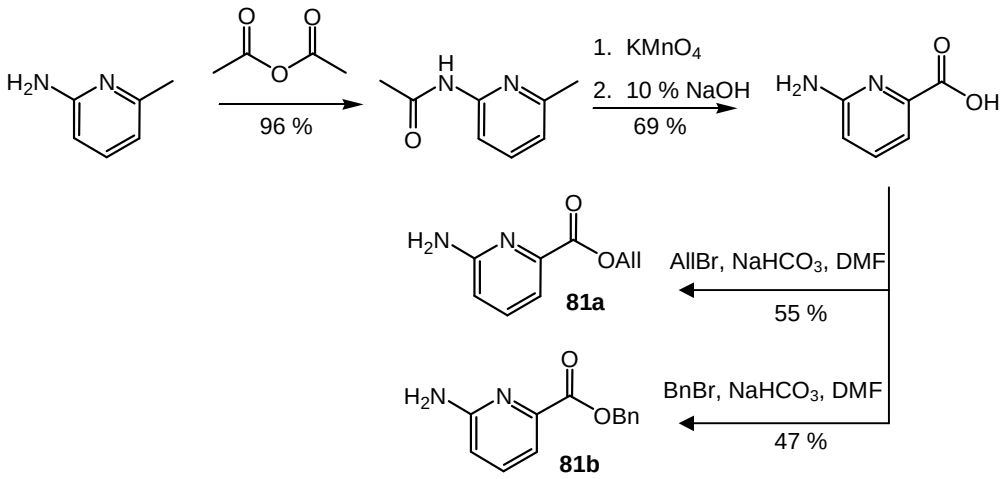


Chart 1 (a) ^{1}H-NMR spectra of main product *4S*-**78b** and its epimer *4R*-**78b** recorded in DMSO after chromatographic purification (400 MHz, 100 °C) and (b) HPLC-Chromatogramm of compounds *4S*-**78b** (Rt 32.5 min) and *4R*-**78b** (Rt 34.9 min) recorded before recrystallization at 254 nm, 25 °C in water/methanol as mobile phase.

In the final step of the synthesis, nitrile *4S*-**78b** was hydrogenolytically deprotected at the carboxylate group affording Boc-(*4S)*-4-cyanoproline **80** in quantitative yield. Attempts to

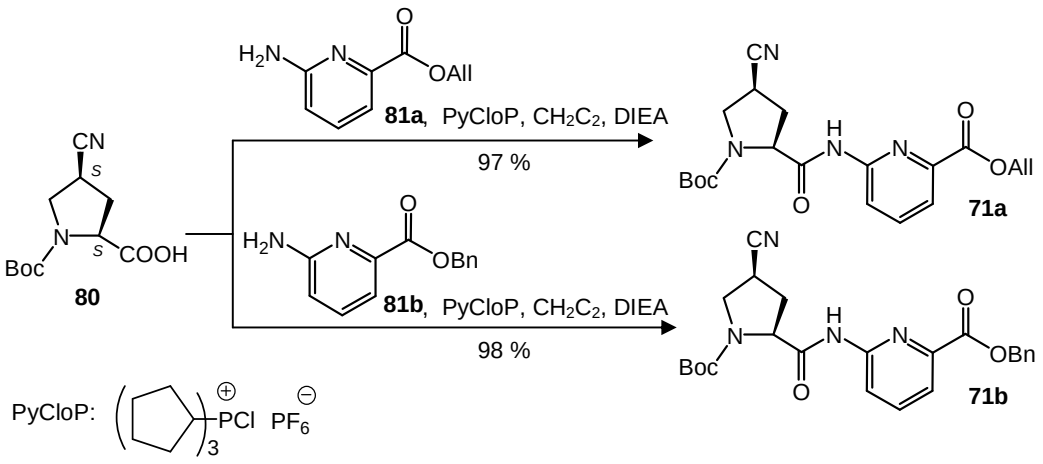
deprotect the benzyl group in 9:1 (v/v) solution of 1,4-dioxane/water required much longer reaction times and traces of educt were still detected after 4 days of stirring. The nitrile group was not affected under these conditions as clearly evidenced by the presence of an absorption band at 2248 cm^{-1} in the IR spectrum of **80**.

Compound **80** was then used as a building block for the preparation of dipeptide **71**, which requires coupling with 6-aminopicolinic acid benzyl or allyl ester (**81a** or **81b**). Preparation of the latter derivatives from 2-amino-6-methylpiridine proceeds in a known four-step reaction sequence outlined in Scheme 25.[199, 206, 209] Acetic anhydride was used to protect the free amino group in the starting material. The obtained acetamide was then oxidized to the corresponding acid with $KMnO_4$. Treatment of the product with NaOH, followed by reaction of the free amino acid with benzyl or allyl bromide afforded, respectively, allyl (**81a**) or benzyl 6-aminopicolinate (**81b**). In the initial procedure of the preparation of the esters, the heterogeneous mixture was stirred for 7 days at room temperature. I found that stirring only for 48 h followed by ultrasound irradiation caused a significant reduction of reaction time without compromising the reported yields.



Scheme 25 Synthesis of 6-aminopicolinic acid benzyl or allyl ester **81a** and **81b**.

Coupling of **80** to **81a** or **81b** to give the respective dipeptides **71a** and **71b** was performed by using standard procedures established in the group by means of PyCloP (chlorotripyrrolidino-phosphonium hexafluorophosphate) as coupling reagent (Scheme 26).

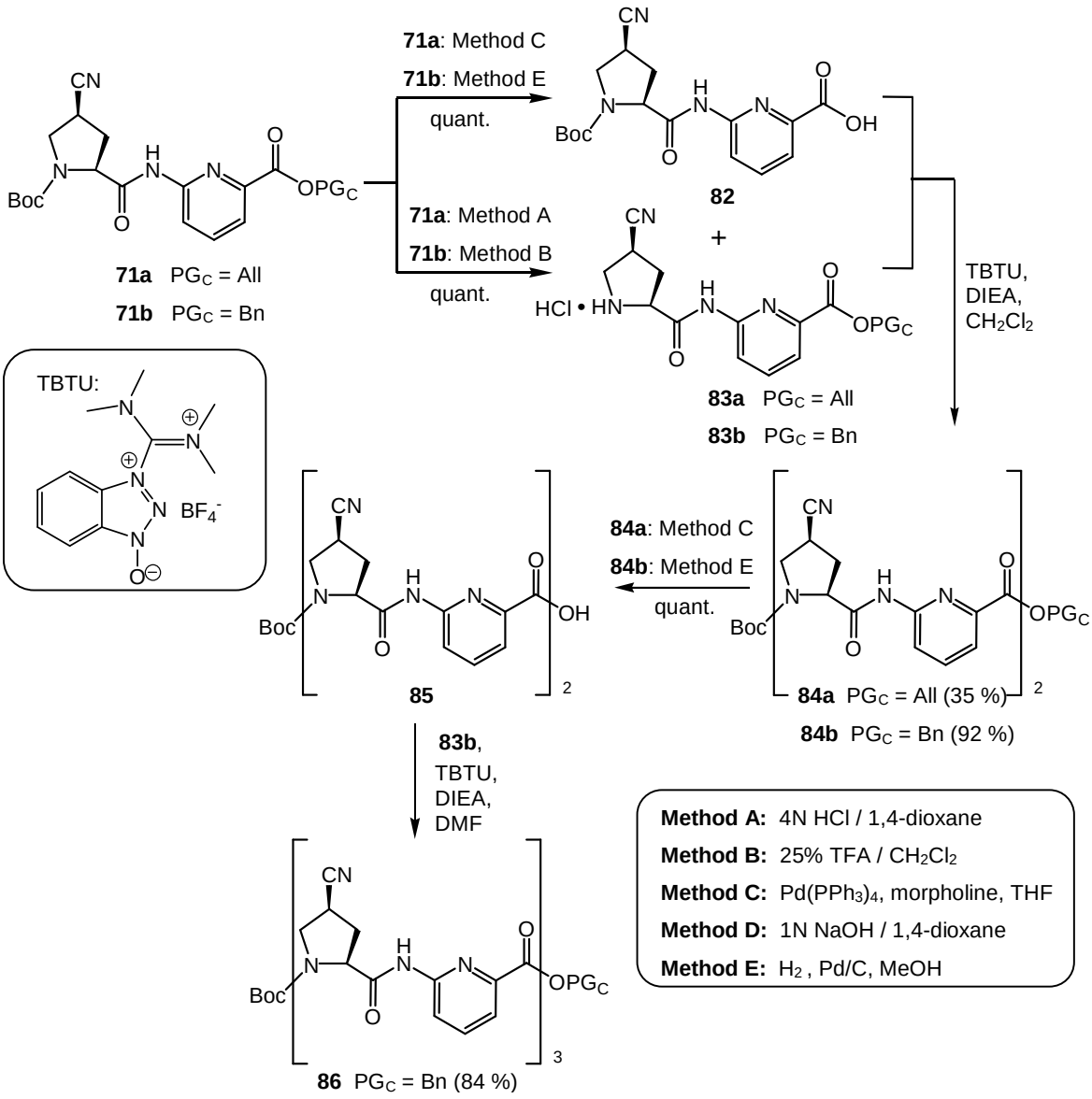


Scheme 26 Synthesis of dipeptides **71a** and **71b**.

4.1.2 Synthesis of cyclopeptide trinitrile 67

The synthetic pathways shown in Scheme 27 were envisioned for chain-elongation of dipeptides **74a** and **74b** to the corresponding linear hexapeptides.

Dipeptides **71a** and **71b** contain two orthogonal protecting groups that can be cleaved independently. The Boc-group can be cleaved under acidic conditions by using a solution of HCl in 1,4-dioxane or TFA in methylene chloride. The allyl ester, on the other hand, can be cleaved under inert conditions by means of catalytic amounts of tetrakis[triphenylphosphine] palladium(0) ($Pd(PPh_3)_4$) and a nucleophilic scavenger for allyl cations, morpholine.[210] Alternatively, the allyl ester can also be removed under basic conditions by stirring the ester in a mixture of 1,4-dioxane and aqueous NaOH. The benzyl ester in **71b** and related chain-elongated analogs is cleaved hydrogenolytically. Coupling steps are usually carried out by following established procedures using TBTU (*O*-(benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium tetrafluoroborate) as coupling agent (Scheme 27).



Scheme 27 Synthesis of nitrile functionalized tetrapeptides and hexapeptides.

When allyl protected dipeptide **71a** was used as starting material for the synthesis of the tetrapeptide two products were isolated after coupling of **83b** to **82**. The target tetrapeptide **84a** was only obtained in 35 % yield. In addition, chromatographic purification yielded a fraction that contained another product contaminated with benzotriazole. Comparison of the ^{1}H-NMR spectra of the obtained products showed that the shifts of the protons in the region between ca. 4.6 - 11.0 ppm (besides the signals of benzotriazole) were almost identical, while considerable differences in the aliphatic part of the spectrum up to 4.5 ppm were noticed (Chart 2). Furthermore, the

different retention times in the TLC and HPLC analysis of the obtained products indicated that this minor product is an impure isomer of **84a**.

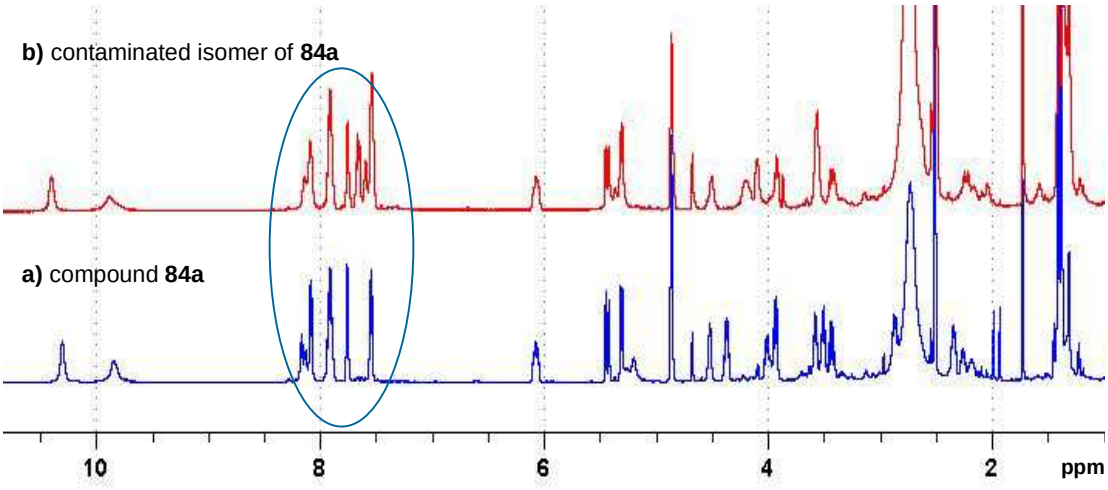


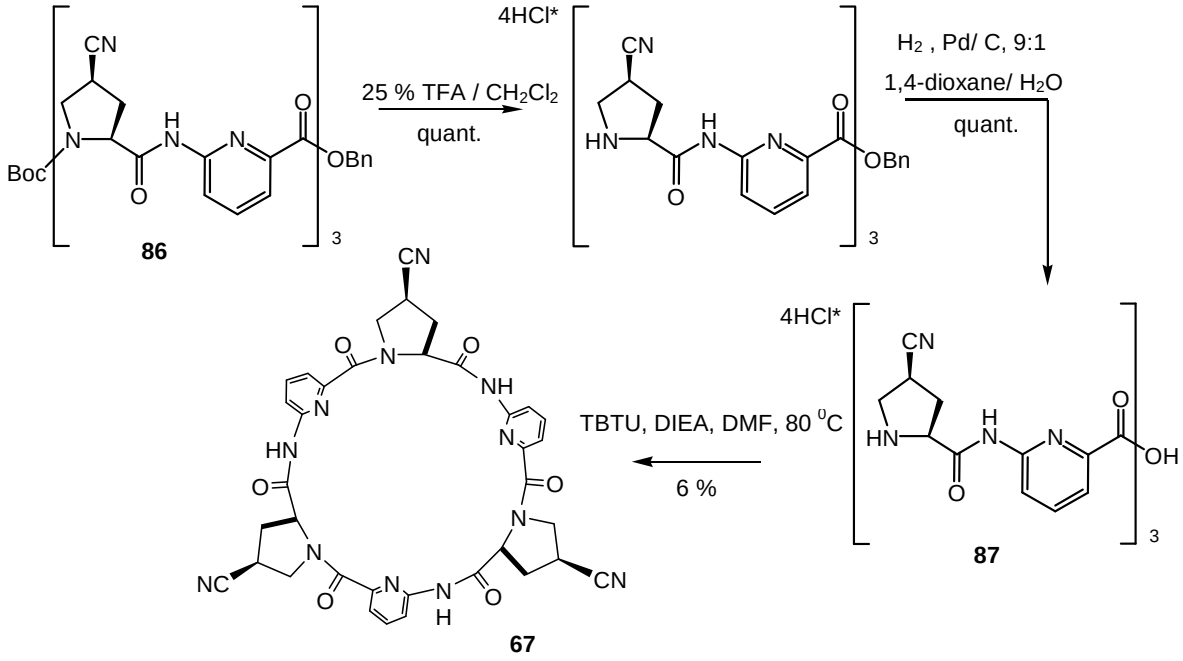
Chart 2 ^{1}H-NMR of pure **84a** (a) and of side product containing benzotriazole as contaminant (b) recorded in DMSO after chromatographic purification (400 MHz, 100 °C).

Test reactions were therefore set up to see whether other coupling reagents such as PyCloP, PyBoP (benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate), HATU (2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate), HCTU (2-(6-chloro-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate) or EDC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride) would be better suited for the corresponding transformation. Unfortunately, neither reagent yielded tetrapeptide **84a** in better yields or in pure form.

To overcome these complications the protecting group strategy was changed, i.e. the benzyl protected dipeptide **71b** was used as starting material. In this case, tetrapeptide **84b** was obtained in pure form and in excellent yields of 92 % when using TBTU as coupling reagent. No significant improvement in the yield was observed when PyCloP was used in the coupling step instead.

Next, one equivalent of tetrapeptide **84b** was deprotected at the C-terminus and coupled with one equivalent of N-deprotected dipeptide **83b** to give hexapeptide **86** in 84 % yield. (Scheme 27). This reaction was carried out in DMF because of the generally lower solubility of such hexapeptides in CH_2Cl_2. It was noted that the isolated yield of **86** depended dramatically on the work-up. The usual work-up involves precipitation of the product by pouring the reaction mixture into water. Because of the higher water solubility of **86** with respect to previously prepared hexapeptides this procedure is unsuitable for the isolation of **86**. Instead, **86** was isolated chromatographically.

Prior to cyclization, the hexapeptide precursor **86** was deprotected at both ends: first at the terminal amino group, and then at the opposite carboxylate group (Scheme 28). An IR-spectroscopic analysis was performed to confirm the presence of a nitrile absorption band at 2246 cm^{-1} in the spectrum of **87**. Fully deprotected **87** was then subjected to cyclization under the pseudo-high dilution conditions usually employed in the group for this type of reactions.[211] Specifically, a solution of **87** was slowly added dropwise to a solution of TBTU and *N,N*-diisopropylethylamine (DIEA) in DMF at 80 °C.



Scheme 28 Synthesis of cyclopeptide **67** with three nitrile groups from hexapeptide **86**.

After the usual chromatographic work-up cyclopeptide trinitrile was isolated in a disappointingly low yield of 6 %. It was unclear if the cyclization reaction itself was responsible for the low yield or the extensive purification steps. These steps involved chromatographic purification on a silica, then on a reversed phase column and finally on another silica column with a different eluent. Afterwards, preparative HPLC was used to separate the remaining impurities. According to spectroscopic analysis the cyclopeptide **67** thus obtained was nearly pure (3 % impurities). It was characterized by ^{1}H-NMR and MALDI-TOF MS (Chart 3). Unfortunately, the overall amount obtained was just 30 mg, barely enough for some preliminary complexation studies with palladium complex **52a**. For reasons of time, no further attempts to synthesize more material were performed.

Cyclopeptide trinitrile **67** dissolves in polar aprotic solvents such as acetonitrile, DMF, or DMSO. Among polar protic solvents **67** was only partially soluble in methanol and not in water.

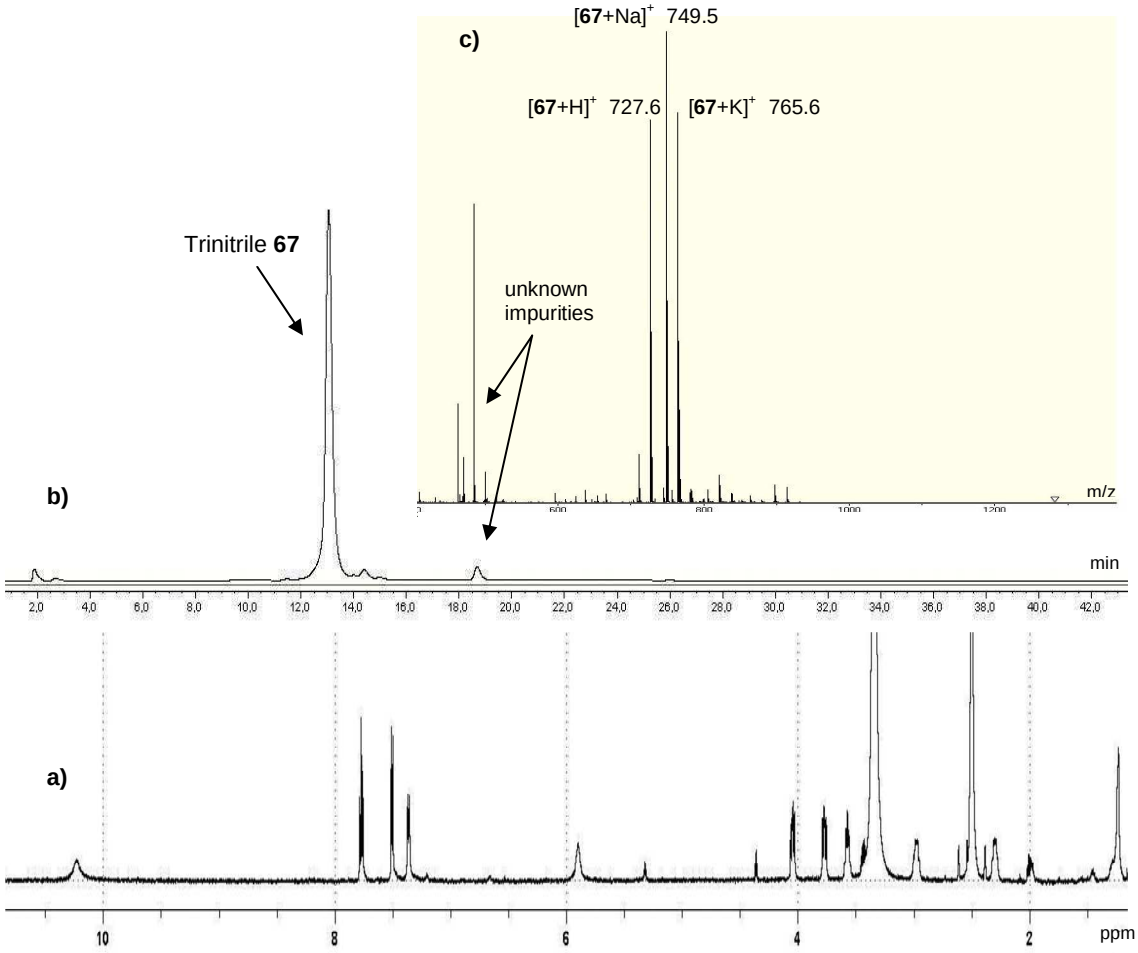
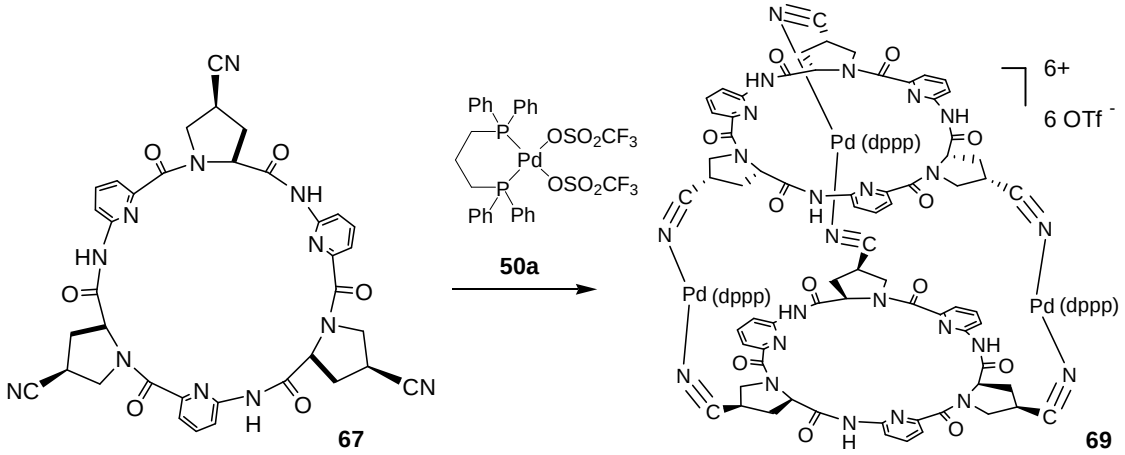


Chart 3 Characterization of cyclopeptide **67**. (a) ^{1}H-NMR in DMSO-d_6 (400 MHz, 25 °C), (b) HPLC chromatogram (Rt 13.06 min) recorded at 25 °C in water/methanol as mobile phase, and (c) MALDI-TOF MS spectrum (positive mode).

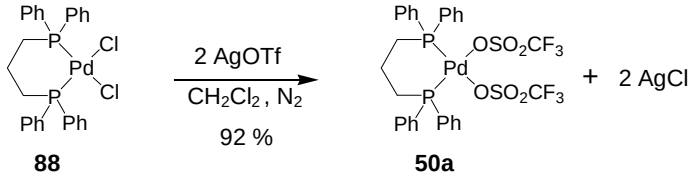
4.1.3 Attempts to assemble the coordination cage 69

Mixing trinitrile **67** with *cis*-protected palladium(II) precursor **50a** in 1:1.5 molar ratio could lead to coordination cage **69** as shown in Scheme 29. According to literature information such cages can often be isolated from the reaction mixture either by precipitation or after solvent evaporation. Complex Pd(dppp)OTf$_2$ was selected among other possible palladium complexes for the attempted synthesis of **69** because of the known coordination of palladium(II) centers to nitrile groups, the easily exchangeable *cis*-configured triflate ions, the bulky blocking loop (dppp), and the straightforward synthesis. Moreover, other researchers have successfully used this building block for the assembly of coordination macrocycles and cages.[42, 177, 179a, 181, 186]



Scheme 29 Synthetic strategy toward assembly of coordination cage **69**.

The preparation of **50a** is outlined in Scheme 30.[175] It includes replacement of two chlorides with triflate anions in the commercially available complex $Pd(dppp)Cl_2$ by treatment with an excess of silver triflate. The precipitated complex was isolated in analytically pure form after filtration, evaporation of the solvent, and washing of the residue with diethyl ether. The obtained product is light sensitive; therefore it was freshly prepared immediately before the self-assembly experiments. It should be noted that **50a** reacts with water molecules in wet solvents forming a small amount of bis-aquo complex $[Pd(dppp)(H_2O)_2]OTf_2$. Therefore, **50a** was used in a slight excess when setting up metal-ligand exchange reactions.[175]



Scheme 30 Synthesis of **50a** from dichloro-complex **88**.

Next, **50a** and trinitrile **67** were mixed in a ratio of 1.6:1 in a micro NMR tube to obtain information by ^{1}H-NMR spectroscopy whether both components react and yield the desired coordination cage **69**. The ^{1}H-NMR spectrum of a highly symmetrical species such as cage **69** should be quite simple and similar to that of trinitrile **67**. Expected diagnostic shifts for nitrile coordination to a metal center are those corresponding to the signals for proline H(γ) protons, and to a smaller extent, to the signals of the proline H(β) and H(δ) protons, located in close proximity to the position of coordination. On the other hand, if the triflate anion acts as template and enters the bis(cyclopeptide) cavity, shifts of the signals of the amide protons and especially of the proline H(α) protons should be visible as previously reported for other bis(cyclopeptide)-based cages templated with inorganic anions.[191]

Such self-assemblies proceed generally well in the following solvents: C_6H_6, $CHCl_3$, $C_2H_2Cl_4$, CH_2Cl_2, CH_3COCH_3, CH_3OH, CH_3NO_2 as well as in polar solvent mixtures such as CH_3NO_2/$CHCl_3$ or $CHCl_3$/DMSO. In some cases, the exchange reactions were even performed in acetonitrile, DMSO or in pure water. Considering the solubility of **67**, nitromethane, or acetonitrile could not be used as solvents. DMSO seems to be not an optimal choice because it strongly interacts with the hydrogen-bond donors on the cyclopeptide thus weakening possible template effects of anions. Hence, methanol was initially used for the reaction. Although the trinitrile **67** is only moderately soluble in CD_3OD, addition of 1.6 equiv. of methanolic Pd(dppp)OTf_2 solution sometimes resulted in complete dissolvation. Unfortunately, this result was not well reproducible and in some cases solid material remained.

^{1}H-NMR spectra were recorded immediately after the addition of **50a** (ca. 10 min) and after 18 h. Disappointingly, the signal patterns of the spectra of the reaction mixture direct after addition of **50a** and after 18 h were identical. Unfortunately, the limited solubility of **67** in CD_3OD did not allow recording a reference spectrum in this solvent. To allow comparison, the ^{1}H-NMR of **67** was first recorded in DMSO-d_6 (Chart 4). Then a drop DMSO-d_6 was added to the reaction mixture containing **67** and **50a** in CD_3OD. The obtained ^{1}H-NMR spectrum of this solution was almost identical with the one obtained for **67** in DMSO-d_6. Although minor signal shifts were detected for the signals of the proline H(α), and H(γ) proton, NMR spectroscopy provided no conclusive information if a reaction between **67** and **50a** had occurred.

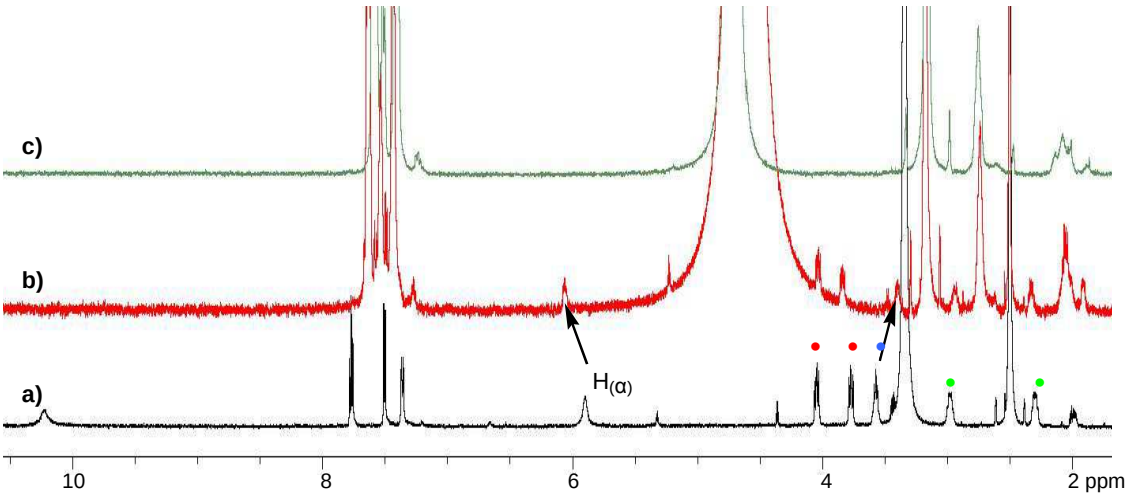


Chart 4 ^{1}H-NMR spectra (600 MHz, 25 °C) of cyclopeptide **67** in DMSO-d_6 (a); a mixture of **67** and **50a** in a ratio of 1:1.6 in DMSO-d_6 /CD_3OD (4:96) (b), and of **50a** in CD_3OD (c). Signals for the proline protons H(δ), H(γ), and H(β) are marked as red, blue, and green dots, respectively.

^{31}P-NMR and ^{19}F-NMR spectra were also recorded of the reaction mixture to see whether shifts of the signals in the corresponding spectra with respect to the spectra of pure complex **50a** would provide information about the potential coordination of the nitrile groups to the metal center in the

palladium complex. The ^{31}P-NMR spectrum of the reaction mixture recorded after 18h of equilibration featured just one singlet at 17.08 ppm, shifted by 0.2 ppm to lower frequencies in comparison with the signal in the spectrum of Pd(dppp)OTf$_2$ (17.27 ppm) (Chart 5a). Also ^{19}F-NMR spectroscopy provided no strong indication for encapsulation of triflate anions in the potential cage **69**. One signal was observed in the ^{19}F-NMR spectrum of the reaction mixture shifted by 0.35 ppm to lower field with respect to the signal in the spectrum of **50a**. Both ^{31}P-NMR and ^{19}F-NMR spectroscopy therefore also gave no clear evidence for the formation of **69**.

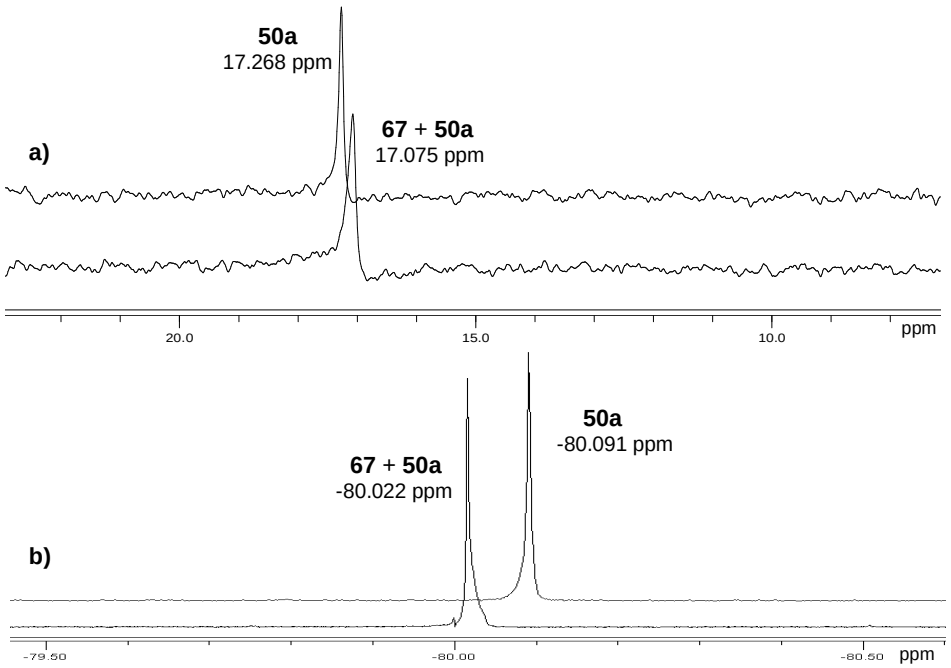


Chart 5 ^{31}P-NMR (a) and ^{19}F-NMR (b) spectra of a mixture of **67** and **50a** in a ratio of 1:1.6 in DMSO-d_6 /CD_3OD (4:96) and of free complex **50a** in CD_3OD.

Finally, mass spectrometric analysis of the reaction mixture was attempted, which was expected to indicate directly whether species of higher mass containing more than one cyclopeptide rings were present in solution. Unfortunately, corresponding ions were neither visible in the MALDI-TOF nor the ESI-TOF MS spectra. Aggregation of **67** into higher complexes such as tetrameric or octameric could thus also be excluded.

Concluding, these preliminary experiments did not provide any evidence for the formation of **69** under the chosen conditions. Further synthetic experiments are necessary involving other solvents, conditions and/or palladium complexes to investigate whether self-assembly is impossible due to principal reasons or whether the correct conditions still need to be identified. To this end, the improvement of the synthesis of **69** and the isolation of more of the cyclopeptide is a necessary prerequisite. Another possibility is replacement of palladium(II) ions as connecting

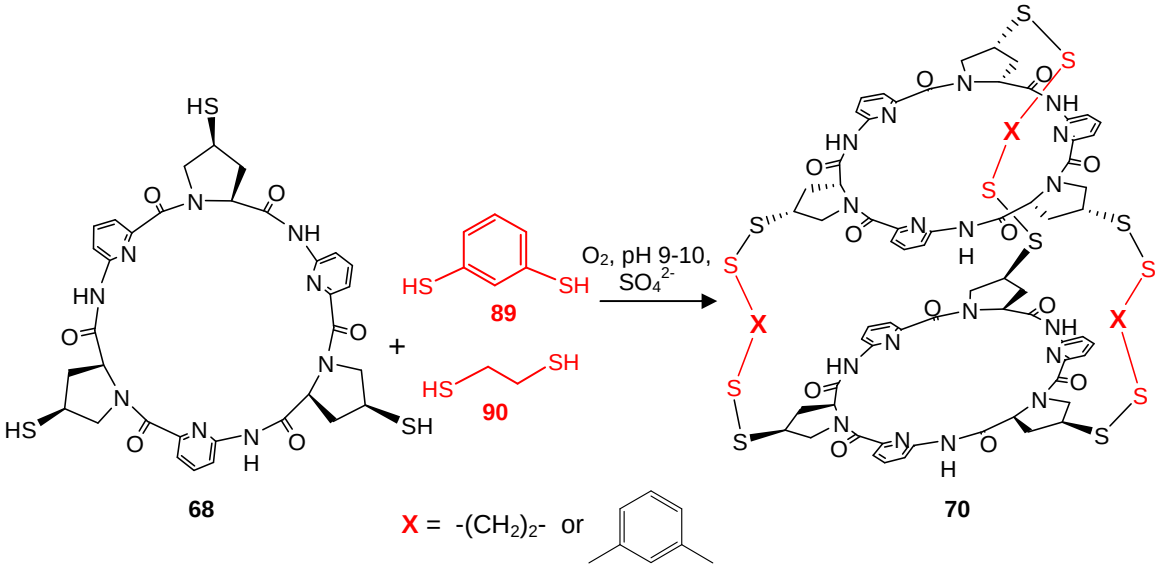
metal ions with platinum(II) ions that form kinetically more inert complexes with nitrile groups. Also the systematic screening of potential anionic templates that could induce cage formation still needs to be performed. Last but not least, structural variation of the cyclopeptide could also be considered to change the bite angle of the Lewis-basic groups on the macrocyclic cage components. One possibility in this respect could be the replacement of the nitrile group in γ-position of the proline rings with 4-benzonitrile or pyridine groups.

4.2. Triply-linked covalently assembled bis(cyclopeptide) cage

4.2.1 Building blocks for the covalent cage 70

The second part of my PhD thesis concerned the preparation of bis(cyclopeptidic) cages in which the two cyclopeptide halves are linked covalently via three linkages. So far, investigations on singly-and doubly-linked bis(cyclopeptides) demonstrated that anion affinity of these hosts improved significantly in aqueous solvent mixtures with increasing number of linkages.[198] This trend is a consequence of the improved preorganization of the receptor subunits. Improved anion selectivity is to be expected upon incorporation of a third spacer. Utilization of different linkers gives access to structurally diverse neutral hosts. In addition, depending on the size or length of the linkers one could realize permanent or reversible encapsulation of an anion.

Previous results demonstrated that DCC using disulfide exchange as a reversible reaction is a particularly useful strategy to optimize the linker structure. For bis(cyclopeptides) connected via one and two linkers this approach provided 1,3-benzenedithiol (**89**) and 1,2-ethanedithiol (**90**) as the most suitable linking units, while the best guests were sulfate and iodide for DCLs generated in 2:1 acetonitrile/water solution.[64n] Therefore, these spacers and the sulfate anion as template were selected to build up the triply-linked receptor **70** (Scheme 31). To make use of the disulfide exchange reaction, the cyclopeptide precursor required for the assembly of **70** is trithiol **68**. After successful synthesis of this new building block it has to be equilibrated with dithiol linkers **89** and **90** and appropriate templates in 2:1 acetonitrile/water mixtures to generate the desired product.

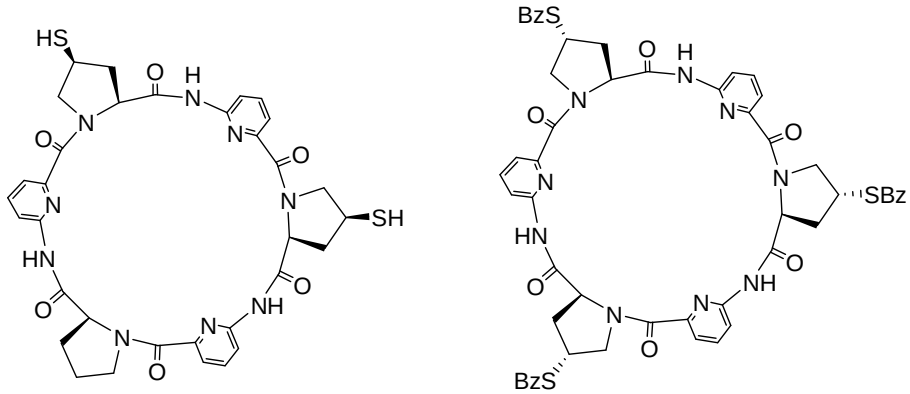


Scheme 31 Synthesis of cages of general structure **70** from trithiol **68** under thermodynamic control. Inorganic sulfate salts were used as template.

Products should be isolated, purified, and their receptor properties toward substrates characterized. The results, should provide information about anion affinity and selectivity as well as details about the mechanism of complex formation.

4.2.2 Synthesis of cyclopeptide trithiol 68

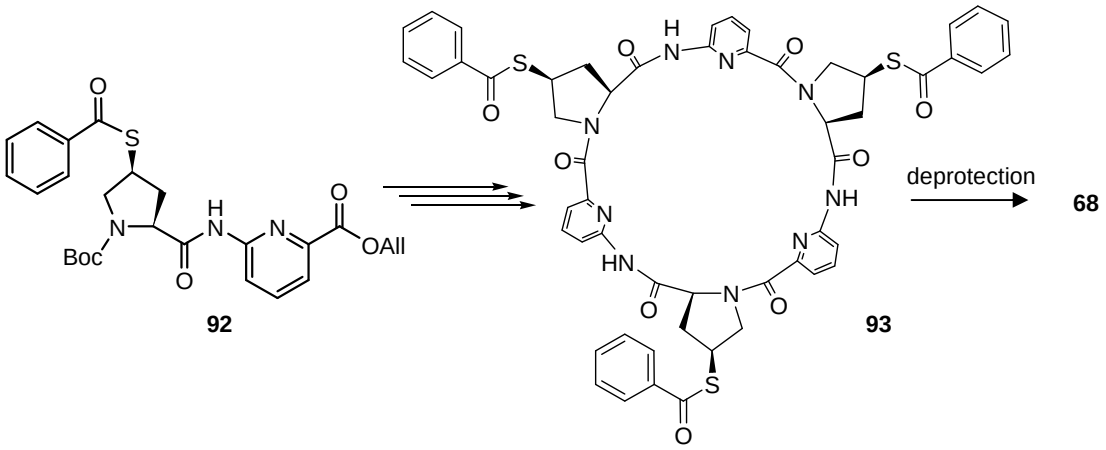
Synthesis of the cyclic hexapeptide containing three thiol groups in γ-position of the proline units was performed analogously to the cyclopeptide synthesis established in the group. At the beginning of my PhD the preparation of cyclopeptide **61**[188a] with two (4*S*)-configured 4-thioproline groups and of cyclopeptide **91**[199] containing three benzoyl protected (4*R*)-4-thioproline units, the latter having different configurations at the proline C(γ) positions than **68**, was already known (Scheme 32). In compound **91** benzoyl protection was used because acetate groups proved to be unstable under the conditions required for the cyclization of the linear precursor.[188a]



61 **91**

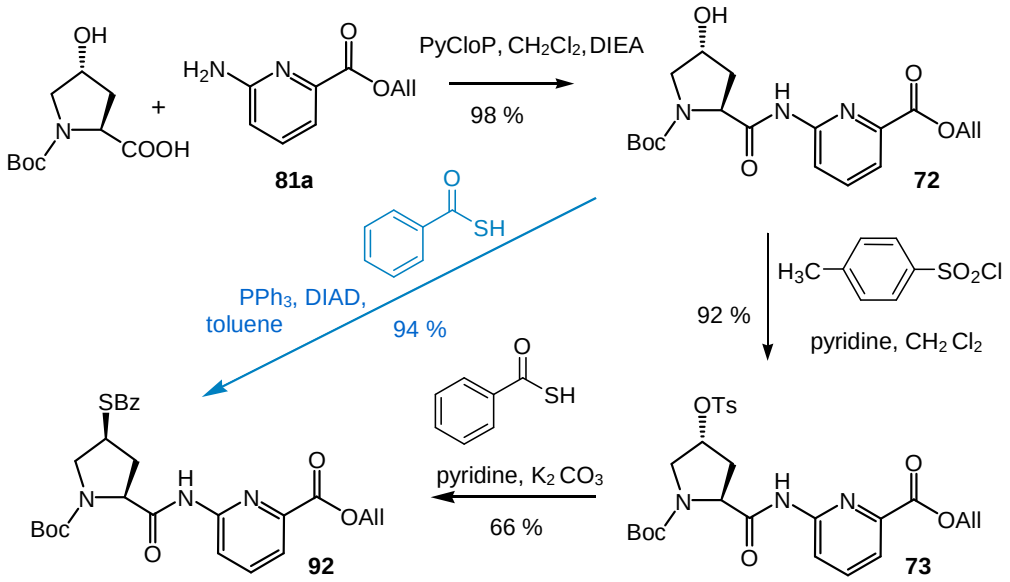
Scheme 32 Previously reported thiol containing cyclopeptides **61** and **91**.

Preparation of **68** was conducted by following synthetic protocols developed by C. Reyheller for the synthesis of cyclopeptide **61**. Synthesis of benzoyl-protected derivative **93** requires dipeptide **92** with the required *S*-configuration at the C(γ) atom of the proline unit (Scheme 33). The presence of the sulfur atom in **92** made it necessary to use the allyl ester group to block the carboxylate terminus. Benzyl esters used in other peptide syntheses have to be cleaved by hydrogenation under palladium(0) catalysis, which fails when the substrate contains sulfur atoms. The allyl ester and Boc-group in dipeptide **92** can be cleaved independently by palladium catalysis or treatment with acids, respectively.



Scheme 33 Dipeptide **92** was used as building block to prepare trithiobenzoate **93**, which should give trithiol **68** after deprotection.

Synthesis of dipeptide **92** started by coupling 6-aminopicolinic acid allylester (**81a**) to Boc-L-hydroxyproline in the presence of PyCloP (Scheme 34). Then, standard synthetic methods were used to convert the hydroxyl group into a tosylate (**73**) and subsequently into a thioester with concomitant configurational change at C(γ) of the proline ring.



Scheme 34 Synthesis of dipeptide **92**.

In order to reduce the synthetic efforts an alternative strategy for the synthesis of **92** was tested. Specifically, introduction of the thiobenzoate group in C(γ) position of the acohol **72** by means of a Mitsunobu reaction comprising treatment of **72** with thiobenzoic acid, triphenylphosphine, and diisopropyl azodicarboxylate (DIAD) could directly yield **92** (Scheme 34 in blue). In

tetrahydrofuran product formation did not take place because of the poor solubility of **72** in this solvent. Therefore, the reaction was conducted in freshly distilled dry toluene under exclusion of air. The reaction progress was controlled by TLC and MALDI-TOF MS. After work-up and chromatographic purification dipeptide **92** was isolated in a crude yield of 94 %. The subsequent characterization indicated that the isolated product was still impure most likely containing hydrazide byproducts and triphenylphosphine oxide. These impurities could not be separated by further chromatographic steps or recrystallization. Therefore, the Mitsunobu reaction proved to be unsuitable to obtain **92**.

Next, typical peptide synthesis was used to transform **92** into the corresponding tetrapeptide **96** (Scheme 35). The linear hexapeptide **97** was obtained by coupling of the amino-deprotected tetrapeptide **95** to carboxy-deprotected dipeptide **94**. Allyl deprotection is usually accompanied with some loss of material due to the work-up. Therefore, deblocking at the N-terminus of the tetrapeptide **96** was preferred. Furthermore, it was noticed that allyl deprotection proceed slowly on the stage of hexapeptide requiring addition of more catalyst and morpholine and prolonged reaction times. After deprotection of hexapeptide **97** at both ends, cyclization under pseudo-high dilution conditions, and several chromatographic steps cyclopeptide **93** was obtained in 49 **%** yield in analytically pure form. The ^{1}H-NMR spectrum, MALDI-TOF MS spectrum and chromatogram of cyclopeptide **93** are depicted in Chart 6.

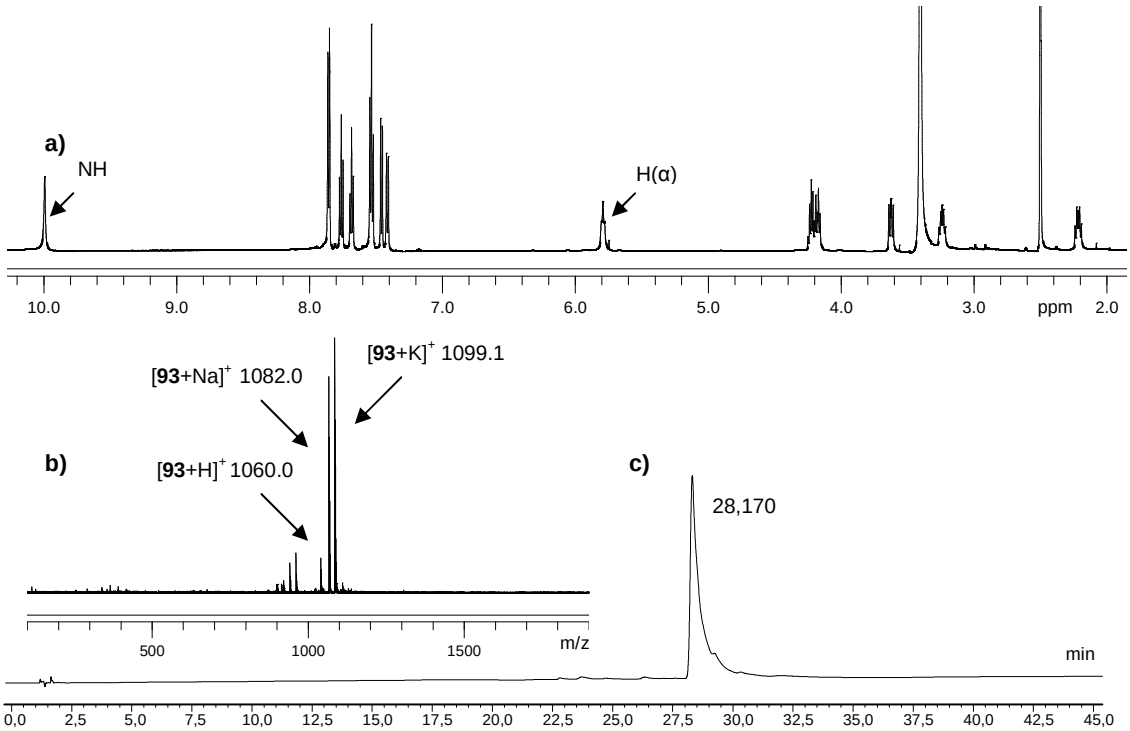
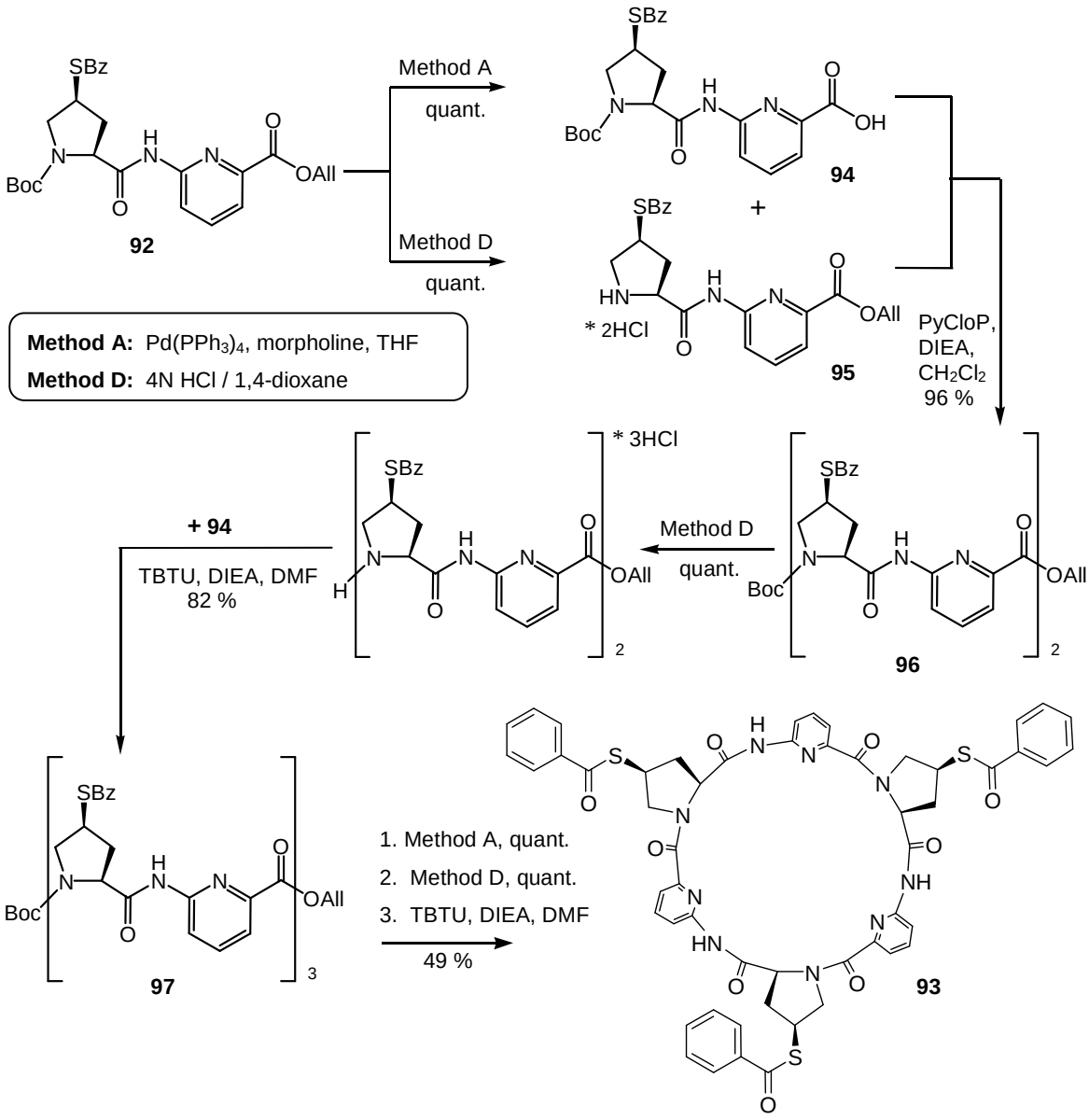
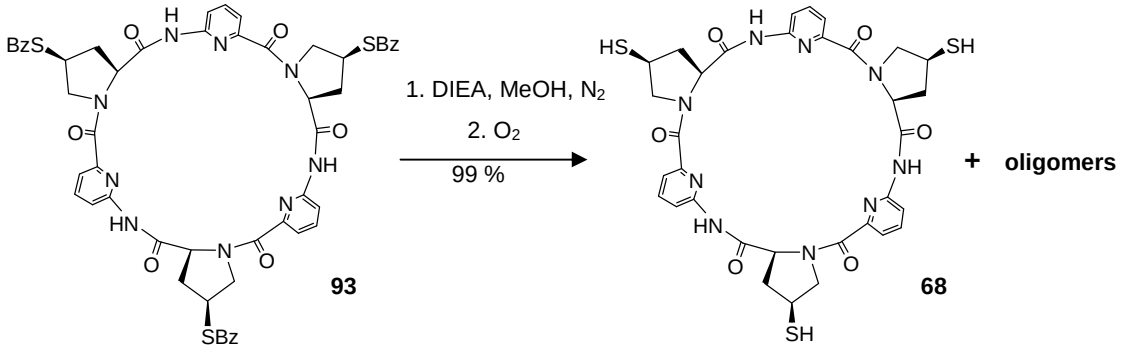


Chart 6 Characterization of cyclopeptide **93**. (a) ^{1}H-NMR in DMSO-d_6 (600 MHz, 25 °C); (b) MALDI-TOF MS spectrum (positive mode), and (c) HPLC chromatogram recorded at λ 254 nm (40 °C, mobile phase: acetonitrile + 0.1 vol % TFA / water + 0.1 vol % TFA).



