Ammonium Ion Recognition:

Luminescent Amino Acid and Peptide Receptors

Modification of Amino Acids:

Artificial Amino Acids, Cyclopeptides and Guanidinium-bis-carboxylates

Dissertation

Zur Erlangung des Doktorgrades der Naturwissenschaften
(Dr. rer. nat.)
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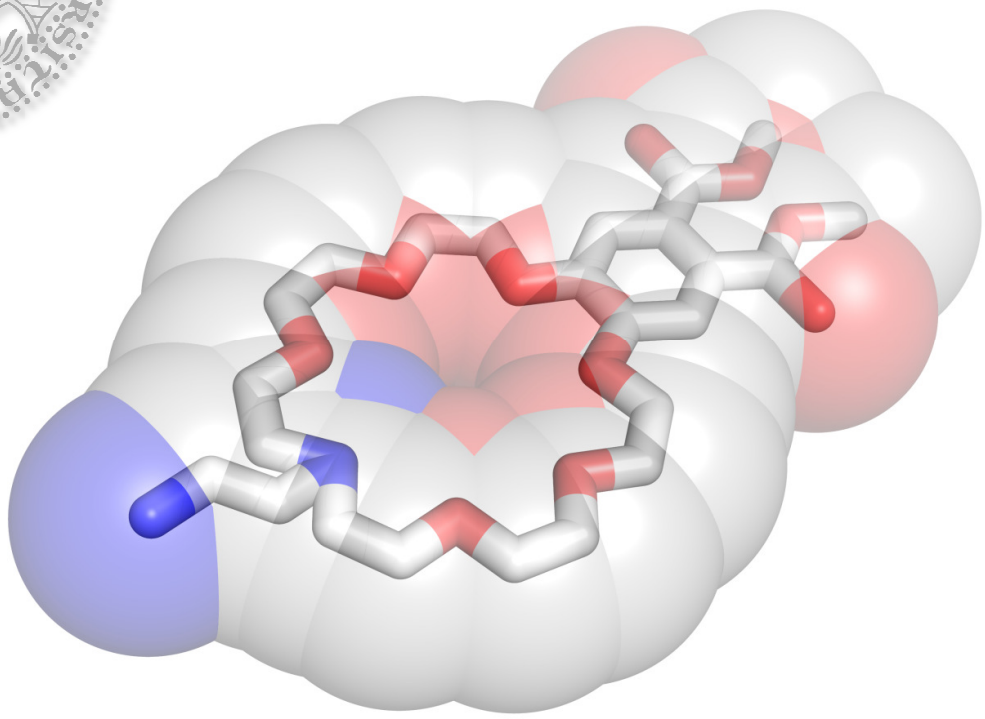

vorgelegt von

Andreas Späth

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The experimental part of this work was carried out between October 2005 and October 2009 at the Institute for Organic Chemistry, University of Regensburg, Germany and the Shanghai Institute for Organic Chemistry, Shanghai, P. R. China under the supervision of *Prof. Dr. B. König*.

The PhD – thesis was submitted on:		26. February 2010
The colloquium took place on:		24. March 2010
Board of Examiners:	Prof. Dr. J. Daub	(Chairman)
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	Prof. Dr. H. A. Wagenknecht	(2nd Referee)
	Prof. Dr. H. Brunner	(Examiner)

Für

Meine Eltern, Meinen Bruder

&

Marlene

Willst du dich am Ganzen erquicken,

so musst du das Ganze im Kleinsten erblicken.

(Johann Wolfgang von Goethe)

To live is the rarest thing in the world.

Most people exist, that is all.

(Oscar Wilde)

Danksagung

Mein besonderer Dank gilt Herrn Prof. Dr. B. König für die Überlassung des überaus interessanten und vielseitigen Themas, sowie für die Förderung und die mit Anregungen und Diskussionen verbundene Unterstützung dieser Arbeit.

Ich bedanke mich bei bei Prof. Dr. Dawei Ma für die Möglichkeit eines zweimonatigen Aufenthaltes am Shanghai Institute of Organic Chemistry (SIOC) in China. Ein besonderer Dank gilt allen Mitgliedern der Arbeitsgruppe für die gute Zusammenarbeit in jederzeit freundschaftlicher Arbeitsatmosphäre, vor allem Dr. Sascha Breeger für seine Unterstützung,

Den Mitarbeitern der Zentralen Analytik der Fakultät für Chemie und Pharmazie danke ich für die schnelle und gewissenhafte Durchführung der analytischen Messungen. Insbesondere Herrn Dr. T. Burgemeister, Herrn F. Kastner, Frau N. Pustet, Frau A. Schramm und Frau G. Stühler für die Aufnahme der NMR-Spektren, ebenso wie Herrn Dr. K. K. Mayer, Herrn J. Kiermaier und Herrn W. Söllner für die Messung und Auswertung der Massenspektren und Herrn Dr. Zabel und Fr. Stempfhuber für alle durchgeführten Kristallstrukturanalysen.

Des Weiteren danke ich den Arbeitskreisen von Prof. Dr. O. Reiser für die gute Zusammenarbeit und die Möglichkeit der Benutzung des IR-Spektrometers und von Prof. Dr. A. Scheer für die Benutzung der Glovebox.

Herrn Dr. W. Braig, Frau Dr. C. Braig, Frau E. Liebl, Frau B. Badziura, Frau R. Hoheisel, Frau S. Strauß, Herrn E. Lautenschlager, Frau S. Graetz, Frau S. Schulze und Herrn Dr. R. Vasold danke ich für ihre Unterstützung.

Dr. Anna Berlicka, Dr. Tatjana Mitkina, Dr. Evgeny Katayev, Alexandra Bila und Patrina Pellet danke ich für Anregungen und einige gute Hinweise, wodurch mir das Erstellen des umfangreichen Reviews zum Thema Ammoniumionenerkennung erleichtert wurde.

Ich bedanke mich bei Robert Lechner, Dr. Anna Berlicka, Dr. Evgeny Katayev, Peter Raster und Stefan Weiss für das Korrekturlesen der Kapitel meiner Dissertation.

Allen Mitarbeitern und allen ehemaligen Kollegen am Lehrstuhl danke ich für die gute Zusammenarbeit und das angenehme Arbeitsklima – vor und nach Feierabend. Besonderen Dank an:

Karin Lehner für die vielen fantastische Gaumenfreuden, so manchen kulinarischen Ausflug, die schönen gemeinsamen Erlebnisse und die gemütlichen Abende.

Stefan Weiss und Peter Raster für viele interessante Diskussionen „all around chemistry“ und über allerelei erheiternde, völlig fachfremde Themen, sowie für viele gemeinsame Unternehmungen.

Der Karategruppe des Reebok Centers Regensburg und Markus Gierl für die schöne Zeit während des absolut notwendigen sportlichen Ausgleichs.

Dr. Andreas Grauer für die schöne gemeinsame Zeit in China und Münster, die vielen lustigen Abende, sowie einige interessante Gespräche und Diskussionen.

Dr. Jiri Svoboda für den jederzeit interessanten Austausch über Heavy Metal Musik, die schöne gemeinsame Zeit am Lehrstuhl und einen tollen Therion-Konzertabend in Prag, sowie bei Dr. Jehns Geduhn für unzählige spannende Impressionen aus Jazz, Rock und Percussionmusik. Rock on!

Meinen Laborkollegen Michael Dobmeier, Dr. Jens Geduhn, Dr. Maity Prantik, Christian Ochoa Puentes, Susanna Schmidbauer und Dr. Anna Berlicka für die gute Zusammenarbeit und angenehme, freundliche Atmosphäre im Labor.

Meinen Forschungspraktikanten Christoph Schwarzmeier, Dominik Meinel, Matthias Zwick, Markus Daerr, Eva-Maria Rummel, Nicole Berner, Malte Hansen, Sabine Eiblinger und ganz besonders Allison Cheung und meinen beiden Bachelorstudentinnen Carina Koch und Nina Gonschor danke ich für ihr Engagement.

Für finanzielle Unterstützung gilt mein Dank dem Graduiertenkolleg Photoreceptors (GRK 640) für die Vergabe von Reisekostenmittel im Rahmen der Konferenzen in Dublin und Kiew, sowie für die Finanzierung meines Fluges nach China im Rahmen meines Forschungsaufenthaltes am Shanghai Institute for Organic Chemistry. Für kurzzeitige

finanzielle Förderung am Ende meiner Doktorarbeit danke ich der deutschen Bundesstiftung Umwelt, sowie dem Graduiertenkolleg Photoreceptors und vor allem Herrn Prof. Dr. B. Dick. Ich danke dem Asia Link Medicinal Chemistry für die Möglichkeit der Teilnahme an der Summerschool Medicinical Chemistry 2007 in Saigon, sowie für die größzügige Vergabe eines Reisekosten Stipendiums im Rahmen des „Asia-Link“-Programms.

Marlene Ernst danke ich für ihre Liebe, ihre Unterstützung und Geduld und für die unzähligen Momente, in denen du mir auf deine wunderbare Art und Weise gezeigt hast, dass es auch noch etwas anderes gibt als Chemie.

Zuletzt, aber vor allem und ganz besonders, danke ich meiner Familie für ihre großartige Unterstützung und den Rückhalt während der Zeit der Promotion.

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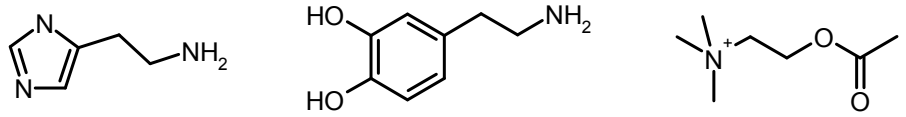
I. Binding Organic Ammonium Ions in Solution using Synthetic Receptors[i]

I. 1. Introduction

The amino group is one of the most important functional groups in molecules of biological relevance. Examples of physiologically active amines are histamine (**1**), dopamine (**2**) or quaternary ammonium ions, such as acetylcholine (**3**). Amino acids have amino groups, as peptides and proteins. Under physiological conditions the amino group is usually protonated as an ammonium ion.



1 **2** **3**

Figure 1: Biologically important amines and quaternary ammonium salts: histamine (**1**), dopamine (**2**) and acetylcholine (**3**)

The interaction of small ammonium-ion bearing compounds with protein receptors is important for biological signal transduction processes. Like in all biological regulatory processes, selectivity of recognition is of key importance for subsequent steps and cellular response. An example is the binding of histamine (**1**) to the human H_1 receptor, which results in lower blood pressure and dilatation of blood vessels or plays a primary role for allergic response. [1] Also inhibition of biological processes is addressed by molecular recognition involving amino acids and peptides: The antibiotic vancomycin binds selectively with its terminal lysyl-*R*-alanyl-*R*-alanine residues in bacterial cells through several hydrogen bonds.[2] Once it has bound to these particular peptides they are unavailable for construction of the bacteria's cell wall causing their cell death.

[i] A. Späth, B. König, *Beilstein J. Org. Chem.* **2010**, *6*, No. 32.