Scheme 35 Synthesis of cyclopeptide **93**.

Cleavage of the ester groups in **93** was accomplished by stirring of **93** in a mixture of DIEA in dry methanol.[130a, 195] The reaction proceeded only slowly. Mass spectrometry indicated full deprotection after 9 - 11 days (Scheme 36). After the reaction went to completion the reaction flask was opened to air to induce oxidation of the thiol groups to disulfides and stirred for another 2 hours prior to work-up. The product was obtained in a yield of 99 %. HPLC analysis showed only one broad peak in the chromatogram, indicating polymerization due to disulfide formation rather than formation of a distinct product (Chart 7a). MALDI-TOF MS studies confirmed that the isolated material is a mixture of monomer to hexamers (Chart 8a).



Scheme 36 Deprotection of **93** yielded trithiol **68** and corresponding oligomers due to oxidative disulfide formation.

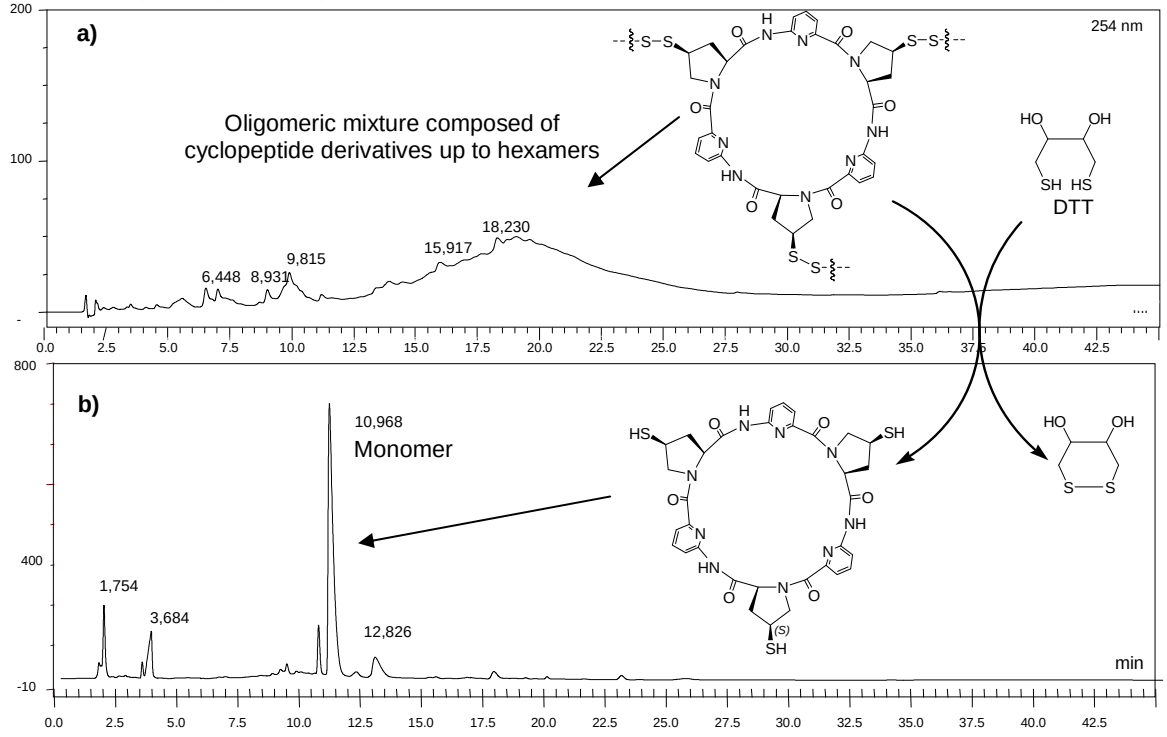


Chart 7 Comparison between the HPLC chromatograms obtained for (a) the isolated oligomeric mixture of **68** and (b) for a stock solution of **68** containing 5 equiv. of DTT (λ 254 nm, 40 °C, mobile phase: acetonitrile + 0.1 vol % TFA / water + 0.1 vol % TFA).

Chromatographic analysis of the product mixture obtained after deprotection of **93** showed that the chromatogram simplified considerably after addition of dithiothreitol (DTT) to the solution (Chart 7b).[212] A major peak was observed in the resulting chromatogram that, according to MALDI-TOF MS, represents the fully reduced thiol **68** (Chart 8b). This experiment indicated that

DTT allows depolymerization of the obtained polymeric product releasing monomer **68**, the building block required for the preparation of bis(cyclopeptide) **70**.

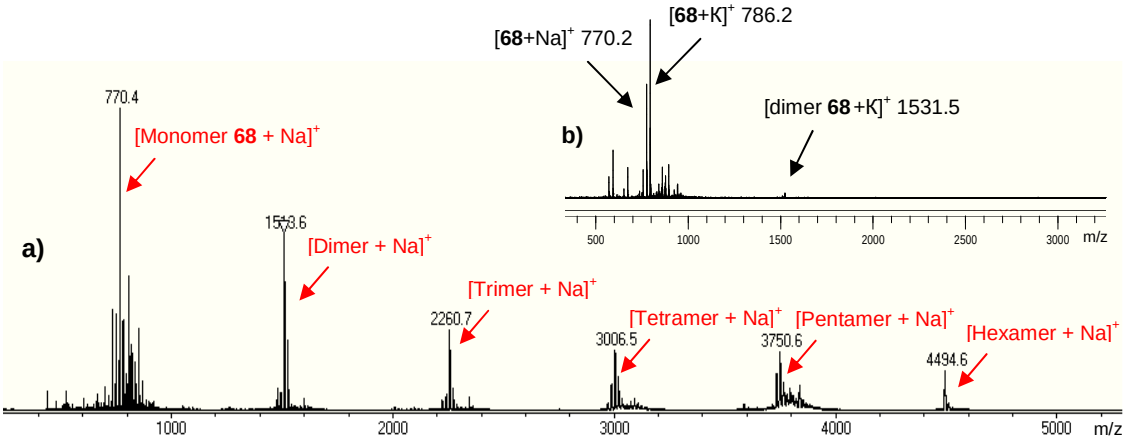
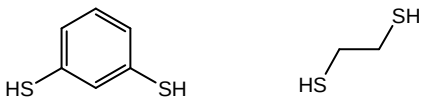


Chart 8 MALDI-TOF MS spectra of (a) the oxidized mixture obtained after deprotection of **93** and of (b) the product **68** obtained after reduction with DTT (positive mode).

4.3 Dynamic libraries

4.3.1 Sulfate anions as templates

The oligomeric mixture of **68** was next used to set up dynamic libraries. Two dithiols were chosen as linkers that were shown in previous investigations to yield singly- and doubly-linked bis(cyclopeptides) with high affinity for inorganic anions, namely 1,3-benzenedithiol (**89**) and 1,2-ethanedithiol (**90**) (Scheme 37). Both differ in their rigidity and the number of carbon atoms between the thiol groups. Since our bis(cyclopeptide)-derived anion receptors generally exhibit highest affinity for sulfate anions, sodium sulfate was initially used as template.[64n, 130a, 191]



89 **90**

Scheme 37 Dithiol linkers equilibrated in the dynamic libraries with **68**.

DCLs were set up under analogous conditions as previously reported by Otto and Kubik.[130a, 198] Stock solutions of compound **68** and a dithiol linker (**89** or **90**) were dissolved in 2:1 (v/v) acetonitrile/water at pH 9 (Scheme 31). The resulting mixture consists of **68** and **89** or **90** in a 1:1.5 molar ratio was divided into two samples to one of which a solution of sodium sulfate was added (templated library). The other sample was made up to the same volume with water (non-templated library). The reaction course was followed by using HPLC. To obtain sharp HPLC peaks the eluent contained 0.1 vol % of TFA. Use of formic acid instead of TFA gave no

satisfactory chromatograms. When no further change in library composition was detected (usually within one week) the reaction was considered to have reached thermodynamic equilibrium. To evaluate the effects of templation the chromatograms of the templated and the non-templated library were compared. The nature of the amplified compounds was initially analyzed by mass spectrometry. In addition, ^{1}H-NMR spectroscopy was used for structural characterization after isolation of the products on a preparative scale.

The initial experiments involved direct use of the mixture of oligomeric products obtained after deprotection of **93**. Although HPLC analysis indicated in the case of 1,3-benzenedithiol the consumption of the linker during equilibration, the only new product that could be observed in the untemplated library after 7 days of equilibration was monomer **68** (Chart 9). In the templated library no other compounds appeared that could be assigned to a bis(cyclopeptide). Change of the solvent from 2:1 CH_3CN/H_2O to *i*-PrOH/H_2O and THF/H_2O mixed in the same ratio as well as use of more flexible linker, namely 1,3-(*n*-propane)dithiol, did not alter the outcome of the reaction. A possible explanation of this result is that the oligomers of **68** are too stable to participate in disulfide exchange.

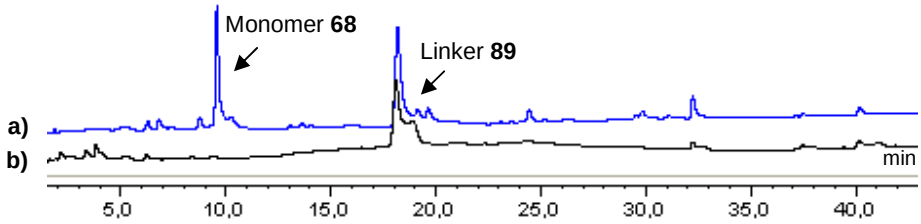


Chart 9 HPLC traces of libraries containing trithiol **68** and linker **89** after 7 days of equilibration. (a) Untemplated library, (b) library templated with Na_2SO_4 (10 mM) (λ 254 nm, 40 °C, mobile phase: acetonitrile + 0.1 vol % TFA / water + 0.1 vol % TFA).

A remedy was to add DTT to the libraries. As already demonstrated this agent causes depolymerization of oligomers **68** to give the corresponding monomer. Experiments conducted with different amounts of DTT (2.25; 3.0; 5.0 and 10.0 equivalents) revealed that addition of 5 equivalents to the library is optimal. Under these conditions, a clear peak of monomer **68** was observed in the chromatogram shortly after starting the reaction. Libraries generated upon addition 2.25 and 3.0 equivalents liberated monomer **68** either too slowly or incompletely. Optimized conditions for setting up a DCL with cyclopeptide **68** on analytical scale are summarized in Tables 23 and 27 in the experimental part.

When using 1,3-benzenedithiol **89** as linker amplification of a new peak was observed in the HPLC chromatogram of the sulfate templated library after ca. nine days at 22.4 min, indicating formation of a new compound (Figure 31). Prolonged reaction times did not change product distribution. The recorded chromatograms are depicted in Chart 10. A small amount of this compound was isolated using HPLC and analyzed by MALDI-TOF MS. The detected mass ion

matched with the one of the expected triply-linked bis(cyclopeptide) **98**. In addition, a further minor peak was observed in the chromatogram at slightly larger retention time (28.4 min). This compound was subsequently assigned to a capsule containing two cyclopeptide rings and four linkers (**99**). The ratio of **98** and **99** according to the relative peak areas in the chromatogram of the templated library of these compounds is 13:1, which is far away from the statistical pattern. The non-templated library predominantly contained monomer **68**, polymerized linker, and some by-products after the same period of equilibration.

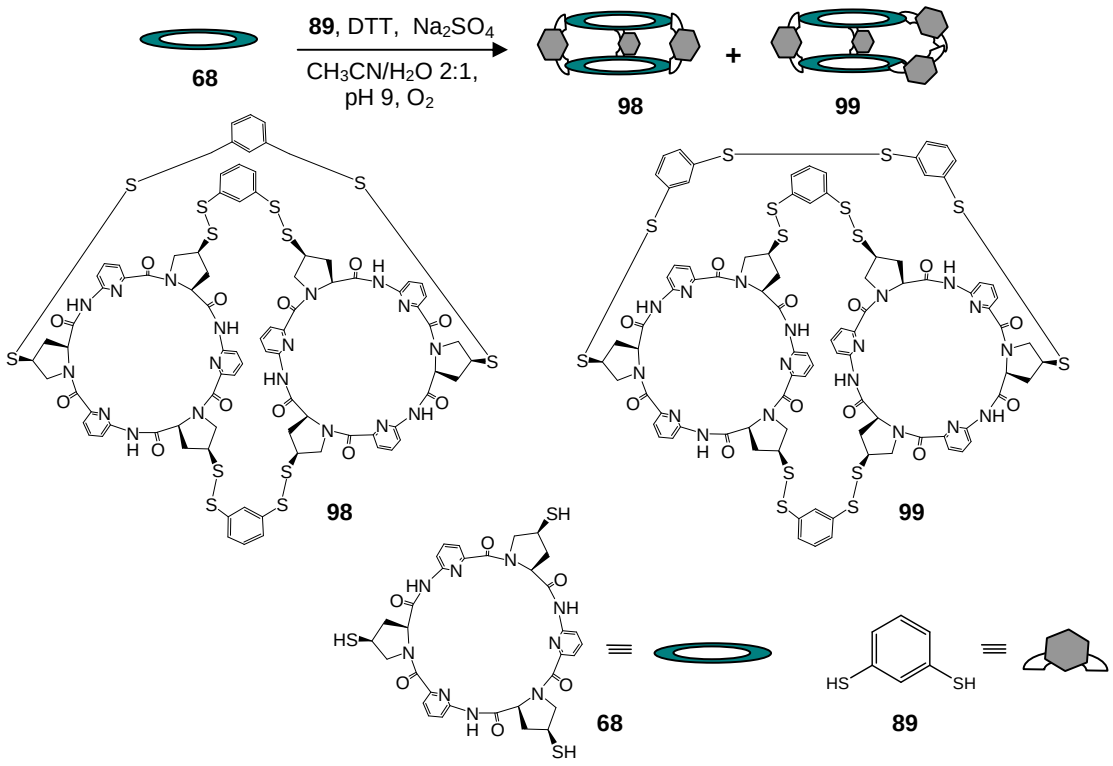


Figure 31 Preparation of triply-linked bis(cyclopeptides) **98** and **99**, containing linker **89**.

When 1,2-ethanedithiol **90** was used as linker formation of triply-linked bis(cyclopeptides) was also observed in the templated libraries (Chart 11). In this case, thermodynamic equilibrium was reached faster (within a week) in comparison with the libraries containing compound **89**. According to HPLC, amplification of the desired receptor was nearly quantitative. LC-MS of the library and MALDI-TOF MS confirmed that the main peak in the chromatogram at 16.8-17.4 min represents the triply-linked compound **100** with three linkers, while the minor peak at 19.3-20.4 min was the capsule **101** containing four linkers (Chart 12). These products were formed in a ratio of 6:1 in favor of **100**.

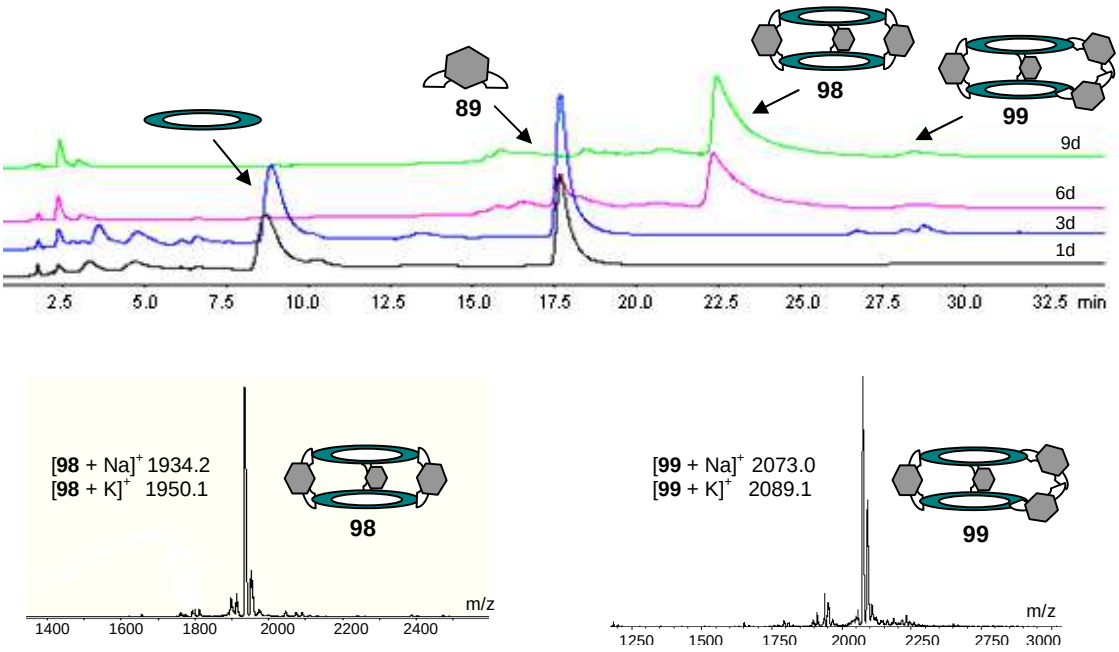


Chart 10 (a) Evolution of the HPLC chromatogram (λ 254 nm, 40°C, mobile phase: acetonitrile + 0.1 vol % TFA / water + 0.1 vol % TFA + 0.1 wt % Na_2SO_4) of a library containing **68**, DTT and 1,3-benzenedithiol linker after 1 day, 3 days, 6 days, and 9 days. (b) MALDI-TOF MS of the amplified hosts **98** and **99** (positive mode).

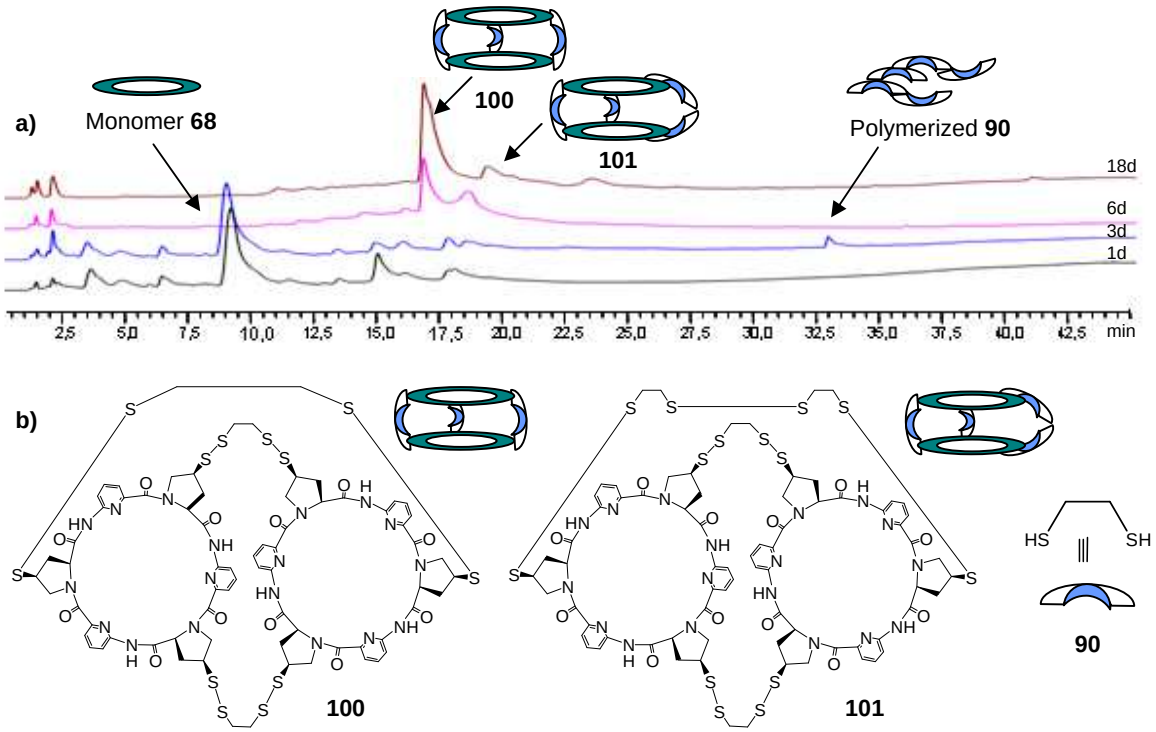


Chart 11 (a) HPLC chromatograms of sulfate templated library containing **68**, DTT and 1,2-ethanedithiol linker recorded over a period of 18 days (λ 254 nm, 40 °C, mobile phase: acetonitrile + 0.1 vol % TFA / water + 0.1 vol % TFA + 0.1 wt % Na_2SO_4). (b) Schematic representation of the amplified triply-linked bis(cyclopeptides) **100** and **101**, containing three or four linkers, respectively.

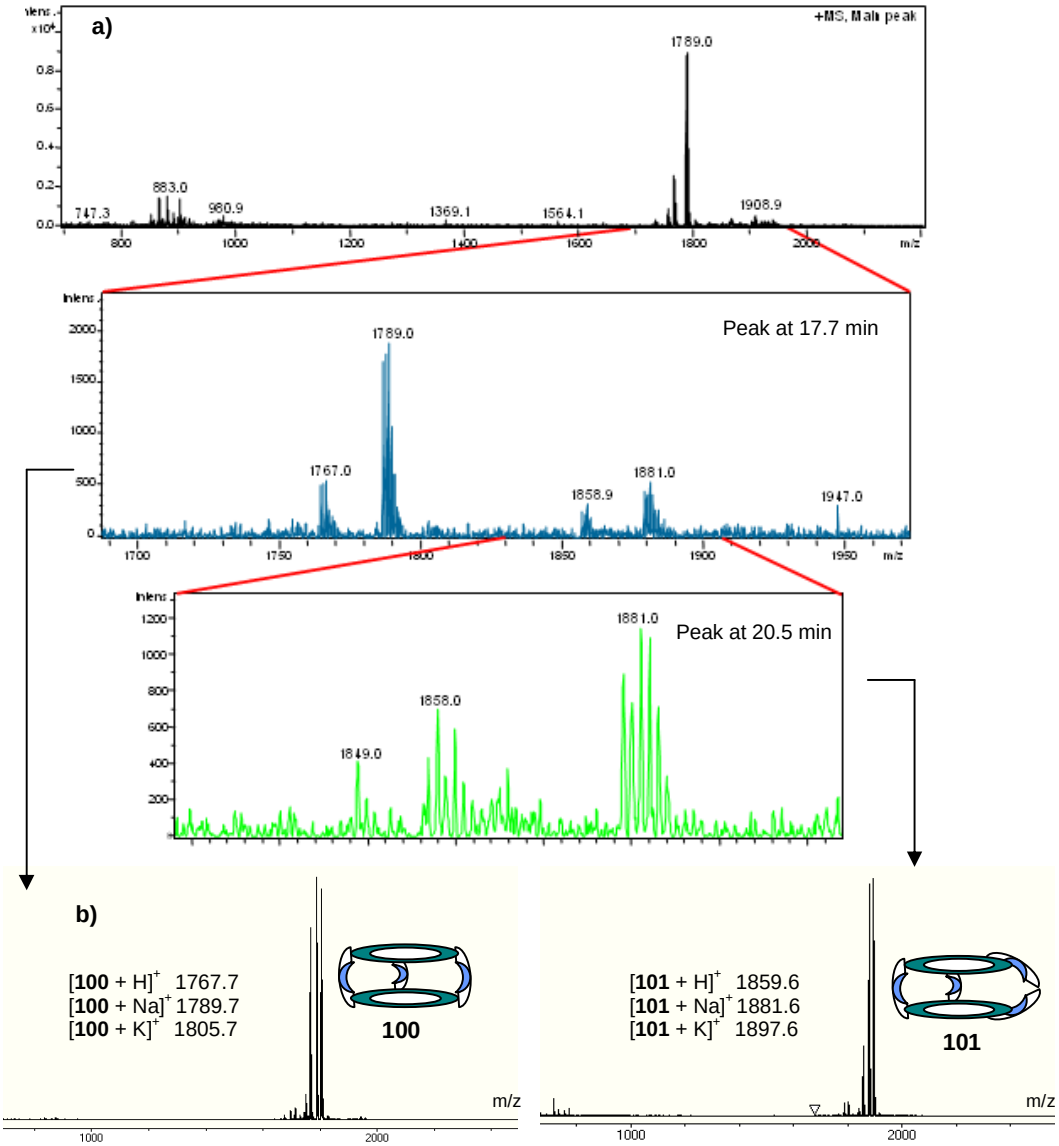


Chart 12 (a) LC-MS of a library containing compound **68**, DTT and 1,2-ethanedithiol linker recorded after 7 days of equilibration (λ 254 nm, 40 °C, mobile phase: acetonitrile + 0.1 vol % TFA / water + 0.1 vol % TFA + 0.1 wt % Na_2SO_4). (b) Expansion of the MALDI-TOF spectrum showing the singly charged receptor ions that correspond to **100** and **101** (positive mode).

To simplify the procedure it was tested whether syntheses of compounds **98**-**101** can be achieved by starting from the protected hexapeptide **93** and thus combining deprotection and bis(cyclopeptide) formation in one step. To this end, **93** was treated with a disulfide linker in the presence of sodium sulfate in 2:1 CH_3CN/H_2O at pH 11. Both linkers **89** and **90** were tested, but only 1,2-ethanedithiol yielded the corresponding triply-linked bis(cyclopeptides) **100** and **101** (Figure 32).

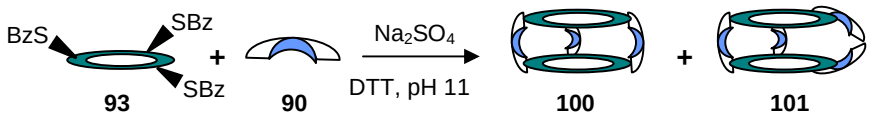


Figure 32 Preparation of **100** and **101** by in situ deprotection of **93** and equilibration with linker **90**.

Libraries containing rigid linker **89** did not furnish the desired product even after 30 days or when increasing the pH to 13. This outcome could indicate that the bis(cyclopeptides) resulting from linker **90** are thermodynamically more stable. A similar result was obtained by Kubik and Otto for bis(cyclopeptides) containing two linkers and was attributed to the higher conformational adaptability of **90**.[198]

HPLC analysis of the library containing linker **90** showed the same pattern as that observed for the two-step process (Chart 13). The one-step procedure, however required more time for reaching equilibrium, namely 20 - 40 days rather than 7 - 9 days. Its advantage is that isolation of **68** and addition of DTT can be avoided. Taking into account that deprotection of **93** requires ca. 10 days the overall reaction time of both methods is similar.

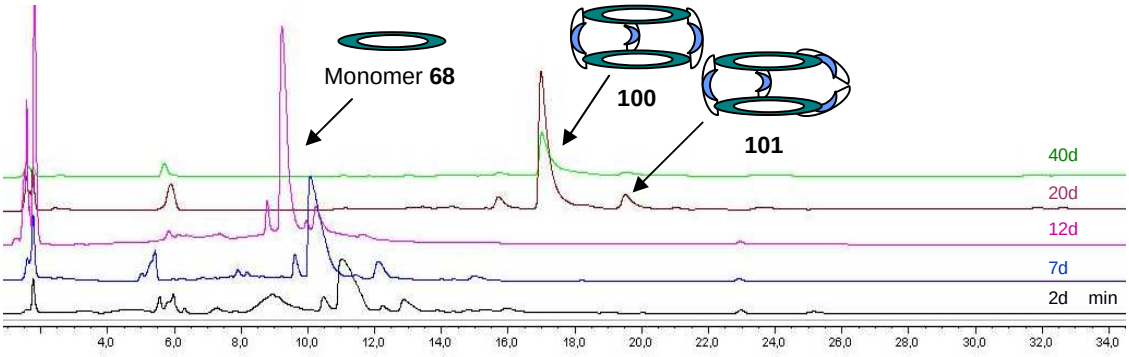


Chart 13 HPLC chromatograms of sulfate templated library containing **93**, DTT and 1,2-ethanedithiol recorded over a period of 40 days (λ 254 nm, 40 °C, mobile phase: acetonitrile + 0.1 vol % TFA / water + 0.1 vol % TFA + 0.1 wt % Na_2SO_4).

4.3.2 Halide anions as templates

Once suitable conditions for the dynamic libraries were found, the next step was to study the effects of other templates. Previous studies suggested that singly- and doubly-linked bis(cyclopeptides) also bind efficiently to spherical anions such as halides.[54, 130a, 190-191, 193] Therefore, the ability of these anions was studied to amplify triply-linked bis(cyclopeptides) in dynamic libraries. For this purpose, DCLs were generated with 1,2-ethanedithiol, trithiol **68**, and

DTT in presence of bromide, chloride, and iodide salts. The effect of these salts on product distribution is summarized in Table 6.

template	ionic radius / Å	amplification of bis(cyclopeptide) 100
Chloride	1.81	⊖
Bromide	1.95	⊖
Iodide	2.20	⊕
Sulfate	2.30	⊕

Table 6 Effects of halides on the reaction between **68**, linker **90**, and DTT. The templates were introduced as sodium salts. Formation of bis(cyclopeptide) **100** is denoted as ⊕ , and the absence of an effect with ⊖.[4a, 213]

Libraries containing a bromide and a chloride salt did not exhibit formation of bis(cyclopeptides). The progress of these reactions was slow and after two weeks of equilibration the libraries still contained starting material. These anions are probably too small to efficiently template the formation of the triply-linked bis(cyclopeptides), i.e. the corresponding complexes are not stable enough to overcome the entropic penalty associated with bis(cyclopeptide) formation. Considering that the atomic radius of fluoride is even smaller, experiments with this anion as template were not carried out. Iodide anions in contrast, were able to induce amplification of compound **100** (Chart 14). Interestingly, clear HPLC detection of the amplified product required the addition of KI to the eluent. In general, amplification of **100** induced by sulfate was stronger, indicating that sulfate anions are the better templates for this receptor.

In additional experiments, the sulfate anions were introduced to the library in the form of tetra-*n*-butylammonium (TBAS) or tetramethylammonium (TMAS) salts. As expected, after 8 days of equilibration formation of **100** was also observed and no strong effect of the counterion on product distribution (**100** vs. **101**) or product yield was detected.

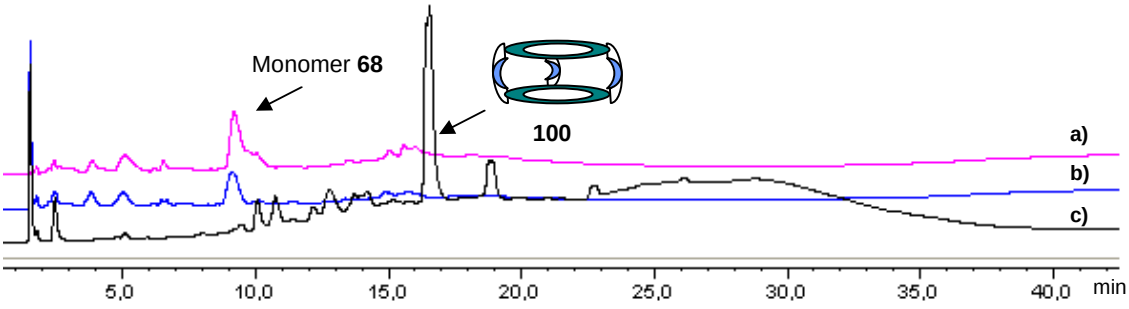
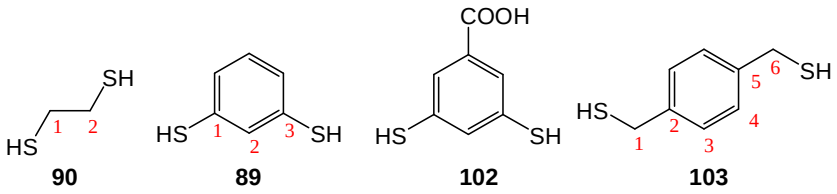


Chart 14 HPLC traces for DCLs equilibrated for 8 days containing **68**, **90**, and DTT in the presence of (a) chloride, (b) bromide, and (c) iodide anions (λ 254 nm, 40 °C, mobile phase: acetonitrile + 0.1 vol % TFA / water + 0.1 vol % TFA). Template salt was added to the water eluent used for the HPLC analyses to obtain better chromatograms.

4.3.3 Spacer competition experiments

To obtain information whether capsules containing different linkers form more stable anion complexes than capsules with just one linker type, dynamic libraries were set-up containing mixtures of dithiols. To reduce the complexity of the generated product mixtures only two different dithiols were used in each library.

The structures of the dithiols used in these experiments are depicted in Scheme 38. Some of these spacers are commercially available, some were kindly provided by our collaboration partner S. Otto. Besides **89** and **90**, 3,5-dimercaptobenzoic acid **102** was selected because the investigations involving bis(cyclopeptides) with one and two linkers indicated that incorporation of this inker into bis(cyclopeptides) could afford water soluble products. Rigid linkers **89** and 1,4-benzenedimethanethiol **103** were used to study how the increase of the number of C-atoms in the linker influences the product distribution of triply-linked bis(cyclopeptides). The corresponding 1,4-positional isomer of **89** was not included in this survey because previous studies on doubly-linked bis(cyclopeptides) demonstrated already that it is not incorporated into the products.



90 **89** **102** **103**

Scheme 38 Linker molecules used for the mixed libraries.

DCLs used for the screening of potential spacer molecules were composed of two different linkers, cyclopeptide **68**, and DTT in 2:1 CH_3CN/H_2O at pH 9. Libraries were incubated for a period of 7 days and reaction progress was monitored by diode array HPLC and MALDI-TOF MS. Products containing cyclopeptide moieties were detected according to their characteristic UV spectra (absorption at 254 nm and 290 nm) with the diode array technique. The combinations of tested linkers and the obtained results together with the retention times (R_t) of the amplified species containing cyclopeptide building blocks are depicted in Table 7.

entry	combination of linkers	amplified new species, R_t / min
1	**90 + 102**	3 new peaks: 17.6, 20.1, 26.5
2	90 + 103	precipitation
3	**90 + 89**	3 new peaks: 18.4, 20.8, 22.6
4	89 + 103	precipitation
5	**89 + 102**	1 new peak: 20.5
6	102 + 103	precipitation

Table 7 Combinations of dithiols **89**, **90**, **102**, and **103** used for the mixed libraries and retention times of amplified products. Entries depicted in bold text gave the best results.

These experiments provided qualitative information about the dependence of the receptor binding affinity towards sulfate on the length and rigidity of the linker. Libraries with linker **103** that encloses six carbons between the thiol groups rapidly precipitated either on the first day or after a maximum of 5 days after mixing (entries 2, 4 and 6). This precipitation was attributed to polymerization of the linker(s) or oligomerization of bis(cyclopeptide) units. No definite product species could be detected. Libraries with compound **102** containing a carboxyl group turned out to be more promising (entries 1 and 5) unless **102** was combined with **103**. When the two linkers **89** and **102** were mixed precipitation was also observed after 5 days but one new peak emerged. According to its UV-trace this peak, with a retention time of 20.5 min, derived from a cyclopeptide. However, further structural characterization of this compound from the library mixture failed. Libraries containing **102** or **89** and aliphatic linker **90** reached equilibrium within 7 days after which three new peaks were observed in the chromatogram all containing cyclopeptide moieties (entries 1 and 3). These DCLs needed much time to reach equilibrium (up to 14 days) and no polymerization was monitored. As an example, HPLC-traces of the DCL with linkers **89** and **90** after 9 days of equilibration are shown in Chart 15.

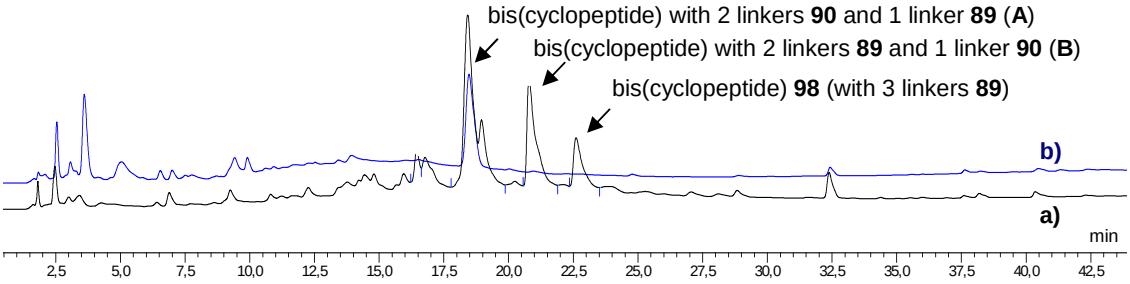


Chart 15 HPLC-traces of the product distribution obtained by equilibrating **63**, **89** and **90** in the presence (a) and absence (b) of sodium sulfate.

To determine the structure of compounds amplified in the mixtures, LC-MS measurements were carried out for entries 1, 3 and 5. Unfortunately, I could not find the expected bis(cyclopeptide) masses in the corresponding mass spectra neither in the positive nor in the negative mode even under different LC-MS conditions. It was only possible to characterize the library containing linkers **89** and **90** (Chart 15) by collecting fractions during an HPLC run and measuring MALDI-TOF MS spectra in the positive mode thereafter. These spectra confirmed that all three new products represented bis(cyclopeptides). Interestingly, two of the bis(cyclopeptides) contained different kinds of linkers. The peak at 17.6 min corresponds to a bis(cyclopeptide) (**A**) with two linkers deriving from **90** and one from aromatic linker **89**, while the peak at 20.1 min derives from bis(cyclopeptide) (**B**) with two aromatic and one aliphatic linker. The *m/z* ratio recorded for the third peak (26.5 min) matched with bis(cyclopeptide) **98** containing three linkers derived from **89**. Inspection of the relative peak areas of the formed bis(cyclopeptides) shows that the sulfate anion

induces a distribution **A**:**B**:**98** close to 3:1.5:1. Thus, bis(cyclopeptide) **A** containing two aliphatic linkers is predominantly formed, followed by **B** and finally compound **98**. The fact that compound **A** has the largest amplification factor does not necessarily mean that it is also a better sulfate receptor than bis(cyclopeptides) **B**, **98**, or **100**, which was not amplified at all. Corbett et al. recently pointed out that in mixed libraries members that require a smaller number of a particular building block have a competitive advantage over those library members that contain more of the same building block.[86] This means that the chances of forming mixed bis(cyclopeptides) with two and one linker molecules of **89** and **90** are higher than those of forming bis(cyclopeptides) in which the three linkers are of only one type if anion affinity of the products is not too different. However, analysis of the relative amounts of each bis(cyclopeptide) present in the library shows that species containing aliphatic linker **90** in their structure are favored. This suggests that bis(cyclopeptides) that include more units derived from **90** are better sulfate receptors than bis(cyclopeptides) containing the aromatic linker. This assumption is consistent with previous work on singly- and doubly-linked bis(cyclopeptides) and with the higher ease of forming triply-linked bis(cyclopeptide) **100** in comparison to **98**.

On the basis of this study it may be concluded that forming triply-linked bis(cyclopeptides) containing different types of linkers are accessible via DCC. The combination of rigid and more flexible linkers in these bis(cyclopeptides) could give rise to interesting binding properties that has to be evaluated in more detail in future studies.

4.4 Isolation of triply-linked bis(cyclopeptides)

Subsequently, isolation and purification of triply-linked bis(cyclopeptides) **98**-**101** was undertaken. For this purpose, sulfate templated libraries containing trithiol **68** as starting material and linkers **89** or **90** were scaled-up.

Using more than 20 mg of **68** in these syntheses turned out to be unpractical, which is why syntheses of bis(cyclopeptides) were usually performed on this scale. When using linker **90** the reactions reached equilibrium after approximately the same period of time as in the small-scale reactions (7 - 9 days). Reaction mixtures containing **89**, on the other hand, required longer reaction times (10 - 11 days) than in the previous experiments. To halt the disulfide exchange, the mixtures were acidified with diluted sulfuric acid to a pH of 3. After evaporation of the solvent and dissolution of the residue in DMSO aliquots of the resulting solution were subjected to semi-preparative HPLC purification. Under the conditions of preparative reverse-phase (RP) chromatographic purification it turned out to be impossible to separate compounds containing three linkers from those containing four linkers. Another problem in the isolation of bis(cyclopeptides) **98** and **100** consisted in the removal of the sulfate salts which could not be fully separated during the HPLC runs. To remove these salts sulfate for chloride exchange was attempted by addition of an excess of $BaCl_2$ prior to the chromatographic purification. Still, the

sulfate complexes of **98** and **100** were obtained (**98**•SO_4 and **100**•SO_4, respectively). Using ^{1}H-NMR and MALDI-TOF MS analyses I could demonstrate that the materials thus obtained contained sulfate anions most likely enclosed in the cavity formed between the peptide rings. This result can be attributed to the high sulfate affinity of the corresponding bis(cyclopeptides). Therefore, I decided to isolate bis(cyclopeptides) **98** and **100**, and if possible the minor products **99** and **101**, as the corresponding sulfate complexes.

The corresponding protocol for isolation of the sulfate complexes of bis(cyclopeptides) **98**-**101** involved RP chromatographic purification using semi-preparative HPLC and an acetonitrile/water eluent that contained not only TFA (0.05 vol %) but also Na_2SO_4 (0.1 wt %) as additive. These additives improved separation, sharpened the chromatogram peaks and allowed for increase of the injected volume. The combined fractions of pure product were further worked-up by evaporation, filtration, washing and centrifugation to remove residual TFA and sulfate salts. Thus, a yield of the sulfate complexes of **98** and **100** containing three linker molecules of 19 % (5 mg) and 24 % (6 mg), respectively, could be achieved while sulfate complexes of minor compounds **99** and **101** enclosing four linkers between the two cyclopeptide moieties were obtained in only 3 % yield (ca. 1 mg each). All complexes most likely contain sodium ions as counterions.

Unfortunately, I could not find a satisfactiory explanation why libraries that proceed almost quantitatively on the analytical scale give so poor yields when scaled-up (19 - 24 %). Yet, the yield of doubly-linked bis(cyclopeptides) isolated under similar conditions was low as well (14 - 52 %).[197] Partially, I could attribute this outcome to the extensive work-up prior and after the chromatographic purification. Another reason may be the fact that peaks for products containing three and four linkers have similar retention times, separated by 2 - 3 min (**98** vs. **99** and **100** vs. **101**), and isolation is therefore associated with some loss of material.

Although the amount of isolated bis(cyclopeptides) **98** and **100** was small preliminary investigations and anion binding studies by means of ^{1}H-NMR spectroscopy could be performed.

4.5 Characterization of triply-linked bis(cyclopeptides)

Among the bis(cyclopeptides) that could be isolated, my attention was mainly directed to **48**•SO_4 because this compound could be obtained in largest amounts. Prior to characterization molecular modeling studies were performed.

The calculated structures of capsule **48** containing a sulfate anion are depicted in Figure 33. Structures show that these anions nicely fit inside the cavity of **48** where they are completely surrounded by the host. Furthermore, the space filling representation shows that the cyclopeptide units come into close contact as observed in related bis(cyclopeptide) anion complexes.[130b]

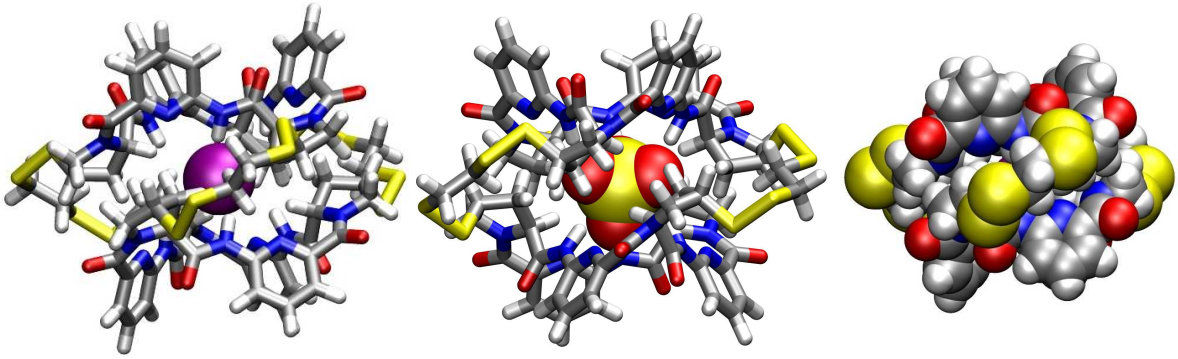


Figure 33 Molecular modeling of cage **100** containing three 1,2-ethanedithiol linkers and iodide (a) or sulfate (b and c) as the bound anion. The sulfate complex is shown as a stick and a space filling model (MMFF force field, gas phase, Mac Spartan, Wavefunction, Inc).

Unfortunatelly, attempts to grow crystals from Na_2SO_4 or tetrabutylammonium sulfate (TBAS) containing solutions of the sulfate complex of **100** failed. Therefore, the results of the calculations could not be supported by experimental evidence.

^{1}H- and ^{13}C-NMR spectra of **100** in 2:1 (v/v) $CDCN_3/D_2O$ show only one signal for each chemically identical proton or carbon atom consistent with a C_3 symmetrical structure. The resonance at ca. 7 ppm of the proline H(α) protons is a typical indication that the sulfate anion is bound between the cyclopeptide rings (Chart 16). Previous investigations have shown that anion binding of such bis(cyclopeptides) causes the proline H(α) signals to shift downfield from ca. 5.5 ppm for the non-complexed form to ca. 6.5-7.0 ppm for the sulfate complex.[54, 198] The reason is that the proline H(α) protons are located inside the bis(cyclopeptide) cavity where their resonance in the ^{1}H-NMR is influenced by the bound anion. The fact that addition of Na_2SO_4 to the NMR sample of **100** causes no further downfield shift indicated that the equilibrium already lies completely on the side of the complex suggesting a high affinity of the sulfate complex of **100**.

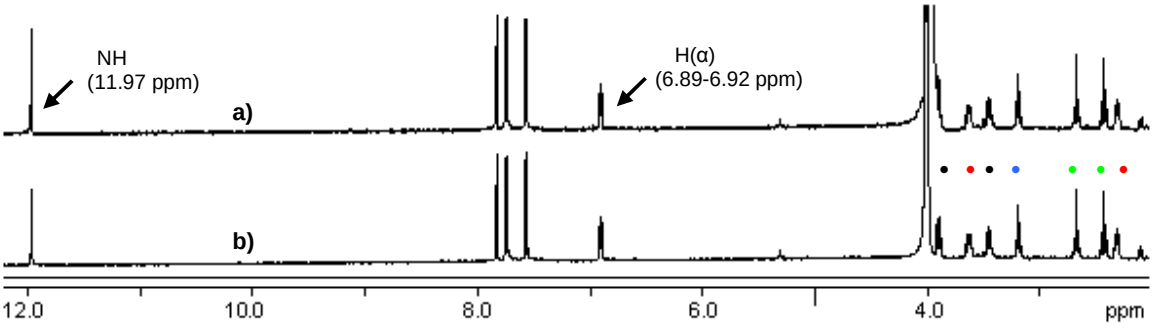


Chart 16 ^{1}H-NMR spectra of isolated **100** (0.66 mM, 2:1 (v/v) CD_3CN/D_2O) in the absence (a) and in the presence (b) of 2.0 equiv. of Na_2SO_4. Aliphatic protons are marked with dots in different colors (red for H(β), blue for H(γ), black for H(δ), and green for CH_2 from spacer protons).

Notably, also the signals for the NH protons are visible in the ^{1}H-NMR spectrum as a sharp singlet despite of the fact that the spectrum was recorded in a protic solvent mixture where H/D (proton-deuterium) exchange is usually very fast. This indicates that the NH protons, which are also located in the cavity of the bis(cyclopeptide) and involved in hydrogen-bonds with the sulfate anion, are well shielded from the surrounding solvent.

Mass spectrometric characterization confirmed that the product isolated indeed represents the sulfate complex of **100**.The ESI-TOF MS spectrum of **100** recorded in the negative mode essentially only contains a single peak at 930.8 *m/z* (Chart 17). The isotopic pattern and the *m/z* ratio of this peak are consistent with the doubly-negatively charged sulfate complex of **100**. Minor peaks are also visible that correspond to the empty deprotonated cage, and to the bis(cyclopeptide) associated with $NaSO_4^-$, HSO_4^- and Cl^-. Fragmentation of the molecular ion in the ESI-MS/MS was also possible in the negative mode. Detected species correspond to fragments formed by loss of one C_2H_4 and one $C_2H_6S_4$ group. Since naked sulfate anions are unstable in the gas phase, the detection of the sulfate complex of **100** in the gas phase indicates a stabilization of the anion through multiple hydrogen bonds.[214] The spectrum in the positive mode showed the existence of species of the capsule containing additional sodium cations and protons (**100**•SO_4 + 2Na^+ + H^+ and **100** + Na^+ + H^+).

Analogously, characterization of cage **98** containing 1,3-benzenedithiol linkers was performed. ^{1}H-NMR spectra of bis(cyclopeptide) **98** recorded in the same solvent mixture as **100** showed one set of signals for the chemically identical protons from the two cyclopeptide rings illustrating that **98** also has C_3 symmetry (Chart 18). Comparison of the ^{1}H-NMR spectra of **98** obtained in the absence and the presence of an excess of Na_2SO_4 showed no further shifts of the resonances of the NH and proline H(α) protons. The result indicates that also in this case the sulfate complex **98** was isolated. Addition of a sulfate salt to the NMR sample of **98** only caused sharpening of the signals and improved the quality of the recorded spectrum. This effect was attributed to the existence of a minor amount of non-complexed bis(cyclopeptide) in the solution that was converted into the corresponding sulfate complex when Na_2SO_4 was added. Subsequent ESI-TOF MS experiment conducted with the NMR sample of **98** confirmed the composition of the 1:1 sulfate complex of **98** by the presence of a single peak at 1003.1 *m/z*. Note that in the MS spectrum obtained for the sulfate complex of **98** two of the amide protons were exchanged with deuterium.

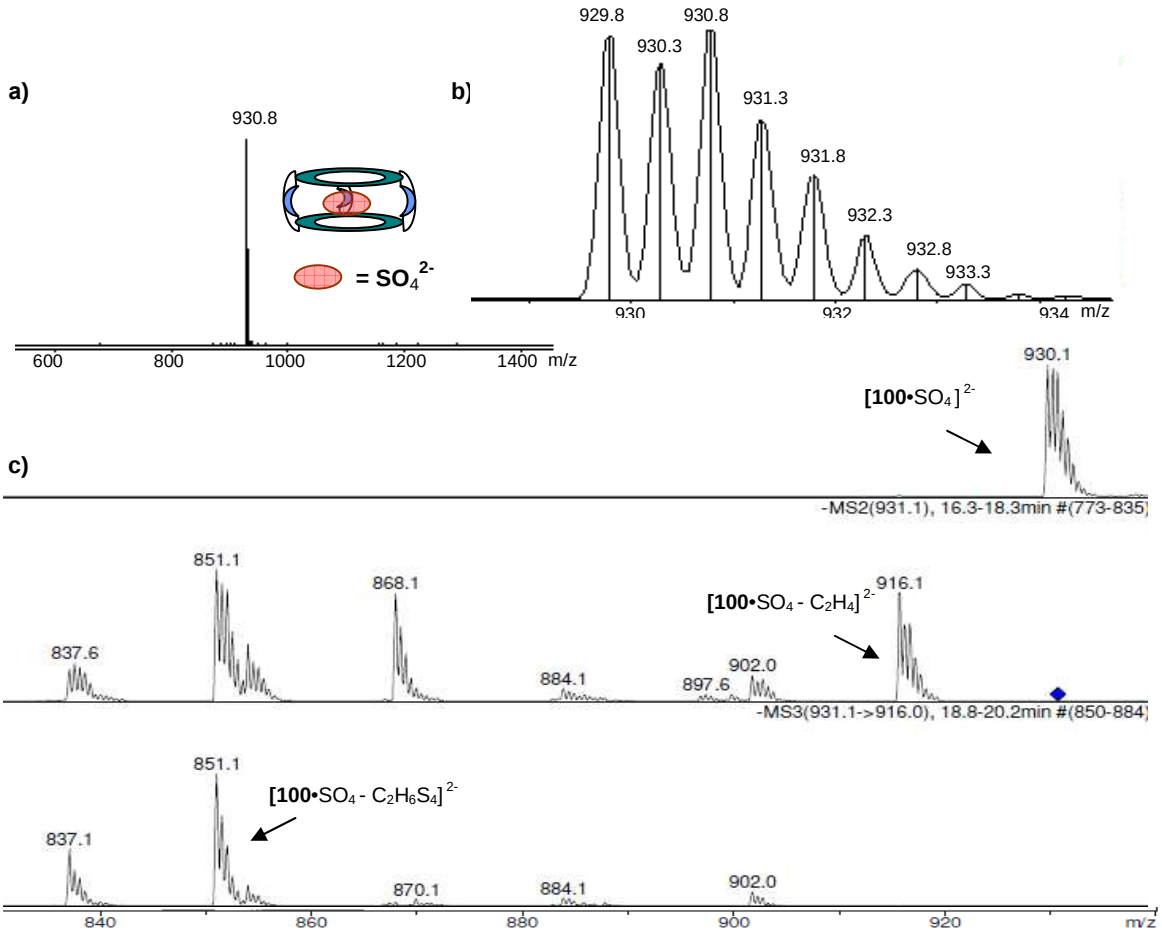


Chart 17 ESI-TOF MS spectrum of **100** in 2:1 (v/v) CH_3CN/H_2O. The *m/z* ratio (a) and the isotopic pattern (b) correspond to half the mass of the sulfate complex (*m/z* calcd. $C_{72}H_{72}N_{18}O_{12}•SO_4^{2-}$ 930.09). (c) ESI-MS/MS fragmentation of the peak at 930.8 *m/z* (negative mode).

Hence, one can conclude that both complexes **98** and **100** form cages with rather similar structures in which a sulfate anion is encapsulated. However, when ^{1}H-NMR spectra of **98**•SO_4 and **100**•SO_4 were compared higher resonances of the amide NH and proline H(α) signals were observed (0.2 ppm for NH and 0.5 ppm for H(α)) for **100**•SO_4 than for **98**•SO_4. These spectral differences could account for a tighter binding of the sulfate anion in the cavity of **100**.

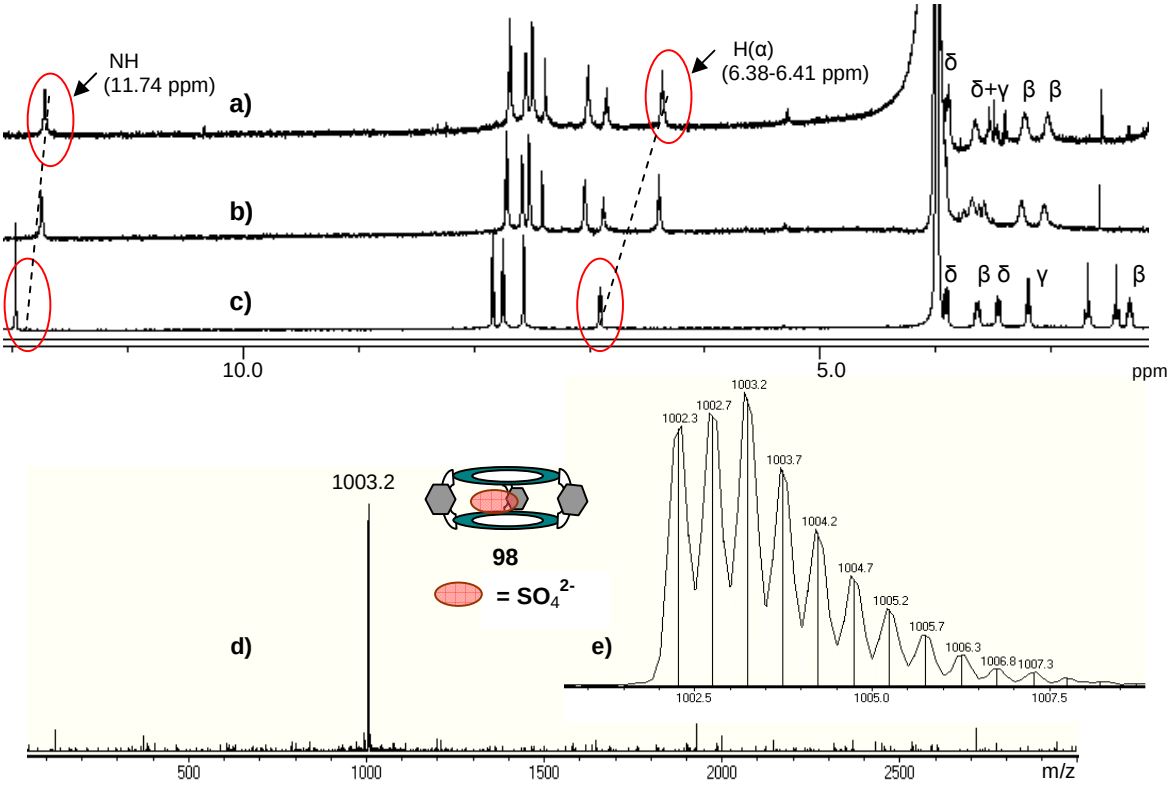


Chart 18 ^{1}H-NMR spectra of the sulfate complex of **98** recorded in 2:1 (v/v) CD_3CN/D_2O (a) before and (b) after the addition of 6.8 equiv. of Na_2SO_4. (c) Reference spectrum of cage **100** with 2.0 equiv. of Na_2SO_4. (d, e) ESI-TOF MS of complex **98** with sulfate and its isotopic pattern (*m/z* calcd. $C_{84}H_{70}D_2N_{18}O_{12}S_{12}\bullet SO_4^{2-}$ 1003.1).

Some structural information was also obtained for the side products observed in the DCLs, namely bis(cyclopeptides) **99** containing linker **89** and **101** containing linker **90**. According to MS spectra both compounds contain four linking units, presumably because two linking units are incorporated in sequence at one linkage between the two cyclopeptide rings. Although only a small amount of pure **101** could be obtained a ^{1}H-NMR spectrum could be recorded (Chart 19). This spectrum clearly shows that the structure of this bis(cyclopeptide) is unsymmetrical, consistent with the structural differences in the linkages. The resonance of the signals of the proline H(α) protons is consistent with the presence of a sulfate anion in the cavity of this compound. This observation was subsequently confirmed by the ESI-TOF MS spectrum of **101** recorded in deuterated solvents. It showed a single peak corresponding to the doubly negatively charged sulfate complex of **101**. The small amount obtained of bis(cyclopeptide) **99** prevented a similar NMR spectroscopic characterization. It was not the primary goal of this thesis to study derivatives **99** and **101**, but isolation of sufficient amounts to allow subsequent comparison of the anion binding affinities with hosts containing three linker units (**98** and **100**) would be interesting.

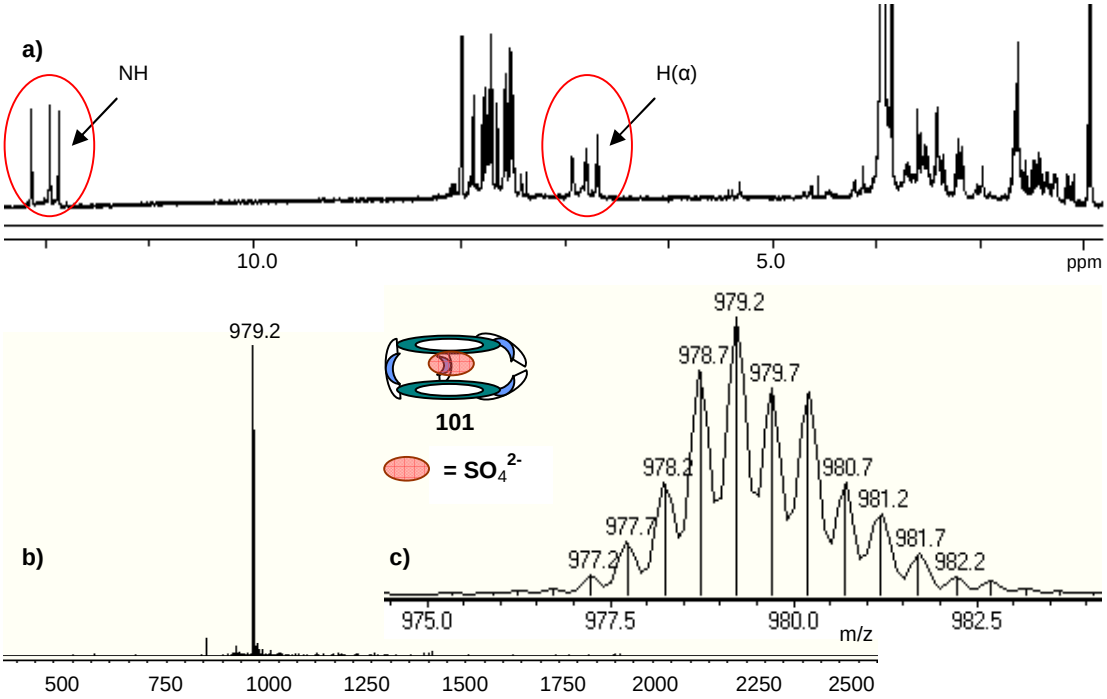


Chart 19 ^{1}H-NMR (a) and ESI-TOF MS (b) spectra of the sulfate complex of **101** recorded in 2:1 (v/v) CD_3CN/D_2O in the presence of 0.14 equiv. of Na_2SO_4 (*m/z* calcd. $C_{74}H_{70}D_6N_{18}O_{12}S_{14}•SO_4^{2-}$ 979.1, negative mode).

4.6 Assessment of purity of sulfate complexes 100 and 98

Because of the small amounts obtained of bis(cyclopeptides) **98** and **100** no attempts to assess purity by elemental analysis were undertaken. Instead purity was evaluated by using ^{1}H-NMR spectroscopy. For this purpose a known amount of an organic salt, namely tetramethylammonium iodide (TMAI), was added to three samples containing a known amount of **100**. TMAI was chosen because it is inert and exhibits a sharp signal for the tetramethylammonium cation in the ^{1}H-NMR that does not overlap with signals of the bis(cyclopeptides). ^{1}H-NMR spectra were then recorded and the areas for the proline H(α) signals and for the tetramethylammonium signal integrated. The proline H(α) signals were used as a reference and their integral was set to 6. From the known amount of TMAI present in the sample and the integral ratio of bis(cyclopeptide) H(α) signals to tetramethylammonium signal it was possible to calculate the absolute amount of **100**•SO_4. Based on this calculation a purity of 70 % for compound **100**•SO_4 was estimated with the residual 30 % corresponding presumably to inorganic impurities.

In the case of **98**•SO_4 similar measurements were conducted. The difference is that aliquots of TMAI were added to three samples containing the iodide complex of **98** (**98**•I). Because of overlap of the signals of proline H(β) protons in **98**•SO_4 with the signal of the TMAI methyl

protons, **98**•SO_4 had to be converted into the corresponding iodide complex prior to measurement. In this case, the purity of **98**•SO_4 is only 59 % with 41 % of impurities.
Unfortunately, the majority of the experiments described in the following sections were already performed when these results were obtained. All experimental conditions were therefore recalculated on the basis of the NMR spectroscopically determined composition of **98**•SO_4 and **100**•SO_4. The presence of impurities in the isolated products also precluded quantitative evaluation of anion affinity of both bis(cyclopeptides).

4.7 Binding studies

Compounds **98** and **100** are the first members of a new family of triply-linked bis(cyclopeptides). The high affinity of these compounds for sulfate anions became already evident during their isolation. As pointed out, isolation of the free bis(cyclopeptides) without a bound anion turned out to be not possible. Another remarkable result was the slow hydrogen-deuterium (H/D) exchange observed for the NH protons of **100** in the ^{1}H-NMR spectrum. The following experiments addressed the questions if the anion is permanently entrapped within the cavity of the bis(cyclopeptides) or whether it could be exchanged for other anions. In addition, the kinetics of H/D exchange in the presence of different anions in solution was studied. These investigations eventually yielded some qualitative results on anion affinity as well as information about the mechanism of guest exchange.
The major part of the experiments in this section was performed with cage **100**, and only selected experiments with **98** because the latter compound was obtained in smaller amounts.

4.7.1 Release of the bound sulfate

The aim of this study was to gain information whether bis(cyclopeptide) **100**•SO_4 is a carcerand, which permanently entraps the sulfate anion, or a hemicarcerand, whose anion-binding is dynamic. To this end, the ability of **100**•SO_4 to release the bound anion under external stimuli was first tested. As a trigger for sulfate release DTT was used that should induce reductive cleavage of the disulfide bridges in **100**•SO_4. By addition of DTT (in excess) to **100**•SO_4 (0.59 mM in 2:1(v/v) CD_3CN/D_2O) and adjustment of the pH to 9 disulfide exchange was initiated. ^{1}H-NMR spectra represented in Chart 20 clearly show that **100**•SO_4 completely disassembles under these conditions. The spectrum of the reaction mixture recorded after addition of DTT exhibits a new set of signals whose pattern resembles the one of the symmetric cyclopeptide precursor (monomer **68**). Unfortunately, structural assignment of this new compound was not possible because the reaction mixture re-equilibrated rapidly. Another notable change is the pronounced upfield shift of the proline H(α) signal (from ca. 6.9 to 4.9 ppm) that indicates a substantially weaker interaction

of the product with the anion with respect to **100**•SO_4. In addition, signals of impurities are visible in the spectrum that could not be assigned. Interestingly, in further spectra recorded after 2 days and after one month, the signals corresponding to **100**•SO_4 reappear indicating that the sulfate complex of this bis(cyclopeptide) is slowly re-formed.

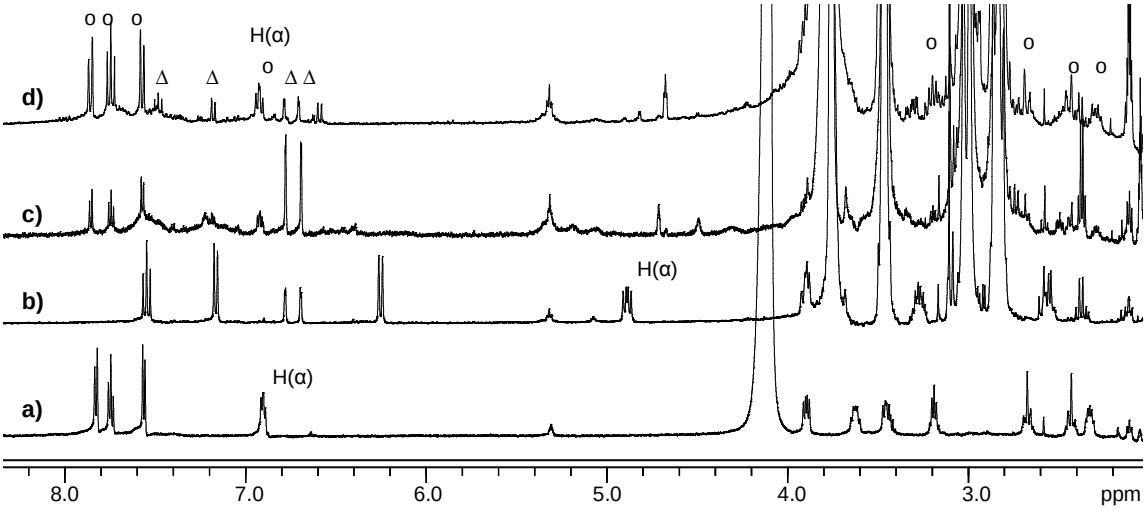


Chart 20 ^{1}H-NMR spectra of **100**•SO_4 (0.59 mM in 2:1(v/v) CD_3CN/D_2O) recorded in the absence (a) and in the presence (b) of DTT (31 equiv.) / NaOH at pH 9. Spectra c) and d) correspond to the reaction mixture recorded after 48h and 1 month, respectively. Signals deriving from **100**• SO_4 and from an unknown compound are marked with, respectively, o and Δ in the upper spectrum. H(α) signals are also assigned.

To gain information whether the sulfate anion can be removed from the intact cage, $BaCl_2$ (0.86 equiv.) was added to a solution of **100**•SO_4 (0.59 mM) in 2:1(v/v) CD_3CN/D_2O and ^{1}H-NMR spectra were recorded. After addition of $BaCl_2$ a new set of signals emerged upfield shifted with respect to those deriving form **100**•SO_4 (Chart 21). During 24 h the intensity of these signals increased at the expense of the intensity of the signals for **100**•SO_4. These signals were assigned to the chloride complex of **100** (**100**•Cl) that formed in small amounts (12 %). Interestingly, this amount is significantly smaller than expected under the assumption that all Ba^{2+} ions present in solution would remove the corresponding amount of sulfate ions by precipitation of $BaSO_4$. Still, the results clearly indicate that sulfate anions can be removed from the cage and exchanged for other anions rendering **100** a hemicarcerand.

Anion exchange is slow on the NMR time-scale as separate signals were observed in the ^{1}H-NMR spectrum deriving from the sulfate and the chloride complexes of **100**. In addition, anion exchange also seems to be slow on the human time-scale requiring ca. 24 h to obtain at about 12 % of **100**•Cl. As expected, reaction becomes faster at elevated temperatures. If one records the

spectrum at 40 °C or heats the sample to 40 °C and then measures the NMR at ambient temperatures sulfate for chloride exchange increased to 47 %.

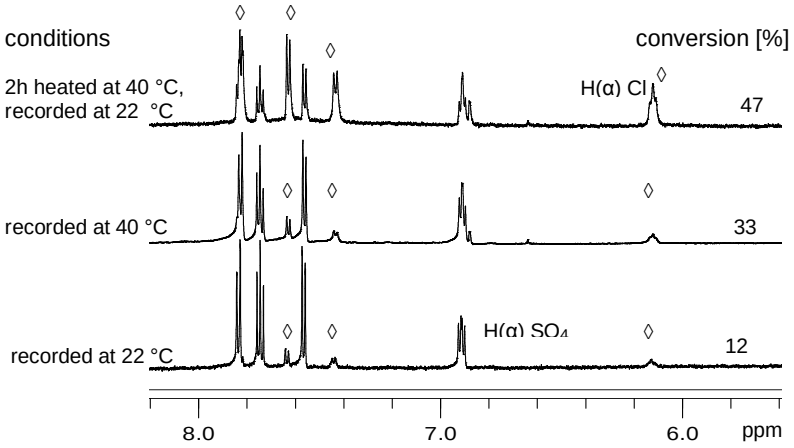


Chart 21 ^{1}H-NMR spectra showing sulfate for chloride exchange of **100**•SO_4 (**100**, 0.59 mM in 2:1(v/v) CD_3CN/D_2O, 0.86 equiv. $BaCl_2$) and the effect of temperature on the extent of exchange. Signals deriving from **100**•Cl are marked with rhombs.

The conclusion of these experiments is that bis(cyclopeptide) **100**•SO_4 is a hemicarcerand able to exchange the bound sulfate with other anions (for example, with chloride). Moreover, the sulfate anion can also be released by reducing **100**•SO_4 with DTT.

4.7.2 Results

4.7.2.1 Anion exchange

As I could demonstrate that sulfate is not permanently entrapped in the cavity of bis(cyclopeptide) **100**, I was interested to study anion exchange of **100**•SO_4 systematically. To this end, the bound sulfate anion in **100**•SO_4 was replaced by other anions of different size, shape and charge under appropriate conditions. The amounts of the new complexes formed were quantified ^{1}H-NMR spectroscopically. In addition, NMR spectroscopy provided information about the extent to which **100** interacts with anions by following the characteristic shift of the proline H(α) signals. Because of the inorganic impurities found in compounds **98**•SO_4 (30 %) and **100**•SO_4 (41 %), these experiments could only be qualitative. However, they still provided information about the versatility of **100** as anion receptor.

Anions used for these experiments include tetrahedral anions of different charge (ReO_4^-, BF_4^-, SO_4^{2-}, SeO_4^{2-}, and PO_4^{3-}) some of which contain bulky groups (BPh_4^-). Anions varied in their structure (halides, NO_3^-, CO_3^{2-}, and PF_6^-) and in their size. In addition, large organic anions such

as tosylate (TsO^-) were also considered. Anions used for the binding studies are summarized in Table 8 grouped in accordance to their geometry and size.

shape	anions (ordered according to their size)
spherical	$I^- > Br^- > Cl^-$
trigonal planar	$NO_3^- \sim CO_3^{2-}$
tetrahedral	$BPh_4^- > ReO_4^- > SeO_4^{2-} > HPO_4^{2-} / H_2PO_4^- > BF_4^- > SO_4^{2-}$
octahedral	PF_6^-
more complex	4-$CH_3C_6H_4SO_3^-$ (TsO^-)

Table 8 Anions used for the binding studies arranged by geometry and size.

For anion exchange experiments samples of compound **98** (0.5 mM in 2:1(*v/v*) CD_3CN/D_2O) or **100** (0.59 mM in 2:1(*v/v*) CD_3CN/D_2O) were prepared at fixed concentrations containing an additional small amount of sodium sulfate (0.17 equiv. and 0.14 equiv. for **98** and **100**, respectively). Addition of Na_2SO_4 prior to the experiment was necessary to transform species in solution other than the sulfate complexes of these bis(cyclopeptides) into the corresponding sulfate-bound forms.

Because of the high affinity of the bis(cyclopeptides) for sulfate anions, direct replacement of the bound anion by addition of an excess of a salt containing another potential anionic substrate proved to be impossible. Of all studied anions, solely selenate, which has a similar geometry, charge, and size as sulfate, allowed direct replacement. In the presence of 11.3 equiv. of Na_2SeO_4 44 % of **100**•SO_4 could be converted into the corresponding selenate complexes (**100**•SeO_4) as shown by integrating characteristic signals of the sulfate and selenate complexes of **100** in the ^{1}H-NMR (Chart 25).

For sulfate to be replaced by other anions a sequential exchange therefore had to be used. A schematic representation of the general procedure of these NMR experiments is shown in Chart 22. Accordingly, **100**•SO_4 was initially converted into a complex containing a weakly bound anion (i.e. iodide or chloride complexes) which was subsequently replaced by a more strongly bound anion upon addition of another salt. Furthermore, effects of the formation of insoluble salts on the complexation equilibria were utilized.

Specifically, the sulfate complex of **100** was initially transformed into the iodide complex (**100**•I) by addition of BaI_2 to the solution of **100**•SO_4. The small solubility product of $BaSO_4$ ($K_{sp\ (BaSO4)}$ = 1.1 x 10^{-10} M^2 at 25 °C in water), which forms under these conditions, should prevent sulfate from participating further in the exchange process. This reaction is schematically depicted in Figure 34.[213] Next, silver salts were added to cause precipitation of AgI ($K_{sp\ (AgI)}$ = 8.5 x 10^{-17} M^2 at 25 °C in water) and complexation of the anion of the silver salt. If no silver salt of an anion in question was available, **100**•SO_4 was first converted to the chloride complex, which is less stable than the

iodide complex. In this case, bound chloride anions could easily be replaced by more strongly bound anions by adding respective sodium salts to these solutions.

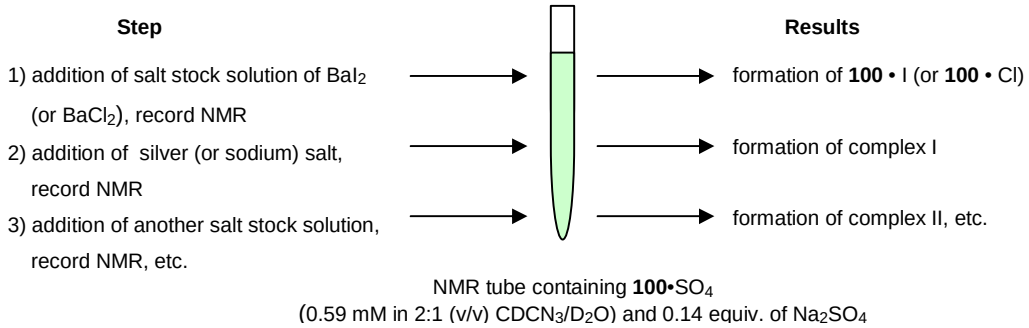


Chart 22 Sequential anion exchange experiments with **100**•SO_4 conducted in an NMR-tube.

Qualitative assessment of anion binding of **100** was made by following the chemical shifts of the proline H(α) and (if possible) amide NH signals and/or comparing the integrals of signals belonging to different complexes as a function of anion concentration in the ^{1}H-NMR spectra. A summary of the results of all anion exchange experiments is depicted in the Experimental part (Chart 35). ^{1}H-NMR spectra of the different anion complexes thus obtained, arranged by the chemical shifts of the proline H(α) signals, are shown in Chart 23. Additionally, the chemical shifts of these signals and, where available, of the NH signals together with the size of the anionic guests are summarized in Table 9.

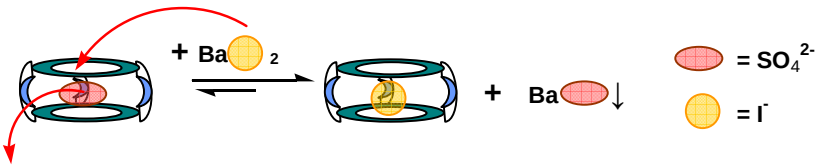


Figure 34 Initial step of an anion exchange experiment consisting in replacement of the sulfate anion by an iodide anion and precipitation of $BaSO_4$.

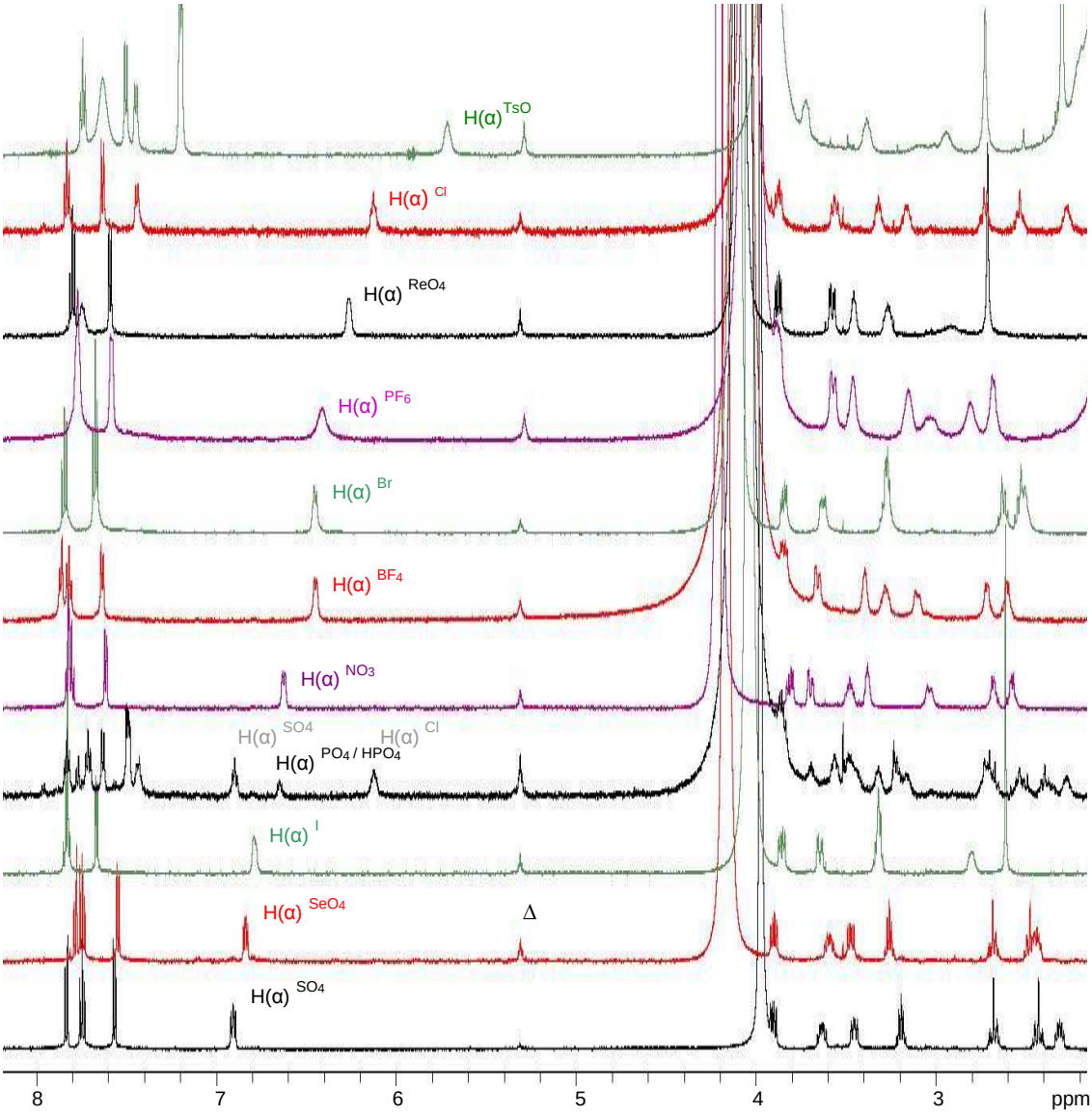


Chart 23 ^{1}H-NMR spectra of various anion complexes of **100** obtained from **100**•SO_4 (0.59 mM in 2:1 (v/v) CD_3CN/D_2O, Na_2SO_4, 0.14 equiv.) arranged by the resonances of the proline H(α) protons. The signal denoted Δ was assigned to an impurity that was introduced with the addition of D_2O.

complex of 100 with	anion radius / Å	δ H (α) / ppm	δ NH / ppm
SO_4^{2-}	2.30	6.89-6.92	11.97
SeO_4^{2-}	2.43	6.81-6.87	11.93
I^-	2.20	6.72-6.85	9.09
CO_3^{2-}	1.78	6.67-6.70	n.a.
PO_4^{3-}/ HPO_4^{2-}	2.38/2.00	6.60-6.68	n.a.
NO_3^-	1.79	6.58-6.67	n.a.
BF_4^-	2.32	6.40-6.50	n.a.
Br^-	1.96	6.40-6.50	9.25
PF_6^-	2.46	6.30-6.50	n.a.
ReO_4^-	2.60	6.22-6.30	n.a.
Cl^-	1.81	6.12-6.15	9.58
4-$CH_3C_6H_4SO_3^-$ (TsO^-)	~ 1.20	5.65-5.80	n.a.
BPh_4^-	4.20	[a]	

Table 9 Size of anions used for the binding studies and resonances of the proline H(α) and NH protons observed in the corresponding complexes of **100**. Chemical shifts were obtained from ^{1}H-NMR spectra of **100**•SO_4 (0.59 mM in 2:1 (v/v) CD_3CN/D_2O, Na_2SO_4, 0.14 equiv.) after anion exchange (n.a. - not available, [a] - no complexation with this anion was observed).

Exchange with halides in 100

The extent to which the sulfate complex of **100** could be converted into the corresponding halide complexes by using different amounts of barium halides was investigated by using two different experiments. First, the amount of halide complexes formed after equilibrium was reached was quantified when adding the same amounts of $BaCl_2$, $BaBr_2$, BaI_2 (1.72 equiv.) to the stock solution of **100**•SO_4 (0.59 mM in 2:1 (v/v) CD_3CN/D_2O, Na_2SO_4, 0.14 equiv.). Under these conditions, complete anion exchange was only observed in the case of BaI_2, while 85 % of the bromide complex was formed and only 79 % of the chloride complex (Table 10).

	% conversion after	
salt	**24h**	**48h**
BaI_2	100%	100%
$BaBr_2$	78%	85%
$BaCl_2$	79%	79%

Table 10 Extent of conversion of **100**•SO_4 into halide complexes induced by addition of 1.72 equiv. of BaI_2, $BaBr_2$ or $BaCl_2$ to **100**•SO_4 (0.59 mM in 2:1 (v/v) CD_3CN/D_2O, Na_2SO_4, 0.14 equiv.).

Chart 24 shows the evolution of the ^{1}H-NMR spectra over time when BaI_2 was used as guest. Interestingly, the amide NH signal of the bis(cyclopeptide) is still visible after 24 h when exchange is complete. In the intermediate region both NH signals for the sulfate and the iodide complex can be observed.

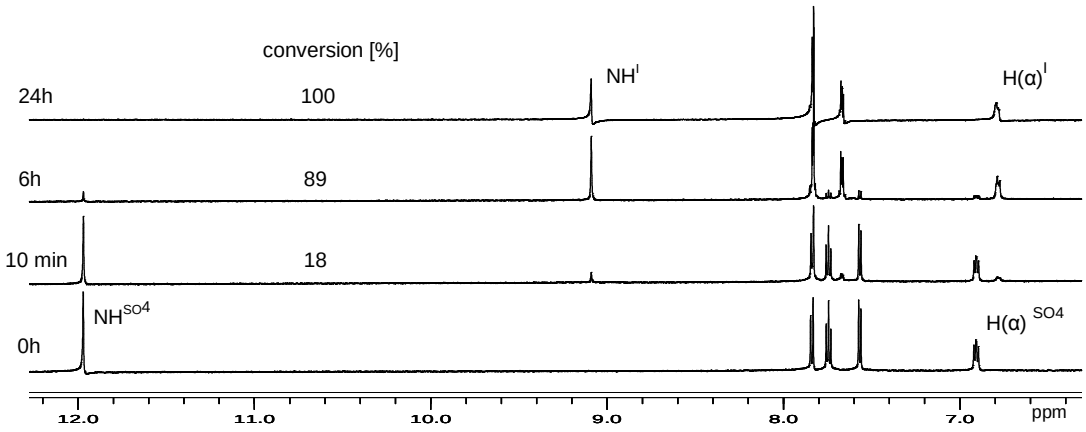


Chart 24 Time-dependence of the ^{1}H-NMR spectrum of **100**•SO_4 (0.59 mM in 2:1 (v/v) CD_3CN/D_2O, Na_2SO_4, 0.14 equiv.) after the addition of 1.72 equiv. of BaI_2.

A second experiment aimed at the determination of the amount of barium halide needed for complete conversion of **100**•SO_4 into the corresponding halide complex (**100**•X). To this end, increasing amounts of BaI_2, $BaBr_2$ or $BaCl_2$ were added to **100**•SO_4 (0.59 mM in 2:1 (v/v) CD_3CN/D_2O, Na_2SO_4, 0.14 equiv.). The NMR spectra thus obtained are depicted in Chart 32 and the results are summarized in Table 11. It is evident that the amount of barium salt for 100 % conversion decreases from chloride over bromide to iodide.

salt	equiv. of salt required for 100 % conversion
BaI_2	1.72
$BaBr_2$	3.43
$BaCl_2$	5.15

Table 11 Equivalents of barium salts needed to achieve complete conversion of **100**•SO_4 into halide complexes of **100** (**100**•X).

Exchange with oxyanions and other anions in 100

In the course of the experiments it became evident that exchange of sulfate by selenate is possible by addition of Na_2SeO_4 to the solution of **100**•SO_4 (0.59 mM in 2:1 (v/v) CD_3CN/D_2O, Na_2SO_4, 0.14 equiv.) (Chart 25).

However, complete conversion into **100**•SeO_4 by adding an excess of Na_2SeO_4 was not achieved. In the presence of 11.29 equiv. of Na_2SeO_4 the conversion amounted to only 44 %. It should be noted that the amide signals of both complexes **100**•SO_4 and **100**•SeO_4 appeared as sharp singlets at the beginning of anion exchange but became broader with time and split into

several peaks. Complete conversion into **100**•SeO_4 can be achieved by adding Na_2SeO_4 (5.72 equiv.) to **100**•Cl.

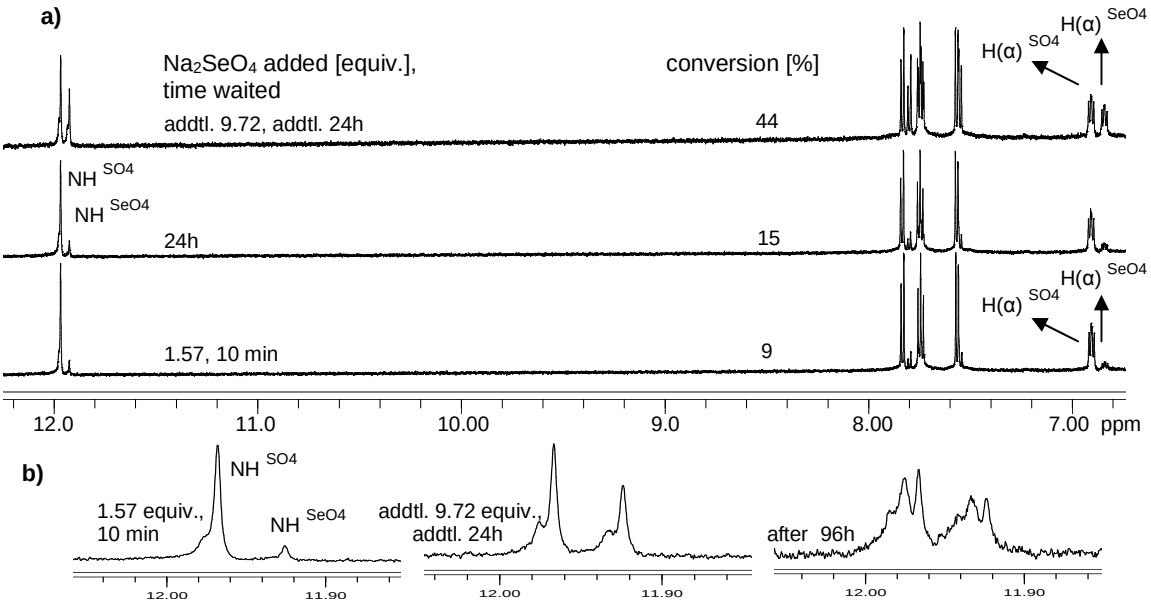


Chart 25 (a) ^{1}H-NMR spectra showing the formation of **100**•SeO_4 after addition of 1.57 equiv. and another 9.72 equiv. (altogether 11.29 equiv.) of Na_2SeO_4 to **100**•SO_4 (0.59 mM in 2:1(v/v) CD_3CN/D_2O, Na_2SO_4, 0.14 equiv.). (b) Development of amide NH signals of **100**•SO_4 and **100**•SeO_4 with time.

The fact that **100**•SO_4 and **100**•SeO_4 are in equilibrium in the presence of an excess of Na_2SeO_4 without having to form insoluble salts that shift the equilibrium allows one to estimate the ratio of the complex stabilities of both complexes K_a^{SO4} / K_a^{SeO4}. The underlying equations are summarized in Scheme 39.

The two mass action laws of forming the sulfate and the selenate complexes must be considered (**1**) and (**2**). The ratio K_a^{SO4} / K_a^{SeO4} can then be expressed with equation (**3**). The mass balances (**4**) and (**5**) allow calculation of the concentrations of [SeO_4] and [SO_4] from the ratio of the two complexes in the equilibrium derived from integration of the respective proline H(α) signals in the ^{1}H-NMR of the mixture and the known overall concentrations of $[SeO_4]_0$ and $[SO_4]_0$.

According to the ^{1}H-NMR spectrum of the reaction mixture 15 % of **100**•SeO_4 and 85 % of **100**•SO_4 are formed in the presence of 1.57 equiv. of Na_2SeO_4, yielding concentrations of both complexes of 8.8 x 10^{-5} M and 4.99 x 10^{-4} M, respectively. Using these values in equation (**6**) provides a K_a^{SO4} / K_a^{SeO4} ratio of 28. Applying the same calculations for the reaction mixture in the presence of 11.29 equiv. of Na_2SeO_4 (concentrations of both complexes **100**•SeO_4 (44 %) and **100**•SO_4 (56 %) are, respectively, 2.58 x 10^{-4} M and 3.29 x 10^{-4} M) one obtains a K_a^{SO4} / K_a^{SeO4}

ratio in the same order but slightly smaller amounting to 24. Hence, the affinity of **100** to the sulfate anion is on average ca. 26 times higher than to the selenate anion.

$$\mathbf{100} + SO_4 \rightleftharpoons \mathbf{100}\bullet SO_4 \qquad \mathbf{100} + SeO_4 \rightleftharpoons \mathbf{100}\bullet SeO_4$$

(**1**) $K_a^{SO4} = \dfrac{[\mathbf{100}\bullet SO_4]}{[\mathbf{100}][SO_4]}$ (**2**) $K_a^{SeO4} = \dfrac{[\mathbf{100}\bullet SeO_4]}{[\mathbf{100}][SeO_4]}$ (**3**) $\dfrac{K_a^{SO4}}{K_a^{SeO4}} = \dfrac{[\mathbf{100}\bullet SO_4][SeO_4]}{[SO_4][\mathbf{100}\bullet SeO_4]}$

(**4**) $[SeO_4] = [SeO_4]_0 - [\mathbf{100}\bullet SeO_4]$ $[SeO_4]_0 = 9.25 \times 10^{-4}$ M (**7**) $[\mathbf{100}\bullet SeO_4] = 8.80 \times 10^{-5}$ M

(**5**) $[SO_4] = [SO_4]_0 - [\mathbf{100}\bullet SO_4]$ $[SO_4]_0 = 6.66 \times 10^{-4}$ M (**8**) $[\mathbf{100}\bullet SO_4] = 4.99 \times 10^{-4}$ M

(**6**) $$\frac{K_a^{SO4}}{K_a^{SeO4}} = \frac{[\mathbf{100}\bullet SO_4]\,([SeO_4]_0 - [\mathbf{100}\bullet SeO_4])}{[\mathbf{100}\bullet SeO_4]\,([SO_4]_0 - [\mathbf{100}\bullet SO_4])} = 28$$

Scheme 39 Estimation of sulfate versus selenate affinity for bis(cyclopeptide) **100**. Charges of anions were omitted for clarity.

Oxyanions that were found to bind to **100** are selenate, nitrate, perrhenate, tosylate, and to some extent phosphate (Chart 30). While complexes with selenate, nitrate, perrhenate, and tosylate were quantitatively formed from the corresponding **100**•Cl or **100**•I complexes, the behavior of phosphate was different. Introduction of an amount of Na_3PO_4 (5.72 equiv.) expected to be sufficient to fully convert **100**•Cl into the corresponding phosphate complex resulted in only 22 % of phosphate complex, accompanied by 45 % of **100**•Cl and 33 % of **100**•SO_4. After 24 h the reaction mixture was predominantly composed of **100**•SO_4 and no phosphate complexes were detected. It should be pointed out that under the conditions of the experiment it can be expected that HPO_4^{2-} and $H_2PO_4^-$ are the predominant species of the phosphate anion in solution. A similar decrease of the initially formed anion complex with time was also observed for other anions (BF_4^- and PF_6^-)

An oxyanion that caused only insignificant changes to the ^{1}H-NMR spectrum of **100**•Cl is carbonate. Even added in great excess (12.5 equiv. of Na_2CO_3) to the chloride complex of **100**, the carbonate anion was not able to induce formation of **100**•CO_3. Only slow formation of **100**•SO_4 was observed, most likely due to formation of $BaCO_3$ ($K_{sp\ (BaCO3)}$ 8.0×10^{-9} M ^{2}).

Minor changes in the ^{1}H-NMR spectrum of **100**•I were observed after addition of 3.58 equiv. of $AgBPh_4$ which is usually enough to displace iodide from the complex. Again slow formation of **100**•SO_4 (3 %) was monitored at the expense of **100**•I (77 %). It should be noted that the low solubility of $AgBPh_4$ in the used solvent mixture (2:1(v/v) CD_3CN/D_2O) caused its precipitation and precluded further analyses. In this case, precipitation of AgI was not observed.

Another interesting effect was observed in the presence of BF_4^- and PF_6^- anions. When the silver salt (3.58 equiv.) of either of these anions was added to **100**•I quantitative formation of **100**•BF_4 or **100**•PF_6 was observed. With time the spectra changed and the **100**•BF_4 or **100**•PF_6 complexes were slowly replaced by the sulfate complex of **100**. This exchange was followed over 7 days in the case of **100**•BF_4. At that time the amount of **100**•BF_4 in the sample decreased to 91 % and the amount of **100**•SO_4 increased to 9 %. In comparison, conversion of **100**•PF_6 into **100**•SO_4 is more pronounced. After 7 days ca. 45 % of **100**•SO_4 were present and the amount of **100**•SO_4 increased to 70 % after 30 days. This process is illustrated in Chart 26. The slow dissociation of **100**•PF_6 was additionally followed by ^{19}F-NMR. With time a continuous upfield shift and sharpening of the two ^{19}F-signals of the PF_6^- anions were observed, indicative for the release of the guest from the cavity of the bis(cyclopeptide). A comparison between the ^{1}H-NMR spectra of **100**•BF_4 and **100**•PF_6 is depicted in Chart 34 of the discussion.

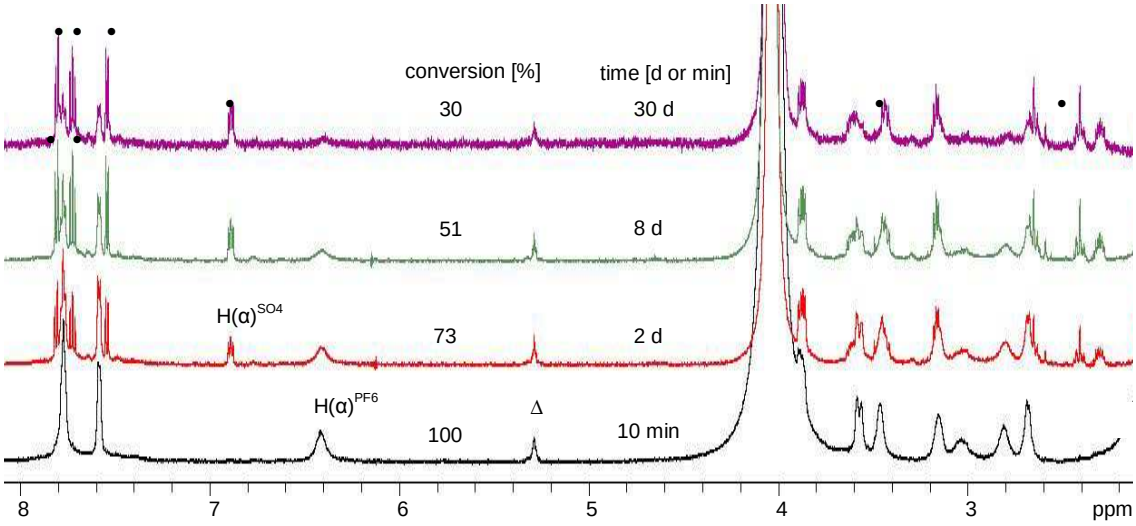


Chart 26 ^{1}H-NMR spectra demonstrating the slow release of PF_6^- form the cavity of **100**•PF_6 and its substitution by SO_4^{2-} with time. Signals of **100**•SO_4 (0.59 mM in 2:1 (v/v) $CDCN_3/D_2O$, Na_2SO_4, 0.14 equiv.) are marked with • . The impurity denoted Δ was introduced by addition of D_2O.

Exchange with halides in 98

Due to the small amount obtained from **98**•SO_4, this compound was used only for binding studies with halide anions. Bis(cyclopeptide) **98** was shown to release the bound sulfate anion and to bind halide anions after addition of barium halides to a solution of **98**•SO_4 in 2:1 (v/v) CD_3CN/D_2O. Equilibrium was reached invariably within 24 h. Table 12 shows the amount of barium salt needed to achieve full exchange in these experiments.

salt	equiv. of salt required for 100 % conversion
BaI_2	2.03
$BaBr_2$	> 2.03
$BaCl_2$	3.04

Table 12 Equivalents of barium salts needed to achieve complete conversion of **98**•SO_4 to the corresponding halide complexes (**98**•X).

The spectra obtained for stepwise formation of the chloride complex of **98** by addition of increasing amounts of $BaCl_2$ are depicted in Chart 27. These spectra show that to fully displace the sulfate in **98**•SO_4 by a chloride anion, 3.04 equiv. of $BaCl_2$ are necessary. In comparison, full conversion into **98**•I requires only 2.03 equiv. of BaI_2. Studies conducted with $BaBr_2$ were not well reproducible, unfortunately, but the amount of $BaBr_2$ was similar or larger as the amount of BaI_2 needed for full conversion. Observed resonances of proline H(α) signals in the anion complexes of **98** are summarized in Table 13.

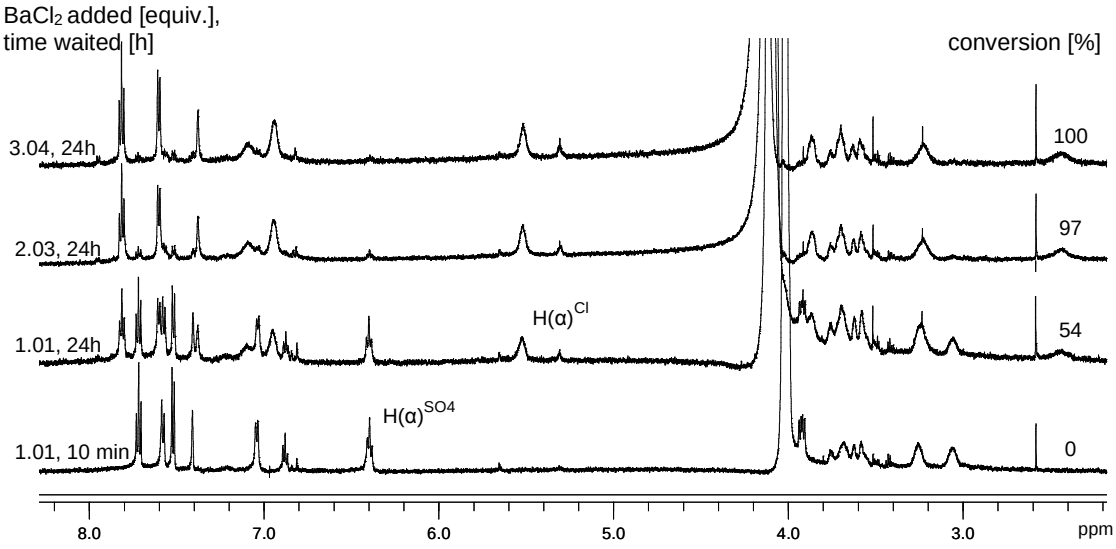


Chart 27 ^{1}H-NMR spectra recorded of **98**•SO_4 (0.50 mM, 0.12 µmol in 2:1(v/v) CD_3CN/D_2O, Na_2SO_4, 0.02 µmol, 0.17 equiv.) in the presence of increasing amounts of $BaCl_2$. Each spectrum was recorded 24 h after salt addition.