Malfunction of dopamine-responsive neurons has been implicated in a number of disease states including Parkinson's disease.[3] The understanding of alkylammonium recognition in the dopamine (**2**) class of neurotransmitters is central for the development of tools to study these systems. The investigation of ammonium ion recognition is therefore of considerable fundamental and practical interest.[4,70]

Selective ligand-protein receptor binding relies typically on a portfolio of many specific interactions between two or more molecules. For the recognition of ammonium-ions three interactions, mostly acting simultaneous, are typically the most important:

1) Hydrogen bonds[5]

Hydrogen bonds are formed from the strongly polarized N^+-H bonds to a free electron pair of an electronegative atom (O, N, F). Crystal structures show mainly a linear arrangement of the three atoms, but bifurcated hydrogen bonds are observed, too.[6] If exposed to a competing solvent, a single hydrogen bond cannot contribute much binding energy. Gas phase energies range from 22 kJ/mol (neutral hydrogen bonds between water molecules) up to 163 KJ/mol (anionic F-H-F^- complex).[7] Quaternary ammonium ions cannot be bound by hydrogen bonds.

2) Cation-π-interaction[8]

First experimental evidence of interactions between cations and aromatic π-systems came from Kebarle et al., who showed that binding of potassium ions to benzene and water in the gas phase is of similar energy.[6, 9] Ammonium-π-interactions were investigated in detail experimentally and by *ab initio* calculations and are mainly based on electrostatic interactions. The binding energies are between 42 and 92 KJ/mol in the gas phase. The cation-π–bond is an important motif for the recognition of quaternary ammonium ions. A relevant example is the binding of acetylcholine (**3**) in biological systems.[10]

3) Ion pairs and salt bridges

Coulomb interaction attracts cations and anions. In salt bridges, additional hydrogen bonds are formed.[11] A typical example of a salt bridge is the ammonium-ion carboxylate ion pair. The strength of cation – anion affinity depends on the distance, the polarity of the solvent and the ionic strength. Extrapolated to an ionic strength of zero, most coulomb interactions level at a value of about 8 KJ/mol.[12] The binding energy is mainly independent on geometry, polarizability of the ions or formation of a salt bridge.

In addition the selective recognition of ammonium ions depends on steric and molecular complementarity and the pre-organization[13] of interacting functional groups. Already in 1890 Fischer suggested that enzyme-substrate interactions are working like a "lock and key" of a pre-empty host and a guest exhibiting molecular complementarity.[14]

Until today, studies of non-covalent interactions mainly by artificial model structures and receptors led to a far better understanding of many biological processes. In return they are often the inspiration for supramolecular research, including self-assembly, mechanically-interlocked molecular architectures and molecular recognition in host-guest chemistry.[15] Analogous to biological systems, the formation and function of such supramolecular complexes occurs through a multiplicity of often difficult to differentiate non-covalent forces: Di- or polytopic receptors are used to further enhance the binding and selectivity with a binding mechanism that can be understood on the combined efforts of several non-covalent interactions such as hydrogen bonding, electrostatic interactions, hydrophobic interactions[16], cation–π interactions, π–π staking interactions[17] and steric complementarity.[18] The crucial interaction mechanisms have been comprehensively summarized;[19] basic rules for receptors and design have been outlined.[20]

As found in nature, molecular recognition can be either static, a complexation reaction with defined stoichiometry between a specific host and guest, or dynamic, where the binding of the first guest to the first binding site of a receptor affects the association constant of a second guest with the second binding site. Either positive allosteric binding - the first guest increases the association constant of the second guest - or negative allosteric binding - the first guest decreases the association constant with the second - can occur.[21] Obviously, a most successful synthetic receptor concept should feature positive allostery or cooperativity.[22] In most cases the host forms a cavity in which molecules of the guest are complexed as „key“ in the complementary binding site or an inclusion compound. This host pre-organization is leading to major enhancement to overall energy of guest complexation. The binding is energetically favoured: Both enthalpic - a less solvent accessible area leads to a less strongly solvated guest and fewer solvent-ligand bonds that have to be broken - as well as entropic - macrocycles[23] or cavities[24] being less conformationally flexible so losing fewer degrees of freedom upon complexation, due to the reorganization energy being already paid in advance in the synthesis. In a few examples, guest molecules are enclosed on all sides by the receptor being ‘trapped’ as in a cage forming clathrates.[25] Binding of the amino group to a planar surface of the receptor is found in metal complexes or metalla-porphyrins. The molecular environment and the solvent determine the stability of the assembly: Competitive solvents building strong

hydrogen bonds or having electrostatic and charge-transfer capabilities interfere with the ammonium ion binding and may even inhibit the complex formation completely. Especially recognition in water is a challenging topic with growing interest. New efforts in a general view have been recently summarized.[26]

Many types of synthetic ammonium ion receptors are available, reaching from crown ethers, calixarenes, porphyrins, cucurbiturils, cyclodextrins and cyclopeptides to tweezer ligands, sterically geared tripods and several types of metal complexes. The most important methods used for evaluating ammonium ion binding processes are direct absorption and emission measurements utilizing chromophors in the receptor or analyt molecule, displacement assays with suitable dyes, NMR titration experiments, isothermal titration calorimetry and transport through an organic phase monitored by HPLC, NMR[27] or UV-vis absorption.[28]

I. 2. Scope and Limitations of this Survey

Synthetic receptors for ammonium ions may help to understand the individual contributions of the different forces involved in ammonium ion binding better. In addition, they are valuable tools as chemosensors for the analytical detection of drugs or biogenic amines. Most of them possess a chiral structure. Enantiomeric recognition is an essential process in living organisms frequently involving ammonium ion compounds, especially in enzyme-substrate interactions,[29] as well as in artificial systems e.g. for separation science[30] and the design of enzyme mimetics.[31]

In this review, we discuss the different structures of ammonium-ion receptors using typical examples from the recent literature. Where available, examples of enantioselective recognition of chiral ammonium ion guests will be covered. The recognition of guanidinium ions and metal cations[32] is not included. Ion pair recognition will be only discussed briefly if relevant for ammonium recognition purposes. A comprehensive review on this topic has been published by Sessler et al..[33] We discuss the substance classes that were mostly used in ammonium ion recognition: crown ethers, calixarenes[34], cyclodextrins[35,36], cucurbiturils, porphyrins, phosphonate based receptors, tripodal receptors, tweezer ligands, clefts, cyclopeptides and metal complexes. We have not included rotaxanes,[37,38] catenanes,[37,39] spherands,[40] cryptophanes,[41] as well as switching devices[42], self assembly systems[43] or carcerands,[44,45] as these structures are less frequently used for ammonium ion binding or their

binding is based on similar interactions as in the previous receptor classes. Comprehensive information on the recognition properties of the compounds is available in the cited literature. We will start every chapter with a short discussion of fundamental properties like selectivity and complementarity. Beginning with structurally simple examples we will increase complexity to higher substituted moieties and combinations of recognition sites to ditopic or oligomeric receptor types of the class. Synthetic receptors bearing binding sites from different compound classes are classified by their amine recognition moiety.

We present selected results covering complexation, solvent extraction and transport of ammonium ions in solution, thus excluding polymer[46] or other solid phase[47] materials and gas phase measurements, without attempting to cover all available references. Representative molecules for application in ion selective electrodes (ISE)[48] are briefly discussed. The scope of the review unfortunately cannot cover the topic of artificial ammonium-ion receptors comprehensively. It is rather the intention to illustrate the scope and the limitations of a binding motif using typical examples.

I. 3. Crown Ethers

This chapter discusses recent reports on ammonium ion recognition using crown ethers and their derivatives. First the properties of the substance class is illustrated by simple examples followed by more complex crown ethers and related systems. The next part discusses molecules capable to differentiate enantiomeric ammonium ions, followed by receptors for diammonium ions, such as ditopic crown ether compounds. Finally we discuss the simultaneous recognition of ammonium ions and a second functional group as for example in amino acids.

I. 3.1. Ammonium Ion Binding by Simple Crown Ethers

Already in his first publication, Petersen,[49] who discovered the compound class and later received the Nobel Prize for it, mentioned the use of crown ethers for the recognition of ammonium-ions.[50] Later, Cram[51] and co-workers concluded after extensive studies on *tert*-butylammonium thiosulfate and different crown ethers, that two factors are important to achieve high binding constants:[52] The principle of complementary binding sites must be fulfilled. Receptor and guest binding sites should be in close proximity, of complementary geometry and fit without generating steric strain. Secondly, receptors which are suitably pre-organized for guest binding will lead to the more stable complex. Crown ether ammonium-ion binding occurs by hydrogen bonding between oxygen atoms (or nitrogen, sulphur or other free electron pair in hetero crown ethers) and N^+-H bonds.[53] The cyclic arrangement leads to a pre-organization of the host,[54] whereby selectivity is determined by the ring size. Primary ammonium-ions are complexed with highest affinity by 18-crown-6 derivatives.[6]

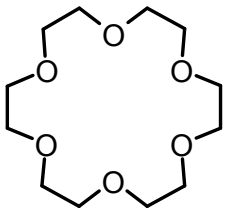


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Figure 2: Crown ether 18-crown-6.

Table 1 exemplarily summarizes the affinity of benzylammonium chloride and 18-crown-6 in several solvents for comparison with other examples of this review. The given data were determined by isothermal titration calorimetry.[6]

Solvent	**log K**
Water	1.44
Methanol	4.22; 4.43*
Isopropanol	4.14
n-Octanol	3.25
Dimethylformamide	2.50
Dimethylsulfoxide	1.34

* Determined by ion-selective electrode

Table 1: Binding constants of 18-crown-6 and benzyl ammonium chloride in several solvents

These data show that crown ethers bind ammonium ions in different solvents which compete for hydrogen bonds, such as dimethylsulfoxide, a very good hydrogen bond acceptor, and water, which is a poorer hydrogen bond acceptor than methanol, but very good hydrogen bond donor. The more competitive the solvent behaves the lower is the observed binding constant. Additionally the binding ability is strongly affected by the polarity of the solvent.[55] The conformation of crown ethers in non-polar organic solvents reflects a "droplet of water in oil" with the lone pairs pointing to its interior in advantageous manner for ion coordination (fig. 3). In water, or generally speaking hydrophilic media, the lone pairs are oriented to the exterior. Upon guest coordination the crown ether has to be reorganized, which is energetically less favourable. Therefore, highest affinities for polar solvents are observed in methanol, in chloroform the values are even higher.[56]

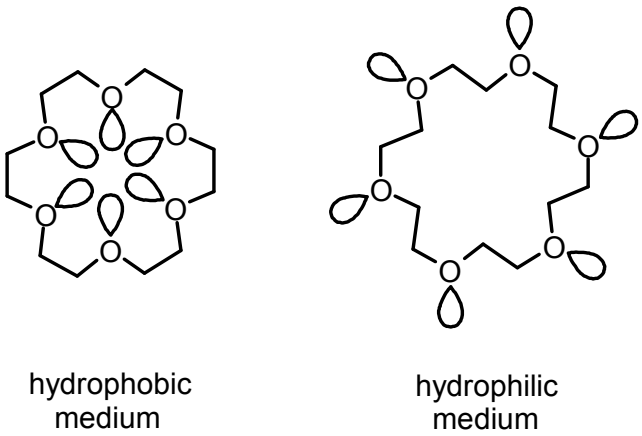

Figure 3: Conformations of 18-crown-6 (**4**) in solvents of different polarity

Table 2 shows the effect of the crown ethers size and constitution on the binding constant in methanol. The data were determined using an ion-selective electrode.

Crown ether	Cavity size	Guest	log K
12-Crown-4	120 - 150 pm	$BnNH_3Cl$	0.80
15-Crown-5	170 - 220 pm	$BnNH_3Cl$	2.74
18-Crown-6	260 - 320 pm	$BnNH_3Cl$	4.43

Table 2: Binding constants of three crown ethers to benzylammonium chloride in methanol.

Depending on the ratio of the crown ether ring size[55] and the diameter of the cation complex different 1:1 topologies are observed reflecting differently strong coordination and complex stability.[57,58]

Figure 4: Binding topologies of the ammonium ion depending on the crown ring size

The ionic diameter of an ammonium ions is 286 pm, very similar to potassium ions with 266 pm. Important to note is, that ammonium ions prefer a tetrahedral, potassium ions need an octahedral coordination for strong binding. By reducing the coordination points (see **7b**)[59] or changing the coordination sphere, the selectivity of a coronand system can be directed towards ammonium ion binding.

As mentioned, 18-crown-6 type structures show typically the highest ammonium ion affinity. Exceptions are secondary ammonium ions, which prefer larger crown ethers.[60] The ammonium ion slips through the crown ether ring forming "pseudorotaxane" like structures.

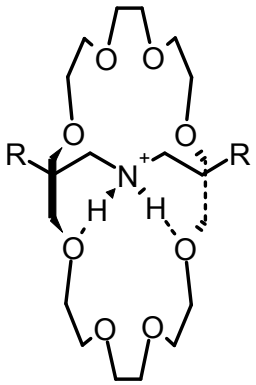

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Figure 5: A pseudorotaxane structure consisting of 24-crown-8 and a secondary ammonium-ion (**5**); R = Ph

The structural variability of crown ethers is very large. This allows varying the ring size, introducing substituents and changing the donor sites from oxygen atoms, to nitrogen atoms (aza crowns) or sulphur, phosphorus and arsenic. Crown ether oxygen atoms as the donor site prefer harder cations of main group elements as guests, while for crown ethers with sulphur atoms as the donor site the complexation of softer transition metals, e.g. Ag^{+}, Cu^{2+}, Hg^{2+}, is particularly suitable.[61]

Important heterocrowns are macrocycles like cyclens (**6**) and cyclams (**7**), which show excellent complexation properties towards transition metal ions.[62] Special classes of crown ethers are pyridino crowns (**9**), having one or more oxygen motifs replaced by pyridino moieties in the polyether chain, or azacrown ethers **8**, bearing a certain number of nitrogen atoms instead of oxygen in the macrocycle.

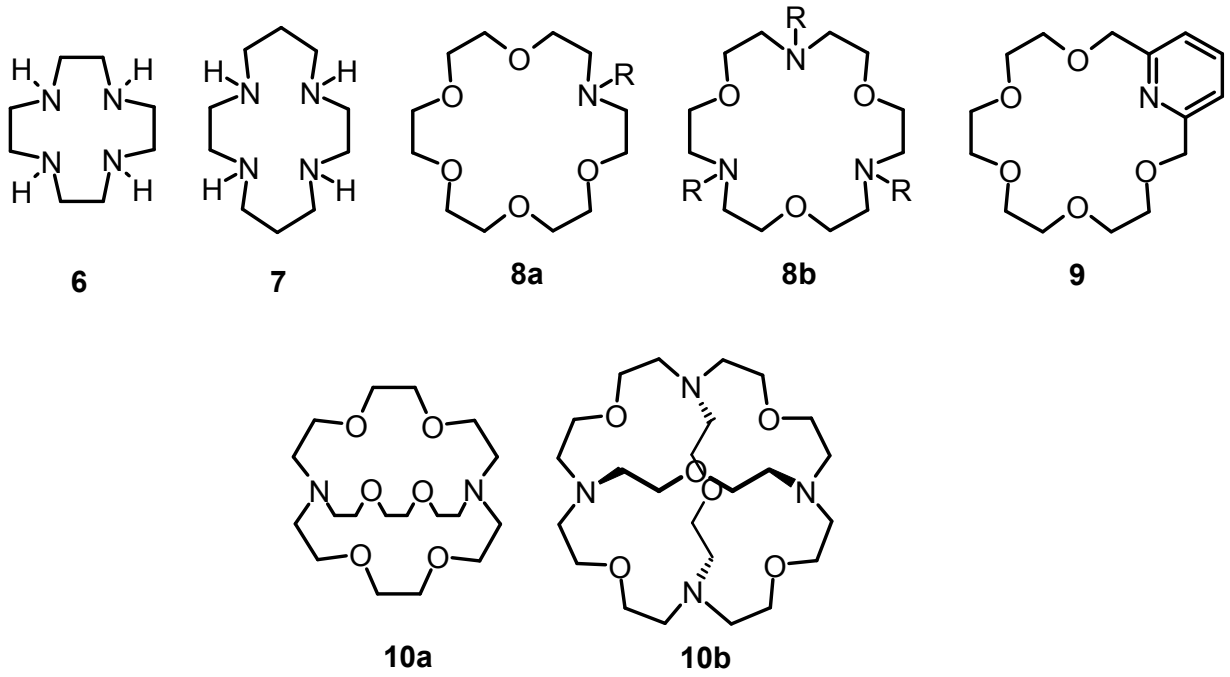


Figure 6: Typical examples of azacrown ethers, cryptands and related aza macrocycles.

A combination of both, triaza-crown ether with alternating nitrogen and oxygen atoms in the ring (**8b**), can be employed to enhance the selectivity for ammonium ions in comparison to potassium ions. It provides a sufficient number of binding sites for ammonium ions, but too less for potassium ions compared to 18-crown-6. The interaction is particularly advantageous when the number of complementary binding sites is maximal (**10b**).

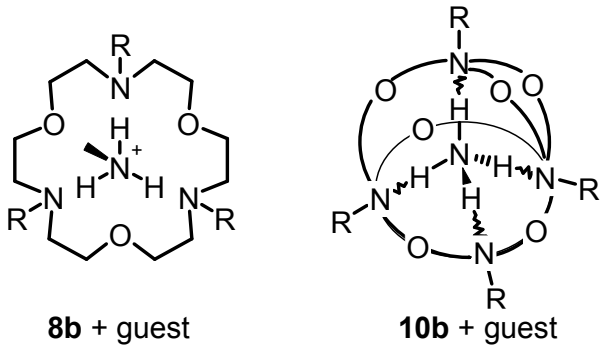


Figure 7: Binding of ammonium to azacrown ethers and cryptands[63]

Azacrown ethers with an additional side arm attached on the nitrogen of the macrocyclic ring may have, compared to the related parent crown ether, enhanced cation-binding. Crown ethers with linear or branched heteroatom-containing podand arms – depending on the connection point either *N*-pivot or *C*-pivot lariat ethers – exhibit increased guest specificity.[58,64] This

argument holds for polyether compounds with two podand arms, bibraccial lariat ethers. Bridging the ring with the arm leads to cryptands, bicyclic (**10a**) or polycyclic (**10b**) crown ethers.[65] If the moiety is „tricyclic closed“ via the two nitrogens the resulting cryptand **10a** is permitting a cation to be encapsulated.[66] Upon inclusion in the cavity of the cryptand, the guest is shielded by three or more polyether bridges. As a result of this encapsulation cryptands form more stable complexes than coronands ($K_a = 10^6$ for NH_4^+ in methanol at 25°C). Solution thermodynamics of amino acids with **4** and **10a** in comparison underline the discussed facts and supply interesting views on their applicability.[67]

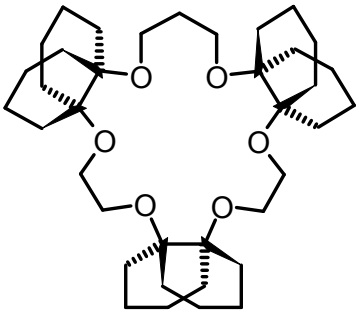
Macrotricyclic cryptand **10b** exhibits a substantial enhancement in ammonium vs. potassium selectivity in comparison to crown and azacrown ethers, as determined by calorimetric[56] and NMR studies.[68] The high selectivity over potassium ions has been attributed to the tetrahedral binding site geometry that favours complexation of the tetrahedral ammonium ion over that of the spherically symmetrical potassium ion, underlining the particular importance of hydrogen bonding and symmetry considerations in the design of ammonium ion recognition sites. Differences between these types of ligands also show up in the kinetics of complex formation. The conformationally rigid cryptands complex slower than coronands and these in turn are slower complexing than the flexible podands. In contrast to crown ethers, the three dimensional cryptands display peak selectivity in cation binding. The cavities are more rigid and unable to adapt to bind cations that are too small or too large for the cavity.

The large body of published work on crown ether synthesis[69] and crown ether ammonium ion binding[70] cannot be covered comprehensively in this review and we refer to recent overviews. Very recent publications of cryptands for ammonium ion recognition are rare. Crown ethers and azacrowns are widely used and we will therefore focus on these two moieties. An excellent review covering concepts, structure and ammonium ion binding of crown compounds is available. [71] For the highly dynamic motion of 18-crown-6 in complexation/decomplexation processes[72] and an interesting closer view on the binding of ammonium ions to 18-crown-6 and its competition with potassium ions[73] we refer to the articles of Schalley and Kimura.

In the following we discuss recent examples of ammonium ion binding compounds, which contain crown ether substructures, but are more complex in structure than the parent compounds.

I. 3.2. Ammonium Ion Binding by More Complex Crown Ethers

An ammonium ionophore with better sodium selectivity than the natural antibiotic nonactin was developed based on a 19-membered crown compound (**11**). Increased selectivity for ammonium ions over smaller and larger cations[74] was achieved by the introduction of decalino subunits. They prevent folding of the receptor to coordinate smaller cations and add bulkiness to block larger cations from entering the cavity. This compound was found to exhibit a high ammonium ion selectivity over K^+, similar to nonactin, and over Na^+ [log $K_{NH4+,\ K+}$ = -1.0 (nonactin –1.0), log $K_{NH4+,\ Na+}$ = -3.5 (nonactin –2.6)[75]] in an ion selective electrode (ISE). It revealed a nearly Nernstian response (58.1 mV/decade) in the range $5*10^{-6}$ – 10^{-1} M ammonium ion activity, reflecting a similar detection limit as nonactin.