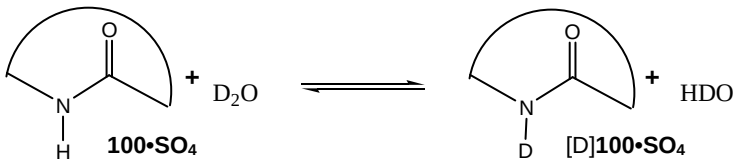
As visible in Table 13 and depicted in Charts 32 and 33, the resonances of the proline H(α) signals in the halide complexes of **98** are all lower than those of the corresponding signals in the halide complexes of **100**.

complex of 98 with	anion radius / Å	δ H(α) / ppm
SO_4^{2-}	2.30	6.33-6.42
I^-	2.20	6.07-6.12
Br^-	1.96	5.73-5.79
Cl^-	1.81	5.55-5.50

Table 13 Resonances of proline H(α) protons observed in the sulfate and halide complexes of **98**, and anion radii of the anions. Chemical shifts were obtained from ^{1}H-NMR spectra of **98**•SO_4 (0.50 mM in 2:1 (v/v) CD_3CN/D_2O, Na_2SO_4, 0.17 equiv.) after addition of barium salts.

4.7.2.2 H/D exchange

It was observed that amide NH protons buried in the interior of the cavity of **100**•SO_4 exchange with deuterium from the solvent very slowly in 2:1(*v/v*) CD_3CN/D_2O. The exchange of hydrogen by deuterium at one NH group of bis(cyclopeptide) **100**•SO_4 is shown schematically in Scheme 40. With time the signal for the six amide protons observed in the ^{1}H-NMR spectrum of **100**•SO_4 shifted downfield (from 11.96 to 12.02 ppm) and transformed from a sharp singlet into a broad signal exhibiting several individual peaks (Chart 28a). Complete disappearance of the NH signals required more than 46 days.



Scheme 40 Schematic representation of H/D exchange within **100**•SO_4 in protic deuterated solvent mixtures.

Splitting of the signals was attributed to the presence of different deuterated states of the bis(cyclopeptide) in solution that could be detected individually by the distinct shift of the NH protons. In the sample of **100**•SO_4 containing additional 0.14 equiv. of Na_2SO_4 only the complex containing one deuterium could be observed after one day beside a major fraction of the undeuterated bis(cyclopeptide) (Chart 28a). Subsequently, the signal of the undeuterated complex decreased and that of the monodeuterated one increased. A signal of a complex containing two deuterium atoms became visible after 6 days. After 10 days, species containing one to five deuterium atoms were visible. At this stage there was still completely undeuterated bis(cyclopeptide) in solution although in very small amounts. After 46 days the NH signal was very small and presumably only reflected species with higher deuteration states.
Chart 28b shows that deuteration is significantly slower in the presence of 1.86 equiv. of Na_2SO_4 and after 10 days only species containing up to 3 deuterium atoms can be observed in solution.

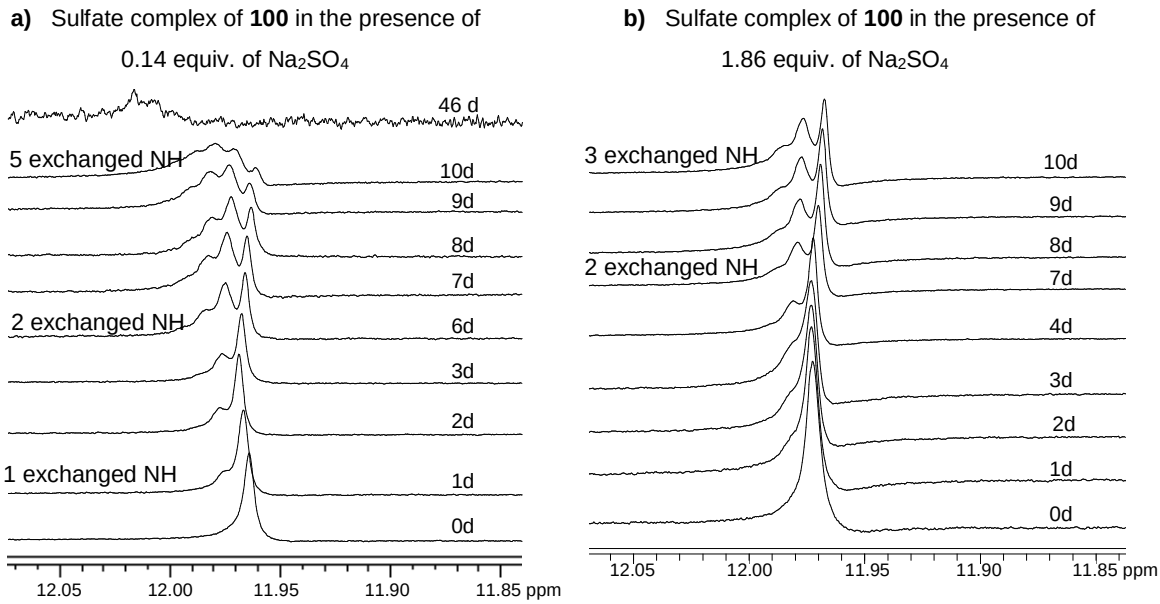


Chart 28 Section of the ^{1}H-NMR spectra (a) of **100**•SO_4 (0.59 mM in 2:1(v/v) CD_3CN/D_2O) in the presence of 0.14 equiv. of Na_2SO_4 (0.02 µmol) recorded over 46 days (a) and in the presence of 1.86 equiv. of Na_2SO_4 (0.27 µmol) recorded over 10 days (b).

Hydrogen-deuterium (H/D) exchange is a second-order reaction whose rate can be described on the basis of the following mathematical expressions (Scheme 41). Equation **7** shows that the rate depends on the concentrations of **100**•SO_4 and D_2O ([**100**] and [D_2O], respectively). Since only a small amount of D_2O is consumed during the reaction, the concentration of D_2O can be regarded as constant. This allows simplifying the rate expression in the form of a pseudo-first-order kinetics (equation **10**). The kinetic parameters k_{ps} and the half-time ($t_{1/2}$) can be determined by plotting ln [**100**] / [**100**]$_0$ against time (t) and determining the slope of the resulting line (equations **11**-**13**). The change of the concentration [**100**] / [**100**]$_0$ can be correlated with the decrease of the integral of the NH signal ($\int$NH / $\int$NH$_0$) in the ^{1}H-NMR spectrum with time. When k_{ps} is known, the half-life time $t_{1/2}$ can be calculated according to equation **13**. This value is the time needed for **100** to exchange half of the NH protons.

The H/D exchange rates for the sulfate complexes of **98** and **100** were determined in 2:1(*v/v*) CD_3CN/D_2O. For the kinetic measurements, samples were prepared containing compound **98**•SO_4 (0.5 mM in 2:1(*v/v*) CD_3CN/D_2O) or **100**•SO_4 (0.59 mM in 2:1(*v/v*) CD_3CN/D_2O) and a defined amount of sodium sulfate. In the case of **100**•SO_4, the rate of H/D exchange was determined for samples containing 0.14 equiv. and 1.86 equiv. of Na_2SO_4. The rate of H/D exchange of **98**•SO_4 was only determined in the presence of 0.17 equiv. of Na_2SO_4. In addition, experiments were performed with **100**•SO_4 where the samples contained 0.14 equiv. of Na_2SO_4

and 1.72 equiv of BaI_2 or $BaCl_2$. Measurements were also conducted with a sample of **100**•SO_4 containing 0.14 equiv. of Na_2SO_4 and smaller amount of $BaCl_2$ (0.86 equiv.). In the presence of BaI_2 the samples were first allowed to equilibrate for 24 h to allow full conversion into the corresponding iodide complex (**100**•I). Afterwards, the decrease of the area of the NH signal was followed. Adding 0.86 equiv. or 1.72 equiv.of $BaCl_2$ to **100**•SO_4 does not lead to full conversion of the sulfate complex into the chloride complex. Conversion after 24 h amounted to, respectively, 12 % and 79 %. Under both conditions only amide protons deriving from **100**•SO_4 were observed. Therefore, the decrease of the area of the NH signal was followed directly after addition of the barium salt. While impurities in the samples of **98**•SO_4 and **100**•SO_4 have no influence on the practical aspects of the measurements it is unclear how the impurities affect the outcome of the measurements. Still, comparison of the measurements performed under analogous conditions should be feasible. The results of the H/D exchange studies are summarized in Table 14.

(7) $v = k\,[\mathbf{100}]\,[D_2O]$ **(8)** $-\dfrac{d\,[\mathbf{100}]}{d\,t} = k\,[D_2O]\,[\mathbf{100}]$ **(9)** $k_{ps} = k\,[D_2O]$

(10) $-\dfrac{d\,[\mathbf{100}]}{[\mathbf{100}]} = k_{ps}\,dt$ **(11)** $\ln([\mathbf{100}] / [\mathbf{100}]_0) = -k_{ps}\,t$

(12) $k_{ps}\,t_{1/2} = \ln\dfrac{[\mathbf{100}]_0}{[\mathbf{100}]_0 / 2} = \ln 2$ **(13)** $t_{1/2} = \ln 2 / k_{ps} = 0.693 / k_{ps}$

Scheme 41 Mathematical expressions describing the rate of H/D exchange on the basis of the kinetic parameters reaction rate v, rate constant *k*, pseudo-first order rate constant k_{ps} and half-life time $t_{1/2}$.

entry	sample	k_{ps} / min^{-1}	$t_{1/2}$ / h	k_{ps} / k_0
1	**100**•SO_4, 1.86 equiv. of Na_2SO_4	$(1.30 \pm 0.01) \times 10^{-5}$	889	0.44
2	**100**•SO_4, 0.14 equiv. of Na_2SO_4	$(2.92 \pm 0.00) \times 10^{-5}$	396	1
3	**100**•SO_4, 0.14 equiv. of Na_2SO_4, 1.72 equiv. of BaI_2	$(9.30 \pm 0.03) \times 10^{-5}$	124	3
4	**98**•SO_4, 0.17 equiv. of Na_2SO_4	$(4.52 \pm 0.11) \times 10^{-4}$	25	16
5	**100**•SO_4, 0.14 equiv. of Na_2SO_4, 0.86 equiv. of Ba_2Cl_2	$(2.42 \pm 0.01) \times 10^{-3}$	4.8	83
6	**100**•SO_4, 0.14 equiv. of Na_2SO_4, 1.72 equiv. of $BaCl_2$	$(4.67 \pm 0.37) \times 10^{-3}$	2.5	159

Table 14 Effects of sodium sulfate and barium halides on the kinetics of the H/D exchange of **100**•SO_4 (0.59 mM in 2:1(v/v) CD_3CN/D_2O) and **98**•SO_4 (0.50 mM in 2:1(v/v) CD_3CN/D_2O). k_{ps} pseudo-first order rate constant, k_0 rate constant for entry 2, $t_{1/2}$ half-life time.

Table 14 shows that H/D exchange of **100**•SO_4 becomes significantly slower when the concentration of Na_2SO_4 increases in solution (entries 1 and 2). When 1.72 equiv. of BaI_2 are

added to the solution, the sulfate complex of **100** is first completely converted into **100**•I. Entry 3 therefore reflects the rate of H/D exchange of the iodide complex, which is obviously faster than the rate of the sulfate complex. In the cases when $BaCl_2$ was added there is significant increase in the rate of H/D exchange, which becomes the higher, the more $BaCl_2$ is added (entries 5 and 6). When using more than 3.43 equiv. of $BaCl_2$ the rate becomes so fast that no meaningful rate constant could be calculated from following the change of the area of the NH signal in the ^{1}H-NMR spectra.

Rate of H/D exchange of **98**•SO_4 is higher than that of **100**•SO_4 under similar conditions (entry 4 vs. 2). Interestingly, the splitting of the NH signal is not observed when following the time-dependent decrease of the NH signal of the sulfate complex of **98** or iodide complex of **100** (Chart 29). In both cases the signal only exhibits a slight but significant downfield shift. In the presence of a small amount of $BaCl_2$ (0.86 equiv.) added to **100**•SO_4 splitting of the NH signal deriving from monodeuterated **100**•SO_4 was detected during the first 2-3 hours after starting the measurements.

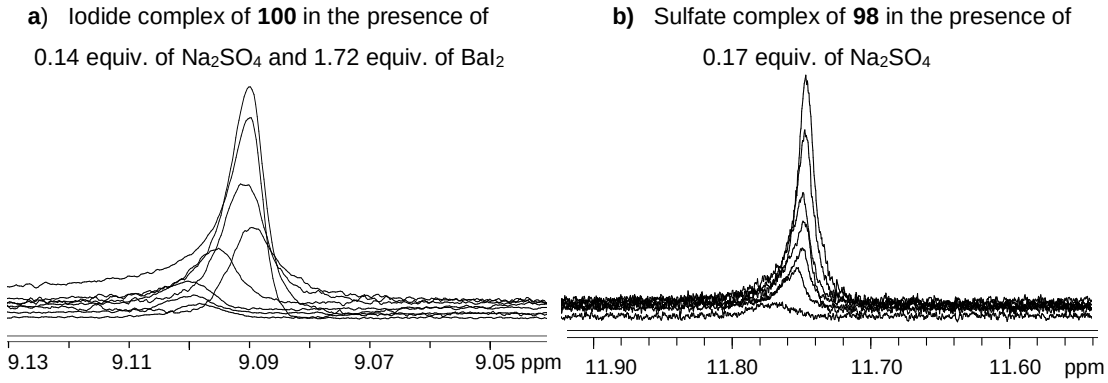


Chart 29 Region of the ^{1}H-NMR spectra showing the time-dependent decrease of the NH signal of **100**•SO_4 (0.59 mM in 2:1(v/v) CD_3CN/D_2O, Na_2SO_4, 0.02 µmol, 0.14 equiv.) recorded 24 h after adding 1.72 equiv. of BaI_2 (0.25 µmol) over 13 days (a) and of **98**•SO_4 (0.50 mM, in 2:1(v/v) CD_3CN/D_2O, Na_2SO_4, 0.02 µmol, 0.17 equiv.) recorded directly after salt addition over 7 days (b).

4.7.3 Discussion

4.7.3.1 Anion exchange

On the basis of the molecular modeling studies, and the NMR spectroscopic and mass spectrometric results I assume that both triply-linked bis(cyclopeptides) **98** and **100** use the same binding mode for anion complexation as corresponding singly- and doubly-linked bis(cyclopeptides). Complexes of **98** and **100** possess 1:1 stoichiometry and anion coordination

takes place in the cavity between the two peptide rings by means of hydrogen bonding. The strong deshielding of the amide NH and proline H(α) protons in the sulfate complexes of **98** and **100** accounts for strong interactions of these bis(cyclopeptides) with the sulfate anion. This assumption is supported by the pronounced templation effects of sulfate anions on the synthesis of these receptors. No other anions were found that cause similar strong effects on the protons residing in the bis(cyclopeptide) cavity.

Although this result could indicate that none of these anions forms complexes that are as stable as those of sulfate anions, the direct correlation between the extent of deshielding of the proline H(α) protons with binding strength is presumably oversimplified because deshielding also depends on the polarizability of the anion and the distance of the anion to the H(α) protons. The observed trends can best be described by dividing the investigated anions into the following categories: oxyanions, halides, anions that only interact weakly with the bis(cyclopeptides), and anions that presumably do not bind at all.

Complexation with oxyanions

The extent to which oxyanions affect the resonance of the H(α) protons of 100 decreases in the order SO_4^{2-} > SeO_4^{2-} > NO_3^- > ReO_4^- > TsO^- (Chart 30).

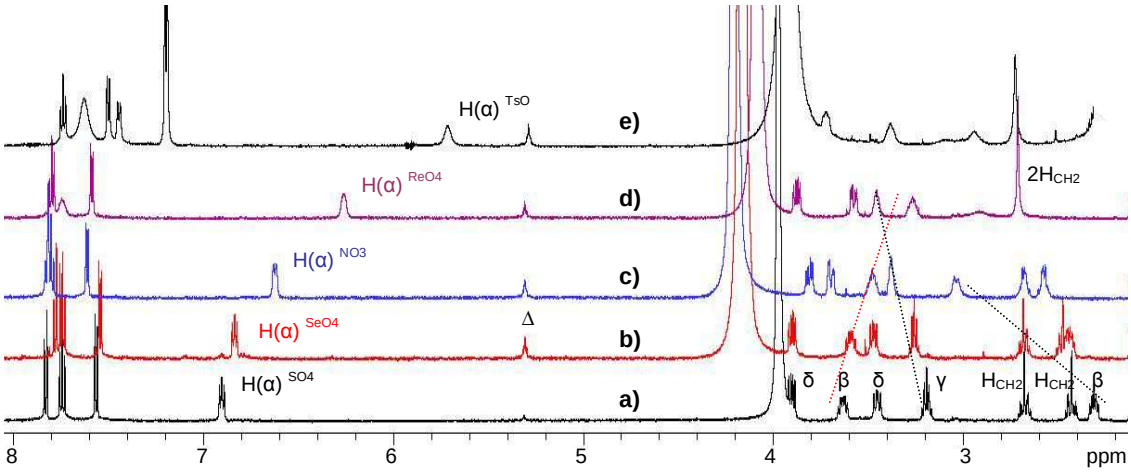


Chart 30 ^{1}H-NMR spectra of complexes of **100** (0.59 mM in 2:1 (v/v) $CDCN_3/D_2O$) with (a) sulfate, (b) selenate, (c) nitrate, (d) perrhenate and (e) tosylate. The impurity denoted Δ was introduced by addition of D_2O.

The two doubly-charged anions SO_4^{2-} and SeO_4^{2-} have a stronger effect on the resonance of the proline H(α) signal than singly-charged anions NO_3^-, ReO_4^-, TsO^-. This effect is consistent with the stronger electrostatic effects of ions with a higher charge. Despite the larger size of the selenate anion (r_{SeO4} = 2.43 Å, r_{SO4} = 2.30 Å), the effect of the sulfate anion is stronger. Thus, the

stronger effect that could be expected for the selenate complex as a result of the smaller distance between the H(α) protons and the oxygen atoms of the anion is probably counterbalanced by the higher polarizability of the selenate anion. A similar trend is observed for the singly-charged anions NO_3^- and ReO_4^-. In the case of the ReO_4^-, which is the largest anion found to be able to enter the cavity of **100**, this effect is accompanied by a conformational re-adjustment of the bis(cyclopeptide) units as visible from the changes emerging in the aliphatic region of the spectrum of **100**•ReO_4. An indication for the still relatively strong binding of selenate anions by **100** is the fact that SeO_4^{2-} anions are the only anions beside SO_4^{2-} that allow individual deuteration states of the bis(cyclopeptide) to be observed upon H/D exchange. The stronger coordination ability of the sulfate anion is most likely also the reason for the ca. 26 fold higher stability of the sulfate complex in comparison to the selenate complex observed in the direct competition experiments. The same reason explains the anion selectivity of the SBP, which binds sulfate 42 times stronger than selenate.[170c]

The structure of SBP-sulfate complex around the active site is schematically represented in Figure 35.[5] The sulfate anion that is completely encapsulated by the protein and inaccessible to solvent molecules, is held by a total of seven directional hydrogen bonds, donated by five main-chain peptide NH groups, a serine hydroxyl, and a tryptophane NH group. In this structure all of the sulfate oxygens except one have two hydrogen bonds linking them to the seven hydrogen-bond donors.[215] The difference in binding affinity of SBP to the SO_4^{2-} anion in comparison to the SeO_4^{2-} anion has been attributed to the larger length of Se-O bond (1.65 Å) in comparison to the length of S-O bond (1.49 Å).[170b] This causes minor deformations of the optimal coordination geometry around the SeO_4^{2-} anion in the binding site of the protein. According to molecular modeling studies, this deformation has to be compensated by local conformational readjustment and/or changes in the relative orientation of domains of the protein.

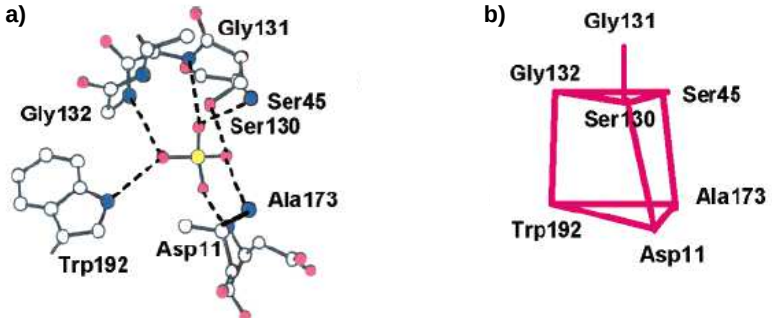


Figure 35 X-Ray crystal structure of SBP-sulfate complex (a) and distorted trigonal prismatic seven-coordinated structure of the sulfate-binding protein (b).

When comparing the binding mode of SBP-sulfate complex and **100**•SO_4 some similarities are evident. In both complexes, the sulfate anion is bound in a cavity via several directional hydrogen

bonds formed with at least six amino acid residues and the substrate is shielded from the bulk solvent. Main differences between the natural and the synthetic receptor which are presumably responsible for an increased sulfate affinity and better SO_4^{2-} / SeO_4^{2-} selectivity in the case of the SBP are the number and type of amino acid residues (7 vs. 6), the number, and type of hydrogen bonds (7 vs. 6 or more, and presence vs. absence of O-H···O hydrogen bond from serine hydroxyl group), receptor geometry (α-helical vs. capsular), size, and scaffold flexibility.

The effect of the tosylate anions on the shift of the proline H(α) protons was surprising because this anion is too large to fully fit the cavity of **100**. Molecular modeling indicated that the sulfonate group of tosylate anion can still fit into the cavity and interact with the NH protons while the aromatic residue of the anion resides outside. The corresponding mode of action requires a significant deformation of the bis(cyclopeptide), which is presumably reflected in the broadening of several of the bis(cyclopeptide) signals in the ^{1}H-NMR spectrum of the tosylate complex of **100**. Comparing the ^{1}H-NMR spectra of the sulfate complexes of **98** and **100** shows that the extent to which the NH and H(α) signals are deshielded in both spectra is significantly larger for **100**•SO_4. Specifically, the proline H(α) protons absorb at 6.89-6.92 ppm (and NH protons at 11.97 ppm) in **100**•SO_4 while the chemical shift of the same protons amount to 6.38-6.41 ppm (and of the NH protons to 11.74) in **98**•SO_4 (Chart 31). The cavity size and strength of interaction is therefore clearly affected in both bis(cyclopeptides) by the structure of the linkers. Consistent with the results obtained for corresponding singly- and doubly-linked bis(cyclopeptides), **100** seems to be the better sulfate receptor.[64n]

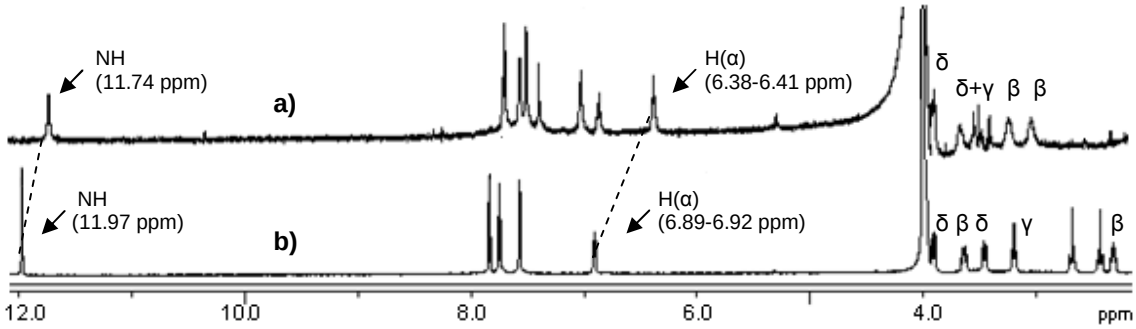


Chart 31 ^{1}H-NMR spectra of the sulfate complexes of **100** (a) and **98** (b) recorded in 2:1 (v/v) CD_3CN/D_2O.

Complexation with halides

The binding studies involving halide anions allow correlation of the coordinating ability of the anion and its ionic radius with the NMR spectroscopic changes independent of other geometric parameters as all halide ions are spherical. Coordinating ability decreases when going from the large iodide anion to the small chloride anion. As clearly evident in Chart 32, the extent to which the H(α) protons are deshielded in **100** correlates with the size of the anion increasing from

chloride over bromide to iodide. This order parallels the one observed for singly-linked bis(cyclopeptides) that usually bind stronger to iodide than to the smaller chloride.[130a, 191] This observation was attributed to the better fit of the larger iodide anion in the cavity of the bis(cyclopeptide) that allows simultaneous interactions with all six peptide NH groups. The smaller halides, although more strongly coordinating as visible from the shifts of the amide NH protons of **100**, which increase in the order $\delta NH^{I} < \delta NH^{Br} < \delta NH^{Cl}$, cannot bind to all bis(cyclopeptide) NH groups with similar efficiency (Table 9).

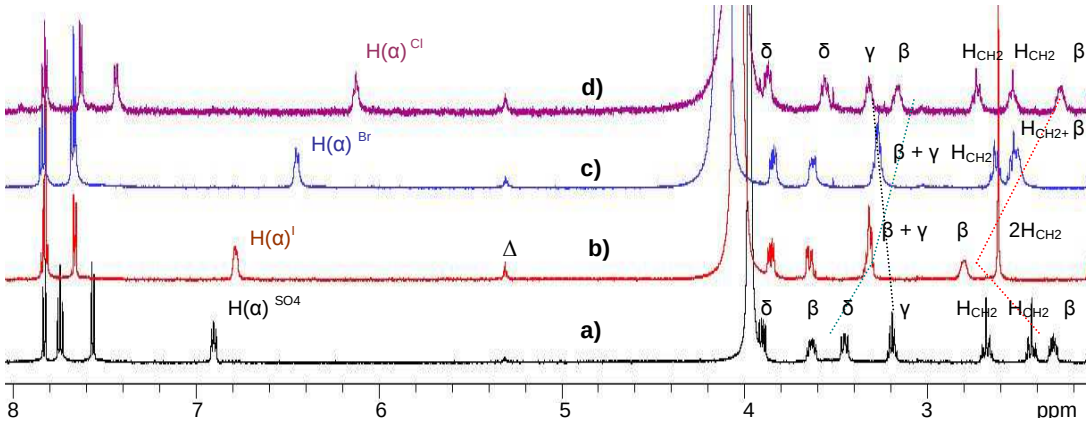


Chart 32 Complexes of **100** (0.59 mM in 2:1 (v/v) $CDCN_3/D_2O$) with (a) sulfate, (b) iodide, (c) bromide, and (d) chloride. The impurity denoted Δ was introduced by addition of D_2O.

Chart 32 also shows that not only the shifts of the H(α) and NH signals are affected by anion complexation but also the shifts of the aromatic protons and other proline protons. While the shifts of the aromatic protons are most likely affected by through-bond effects upon anion binding those of the proline protons can be correlated with through-space effects. For example, close inspection of the calculated structure in Figure 33 shows that also the H(β) protons of the proline rings are orientated toward the interior of the cavity. They therefore also experience a downfield shift upon anion binding, which is the larger the stronger the interactions with the anion are, increasing from chloride over bromide to iodide. The proline H(δ) protons, on the other hand, reside on the outside of the cavity and are only weakly affected by anion binding. The anion also has an influence on the shift of the linker protons. While all protons of the ethylene unit in the iodide complex of **100** are chemically equivalent, the diastereotopic protons on the individual CH_2 groups absorb at increasingly different frequences as the halide anion becomes smaller. Whether this effect accounts for a larger flexibility of the linker groups in **100**•I can currently not be estimated.

Qualitatively, the effects of halide anions on the shifts of receptor protons of **98** are similar (Chart 33). In this case, a large deshielding of the larger halide ions is also observed for the proton in 4-position (L_4) of the aromatic linker. It is possible that this proton is arranged close to the anion in

the complex of **98**, although this assumption could so far not be confirmed on the basis of molecular modeling studies.

Another important observation is the fact that different amounts of barium halides are required to convert **100**•SO_4 or **98**•SO_4 fully into the corresponding halide complexes. Table 15 shows, for example, that addition of 1.72 equiv. of BaI_2 is sufficient to fully replace the sulfate anion in **100**•SO_4 with an iodide anion. In the case of sulfate to bromide exchange 3.43 equiv. have to be added to **100**•SO_4 and for the sulfate to chloride exchange even 5.15 equiv. Since in all cases $BaSO_4$ is formed, precipitation of the latter is not sufficient to fully shift the equilibrium. This result clearly indicates that **100**•SO_4 is able to compete with $BaSO_4$ precipitation and the affinity of **100** to the anions present in solution also determine the extent of the exchange. When iodide is introduced, for which **100** presumably also possesses appreciable affinity, less iodide anions need to be present in solution to replace the bound sulfate than in the case of anions that form much weaker complexes with **100**, such as chloride and bromide. Although qualitative, this result shows that **100** must possess high anion affinity presumably in the order of the solubility product of $BaSO_4$, i.e. in the submillimolar range.

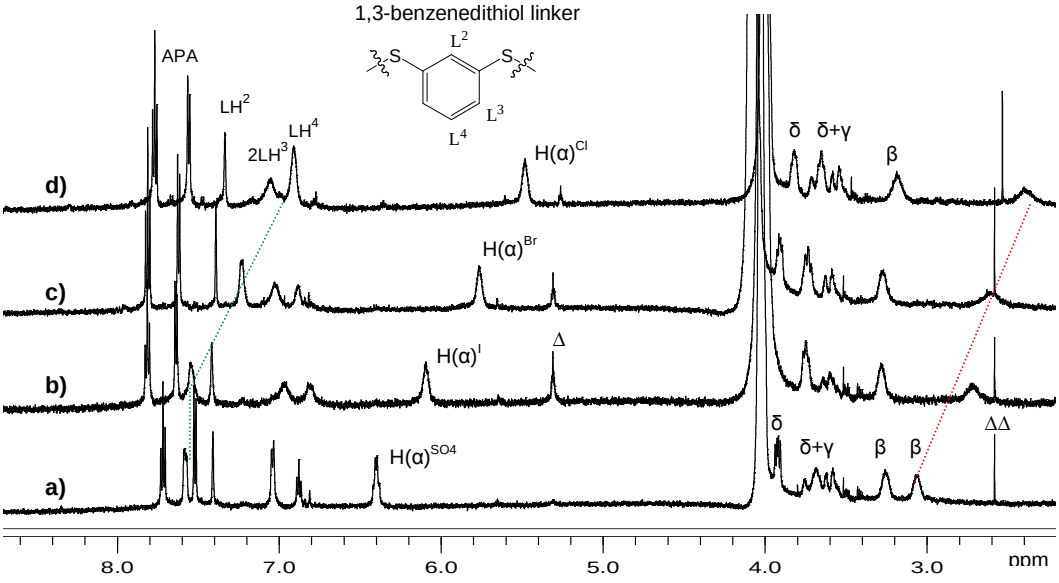


Chart 33 ^{1}H-NMR spectra of complexes of **98** (0.50 mM in 2:1(v/v) CD_3CN/D_2O) with (a) sulfate, (b) iodide, (c) bromide, and (d) chloride. Signals denoted Δ and ΔΔ derive from impurities in D_2O or from residual solvent, respectively. Protons deriving from aminopicolinic acid subunits and linker are marked with APA and LH^2, LH^3, and LH^4, respectively.

Table 15 shows also that the amount of barium halides needed to achieve full conversion of **98**•SO_4 into the corresponding halide complexes is much less for **98**•SO_4 than for **100**•SO_4 (except for iodide). For example, the amount of $BaCl_2$ used to completely remove sulfate from the cavity

of **100**•SO_4 is almost 1.7 times larger than the amount used in the case of **98**•SO_4. This is an additional indication for the higher stability of the sulfate complex of **100** with respect to the sulfate complex of **98**. In addition, the fact that exchange of sulfate with halides for bis(cyclopeptide) **98** takes approximately 24 h to achieve full conversion reveals a very slow sulfate decomplexation process.

bis(cyclopeptide)	BaI_2 / equiv.	$BaBr_2$ / equiv.	$BaCl_2$ / equiv.
98•SO_4	2.03	> 2.03	3.04
100•SO_4	1.72	3.43	5.15

Table 15 Amounts of barium salts needed to cause complete conversion of **98**•SO_4 and **100**•SO_4 into the corresponding halide complexes.

Finally, it should be noted that also the kinetics of halide complexation/decomplexation of **100** differs profoundly from that of the sulfate complex of **100**. Replacement of sulfate anions from the cavity of **100** upon addition of barium halides requires 24 h to reach thermodynamic equilibrium. Replacing halides, such as iodide or chloride with other anions including sulfate is, however, very fast, usually complete within 10-40 min after salt addition. This indicates that k_{on} (association rate constant) of the sulfate complex of **100** is fast while k_{off} (dissociation rate constant) is slow, again accounting for the high stability of **100**•SO_4. Complexes of other anions are less stable since k_{off} is considerably faster.

Complexes of 100 with weakly bound anions

Anions that belong to this group are phosphate, tetrafluoroborate, and hexafluorophosphate (Chart 34).

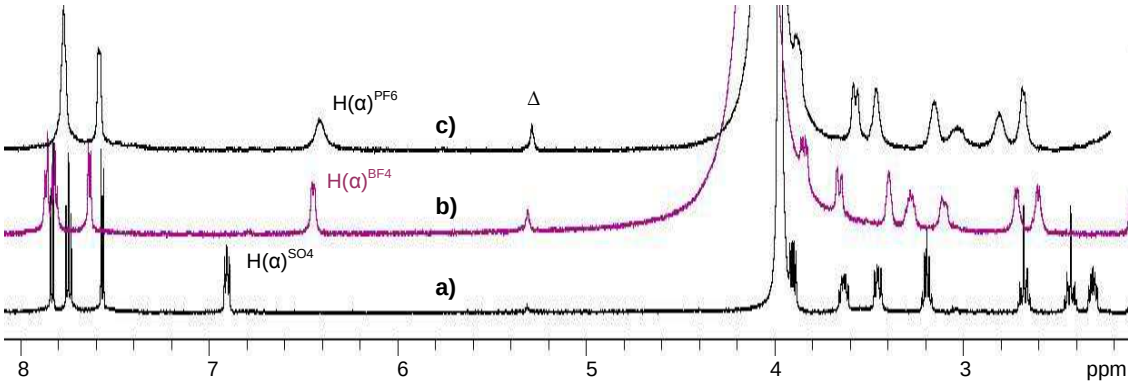


Chart 34 ^{1}H-NMR spectra of complexes of **100** (0.59 mM in 2:1 (v/v) $CDCN_3/D_2O$) with (a) sulfate, (b) tetrafluoroborate, (c) hexafluorophosphate. The impurity denoted Δ was introduced by addition of D_2O.

Complexes formed between these anions and **100** seem to be not very stable and their concentration was found to decrease with time leading to reappearance of the sulfate complex of **100**. Thus, directly after adding $AgBF_4$ or $AgPF_6$ to the iodide complex of **100** quantitative formation of the corresponding complexes **100**•BF_4 and **100**•PF_6 was observed. Addition of Na_3PO_4 to **100**•Cl only leads to incomplete formation of the phosphate complexes (22 %).
Subsequently, the signals of the newly formed complexes disappeared again and the signals of **100**•SO_4 reappeared. In the case of **100**•BF_4 no further change of the ^{1}H-NMR spectrum was observed once ca. 9 % of **100**•SO_4 was formed. A more pronounced effect was observed in the case of **100**•PF_6 where the ^{1}H-NMR spectrum indicated the presence of ca. 70 % of **100**•SO_4 after 30 days of equilibration. The fact that complexes of **100** containing weakly bound anions are able to dissolve the solid $BaSO_4$ present in each sample is yet another indication for the exceedingly high sulfate affinity of **100**. The dissolvation of $BaSO_4$ can be described according to the equilibrium shown in Figure 36.

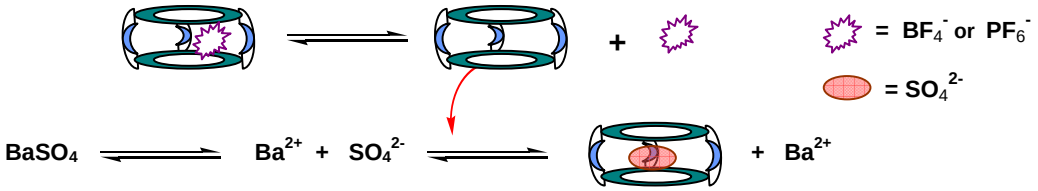


Figure 36 Potential reaction that takes place in solution after complexes of **100** with weakly coordinating anions (BF_4^- or PF_6^-) have been formed.

Apparently, the dissociation of the complexes of **100** with BF_4^- or PF_6^- affords sufficient amounts of empty / solvent-filled receptor in solution. The latter empty cages of **100** can then efficiently compete with Ba^{2+} ions for binding to sulfate anions present in solution deriving from the dissociation of $BaSO_4$. This concentration is small because of the small solubility product of $BaSO_4$ ($K_{sp\ (BaSO4)}$ = 1.1 x 10^{-10} M^2 at 25 °C in water) amounting to ca. 0.1 µM.
In the case of the experiment with Na_3PO_4 one has to consider that the solubility product of $Ba_3(PO_4)_2$ is even smaller than that of $BaSO_4$ amounting to 3.4 x 10^{-23} M^5. Thus, $BaSO_4$ should partly dissolve also in the absence of **100** when Na_3PO_4 is added to the solution because the sulfate and phosphate concentrations above, respectively, solid $BaSO_4$ and $Ba_3(PO_4)_2$ are similar amounting to ca. 1 x 10^{-5} M and 2.5 x 10^{-5} M.

Anions that do not bind to 100

No notable changes in the ^{1}H-NMR spectra were observed when $NaBPh_4$ or Na_2CO_3 were added to solutions of **100**•Cl in 2:1 (v/v) $CDCN_3/D_2O$ even if these salts were added in large excess (ca. 12.5 equiv.). The obvious inability of **100** to bind carbonate anions was surprising because I

expected these anions to bind in the similar fashion as NO_3^-. One possible reason for the outcome of this experiment is the lack of pH control. Since CO_3^{2-} is a basic anion that is protonated to a considerable extent in aqueous solution I only observed the inability of HCO_3^- to enter the cavity of **100**. Binding of **100** to phosphate has a similar problem and also in this case only very weak binding was observed. The inability of BPh_4^- to enter the cavity of **100** is due to the large size of this anion and the low solubility of the added $AgBPh_4$ in the solution (2:1 (v/v) $CDCN_3/D_2O$) in which these experiments were performed.

To conclude, the interaction of bis(cyclopeptide) **100** with anions of different size, geometry and charge was studied. The extent of the shift of the proline H(α) protons of **100** in the ^{1}H-NMR was correlated with the size, geometry, charge, and polarizability of the anions. Bis(cyclopeptide) **100** binds best to anions that are able to fill the cavity between the cyclopeptide rings. Anions of similar size bind better if they have a higher charge ($SO_4^{2-} > I^-$). Anions of similar size and same charge bind better if they are more strongly coordinating ($SO_4^{2-} > SeO_4^{2-}$). The exchange experiments also provide information about the relative complex stabilities: the more of a barium salt is required to fully replace the sulfate anion from the cavity of the bis(cyclopeptides) the less stable the formed complexes are. The low solubility of $BaSO_4$, which is invariably formed during this exchange, is not the only factor controlling the extent of exchange. This indicates that both bis(cyclopeptides) **98** and **100** have a very large sulfate affinity with the one of **100** being most likely larger than the one of **98**.
Because of the impurities present in both bis(cyclopeptides) and because I did not succeed in obtaining an absolute estimate of complex stability for the anion complexes of **98** and **100** these results, unfortunately, have to remain qualitative. Only one experiment allowed showing that sulfate affinity of **100** is on average 26 times higher than selenate affinity which parallels the behavior of the sulfate-binding protein.

4.7.3.2 H/D exchange

The rate of H/D exchange gives information about solvent accessibility to various parts of a molecule, for example a protein. Protons inaccessible to the solvent or involved in intramolecular hydrogen bonds exchange slowly if at all, while amide protons located on solvent exposed sites of a molecule exchange rapidly. The exchange rate is therefore a function of two parameters, solvent accessibility and involvement in hydrogen bonding. When both parameters contribute to the rate of exchange, it is difficult to correlate the observed rate with one or the other effect. In addition, the rate of amide H/D exchange is highly solvent, temperature and pH dependent with a minimum usually in the pH range of 2.4 - 3 and a ca. 10-fold increase for each pH unit above 3-4.[216] An increase of the temperature of 10 °C increases exchange rates about 3-fold.[217] Detailed

characterization of H/D exchange rates is a valuable means of obtaining information about protein structure. Protein domains for which very slow H/D exchange is usually observed are tightly folded secondary or tertiary structural motifs.[218] Typical protein H/D exchange constant may vary over many orders of magnitude depending on the amide environment but most are usually in the range of 10^4-10^1 mol^{-1} s^{-1}.[219] In a protein system, the rate of amide H/D exchange can decrease due to hydrogen bonding by a factor of ca. 100.[218]

In the case of bis(cyclopeptides) **98** and **100**, the rates of H/D exchange provide information about the accessibility of the cavity for D_2O molecules. Kinetic experiments revealed a half-time of H/D exchange amounting to ca. 16 days and 1 day for **100** and **98**, respectively, showing that the H/D exchange is not totally suppressed by the presence of sulfate anion in these receptors. In the case of **100**, the very slow H/D exchange was accompanied not only by decreasing of the intensity of the amide NH signal but also by splitting of this signal and a downfield shifting.

Since H/D exchange can only proceed when a D_2O molecule approaches an NH group, the slow H/D exchange observed for **100**•SO_4 and **98**•SO_4 indicates that D_2O molecules cannot easily enter the cavity of these bis(cyclopeptides) when an anion is bound. In case the cavity is large enough to accommodate an anion and a D_2O molecule simultaneously, the rate of H/D exchange provides information about the strength of the hydrogen-bonding interactions between the anion and the amide NH groups. If, however, the cavity of the bis(cyclopeptide) is not spacious enough to host a D_2O molecule and an anion one must assume that H/D exchange is only possible if the anion is not bound so that solvent molecules can enter the cavity. In both cases the influence of externally added salts on the rate of H/D exchange should differ. Since externally added sulfate anions should not affect the intrinsic strength with which a sulfate anion binds to the bis(cyclopeptide), no pronounced effect is expected on the rate of H/D exchange when the sodium sulfate concentration is varied in solution. If, however, H/D exchange can only take place upon dissociation of the complex, one can assume that it should become the slower the lower the concentration of the dissociated complex is in solution.

My experiments demonstrated that an increase of the sulfate concentration considerably slows down the H/D exchange rate. Since increasing the concentration of the guest in solution shifts the equilibrium toward the complex and decreases the concentration of the free receptor according to Le Châtelier's principle, one can conclude that H/D exchange most likely requires "empty" (solvent-filled) bis(cyclopeptides). The lower the concentration of "empty" bis(cyclopeptides) in solution the slower the H/D exchange process.

Consistent with this interpretation is the observation that H/D exchange is faster of the iodide complex of **100**. In this case, the intensity of the NH signal decreases relatively rapidly ($t_{1/2}$ of **100** in the presence of 1.72 equiv. of BaI_2 amounts to 5 days). Since the iodide complex is less stable than the sulfate complex of **100** according to the binding studies its complexation/decomplexation equilibrium involves a higher concentration of dissociated species ("empty" **100**). Even faster is

the H/D exchange observed for the chloride complex of **100** ($t_{1/2\ (100\bullet SO4)} > t_{1/2\ (100\bullet I)} > t_{1/2\ (100\bullet Cl)}$), although the rate could only be quantified when a fraction of **100**•SO_4 was converted into the corresponding chloride complex (12 % or 79 % of **100**•Cl). The larger this fraction, the faster the exchange rate (Table 14). The higher rate of H/D exchange observed for **98**•SO_4 in comparison to **100**•SO_4 again indicates that the bis(cyclopeptide) containing the 1,3-benzenedithiol linker forms a less stable complex.

The results obtained about the H/D exchange also provide some information about the mechanism of anion exchange. As H/D exchange seems to require the dissociated complex it is likely that exchange of bound anions involved the “empty” (solvent-filled) bis(cyclopeptide) as an intermediate stage as shown schematically in Figure 37.

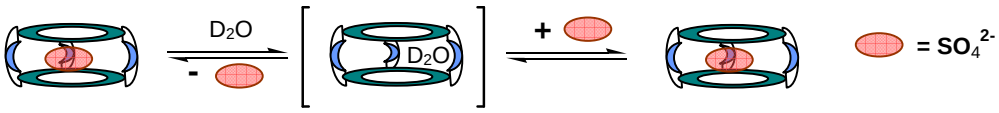


Figure 37 Potential mechanism of the decomplexation/complexation equilibrium of **100**•SO_4.

This mechanism of anion exchange is reminiscent of the mechanism of an S_N1 reaction and was therefore termed SS_g1 by Rebek (unimolecular guest substitution, supramolecular).[48c] The alternative SS_g2 mechanism involves direct replacement of a guest by another guest. This reaction should become faster the higher the external concentration of the guest is. However, if direct anion exchange is not accompanied by H/D exchange, which is very likely, the H/D exchange experiments provide no information whether this alternative mechanism has also to be considered. It should be noted that the majority of exchange reactions of other molecular cages or capsules proceed along the SS_g2 mechanism.[220]

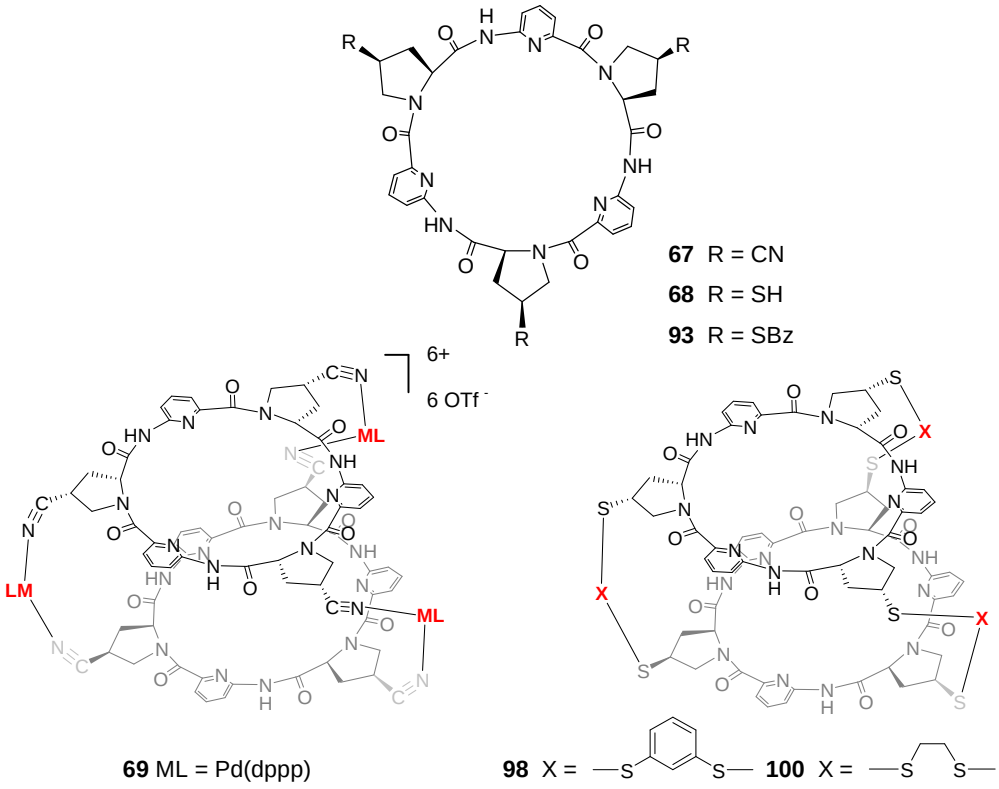
The splitting of the NH signal observed during H/D exchange that allows one to detect the individual deuteration stages of **100**•SO_4 is unusual and has so far been only rarely described for synthetic receptors.[221] The fact that only one signal for each deuteration step is observed indicates that there is fast scrambling of the H and D atoms on the amide groups inside the cage, presumably caused by tumbling of the anion in the cavity, so that every intermediate becomes symmetrical on average. The increasing downfield shift of the signal of the remaining hydrogen atoms for increasing extent of deuteration is in agreement with the results of other investigations where splitting of NH signals accompanying deuteration has been observed.[222]

Most closely related to the isotope effects detected in my H/D exchange experiments are those observed for the increasing deuteration of ammonium ions in corresponding ammonium salts.[222] Also in this case, a downfield shift of the NH signal was monitored ^{1}H-NMR spectroscopically with increasing degree of deuteration. This shift was explained by the difference in hydrogen-bonding interactions between the counterion of the ammonium salt and the N-D and N-H groups. As hydrogen bonds to deuterium are generally weaker than to hydrogen atoms the interactions

between the anion and the remaining N-H group become increasingly stronger as the degree of deuteration of the ammonium ion increases.[223] This increasing hydrogen-bond strength causes the downfield shift of the signal of the NH protons in the ^{1}H-NMR spectrum. Importantly, this effect should only be visible if the anion forms strong directed hydrogen bonds to the ammonium ion.[224] Consistent with this explanation, a shift and a splitting of the NH signal is only observed for complexes of **100** with sulfate and selenate anions. Deuteration of **100** should cause the strength of the interactions between the included anion and the remaining NH groups of the bis(cyclopeptide) to become stronger, as demonstrated by the higher resonance of the NH signal of complex species with a larger degree of deuteration. No such effect was observed for the iodide complex of **100**, showing that it is restricted to anions that form directed hydrogen bonds with NH groups. The fact that effects of deuteration are only visible for the sulfate (and selenate) complexes of **100** and not for corresponding complexes of **98** agrees with the lower anion affinity of the latter bis(cyclopeptide), i.e. the weaker interactions of the NH groups of **98** with anions included into the cavity.

5. Summary and perspectives

The objective of my thesis was to develop new anion receptors based on triply-linked bis(cyclopeptides) and study the binding properties of these compounds. My research is divided into two major parts. The first section is related to the syntheses of bis(cyclopeptides) in which the two cyclopeptide rings are connected through coordinative interactions. The second part of my research involves covalent triply-linked bis(cyclopeptides) containing three linkers derived from aromatic or aliphatic dithiols. This type of bis(cyclopeptides) was prepared by using disulfide exchange under thermodynamic control. The structures of the building blocks and target compounds of my work are shown in Scheme 42.



Scheme 42 Cyclopeptide building blocks and general structures of triply-linked bis(cyclopeptides) targeted in my work.

The building bocks required for the synthesis of the two types of bis(cyclopeptides) shown in Scheme 42 are hexapeptides **67** and **68** composed of alternating L-proline and 6-aminopicolinic acid subunits.

Assembly of a coordination cage **69** from the trinitrile **67** was expected to proceed by coordination of the nitrile groups to suitable metal centers in square-planar palladium (II) complexes with labile

ligands. To investigate this concept, a synthesis of the new cyclopeptide **67** was first developed. Crucial steps in the preparation are the introduction of the nitrile groups in properly functionalized proline derivatives and the correct protecting group strategy. After evaluating different strategies I identified reaction conditions to obtain the (4*S*)-cyanoproline building block required for the synthesis of **67** and found a suitable combination of protecting groups for the assembly of the cyclopeptide. Unfortunately, cyclization afforded the desired cyclopeptide in unsatisfactory yields. With small amounts of compound **67** in hand, I could only perform some qualitative experiments to assemble a bis(cyclopeptide) **69** from **67**. To this end, **67** was mixed with a palladium (II) complex, namely Pd(dppp)OTf_2, and the possible complex formation was followed with different NMR-spectroscopic techniques. Unfortunately, none of the experiments provided conclusive evidence that self-assembly took place or that the desired bis(cyclopeptide) **69** was formed.

In a second part of my work, the synthesis of disulfide-containing triply-linked bis(cyclopeptides) was studied. At the onset of this project, the new building block **68** containing three thiol groups in 4-position of the proline subunits was synthesized. The corresponding tris(benzoyl)-protected derivative of this compound (**93**) was prepared in satisfactory yield and in analytically pure form. Deprotection of **93** resulted in a mixture of oligomers, which was not characterized further, but could be used for the subsequent formation of corresponding bis(cyclopeptides).

These bis(cyclopeptides) were obtained by equilibrating **68** with suitable dithiols in 2:1 acetonitrile/water at pH 9 in the presence of template salts. As dithiols, mainly 1,2-ethanedithiol (**90**) was used as well as 1,3-benzenedithiol (**89**), compounds that were shown in previous studies to yield potent anion receptors derived from singly- and doubly-linked bis(cyclopeptides). Mainly sulfate salts were used as templates whose anions are known to strongly bind to such bis(cyclopeptides). I could show that in the presence of either linker **89** or **90** the desired triply-linked bis(cyclopeptides) **98** and **100**, respectively, are formed. In addition, minor products were found in the reaction mixture comprising bis(cyclopeptides) with four linker molecules. These compounds presumably contain a sequence of two linkers in one position.