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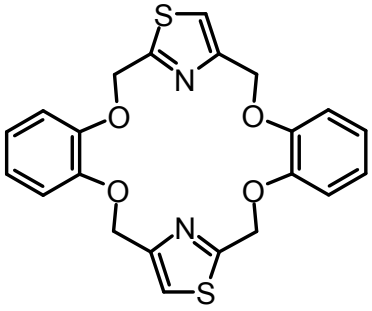


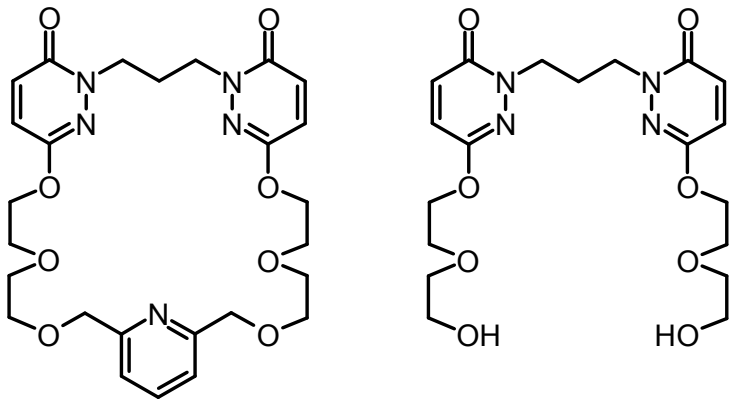
12

Figure 8: A 19-crown-6-ether with decalino blocking groups (**11**) and a thiazole-dibenzo-18-crown-6-ether (**12**)

Similarly, Kim et al. investigated the use of a thiazole containing dibenzo-18-crown-6 derivative (**12**) as ammonium ionophore in an ISE sensor and reported a strongly enhanced selectivity for ammonium ions over sodium ions and a slightly higher selectivity versus potassium ions in comparison to nonactin[76] [log $K_{NH4+,\ K+}$ = -1.3 (nonactin –1.0), log $K_{NH4+,\ Na+}$ = -3.9 (nonactin –2.6)[75]]. This ionophore exhibited a similar detection limit of ~ $3*10^{-6}$ M compared to nonactin ($1*10^{-6}$ M)[77] in an ISE sensor format. This design was primarily based on size-fit factors. In addition, the aromatic units increase rigidity and the thiazoles provide hydrogen bonding sites.

Campayo et al. presented acyclic compounds containing the 1,3-bis(6-oxopyridazin-1-yl)-propane and the corresponding heteroaromatic macrocycles containing pyridine units.[78] The cyclic receptor **13** is a most effective carrier of ammonium ions (ν = 57 μM h^{-1}) and exhibits

an excellent selectivity for NH_4^+ in relation to the three metal cations evaluated (NH_4^+/Na^+ = 9.2, NH_4^+/K^+ = 9.5, NH_4^+/Ca^{2+} = 11.8). The acyclic intermediate **14** shows efficient carrier properties for NH_4^+ ions and excellent selectivity in NH_4^+ transport in relation to K^+ (NH_4^+/K^+ = 73), which was almost seven times higher than that for nonactin.[75] An impressive selectivity in relation to Ca^{2+} (NH_4^+/Ca^{2+} = 146) was also observed. The formation of a pseudocavity by intramolecular hydrogen bonding in **14** and contribution to the binding of the host's oxyimino part were suggested by molecular modelling of the ammonium complex.



13 **14**

Figure 9: 1,3-Bis(6-oxopyridazin-1-yl)-propane derivatives **13** and **14** by Campayo *et al.*

In ammonium ions, which are substituted by organic residues, the substituent will influence the binding. The coordination of primary ammonium ions salts with varying steric demand was investigated. The sensing ability of fluorescently labelled 1,10-diaza-18-crown-6 (**16**) was compared to the analogous monoaza-18-crown-6 coumarin sensor (**15**).[79] The coordination experiments were pursued by fluorescence and ^{1}H-NMR spectroscopy in $CH_2Cl_2/CDCl_3/CD_3OD$ 90/9/1 vol/vol/vol %. According to the NMR titrations sensor **15** shows the highest affinity, two orders of magnitude above that of **16a** (table 3). The stoichiometry of the complexes with *n*-butylammonium perchlorate was established as 1:1 in all cases. For ammonium salts of increased steric demand the binding values generally decrease.

Perchlorate of	**Log K_{ass} (15)**	**Log K_{ass} (16a)**	**Log K_{ass} (16b)**
n-butylamine	6.0	3.5	4.5
tert-butylamine	4.6	2.8	4.5
neopentylamine	5.2	2.8	5.1

Table 3: Binding constants of **15** and **16**

The 18-crown-6 based PET sensors output was linked to the changes in the sensors' conformational dynamics on complexation. The fluorescence enhancements upon guest addition of the diaza compounds **16** (140- to 170-fold) were 3 to 4 times higher than that of the monoaza receptor **15** (only 40-fold increase). The changes in the conformational mobility of these sensors induced by guest binding have a profound effect on their signalling.

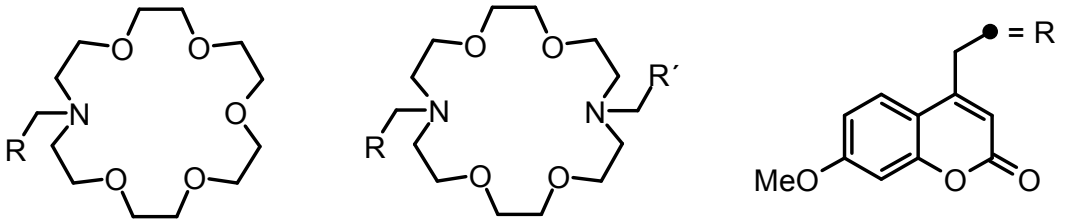


Figure 10: Fluorescent azacrown-PET-sensors based on coumarin

I. 3.3. Enantioselective Recognition of Chiral Ammonium Ions by Crown Ethers

Chiral ammonium salts are found in most biologically active molecules. The enantioselective discrimination of such molecules is of interest, as the biological properties of enantiomers may differ.[80] Since Cram et al. synthesized BINAP-crown ethers, which were the first enantioselective receptors for primary organoammonium salts[81] leading to a novel separation technique,[82] a great number of attempts have been made to distinguish chiral ammonium ions by chiral crown ethers.[83] Particularly amino acids and their derivatives are of interest.[80] Chiral macrocyclic ethers and their derivatives are typical receptors for enantioselective recognition of primary organoammonium salts.[84,85,86,87] Recent examples will be discussed.

Pyridino crown receptors were extensively studied for this purpose by Huszthy et al.[88] and Izatt, Bradshaw and co-workers:[80, 89] An achiral (**17**) and a chiral pyridine-based macrobicyclic clefts (**18**) were prepared[90] and compared to pyridine-18-crown-6 without the additional podand bridge (**19**).[91] Compound **17** formed complexes in $CH_3OH/CHCl_3$ (1:1, vol/vol) with primary ammonium salts with binding strengths around 10^3 M^{-1} as evidenced by a significant change in the ^{1}H-NMR spectrum. The strong intermolecular binding observed is attributed to the 3-point hydrogen bonding of the ammonium hydrogen atoms to the pyridine

nitrogen atom and two of the oxygen atoms within the ring.[92] Binding strengths for **18** are slightly higher than for **17**. Compared to (*S,S*)-**19**, macrobicyclic (*S,S,S,S*)-**18** shows an improved stereoselective recognition towards NEA (1-naphthyl-ethyl-ammonium salt, **20a**) in its three-dimensional cavity. A large difference in stabilities between the complexes of (*R*)- and (*S*)-NEA with (*S,S,S,S*)-**18** (Δ log K_{ass} = 0.85) is observed in a 2:8 (vol/vol) EtOH/$C_2H_4Cl_2$ solvent mixture, while the Δ log K_{ass} value for (*R*)- and (*S*)-NEA interactions with (*S,S*)-**19** is 0.46 in the same solvent mixture. This high degree of enantiomeric recognition was attributed to an increase in molecular rigidity by introducing a second macrocyclic ring on the monocyclic pyridinocrown ligand. Positive values of entropy changes for **18**-NEA interactions, as compared to **19**-NEA interactions, which show negative values of entropy changes, suggest a smaller conformational change of ligand **18** during the complexation.

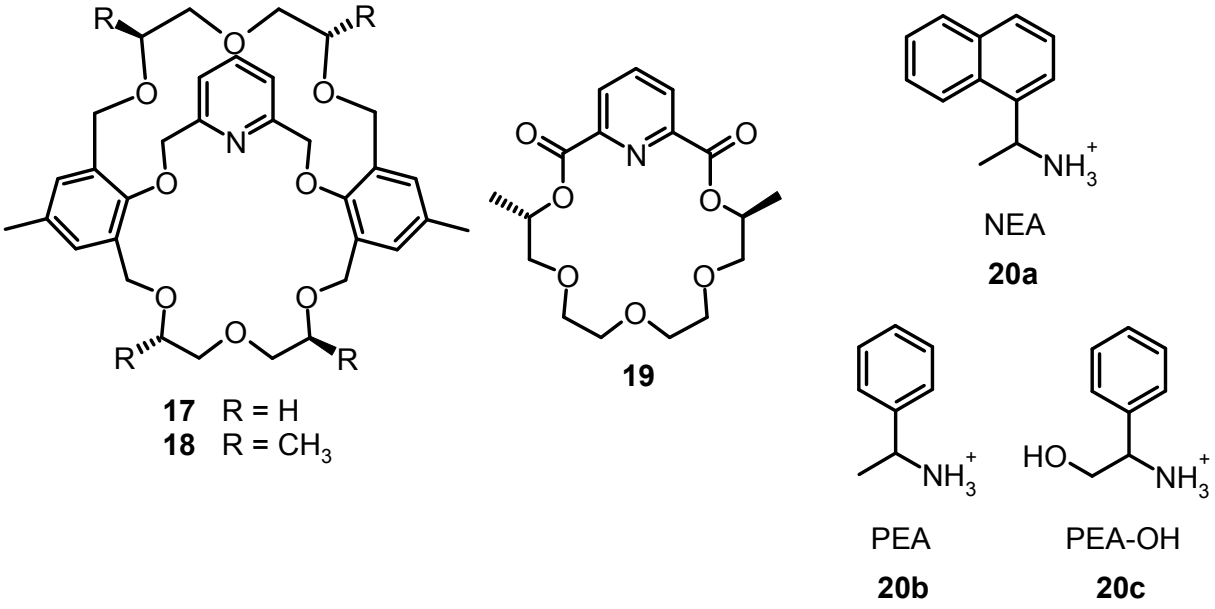
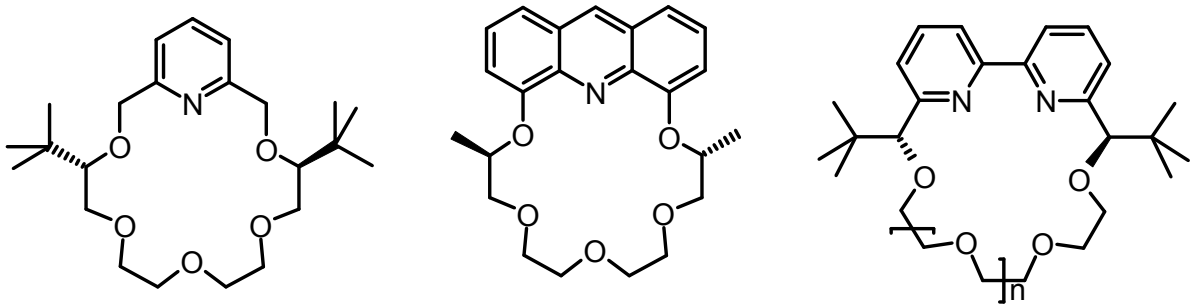


Figure 11: Two different pyridino-cryptands (**17** and **18**) compared to a pyridino-crown (**19**); chiral ammonium ions as guests (**20**).

Pyridino-crown systems proved to be advantageous in enantiodiscrimination in the extensive studies of Izatt and Bradshaw. Other groups employed the principle for the preparation of other chiral receptors: A series of enantiomerically pure chiral pyridino-18-crown-6 ligands were prepared by Samu et al.[93] Their ability to act as enantioselective hosts for primary ammonium salts was demonstrated with the two enantiomers of NEA.[94] The equilibrium constants were measured in a $CD_3OD/CDCl_3$ mixture by NMR spectroscopy. The best example *(R,R)*-**21** (R = ^{t}Bu) shows a four times higher log K_{ass} for the *S*-enantiomer in favour

over the *R*-enantiomer of the guest, being more selective as the former examples, but a weaker binder (log $K_{ass} < 10^3$ M^{-1}).



21 **22** **23** n = 1 - 4

Figure 12: Pyridino-18-crown-6 ligand (**21**), a similar acridino-18-crown-6 ligand (**22**) and a structurally related bispyridyl (bpy)-18-crown-6 receptor **23**

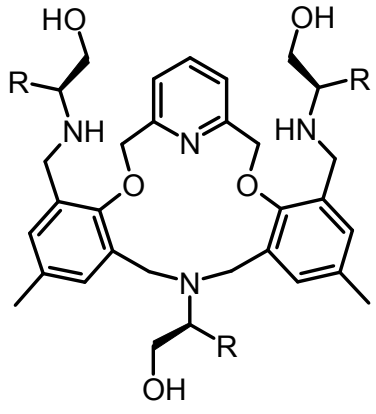
Structurally similar acridino-18-crown-6 ligands like **22** were studied by the same group monitoring the association process between ligands and organic ammonium ions by changes in their photophysical properties in acetonitrile.[95] With the enantiomerically pure *(R,R)*-ligand good binding and enantiodiscrimination in favour of the *S*-enantiomers of PEA (**20b**)[94] (K_{ass} = 2.3*10^6 M^{-1}) and NEA (K_{ass} = 1.7*10^6 M^{-1}) over the corresponding *R*-enantiomers (K_{ass} = 4.4*10^5 M^{-1} and K_{ass} = 3.4*10^5 M^{-1} respectively) was observed.

This optically active dimethylacridino-18-crown-6 ether *(R,R)*-**22** showed higher enantioselectivity towards NEA (**20a**) and PEA (**20b**)[94] than its comparable pyridino analogue *(S,S)*-**21** (R = Me instead of ^{t}Bu).[95] The higher enantioselectivity was rationalized by the stronger π–π-interaction of the extended π-system of the acridine unit and the more rigid conformation of host molecule. An interesting application was demonstrated by Lakatos: Molecule **22** was attached to a silica gel surface to get a stationary phase for enantioseparation of racemic protonated primary arylalkyl amines.[96]

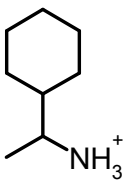
Comparable enantioselectivities with a stronger coordinating ligand can be achieved using a crown ether bearing a bispyridyl (bpy) unit in the ring (**23**). A series of these C2-symmetric 2,2-bipyridine-containing crown macrocycles have been developed by Lee et al.[97] They have studied their enantiomeric recognition properties towards a number of amino acid derivatives and chiral organic ammonium salts using UV–vis and NMR methods. The macrocycles were found to be strong chelating agents for primary organic ammonium salts with binding affinities K_{ass} up to 4.8*10^5 M^{-1} in CH_2Cl_2 with 0.25 % CH_3OH. The bpy-crown macrocycle with n = 1, reflecting the pseudo 18-crown-6 type structure, exhibited the best properties and

the highest enantioselectivity towards the *S*-enantiomer of phenylglycine methyl ester hydrochloride with a *K(S)* to *K(R)* ratio of 2.1 ($\Delta\Delta G_0$= -1.84 kJ mol^{-1}). The Job's plot analysis supported the 1:1 stoichiometry of the host-guest complex. An analysis of the structure-binding relationship showed that the aromatic subunit and the ester group of the ammonium guests are both important for achieving high enantioselectivity.

The enantiomeric recognition of a different pyridino-crown type ligand bearing aminoalcohol subunits on the exterior were investigated by UV titration in chloroform.[98] The hosts formed very stable 1:1 complexes with α-phenylethylamine hydrochloride (**20b**) and α-cyclohexyl-ethylamine hydrochloride (**25**) with relatively similar binding constants (10^4 M^{-1}) as calculated according to a modified Benesi–Hildebrand equation. A preference for enantiomers with an absolute configuration of (S) for both amine salts was found: Host **24a** bearing isobutyl groups shows an enantiomer recognition factor of 2.0 and 5.0 (K_S/K_R), which corresponds to approximately 33 % and 67 % *ee* for **20b** and **25**, respectively. For the host bearing a phenyl residue (**24b**) similar factors of 2.1 and 5.0 (K_S/K_R) corresponding to approximately 36 % and 67 % *ee* for **20b** and **25**, were observed. With the benzyl substituted moiety (**24c**) a far weaker discrimination was found. Hydrogen bonding of the alcohols combined with π–π staking, π–charge interaction and steric complementarity were assumed as being responsible for the enantioselective recognition.



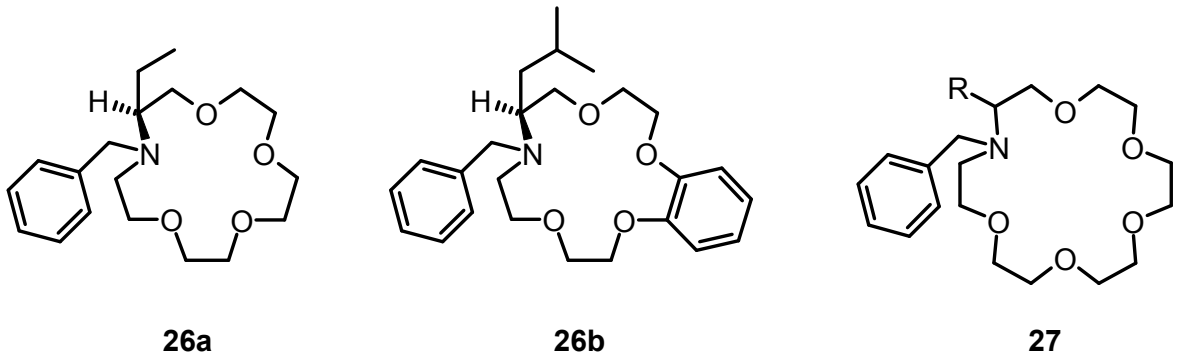
24a R = iBu
24b R = Ph
24c R = Bn



25

Figure 13: Chiral pyridino-azacrown ether receptors **24**

Even better enantioselectivities as with pyridino-crowns were observed with chiral azacrown compounds, but the binding constants were for comparable cases approximately one order of magnitude lower. Togrul et al.[99] and Turgut et al.[100] presented several chiral monoaza-15-crown-5 ethers based on chiral aminoalcohols and investigated the effect of the substituent at the stereogenic centre on the enantioselectivity. The benzocrown derivative of *S*-leucinol and the 15-crown-5 prepared from (*R*)-(–)-2-amino-1-butanol were found to be the most effective examples.[101] Both molecules show enantioselectivity towards (*R*)-**20b** perchlorate compared to (*S*)-**20b** perchlorate:[94] The aggregate was for **26b** 4.76 times more stable for the *R*-enantiomer than with the *S*-form ($\Delta\Delta G_0$= -1.73 kJ mol^{-1}; $K_{ass,R}$ = 9.8*10^4 dm^3 mol^{-1}, $K_{ass,S}$ = 2.2*10^4 dm^3 mol^{-1}). In the case of **26a** they achieved a ratio of K_R/K_S = 4.46 ($\Delta\Delta G_0$ = -3.7 kJ mol^{-1}; $K_{ass,R}$ = 9.5*10^3 dm^3 mol^{-1}, $K_{ass,S}$ = 4.8*10^3 dm^3 mol^{-1}).



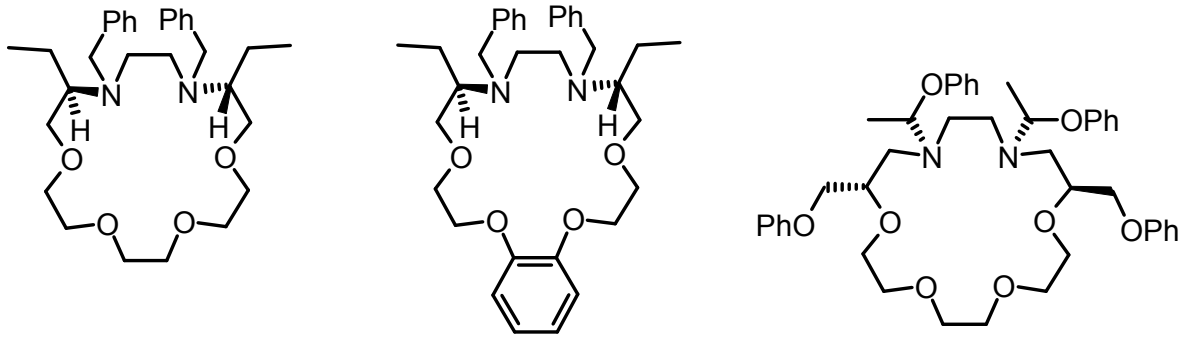
26a **26b** **27**

Figure 14: Chiral 15-crown-5 receptors **26** and an analogue 18-crown-6 ligand **27** derived from amino alcohols

Enantiomeric recognition of chiral primary ammonium perchlorate salts was investigated with analogue chiral mono aza-18-crown-6 derivatives like **27**.[102] For the isobutyl substitution (**27**, R = ^{i}Bu) the respective host exhibited the highest binding constant and the best enantiomeric selectivity ability towards 1-phenylethylammonium perchlorate isomers (**20b**): The complex with the *R*-isomer (K_a = 3.3*10^4 dm^3 mol^{-1}) was 2.5 times more stable than the one with the *S*-configuration (K_a = 1.3*10^4 dm^3 mol^{-1}).[101]

Turgut et al. published the corresponding C2-symmetric chiral diaza-18-crown-6 ethers **28a** and **28b** derived from chiral (*R*)-(-)-2-amino-1-butanol.[103] The association constants, measured by UV–vis spectroscopy in methanol/chloroform solvent mixture, revealed for *S*-, *R*-Ala-OMe hydrochloride the highest value for both macrocycles (K_a = 1.5*10^4 dm^3 mol^{-1}) as calculated according to the modified Benesi-Hildebrand equation, but without pronounced

chiral discrimination. The highest enantioselectivity was observed in the case of Trp-OMe hydrochloride (K_R/K_S = 12.5) with a binding strength in the same order of magnitude as observed for the alanine ester. This was the highest factor found to date for such systems. The authors reasoned that steric and π–π-interactions with the crowns phenyl substituents are decisive for the enantioselective recognition.