Investigating the effects of different parameters on product formation provided information about the behavior of the system: sulfate salts induce bis(cyclopeptide) formation more efficiently than other investigated template salts, indicating that the bis(cyclopeptides) possess highest affinity for the sulfate anion. Iodide anions also template the formation of bis(cyclopeptides) to some extent while addition of NaBr and NaCl showed no effect on the outcome of the reaction. The counterions seem to have no effect on bis(cyclopeptide) formation.

Templation is always more efficient in the case of bis(cyclopeptide) **100** containing 1,2-ethanedithiol as linker. This is an indication that **100** is a more stable compound or a better sulfate receptor than **98**.

When different types of dithiols are present in the reaction mixtures, bis(cyclopeptides) containing mixed linkers are observed. These compounds are predominantly formed in the presence of 1,2-

ethandithiol, but bis(cyclopeptides) containing different linkers are also accessible when the linkers are structurally related as in the case of 1,3-benzenedithiol and 3,5-dimercaptobenzoic acid.

Subsequently, bis(cyclopeptides) **98** and **100** were synthesized on a larger scale. Both compounds were isolated as their Na_2SO_4 complexes. ^{1}H-NMR spectroscopic analyses showed that both products contained ca. 30 - 40 % of impurities, which were invisible in the NMR and mass spectra. These impurities were presumably introduced in the chromatographic purification step and they could not be removed. Therefore, only qualitative anion binding studies could be performed. These binding studies mainly addressed the questions whether the sulfate ion present in the cavity of **98** and **100** could be exchanged by other anions, how fast anion complexation/decomplexation processes are, and according to which mechanism they proceed.

In the course of these experiments it became evident that the sulfate anions in the cavities of bis(cyclopeptides) **98** and **100** are not permanently entrapped but can be replaced by other anions. For example, adding $BaCl_2$ or BaI_2 to the sulfate complex of **100** converts **100**•SO_4 into, respectively, the iodide or the chloride complex. This step benefits from the low solubility of $BaSO_4$, which shifts the equilibrium of the anion exchange. Since the iodide and the chloride complexes of **100** are less stable than the sulfate complex further anion exchange experiments leading to a variety of other anion complexes could be performed. These complexes were mainly characterized by means of ^{1}H-NMR spectroscopy. The corresponding investigations provide information about the structure and the relative stability of the complexes. In addition, they show that sulfate exchange is a slow process requiring ca. 24 h to reach thermodynamic equilibrium. Exchange of other anions from the cavity of **100** is significantly faster. Bis(cyclopeptide) **98** generally form less stable complexes then **100**. In terms of structure, anion complexes of **98** and **100** are related to corresponding complexes of singly-linked bis(cyclopeptides) with the anion residing between the two cyclopeptide rings and bound to the NH groups by hydrogen bonding.

Several experiments provided qualitative information that sulfate affinity of **100** is very high, possibly exceeding that of previously described doubly-linked bis(cyclopeptide), which proved to be in the nanomolar range. For example, equilibrating a complex of **100** with a weakly bound anion such as BF_4^- or PF_6^- in the presence of solid $BaSO_4$ resulted in dissolvation of $BaSO_4$. Unfortunately, no absolute data for the stability of the anions of **100** and **98** could be obtained.

Information about the mechanism of anion exchange could be derived from following the rate with which protons on the amide NH groups of **100** or **98** are replaced by deuterium atoms in protic deuterated solvents by ^{1}H-NMR spectroscopy. These experiments showed that H/D exchange of **100**•SO_4 is very slow, possessing a half-time of 16.5 days. The fact that H/D rate decreases when the external sulfate concentration is increased indicates that sulfate exchange proceeds via the dissociated complex. Accordingly, for complexes with a lower stability whose complexation equilibria involve a larger fraction of unbound species, H/D exchange is significantly faster.

Another interesting feature of **100**•SO_4 is that the individual deuteration steps can be followed by ^{1}H-NMR spectroscopy as each one is associated with an individual NH signal. This feature was also observed for complexes of **100** with selenate and highlights the well defined complex structures of **100**•SO_4 and **100**•SeO_4 comprising sulfate and selenate anions most tightly bound between the two cyclopeptide rings.

In this work, I have successfully developed a strategy to access new triply-linked bis(cyclopeptides) using disulfide exchange. Anion affinity of these compounds in aqueous media is so high that all attempts to isolate them in the anion-free form failed. Qualitative binding studies showed that highest affinity is observed for sulfate anions which are bound with binding constants at least in the submillimolar range. In another part of my work, preliminary investigations were undertaken to access triply-linked coordination cages on the basis of cyclopeptides. A synthetic strategy was developed to obtain the required cyclopeptide precursor. Attempts to use this compound to assemble the corresponding coordination cage failed, unfortunately.

Outlook

The results obtained in this work show that triply-linked bis(cyclopeptides) **98** and **100** are highly promising neutral anion receptors operating in aqueous solvent mixtures. So far, my results provide only qualitative information about anion affinity. Therefore, subsequent studies have to aim at measuring anion affinity quantitatively. To this end, efforts have to be made to obtain the corresponding bis(cyclopeptides) **98** and **100** in pure form. Further binding studies should also address comparison of the sulfate affinity of **98** and **100** with that of the sulfate-binding protein. It would be interesting to see whether these synthetic systems have binding properties similar or even exceeding those of the natural protein. Other attractive target compounds are bis(cyclopeptides) with different types of linkers and bis(cyclopeptides) containing polar substituents in their linkers, such as 3,5-dimercaptobenzoic acid **102**. The latter substituents may induce water solubility thus allowing anion binding to be studied in water. Work in this area is ongoing in the Kubik group.

6. Experimental part

6.1 Abbreviations

Ac	Acetyl
AgOTf	Silver trifluoromethanesulfonate
All	Allyl
APA	6-Aminopicolinic acid
Apro	(2*S*,4*S*)-4-Aminoproline
Ar	Aryl
BDT	1,4-Butanedithiol
Bn	Benzyl
t-Boc	*tert*-Butoxy carbonyl
Bu	Butyl
tBu	*tert*-Butyl
Bz	Benzoyl
Cat.	Catalyst
(4*S*)Cpro	(2*S*,4*S*)-4-Cyanoproline
(4*R*)Cpro	(2*S*,4*R*)-4-Cyanoproline
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	Dynamic combinatorial chemistry or *N,N'*-Dicyclohexylcarbodiimide, depending on the context
DCL	Dynamic combinatorial library
DIEA	*N,N*-Diisopropylethylamine
DMAP	Dimethylaminopyridine
DMF	*N,N*-Dimethylformamide
DMSO	Dimethylsulfoxide
dppp	1,3-Bis(diphenylphosphino)propane
DTT	DL-Dithiothreitol
equiv.	Equivalent
EDC	1-Ethyl-3-(3-dimethyllaminopropyl)carbodiimide hydrochloride
ESI-MS	Electrospray ionization mass spectrometry
Et	Ethyl
Fw	Formula weight
ΔG	Gibbs free energy
HATU	*O*-(7-Azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate
HBTU	*O*-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate
HPLC	High performance liquid chromatography

Hyp	(2*S*,4*R*)-4-Hydroxyproline
ITC	Isothermal titration microcalorimetry
K	Degrees Kelvin or equilibrium constant, depending on the context
Ka	Binding constant
L	Ligand
LC-MS	Liquid chromatography-mass spectrometry
mAu	Milli absorbance units
MALDI-MS	Matrix-assisted laser desorption/ionization-mass spectrometry
M	Metal atom
Me	Methyl
MP	Melting point
MS	Mass spectrometry
m/z	Mass to charge ratio
MW	Microwave heating or molecular weight, depending on the context
NOESY	Nuclear Overhauser effect spectroscopy
PBP	Phosphate-binding protein
$Pd(dppp)Cl_2$	(1,3-Bis(diphenylphosphino)propane)palladium(II) chloride
$Pd(dppp)OTf_2$	(1,3-Bis(diphenylphosphino)propane)palladium(II) trifluoromethanesulfonate
Ph	Phenyl
*i*Pr	Isopropyl
Pro	(2*S*)-Proline
PyCloP	Chlorotripyrrolidinophosphonium hexafluorophosphate
RP	Reversed phase
R_t	Retention time
SBP	Sulfate-binding protein
TBACN	Tetra-*n*-butylammonium cyanide
TBAS	Tetrabuthylammonium sulfate
TBTU	*O*-(1*H*-Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMAI	tetramethylammonium iodide
TOF	Time of flight
Tpro	(2*S*,4*S*)-4-Thioproline
Ts	*p*-Toluolsulfonyl
X	Halide or linker, depending on the context

IR-Spectroscopy

m	Medium
v	Wavelength
s	Strong
w	Weak

NMR-Spectroscopy

δ	Chemical shift relative to tetramethylsilane in ppm
brs	Broad singlet
d	Doublet
dd	Doublet of doublets
Hz	Hertz
J	Coupling constant in Hz
m	Multiplet
mc	Symmetrical multiplet
s	Singlet
t	Triplet

6.2 General information

^{1}H-NMR spectra were recorded on Bruker DPX 400 or Bruker Avance 600 spectrometers at 400 MHz and 600 MHz, respectively. ^{13}C-NMR spectra were recorded on the same spectrometers at 101 MHz or 151 MHz. Chemical shifts are reported in parts per million (ppm, *δ*) using TMS (0.0 ppm), CD_3CN or DMSO (1.94 and 2.54 ppm, respectively) as internal standards for proton spectra. ^{13}C-NMR chemical shifts are reported in ppm relative to the central line of the CD_3CN (δ 1.3), $(CD_3)_2SO$ (δ 39.5) or $CDCl_3$ (δ 77.2). Signal ^{1}H-NMR splitting patterns with observed first-order couplings are denoted as singlet (s), doublet (d), triplet (t), or quartet (q). Splitting patterns that could not be interpreted or easily visualized are denoted as broad (br) or multiplet (m). Elemental analyses for the new compounds were obtained in limits not exceeding ± 0.4 %. Mass spectra were measured either on an Ultraflex TOF/TOF instrument (Bruker Daltonics, Germany) or were obtained using an electrospray ionization quadrupole mass spectrometer (Bruker Daltonics Esquire 3000). Melting points were measured on a Büchi 510 capillary apparatus and were not corrected. Analytical thin layer chromatography (TLC) was performed on pre-coated silica gel 60 F-254 plates. Visualization was accomplished using either UV light detection or ninhydrin staining. In the latter case, plates were sprayed with an ethanolic ninhydrin solution and

heated. Column (flash) chromatographic separations were performed on silica gel 60 (230-400 mesh). Solvent mixtures used for TLC and column chromatography are specified in *v/v* ratios. Evaporation of solvents was, except otherwise stated, performed by using a rotary evaporator under reduced pressure.

Commercially available reagents and solvents were used as received from suppliers without further purification. Where necessary, solvents were purified and dried following standard general procedures.[225] Dry THF was obtained by pre-drying of commercial THF with potassium hydroxide, treatment with a sodium metal, and distillation. Triethylamine and dichloromethane were distilled from calcium hydride (CaH_2) or calcium chloride ($CaCl_2$). Acetonitrile, pyridine, and toluene were dried over CaH_2 (or over sodium metal for CH_3CN), distilled and stored over 4 Å molecular sieves. DMF and DMSO were stirred over CaH_2 for 18 h, filtered, and then distilled at low pressure. Dried DMF and DMSO were stored over 4 Å molecular sieves. Water for HPLC analysis was purified using a tap-fed water purification system Barnstead EASY pure RoDi (model D13321).

Cleavage of *N*-tert-butoxycarbonyl group required preparation of 6 N HCl solution in 1,4-dioxane. To this end, hydrogen chloride was bubbled through dry 1,4-dioxane under stirring and cooling with an ice bath. Concentration was checked by titration with 1M NaOH and phenolphthaleine as indicator in regular intervals. Addition of HCl was terminated when concentration reached 6 N.

Synthesis of tetrakis[triphenylphosphine]palladium(0) for the cleavage of allyl esters was performed as described.[210] The coupling reagent chlorotripyrrolidinophosphonium hexafluorophosphate (PyCloP) was synthesized analogously to the procedure described by Coste and Yvernault.[226] Commercially available coupling reagents TBTU, HATU, HCTU, and EDC were purchased.

6.3 Solvents and chemicals

ABCR	*N,N'*-Dicyclohexylcarbodiimide
Acros	Acetic anhydride
	Chloroacetic acid
	Potassium permanganate p.a.
	Phosphoryl chloride
	Phosphorus pentoxide
	Palladium-tetrakis(triphenylphosphine)
	4-Toluenebenzoic acid
	4-Toluenesulfonyl chloride

Aldrich	1,3-Benzenedimethanethiol, 95 %
	1,4-Benzenedimethanethiol, 98 %
	1,3-Benzenedithiol, 97 %
	1,4-Benzenedithiol, 97 %
	(1,3-Bis(diphenylphosphino)propane)palladium(II)chloride
	4-Dimethylaminopyridine
	N,*N*-Diisopropylethylamine
	DL-Dithiothreitol, ≥ 98 %
	Methanesulfonic acid
	Palladium hydroxide on carbon 20 wt %
	Potassium hexafluorophosphate, 98 %
	Silver nitrate
	Sodium tetraphenylborate
	Tetra-*n*-butylammonium cyanide, ≥ 95%
	Tetra-*n*-butylammonium iodide, ≥ 98 %
	Tetra-*n*-butylammonium sulfate, 50 wt % aqueous solution
	Tetra-*n*-methylammonium sulfate
	4-Toluenesulfonate, ≥ 99%
BASF	1,4-Dioxane
	Methanol
Berkel	Ethanol p.a.
Brenntag	Acetone
	Ethyl acetate p.s.
	Toluene p.s.
Degussa-Hüls	Diethyl ether p.a.
	Dimethyl sulfoxide-d_6
Euriso-top	Acetonitrile- d_3
	Chloroform-d_1
	Deuterium oxide
	Dimethyl sulfoxide-d_6
	Methanol-d_4

Fluka	2-Amino-6-methylpyridine
	Chloroform
	Celite® Filter Gel
	Di-*tert*-butyl-dicarbonate
	4*R*-Hydroxy-L-proline-methylester hydrochloride
	Potassium bisulfate
	Palladium on carbon 10 wt %
	Pyrrolidine p.a.
	Silver trifluoromethanesulfonate, ≥ 98 %
	TBTU
	Tetra-*n*-butylammonium cyanide
	Tetra-*n*-butylammonium hydroxide, 20 wt % aqueous solution
	Trifluoroacetic acid, ≥ 99 %
T.J. Baker	Acetonitrile, HPLC-grade
	Hydrochloric acid, 37 % p.a.
	n-Pentane
	Sodium hydroxide
	Tetrahydrofuran p.s.
Merck	Barium chloride
	Dimethylsulfoxide
	Hydrochloric acid 1N
	Iodine
	N-Ethyldiisopropylamine
	N,N-Dimethylformamide p.s.
	Nitric acid
	Sodium bicarbonate, anhydrous, ≥ 98 %
	Sodium carbonate, anhydrous
	Sodium chloride p.a.
	Sodium sulfate, anhydrous p.a.
	Sodium hydroxide
	Sulfuric acid
	Tetramethylsilane
	Triethylamine
	Trifluoroacetic acid, ≥ 99 %
MP SiliTech	Silica gel 63 – 200 (60 Å)

Nova-Biochem	4*R*-Hydroxy-L-proline
	Boc-4*R*-Hydroxy-L-proline
	EDC
	HATU
	HBTU
	TBTU
Riedel-de-Haën	Morpholine
	Pyridine
Roth	*n*-Pentane
VWR	Acetonitrile, HPLC-grade
	Dichloromethane
	Ethylacetate
	i-Propanol, HPLC-grade
	Methanol, HPLC-grade
	n-Hexane

6. 4 Analytical methods

^{1}H-NMR	Bruker Avance 600 (600 MHz)
	Bruker DPX 400 (400 MHz)
^{13}C-NMR	Bruker Avance 600 (151 MHz)
	Bruker DPX 400 (101 MHz)
Mass spectrometry	MALDI-TOF MS: Bruker Ultraflex TOF/TOF
	2,5- Dihydroxybenzoic acid as solid matrix, 20 kV and
	25 kV pulsed voltage was used for measurements in negative
	and positive mode, respectively.
	ESI Quadrupole MS: Bruker Daltonics Esquire 3000
Elemental analysis	Perkin-Elmer elemental analyzer EA 2400 CHN
	Elementar Vario MICRO cube
	Analytic Department, Organic Chemistry, TU Kaiserslautern

Melting Point	Müller SPM-X 300
IR	Perkin Elmer FT-IR Spectrometer, Spectrum 1000
HPLC	Dionex: P680 HPLC pump, ASI-100 Autosampler, TCC-100 column oven, UVD 170U UV/Vis Detector, Chromeleon V 6.70 Software
HPLC-Columns	Acclaim® 120-C18, 4.6 x 150 mm, 5µm, 120 Å Chromolith® SemiPrep 100-10 mm RP-18 endcapped LiChroCART® 250-10, 5 µm Purospher®STAR NH_2 RP-18 endcapped, 5 µm Zorbax Eclipse XDB-C8, 4.6 x 150 mm, 5 µm Zorbax Eclipse XDB-C8, 9.4 x 250 mm, 5 µm
Column chromatography	Silica gel 63-200 (60 Å) from MP SiliTech Silica gel 60 (230-400 Mesh ASTM) from Merck Lobar® Column from Merck, size B (310-25) Lichroprep® RP-8 (40-63 µm) Lobar® Column from Merck, size A (240-10) Lichroprep® RP-18 (40-63 µm)
Thin layer chromatography	Silica gel 60 F 254 aluminum foil sheet (Merck) RP-8 F254 glass foil sheet (Merck) RP-18 F254 glass foil sheet (Merck)

6.5 Characterization

6.5.1 ^{1}H-NMR spectroscopic characterization of bis(cyclopeptides) and binding studies

The different salts of anions, spacer compounds, and receptors were weighted using a high precision analytical balance and, where needed, dissolved to known volume.

The ^{1}H-NMR spectra of compounds **98-101** were acquired in a 2:1 (*v/v*) mixture of CD_3CN and D_2O. To this end, stock solutions of bis(cyclopeptides) **98** (0.50 mM, 1.00 µmol in 2 mL 2:1 CD_3CN/D_2O) and **100** (0.59 mM, 1.18 µmol in 2 mL 2:1 CD_3CN/D_2O) were prepared, stirred for 10-15 minutes at room temperature until fully dissolved, and filtered. An aliquot of 250 µL (0.12 µmol for **98** and 0.15 µmol for **100**) was transferred into an NMR tube (Bruker MATCH^{TM}, 3 x 100

mm) and ^{1}H-NMR spectra were recorded at 600 MHz frequency (512 or 256 scans). Anion binding studies with hosts **98** and **100** were performed by adding a 5 µL aliquot from a salt stock solution to 250 µL of the stock solution containing the bis(cyclopeptide). Information about the composition of salt stock solutions added to **98** and **100** is available in Tables 16 and 17, respectively. A syringe equipped with a long needle was used to mix the solutions and ^{1}H-NMR spectra were recorded direct after salt addition and in regular intervals using the same number of scans and frequency.

For experiments in the context of successive anion exchange samples were prepared containing bis(cyclopeptides) **98** or **100**. Concentration of **98** is 0.5 mM in 2:1(*v/v*) CD_3CN/D_2O to which 0.17 equiv. of Na_2SO_4 was added. Concentration of **100** is 0.59 mM in 2:1(*v/v*) CD_3CN/D_2O to which 0.14 equiv. of Na_2SO_4 was added. To fully convert **100**•SO_4 into the corresponding iodide or chloride complexes 1.72 equiv. of BaI_2 or 5.15 equiv. of $BaCl_2$ were added, respectively and the mixture was allowed to equilibrate at room temperature. Further anion exchange was achieved either by adding different silver salts containing the anion under study (e.g. AgTs, $AgBF_4$ and $AgNO_3$) to the resulting iodide complex of **100** or by adding sodium salts containing the anion under study (e.g. Na_2SeO_4, $NaReO_4$ and Na_3PO_4) to the resulting chloride complex of **100**. In the case of **98**•SO_4 the corresponding halide complexes were prepared by addition of 3.04 equiv. of $BaCl_2$, more than 2.03 equiv. of $BaBr_2$ or 2.03 equiv. of BaI_2 to the above mentioned sample containing **98** and 0.17 equiv. of Na_2SO_4. The experiments performed during the binding studies and the outcomes are shown schematically in Chart 35.

salt	equiv.	composition of stock solution
$BaCl_2$ •2 H_2O	1.01	1.5 mg of $BaCl_2$ in 250 µL of D_2O
	2.03	3.1 mg of $BaCl_2$ in 250 µL of D_2O
	3.04	4.6 mg of $BaCl_2$ in 250 µL of D_2O
$BaBr_2$ •2 H_2O	1.01	2.1 mg of $BaBr_2$ in 250 µL of D_2O
	2.03	4.2 mg of $BaBr_2$ in 250 µL of D_2O
	6.08	12.6 mg of $BaBr_2$ in 250 µL of D_2O
BaI_2 •2 H_2O	1.01	2.6 mg of BaI_2 in 250 µL of D_2O
	2.03	5.2 mg of BaI_2 in 250 µL of D_2O
	4.06	10.5 mg of BaI_2 in 250 µL of D_2O
Na_2SO_4	0.17	0.6 mg of Na_2SO_4 in 1000 µL of D_2O
	6.76	11.9 mg of Na_2SO_4 in 500 µL of D_2O
Me_4NI	0.84	2.1 mg of MeN_4I in 500 µL of D_2O
	1.86	2.3 mg of MeN_4I in 250 µL of D_2O

Table 16 Composition of salt stock solutions added to bis(cyclopeptide) **98**.

salt	equiv.	composition of stock solution
$AgNO_3$	1.72	4.3 mg of $AgNO_3$ in 500 µL of D_2O
$AgBF_4$	3.56	5.1 mg of $AgBF_4$ in 250 µL of D_2O
$AgBPh_4 \bullet H_2O$	2.86	5.6 mg of $AgBPh_4$ in 150 µL of D_2O
	3.56	7.0 mg of $AgBPh_4$ in 150 µL of D_2O
$AgPF_6$	3.56	2.7 mg of $AgPF_6$ in 100 µL of D_2O
AgTs	3.56	2.8 mg of AgTs in 100 µL of CD_3CN
$BaCl_2 \bullet 2\ H_2O$	0.86	3.1 mg of $BaCl_2$ in 500 µL of D_2O
	1.72	3.1 mg of $BaCl_2$ in 250 µL of D_2O
	2.57	4.6 mg of $BaCl_2$ in 250 µL of D_2O
	3.43	6.2 mg of $BaCl_2$ in 250 µL of D_2O
	5.15	5.5 mg of $BaCl_2$ in 150 µL of D_2O
$BaBr_2 \bullet 2\ H_2O$	0.86	4.2 mg of $BaBr_2$ in 500 µL of D_2O
	1.72	4.2 mg of $BaBr_2$ in 250 µL of D_2O
	2.57	3.8 mg of $BaBr_2$ in 150 µL of D_2O
	3.43	5.0 mg of $BaBr_2$ in 150 µL of D_2O
	5.15	7.6 mg $BaBr_2$ in 150 µL of D_2O
$BaI_2 \bullet 2\ H_2O$	1.72	5.4 mg of BaI_2 in 250 µL of D_2O
$NaI \bullet 2\ H_2O$	1.57	4.3 mg of NaI in 500 µL of D_2O
	1.72	4.7 mg of NaI in 500 µL of D_2O
NaI	1.57	3.5 mg of NaI in 500 µL of D_2O
	1.72	3.8 mg of NaI in 500 µL of D_2O
Na_2SO_4	0.14	0.6 mg of Na_2SO_4 in 1000 µL of D_2O
	1.72	3.6 mg of Na_2SO_4 in 500 µL of D_2O
	1.86	3.9 mg of Na_2SO_4 in 500 µL of D_2O
	3.43	3.6 mg of Na_2SO_4 in 250 µL of D_2O
	5.15	3.2 mg of Na_2SO_4 in 150 µL of D_2O
Na_2SeO_4	1.57	4.4 mg of Na_2SeO_4 in 500 µL of D_2O
	9.72	8.1 mg of Na_2SeO_4 in 150 µL of D_2O
Me_4NI	0.55	1.1 mg of MeN_4I in 350 µL of D_2O
	1.56	2.3 mg of MeN_4I in 250 µL of D_2O

Table 17 Composition of salt stock solutions added to bis(cyclopeptide) **100**.

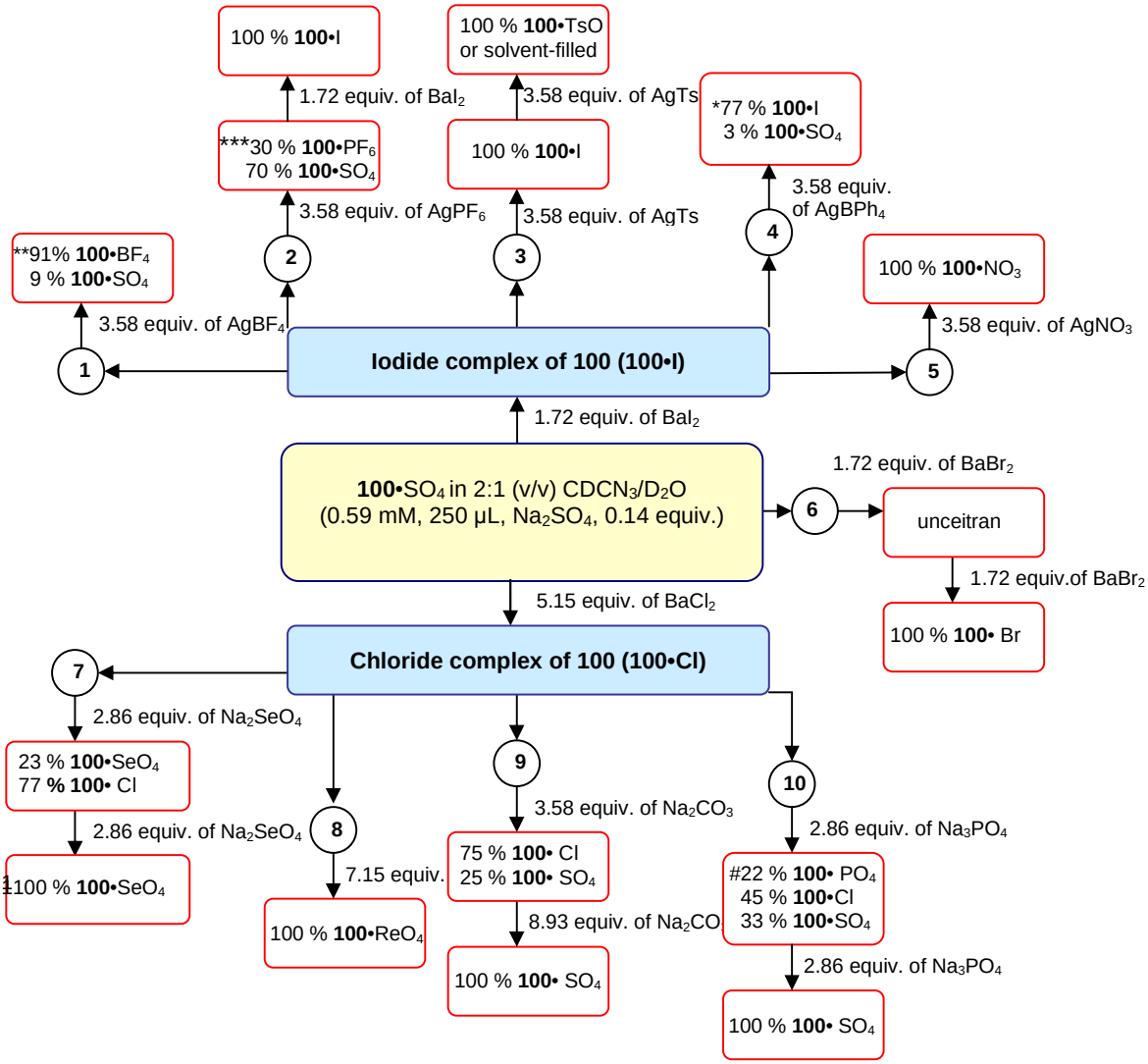


Chart 35 Summary of the anion exchange experiments performed with **100** and the obtained results. The observed ratios of the anion complexes after establishing the equilibrium (24h) are shown in red boxes. Samples that were equilibrated for 96 h, 168 h or 30 days are marked with *, **, or ***, respectively. # The nature of the bound phosphate anion is unclear (HPO_4^{2-} / $H_2PO_4^-$).

6.5.2 H/D exchange experiments

Hydrogen-deuterium exchange was followed by means of ^{1}H-NMR spectroscopy. Series of spectra were recorded starting directly after salt addition (ca. 10 min) and then in regular intervals. Initially, samples containing compounds **98** and **100** were prepared.

Compound **98** (0.5 mM, 0.12 µmol) was dissolved in 2:1(*v/v*) CD_3CN/D_2O and 5 µL of a salt stock solution of Na_2SO_4 (0.02 µmol, 0.17 equiv. in the sample) was added. The solution was thoroughly mixed, filtered and transformed into small NMR tube. The tube was closed with a cap, which was carefully wrapped with parafilm and NMR spectra were recorded. Compound **100** (0.59 mM, 0.15 µmol) was dissolved in 2:1(*v/v*) CD_3CN/D_2O and 5 µL of a salt stock solution of Na_2SO_4 (0.02 µmol, 0.14 equiv. in the sample) was added. Sample preparation was than the same as in the case of **98**.

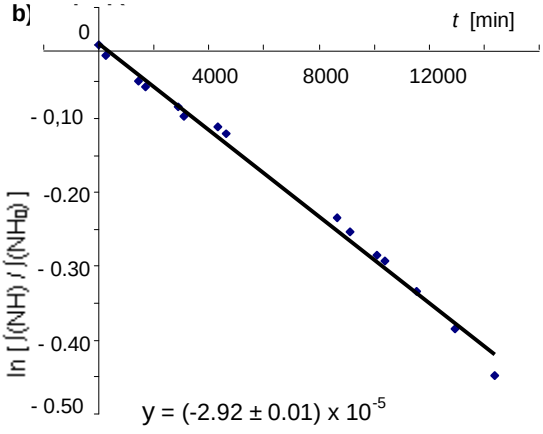
To the sample of compound **100** was added a 5 µL aliquot from a salt stock solution of either Na_2SO_4 (1.72 equiv. in sample), BaI_2 (1.72 equiv.), $BaCl_2$ (1.72 equiv.) or $BaCl_2$ (0.86 equiv.). The solution was thoroughly mixed by means of a syringe with a long needle. The NMR tube was closed with a cap and carefully wrapped with parafilm. NMR spectra were recorded.

For the NMR analyses the integral of the NH peak was determined in a defined ppm range. To allow comparison of different spectra the integral of the signal of the H(α) protons (or the sum of the signals of the H(α) protons in case more than one anion complex is present in solution) was used for calibration. The rate of hydrogen-deuterium exchange was followed in regular time intervals for up to 45 days in the case of bis(cyclopeptide) **100**. The sample containing **98** was followed over 7 days. Each sample was stored at ambient temperature and measured under identical conditions (600 MHz machine, 512 scans and same integrated areas). Relaxation delay was not taken into account. The results and calculations obtained are summarized in Charts 36-39.

a)

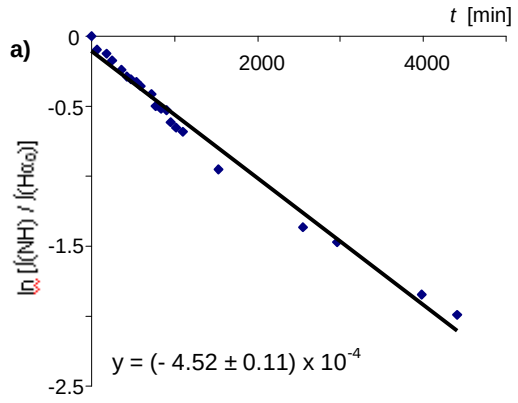
t [min]	∫NH	∫NH / ∫NH$_0$	ln [∫NH/∫NH$_0$]
10	1.0701	1.00000	0.00000
246	1.0543	0.98598	-0.01412
1439	1.0187	0.95171	-0.04949
1680	1.0097	0.94393	-0.05771
2878	0.9827	0.91900	-0.08447
3086	0.9712	0.90810	-0.09640
4322	0.9574	0.89408	-0.11196
4619	0.9485	0.88629	-0.12071
8638	0.8474	0.79128	-0.23411
9135	0.8311	0.77570	-0.25399
10100	0.8050	0.75234	-0.28457
10379	0.7992	0.74611	-0.29289
11539	0.7669	0.71651	-0.33336
12933	0.7278	0.68069	-0.38466
14369	0.6840	0.63863	-0.44843

b)



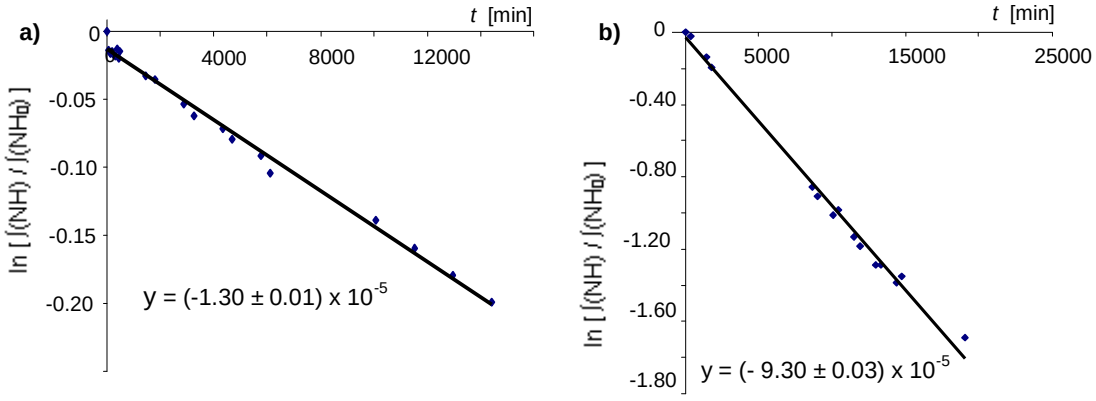
c) k_{ps} = (2.92 ± 0.01) x 10^{-5} min^{-1} $t_{1/2}$ = 16.5 d

Chart 36 H/D exchange rate of **100**•SO_4 (0.59 mM in 2:1(v/v) CD_3CN/D_2O) in the presence of 0.14 equiv. of Na_2SO_4. (a) Dependence of the area of the NH signal (∫NH) on time, and calculation of ln ∫NH/ ∫NH_0. (b) Graphical representation of the results. (c) Results.



b) k_{ps} = (4.52 ± 0.11) x 10^{-4} min^{-1}
$t_{1/2}$ = 1.06 d = 25.5 h

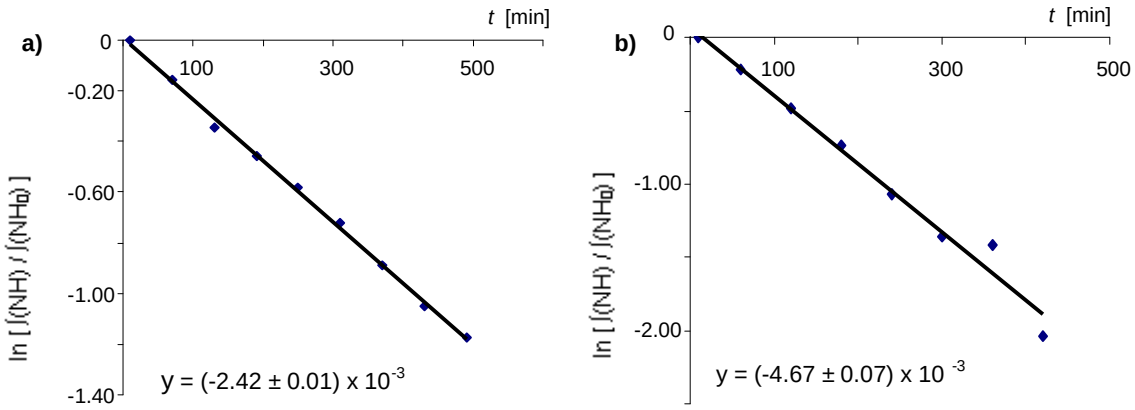
Chart 37 H/D exchange rate of **98**•SO_4 (0.50 mM in 2:1(v/v) CD_3CN/D_2O) in the presence of 0.17 equiv. of Na_2SO_4. (a) Graphical representation of the results. (b) Results.



c) k_{ps} = (1.30 ± 0.01) x 10^{-5} min^{-1}
$t_{1/2}$ = 37.0 d

d) k_{ps} = (9.30 ± 0.03) x 10^{-5} min^{-1}
$t_{1/2}$ = 5.2 d

Chart 38 H/D exchange rate of **100**•SO_4 (0.59 mM in 2:1(v/v) CD_3CN/D_2O) in the presence of 0.14 equiv. of Na_2SO_4 and additional 1.72 equiv. of Na_2SO_4 (a, c) or 1.72 equiv. of BaI_2 (b, d).



c) k_{ps} = (2.42 ± 0.01) x 10^{-3} min^{-1}
$t_{1/2}$ = 0.20 d = 4.8 h

d) k_{ps} = (4.67 ± 0.07) x 10^{-3} min^{-1}
$t_{1/2}$ = 0.10 d = 2.5 h

Chart 39 H/D exchange rate of **100**•SO_4 (0.59 mM in 2:1(v/v) CD_3CN/D_2O) in the presence of 0.14 equiv. of Na_2SO_4 and additional 0.86 equiv. of $BaCl_2$ (a, c) or 1.72 equiv. of $BaCl_2$ (b, d).

6.5.3 HPLC- und LC/MS-Analysis

HPLC purifications were carried out using an Agilent 1100 series machine coupled with a UV/Vis-multi wavelength detector. Data was processed using the Chromeleon software Version 6.70. As stationary phase a double end capped Zorbax Eclipse XDB-C8 column (for analytical HPLC: 4.6 x 150 mm, pore size 5 µm; for preparative HPLC: 9.4 x 250 mm, pore size 5 µm), thermostatted to 40 °C was used. The injected volume for an analytical measurement was 10 µL (unless specially noted) and the flow rate of elution 1 ml/min. The separation was performed with a mixture of water/acetonitrile as mobile phase, both containing 0.1 vol % of trifluoroacetic acid (TFA). In order to obtain better chromatographic separation, deprotected thiol containing cyclic peptides were sometimes eluted with a water mobile phase containing 0.1 wt % of Na_2SO_4 and 0.1 vol % of TFA when sulfate salts were used as template in the DCLs. Likewise, 0.1 wt % of NaI was used when iodide acted as template. Adding 0.1 wt % of the template salt to the water mobile phase was used to analyze mixed libraries as well as to isolate products on a preparative scale. The relative rates of eluents were gradually changed with time. The HPLC gradient program for analytical HPLC is summarized in Table 18. More details about HPLC parameters for analytical and preparative separations are available in Section 4.3. Detection was performed by measuring the UV absorbtion at 254 nm and 290 nm.

time / min	A / vol %	B / vol %
0	25	75
20	50	50
25	60	40
35	90	10
40	97	3
48	97	3
50	25	75
60	25	75

Table 18 Gradient program for HPLC analysis (mobile phase A: acetonitrile + 0.1 vol % TFA; B: water + 0.1 vol % TFA (+ 0.1 wt % of template salt if necessary)).

LC-MS was performed using the same column and gradient program. Detection was achieved by using a diode array UV detector and an Agilent XCT ion trap mass spectrometer. These measurements were performed in the Department of Chemistry at the University of Cambridge, UK. A 1:20 splitter to reduce the flow exiting the UV flowcell from 1 mL/min to 50 µL/min was installed prior to the electrospray ionization chamber. Thus, better ionization efficiency was achieved. Data were processed using Agilent LCMSD trap data analysis software. Addition of trifluoroacetic acid may cause problems during the MS-detection. In order to prevent contamination, the use of formic acid was preferred or changing of the tubes, capillaries, splitter and extensive cleaning of the machine was necessary after the measurements.

6.5.4 ESI-MS

The accuracy of the MS equipment immediately after calibration was ± 0.3 m/z. The MS data shown in Sections 4.2 and 4.3 were -0.5 *m/z* offset with respect to the exact masses of the protonated receptors or their adducts with sodium. Some of the MS spectra recorded for bis(cyclopeptides) **98-101** were acquired in deuterated solvents usually in a 2:1 (*v/v*) mixture of CD_3CN and D_2O if not otherwise specified.

MS Parameters: Mass range mode: Standard enhanced
Ion polarity: Negative mode (if not specified)
Ion Source: ESI
Dry temperature: 325 °C
Nebulizer pressure: 15.00 psi
Dry gas flow: 5 L/min
HV capillary: 4000 V
Scan range: 700-2200

6.6 Nomenclature

The atom numbering within cyclopeptides does not follow the IUPAC rules. Functional groups in the products are abbreviated as follows:

• Amino acids are abbreviated with a three or four letters code:

APA = 6-aminopicolinic acid, Pro = (2*S*)-proline,

Hyp = (2*S*,4*R*)-4-hydroxyproline, Tpro = (2*S*, 4*S*)-4-thioproline

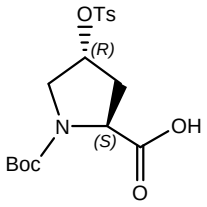
(4*S*)Cpro = (2*S*,4*S*)-4-cyanoproline, (4*R*)Cpro = (2*S*,4*R*)-4-cyanoproline,

• Position and configurations of proline substituents are denoted in round brackets, for example:

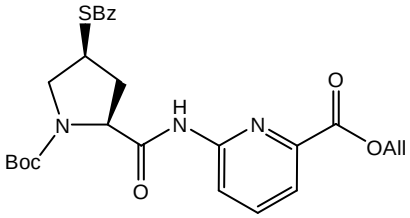
(4*R*-Ms)Hyp, (4*R*-Ts)Hyp, (4*S*)Cpro

• Peptide sequences are specified from N- to C-terminus, where H- and OH- represent the non-protected amino acids, while other abbreviations denote the respective protecting group.

Example:



Boc-(4*R*-Ts)Hyp-OH

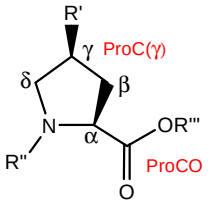


Boc-(Bz)Tpro-APA-OAll

• For the assignment of the chemical shifts of protons and carbon atoms collected from the NMR-spectroscopic data the following nomenclature is used:

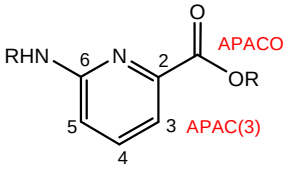
Amino acids:

Proline compounds (Pro)



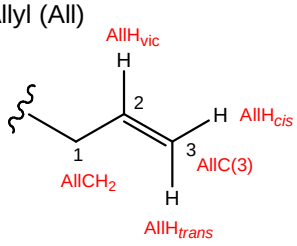
R' = H (Pro)
R' = OH (Hyp)
R' = CN (Cpro)
R' = SR (Tpro)

Aminopicolinic acid derivatives (APA)

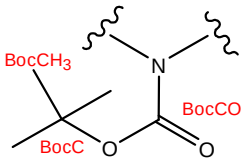


Protective groups:

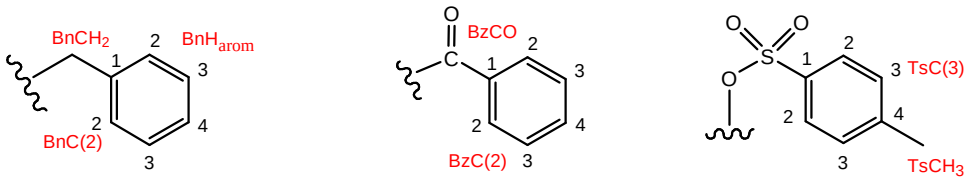
Allyl (All)



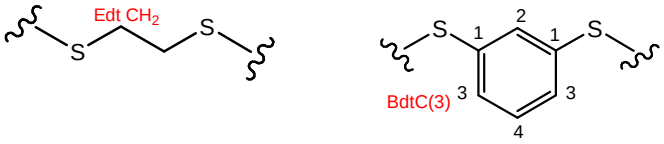
N-tert-Butyloxycarbonyl (Boc)



Benzyl (Bn) Benzoyl (Bz) Tosyl (Ts)



Linkers: 1,2-Ethanedithiol 1,3-Benzenedithiol



6.7 Cleavage of the protective groups

Cleavage of allyl (All) esters

The allyl ester was dissolved in dry and freshly degassed THF (20 mL/mmol) under inert conditions. Heating of the flask is not recommended because of the thermolability of *N*-tert-butyloxycarbonyl-protective groups. Tetrakis(triphenylphosphine)palladium(0) (5 mg) and freshly distilled morpholine (3 mmol) were added, and the reaction mixture was stirred at room temperature. Progress of the reaction was checked by TLC and MALDI/TOF MS after 5 min. In case starting material was still detectable additional catalyst (5 mg) and morpholine (0.2 mmol) were added. Stirring was continued until no traces of starting material were observed. Solvent was evaporated to dryness and the residue was dissolved in ethyl acetate (in the case of linear hexapeptides containing thiol groups methylene chloride was used). The organic layer was washed three times with 10 % aqueous $KHSO_4$ and twice with water. The solvent was evaporated. The product was dried *in vacuo* and used for the following step without further purification.

Cleavage of benzyl (Bn) esters

Method A: The ester was dissolved in methanol (50 mL/mmol) and 10 % Pd/C (100 mg/mmol) was added. The reaction mixture was subjected to hydrogenation at 1 atm for 2 h (7 h for linear hexapeptides containing nitrile groups). Progress of the reaction was checked by TLC and MALDI-TOF MS. The catalyst was filtered off through a layer of Celite and washed with methanol. The filtrate and the washings were concentrated to dryness *in vacuo*. The product obtained was triturated with diethyl ether and used for the next step without purification.

Method B: The ester was dissolved in 9:1 (v/v) of 1,4-dioxane/water (50 mL/mmol) and 10 % Pd/C (100 mg/mmol) was added. The reaction mixture was subjected to hydrogenation at 1 atm

for 2 h (72 h for linear hexapeptides containing nitrile groups). Work-up was performed as described for Method A.

Cleavage of benzoyl (Bz) esters

The reaction was conducted under inert conditions. The thiobenzoate (0.1 mmol) was suspended in dry and freshly degassed methanol (82 µL, 0.1 mmol). DIEA (0.8 mmol) was injected until the pH of the reaction mixture reached ca. 9-10. The suspension was stirred for 9 - 11 days under nitrogen at room temperature, whereby the starting material slowly dissolved. Reaction progress was checked by MALDI-TOF MS. After full deprotection, the flask was opened to the air for 2 h. The mixture was concentrated to ca. 1 - 2 mL. Addition of diethyl ether (ca. 50 mL) to the residue caused formation of a white precipitate. The product was filtered off, thoroughly washed with diethyl ether, and dried *in vacuo*.

Cleavage of *N*-tert-butyloxycarbonyl (Boc) groups

Method A: A suspension of the carbamate in 1,4-dioxane (20 mL/mmol) was cooled in an ice bath and a 6 N solution of HCl in 1,4-dioxane (40 mL/mmol) was added dropwise. The reaction mixture was stirred for 2 h at 0 - 5 ºC (3 - 4 h for hexapeptides containing thiol groups). Completion of the reaction was controlled by TLC and MALDI-TOF MS. After full deprotection, the solvent was evaporated and the residue triturated with diethyl ether. The crude product was filtered off, washed with diethyl ether, dried *in vacuo*, and used for the next step without further purification.

Method B: A solution of trifluoroacetic acid in dry methylene chloride (1:1 (v/v), 3 mL/mmol) was added dropwise to an ice-cold mixture of the carbamate in dry methylene chloride (3 mL/mmol). The reaction was stirred for 1 h at 0 - 5 ºC and for another 2 h at room temperature (3 - 4 h for hexapeptides containing nitrile groups). Completion of the reaction was controlled as described for Method A, and then the solution was concentrated to dryness *in vacuo*. The residue was dissolved in methylene chloride. The solution was washed twice with 10 % aqueous Na_2CO_3 and the combined aqueous extracts three times with methylene chloride. The combined organic layers were dried over $MgSO_4$, and the solvent was evaporated *in vacuo*. The resulting crude product was triturated with diethyl ether, dried *in vacuo*, and used without further purification.

6.8 Syntheses

6.8.1 Preparation of salts for ^{1}H-NMR spectroscopic characterization of bis(cyclopeptides) and binding studies

Synthesis of Silver tetraphenylborate ($AgBPh_4$)[227]

Silver tetraphenylborate prepared from sodium tetraphenylborate has the tendency to decompose, turning brown. Therefore, the silver salt was prepared by metathesis of exactly stoichiometric amounts of aqueous potassium tetraphenylborate (53.7 mg, 0.15 mmol) and silver nitrate (25.3 mg, 0.15 mmol) in a minimum amount of acetone (1.5 mL). After stirring at room temperature for 1 h the solvent was decantated, and the salt thoroughly washed, first with hot distilled water to remove nitrate impurities, then with toluene/ isopropanol/ water 50.0 / 49.5 / 0.5 vol %, and finally dried *in vacuo*. The product is sensitive to light.

Yield 24.0 mg, 36 % (white solid)

^{1}H-NMR [400MHz, CD_3OD-d_4/DMSO-d_6, 22 ºC]: δ = 6.76-6.89 (m, 4H, BnH_{arom}), 6.89-7.06 (t, 8H, ^3J= 7.6 Hz BnH_{arom}), 7.24-7.33 (m, 8H, BnH_{arom})

C, H, N Calcd C: 64.76 % H: 4.98 %
Found C: 65.35 % H: 4.86 %
M_r ($C_{24}H_{20}AgB•H_20$) 445.11 g/mol

Synthesis of (1,3-Bis(diphenylphosphino)propane) palladium(II)trifluoromethane-sulfonate ($Pd(dppp)OTf_2$, 50a)[175]

The reaction was conducted under nitrogen in a flask wrapped with alumina foil due to the light sensitivity of the product and the air sensibility of the educt. Silver trifluoromethanesulfonate (30.83 mg, 0.12 mmol) was added to a solution of (1,3-bis(diphenylphosphino)-propane)palladium(II)chloride (23.59 mg, 40 µmol) in dry, degassed methylene chloride (4 mL) and the reaction mixture was stirred overnight at room temperature. The heterogeneous suspension was filtered and the filtrate concentrated to 1 mL under reduced pressure. Diethyl ether (p.a.) was added to precipitate the complex. The product was isolated by filtration, washing with diethyl ether, and drying *in vacuo*.

Yield 30.09 mg, 92 % (yellow powder)

^{1}H-NMR [600MHz, CD_3OD-d_4, 22 ºC]: δ = 2.14-2.31 (m, 2H, $dpppCH_2$), 2.95-2.85 (m, 4H, $dpppCH_2$), 7.51-7.8 (m, 20H, BnH_{arom})

^{19}F-NMR [600MHz, CD_3OD-d_4, 22 ºC]: δ = -80.09

^{31}P-NMR [600MHz, CD_3OD-d_4, 22 ºC]: δ = 17.27; 26.79

C, H, N Calcd	C: 39.99 %	H: 3.70%	S: 7.36%
Found	C: 40.25 %	H: 3.53 %	S: 7.32 %
M_r	($C_{29}H_{26}F_6O_6P_2PdS_2$•3 H_20)		871.01 g/mol

6.8.2 Preparation of amino acid derivatives

Synthesis of 6-Aminopicolinic acid allyl ester (81a)[54, 209a, 228]

6-Aminopicolinic acid (6.90 g, 50 mmol) and $NaHCO_3$ (8.40 g, 100 mmol) were suspended in DMF (300 mL) and 3-bromopropane (13.1 mL, 150 mmol) was added dropwise. The reaction mixture was stirred for 48 h at room temperature, and then sonicated for another 4-5 h at 25 ºC. The solvent was removed *in vacuo* and ethyl acetate was added. The organic layer was washed three times with 10 % aqueous Na_2CO_3 and once with water, dried with Na_2SO_4, and evaporated. The residue was dissolved in a small amount of ethyl acetate and the product was precipitated by addition of pentane. Additional product can be isolated from the filtrate by evaporation and precipitation by column chromatography (SiO_2, ethyl acetate/pentane 1:1 v/v). The excess of 6-aminopicolinic acid can be precipitated from the aqueous washings directly by acidification with 1 M HCl (pH 6 - 7) and used for other reactions.