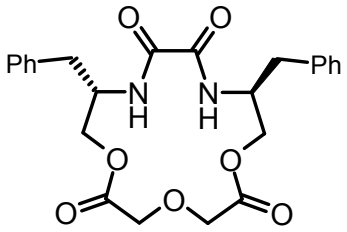
28a **28b** **29**

Figure 15: C2-symmetric chiral 18-crown-6 amino alcohol derivatives **28** and related macrocycles

Recently Turgut et al. reported a comparable series of C2-symmetric chiral aza crown ether macrocycles (**29**) based on (*S*)-3-phenyloxy-1,2-propanediol and (*S*)-1-methyl-1,2-propanediol for the enantiomeric recognition of amino acid ester derivatives.[104] The four similar macrocycles have been shown to be complexing agents for primary organic ammonium salts by ^{1}H-NMR titration. The best example, the depicted host **29**, exhibited enantioselective bonding toward the *R*-enantiomer of phenylalanine methyl ester hydrochloride with K_R/K_S of 6.87 in $CDCl_3$ with 0.25 % CD_3OD. The binding constants are far lower as in the former examples.

Related macrocycles **30** with diamide-diester groups derived from dimethyloxalate and amino alcohols also showed a considerable binding affinity and enantiomeric discrimination of aromatic amine salts.[105] The binding properties were evaluated by ^{1}H-NMR titration in acetonitrile. For the *(R,R)*- and *(S,S)*-configurated host with a phenyl residue the highest differences in the K_{ass} values were observed: (*R*)-NEA and (*S*)-NEA (**20a**)[94] to (*S*,*S*)-**30** and (*R*,*R*)-**30** (R = Ph) show ratios of K_S/K_R = 5.55 and K_R/K_S = 3.65, respectively. A general tendency for the host to include the guests with the same absolute configuration was found. The amide and ester groups ensure a high rigidity of the host. The highest binding constant of

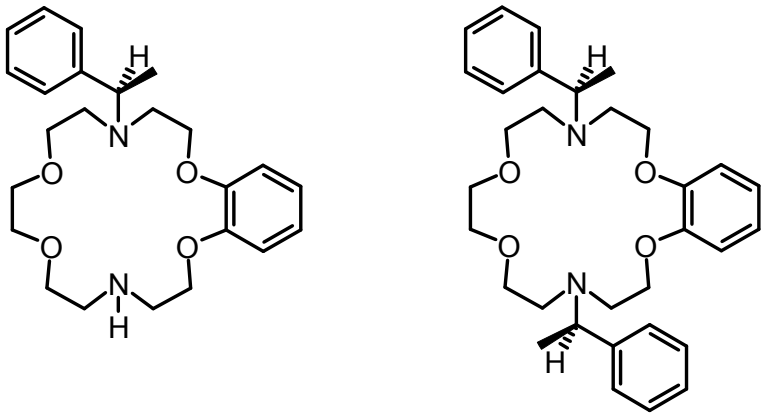
7.8*10^3 M^{-1} was found for the complex of phenyl substituted *(R,R)*-**30** with the *R*-enantiomer of the guest.



30

Figure 16: Macrocycles with diamide-diester groups (**30**)

Chiral side arms derived from phenethylamine attached to diaza-18-crown-6 ethers **31** enable effectively the molecular recognition of aromatic amino acid potassium and sodium salts[106] as shown in the selectivity order Phe > Thr > Ala. The abilities of the crown ethers to coordinate to the salts were investigated using UV-vis titration in a solution of acetonitrile/water (50:1). The highest affinities of 4*10^4 M^{-1} were obtained with the monoaromatic ring system **31a** for the potassium salt of *S*-Phe. The cavity of the macrocycle plays an important role in recognition: A dibenzo substitution on the diazacrown ether may close the cavity due to steric hindrance of the arene units on the ring and the resulting π–π-interaction between the two aromatic moieties on the ring. However, π-stacking interactions between the aromatic moiety and aromatic part of the amino acid contributes to the overall binding strength of the receptor.



31a **31b**

Figure 17: C2-symmetric chiral aza-18-crown-6 ethers (**31**) with phenethylamine residues

In transport experiments chiral lariat ethers show an increased flux of amino acids or their carboxylate salts and provide enantiomeric discrimination (fig. 18, table 4): With preference for the *R*-enantiomers, the benzo- and naphtho-18-crown-6 **33a** and **33b** generally revealed a larger flux of the aromatic amino acids or their salts than hosts **32a** and **32b**.[107] This was attributed to a strong π–π stacking interaction. The highest flux values and enantiomeric selectivities were obtained for the *R*-enantiomers of tyrosine and its potassium salt. The more pronounced enantioselectivity of tyrosine may be explained by hydrogen bonding and the favourable π–π interaction between the hosts' side arm and the aromatic moiety of guests. The higher enantioselectivity of potassium salts in comparison to other salts were explained by apical-π or a sandwich-type supramolecular complex due to the ions larger size.

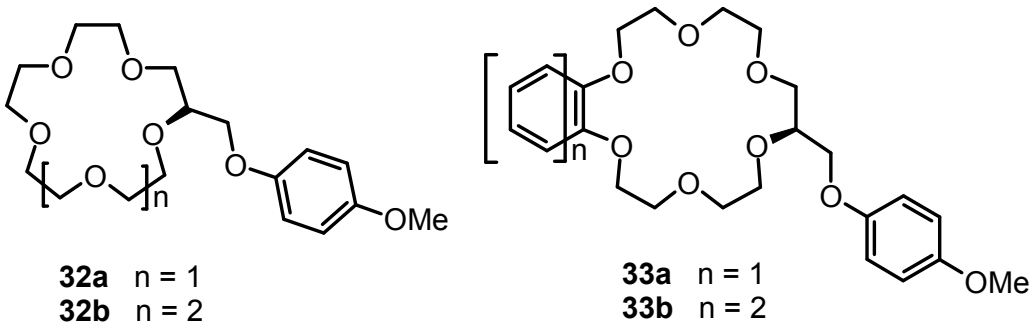


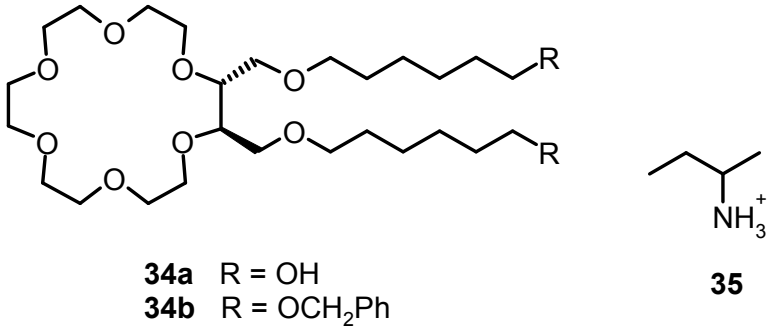
Figure 18: Chiral *C*-pivot *p*-Methoxy-phenoxy-lariat ethers

Crown / Guest	*32a*		*32b*		*33a*		*33b*	
	f_{72} *10^8 (mol m^{-2} s^{-1})	α_T	f_{72} *10^8 (mol m^{-2} s^{-1})	α_T	f_{72} *10^8 (mol m^{-2} s^{-1})	α_T	f_{72} *10^8 (mol m^{-2} s^{-1})	α_T
S-Tyr	3.05	13.7	11.01	3.5	7.96	4.9	2.56	15.5
R-Tyr	41.87		38.04		38.73		39.81	
S-Tyr K$^+$	4.62	8.3	10.81	3.5	7.18	5.2	2.75	14.1
R-Tyr K$^+$	38.34		37.65		37.45		38.83	

Table 4: Fluxes and enantiomeric selection behaviour of substance class **32** and **33**

The approach to introduce chirality for a similar function by the introduction of *C*-pivot podand arms, resulting in stereogenic centres, was presented by Colera et al.[108] The properties of the compounds were evaluated with two different chiral alkylammonium picrates, (+)-(*S*)- and (-)-(*R*)-**35** (AmI) and (+)-(*R*)- and (-)-(*S*)-**20b** (AmII) in acetonitrile. The ligands *(R,R)*-

34b and *(R,R)*-**34a** showed enantioselective binding: *(R,R)*-**34b** favoured (*R*)-AmI over (*S*)-AmI and (*R*)-AmII over (*S*)-AmII by a $\Delta\log K_{ass}$ of 2.06 and 3.23, respectively. Similar results were observed with *(R,R)*-**34a** with $\Delta\log K_{ass}$ = 2.64 and 2.43 for AmI and AmII. These results indicated that the presence of the phenyl rings in ligand *(R,R)*-**34b** not only gives rise to higher complexation constants with (*R*)-AmII than with (*R*)-AmI (log K_{ass} = 5.42 and = 4.61, respectively) but also increases the enantioselective recognition. In addition, racemic aqueous solutions of the ammonium salts have been enriched in the *R*-enantiomer after extraction experiments with the best results for (±)-AmII with an *ee* of 33 %.



34a R = OH
34b R = OCH_2Ph

35

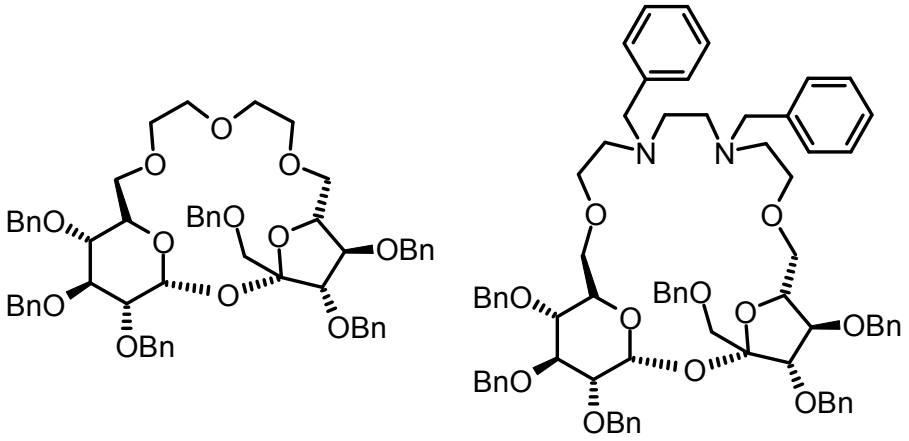
Figure 19: Chiral lariat crown ether **34**

The results of the last examples are difficult to compare, their properties were investigated in different solvent mixtures and by different methods. However, this underlines the versatility of the systems published: For different conditions and separation problems approaches are available.

A general trend is observable: 18-crown-6-systems reveal higher binding constants then 15-crown-5-systems, due to the better size fit of the guest ion. Aromatic substituents lead to better recognition and enantiomeric excess (up to 70 %) with aromatic guests like NEA (**20a**) or phenylglycinol (**20c**). For tryptophan (**81b**) the best results were achieved with selection factors of one enantiomer over the other up to 13-fold, corresponding to over 90 % *ee*. This is explained by π-π-interactions.

Besides chiral substituents on the crown ether ring, chiral groups in the ring can be employed for enantioselection of guest ions: Stoddart determined the stability of complexes of *D*-mannitol based crown ethers with ammonium cations using NMR spectroscopy.[109] Another example uses fructopyrano-crown ethers with different ring sizes.[110]

The chiral azacoronands **36a** and **36b** based on sucrose display high enantioselectivity in the complexation of phenylethylammonium chlorides.[111] The stability constants of these receptors in acetone towards ammonium cations (NMR titration of NH_4SCN) were 560 M^{-1} for **36a** and 230 M^{-1} for **36b**.[112] In NMR titration experiments in chloroform the receptors showed the preferential complexation of the (*S*)-ammonium salt with the highest value (K_{ass} = 1244 M^{-1}) for the complex of compound **36a** with α-phenylethylammonium chloride. The complex with the (*R*)-amine was of lower affinity (K_a = 837 M^{-1}, K_S/K_R = 1.84). Although the stability constants of **36b** with the (*S*)-amine were lower than for **36a** (K_{ass} = 945 M^{-1}) it has interesting complexing abilities: The macrocycle did not complex the (*R*)-enantiomer of α-phenylethyl-amine. In all cases a Job´s plot confirmed a 1:1 stoichiometry of the aggregates.

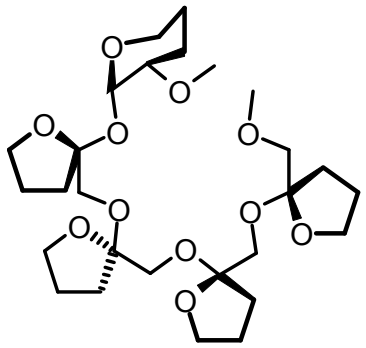


36a **36b**

Figure 20: Sucrose based chiral crown ether receptors **36**

The use of cyclodextrin type structures in chiral discrimination is well documented.[113] In a recent example Shizuma and Sawada demonstrated a high degree of chiral discrimination between amino acid ester salts with a permethylated fructooligosaccharide (pentasaccharide) by an induced-fitting chiral recognition mechanism with amino acid ester salts:[114] ValOPr$_i$ gave $I_R/I_{S\text{-}Dn}$ = 0.14 corresponding to $\Delta\Delta G_{enan}$ = 1.2 kcal mol^{-1} with *S*-selectivity and PheOPr$_i$ led to $I_R/I_{S\text{-}Dn}$ = 0.18 corresponding to $\Delta\Delta G_{enan}$ = 1.0 kcal mol^{-1}, also with *S*-selectivity. It was assumed that a pseudo-18-crown-6-ring structure surrounding the ammonium ion was formed by the acyclic methylated pentasaccharide in the complexation. The chiral discrimination was ascribed to the steric effect of the fructofuranose rings of the pentasaccharide and the substituent of a given amino acid ester salt (complexation-induced selectivity). The binding ability of compound **44** in solution ($CHCl_3$) was determined by UV–vis spectrometry using a

picrate anion probe. This is one of the rare examples of podands used for enantioselective recognition.

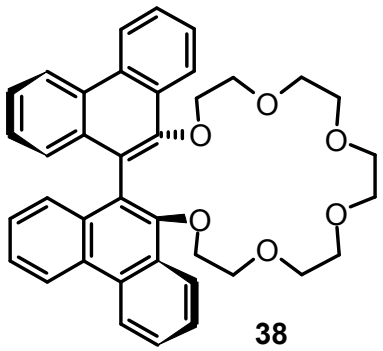


37

Figure 21: Permethylated fructooligosaccharide **37** showing induced-fit chiral recognition

The pioneering work on this topic has been carried out in the 1970s by Cram et al.[84] They studied the chiral recognition ability for binaphtol based chiral macrocycles using the picrate salt extraction method.[115]

Many examples of chiral receptors have been reported, which exhibit chiral recognition towards cations derived from phenylethylamine. The biphenantryl-18-crown-6 derivative **38** presented by Yamamoto et al.[116] displayed one of the highest enantioselectivities towards one enantiomer of phenylethylamine hydrochloride as was demonstrated by liquid/liquid extraction experiments [the respective *ee* values are 42 % (*R*) and 45 % (*S*)].



38

Figure 22: Biphenantryl-18-crown-6 derivative **38**

Fuji et al.[117] have developed the related chiral lariat crown ether **39**. Its phenolic hydroxyl group converts basic amines into ammonium ions, which are bound more tightly. A salt bridge between the ammonium and the phenolate ions supports the binding process. From UV and NMR titration experiments the authors derive binding constants for hexylamine of 14 M^{-1}

in THF and > 10^5 M^{-1} in DMSO. This is surprising, because an increased ability of the solvent to act as a hydrogen bond acceptor typically leads to decreased binding constants. A significant contribution of the phenolate-ammonium salt bridge or from π-cation interactions is likely. The best enantioselective binding of chiral ammonium ions was observed using phenylglycinol: The *R*-enantiomer (K_{ass} = 30 M^{-1}) was bound preferentially over the *S*-enantiomer (K_{ass} = 9 M^{-1}) by a factor of 3.2 in a methanol/acetonitrile solvent mixture.

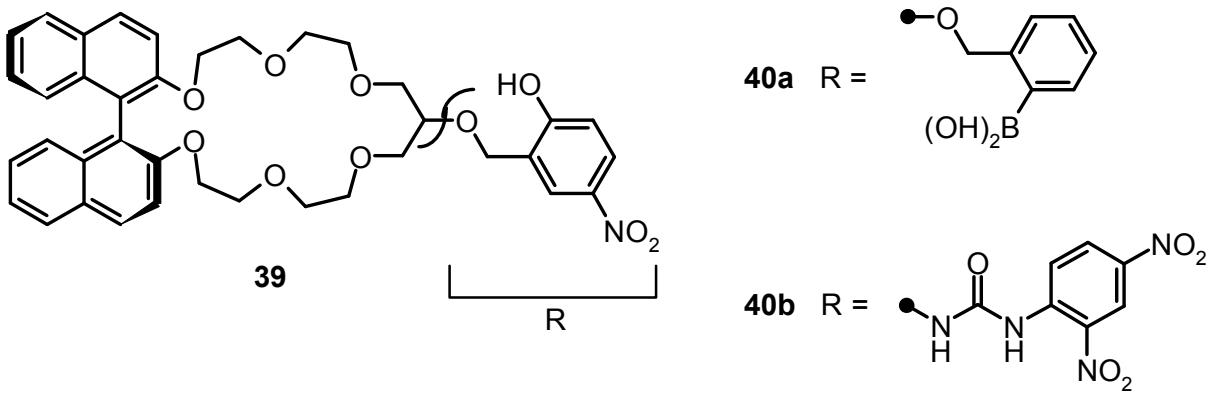


Figure 23: Chiral lariat crown ethers derived from binol by *Fuji et al.*

The authors expanded their approach by two similar binaphthyl crown recognition systems containing phenylboronic acid **40a** and 2,4-dinitrophenylurea **40b** as lariat parts.[118] Host **40a** had 30 % extraction efficiency for γ-aminobutyric acid (GABA) by a solid–liquid extraction in DMSO, but showed only small selectivities for α-amino acids: Boc-*R*-Lys-OH (18.5 %), Boc-*S*-Lys-OH (14.1 %) and H-*R*-Asp-NH_2 (8.2 %), H-*S*-Asp-NH_2 (4.3 %). The chromogenic host **40b** discriminated amino acids by their length. After extraction, the solvents colour changed from colourless to yellow due to an increased absorbance around 460 nm. The extent of the colour change correlates with the affinity to the guest amino acid. ω-Aminohexanoic acid caused the most significant change. Although the colour change can be seen by the naked eye, the maximum extracted amount of 3 % was small.

Homochiral phenolic crown ethers with "aryl chiral barriers" were investigated and published already in 1998 by the group of Naemura.[119] This system displayed, upon investigation by UV-vis spectroscopy in chloroform a good enantiodiscrimination ability in favour of *R*-phenylalaninol with an $\Delta_{R\text{-}S}\Delta G$ = 6.4 kJ mol^{-1}. In succession, Steensma et al. investigated thermodynamic data and conditions for chiral separation of amines and amino alcohols.[120] The azophenolic crown ether was a versatile and a highly enantioselective host for their chiral

separation by reactive extraction. Transport from a basic aqueous solution of the racemic mixture in CH_2Cl_2 and toluene was followed by UV-vis titration. **41** showed the highest affinity for phenylglycinol (**42b**) with association constants of $K_{ass} = 1.5*10^5\ M^{-1}$ in CH_2Cl_2 and $K_{ass} = 8.0*10^4\ M^{-1}$ in toluene with a 10-fold higher binding constant to the *R*-enantiomer. Also norephedrine (**42c**) and 2-aminobutanol (**42a**) could be separated in an acceptable ratio. The extractant could be reused for chiral separation without loss of activity or selectivity. Ammonium ion binding by chiral azophenol crowns and of diamines by bisazophenol crown ethers has been summarized in a special review.[121]

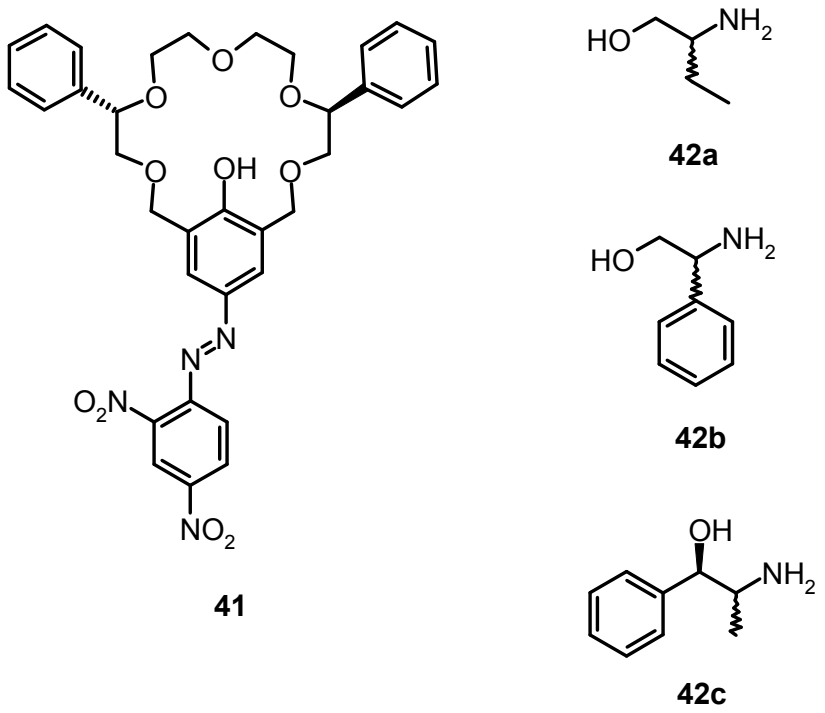
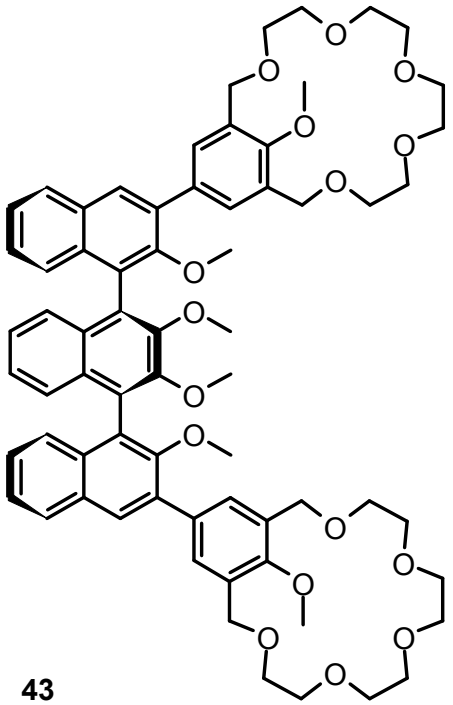