Yield 4.90 g, 55 % (white to light yellow solid)

MS MALDI-TOF *m*/*z* (rel. Int.): $[M+H]^+$ 178.66 (100 %), $[M+Na]^+$ 200.67 (99 %), $[M+K]^+$ 216.68 (95 %)

Synthesis of 6-Aminopicolinic acid benzyl ester (81b)[190]

6-Aminopicolinic acid (6.70 g, 48.47 mmol) and $NaHCO_3$ (5.70 g, 67.86 mmol) were suspended in DMF (290 mL) and benzyl bromide (8 mL, 67.90 mmol) was added dropwise. The reaction mixture was stirred for 48 h at room temperature, and then sonicated for another 4 - 5 h at 25 ºC. The excess of 6-aminopicolinic acid was filtered off. After washing with water, acetone, and diethyl ether it can be used for further reactions. The filtrate was concentrated *in vacuo* to approximately 80 mL and after the addition of 10 % aqueous Na_2CO_3 (190 mL) the mixture was extracted three times with ethyl acetate. The combined organic layers were washed three times with water and dried over Na_2SO_4. The solvent was evaporated, and the crude product was triturated with hexane. Finally, the product was recrystallized from hexane/ethyl acetate.

Yield 5.20 g, 47 % (white solid)

MS MALDI-TOF *m*/*z* (rel. Int.): $[M+H]^+$ 229.09 (100 %), $[M+Na]^+$ 251.26 (86 %), $[M+K]^+$ 267.26 (76 %)

Synthesis of Boc-Hydroxyproline-benzyl ester (Boc-(4*R*-Hyp)-OBn, 76b)[188a]

Hydroxyproline benzyl ester hydrochloride (10.81 g, 42.0 mmol) was suspended in 2:1 (v/v) 1,4-dioxane/water (126 mL) and the mixture was cooled in an ice bath. After the addition of triethylamine (5.8 mL, 42.0 mmol), a solution of di-*tert*-butyl dicarbonate (10.08 g, 46.2 mmol) in 1,4-dioxane (84 mL) was added dropwise. The reaction mixture was then stirred for 2 h at room temperature. The 1,4-dioxane was evaporated, and the residue was extracted three times with ethyl acetate. The combined organic layers were washed with water, dried over Na_2SO_4, and the solvent was removed *in vacuo* to afford pure product.

Yield 13.24 g, 98 % (white solid)

TLC R_f = 0.35 (ethyl acetate/pentane 2:1 v/v), detection by staining with ninhydrin

m.p. 72 - 74 ºC

^{1}H-NMR [600MHz, DMSO-d_6, 100 ºC]: δ = 1.37 (s, 9H, BocCH_3), 1.98-2.02 (m, 1H, Hyp(β)H), 2.15-2.19 (m, 1H, Hyp(β)H), 3,31-3,33 (d, 1H, ^2J= 10.8 Hz, Hyp(δ)H), 3.44-3.47 (dd, 1H, ^2J= 10.8 Hz, ^3J= 4.8 Hz, Hyp(δ)H), 4.30-4.31 (m, 1H, Hyp(γ)H), 4.35 (t, 1H, ^3J= 7.8 Hz, ^3J= 7.2 Hz, Hyp(α)H), 4.63 (br s, 1H, OH), 5.13-5.17 (q, 2H, ^2J= 12.6 Hz, BnCH_2), 7.31-7.37 (m, 5H, BnH_{arom})

^{13}C-NMR [151 MHz, DMSO-d_6, 100 ºC]: δ = 27.4 (BocCH_3C), 37.9 (ProC(β)), 53.9 (HypC(γ)), 57.3 (HypC(α)), 65.2 (HypC(δ)), 67.5 (Bn-CH_2C), 78.4 (BocC), 127.0 (BnC(2)), 127.2 (BnC(4)), 127.6 (BnC(3) + BnC(5)), 135.4 (BnC(1)), 152.9 (BocCO), 171.6 (HypCO)

MS MALDI-TOF *m*/*z* (rel. Int.): $[M+Na]^+$ 344.4 (100 %), $[M+K]^+$ 360.4 (69 %)

Synthesis of Boc-(4*R*)-4-Mesylhydroxyproline benzyl ester (Boc-(4*R*-Ms)Hyp-OBn, 79)

To a mixture of Boc-(4*R*-Hyp)-OBn (13.2 g, 41.07 mmol) and pyridine (40 mL, 0.49 mol) in dry methylene chloride (44 mL) methanesulfonyl chloride (15.5 mL, 0.20 mol) was added dropwise. The solution was stirred for 18 h at room temperature and the solvent was then removed *in vacuo*. The residue was redissolved in ethyl acetate, the solution extracted three times with 10 %

aqueous $NaHCO_3$ and finally washed twice with water. After drying over Na_2SO_4, the solvent was removed *in vacuo*, and the product was purified by column chromatography (SiO_2, ethyl acetate/pentane 2:1 v/v). The obtained oily substance was treated with a small amount of methylene chloride to afford a white solid.

Yield 15.25 g, 93 % (white solid)

TLC R_f = 0.68 (ethyl acetate/pentane 2:1 v/v), detection by staining with ninhydrin

m.p. 71 - 72 ºC

^{1}H-NMR [600MHz, DMSO-d_6, 100 ºC]: δ = 1.37 (s, 9H, BocCH_3), 2.29-2,36 (m, 1H, Hyp(β)H), 2.57-2,60 (m, 1H, Hyp(β)H), 3.17 (s, 3H, MsCH_3), 3.65-3.71 (m, 2H, Hyp(δ)H), 4.42 (t, 1H, ^3J= 7.8 Hz, Hyp(α)H), 5.16-5.20 (q, 2H, ^2J= 13.2 Hz, BnCH_2), 5.26 (br s, 1H, Hyp(γ)H), 7.32-7.38 (m, 5H, BnH_{arom})

^{13}C-NMR [151 MHz, DMSO-d_6, 100 ºC]: δ = 22.9 (HypC(β)), 27.3 (BocCH_3C), 37.6 (OMs-CH_3C), 51.6 (Hyp C(δ)), 56.7 (HypC(α)), 65.6 (Bn-CH_2C), 79.2 (BocC + HypC(γ)), 127.1 (BnC(2)), 127.4 (BnC(4)), 127.7 (BnC(3)), 135.2 (BnC(1)), 152.4 (BocCO), 170.7 (HypCO)

IR [KBr, cm^{-1}] 3485 (b), 3010 (w), 2972 (w), 2926 (w), 2878 (w), 1752 (s), 1705 (s), 1404 (m), 1385 (m), 1364 (s), 1350 (m), 1185 (s), 1127 (m), 955 (s), 928 (m), 901 (s), 758 (w)

MS MALDI-TOF *m/z* (rel. Int.): $[M+Na]^+$ 422.2 (95 %), $[M+K]^+$ 438.2 (100 %)

C, H, N Calcd	C: 54.12 %	H: 6.31 %	N: 3.51 %	S: 8.03 %
Found	C: 54.15 %	H: 6.11 %	N: 3.73 %	S: 7.88 %
M_r	($C_{18}H_{25}NO_7S$)		399.46 g/mol	

Synthesis of Boc-(4*R*)-4-Tosylhydroxyproline methyl ester (Boc-(4*R*-Ts)Hyp-OMe, 77a)[188a, 207]

To a mixture of commercially available Boc-(4*R*-Hyp)-OMe (1.25 g, 5.1 mmol) and pyridine (4.9 mL, 61.2 mmol) in dry methylene chloride (5 mL), toluenesulfonyl chloride (4.9 g, 25.5 mmol) was added. The solution was stirred for 18 h at room temperature and the solvent was then removed *in vacuo*. The residue was dissolved in ethyl acetate, the solution extracted three times with 10 %

aqueous Na_2CO_3, and finally washed three times with water. After drying over Na_2SO_4 the solvent was removed *in vacuo* and the product was purified by column chromatography (SiO_2, ethyl acetate/pentane 1:2 v/v). The obtained oily substance was treated with a small amount of methylene chloride to afford a white solid.

Yield 1.87 g, 92 % (white solid)

TLC R_f = 0.62 (ethyl acetate/pentane 1:2 v/v), detection by staining with ninhydrin

^{1}H-NMR [600MHz, $CDCl_3$-*d*, 22 ºC]: δ = 1.38-1.41 (m, 9H, BocCH_3), 2.07-2.17 (m, 1H, Hyp(β)H), 2.37-2.55 (m, 4H, TsCH_3 + Hyp(β)H), 3.54-3.63 (m, 2H, Hyp(δ)H), 3.71 (s, 3H, CH_3), 4.36 (q, 1H, ^3J= 12.0 Hz, Hyp(γ)H), 5.01-5.04 (m, 1H, Hyp(α)H), 7.34-7.37 (dd, 2H, ^3J= 12.0 Hz, ^4J= 5.4 Hz, TsH), 7.77 (d, 2H, ^3J= 12.6 Hz, TsH)

MS MALDI-TOF *m*/*z* (rel. Int.): $[M+Na]^+$ 422.1 (73 %), $[M+K]^+$ 438.1 (100 %)

Synthesis of Boc-(4*S*)-4-Cyanoproline benzyl ester (Boc-(4*S*)Cpro-OBn, 78b)[203-204, 229]

Method A: Boc-(4*R*-Ts)Hyp-OBn (476 mg, 1 mmol) was dissolved in dry DMF (8 mL) and finely grinded NaCN (93 mg, 1.9 mmol) was added at room temperature under stirring. The reaction mixture was stirred for 72 h at 90 ºC, then cooled to 25 °C and finally poured into ice water. The resulting suspension was extracted three times with ethyl acetate, and the combined organic extracts were washed three times with water, dried over $MgSO_4$ and concentrated *in vacuo*. TLC of the residue showed formation of the product as well as of several by-products. Chromatographic purification (SiO_2, ethyl acetate/pentane 1:2 v/v) was performed to obtain a small amount of Boc-(4*S*)-4-cyanoproline benzyl ester.

Yield 23 mg, 7 % (light yellow solid)

Method B: Boc-(*4R*-Ms)Hyp-OBn (15.24 g, 38.2 mmol) was dissolved in DMSO (65 mL) and finely grinded NaCN (3.74 g, 76.4 mmol) was added under stirring at room temperature. The reaction mixture was stirred for 72 h at 90 ºC, then cooled to room temperature, and finally poured into ice water. The resulting suspension was extracted three times with ethyl acetate, and the combined organic extracts were washed three times with water, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2, ethyl acetate/pentane 1:3 v/v) to obtain Boc-(4*S)*Cpro-OBn (**4*S*-78b**) as the major product. This compound was crystallized from *n*-pentane at 4 ºC. A minor product was also isolated representing Boc-(4*R*)-4-cyanoproline benzyl ester (**Boc-(4*R)*Cpro-OBn**, **4*R*-78b**).

Characterization of Boc-(4*S*)Cpro-OBn (4*S*-78b)

Yield 3.85 g, 42 % (white solid)

TLC R_f = 0.52 (ethyl acetate/pentane 1:3 v/v), detection by staining with ninhydrin

HPLC Zorbax Eclipse XDB-C8, mobile phase: water /methanol; flow: 1 mL/min; injection volume: 20 µL, gradient elution: 75/25 to 3/97; column oven 25 °C; λ = 254 nm, R_t 32.4 - 32.6 min (Table 19).

time / min	A / vol %	B / vol %
0	25	75
20	50	50
25	60	40
35	90	10
40	97	3
48	97	3
50	25	75

Table 19 Gradient program for HPLC analysis of compounds **4*S*-78b** and **4*R*-78b** (mobile phase A: methanol; B: water).

m.p. 52 - 55 ºC

^{1}H-NMR [600MHz, DMSO-d_6, 100 ºC]: δ = 1.36 (s, 9H, BocCH_3), 2.15-2.18 (m, 1H, Cpro(β)H), 2.72-2.77 (m, 1H, Cpro(β)H), 3.44-3.50 (m, 2H, Cpro(δ)H + Cpro(γ)H), 3.85 (t, 1H, ^3J= 7,2 Hz, Cpro(δ)H), 4.37-4.39 (dd, 1H, ^3J= 5.4 Hz, ^3J= 8.4 Hz, Cpro(α)H), 5.15 (d, 1H, ^2J= 12.6 Hz, BnCH_2), 5.22 (d, 1H, ^2J= 12.6 Hz, BnCH_2), 7.33-7.38 (m, 5H, BnH_{arom})

^{13}C-NMR [151 MHz, DMSO-d_6, 100 ºC]: δ = 25.4 (CproC(γ)), 27.3 (BocCH_3C), 32.8 (CproC(β)), 48.7 (CproC(δ)), 57.6 (CproC(α)), 65.7 (Bn-CH_2C), 79.3 (BocC), 119.3 (CproCN), 127.2 (BnC(2)), 127.3 (BnC(4)), 127.6 (BnC(3)), 135.2 (BnC(1)), 152.1 (BocCO), 170.3 (CproCO)

IR [KBr, cm^{-1}] 3428 (b), 3034 (w), 2974 (m), 2935 (w), 2888 (w), 2249 (w), 1745 (s), 1700 (s), 1457 (m), 1403 (s), 1366 (s), 1258 (m), 1154 (s), 1117 (m), 1025 (m), 889 (w), 743 (m), 699 (m), 599 (w)

MS	MALDI-TOF *m/z* (rel. Int.): $[M+Na]^+$ 353.0 (67 %), $[M+K]^+$ 369.0 (100 %)			
C, H, N	Calcd	C: 65.44 %	H: 6.71 %	N: 8.48 %
	Found	C: 65.28 %	H: 6.72 %	N: 8.26 %
	M_r	($C_{18}H_{22}N_2O_4$)		330.38 g/mol

Characterization of Boc-(4*R*)Cpro-OBn (4*R*-78b)

Yield	1.39 g, 11 % (white solid)
TLC	R_f = 0.75 (ethyl acetate/pentane 1:3 v/v), detection by staining with ninhydrin
HPLC	Zorbax Eclipse XDB-C8, mobile phase: water /methanol; flow: 1 mL/min; injection volume: 20 µL, gradient elution: 75/25 to 3/97; column oven 25 °C; λ = 254 nm, R_t 34.4 - 34.9 min
m.p.	83 - 86 ºC
^{1}H-NMR	[600MHz, DMSO-d_6, 100 ºC]: δ = 1.38 (s, 9H, $BocCH_3$), 2.33-2.38 (m, 1H, Cpro(β)H), 2.54-2.59 (m, 1H, Cpro(β)H), 3.43 (q, 1H, ^3J= 7.2 Hz, Cpro(γ)H), 3.62 (t, 1H, ^2J= 10.2 Hz, ^3J= 6.0 Hz, Cpro(δ)H), 3.71 (t, 1H, ^2J= 9.6 Hz, ^3J= 8.4 Hz, Cpro(δ)H), 4.43 (t, 1H, ^3J= 4.2 Hz, Cpro(α)H), 5.17 (s, 2H, $BnCH_2$), 7.33-7.34 (m, 5H, BnH_{arom})
^{13}C-NMR	[151 MHz, DMSO-d_6, 100 ºC]: δ = 25.9 (CproC(γ)), 27.3 ($BocCH_3C$), 32.9 (CproC(β)), 48.7 (CypC(δ)), 57.3 (CproC(α)), 65.7 (Bn-CH_2C), 79.4 (BocC), 119.3 (CproCN), 127.1 (BnC(2)), 127.3 (BnC(4)), 127.6 (BnC(3)), 135.2 (BnC(1)), 152.2 (BocCO), 170.4 (CproCO)
IR	[KBr, cm^{-1}] 3477 (b), 3054 (w), 2973 (m), 2936 (w), 2885 (m), 2242 (w), 1749 (s), 1706 (s), 1499 (m), 1476 (m), 1455 (m), 1386 (s), 1362 (s),1280 (s), 1199 (s), 1156 (s), 1123 (s), 986 (m), 925 (m), 870 (m), 751 (s), 699 (m), 541 (w)

MS	MALDI-TOF *m/z* (rel. Int.): $[M+Na]^+$ 353.0 (80 %), $[M+K]^+$ 369.0 (100 %)			
C, H, N	Calcd	C: 65.44 %	H: 6.71 %	N: 8.48 %
	Found	C: 65.71 %	H: 6.53 %	N: 8.16 %
	M_r	($C_{18}H_{22}N_2O_4$)		330.38 g/mol

Synthesis of Boc-(4*S*)-4-Cyanoproline (Boc-(4*S*)Cpro-OH, 80)

Boc-(4*S*)Cpro-OBn (3.66 g, 11.1 mmol) was subjected to hydrogenation according to the general procedure for the cleavage of benzyl ester groups (Method A).

Yield 2.66 g, quantitative (white solid)

TLC R_f = 0.0 (ethyl acetate/pentane 1:3 v/v), detection by staining with ninhydrin

MS MALDI-TOF *m*/*z* (rel. Int.): $[M+Na]^+$ 262.8 (100 %), $[M+K]^+$ 278.8 (77 %)

IR [KBr, cm^{-1}] 3422 (b), 2980 (m), 2248 (w), 1701 (s), 1406 (s), 1368 (s), 1258 (m), 1162 (s), 1127 (m), 771 (w)

6.8.3 Synthesis of cyclopeptide trinitrile *cyclo*-[(4*S*)Cpro-APA]$_3$ 67

Synthesis of dipeptide Boc-(4*S*)Cpro-APA-OAll (71a)

Boc-(4*S*)Cpro (2.4 g, 10.0 mmol), 6-amino-picolinic acid allyl ester (1.19 g, 6.7 mmol), and PyCloP (4.21 g, 10.0 mmol) were dissolved in dry methylene chloride (240 mL) and DIEA (3.50 mL, 20.0 mmol) was added dropwise. If necessary, the pH of the reaction mixture was adjusted to ca. 9 by addition of more DIEA. The mixture was then stirred for 6 days at room temperature. The solvent was evaporated *in vacuo*, and the product was purified by column chromatography (SiO_2, ethyl acetate/pentane 3:1 v/v).

Yield 2.6 g, 97 % (white solid)

TLC R_f = 0.63 (ethyl acetate/pentane 2:1 v/v)

m.p. 84 - 90 ºC

^{1}H-NMR [600MHz, DMSO-d_6, 100 ºC]: δ = 1.38 (s, 9H, BocCH_3), 2.19-2.23 (m, 1H, Cpro(β)H), 2.74 (m, 1H, Cpro(β)H), 3.41-3.47 (m, 1H, Cpro(γ)H), 3.57 (t, 1H, ^2J= 10.2 Hz, ^3J= 7.8 Hz, Cpro(δ)H), 3.91 (t, 1H, ^2J= 10.2 Hz, ^3J= 7.8 Hz, Cpro(δ)H), 4.57 (t, 1H, ^3J= 7.8 Hz, Cpro(α)H)), 4.85 (d, 2H, ^3J= 4.2 Hz, AllCH_2), 5.30 (d, 1H, ^3J= 10.2 Hz, AllH_{cis}), 5.42 (d, 1H, ^3J= 17.4 Hz, AllH_{trans}), 6.02-6.09 (m, 1H, AllH_{vic}), 7.77 (d, 1H, ^3J= 7.8 Hz, APAH(3)), 7.96 (t, 1H, ^3J= 7.8, ^3J= 8.4 Hz, APAH(4)), 8.24 (d, 1H, ^3J= 7.8 Hz, APAH(5)), 10.38 (br s, 1H, NH)

^{13}C-NMR [151 MHz, DMSO-d_6, 22 ºC]: δ = 25.7 (CproC(γ)), 27.8 (BocCH$_3$C), 34.0 CproC(β)), 49.3 (CroC(δ)), 59.3 (CproC(α)), 65.7 (All-C(1)), 79.5 (BocC), 117.5 (AllC(3)), 118.7 (APAC(3)), 120.2 (APAC(5)), 120.8 (CproCN), 132.4 (AllC(2)), 139.9 (APAC(4)), 145.9 (APAC(2)), 151.9 (APAC(6)), 152.6 (BocCO), 164.0 (APACO), 171.4 (CproCO),

IR [KBr, cm^{-1}] 3293 (m), 2978 (s), 2249 (w), 1702 (s), 1580 (s), 1540 (s), 1457 (m), 1404 (s), 1303 (s), 1259 (s), 1160 (m), 990 (m), 845 (s), 768 (s), 558 (w)

MS MALDI-TOF *m*/*z* (rel. Int.): $[M+H]^+$ 401.0 (65 %), $[M+Na]^+$ 423.0 (100%), $[M+K]^+$ 439.0 (91 %)

C, H, N Calcd	C: 57.41 %	H: 6.26 %	N: 13.39 %
Found	C: 57.14 %	H: 6.21 %	N: 12.94 %
M_r	($C_{24}H_{26}N_4O_5$•H_20)		418.44 g/mol

Synthesis of tetrapeptide Boc-[(4*S*)Cpro-APA]$_2$-OAll (84a)

Prior to coupling, equimolar amounts of dipeptide Boc-(4*S*)Cpro-APA-OAll (1.20 g, 3.0 mmol) were deprotected at the terminal amino group and the terminal carboxyl group following, respectively, the general procedures for the cleavage of Boc- (Method B) and allyl ester groups.
Both dipeptides 2HCl•H-(4*S*)Cpro-APA-OAll and Boc-(4*S*)Cpro-APA-OH as well as TBTU (1.15 g, 3.6 mmol) were dissolved dry methylene chloride (109 mL). DIEA (2.3 mL, 13.2 mmol) was added dropwise and the pH was adjusted to ca. 9 by addition of more base if necessary. The reaction mixture was then stirred for 18 h at room temperature. Afterward, the solvent was removed *in vacuo*, and the product was isolated from the residue by chromatographic purification (SiO_2, ethyl acetate/pentane 1:1 v/v). Two fractions were isolated. One representing the target tetrapeptide Boc-[(4*S*)Cpro-APA]$_2$-OAll, **84a** (a) and the other one presumably an isomer of the product containing benzotriazole as a contaminant (b). Fraction b) was chromatographed for a second time (SiO_2, ethyl acetate/pentane 1:1 v/v) and finally recrystallized (ethyl acetate/pentane) but no pure compound could be obtained. Test reactions to obtain the product using other coupling reagents such as HATU, HCTU, EDC, PyCloP, or PyBoP (each 3.6 mmol) were performed but resulted in no improvement.

Yield a) 675 mg, 35 % (white solid of tetrapeptide **84a**)
b) 289 mg, 15 % (yellow oil of isomer of tetrapeptide **84a** contaminated with benzotriazole)

TLC R_f (a) = 0.41 (ethyl acetate/pentane 1:1 v/v)
R_f (b) = 0.64 (ethyl acetate/pentane 1:1 v/v)

HPLC Eclipse-C8 column, mobile phase: water/methanol; flow: 1 mL/min; injection volume: 10 µL, gradient elution: 75/25 to 40/60; column oven 25 °C; λ = 254 nm two peaks: R_t (a) 27.6 - 28.4 min; R_t (b) 28.2 - 28.9 min.

time / min	A / vol %	B / vol %
0	25	75
20	50	50
25	60	40
40	60	40
45	25	75

Table 20 Gradient program for HPLC analysis of Boc-[(4*S*)Cpro-APA]$_2$-OAll (mobile phase A: methanol; B: water).

MS MALDI-TOF *m*/*z* (rel. Int.): a) $[M+H]^+$ 643.5 (100 %), $[M+Na]^+$ 665.5 (%), $[M+K]^+$ 681.5 (%), b) $[M+H]^+$ 643.5 (%), $[M+Na]^+$ 665.5 (100%), $[M+K]^+$ 681.5 (%)

Synthesis of dipeptide Boc-(4*S*)Cpro-APA-OBn (71b)

Boc-(*(*4*S*)Cpro (3,63 g, 15.12 mmol), 6-aminopicolinic acid benzyl ester (2.30 g, 10.08 mmol) and PyCloP (6.37 g, 15.12 mmol) were dissolved in dry methylene chloride (120 mL). DIEA (5.30 mL, 30.24 mmol) was added dropwise. If necessary, the pH of the reaction mixture was adjusted to ca. 9 by adding more DIEA. The reaction mixture was stirred for 7 days at room temperature. The solvent was evaporated *in vacuo*, and the product was purified by column chromatography (SiO_2, ethyl acetate/pentane 2:1 v/v).

Yield 4.45 g, 98 % (white solid)

TLC R_f = 0.71 (ethyl acetate/pentane 2:1 v/v)

m.p. 101 - 106 ºC

^{1}H-NMR [600MHz, DMSO-d_6, 100 ºC]: δ = 1.37 (s, 9H, BocCH_3), 2.19 (q, 1H, ^3J= 6.6 Hz, Cpro(β)H), 2.72-2.77 (m, 1H, Cpro(β)H), 3.40-3.45 (m, 1H, Cpro(γ)H), 3.56 (dd, 1H, ^2J= 10.2 Hz, ^3J= 7.8 Hz, Cpro(δ)H), 3.90 (dd, 1H, ^2J= 10.2 Hz, ^3J= 7.8 Hz, Cpro(δ)H), 4.57 (t, 1H, ^3J= 7.5 Hz, Cpro(α)H), 5.40 (s, 2H, BnCH_2), 7.36 (t, 1H,

^3J= 7.2 Hz, BnH(4)), 7.40 (t, 2H, ^3J= 7.4 Hz, BnH(3)+ BnH(5)), 7.47 (d, 2H, ^3J= 7.6 Hz, BnH(2)+ BnH(6)), 7.79 (d, 1H, ^3J= 7.5 Hz, APAH(3)), 7.97 (t, 1H, ^3J=7.9 Hz, APAH(4)), 8.25 (d, 1H, ^3J= 8.3 Hz, APAH (5)), 10.42 (br s, 1H, NH)

^{13}C-NMR [151 MHz, DMSO-d_6, 100 ºC]: δ = 25.4 (CypC(γ)), 27.4 (BocCH$_3$C), 33.3 (CproC(β)), 49.1 (CproC(δ)), 59.0 (CproC(α)), 66.0 (Bn-CH$_2$C), 79.2 (BocC), 116.8 (CproCN), 119.18 (APAC(3)), 119.8 (APAC(5)), 127.4 (BnC(2)+ BnC(4)), 127.7 (BnC(3)), 135.4 (BnC(1)), 138.6 (APAC(4)), 145.8 (APAC(2)), 151.2 (APAC(6)), 152.3 (BocCO), 163.6 (BnCO), 170.3 (CproCO)

IR [KBr, cm^{-1}] 3286 (w), 2976 (m), 2247 (w), 1703 (s), 1580 (s), 1536 (s), 1455 (s), 1401 (s), 1301 (s), 1254 (s), 1163 (s), 990 (w), 828 (w), 768 (s), 698 (m)

MS MALDI/TOF *m*/*z* (rel. Int.): $[M+H]^+$ 451.3 (57 %), $[M+Na]^+$ 473.3 (80 %), $[M+K]^+$ 489.4 (100 %)

C, H, N Calcd	C: 61.53 %	H: 6.02 %	N: 11.96 %
Found	C: 61.69 %	H: 6.22 %	N: 12.16 %
M_r	($C_{24}H_{26}N_4O_5$•H_20)		468.51 g/mol

Synthesis of tetrapeptide Boc-[(4*S*)Cpro-APA]$_2$-OBn (84b)

Prior to coupling, equimolar amounts of dipeptide Boc-(4*S*)Cpro-APA-OBn (1.28 g, 2.84 mmol) were deprotected at the terminal amino group and the terminal carboxyl group following, respectively, the general procedures for the cleavage of Boc- (Method B) and benzyl ester groups (Method A).

Both dipeptides, 2HCl*H-(4*S*)Cpro-APA-OBn and Boc-(4*S*)Cpro-APA-OH, as well as TBTU (1.1 g, 3.41 mmol) were dissolved dry methylene chloride (86 mL). DIEA (2.17 mL, 12.50 mmol) was added dropwise (if necessary the pH was adjusted to ca. 9 - 10 by addition of more base) and then the reaction mixture was stirred for 18 h at room temperature. Afterward, the solvent was removed *in vacuo*, and the product was isolated from the residue by chromatographic work-up (SiO_2, ethyl acetate/pentane 3:1 v/v).

Yield 1.80 g, 92 % (white solid)

TLC R_f = 0.30 (ethyl acetate/pentane 3:1 v/v)

^{1}H-NMR [600MHz, DMSO-d_6, 100 ºC]: δ = 1.34 (s, 9H, BocCH$_3$), 2.20-2.27 (m, 2H, Cpro(β)H), 2.71-2.74 (m, 2H, Cpro(β)H), 3.45-3.55 (m, 4H, Cpro(δ)H), 3.90-4.00

(m, 2H, Cyp(α)H) + Cyp(γ)H), 4.31-4.50 (m, 2H, Cyp(α)H) + Cyp(γ)H), 5.40 (s, 2H, $BnCH_2$), 7.36-7.52 (m, 5H, BnH_{arom}), 7.91-8.18 (m, 6H, APAH(3)+ APAH(4)+ APAH(5)), 10.14 (br s, 1H, NH), 10.53 (br s, 1H, NH)

IR [KBr, cm^{-1}] 3274 (m), 2960 (m), 2247 (w), 1707 (s), 1577 (s), 1457 (s), 1399 (s), 1302 (s), 1259 (m), 1157 (s), 1063 (m), 765 (m)

MS MALDI-TOF *m*/*z* (rel. Int.): $[M+H]^+$ 693.3 (74 %), $[M+Na]^+$ 715.3 (100 %), $[M+K]^+$ 731.4 (58 %)

Synthesis of hexapeptide Boc-[(4*S*)Cpro-APA]$_3$-OBn (86)

Prior to coupling the dipeptide Boc-(4*S*)Cpro-APA-OBn was deprotected at the terminal amino group following the general procedure for the cleavage of Boc groups (Method B). An equimolar amount of tetrapeptide Boc-[(4*S*)Cpro-APA]$_2$-OBn was deprotected at the C-terminus following the general procedure for the cleavage of benzyl ester groups (Method A).

The resulting products (2.05 mmol each) were dissolved in DMF p.a. (62 mL) and TBTU (0.72 g, 2.24 mmol) was added followed by DIEA (1.50 mL, 8.57 mmol). If necessary, the pH of the reaction mixture was adjusted to ca. 9. The solution was stirred for 3 h at room temperature, and then the solvent was removed *in vacuo*. The product was initially isolated from the residue by chromatographic purification with acetone, and then the obtained material was chromatographed with a mixture of methylene chloride/methanol as eluent (SiO_2, 20:1 v/v).

Yield 1.60 g, 84 % (yellow solid)

TLC R_f = 0.46 (methylene chloride/methanol 20:1 v/v)

^{1}H-NMR [600MHz, DMSO-d_6, 100 °C]: δ = 1.38 (s, 9H, $BocCH_3$), 2.21-2.27 (m, 3H, Cpro(β)H), 2.71-2.88 (m, 2H, Cpro(β)H signal beneath water), 3.42-3.56 (m, 6H, Cpro(δ)H), 3.91-4.09 (m, 3H, Cpro(α)H) + Cpro(γ)H), 4.30-4.55 (m, 3H, Cpro(α)H) + Cpro(γ)H), 5.40 (dd, 2H, ^2J= 10.92 Hz, $BnCH_2$), 7.36-7.50 (m, 5H, BnH_{arom}), 7.78-8.23 (m, 9H, APAH(3)+ APAH(4)+ APAH(5)), 10.16 (br s, 2H, NH), 10.57 (br s, 1H, NH)

IR [KBr, cm^{-1}] 3280 (m), 2931 (m), 2246 (w), 1706 (s), 1576 (s), 1400 (s), 1301(s), 1246 (s), 1163 (s), 1080 (m), 991 (m), 825 (m), 763 (s), 698 (m)

MS MALDI-TOF *m*/*z* (rel. Int.): $[M+H]^+$ 935.5 (83 %), $[M+Na]^+$ 957.5 (100 %), $[M+K]^+$ 973.5 (64 %)

Synthesis of *cyclo*[(4*S*)Cpro-APA]$_3$ (67)

Prior to coupling the linear hexapeptide Boc-[(4*S*)Cpro-APA]$_3$-OBn (0.65 g, 0.7 mmol) was deprotected first at the terminal amino group using the general Method B for the cleavage of Boc groups. Afterwards, the benzyl protective group was removed according to the general procedure for the cleavage of benzyl esters (Method B).

The cyclization reaction was conducted with strict exclusion of air using only dry solvents. To a degassed solution of TBTU (1.12 g, 3.5 mmol) in DMF (138 mL), DIEA (0.29 mL, 1.68 mmol) was added. The solution was then heated to 80 ºC. A second solution containing the completely deprotected hexapeptide, DMF (27 mL) and DIEA (0.44 mL, 2.52 mmol) was added dropwise within 4h. The pH of the reaction mixture was adjusted to ca. 9 by addition of more DIEA. Stirring was continued for 2 h at 80 ºC and then the solvent was evaporated *in vacuo*. The product was subjected to an initial chromatographic purification step on a silica column using acetone as eluent. The isolated material was further purified on a RP-8 column. To this end, it was dissolved in small amount of acetone and applied to the column conditioned with 1:50 (v/v) 1,4-dioxane/water. Slow gradual change of the eluent composition to 1:2 (v/v) 1,4-dioxane/water resulted in the elution of the product. Fractions containing product were combined and the solvent was removed. The compound thus obtained was still impure; therefore it was once more chromatographed (SiO_2, 3:1 (v/v) ethanol/hexane). Fractions containing product were combined, and the solvent was slowly concentrated to 5 mL. The precipitate was filtered off, washed thoroughly with cold ethanol and dried *in vacuo*.

The isolated material still contained 9 % of impurities according to HPLC. Final purification was achieved by preparative HPLC (Table 21). For this, the residue was dissolved in DMSO (500 µL, p.a.) and immediately purified (stationary phase: Zorbax Eclipse XDB-C8, mobile phase: water/methanol; flow: 3 mL/min; injection volume: 100 µL, gradient elution: 30/70 to 60/40; column oven 25 °C; R_t 20.6 - 21.0 min). Pure fractions were combined, the solvent was evaporated, and the product was dried *in vacuo*. The cyclopeptide thus obtained contained 3% impurities according to analytical HPLC.

Yield 30.5 mg, 6 % (white solid)

RP TLC R_f = 0.28 (1,4-dioxane / water 1:2 v/v)

HPLC Zorbax Eclipse XDB-C8, mobile phase: water /methanol; flow: 1 mL/min; injection volume: 10 µL, gradient elution: 75/25 to 40/60; column oven 25 °C; λ = 254 nm, R_t 12.4 - 13.1 min.

Semi prep. HPLC Zorbax Eclipse XDB-C8, mobile phase: water /methanol; flow: 3 mL/min; injection volume: 100 µL, same gradient program, R_t 20.6 - 21.0 min.

a) time / min	A / vol %	B / vol %	b) time / min	A / vol %	B / vol %
0	25	75	0	30	70
20	50	50	20	40	60
25	60	40	36	50	50
40	60	40	37	60	40
45	25	75	42	25	75

Table 21 Gradient programs for HPLC analysis of *cyclo*[(4*S*)Cpro-APA]$_3$ **67** (a) and for its isolation on a preparative scale (b) (A: methanol; B: water).

^{1}H-NMR [600MHz, DMSO-d_6, 22 ºC]: δ = 2.28-2.32 (m, 3H, Cpro(β)H), 2.96-3.00 (m, 3H, Cpro(β)H), 3.57 (q, 6H, ^3J= 7.5 Hz, Cpro(γ)H), 3.77 (dd, 3H, ^2J= 11.4 Hz, ^3J= 7.8 Hz, Cpro(δ)H), 4.05 (dd, 3H, ^2J= 11.4 Hz, ^3J= 7.8 Hz, Cpro(δ)H), 5.87-5.92 (m, 6H, Cpro(α)H), 7.36 (d, 6H, ^3J= 8.4 Hz, APAH(3)), 7.51 (d, 6H, ^3J= 7.8 Hz, APAH (5)), 7.77 (t, 6H, ^3J= 7.8 Hz, APAH(4)), 10.24 (br s, 6H, NH)

MS MALDI-TOF *m*/*z* (rel. Int.): $[M+H]^+$ 727.6 (43 %), $[M+Na]^+$ 749.5 (100%), $[M+K]^+$ 765.5 (42 %)

6.8.4 Preparation of cyclopeptide trithiol *cyclo*[Tpro-APA]$_3$ 68

Synthesis of dipeptide Boc-Hyp-APA-OAll (72)[54, 64n, 188a, 199]

Boc-Hydroxyproline (6.24 g, 27.0 mmol), 6-aminopicolinic acid allyl ester (3.21 g, 18.0 mmol), and PyCloP (11.39 g, 27.0 mmol) were dissolved in dry methylene chloride (216 mL). DIEA (9.40 mL, 54.0 mmol) was added dropwise. If necessary, the pH of the reaction mixture was adjusted to ca. 9 by adding more DIEA and stirring was continued for 7 days at room temperature. The solvent was evaporated *in vacuo*, and the product was purified by column chromatography (SiO_2, ethyl acetate/pentane 3:1 v/v).

Yield 6.89 g, 98 % (white solid)

^{1}H-NMR [600MHz, DMSO-d_6, 100 ºC]: δ = 1.35 (s, 9H, BocCH_3), 1.98-2.02 (m, 1H, Hyp(β)H), 2.16-2.0 (m, 1H, Hyp(β)H), 3.33 (d, 1H, ^2J=11.4 Hz, Hyp(δ)H), 3.52

(dd, 1H, ^{2}J= 11.4 Hz, ^{3}J= 4.8 Hz, Hyp(δ) H), 4.32-4.34 (m, 1H, Hyp(γ)H), 4.58 (t, 1H, ^{3}J= 7.4 Hz, Hyp(α)H)), 4.71 (br s, 1H, OH), 4.84 (d, 2H, ^{3}J= 6,0 Hz, AllCH$_2$), 5.29 (d, 1H, ^{3}J= 10,2 Hz, AllH$_{cis}$), 5.42 (d, 1H, ^{3}J= 16.8 Hz, AllH$_{trans}$), 6.02-6.08 (m, 1H, AllH$_{vic}$), 7.75 (d, 1H, ^{3}J= 7.2 Hz, APAH(3)), 7.94 (t, 1H, ^{3}J= 7.8 Hz, APAH(4)), 8.25 (d, 1H, ^{3}J= 8.4 Hz, APAH(5)), 10.45 (br s, 1H, NH)

MS MALDI-TOF *m*/*z* (rel. Int.): [M+Na]$^{+}$ 415.0 (100 %), [M+K]$^{+}$ 430.0 (99 %)

Synthesis of dipeptide Boc-(4*R*-Ts)Hyp-APA-OAll (73)[64n, 188a, 199]

Boc-Hyp-APA-OAll (6.25 g, 16.0 mmol) and pyridine (15.6 mL) were dissolved in dry methylene chloride (15.6 mL). 4-Toluensulfonyl chloride (15.25 g, 80.0 mmol) was added. The mixture was stirred overnight at room temperature and the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate, washed three times with 10 % aqueous Na_2CO_3, three times with water, and dried over Na_2SO_4. The solvent was evaporated to dryness and the residue was purified chromatographically (SiO_2, ethyl acetate/pentane 1:1 v/v).

Yield 8.03 g, 92 % (light yellow solid)

^{1}H-NMR [600MHz, DMSO-d_6, 100 ºC]: δ = 1.38 (s, 9H, BocCH$_3$), 2.04-2.09 (m, 1H, Hyp(β)H), 2.30-2.35 (m, 4H, Hyp(β)H + TsCH$_3$), 3.40 (t, 1H, ^{3}J= 9.6 Hz, Hyp(δ) H)), 4.07 (t, 1H, ^{3}J= 9.6 Hz, Hyp(δ) H)), 4.13-4.18 (m, 1H, Hyp(γ)H), 4.63 (t, 1H, ^{3}J= 7.2 Hz, Hyp(α)H), 4.84 (d, 2H, ^{3}J= 5.4 Hz, AllCH$_2$), 5.29 (d, 1H, ^{3}J= 10.2 Hz, AllH$_{cis}$), 5.42 (d, 1H, ^{3}J= 16.8 Hz, AllH$_{trans}$), 6.02-6.08 (m, 1H, AllH$_{vic}$), 7.53 (d, 2H, ^{3}J= 7.8 Hz, TsH(3)), 7.77 (d, 1H, ^{3}J= 7.8 Hz, APAH(3)), 7.87 (d, 2H, ^{3}J= 7.8 Hz, TsH(2)), 7.97 (t, 1H, ^{3}J= 7.8 Hz, APAH(4)), 8.26 (d, 1H, ^{3}J= 7.8 Hz, APAH(5)), 10.53 (br s, 1H, NH)

MS MALDI-TOF *m*/*z* (rel. Int.): [M+H]$^{+}$ 546.2 (100 %), [M+Na]$^{+}$ 568.2 (20 %), [M+K]$^{+}$ 584.2 (35 %)

Synthesis of dipeptide Boc-(Bz)Tpro-APA-OAll (92) [64n, 188a, 199]

Method A: The reaction was conducted with strict exclusion of air using dry solvents. Diisopropylazodicarboxylate (660 µL, 3.37 mmol) was added dropwise to an ice-cool solution of triphenylphosphine (0.8 g, 3.06 mmol) in anhydrous toluene (3 mL) under nitrogen. The mixture was stirred continuously while it was warmed to room temperature. A solution of dipeptide Boc-Hyp-APA-OAll (0.6 g, 1.53 mmol), thiobenzoic acid (90 %, 6.34 mL, 5.4 mmol) and toluene (1.2 mL) was added. The reaction mixture was stirred for another 18h at room temperature. Progress

of the reaction was followed by TLC. In case starting material was still detectable after 18 h, further triphenylphosphine (0.5 equiv.) and diisopropylazodicarboxylate (0.5 equiv.) were added and stirring was continued for another 2 h. The solvent was removed *in vacuo* and ethyl acetate was added. The organic solution was washed three times with water, dried over Na_2SO_4, and evaporated. The product was isolated chromatographically (SiO_2, ethyl acetate/pentane 1:1 v/v). Separation of diisopropylazodicarboxylate and triphenylphosphine oxide proved to be very difficult chromatographically. Therefore, recrystallization from ethylacetate/pentane was performed. However, subsequent HPLC and ^{1}H-NMR analyses showed that the product thus obtained was still contaminated. Further purification was not undertaken and Method B was used for preparation of compound Boc-(Bz)Tpro-APA-OAll (**92**).

Method B: A suspension of Boc-(*4R*-Ts)Hyp-APA-OAll (7.80 g, 14.3 mmol), thiobenzoic acid (90%, 6.96 g, 50.34 mmol), and K_2CO_3 (5.93 g, 42.9 mmol) in DMF (321 mL) was heated to 80 °C for 5h. The solvent was evaporated *in vacuo* and ethyl acetate was added. The product was isolated from the resulting mixture analogously to Method A. Purification was achieved chromatographically (SiO_2, ethyl acetate/pentane 1:1 v/v).

Yield 4.83 g, 66 % (white powder)

m.p. 70 - 75 °C

^{1}H-NMR [600MHz, DMSO-d_6, 100 °C]: δ = 1.41 (s, 9H, BocCH_3), 2.03-2.08 (m, 1H, Tpro(β)H), 2.88-2.92 (m, 1H, Tpro(β)H), 3.38-3.41 (m, 1H, Tpro(γ)H), 4.05-4.08 (m, 1H, Tpro(δ) H), 4.15 (q, 1H, ^3J= 7.8 Hz, Tpro(δ) H), 4.62 (t, 1H, ^3J= 7.5 Hz, Tpro(α)H), 4.84 (dt, 2H, ^3J= 5.4 Hz, ^4J= 1.8 Hz, AllCH_2), 5.30 (dq, 1H, ^3J= 10.8 Hz, ^4J= 1.2 Hz, AllH_{cis}), 5.42 (dq, 1H, ^3J= 17.4 Hz, ^4J= 1.8 Hz, AllH_{trans}), 6.02-6.08 (m, 1H, AllH_{vic}), 7.54 (t, 2H, ^3J= 8.4 Hz, BzH (3)), 7.67 (t, 1H, ^3J= 7.2 Hz, BzH (4)), 7.77 (d, 1H, ^3J= 7.2 Hz, APAH(3)), 7.88 (dd, 2H, ^3J= 8.4 Hz, ^4J= 1.2 Hz, BzH (2)), 7.97 (t, 1H, ^3J= 7.8 Hz, APAH(4)), 8.28 (d, 1H, ^3J= 7.8 Hz, APAH(5)), 10.55 (br s, 1H, NH)

IR [KBr, cm^{-1}] 3396 (w), 3062 (w), 2977 (w), 2973 (w), 1701 (s), 1669 (s), 1579 (m), 1456 (s), 1399 (m), 1245 (m), 1210 (m), 1160 (s), 1078 (w), 989 (w), 908 (m), 768 (m), 689 (m)

MS MALDI-TOF *m*/*z* (rel. Int.): $[M+H]^+$ 512.19 (100 %), $[M+Na]^+$ 534.19 (55 %), $[M+K]^+$ 550.20 (52 %)

C, H, N Calcd	C: 61.04 %	H: 5.71 %	N: 8.21 %
Found	C: 60.83 %	H: 5.66 %	N: 8.08 %
M_r	($C_{26}H_{39}N_3O_6S$)		511.59 g/mol

Synthesis of tetrapeptide Boc-[(Bz)Tpro-APA]$_2$-OAll (96)[64n, 188a]

Prior to the coupling, two equimolar amounts of dipeptide Boc-(Bz)Tpro-APA-OAll (1.79 g, 3.5 mmol) were deprotected at the terminal amino group and the terminal carboxy group following, respectively, the general procedures for the cleavage of Boc groups (Method A) and allyl esters. The resulting dipeptides, 2HCl*H-(Bz)Tpro-APA-OAll and Boc-(Bz)Tpro-APA-OH as well as PyCloP (1.77 g, 4.2 mmol) were dissolved in dry methylene chloride (90 mL). At room temperature, DIEA (2.68 mL, 15.4 mmol) was added dropwise. If necessary, the pH was adjusted to 9 - 10 upon addition of more base, and then the reaction was stirred for 18 h. The solvent was evaporated, and the product was isolated by chromatographic purification (SiO_2, ethyl acetate/pentane 2:1 v/v).

Yield 3.0 g, 96 % (white solid)

m.p. 120 - 123 ºC

MS MALDI-TOF *m*/*z* (rel. Int.): $[M+H]^+$ 865.2 (30 %), $[M+Na]^+$ 887.2 (100 %), $[M+K]^+$ 903.2 (55 %)

Synthesis of hexapeptide Boc-[(Bz)Tpro-APA]$_3$-OAll (97)

Prior to coupling the terminal amino group of tetrapeptide Boc-[(Bz)Tpro)-APA]$_2$-OAll (2.25 g, 2.6 mmol) was deprotected according to Method A for the cleavage of Boc groups. An equimolar amount of dipeptide Boc-(Bz)Tpro-APA-OAll (1.33 g, 2.6 mmol) was deprotected at the carboxyl group according to the general procedure for the deprotection of allyl esters. The deprotected tetrapeptide and dipeptide were dissolved in DMF p.a. (78 mL). TBTU (0.92 g, 2.86 mmol) and DIEA (1.9 mL, 10.92 mmol) were added. The pH was adjusted to 9 - 10 and stirring was continued for 2 h at room temperature. The reaction mixture was poured into ice water under stirring. The pH of the suspension was then adjusted to ca. 4 with 1 N HCl and stirred for another 10 min. The white precipitate was filtered off, washed with water, and dried *in vacuo*. Subsequently, filtration through a SiO_2 column with acetone as mobile phase was used to remove non-soluble starting materials. The product was obtained after chromatographic purification (SiO_2, methylene chloride/methanol 30:1 v/v).

Yield 2.6 g, 82 % (white solid)

MS MALDI-TOF *m*/*z* (rel. Int.): $[M+H]^+$ 1218.6 (53 %), $[M+Na]^+$ 1240.7 (86 %), $[M+K]^+$ 1256.7 (100 %)

Synthesis of c*yclo*[(Bz)Tpro-APA]$_3$ (93)

The linear hexapeptide Boc-[(Bz)Tpro-APA]$_3$-OAll (2.43 g, 2.0 mmol) was deprotected first at the terminal carboxyl group and then at the amino group according to the general methods for the cleavage of allyl esters and Boc groups (Method A), respectively. Usually, cleavage of the allyl ester group proceeded slowly at the stage of hexapeptide and addition of more catalyst (5 mg) and morpholine (0.2 mmol) after stirring the reaction mixture for 20 min is recommended.

The cyclization reaction was conducted under strict exclusion of air using dry solvents. The hydrochloride salt of the completely deprotected hexapeptide was dissolved in a mixture of degassed DMF (75 mL) and DIEA (2.09 mL, 12 mmol). This solution was added dropwise within 4 h to a solution of TBTU (3.21 g, 10 mmol) and DIEA (0.83 mL, 4.75 mmol) in degassed DMF (400 mL) at 80 °C. The pH of the reaction mixture was adjusted to ca. 9 upon addition of more DIEA. Stirring was continued for another 1 h hour at 80 ºC and then the solvent was evaporated *in vacuo*. The product was subjected to an initial chromatographic purification step on a silica column with acetone as eluent. The isolated material was further purified on a RP-8 column. For this, it was dissolved in a small amount of DMF and subjected to a column conditioned with 1:10 (v/v) 1,4-dioxane/water. Slow gradual change of the eluent composition resulted in the elution of the product at 2:1 (v/v) 1,4-dioxane/water. Fractions containing product were combined and the solvent was evaporated. In case the material thus obtained was still impure, it was once more chromatographed on a SiO_2 column with acetone as eluent and finally recrystallized (methanol/dichloromethane).

Yield 1.04 g, 49 % (white solid)

HPLC Zorbax Eclipse XDB-C8, mobile phase: water + 0.1 vol % TFA /acetonitrile + 0.1 vol % TFA; flow: 1 mL/min; injection volume: 10 µL, gradient elution: 75/25 to 3/97; column oven 40 °C; λ = 254 nm, R_t 28.3 - 28.9 min.

time / min	A / vol %	B / vol %
0	25	75
20	50	50
25	60	40
35	90	10
40	97	3
48	97	3
50	25	75

Table 22 Gradient program for HPLC analysis of c*yclo*[(Bz)Tpro-APA]$_3$ **93** (A: acetonitrile+ 0.1 vol % TFA; B: water + 0.1 vol % TFA).

m.p. 189 - 197°C

^{1}H-NMR [600MHz, DMSO-d_6, 22 ºC]: δ = 2.19-2.23 (m, 3H, Tpro(β)H), 3.21-3.26 (m, 3H, Tpro(β)H), 3.61-3.64 (dd, 3H, ^3J= 12.0 Hz, ^3J= 7.8 Hz, Tpro(δ)H), 4.16-4.24 (m, 6H, Tpro(γ)H + Tpro(δ)H), 5.79 (t, 3H, ^3J= 7.2 Hz, Tpro(α)H), 7.41 (d, 3H, ^3J= 8.4 Hz, APAH (3)), 7.46 (d, 3H, ^3J= 7.8 Hz APAH (5)), 7.53 (t, 6H, ^3J= 8.4 Hz, BzH(3)), 7.68 (t, 3H, ^3J= 7.8 Hz, BzH(4)), 7.76 (t, 3H, ^3J= 8.4 Hz, APAH(4)), 7.85 (d, 6H, ^3J= 7.8 Hz, BzH(2)), 9.99 (br s, 3H, NH)

^{13}C-NMR [151 MHz, DMSO-d_6, 22 ºC]: δ = 38.26 (TproC(β)), 38.56 (TproC(γ)), 53.31 (TproC(δ)), 61.56 (TproC(α)),115.92 (APAC(3)), 120.12 (APAC(5)), 127.31 (BzC(2)), 129.69 (BzC(3)), 134.72 (BzC(4)), 136.45 (BzC(1)), 139.78 (APAC(4)), 149.27 (APAC(2)), 151.89 (APAC(6)), 166.45 (APACO), 170.50 (TproCO), 191.26 (BzCO)

IR [KBr, cm^{-1}] 3397 (b), 1707 (m), 1663 (s), 1575 (s), 1460 (m), 1397 (s), 1300 (m), 1209 (s), 1175 (m), 908 (s), 822 (w), 759 (m), 689 (s)

MS MALDI-TOF *m/z* (rel. Int.): $[M+H]^+$ 1060.0 (23 %), $[M+Na]^+$ 1082.0 (100%), $[M+K]^+$ 1099.1 (32 %)

C, H, N Calcd	C: 60.15 %	H: 4.39 %	N: 11.69%
Found	C: 60.02 %	H: 4.38 %	N: 11.86 %
M_r	($C_{36}H_{30}N_{12}O_6$•H_2O):		1078.20 g/mol

Synthesis of c*yclo*[Tpro-APA]$_3$ (68)

The reaction was conducted according to the general procedure for the deprotection of benzoyl ester groups using c*yclo*[(Bz)Tpro-APA]$_3$ (53 mg, 50 µmol) as starting material.

Yield 37 mg, 99 % (white solid)

HPLC Zorbax Eclipse XDB-C8, mobile phase: water + 0.1 vol % TFA /acetonitrile + 0.1 vol % TFA; flow: 1 mL/min; injection volume: 10 µL, gradient elution: 75/25 to 3/97; column oven 40 °C; λ = 254 nm, R_t 9.6 - 10.2 min (250 µL of 3 mmol stock solution in 2:1 CH_3CN/H_2O (*v/v*) containing 5 equiv. of DTT). The gradient program used for HPLC analysis of cyclopeptide **68** is the same as the one for compound **93**).

m.p. ≥ 250 °C

MS MALDI-TOF *m/z* (rel. Int.): *monomer* **68** $[M+H]^+$ 754.1 (86 %), $[M+Na]^+$ 770.1 (100 %), $[M+K]^+$ 786.1 (40 %), *dimer* $[M_2-H_4+Na]^+$ 1513.3 (48 %), $[M_2-H_4+K]^+$ 1529.3 (47 %), *trimer* $[M_3-H_6+Na]^+$ 2258.5 (32 %), $[M_3-H_6+K]^+$ 2274.6 (15 %), *tetramer* $[M_4-H_8+Na]^+$ 3004.4 (20 %), $[M_4-H_8+K]^+$ 3021.6 (13 %), *pentamer* $[M_5-H_9+Na]^+$ 3751.8 (22 %), $[M_5-H_9+K]^+$ 3764.8 (7 %), *hexamer* $[M_6-H_{13}+Na]^+$ 4493.1 (5 %), $[M_6-H_{13}+K]^+$ 4508.0 (3 %)

No elemental analysis was performed and no spectroscopic characterization (NMR, IR) was possible because the product consisted of a mixture of oligomers as evident from the MALDI-TOF MS analysis. This mixture was used for setting-up the dynamic libraries without further purification.

An interpretable chromatogram with a single peak for the monomer **68** was obtained after addition of 5 equiv. of DTT (4.6 mg, 30 µmol) to a solution of cyclopeptide **68** (3 mM in 2:1 CH_3CN/H_2O (*v/v*), 6.4 mg, 6 µmol) (Chart 7). Subsequent MALDI-TOF MS confirmed transformation of the oligomers of **68** into the monomer **68** (Chart 8).

MS MALDI-TOF *m/z* (rel. Int.): *monomer* **68** $[M+Na]^+$ 770.2 (60 %), $[M+K]^+$ 786.2 (100 %)

6.8.5 Generation of dynamic combinatorial libraries (DCLs)

The set-up of DCLs required preparation of stock solutions of the corresponding building blocks and of the template salts (NaCl, NaBr, NaI, or Na_2SO_4) as specified in Table 23. The pH of water for all stock solutions was adjusted to ca. 9 - 10 (with 1 M solution of NaOH) when deprotected cyclopeptide c*yclo*[Tpro-APA]$_3$ (**68**) was used and to a pH of ca. 12 when protected cyclopeptide c*yclo*[(Bz)Tpro-APA]$_3$ (**93**) served as starting material. DTT (5 equiv.) was added to the cyclopeptide solution which was stirred for 5 min prior to addition of an aliquot of linker(s). For the mixed libraries, deprotected cyclopeptide **68** and two different dithiol linkers in the presence of DTT were used as building blocks. Among the available dithiols 1,2-ethanedithiol (**90**), 1,3-benzenedithiol (**89**), 3,5-dimercaptobenzoic acid (**102**), and 1,4-phenylenedimethanethiol (**103**), two were combined in a library.

stock solution	concentration	amount	solvent	volume
c*yclo*[(Bz)Tpro-APA]$_3$ (**93**)	3 mM	6 µmol	CH_3CN/H_2O (2:1 *v/v*)	2 mL
c*yclo*[Tpro-APA]$_3$ (**68**)	2.5 mM	5 µmol	CH_3CN/H_2O (2:1 *v/v*)	2 mL
linker for DCLs containing one dithiol	8 mM	16 µmol	CH_3CN	2 mL
linker for DCLs containing two dithiols	15.9 mM	32 µmol	CH_3CN	2 mL
template	0.5 M	1 mmol	H_2O	2 mL

Table 23 Concentrations of stock solutions.

Libraries were set up in a capped 1.5 mL HPLC vial (for libraries on a preparative scale a 50 mL one neck flask was used) equipped with a micro stirrer bar, and allowed to equilibrate under air. Each library was monitored for a period of 7 - 40 days until no further change in product distribution was detectable to ensure equilibrium was reached. The required minimum volume of each library must be at least 250 µL to ensure proper equilibration. In addition, this is a technical requirement to avoid the presence of air in the HPLC syringes.

Analysis of DCLs

Analytical and preparative HPLC was accomplished using an Agilent 1100 series machine coupled with a UV/Vis-multi wavelength detector. The chromatograms were detected at 254 nm and 290 nm (Tables 24 and 25). The software, column, and parameters used are described in detail in Section 6.5.3.

HPLC parameters for libraries on an analytical scale

Column: Zorbax Eclipse XDB-C8 column (4.6 x 150 mm, 5 µm), 45 °C
Injection volume: 10 µL
Flow rate: 1 mL/min

Mobile phase: A: acetonitrile + 0.1 vol % TFA;
B: water + 0.1 vol % TFA (+ 0.1 wt % of template salt if specified)

time / min	A / vol %	B / vol %
0	25	75
20	50	50
25	60	40
35	90	10
40	97	3
48	97	3
50	25	75
60	25	75

Table 24 Gradient program for HPLC analysis of libraries on an analytical scale.

.

HPLC parameters for libraries on a preparative scale

Column: Zorbax Eclipse XDB-C8 column (9.4 x 250 mm, 5 µm), 40 °C
Injection volume: 150 - 250 µL
Flow rate: 6 mL/min
Mobile phase: A: acetonitrile + 0.05 vol % TFA;
B: water + 0.05 vol % TFA + 0.1 wt % of template salt

time / min	A / vol %	B / vol %
20	45	55
30	60	40
40	90	10
45	97	3
50	97	3

Table 25 Gradient program for HPLC analysis of libraries on a preparative scale.

LC-MS was performed by using the same Agilent HPLC equipment and column. More information about the LC and ESI-MS parameters is given in Sections 6.5.3 and 6.5.4.

DCLs using protected c*yclo* [(Bz)Tpro-APA]$_3$ 93

The following procedure combines deprotection of *cyclo*[(Bz)Tpro-APA]$_3$ (**93**) and set-up of a DCL in a single step.

To a stock solution of **93** (315 µL, 3 mM in 2:1 CH_3CN/H_2O, pH 12), acetonitrile (16 µL) and water (133 µL, pH 12) were added. DTT (0.73 mg, 6.61 mM in library) was introduced to the solution, which was then stirred for 5 min. Afterward, an aliquot of a spacer stock solution was

added (251 µL, 8 mM in CH_3CN) and the pH was adjusted to ca. 12 by dropwise addition of 1 M aqueous NaOH. The reaction mixture was stirred for another 10 - 15 min at room temperature. Concentrations and final volumes of the DCL are specified in Table 26.

compound	concentration in DCL	amount	aliquots of stock solution
c*yclo*[(Bz)Tpro-APA]$_3$ (**93**)	1.32 mM	0.94 µmol	315 µL from 3 mM in 2:1 CH_3CN/H_2O
linker	1.98 mM	2.01 µmol	251 µL from 8 mM in CH_3CN
DTT	6.61 mM	4.72 µmol	0.73 mg
CH_3CN			16 µL
H_2O, pH 12			133 µL
resulting volume			715 µL in 2:1 CH_3CN/H_2O

Table 26 Composition of DCLs prepared from **93**.

The solution was divided in two equal portions of 357 µL and transferred to 1.5 mL HPLC vials equipped with a micro insert. Then, a template stock solution (7 µL, 0.5 M Na_2SO_4 in water, 10 mM in the library) was added to one of the samples. Water (7 µL, pH 12) was added to the second one and both were left to equilibrate under air at room temperature over a period of 40 days. HPLC analyses of the library compositions were performed every second day.

DCLs using fully deprotected c*yclo*[Tpo-APA]$_3$ 68

In a 1.5 mL HPLC vial containing DTT (1.03 mg, 6.61 mM in library), an aliquot from a stock solution of c*yclo*[Tpro-APA]$_3$ (**68**) (536 µL, 2.5 mM in 2:1 CH_3CN/H_2O, pH 9 - 10), acetonitrile (67 µL) and water (159 µL, pH 9 - 10) were added and stirred for 5 min. Then, an aliquot from a stock solution of the spacer (251 µL, 8 mM in CH_3CN) was added. The pH of the reaction mixture was adjusted to ca. 9-10 by dropwise addition of 1 M aqueous NaOH. The mixture was then stirred for another 10-15 min at room temperature. Resulting concentrations are summarized in Table 27. Portions of the mixture (253 µL) were transferred to 1.5 mL HPLC vials equipped with a micro insert. Subsequently, different template stock solutions (5 µL, 0.5 M in water, 10 mM in the library) or water (5 µL, pH 9 - 10) were added to the four resulting libraries, which were left to equilibrate at room temperature under air over a period of 7 days before HPLC analysis.

compound	concentration in DCL	amount	aliquots of stock solution
c*yclo*[Tpro-APA]$_3$ (**68**)	1.32 mM	1.34 µmol	536 µL from 2.5 mM in 2:1 CH_3CN/H_2O
linker	1.98 mM	2.01 µmol	251 µL from 8 mM in CH_3CN
DTT	6.61 mM	6.70 µmol	1.03 mg
CH_3CN			67 µL
H_2O, pH 9 - 10			159 µL
resulting volume			1013 µL in 2:1 CH_3CN/H_2O

Table 27 Composition of DCLs prepared from cyclopeptide **68**.

DCLs using fully deprotected c*yclo*[Tpro-APA]$_3$ 68 and two different dithiols

To a mixture of DTT (1.03 mg, 6.61 mM in library) in acetonitrile (67 µL) and water (159 µL, pH 9-10), a stock solution of c*yclo*[Tpro-APA]$_3$ (**68**) (536 µL, 2.5 mM in 2:1 CH_3CN/H_2O, pH 9 - 10) was added and stirred for 5 min. Proportions of stock solutions of dithiols (each 125.5 µL, 15.9 mM in CH_3CN) were added (Table 38). The reaction mixture was stirred for another 10 - 15 min at room temperature and aliquots (253 µL) of the resulting mixture were transferred to 1.5 mL HPLC vials. A template stock solution (5 µL, 0.5 M Na_2SO_4 in water, 10 mM in the library) was added and the vials were equipped with a micro stirrer bar, capped, and allowed to equilibrate at room temperature under air for a period of 10 days. The experiments were monitored each day by HPLC.

compound	concentration in DCL	amount	aliquots of stock solution
c*yclo*[Tpro-APA]$_3$ (**68**)	1.32 mM	1.34 µmol	536 µL from 2.5 mM in 2:1 CH_3CN/H_2O
two dithiols	1.98 mM	2.01 µmol	125.5 µL each from 19.9 mM in CH_3CN
DTT	6.61 mM	6.70 µmol	1.03 mg
CH_3CN			67 µL
H_2O, pH 9 - 10			159 µL
resulting volume			1013 µL in 2:1 CH_3CN/H_2O

Table 28 Composition of DCLs containing more than one dithiol prepared from deprotected cyclopeptide **68**.

6.8.6 Synthesis on a preparative scale and isolation of triply-linked bis(cyclopeptides)

Syntheses of bis(cyclopeptides) 100•Na_2SO_4 and 101•Na_2SO_4

The pH of a solution containing cyclopeptide c*yclo*[Tpro-APA]$_3$ (**68**) (20 mg, 26.74 µmol, 1.32 mM), DTT (20.62 mg, 13.37 mmol, 0.66 M), 1,2-ethanedithiol (3.80 µL, 40.11 µmol, 1.98 mM), Na_2SO_4 (81 µL of a 0.5 M solution in water, 2 mM), water (6.75 mL , pH 9 - 10) and acetonitrile (13.51 mL) was adjusted to ca. 9 - 10 by dropwise addition of NaOH (0.1 M solution in water) and the mixture was stirred at room temperature under air. An aliquot of 100 µL was taken each day for HPLC analysis to check when equilibrium was reached. Amplification of triply-linked bis(cyclopeptide) **100** as the main product as well as formation of a minor product (**101**) containing four linker units was detected on the fourth day. After 7 - 9 days of equilibration no traces of starting material were detectable. Prolonging the reaction time did not change product distribution. The solution was acidified with diluted sulfuric acid (1M solution in water) to reach a pH of 3 and the solvent was concentrated to 2 - 3 mL. Then HPLC-grade water (2 mL) was added

to the suspension and the mixture was stirred for 10 min. The solid was filtered off and washed thoroughly first with water (HPLC-grade) then with diethyl ether (p.a.).

The residue was dissolved in DMSO (1 mL, p.a.) and immediately subjected to semi-preparative HPLC (stationary phase: Zorbax Eclipse XDB-C8, 9.4 × 250 mm, 5 µm; mobile phase: water + 0.1 % TFA /acetonitrile + 0.1 % TFA; flow: 6 mL/ min; injection volume: 150 µL, gradient elution: 55/45 to 3/97; column oven 40 °C; R_t major **100**: 16.8 - 17.4 min, R_t minor **101**: 19.3 - 20.1 min). Aliquots of not more than 150 µL were injected into the HPLC to obtain good separation. Pure fractions containing products were combined and evaporated to dryness *in vacuo*. The isolated white solids were suspended in water and then separated by centrifugation. This step was repeated with diethyl ether. The products thus isolated were dried for 2 days *in vacuo*. According to ^{1}H-NMR spectroscopic and mass spectrometric analysis both products represent the sulfate complexes of compounds **100** and **101** (Chart 31).

An alternative method for direct isolation of the sulfate complexes of **100** and **101** was developed. After the reaction mixture was dissolveded in DMSO, semi-preparative HPLC purification was performed as described above. However, a mixture of water + 0.05 % TFA + 0.1 % of Na_2SO_4 /acetonitrile + 0.05 % TFA was used as mobile phase. The sulfate salt in the eluent sharpened the chromatogram peaks and consequently improved separation. In addition, the injection volume was also increased from 100-150 µL to 250 µL. Pure fractions were combined and evaporated *in vacuo.* The resulting solids were separately suspended in 1.5 mL of water (HPLC-grade) and stirred for 20 min to remove remaining Na_2SO_4. The products were filtered, washed carefully first with water then with diethyl ether. Finally, they were suspended in diethyl ether, separated by centrifugation and dried for 2 days *in vacuo*.

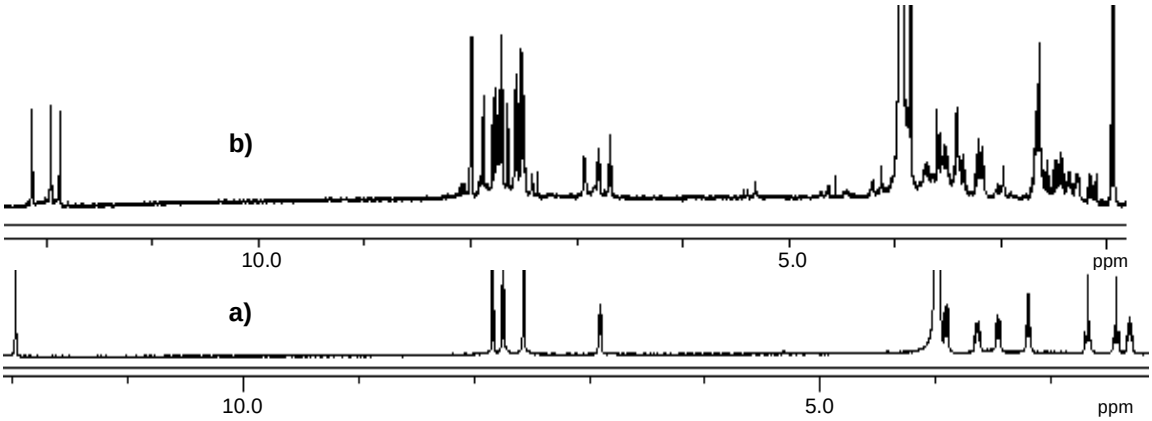


Chart 31 ^{1}H-NMR of triply-linked bis(cyclopeptides) **100**•SO_4 (a) and **101**•SO_4 (b) in CD_3CN/D_2O *(*v/v*)* 2:1 (600 MHz, 25 °C).

Assessment of purity of bis(cyclopeptide) 100•Na_2SO_4

^{1}H-NMR experiments in presence of organic salts were conducted to estimate the purity of bis(cyclopeptide) **100**•Na_2SO_4. Tetramethylammonium iodide as internal reference was chosen

because it is inert to **100**•SO_4 and does not exhibit overlapping of signals with **100**•SO_4 in the ^{1}H-NMR spectrum.

A stock solution of bis(cyclopeptide) **100**•SO_4 (1.28 mg, 0.67 µmol, 0.84 mM) in 800 mL 2:1 *(v/v)* CD_3CN/D_2O was prepared, stirred for 10 - 15 minutes at room temperature until fully dissolved, and filtered. Three aliquots of 250 µL (0.21 µmol) were transferred into NMR tubes. Next, 5 µL of a stock solution of Na_2SO_4 (0.6 mg in 1 mL D_2O, 0.1 equiv.) and 5 µL of a sock solution of tetramethylammonium iodide (2.32 mg, in 250 µL D_2O, 1.1 equiv.) were added to each sample. ^{1}H-NMR spectra were recorded after ca. 10 min and 24 h (512 scans, 600 MHz). The signal areas for the proline H(α) peak and the peak of the tetramethylammonium cation $(CH_3)_4N^+$ were integrated and that of the H(α) signal was set to 6. The ratio between experimentally obtained value for the number of methyl protons and the one calculated on the base of the added quantity of organic salt ($\int(CH_3)_4N^+_{exp.}$ / $\int (CH_3)_4N^+_{calc.}$) was calculated for each experiment. The average value was calculated, which amounted to 1.43. Hence, an aliquot of 250 µL contained 0.28 mg (0.15 µmol, 0.59 mM) of **100**•SO_4 instead of 0.4 mg (0.21 µmol, 0.84 mM). As a consequence, purity of **100**•SO_4 amounts to 70 %. The residual material of 0.12 mg (30 %) most likely corresponds to inorganic impurities (water, silica, and/or inorganic salts) that were not detectable by means of HPLC, ESI-TOF MS and NMR techniques.

Characterization of bis(cyclopeptide) 100•Na_2SO_4

Yield 6 mg, 24 % (white solid, 70 % pure)

RP TLC R_f = 0.65 (1,4-dioxane/water 1:1 v/v)
R_f = 0.41 (dichloromethane/methanol/ CH_3OH 4:1:0.05 v/v/v)

HPLC λ= 254 nm, R_t 16.6 - 17.4 min analytical column Zorbax-C8 with SO_4
λ = 254 nm, R_t 17.0 - 18.7 min analytical column Zorbax-C8
λ = 254 nm, R_t 18.3 - 22.0 min preparative column Zorbax-C8 with SO_4
λ = 254 nm, R_t 22.4 - 27.6 min preparative column Zorbax-C8

^{1}H-NMR [600MHz, CD_3CN/D_20 2:1, 22 ºC]: δ = 2.31 (q, 6H, ^3J=7.2 Hz, Tpro(β)H), 2.39-2.47 (m, 6H, EdtCH_2), 2.65-2.71 (m, 6H, EdtCH_2), 3.19 (q, 6H, ^3J=7.5 Hz, Tpro(γ)H)), 3.45 (dd, 6H, ^2J=12.6 Hz, ^3J=10.8 Hz, Tpro(δ)H)), 3.61-3.66 (m, 6H, Tpro(β)H), 3.90 (dd, 6H, ^2J=12.6 Hz, ^3J=7.2 Hz, Tpro(δ)H)), 6.90 (dd, 6H, ^3J= 8.4 Hz, ^3J= 6.6 Hz, Tpro(α)H), 7.57 (d, 6H, ^3J=7.8 Hz, APAH(3)), 7.75 (t, 6H, ^3J=8.1 Hz APAH(4)), 7.83 (d, 6H, ^3J=8.4 Hz, APAH(5)), 11.97 (br s, 6H, NH)

The ^{13}C-NMR spectrum was acquired over three days but the signal-to-noise ratio was still large. The observed signals are summarized below. Resonances deriving from TproC(γ) and TproC(δ) carbon atoms as well as the one from linker carbon atoms were not detected, presumably because sample concentration was too low. The small amount of available material precluded recording further spectra.

^{13}C-NMR [151 MHz, CD_3CN/D_20 2:1, 22 ºC]: observed signals δ = 40.05 (TproC(β)), 62.92 (TproC(α)), 116.56 (APAC(3)), 120.59 (APAC(5)), 140.45 (APAC(4)), 151.07 (APAC(2/6)), 151.66 (APAC(6/2)), 168.22 (APACO), 173.26 (TproCO).

MS MALDI-TOF *m*/*z* (rel. Int.): $[M+H]^+$ 1765.7 (79%), $[M+Na]^+$ 1789.7 (100%), $[M+K]^+$ 1805.7 (94 %)

The base peak chromatogram and the MALDI-TOF MS spectra of complexes **100** and **101** are shown in Chart 12.

Characterization of bis(cyclopeptide) 101•Na_2SO_4

Bis(cyclopeptide) **101** with four 1,2-ethanedithiol linking units was also obtained as the minor product after semi-preparative HPLC. Satisfactory ^{1}H-NMR spectroscopic data was obtained after addition of 0.1 equiv. Na_2SO_4 (5 µL from solution of 0.6 mg Na_2SO_4 in 1 mL D_2O) to a sample containing **101** (0.838 mM, 0.21 µmol in 250 µL 2:1 CD_3CN/D_2O).

Yield 0.8 mg, 3 % (white solid)

RP TLC R_f = 0.73 (1,4-dioxane/water 1:1 v/v)

HPLC λ = 254 nm, R_t 18.7 - 19.8 min analytical column Zorbax-C8 with SO_4
λ = 254 nm, R_t 24.0 - 26.8 min preparative column Zorbax-C8 with SO_4
λ = 254 nm, R_t 29.3 - 32.7 min preparative column Zorbax-C8

^{1}H-NMR [600MHz, CD_3CN/D_20 2:1, 22 ºC]: δ = 2.08-2.73 (m, 32H, Tpro(β/γ/δ)H+ EdtCH_2), 3.18-3.26 (m, 6H, Tpro(β/γ/δ)H), 3.69-3.79 (m, 2H, Tpro(β/γ/δ)H), 3.83-3.91 (m, 2H, Tpro(β/γ/δ)H), 3.93-4.03 (m, 2H, Tpro(β/γ/δ)H), 6.72 (t, 2H, ^3J= 8.1 Hz, Tpro(α)H), 6.81 (t, 2H, ^3J= 7.8 Hz, Tpro(α)H), 6.94 (t, 2H, ^3J= 7.8 Hz, Tpro(α)H), 7.49 (dd, 4H, ^2J= 17.7 Hz, ^3J= 7.5 Hz, APAH), 7.58 (dd, 2H, ^2J=9,6 Hz, ^3J=7.8 Hz, APAH), 7.68-7.77 (m, 6H, APAH), 7.83 (d, 2H, ^3J=8.4 Hz, APAH(3)), 7.91 (d,

2H, ^3J=8.4 Hz, APAH(3)), 7.97 (d, 2H, ^3J=8.4 Hz, APAH(5)), 11.87 (br s, 2H, NH), 11.95 (br s, 2H, NH), 12.13 (br s, 2H, NH)

MS MALDI-TOF *m*/*z* (rel. Int.): $[M+Na]^+$ 1881.0 (100 %), $[M+K]^+$ 1897.1 (62%)

Synthesis of bis(cyclopeptides) 98•Na_2SO_4 and 99•Na_2SO_4

A solution containing cyclopeptide c*yclo*[Tpro-APA]$_3$ (**68**) (20 mg, 26.74 µmol, 1.32 mM), DTT (20.62 mg, 13.37 mmol, 0.66 M), 1,3-benzenedithiol (5.70 mg, 40.11 µmol, 1.98 mM), Na_2SO_4 (81 µL of a 0.5 M solution in water, 2 mM), water (6.75 mL , pH 9 - 10) and acetonitrile (13.51 mL) was brought to pH 9 - 10 via dropwise addition of NaOH (0.1 M solution in water). The mixture was stirred at room temperature under air, and the progress of the reaction was followed by HPLC analysis every day. Amplification of triply-linked bis(cyclopeptide) **98** as the main product and formation of a minor product (**99**) with four linkers was detected. After 10 - 11 days of equilibration no further change in product distribution was observed. The solution was then acidified to a pH of 3, and product isolation was achieved analogously to bis(cyclopeptide) **100**•Na_2SO_4 using the optimized method for semi-preparative HPLC (stationary phase: Zorbax Eclipse XDB-C8, 9.4 × 250 mm, 5 µm; mobile phase: water + 0.05 % TFA + 0.1 % of Na_2SO_4 /acetonitrile + 0.05 % TFA, flow: 6 mL/min; injection volume: 250 µL, gradient elution: 55/45 to 3/97; column oven 40 °C; R_t major **98**: 21.3 - 22.4 min**,** R_t minor **99**: 27.9 - 28.5 min). Fractions of pure products were combined and the solvent was evaporated *in vacuo*. The resulting solids were suspended in 1.5 mL of water (HPLC-grade), stirred for 20 min, filtered off, and washed with water and then with diethyl ether. They were isolated by suspension in diethyl ether, centrifugation, and subsequent drying *in vacuo*. According to ^{1}H-NMR spectroscopic and mass spectrometric analysis both isolated products correspond to the sulfate complexes of compounds **98** and **99** (Chart 32).

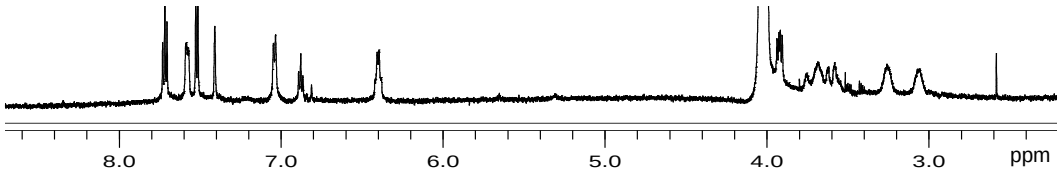


Chart 32 ^{1}H-NMR of triply-linked bis(cyclopeptide) **98**•SO_4 in CD_3CN/D_2O *(v/v)* 2:1 (600 MHz, 25 °C).

Assessment of purity of bis(cyclopeptide) 98•Na_2SO_4

In analogy to **100**•Na_2SO_4, ^{1}H-NMR experiments in the presence of tetramethylammonium iodide were conducted to estimate the purity of bis(cyclopeptide) **98**•Na_2SO_4. Because of overlap of the signal of proline H(β) protons of **98**•Na_2SO_4 and the signal of tetramethylammonium cation,

compound **98**•Na_2SO_4 was converted into the corresponding iodide (**98**•I) complex prior adding the organic salt.

Bis(cyclopeptide) **98**•SO_4 (0.84 mg, 0.41 µmol, 0.84 mM) was stirred for 10 - 15 minutes in 488 mL 2:1 *(v/v)* CD_3CN/D_2O until fully dissolved. The resulting solution was filtered and aliquots of 244 µL (0.2 µmol) were transferred into NMR tubes. Each sample was supplied with 5 µL of a Na_2SO_4 stock solution (0.6 mg in 1 mL D_2O, 0.1 equiv.) and 5 µL of a BaI_2 stock solution (5.2 mg in 250 µL D_2O, 1.2 equiv.). After 24 h 5 µL of a tetramethylammonium iodide solution (2.1 mg in 500 µL D_2O, 0.5 equiv.) were added to each sample. ^{1}H-NMR spectra were recorded after ca. 10 min and 24h (512 scans, 600 MHz).

The signal areas for the proline H(α) peak and the peak of the tetramethylammonium cation $(CH_3)_4N^+$ were integrated and that of the H(α) signal was set to 6. The ratio between experimentally obtained value for the number of methyl protons and the one calculated on the base of the added quantity of organic salt ($\int(CH_3)_4N^+_{exp.}$ / $\int (CH_3)_4N^+_{calc.}$) was calculated for each experiment. The average value was calculated, which amounted to 1.69. Hence, an aliquot of 244 µL contained 0.25 mg (0.12 µmol, 0.5 mM) of **98**•SO_4 instead of 0.42 mg (0.2 µmol, 0.84 mM). As a consequence, purity of the target compound **98**•SO_4 amounts to 59 %. The residual material of 0.17 mg (41 %) most likely corresponds to inorganic impurities (water, silica, and/or inorganic salts).

Characterization of bis(cyclopeptide) 98•Na_2SO_4

Yield 5.2 mg, 19 % (white solid, 59 % pure)

HPLC λ = 254 nm, R_t 22.2 - 23.0 min analytical column Zorbax-C8 with SO_4
λ = 254 nm, R_t 23.9 - 25.0 min analytical column Zorbax-C8
λ = 254 nm, R_t 18.5 - 21.6 min preparative column Zorbax-C8 with SO_4

^{1}H-NMR [600MHz, CD_3CN/D_2O 2:1, 22 ºC]: δ = 3.05-3.09 (m, 6H, Tpro(β)H), 3.22-3.30 (m, 6H, Tpro(δ)H), 3.55-3.57 (m, 6H, Tpro(γ)H), 3.64-3.75 (signal partially beneath the water peak, m, 6H, Tpro(δ)H + Tpro(β)H), 6.40 (t, 6H, ^3J=8.1 Hz, Tpro(α)H), 6.88 (t, 3H, ^3J=7.8 Hz, BdtH(4)), 7.04 (d, 6H, ^3J=7.8 Hz, BdtH(3)), 7.41 (s, 3H, BdtH(2)), 7.52 (d, 6H, ^3J=7.8 Hz, APAH(3)), 7.59 (d, 6H, ^3J=8.4 Hz APAH(5)), 7.71 (t, 6H, ^3J=8.1 Hz, APAH(4)),11.75 (br s, 6H, NH)

MS MALDI-TOF *m/z* (rel. Int.): $[M+Na]^+$ 1934.2 (100 %), $[M+K]^+$ 1950.1 (71%)

Characterization of bis(cyclopeptide) 99•Na_2SO_4

The small amount isolated was not pure enough to obtain satisfactory ^{1}H-NMR data.

Yield 1 mg, 3 % (white solid)

HPLC λ = 254 nm, R_t 27.9 - 28.3 min analytical column Zorbax-C8 with SO_4
λ = 254 nm, R_t 24.2 - 25.8 min preparative column Zorbax-C8 with SO_4

MS MALDI-TOF *m*/*z* (rel. Int.): $[M+Na]^+$ 2073.0 (100 %), $[M+K]^+$ 2089.1 (17%)

6.8.7 Attempted synthesis of bis(cyclopeptide) $[(67)_2(Pd(dppp))_3]^{6+}$•6$OTf^-$ 69

A suspension of *cyclo*[(*4S*)Cpro-APA]$_3$ (**67**) (1.38 mg, 1.89 µmol) in CD_3OD (500 µL) was stirred for 15 - 20 min at room temperature and an aliquot of 250 µL of the solution was transferred into a small NMR tube (Bruker MATCH^{TM}, 3 x 100 mm). Then a Pd(dppp)OTf_2•3 H_2O stock solution (15 µL, 3.3 mg in 75 µL CD_3OD) was added and the solution was thoroughly mixed using a syringe equipped with needle. Addition of the Pd(dppp)OTf_2 solution resulted generally in complete dissolvation of cyclopeptide **67**. DMSO-d_6 (4 vol %) was added to the NMR tube to allow for comparison with the spectrum of cyclopeptide **67**. ^{1}H-, ^{19}F- and ^{31}P-NMR (256 scans) spectra were recorded immediately after addition of the Pd(dppp)OTf_2 solution and after 18 h. The pattern of the spectra did not change significantly. No formation of a new compound was observed.

^{1}H-NMR [600MHz, DMSO-d_6/CD_3OD 4:96, 22 ºC]: δ = 1.99-2.10 (m, 13H, dpppCH2), 2.30-2.35 (m, 6H, Cpro(β)H), 2.69-2.76 (m, 15H, dpppCH_2), 2.91-2.96 (m, 6H, Cpro(β)H), 3.67-3.42 (m, 6H, Cpro(γ)H), 3.82-3.85 (m, 6H, Cpro(δ)H), 4.02-4.05 (m, 6H, Cpro(δ)H), 6.04-6.08 (m, 6H, Cpro(α)H), 7.43-7.65 (m, 72H, APAH(3) + APAH(4) + APAH(5) + dpppBnH_{arom})

^{19}F-NMR [600MHz, DMSO-d_6/CD_3OD 4:96, 22 ºC]: δ = -80.02

^{31}P-NMR [600MHz, DMSO-d_6/CD_3OD 4:96, 22 ºC]: δ = 17.07

6.8.8 Attempts to grow crystals of sulfate complex of bis(cyclopeptide) 100

Salt-free and solutions containing Na_2SO_4 and TBAS, and isolated **100**•SO_4 were slowly evaporated either at room temperature or at -4 °C. A summary of used amount of **100**•SO_4, solvents and conditions is included in Table 29.

Entries 1 and 3-6: The corresponding amount of compound **100**•SO_4 was weighted and dissolved in the given solvent. The sample was filtered and transferred into a 1.5 mL vial. This vial was placed open in a larger vial filled with a sufficient amount of a second solvent to allow diffusion of the vapor of the latter into the 1.5 mL vial containing complex **100**•SO_4. The large vial was supplied with a cap so that no dust could come in contact with the sample. These samples were left to crystallize at room temperature or in the refrigerator for one month.

Entry 2: A known amount of compound **100**•SO_4 was dissolved in acetonitrile/ water (v/v) 2:1 and an aliquot of an aqueous stock solution of TBAS or Na_2SO_4 was added. The sample was filtered and transferred into a 1.5 mL vial supplied with a partially opened cap so that no dust could enter but slow evaporation of the solvent was possible. The sample was left at room temperature for a period of one month.

These experiments failed because the solutions rather precipitated with time than giving crystals suitable for X-ray diffraction.

entry	**100**•SO_4 **dissolved in**	**second solvent**
1	DMSO (0.7 mg of **100**•SO_4 in 200 µL, 1.83 mM, 0.14 equiv. of Na_2SO_4)	dichloromethane
2	acetonitrile/ water (v/v) 2:1 (0.7 mg of **100**•SO_4 in 1.5-2 mL, 3.66 mM) $^{\pm}$	-
3	$CDCl_3$ (1.0-1.4 mg of **100**•SO_4 in 1.5-2 mL, 3.66 mM)	hexane
4	$CDCl_3$ (1.0-1.4 mg of **100**•SO_4 in 1.5-2 mL, 3.66 mM)	ethyl acetate*
5	$CDCl_3$ (1.0-1.4 mg of **100**•SO_4 in 1.5-2 mL, 3.66 mM)	THF*
6	$CDCl_3$ (1.0-1.4 mg of **100**•SO_4 in 1.5-2 mL, 3.66 mM)	1,4-dioxane*

* conducted either at room temperature or at -4°C

$^{\pm}$ either TBAS (1 equiv., 50 wt% aqueous solution) or Na_2SO_4 (0.1 equiv.) was added

Table 29 Experimental conditions to grow crystals of **100**•Na_2SO_4.

7 References

[1] J. T. Davis, P. A. Gale, O. A. Okunola, P. Prados, J. C. Iglesias-Sanchez, T. Torroba, R. Quesada, *Nat. Chem.* **2009**, *1*, 138.

[2] a) S. R. Seidel, P. J. Stang, *Acc. Chem. Res.* **2002**, *35*, 972; b) K. R. West, K. D. Bake, S. Otto, *Org. Lett.* **2005**, *7*, 2615; c) G. V. Oshovsky, D. N. Reinhoudt, W. Verboom, *Angew. Chem. Int. Ed.* **2007**, *46*, 2366

[3] J. L. Sessler, P. A. Gale, W.-S. Cho, *Anion Receptor Chemistry*, Royal Society of Chemistry, Cambridge, **2006**.

[4] a) Y. Markus, H. D. B. Jenkins, L. Glasser, *J. Chem. Soc., Dalton Trans.* **2002**, 3795; b) J. W. Steed, J. L. Atwood, *Supramolecular Chemistry*, Wiley, Chichester, UK, **2000**; c) E. V. Anslyn, D. A. Dougherty, *Modern physical organic chemistry*, University Science Books, Herndon, VA, **2006**.

[5] K. Bowman-James, *Acc. Chem. Res.* **2005**, *38*, 671.

[6] H.-J. Schneider, A. K. Yatsimirsky, *Principles and methods in supramolecular chemistry*, Wiley, Chichester, UK, **2000**.

[7] a) F. Hofmeister, *Arch. Exp. Pathol. Pharmakol.* **1888**, *24*, 247; b) Y. L. Zhang, P. S. Cremer, *Curr. Opin. Chem. Biotechnol.* **2006**, *10*, 658; c) F. Hofmeister, *Arch. Exp. Pathol. Pharmakol.* **1988**, *24*, 247.

[8] a) J. M. Berg, *Acc. Chem. Res.* **1995**, *28*, 14; b) D. W. Christianson, W. N. Lipscomb, *Acc. Chem. Res.* **1989**, *22*, 62; c) J. D. Puglisi, L. Chen, A. D. Frankel, J. R. Williamson, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 3680.

[9] C. Glidewell, *Chem. Ber.* **1990**, *26*, 137.

[10] B. Moss, *Chem. Ind.* **1996**, 407.

[11] a) *Supramolecular chemistry of anionic species, Chem. Soc. Rev.* **2010**, *39*, 3581; b) P. A. Gale, S. E. García-Garrido, J. Garric, *Chem. Soc. Rev.* **2008**, *37*, 151; c) C. Schmuck, V. Bickert, *J. Org. Chem.* **2007**, *72*, 6832; d) P. D. Beer, P. A. Gale, *Angew. Chem. Int. Ed.* **2001**, *40*, 486; e) S. Kubik, *Chem. Soc. Rev.* **2010**, *39*, 3648; f) S. Kubik, *Chem. Soc. Rev.* **2009**, *38*, 585; g) C. H. Lee, H. Miyajia, D.-W. Yoona, J. L. Sessler, *Chem. Commun.* **2008**, 24.

[12] a) D. J. Cram, S. Karbach, Y. H. Kim, L. Baczynskyj, G. W. Kalleymeyn, *J. Am. Chem. Soc.* **1985**, *107*, 2575; b) A. Jasat, J. C. Sherman, *Chem. Rev.* **1999**, *99*, 931; c) J. C. Sherman, *Chem. Commun.* **2003**, 1617; d) D. M. Rudkevich, *Bull. Chem. Soc. Jpn.* **2002**, *75*, 393; e) L. R. MacGillivray, J. L. Atwood, *Angew. Chem., Int. Ed.* **1999**, *38*, 1019; f) F. Hof, S. L. Craig, C. R. Nuckolls, J., Jr. , *Angew. Chem., Int. Ed.* **2002**, *41*, 1488; g) W.-Y. Sun, M. Yoshizawa, T. Kusuawa, M. Fujita, *Curr. Opin. Chem. Biol.* **2002**, *6*, 757; h) A. V. Davis, R. M. Yeh, K. N. Raymond, *Proc. Natl. Acad. Sci. USA* **2002**, *29*, 4793.

[13] H. E. Simmons, C. H. Park, *J. Am. Chem. Soc.* **1968**, *90*, 2428.

[14] a) E. G. J. M. Lehn, *J. Am. Chem. Soc.* **1975**, *97*, 5022; b) B. Dietrich, J. M. Lehn, J. P. Sauvage, *Tetrahedron Lett.* **1969**, *10*, 2889.

[15] a) M. A. Hossain, J. M. Llinares, C. A. Miller, L. Seib, K. Bowman-James, *Chem. Commun.* **2000**, 2269; b) B. Dietrich, J. M. Lehn, J. Guilhem, C. Pascard, *Tetrahedron Lett.* **1989**, *30*, 4125.

[16] T. Fujita, J. M. Lehn, *Tetrahedron Lett.* **1988**, *29*, 1709.

[17] a) C. A. Ilioudis, D. A. Tocher, J. W. Steed, *J. Am. Chem. Soc.* **2004**, *126*, 12395; b) G. Gil-Ramirez, E. C. Escudero-Adan, J. Benet-Buchholz, P. Ballester, *Angew. Chem. Int. Ed.* **2008**, *47*, 4114.

[18] a) V. Amendola, L. Fabbrizzi, *Chem. Commun.* **2009**, 513; b) V. Amendola, L. Fabbrizzi, C. Mangano, P. Pallavicini, A. Poggi, A. Taglietti, *Coord. Chem. Rev.* **2001**, *219-221*, 821; c) V. Amendola, M. Bonizzoni, D. Esteban-Gómez, L. Fabbrizzi, M. Licchelli, F. Sancenón, A. Taglietti, *Coord. Chem. Rev.* **2006**, *250*, 1451; d) J. M. Lehn, S. H. Pine, E. I. Watanabe, A. K. Willard, *J. Am. Chem. Soc.* **1977**, *99*, 6766.

[19] a) J.-M. Chen, W. Wei, X.-L. Feng, T.-B. Lu, *Chem.-Asian J.* **2007**, *2*, 710; b) R. Menif, J. Reibenspies, A. E. Martell, *Inorg. Chem.* **1991**, *30*, 3446; c) Y. Dussart, C. Harding, P. Dalgaard, C. McKenzie, R. Kadirvelraj, V. McKee, J. Nelson, *J. Chem. Soc., Dalton Trans.* **2002**, 1704.

[20] A. D. Bond, S. Derossi, C. J. Harding, E. J. L. McInnes, V. McKee, C. J. McKenzie, J. Nelson, J. Wolowska, *Dalton Trans.* **2005**, 2403.

[21] J. A. Aguilar, T. Clifford, A. Danby, J. M. Llinares, S. Mason, E. Garcia-Espana, K. Bowman-James, *Supramol. Chem.* **2001**, *13*, 405.

[22] a) K. J. Chang, B. N. Kang, M. H. Lee, K. S. Jeong, *J. Am. Chem. Soc.* **2005**, *127*, 12214; b) M. J. Hynes, B. Maubert, V. McKee, R. M. Town, J. Nelson, *J. Chem. Soc., Dalton Trans.* **2000**, 2853; c) B. M. Maubert, J. Nelson, V. McKee, R. M. Town, I. Pal, *J. Chem. Soc., Dalton Tans.* **2001**, 1395; d) D. Farrell, K. Gloe, K. Gloe, G. Goretzki, V. McKee, J. Nelson, M. Nieuwenhuyzen, I. Pal, H. Stephan, R. M. Town, K. Wichmann, *Dalton Trans.* **2003**, 1961; e) T. Clifford, A. Danby, J. M. Llinares, S. Mason, N. W. Alcock, D. Powell, J. A. Aguilar, E. Garcia-Espana, K. Bowman-James, *Inorg. Chem.* **2001**, *40*, 4710.

[23] I. Ravikumar, P. S. Lakshminarayanan, E. Suresh, P. Ghosh, *Inorg. Chem.* **2008**, *47*, 7992.

[24] a) J. Nelson, M. Nieuwenhuyzen, I. Pal, R. M. Town, *Chem. Commun.* **2002**, 2266; b) T. J. Mooibroek, P. Gamez, J. Reedijk, *CrystEngComm* **2008**, *10*, 1501.

[25] a) P. Mateus, R. Delgado, P. Brandao, V. Felix, *J. Org. Chem.* **2009**, *74*, 8638; b) P. Mateus, R. Delgado, P. Brandao, S. Carvalho, V. Felix, *Org. Biomol. Chem.* **2009**, *7*, 4661.

[26] F. P. Schmidtchen, *Angew. Chem. Int. Ed.* **1977**, *16*, 720.

[27] F. P. Schmidtchen, G. Muller, *J. Chem. Soc., Chem. Commun.* **1984**, 1115.

[28] F. P. Schmidtchen, *J. Chem. Soc., Perkin Trans. 2* **1986**, 135.

[29] a) A. P. Bisson, V. M. Lynch, M. K. C. Monahan, E. V. Anslyn, *Angew. Chem. Int. Ed.* **1997**, *36*, 2340; b) S. O. Kang, J. M. Llinares, D. Powell, D. VanderVelde, K. Bowman-James, *J. Am. Chem. Soc.* **2003**, *125*, 10152; c) S. O. Kang, D. Powell, V. W. Day, K. Bowman-James, *Angew. Chem. Int. Ed.* **2006**, *45*, 1921.

[30] a) S. O. Kang, M. A. Hossain, D. Powell, K. Bowman-James, *Chem. Commun.* **2005**, 320; b) M. A. Hossain, J. M. Llinares, D. Powell, K. Bowman-James, *Inorg. Chem.* **2001**, *40*, 2936.

[31] a) P. A. Gale, J. L. Sessler, V. Kral, V. Lynch, *J. Am. Chem. Soc.* **1996**, *118*, 5140; b) P. A. Gale, J. L. Sessler, V. Král, V. Lynch, *J. Am. Chem. Soc.* **1996**, *118*, 5140.

[32] O. D. Fox, T. D. Rolls, M. G. B. Drew, P. D. Beer, *Chem. Commun.* **2001**, 1632.

[33] J. I. Setsune, K. Watanabe, *J. Am. Chem. Soc.* **2008**, 2404.

[34] a) D. W. Yoon, D. E. Gross, V. M. Lynch, C. H. Lee, P. C. Bennett, J. L. Sessler, *Chem. Commun.* **2009**, 1109; b) J. Yoo, M.-S. Kim, S.-J. Hong, J. L. Sessler, C. H. Lee, *J. Org. Chem.* **2009**, *74*, 1065.

[35] D. W. Yoon, H. Hwang, C. H. Lee, *Angew. Chem. Int. Ed.* **2002**, *41*, 1757.

[36] C. H. Lee, J. S. Lee, H. K. Na, D. W. Yoon, H. Miyaji, W. S. Cho, J. L. Sessler, *J. Org. Chem.* **2005**, *70*, 2067.

[37] a) A. Casnati, F. Sansone, R. Ungaro, *Acc. Chem. Res.* **2003**, *36*, 246; b) L. Baldini, A. Casnati, F. Sansone, R. Ungaro, *Chem. Soc. Rev.* **2007**, *36*, 254.

[38] M. Lazzarotto, F. Sansone, L. Baldini, A. Casnati, P. Cozzini, R. Ungaro, *Eur. J. Org. Chem.* **2001**, 595.

[39] F. Sansone, L. Baldini, A. Casnati, M. Lazzarotto, F. Ugozzoli, R. Ungaro, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 4842.

[40] a) K. Moon, A. E. Kaifer, *J. Am. Chem. Soc.* **2004**, *126*, 15016; b) P. S. Mukherjee, N. Das, P. J. Stang, *J. Org. Chem.* **2004**, *69*, 3526; c) C. L. D. Gibb, B. C. Gibb, *J. Am. Chem. Soc.* **2004**, *126*, 11408; d) R. Zadmard, A. Kraft, T. Schrader, U. Linne, *Chem. Eur. J.* **2004**, *10*, 4233; e) T. Amaya, J. Rebek, Jr., *J. Am. Chem. Soc.* **2004**, *126*, 14149; f) H. Xu, D. M. Rudkevich, *Chem. Eur. J.* **2004**, *10*, 5432.

[41] a) J. S. Lindsey, *New J. Chem.* **1991**, *15*, 153; b) A. Pfeil, J. M. Lehn, *J. Chem. Soc., Chem. Commun.* **1992**, 838.

[42] F. Fochi, P. Jacopozzi, E. Wegelius, K. Rissanen, P. Cozzini, E. Marastoni, E. Fisicaro, P. Manini, R. Fokkens, E. Dalcanale, *J. Am. Chem. Soc.* **2001**, *123*, 7539.

[43] a) A. Szumna, *Chem. Eur. J.* **2009**, *15*, 12381; b) J. L. Atwood, L. J. Barbour, A. Jerga, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 4837; c) A. Szumna, *Chem. Commun.* **2009**, 4191.

[44] a) R. L. Paul, Z. R. Bell, J. S. Fleming, J. C. Jeffery, J. A.McCleverty, M. D. Ward, *Heteroat. Chem.* **2002**, *13*, 567; b) I. S. Tidmarsh, B. F. Taylor, M. J. Hardie, L. Russo, W. Clegg, M. D. Ward, *New J. Chem.* **2009**, *33*, 366.

[45] a) C. Brückner, R. E. Powers, K. N. Raymond, *Angew. Chem. Int. Ed.* **1998**, *37*, 1837; b) P. Mal, J. R. Nitschke, *Chem. Commun.* **2010**, *46*, 2417.

[46] Y. S. Park, K. Paek, *Org. Lett.* **2008**, *10*, 4867.

[47] O. Hayashida, A. Shivanyuk, J. Rebek, *Angew. Chem. Int. Ed.* **2002**, *41*, 3423.

[48] a) T. Hainz, D. M. Rudkevich, J. Rebek, Jr., *Nature* **1998**, *394*, 764; b) M. Yamanaka, A. Shivanyuk, J. Rebek, Jr., *J. Am. Chem. Soc.* **2004**, *126*, 2939; c) L. C. Palmer, J. Rebek, Jr., *Org. Biomol. Chem.* **2004**, *2*, 3051.

[49] M. O. Vysotsky, A. Pop, F. Broda, I. Thondorf, V. Böhmer, *Chem. Eur. J.* **2001**, *7*, 4403.

[50] a) T. C. Pochapsky, P. M. Stone, *J. Am. Chem. Soc.* **1990**, *112*, 6714; b) R. Romeo, G. Arena, L. M. Scolaro, M. R. Plutino, *Inorg. Chim. Acta* **1995**, *240*, 81.

[51] a) M. D. Ward, J. S. Fleming, K. L. V. Mann, C.-A. Carraz, E. Psillakis, J. C. Jeffery, J. A. McCleverty, *Angew. Chem. Int. Ed.* **1998**, *37*, 1279; b) K. R. Dunbar, C. S. Campos-Fernandez, R. Clerac, *Angew. Chem. Int. Ed.* **1999**, *38*, 3477; c) S.-T. Cheng, E. Doxiadi, R. Vilar, A. J. P. White, D. J. Williams, *J. Chem. Soc. Dalton Trans.* **2001**, 2239; d) M. D. Ward, R. L. Paul, Z. R. Bell, J. C. Jeffery, J. A. McCleverty, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 4883; e) N. Gimeno, R. Vilar, *Coord. Chem. Rev.* **2006**, *250*, 3161.

[52] G. Cafeo, F. H. Kohnke, L. Valenti, A. J. P. White, *Chem.-Eur. J.* **2008**, *14*, 11593.

[53] A. L. Cresswell, M.-O. M. Piepenbrock, J. W. Steed, *Chem. Commun.* **2010**, *46*, 2787.

[54] T. Fiehn, R. Goddard, R. W. Seidel, S. Kubik, *Chem. Eur. J.* **2010**, *16*, 7241

[55] Y. Li, K. M. Mullen, T. D. W. Claridge, P. J. Costa, V. Felix, P. D. Beer, *Chem. Commun.* **2009**, 7134.

[56] a) C. Olivier, Z. Grote, E. Solari, R. Scopelliti, K. Severin, *Chem. Commun.* **2007**, 4000 ; b) K. Worm, F. P. Schmidtchen, A. Schier, A. Schäfer, M. Hesse, *Angew. Chem. Int. Ed.* **1994**, *33*, 327 ; c) R. Vilar, *Angew. Chem. Int. Ed.* **2003**, *42*, 1460 ; d) E. García-España, P. Díaz, J. M. Llinares, A. Bianchi, *Coord. Chem. Rev.* **2006**, *250*, 2952 ; e) B. Wu, J. Liang, J. Yang, C. Jia, X.-J. Yang, H. Zhang, N. Tang, C. Janiak, *Chem. Commun.* **2008**, 1762

[57] a) S. J. Rowan, S. J. Cantrill, G. R. L. Cousins, J. K. M. Sanders, J. F. Stoddart, *Agew. Chem. Int. Ed.* **2002**, *41*, 898; b) J.-M. Lehn, *Chem. Soc. Rev.* **2007**, *36*, 151.

[58] a) J.-M. Lehn, *Science* **2002**, *295*, 2400; b) J.-M. Lehn, *Science* **1993**, *260*, 1762; c) J.-M. Lehn, *Science* **1985**, *227*, 849; d) D. Seebach, *Angew. Chem. Int. Ed.* **1990**, *29*, 1320; e) J.-M. Lehn, *Supramolecular Chemistry: Concepts and Perspectives*, VCH, New York, Weinheim, **1995**; f) H. J. Schneider, A. Yatsimirsky, *Prinsiples and Methods in Supramolecular Chemistry*, Wiley, Chichester, **2000**.

[59] a) J. R. Nitschke, *Nature* **2009**, *462*, 736; b) B. C. Gibb, *Nat. Chem* **2009**, *1*, 17; c) R. F. Ludlow, S. Otto, *Chem. Soc. Rev.* **2008**, *37*, 101.

[60] J. N. H. Reek, S. Otto, *Dynamic combinatorial chemistry*, Wiley-VCH, Weinheim, **2010**.

[61] G. Aksnes, P. Albriktsen, P. Juvvik, *Acta Chem. Scand.* **1965**, *19*, 920.

[62] S. Anderson, H. L. Anderson, J. K. M. Sanders, *Acc. Chem. Res.* **1993**, *26*, 469.

[63] E. V. Eliel, S. H. Wilen, *Stereochemistry of organic compounds*, Wiley, New York, **1994**.

[64] a) S. J. Rowan, S. J. Cantrill, G. R. L. Cousins, J. K. M. Sanders, J. F. Stoddart, *Angew. Chem. Int. Ed.* **2002**, *41*, 898; b) P. T. Corbett, J. Laclaire, L. Vial, K. R. West, J.-L. Wietor, J. K. M. Sanders, S. Otto, *Chem. Rev.* **2006**, *106*, 3652; c) M. M. Rozenan, B. R. McNaughton, D. R. Liu, *Curr. Opin. Chem. Biol.* **2007**, *11*, 259; d) S. Landame, *Org. Biomol. Chem.* **2008**, *6*, 219; e) R. T. S. Lam, A. M. Belenguer, S. L. Roberts, C. Naumann, T. Jarrosson, S. Otto, J. M. K. Sanders, *Science* **2005**, *308* 667; f) L. Vial, R. F. Ludlow, J. Laclaire, R. Pérez-Fernández, S. Otto, *J. Am. Chem. Soc.* **2006**, *128*, 10253; g) J. M. C. A. Kerckhoffs, M. A. Mateos-Timoneda, D. N. Rinhoudt, M. Crego-Calama, *Chem.-Eur. J.* **2007**, *13*, 2377; h) F. Bulos, S. L. Roberts, R. L. E. Furlan, J. K. M. Sanders, *Chem. Commun.* **2007**, 3092; i) F. A. Aldaye, H. F. Sleiman, *J. Am. Chem. Soc.* **2007**, *129*, 10070; j) K. R. West, R. F. Ludlow, P. T. Corbett, P. Besenius, F. M. Mansfeld, P. A. G. Cormack, D. C. Sherrington, J. M. Goodman, M. C. A. Stuart, S. Otto, *J. Am. Chem. Soc.* **2008**, *130*, 10834; k) M.-K. Chung, C. M. Hebling, J. W. Jorgenson, K. Severin, S. J. Lee, M. R. Gagné, *J. Am. Chem. Soc.* **2008**, *130*, 11819; l) H. Y. Au-Yeung, P. Pengo, G. D. Pantoş, S. Otto, J. K. M. Sanders, *Chem. Commun.* **2009**, 419; m) R. Pérez-Fernández, M. Pittelkow, A. M. Belenguer, L. A. Lane, C. V. Robinson, J. K. M. Sanders, *Chem. Commun.* **2009**, 3708; n) Z. Rodriguez-Docampo, E. Eugenieva-Ilieva, C. Reyheller, A. M. Belenguer, S. Kubik, S. Otto, *Chem. Commun.* **2011**, *47*, 9798.

[65] P. A. Brady, J. M. K. Sanders, *Chem. Soc. Rev.* **1997**, *26*, 327.

[66] a) A. J. Moore, L. Goldenberg, M. R. Bryce, M. C. Petty, A. P. Monkman, S. N. Port, *Adv. Mater.* **1998**, *10*, 395; b) S. Flink, B. A. Boukamp, A. Van den Berg, F. C. J. M. van Veggel, D. N. Reinhoudt, *J. Am. Chem. Soc.* **1998**, *120*, 4652; c) H. Miyaji, J. P. Anzenbacher, J. L. Sessler, E. R. Bleasdale, P. A. Gale, *Chem. Commun.* **1999**, 1723; d) N. Guiusepone, G. Fuks, J.-M. Lehn, *Chem. Eur. J.* **2006**, *12*, 1723.

[67] N. Sreenivasachary, J.-M. Lehn, *Chem.-Asian J.* **2008**, *3*, 134.

[68] a) A. Bugaut, K. Pantoş, J.-L. Wietor, R. Rodriguez, J. K. M. Sanders, S. Balasubramanian, *Angew. Chem. Int. Ed.* **2008**, *47*, 2677; b) O. Ramström, J.-M. Lehn, *Nat. Rev. Drug Discovery* **2002**, *1*, 126; c) B. R. McNaughton, P. C. Gareiss, B. L. Miller, *J. Am. Chem. Soc.* **2007**, *129*, 11306; d) A. Valade, D. Urban, J.-M. Beau, *J. Comb. Chem.* **2007**, *9*, 1; e) M. T. Cancilla, M. M. He, N. Viswanatha, R. L. Simmons, M. Taylor, A. D. Fung, K. Cao, D. A. Erlanson, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3978; f) B. M. R.

Liénard, R. Hüting, P. Lassaux, M. Galleni, J.-M. Frére, C. J. Schofield, *J. Med. Chem.* **2008**, *51*, 684; g) P. C. Gareiss, K. Sobczak, B. R. McNaughton, P. B. Palde, C. A. Thornton, B. L. Miller, *J. Am. Chem. Soc.* **2008**, *130*, 16254.

[69] a) S. Xu, N. Giuseppone, *J. Am. Chem. Soc.* **2008**, *130*, 1826; b) J. W. Sadownik, D. Philp, *Angew. Chem. Int. Ed.* **2008**, *47*, 9965; c) R. Nguyen, L. Allouche, E. Buhler, N. Giuseppone, *Angew. Chem. Int. Ed.* **2009**, *48*, 1093.

[70] J. M. Lehn, *Chem. Eur. J.* **1999**, *5*, 2455.

[71] R. L. E. Furlan, S. Otto, J. K. M. Sanders, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 4801.

[72] a) P. A. Brady, R. P. B.-. Law, S. J. Rowan, C. J. Suckling, J. K. M. Sanders, *Chem. Commun.* **1996**, 319; b) P. A. Brady, J. K. M. Sanders, *J. Chem. Soc., Perkin Trans. 1* **1997**, 3237; c) P. A. Brady, J. K. M. Sanders, *J. Chem. Soc., Perkin Trans. 1* **1998**, 2119.

[73] a) R. Krämer, J.-M. Lehn, A. Marquis-Rigault, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 5394; b) V. S. Smith, J.-M. Lehn, *Chem. Commun.* **1996**, 2733; c) B. Hasenknopf, J.-M. Lehn, B. O. Kneisel, G. Baum, D. Fenske, *Angew. Chem. Int. Ed.* **1996**, *35*, 1838; d) B. Hasenknopf, J.-M. Lehn, N. Boumediene, A. Dupont - Gervais, A. Van Dorsselaer, B. Kneisel, D. Fenske, *J. Am. Chem. Soc.* **1997**, *119*, 10956; e) J.-M. Lehn, *Chem. Eur. J.* **1999**, *5*, 2455.

[74] I. Huc, J.-M. Lehn, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 2106.

[75] a) O. Ramström, J.-M. Lehn, *ChemBioChem.* **2000**, *1*, 41; b) A. M. Whitney, S. Ladame, S. Balasubramanian, *Angew. Chem. Int. Ed.* **2004**, *43*, 1143; c) S. Ladame, A. M. Whitney, S. Balasubramanian, *Angew. Chem. Int. Ed.* **2005**, *44*, 5736.

[76] a) J. H. Hartley, T. D. James, C. J. Ward, *J. Chem. Soc., Perkin Trans. 1* **2000**, 3155; b) R. J. Fitzmaurice, G. M. Kyne, D. Douheret, J. D. Kilburn, *J. Chem. Soc., Perkin Trans. 1* **2002**, 841.

[77] a) B. Brisig, J. K. M. Sanders, S. Otto, *Angew. Chem. Int. Ed.* **2003**, *42*, 1270; b) L. Vial, J. K. M. Sanders, S. Otto, *New J. Chem.* **2005**, *29*, 1001.

[78] R. T. S. Lam, A. Belenguer, S. L. Roberts, C. Naumann, T. Jarrosson, S. Otto, J. K. M. Sanders, *Science* **2005**, *308*, 667.

[79] a) A. Buryak, K. Severin, *J. Comb. Chem.* **2006**, *8*, 540; b) A. Buryak, A. Pozdnoukhov, K. Severin, *Chem. Commun.* **2007**, 2366; c) P. C. Jurs, G. A. Bakken, H. E. McClelland, *Chem. Rev.* **2000**, *100*, 2649.

[80] X.-N. Xu, L. Wang, G.-T. Wang, J.-B. Lin, G.-Y. Li, X.-K. Jiang, Z.-T. Li, *Chem. Eur. J.* **2009**, *15*, 5763.

[81] M. G. Woll, S. H. Gellman, *J. Am. Chem. Soc.* **2004**, *126*, 11172.

[82] J.-M. Lehn, A. Marquis-Rigault, J. Siegel, J. Harrowlield, B. Chevrier, D. Moras, *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 2565.

[83] a) N. Guiseppone, J.-M. Lehn, *Angew. Chem. Int. Ed.* **2006**, *45*, 4619; b) N. Guiseppone, J.-M. Lehn, *Chem. Eur. J.* **2006**, *12*, 1715.

[84] a) S. Tashiro, M. Tominaga, Y. Yamaguchi, K. Kato, M. Fujita, *Chem. Eur. J.* **2006**, *12*, 3211; b) S. Tashiro, M. Tominaga, Y. Yamaguchi, K. Kato, M. Fujita, *Angew. Chem. Int. Ed.* **2006**, *5*, 241.

[85] a) I. Saur, K. Severin, *Chem. Commun.* **2005**, 1471; b) K. Severin, *Chem.-Eur. J.* **2004**, *10*, 2565; c) Z. Grote, R. Scopelliti, K. Severin, *Angew. Chem., Int. Ed.* **2003**, *42*, 3821.

[86] P. T. Corbett, J. K. M. Sanders, S. Otto, *Chem. Eur. J.* **2008**, *14*, 2153.

[87] P. T. Corbett, J. Laclaire, L. Vial, K. R. West, J.-L. Wietor, J. K. M. Sanders, S. Otto, *Chem. Rev.* **2006**, *106*, 3652.

[88] a) P. T. Corbett, S. Otto, J. K. M. Sanders, *Org. Lett.* **2004**, *6*, 1825; b) R. F. Ludlow, S. Otto, *J. Am. Chem. Soc.* **2008**, *130*, 12218; c) J. S. Moore, N. W. Zimmerman, *Org. Lett.* **2000**, *2*, 915; d)S. Otto, R. L. Furlan, J. M. K. Sanders, *Science* **2002**, *297*, 590.

[89] I. Huc, J.-M. Lehn, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 8272.

[90] a) M. C. Calama, R. Hulst, R. Fokkens, N. M. M. Nibbering, P. Timmerman, D. N. Reinhoudt, *Chem. Commun.* **1998**, 1021; b) F. Hof, C. Nickolls, J. Rebek, Jr., *J. Am. Chem. Soc.* **2000**, *122*, 4251; c) D. Hamilton, N. Feeder, S. Teat, J. K. M. Sanders, *New J. Chem.* **1998**, *22*, 1019.

[91] a) B. Hasenknopf, J.-M. Lehn, B. O.Kneisel, G. Baum, D. Fenske, *Angew. Chem., Int. Ed.* **1996**, *35*, 1838; b) B. Klekota, M. H. Hammond, B. L. Miller, *Tetrahedron Lett.* **1997**, *38*, 8639; c) M. Albrecht, O. Blau, R. Frölich, *Chem. Eur. J.* **1999**, *5*, 48; d) S. B. Lee, S. Hwang, D. S. Chung, J.-I. Hong, *Tetrahedron Lett.* **1998**, *39*, 873; e) M. Ziegler, J. J. Miranda, U. N. Andersen, D. W. Johnson, J. A. Leary, K. N. Raymond, *Angew. Chem., Int. Ed.* **2001**, *40*, 733; f) Y. Kubota, S. Sakamoto, K. Yamaguchi, M. Fujita, *Angew. Chem., Int. Ed.* **2001**, *40*, 733; g) M. Albrecht, I. Janser, J. Runsick, G. Raabe, P. Weis, R. Frölich, *Angew. Chem., Int. Ed.* **2004**, *43*, 6662.

[92] a) S. J. Cantrill, S. J. Rowan, J. F. Stoddart, *Org. Lett.* **1999**, *1*, 1363; b) K. Oh, K. S. Jeong, J. S. Moore, *Nature* **2001**, *414*, 889; c) O. Storm, U. Lüning, *Chem. Eur. J.* **2002**, *8*, 793; d) K. S. Chichak, S. J. Cantrill, A. R. Pease, S.-H. Chiu, G. W. V. Cave, J. L. Atwood, J. F. Stoddart, *Science* **2004**, *304*, 1308.

[93] a) J. K. M. Sanders, *Chem. Commun.* **1999**, 1575; b) R. Nguyen, I. Huc, *Chem. Commun.* **2003**, 942.

[94] a) H. Hioki, W. C. Still, *J. Org. Chem.* **1998**, *63*, 904; b) S. Otto, R. L. E. Furlan, J. K. M. Sanders, *J. Am. Chem. Soc.* **2000**, *122*, 12063; c) O. Ramström, J. M. Lehn, *ChemBioChem* **2000**, *1*, 41.

[95] R. Larsson, Z. C. Pei, O. Ramström, *Angew. Chem., Int. Ed.* **2004**, *43*, 3716.

[96] a) M. S. Goodman, V. Jubian, B. Linton, A. D. Hamilton, *J. Am. Chem. Soc.* **1995**, *117*, 11610; b) A. Bilyk, M. M. Harding, *J. Chem. Soc., Chem. Commun.* **1995**, 697; c) B. Hasenknopf, J.-M. Lehn, N. Boumediene, A. Dupont-Gervais, A. Van Dorsselaer, B. Kneisel, D. Fenske, *J. Am. Chem. Soc.* **1997**, *119*, 10956; d) S. Sakai, Y. Shigemasa, T. Sasaki, *Bull. Chem. Soc. Jpn.* **1999**, *72*, 1313; e) B. Klekota, B. L. Miller, *Tetrahedron* **1999**, *55*, 11687; f) V. N. Goral, M. I.; Eliseev, A. V.; Lehn, J.-M. , *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 1347; g) E. C. Constable, C. E. Housecroft, T. Kulke, C. Lazzarini, E. R. Schofield, Y. Zimmermann, *J. Chem. Soc., Dalton Trans.* **2001**, 2864; h) O. Shoji, S. Okada, A. Satake, Y. Kobuke, *J. Am. Chem. Soc.* **2005**, *127*, 2201.

[97] a) P. Vongvilai, M. Angelin, R. Larsson, O. Ramström, *Angew. Chem., Int. Ed.* **2007**, *46*, 948; b) S. M. Turega, C. Lorenz, J. W. Sadownik, D. Philp, *Chem. Commun.* **2008**, 4076; c) N. A. Stephenson, J. Zhu, S. H. Gellman, S. S. Stahl, *J. Am. Chem. Soc.* **2009**, *13*, 10003; d) K. Severin, *Dalton Trans.* **2009**, 5254; e) C. Saiz, P. Wipf, E. Manta, G. Mahler, *Org. Lett.* **2009**, *11*, 3170; f) N. Coskun, C. Aksoy, *Tetrahedron Lett.* **2009**, *50*, 3008; g) A. Paul, S. Ladame, *Org. Lett.* **2009**, *11*, 4894; h) P. Reutenauer, P. J. Boul, J.-M. Lehn, *Eur. J. Org. Chem.* **2009**, 1691.

[98] a) Z. Shaked, R. Szajewski, G. Whitesides, *Bochemistry* **1980**, *19*, 4156; b) W. J. Lees, G. M. Whitesides, *J. Org. Chem.* **1993**, *58*, 642; c) H. F. Gilbert, *Methods Enzymol.* **1995**, *251*, 8.

[99] Y. Furusho, T. Hasegawa, A. Tsuboi, N. Kihara, T. Takata, *Chem. Lett.* **2000**, 18.

[100] T. J. Kucharski, Z. Huang, Q.-Z. Yang, Y. Tian, N. C. Rubin, C. D. Concepcion, R. Boulatov, *Angew. Chem.* **2009**, 7174.

[101] a) A. Meister, M. E. Anderson, *Annu. Rev. Biochem.* **1983**, *52*, 711; b) H. F. Gilbert, *Methods in Enzymology* **1995**, *251*, 8.

[102] R. Singh , G. V. Lamoureux, W. J. Lees, G. M. Whitesides, *Methods Enzymol.* **1995**, *251*, 167.

[103] A. L. Kieran, A. D.Bond, A. M. Belenguer, J. K. M. Sanders, *Chem. Commun.* **2003**, 2674.

[104] S. Otto, R. L. E. Furlan, J. K. M. Sanders, *J. Am. Chem. Soc.* **2000**, *122*, 12063.

[105] a) S. Sando, A. Narita, Y. Aoyama, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2835; b) Y. Krishnan-Ghosh, A. M. Whitney, S. Balasubramanian, *Chem. Commun.* **2005**, 3068.

[106] P. T. Corbett, J. K. M. Sanders, S. Otto, *J. Am. Chem. Soc.* **2005**, *127*, 9390.

[107] K. R. West, K. D. Bake, S. Otto, *Org. Lett.* **2005**, *7*, 2615.

[108] L. A. Ingerman, M. E. Cuellar, M. L. Waters, *Chem. Commun.* **2010**, *46*, 1839.

[109] L. Vial, R. F. Ludlow, J. Leclaire, R. Pérez-Fernández, S. Otto, *J. Am. Chem. Soc.* **2006**, *128*, 10253.

[110] R. Pérez-Fernández, M. Pittelkow, A. M. Belenguer, L. A. Lane, C. Robinson, J. K. M. Sanders, *Chem. Commun.* **2009**, 3708.

[111] a) P. Besenius, F. M. Mansfeld, P. A. Cormack, D. C. Sherrington, J. M. Goodman, M. C. Stuart, S. Otto, *J. Am. Chem. Soc.* **2008**, *130*, 10834; b) L. Raehm, C. Hamann, J.-M. Kern, J.-P. Sauvage, *Org. Lett.* **2000**, *2*, 1991.

[112] a) A. G. Kolchinski, N. W. Alcock, R. A. Roesner, D. H. Busch, *Chem. Commun.* **1998**, 1437; b) T. Doya, S. Choi, A. Yamashita, N. Yui, Y. Sugaya, A. Kano, A. Maruyama, H. Akita, R. Ito, K. Kogure, H. Harashima, *J. Am. Chem. Soc.* **2006**, *128*, 3852

[113] H. Y. Au-Yeung, G. D. Pantoş, J. M. K. Sanders, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 10466.

[114] H. Y. Au-Yeung, G. D. Pantoş, J. M. K. Sanders, *J. Am. Chem. Soc.* **2009**, *131*, 16030.

[115] Z. Rodriguez-Docampo, S. Otto, *Chem. Commun.* **2008**, 5301.

[116] a) P. T. Corbett, J. K. M. Sanders, S. Otto, *Angew. Chem., Int. Ed.* **2007**, *46*, 8858; b) R. F. Ludlow, S. Otto, *Chem. Soc. Rev.* **2008**, *37*; c) R. A. R. Hunt, S. Otto, *Chem. Commun.* **2011**, *47*, 847.

[117] V. Goral, M. I. Nelen, A. V. Eliseev, J.-M. Lehn, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 1347.

[118] J. Leclaire, L. Vial, S. Otto, J. K. M. Sanders, *Chem. Commun.* **2005**, 1959.

[119] A. M. Escalante, A. G. Orrillo, R. L. E. Furlan, *J. Comb. Chem.* **2010**, *12*, 410.

[120] J. M. A. Carnall, C. A. Waudby, A. M. Belenguer, M. C. A. Stuart, J. J. P. Peyralans, S. Otto, *Science* **2010**, *327*, 1502.

[121] a) P. Besenius, P. A. G. Cormack, J. Liu, S. Otto, J. M. K. Sanders, D. C. Sherrington, *Chem. Eur. J.* **2008**, *14*, 9006; b) P. Besenius, P. A. G. Cormack, R. F. Ludlow, S. Otto, D. C. Sherrington, *Chem. Commun.* **2008**, 2809.

[122] Y. Y. Chen, W. Y. He, Y. Wu, C. Q. Niu, G. Liu, *J. Comb. Chem.* **2008**, *10*, 914.

[123] P. Besenius, P. Gormack, R. F. Ludlow, S. Otto, D. C. Sherrington, *Org. Biomol. Chem.* **2010**, *8*, 2414.

[124] Myotonic dystrophy T1 is one type of muscular dystrophy.

[125] R. F. Ludlow, S. Otto, *J. Am. Chem. Soc.* **2010**, *132*, 5984.

[126] a) P. Stang, *Chem. Rev.* **2011**, *111*, 6810; b) T. Kusukawa, M. Fujita, *J. Am. Chem. Soc.* **1999**, 1397; c) S. Hiraoka, M. Fujita, *J. Am. Chem. Soc.* **1999**, *121*, 10239; d) S. Hiraoka, M. Fujita, *Chem. Commun.* **2000**, 1509; e) Y. Kubota, S. Sakamoto, K. Yamaguchi, M. Fujita, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 4854; f) S. Otto, J. K. M. Sanders, in *Supramolecular Libraries, Encyclopedia of Supramolecular Chemistry* (Ed.: M. Dekker), Atwood, J. Steed, J., New York, **2004**, pp. 1427; g) M. Crego-Calama, R. Hulst, R. Fokkens, N. M. M. Nibbering, P. Timmerman, D. N. Reinhoudt, *Chem. Commun.* **1998**, 1021; h) M. Crego-Calama, P. Timmerman, D. N. Reinhoudt, *Angew. Chem. Int. Ed.*

2000, *39*, 755; i) F. Hof, C. Nuckolls, J. Rebek, Jr., *J. Am. Chem. Soc.* **2000**, *122*, 4251; j) C. Naumann, S. Place, J. C. Sherman, *Chem. Commun.* **2002**, 1672.

[127] S.-W. Tam-Chang, J. S. Stehouwer, J. Hao, *J. Org. Chem.* **1999**, *64*, 334.

[128] R. C. Helgeson, A. E. Hayden, K. N. Houk, *J. Org. Chem.* **2010**, *75*, 570.

[129] C. Naumann, S. Place, J. C. Sherman, *J. Am. Chem. Soc.* **2002**, *124*, 16.

[130] a) S. Otto, S. Kubik, *J. Am. Chem. Soc.* **2003**, *125*, 7804; b) Z. Rodriguez-Docampo, S. Pascu, S. Kubik, S. Otto, *J. Am. Chem. Soc.* **2006**, *128*, 11206.

[131] L. Kejči, M. Buděšinsky, J. Vondrášek, J. Vymětal, J. Černy, I. Císařová, J. Brynda, V. Herzig, P. Koutnik, J. Závada, T. Kraus, *J. Org. Chem.* **2009**, *74*, 1082.

[132] L. Kejči, M. Buděšinsky, I. Císařová, T. Kraus, *Chem. Commun.* **2009**, 3557.

[133] L. C. Jones, W. M. L. Y. Vasilyeva, K. Wilson, V. Chechik, *Chem. Commun.* **2009**, 1377.

[134] a) D. L. Caulder, K. N. Raymond, *Acc. Chem. Res.* **1999**, *32*, 975; b) B. J. Holliday, C. A. Mirkin, *Angew. Chem. Int. Ed.* **2001**, *40*, 2022.

[135] a) E. Haeckel, *Zoology* **1887**, *18*, 1803; b) R. W. Horne, *Virus Structure*, Academic Press, New York, **1974**; c) A. J. Cann, *Principles of Molecular Virology, 2nd ed.*, Academic Press., New York, **1997**; d) G. Ercolani, *J. Phys. Chem. B* **1998**, *102*, 5699.

[136] A. Terfort, N. Bowden, G. M. Whitesides, *Nature* **1997**, *386*, 162.

[137] a) G. S. Hanan, D. Volkmer, U. S. Schubert, J.-M. Lehn, G. Baum, D. Fenske, *angew. Chem. Int. Ed.* **1997**, *36*, 1842; b) M. Ruben, J. Rojo, F. J. Romero-Salguero, L. H. Uppadine, J.-M. Lehn, *Angew. Chem., Int. Ed.* **2004**, *43*, 3644.

[138] S. Kubik, *Chem. Soc. Rev.* **2009**, *38*, 585.

[139] a) J. Sanchez-Quesada, C. Seel, P. Prados, J. d. Mendoza, I. Dalcol, E. Giralt, *J. Am. Chem. Soc.* **1996**, *118*, 277; b) H. Juwarker, J. M. Suk, K. S. Jeong, *Chem. Soc. Rev.* **2009**, *38*, 3316.

[140] a) M. Fujita, M. Tominaga, A. Hori, B. Therrien, *Acc. Chem. Res.* **2005**, *38*, 369; b) S. Leininger, B. Olenyuk, P. J. Stang, *Chem. Rev.* **2000**, *100*, 853 ; c) J. J. Rebek, *Acc. Chem. Res.* **1999**, *32*, 278.

[141] a) G. Schill, *Catnanes, Rotaxanes and Knots*, New York, **1971**; b) J.-P. Sauvage, C. Dietrich-Buchecker, *Molecular Catenanes, Rotaxanes and Knots*, Wiley-VCH, Weinheim, **1999**.

[142] S. Leininger, B. Olenyuk, P. J. Stang, *Chem. Rev.* **2000**, *100*, 853.

[143] V. Percec, A. E. Dulcey, V. S. K. Balagurusamy, Y. Miura, J. Smidrkal, M. Peterca, S. Nummelin, U. Edlund, S. D. Hudson, P. A. Heiner, D. A. Hu, S. N. Magonov, S. A. Vinogradov, *Nature* **2004**, *430*, 764.

[144] a) P. D. Tecilla, R. P., G. Slobodkin, D. S. Alavi, D. H. Waldeck, A. D. Hamilton, *J. Am. Chem. Soc.* **1990**, *112*, 9408; b) G. M. Whitesides, M. J. P., C. T. Seto, *Science* **1991**, *254*, 1312.

[145] a) D. S. Lawrence, T. Jiang, M. Levett, *Chem. Rev.* **1995**, *95*, 2229; b) D. Philp, J. F. Stoddart, *Angew. Chem. Int. Ed.* **1996**, *35*, 1154; c) M. Fujita, K. Umemoto, M. Yoshizawa, N. Fujita, T. Kusukawa, K. Biradha, *Chem. Commun.* **2001**, 509; d) M. Yoshizawa, J. K. Klosterman, M. Fujita, *Angew. Chem. Int. Ed.* **2009**, *48*, 3418 ; e) J. Steed, *Chem. Soc. Rev.* **2009**, *38*, 506; f) K. Rissanen, *Molecular encapsulation. Organic reactions in constrained systems*, John Wiley & Sons, Chichester, U. K., **2010**; g) R. Chakrabarty, P. S. Mukherjee, P. J. Stang, *Chem. Rev.* **2011**, *111*, 6810.

[146] a) R. G. Chapman, S. J. C., *J. Am. Chem. Soc.* **1995**, *117*, 9081; b) L. R. MacGillivray, J. L. Atwood, *Nature* **1997**, *389*, 469; c) T. Heinz, D. M. Rudkevich, J. Rebek, Jr., *Nature* **1998**, *394*, 764; d) K. Kobayashi, T. Shirasaka, K. Yamaguchi, S. Sakamoto, E. Horn, N. Furukawa, *Chem. Commun.* **2000**, 41; e) F. Hof, S. L. Craig, C. Nuckolls, J. Rebek, *Angew. Chem. Int. Ed.* **2002**, *41*, 1488; f) M. H. K. Ebbing, M.-J. Villa, J.-M. Valpuesta, P. Prados, J. de Mendoza, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 4962; g) K. Kobayashi, K. Ishii, S. Sakamoto, T. Shirasaka, K. Yamaguchi, *J. Am. Chem. Soc.* **2003**, *125*, 10615; h) F. Sansone, L. Baldini, A. Casnati, E. Chierici, G. Faimani, F. Ugozzoli, R. Ungaro, *J. Am. Chem. Soc.* **2004**, *126*, 6204; i) D. Ajami, J. Rebek, Jr., *J. Am. Chem. Soc.* **2006**, *128*, 5314; j) H. Kitagawa, Y. Kobori, M. Yamanaka, K. Yoza, K. Kobayashi, *Proc. Natl. Acad. Sci. USA* **2009**, 10444.

[147] a) F. Corbellini, R. Fiammengo, P. Timmerman, M. Crego-Calama, K. Versluis, A. J. R. Heck, I. Luyten, D. N. Reinhoudt, *J. Am. Chem. Soc.* **2002**, *124*, 6569; b) F. Corbellini, F. W. B. v. Leeuwen, H. Beijleveld, H. Kooijman, A. L. Spek, W. Verboom, M. Crego-Calama, D. N. Reinhoudt, *New J. Chem.* **2005**, *29*, 243; c) G. V. Oshovsky, D. N. Reinhoudt, W. Verboom, *J. Am. Chem. Soc.* **2006**, *128*, 5270; d) M. D. L. Giles, S., R. L. Emanuel, B. C. Gibb, S. M. Grayson, *J. Am. Chem. Soc.* **2008**, *130*, 14430; e) S. Liu, B. C. Gibb, *Chem. Commun.* **2008**, 3709.

[148] a) A. Ikeda, M. Yoshimura, H. Udzu, C. Fukuhara, S. Shinkai, **1999**, *121*, 4296; b) O. D. Fox, M. G. B. Drew, P. D. Beer, *Angew. Chem. Int. Ed.* **2000**, *39*, 135; c) R. Pinalli, V. Cristini, V. Sottili, S. Geremia, M. Campagnolo, A. Caneschi, E. Dalcanale, *J. Am. Chem. Soc.* **2004**, *126*, 6516; d) K. Kobayashi, Y. Yamada, M. Yamanaka, Y. Sei, K. Yamaguchi, *J. Am. Chem. Soc.* **2004**, *126*, 13896; e) T. Haino, M. Kobayashi, M. Chikaraishi, Y. Fukazawa, *Chem. Commun.* **2005**, 2321; f) S. J. Park, D. M. Shin, S. Sakamoto, K. Yamaguchi, Y. K. Chung, M. S. Lah, J.-I. Hong, *Chem. Eur. J.* **2005**, *11*, 235; g) H. Jude, D. J. Sinclair, N. Das, M. S. Sherburn, P. J. Stang, *J. Org. Chem.* **2006**, *71*, 4155.

[149] a) T. Beissel, R. E. Powers, K. N. Raymond, *Angew. Chem. Int. Ed.* **1996**, *35*, 1084 ; b) R. W. Saalfrank, I. Bernt, *Curr. Opin. Solid State Mater. Sci* **1998**, *3*, 407 ; c) P. N. W. Baxter, J.-M. Lehn, G. Baum, D. Fenske, *Chem. Eur. J.* **1999**, *5*, 102 ; d) D. Fiedler, D. H.

Leung, R. G. Bergman, K. N. Raymond, *Acc. Chem. Res.* **2005**, *38*, 349; e) E. C. Constable, *Chem. Soc. Rev.* **2007**, *36*, 246; f) R. W. Saalfrank, H. Maid, A. Scheurer, *Angew. Chem. Int. Ed.* **2008**, *47*, 8794; g) S. M. Biros, R. M. Yeh, K. N. Raymond, *Angew. Chem. Int. Ed.* **2008**, *47*, 6062; h) P. Mal, B. Breiner, K. Rissanen, J. R. Nitschke, *Science* **2009**, *324*, 1697.

[150] a) J. R. Farrell, C. A. Mirkin, I. A. Guzei, L. M. Liable-Sands, A. L. Rheingold, *Angew. Chem. Int. Ed.* **1998**, *37*, 465; b) N. C. Gianneschi, M. S. M. III, C. A. Mirkin, *Acc. Chem. Res.* **2005**, *38*, 825; c) C. G. Oliveri, P. A. Ulmann, M. J. Wiester, C. A. Mirkin, *Acc. Chem. Res.* **2008**, *41*, 1618.

[151] a) A. W. Maverick, F. E. Klavetter, *Inorg. Chem.* **1984**, *23*, 4129; b) A. W. Maverick, S. C. Buckingham, Q. Yao, J. R. Bradbury, G. C. Stanley, *J. Am. Chem. Soc.* **1986**, *108*, 7430.

[152] P. M. Stricklen, E. J. Volcko, J. G. Verkade, *J. Am. Chem. Soc.* **1983**, *105*, 2494.

[153] a) M. Fujita, K. Ogura, *Coord. Chem. Rev.* **1996**, *148*, 249; b) S. Leininger, B. Olenyuk, P. J. Stang, *Chem. Rev.* **2000**, *100*, 853; c) F. A. Cotton, C. Lin, C. A. Murillo, *Acc. Chem. Res.* **2001**, *34*, 759; d) F. Würthner, C.-C. You, C. R. S.-M. öller, *Chem. Soc. Rev.* **2004**, *33*, 133; e) S. J. Dalgarno, N. P. Power, J. L. Atwood, *Coord. Chem. Rev.* **2008**, *252*, 825; f) B. H. Northrop, Y.-R. Zheng, K.-W. Chi, P. J. Stang, *Acc. Chem. Res.* **2009**, *42*, 1554.

[154] M. Yamanaka, N. Toyoda, K. Kobayashi, *J. Am. Chem. Soc.* **2009**, *131*, 9880.

[155] S. Sato, Y. Ishido, M. Fujita, *J. Am. Chem. Soc.* **2009**, *131*, 6064.

[156] R. V. Eldik, C. D. Hubbard, *Advances in Physical Organic Chemistry, Vol. 41*, Elsevier, Amsterdam, The Netherlands, **2006**.

[157] B. Olenyuk, M. D. Levin, J. A. Whiteford, J. E. Shield, P. J. Stang, *J. Am. Chem. Soc.* **1999**, *121*, 10434.

[158] a) S. B. Lee, J.-I. Hong, *Tetrahedron Lett.* **1998**, *39*, 4317; b) B. F. Abrahams, S. J. Egan, R. Robson, *J. Am. Chem. Soc.* **1999**, *121*, 3535.

[159] M. Fujita, M. Aoyagi, F. Ibukuro, K. Ogura, K. Yamaguchi, *J. Am. Chem. Soc.* **1998**, *120*, 611.

[160] B. Olenyuk, A. Fechtenkötter, P. J. Stang, *J. Chem. Soc. Dalton Trans.* **1998**, 1707.

[161] a) H.-B. Yang, K. Ghosh, B. H. Northrop, P. Stang, *Org. Lett.* **2007**, *9*, 1561; b) Y.-R. Zheng, H.-B. Yang, B. H. Northrop, K. Ghosh, P. Stang, *Inorg. Chem.* **2008**, *47*, 4706; c) B. H. Northrop, H.-B. Yang, P. Stang, *Inorg. Chem.* **2008**, *47*, 11257; d) Y.-R. Zheng, H.-B. Yang, K. Ghosh, L. Zhao, P. Stang, *Chemistry* **2009**, *15*, 7203; e) J.-M. Lehn *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 4769.

[162] M. Fujita, O. Sasaki, T. Mitsuhashi, T. Fujita, J. Yazaki, K. Yamaguchi, K. Ogura, *Chem. Commun.* **1996**, 1535.

[163] M. Fujita, D. Oguro, M. Miyazawa, H. Oka, K. Yamaguchi, K. Ogura, *Nature* **1995**, *378*, 469.

[164] G. R. Van Hecke, D. W. Horrocks, Jr., *Inorg. Chem.* **1966**, *5*, 1968.

[165] a) P. J. Stang, *J. Org. Chem.* **2009**, *74*, 2; b) B. H. Northrop, D. Chercka, P. J. Stang, *Tetrahedron* **2008**, *64*, 11495; c)K. Severin, *Coord. Chem. Rev.* **2003**, *245*, 3.

[166] M. Lofthagen, R. Chadha, J. S. Siegel, *J. Am. Chem. Soc.* **1991**, *113*, 8785.

[167] M. Fujita, K. Ogura, S. Nagao, *J. Am. Chem. Soc.* **1995**, *117*, 1649.

[168] K. Skopek, M. Hershberger, J. Gladysz, *Coord. Chem. Rev.* **2007**, *251*, 1723.

[169] G. H. Clever, S. Toshiro, M. Shionoya, *Angew. Chem.* **2009**, *121*, 7144.

[170] a) S. Kubik, *Chem. Soc. Rev.* **2009**, *38*, 585; b) J. W. Pflugrath, F. A. Quiocho, *J. Mol. Biol.* **1988**, *200*, 163; c) B. L. Jacobson, F. A. Quiocho, *J. Mol. Biol.* **1988**, *204*, 783.

[171] R. Custelcean, J. Bosano, P. V. Bonnesen, V. Kertesz, B. P. Hay, *Angew. Chem. Int. Ed.* **2009**, *48*, 4025.

[172] R. Custelcean, B. A. Moyer, B. P. Hay, *Chem. Commun.* **2005**, 5971.

[173] a) M. D. Pluth, D. W. Johnson, G. Szigethy, A. V. Davis, S. J. Teat, A. G. Oliver, R. G. Bergman, K. N. Raymond, *Inorg. Chem.* **2009**, *48*, 111; b) R. W. Saalfrank, B. Demleitner, H. Glaser, H. Maid, D. Bathelt, F. Hampel, W. Bauer, M. Teichert, *Chem. Eur. J.* **2002**, *8*, 2679; c) M. Albrecht, I. Janser, S. Meyer, P. Weis, R. Frohlich, *Chem. Commun.* **2003**, 2854; d) M. D. Ward, *Chem. Commun.* **2009**, 4487.

[174] a) P. Stang, J. A. Whiteford, **1994**, 3776; b) J. A. Whiteford, C. Lu, P. Stang, *J. Am. Chem. Soc.* **1997**, 2524; c) P. Jacopozzi, E. Dalcanale, *Angew. Chem. Int. Ed.* **1997**, *36*, 613; d) A. I, N. Chaouche, J. Forniés, C. Fortuño, A. Kribii, A. Martin, *Eur. J. Inorg. Chem.* **2005**, 3894.

[175] P. J. Stang, D. H. C. S. Saito, A. M. Arif, *J. Am. Chem. Soc.* **1995**, *117*, 6273.

[176] P. Stang, J. A. Whiteford, *Organometallics* **1994**, *13*, 3776.

[177] T. Weilandt, U. Kiehne, G. Schakenburg, A. Lützen, *Chem. Commun .* **2009**, 2320.

[178] R. Custelcean, P. Remy, P. V. Bonnesen, D. E. Jiang, B. A. Moyer, *Angew. Chem. Int. Ed.* **2008**, *47*, 1866.

[179] a) L. Pirondini, F. Bertolini, B. Cantadori, F. Ugozzoli, C. Massera, E. Dalcanale, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 4911; b) M. Yamanaka, Y. Yamada, Y. Sei, K. Yamaguchi, K. Kobayashi, *J. Am. Chem. Soc.* **2006**, *128*, 1531.

[180] a) E. Menozzi, M. Busi, R. Ramingo, M. Campagnolo, S. Geremia, E. Dalcanale, *Chem. Eur. J.* **2005**, *11*, 3136 ; b) M. Busi, B. Cantadori, F. Boccini, R. D. Zorzi, S. Geremia, E. Dalcanale, *Eur. J. Org. Chem.* **2011**, 2629.

[181] L. Baldini, M. Melegari, V. Bagnacani, A. Casnati, Enrico Dalcanale, F. Sansone, R. Ungaro, *J. Org. Chem.* **2011**, *76*, 3720.

[182] D. Zuccaccia, L. Pirondini, R. Pinalli, E. Dalcanale, A. Macchioni, *J. Am. Chem. Soc.* **2005**, *127*, 7025.

[183] N. Cuminetti, M. H. K. Ebbing, P. Prados, J. de Mendoza, E. Dalcanale, *Tetrahedron Lett.* **2001**, *42*, 527.

[184] S. J. Park, J.-I. Hong, *Chem. Commun.* **2001**, 1554.

[185] S. A. Levi, P. Guatteri, F. C. J. M. v. Veggel, G. J. Vancso, E. Dalcanale, D. N. Reinhoudt, *Angew. Chem. Int. Ed.* **2001**, *40*, 1892.

[186] M. Yamanaka, Y. Yamada, Y. Sei, K. Yamaguchi, K. Kobayashi, *J. Am. Chem. Soc.* **2006**, *128*, 1531.

[187] F. Gruppi, F. Boccini, L. Elviri, E. Dalcanale, *Tetrahedron* **2009**, *65*, 7289.

[188] a) C. Reyheller, PhD thesis, Technische Universität Kaiserslautern **2007**; b) Z. Rodríguez-Docampo, PhD thesis, University of Cambridge **2009**.

[189] T. Fiehn, PhD thesis, Technische Universität Kaiserslautern **2011**.

[190] S. Kubik, R. Goddard, R. Kirchner, D. Nolting, J. Seidel, *Angew. Chem. Int. Ed.* **2001**, *40*, 2648.

[191] S. Kubik, R. Kirchner, D. Nolting, J. Seidel, *J. Am. Chem. Soc.* **2002**, *124*, 12752.

[192] a) S. Kubik, R. Goddard, *J. Org. Chem.* **1999**, *64*, 9475; b) S. Kubik, R. Goddard, *Eur. J. Inorg. Chem.* **2001**, 311; c)E. R. Blout, *Biopolymers* **1981**, *20*, 1901.

[193] S. Kubik, R. Goddard, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 5127.

[194] a) C. Reyheller, B. P. Hay, S. Kubik, *New J. Chem.* **2007**, 2095; b) B. P. Hay, T. K. Firmann, *Inorg. Chem.* **2002**, *41*, 5502; c) V. S. Bryantsev, B. P. Hay, *J. Am. Chem. Soc.* **2006**, *128*, 2035.

[195] C. Reyheller, S. Kubik, *Org. Lett.* **2007**, *9*, 5271.

[196] S. Otto, *Dalton Trans.* **2006**, 2861.

[197] Z. Rodriguez-Docampo, PhD thesis, Fitzwilliam College (Cambridge), **2009**.

[198] Z. Rodriguez-Docampo, E. Eugenieva-Ilieva, C. Reyheller, A. M. Belenguer, S. Kubik, S. Otto, *Chem. Commun.* **2011**, *47*, 9798.

[199] S. Stüwe, PhD thesis, Technische Universität Kaiserslautern **2007**.

[200] S. Otto, S. Kubik, *J. Am. Chem. Soc.* **2002**, *124*, 12752.

[201] T. Tsunoda, K. Uemoto, C. Nagino, M. Kawamura, H. Kaku, S. Ito, *Tetrahedron Lett.* **1999**, *40*, 7355.

[202] Y. Chi, S. H. Gellman, *Org. Lett.* **2005**, *7*, 4253.

[203] T. Kondo, T. Nekado, I. Sugimoto, K. Ochi, S. Takai, A. Kinoshita, Y. Tajima, S. Yamamoto, K. Kawabata, H. Nakai, M. Toda, *Bioorg. Med. Chem.* **2007**, *15*, 2631.

[204] T. R. Webb, C. J. Eigenbrot, *Org. Chem.* **1991**, *56*, 3009.

[205] C. Heindl, H. Hübner, P. Gmeiner, *Tetrahedron Asymm.* **2003**, *14*, 3141.

[206] E. M. Smith, G. F. Swiss, B. R. Neustadt, E. H. Gold, J. A. Sommer, A. D. Brown, P. J. S. Chiu, R. Moran, E. J. Sybertz, T. Baum, *J. Med. Chem.* **1988**, *31*, 875.

[207] a) B. P. Gangamani, V. A. Kumar, K. N. Ganesh, *Tetrahedron* **1996**, *52*, 15017; b) M. Tamaki, G. Han, V. J. Hruby, *J. Org. Chem.* **2001**, *66*, 1038.

[208] B. R. Huck, S. H. Gellman, *J. Org. Chem.* **2005**, *70*, 3353.

[209] a) A. Janecka, T. Janecki, S. Shan, C. Bowers, K. Folkers, *J. Med. Chem.* **1994**, *37*, 2238; b) C. Reyheller, Diplom thesis, Heinrich- Heine Universität (Düsseldorf), **2002**.

[210] a) L.Malatesta, M. Anggoletta, *J. Chem. Soc.* **1957**, 1186; b) D. R. Coulson, L.C.Satek, S.O.Grim, *Inorg. Synth.* **2007**, *13*, 121.

[211] K. Ziegler, *Ber. Dtsch. Chem. Ges.* **1934**, *67A*, 139

[212] W. W. Cleland, *Biochemistry* **1964**, *3*, 480.

[213] F. W. Küster, A. Thiel, *Rechentafel für die Chemische Analytik, 102 Auflage*, de Gruyter, Berlin, **1982**.

[214] T. Becherer, D. Meshcheryakov, A. Springer, V. Böhmer, C. A. Schalley, *J. Mass. Spectrom.* **2009**, *44*, 1338.

[215] J. W. Pflugrath, F. A. Quiocho, *Nature* **1985**, *314*.

[216] L. Wang, D. L. Smith, *Analyt. Biochem.* **2003**, *314*, 46.

[217] Y. Bai, J. S. Milne, S. W. Englander, *Proteins: Struct. Funct. Genet.* **1993**, *17*, 75.

[218] L. G. Laajok, E. Le Breton, G. K. Shooter, J. C. Wallace, G. L. Francis, J. A. Carver, M. A. Kenery, *FEBS Lett.* **1997**, *420*.

[219] D. M. LeMaster, J. S. Anderson, G. Hernández, *Magn. Reson. Chem.* **2007**, *45*, 601.

[220] a) R. Wyler, J. d. Mendoza, J. J. Rebek, *Angew. Chem. Int. Ed.* **1993**, *32*, 1699; b) N. Branda, R. Wyler, J. R. Jr., *Science* **1994**, *263*, 1267; c) R. S. Meissner, J. J. Rebek, J. de Mendoza, *Science* **1995**, *270*, 1485.

[221] P. E. Hansen, *Magn. Reson. Chem.* **2000**, *38*, 10.

[222] a) J. K. M. Sanders, B. K. Hunter, C. J. Jameson, G. Romeo, *Chem. Phys. Lett.* **1988**, *143*, 471; b) P. E. Hansen, A. Lycka, *Acta Chem. Scand.* **1989**, *43*, 222; c) P. E. Hansen, A. E. Hansen, A. Lycka, A. Buvari-Barcza, *Acta Chem. Scand.* **1993**, *47*, 777.

[223] a) S. Schneider, M. Čuma, *J. Am. Chem. Soc.* **1996**, *118*, 1511; b) A. Lycka, P. E. Hansen, *Org. Magn. Reson.* **1984**, *22*, 569; c) P. E. Hansen, *Progress in NMR spectroscopy*, Pergamon, **1988**; d) A. Engdahl, B. Nelander, *J. Chem. Phys.* **1987**, *86*.

[224] M. Munch, A. E. Hansen, T. D. Bouman, *Acc. Chem. Res.* **1992**, *46*, 1065.

[225] D. D. Perrin, W. L. F. Armarego, D. R. Perrin, *Purification of laboratory chemicals* 2nd Ed. ed., Pergamon Press, Oxford **1980**

[226] a) T. Yvernault, G. Yvernault, J. C. Bollinger, *C. R. Acad. Sci. Paris* **1978**, *287*, 519; b) J. Coste, E. Frérot, P. Jouin, *J. Org. Chem.* **1994**, *59*, 2437

[227] O. Popovych, *Sci. Commun.* **1966**, *38*, 117.

[228] T. R. Kelly, F. Lang, *J. Org. Chem.* **1996**, *61*, 4623.

[229] S. Mitsumori, H. Zhang, P. H.-Y. Cheong, K. N. Houk, F. Tanaka, C. F. Barbas, *J. Am. Chem. Soc.* **2006**, *128*, 1040.

LEBENSLAUF

PERSÖNLICHE DATEN

Name	Eugenia Petkova Eugenieva-Ilieva

HOCHSCHULBILDUNG

01.2011-10.2013	Elternzeit
01.2007-12.2010	Promotion in Chemie • Technische Universität (TU) Kaiserslautern, Deutschland • Arbeitskreis von Prof. Dr. S. Kubik • Thema der Dissertation: „Molecular containers for anions based on triply-linked bis(cyclopeptides)"
10.2003-03.2005	Master in Medizinischer Chemie • Universität St. „Kliment Ohridski", Sofia, Bulgarien • Arbeitskreis von Prof. M. Palamareva (Betreuer Assist. Prof. M. Kandinska) • Thema: „Synthese und Stereochemie eines neuen Typs von substituierten Tetrahydroisochinolinen mit einer Peptidbindung in Position 4".
10.1998-10.2003	Bachelor in Anorganischen Chemie • Universität St. „Kliment Ohridski" Sofia, Bulgarien

SCHULISCHE LAUFBAHN

09.1993 – 05.1998	Erwerb der Hochschulreife (Abitur) • Mathematik und Naturwissenschaften Gymnasium St. „Kliment Ohridski", Montana, Bulgarien • Hauptfächer:: Mathematik und Informatik
09.1986 – 06.1993	Erwerb des Grundschulabschlusses • „Petar Beron" Grundschule, Montana, Bulgarien

BERUFSERFAHRUNG

2008 – 2010	Assistent für organische Praktika, Betreuung eines Auszubildenden zum Chemielaboranten, Betreuung einer DAAD Austauschstudentin im AK Kubik.
04.2005 – 12.2006	Wissenschaftliche Mitarbeiter bei BAS, Sofia, Bulgarien • Bulgarische Akademie der Wissenschaften, Institut für Molekularbiologie, Abteilung "Molekulares Design und biochemische Pharmakologie" (BAS) • Arbeitskreis von Prof. E. Golovinsky • Thema: „Synthese von neuen 1N-substituierten Uracil Derivaten".
08.2003	Werkstudent bei „Monbat Plc.", Montana, Bulgarien (zwei Monate) • Laborarbeiten in der analytischen Abteilung

STIPENDIEN

11.2007 – 11.2010	E.U. „Marie Curie“ Research Training Network zur Förderung von Wissenschaftlern (MRTN-CT-2006-035614)
07.2009 – 08.2009	Stipendium der “European Cooperation in Science and Technology” (COST) für den Aufenthalt an der University of Cambridge, Groβbritanien

AUSLANDSAUFENTHALTE

06.2009	Forschungsaufenthalt in Groningen (eine Woche) • Groningen Universität, Groningen, Niederlande • Arbeitskreis von Prof. S. Otto • Ziel des Aufenthalts: „Charakterisierung dynamischer kombinatorischer Bibliotheken“
04.2009	Weiterbildung in Systems Chemistry (eine Woche) • Rijksuniversiteit Groningen, Groningen, Niederlande • Teilnahme an Systems Chemistry Workshop
10.2008 – 11.2008	Forschungsaufenthalt in Groβbritanien (zwei Wochen) • University of Cambridge, Groβbritanien • Arbeitskreis von Prof. S. Otto • Forschungsprojekt: „Entwicklung einer Strategie zur Isolierung und Aufreinigung eines Bis(cyclopeptid) Rezeptors“
06.2001- 05.2002	Au-Pair, Köln, Deutschland • bei Familie Kreitner

SONSTIGE

Sprachen	• Englisch (sehr gut in Wort und Schrift) • Deutsch (sehr gut in Wort und Schrift) • Russisch (Grundkenntnisse)
EDV Kenntnisse	• Microsoft Office • Excel • Power Point
Hobbies	• Arbeit mit Kindern • Schwimmen und Kosmetik • Theater und Filme
Sonstige	• Führerschein (Kategorie B)

Kaiserslautern, Oktober 2013