Figure 24: Chiral phenolic crown ether **41** with "aryl chiral barriers" and guest amines

I. 3.4. Di- and Tritopic Crown Ether Receptors for the Recognition of Bis- and Tris-Ammonium Ions

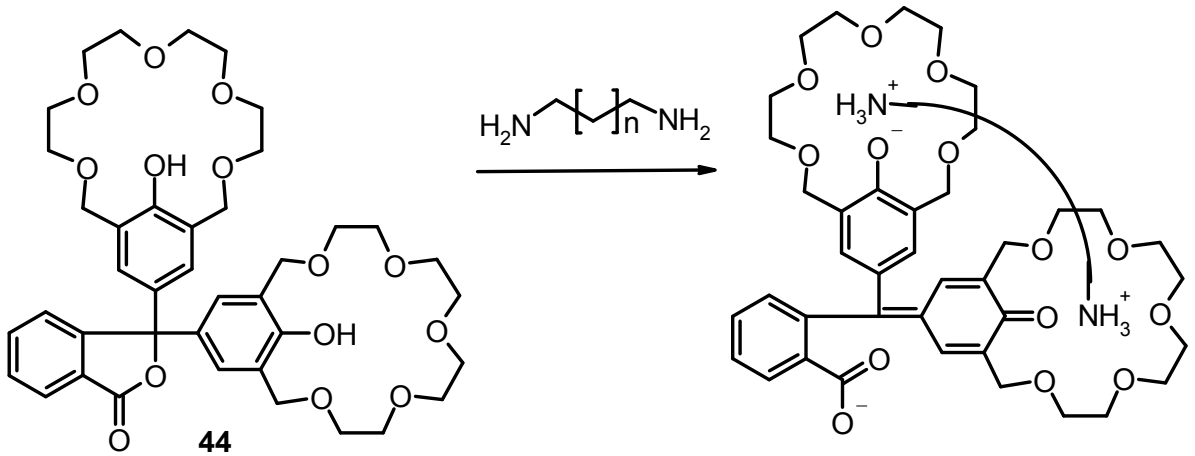
Fuji et al. presented a ditopic receptor **43** to distinguish between the length of α,ω-diamines (fig. 25). The receptor consists of a meso-ternaphthalene backbone and two crown ether rings.[122] Receptor **43** preferably binds and transfers the di-picrate of 1,9-diaminononane and 1,10-diaminodecane from an aqueous solution into $CHCl_3$.



43

Figure 25: Chiral *bis*-crown receptor **43** with a meso-ternaphthalene backbone

The group reported also a colourimetric approach for recognition of such guests, a phenolphthalein core substituted with two crown ether moieties (fig. 26).[123] Upon amine binding, the phenolic hydroxyl groups are deprotonated, which leads to lactone opening and the formation of a coloured quinone conjugated carboxylate structure. The chemosensor discriminated terminal diamines by length: 1,8-diaminooctane (K_{ass} = 1270 M^{-1}) and 1,9-diaminononane (K_{ass} = 2020 M^{-1}) showed the highest binding constants in methanol. Diamines with an alkyl chain length shorter than five carbons were not bound.

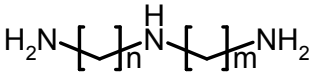


44

Figure 26: Chromogenic pH-dependent *bis*-crown chemosensor **44** for diamines

Investigation of the stoichiometry of the aggregate formation led to a value of 1.2 to 1.3, because one diamine is bound by the two crown ethers and a second diamine is recruited as ammonium counter ion of the carboxylate. Addition of an excess of *N*-ethylpiperidine as base established the expected stoichiometry of the aggregate of 1:1. Control experiments with *N*-ethylpiperidine and phenolphthalein without crown ether moieties confirmed the ammonium ion crown ether interaction being essential for the colour response. Unprotected dipeptides showed an affinity to compound **44** if amino groups were present in a suitable distance, for example as found in dipeptides with a *C*-terminal Lys. Lys-Lys (K_{ass} = 1020 M^{-1}) and Gly-Lys (K_{ass} = 930 M^{-1}) showed the highest affinity constants in methanol/water 10:1.[124]

The same host (**44**) is able to signal the length of a linear triamine in a similar manner. Triamines **45a**-**45c** and spermidine (**45e**) developed a bright purple colour by forming complexes with the host in a 1:1 ratio with the inner imino group capturing the carboxylate after lactone opening. The colour develops in a limited temperature range and therefore can be also used as a visible index of temperature. The association constants (K_a) as well as molar absorption coefficients (ε) were determined by UV-vis titration. For triamine **45c** thermodynamic parameters ΔH = -127.4 ± 6.3 kJ mol^{-1} and ΔS = -362.8 ± 21.3 J mol^{-1} K^{-1} were obtained and temperature dependent measurement of the association constants were conducted (K_{ass} = 14870 ± 880 M^{-1}, ϵ = 5100 ± 30 at 15 °C; K_{ass} = 2270 ± 30 M^{-1}, ϵ = 5080 ± 20 at 25 °C; K_{ass} = 1090 ± 10 M^{-1}, ϵ = 4980 ± 10 at 30 °C). Both K_{ass} and ϵ reach maximum values with triamine **45c**.



45a n = m = 3
45b n = m = 4
45c n = m = 5
45d n = m = 6
45e n = 3; m = 4

Figure 27: Triamine guests for binding to receptor **44**

Based on this phenolphthalein skeleton the host was later developed further for use in visual enantiomeric discrimination.[125] Various types of chiral host molecules were examined in their enantioselective colour effect in complexation with chiral amino acid derivatives in methanol

solution. The methyl substituted compound (*S*,*S*,*S*,*S*)-**46a** showed a particularly prominent selectivity for the alanine amide derivatives with 1,5-pentane diamine and 1,6-hexane diamine: A combination of methyl substituted host (*S*,*S*,*S*,*S*)-**46a** with the *R*-enantiomers developed a purple colour, whereas no colour development was observed with *S*-enantiomers. When Ala-1,6-hexane diamines with different optical purities were added to the host **46a** solution, a linear relationship was observed between the absorbance (λ_{max} = 574 nm) and the *ee* of the added guest. The phenyl substituted compound (*S*,*S*,*S*,*S*)-**46b** showed an even more intensive colour change induced by a wide range of (*S*)-α-amino alcohols compared to the corresponding (*R*)-α-amino alcohols. The function, mechanisms and applicability of phenolphthalein crown systems have been recently summarized by Tsubaki.[126]

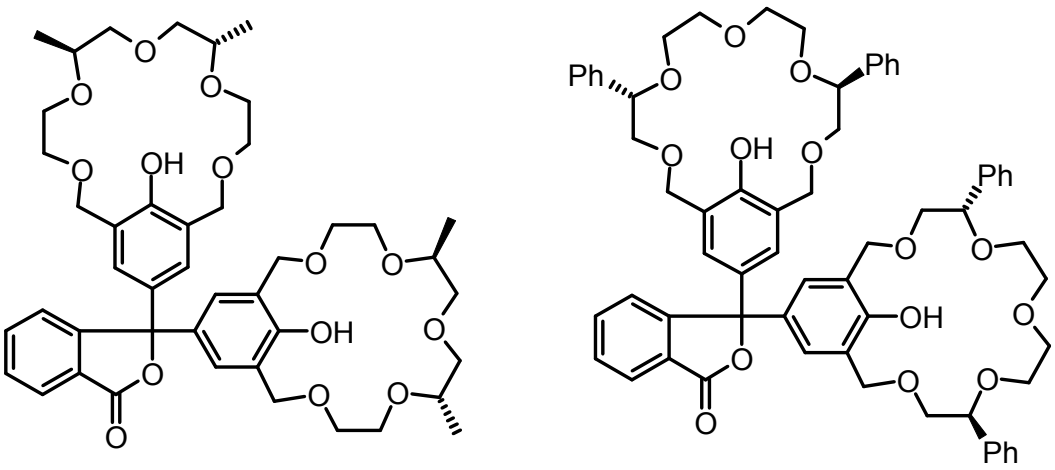


Figure 28: Chiral *bis*-crown phenolphthalein chemosensors **46**

Ditopic receptors can consist of two or more crown ether amino acids. The group of Voyer published crown ether based receptors for diamino and diammonium alkanes.[127] They used crown ether amino acid (CEAA) **19**, which was incorporated twice into an oligo Ala peptide chain.

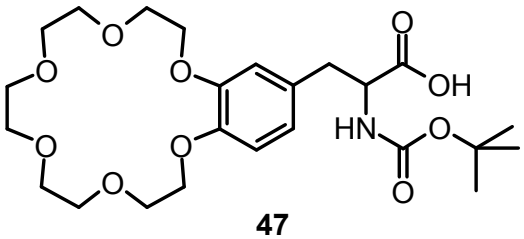
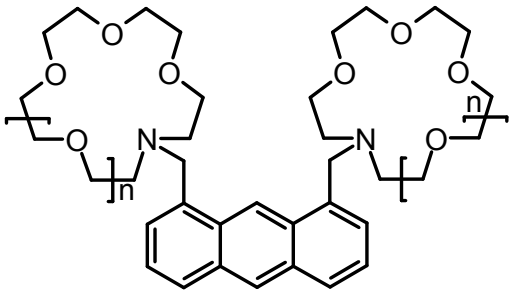


Figure 29: Crown ether amino acid **47**

By variation of the number of Ala residues between the crown ether amino acids from one to two or three the receptors structure is varied: Boc-Ala-Ala-CEAA-$(Ala)_{1-3}$-CEAA-Ala-^{n}Pr. 1,9-diaminononane turns out to be the diamine with highest affinity for all three sequences among all tested diamino alkanes from C_2 to C_9. The binding constants were derived from picrate extraction [128] from water into chloroform showing as highest binding constant $2*10^{10}$ M^{-1}. However, binding constants determined by extraction methods may have larger errors [129] and the binding process includes a phase boundary transition. Therefore binding constants cannot be compared to other systems investigated in homogeneous solutions. Surprisingly, despite the difference in crown ether spacer length of Voyer´s and Fuji´s systems, both preferentially bind 1,9-diaminononane. To match the distance of the phenolphthalein system, the CEAA units must be connected directly. This indicates that the actual binding conformation of the bis-crown ether-diammonium ion aggregates may be more complex under the experimental conditions. Recently they reported the application of a similar peptide forming α-helical amphiphilic peptide nanostructure with cytolytic activity. A potential use of these peptide nanostructures is as prodrugs that may be activated by a specific proteolytic enzyme to selectively target and destroy undesirable cells.[130]

Kim et al. reported two bis(azacrown)anthracene derivatives **48a** and **48b** for the recognition and detection of alkyldiammonium ions in ethanol or a chloroform/methanol mixture (9:1) based on the PET principle.[131] The fluorescence of the anthracene is quenched by the free electron pairs of the nitrogen atoms. When hydrogen bonds are formed by both nitrogen atoms to the bis-ammonium guests, the photoinduced electron transfer (PET) is inhibited and the system shows an enhanced fluorescence. The binding was dependent on the chain length between the two cations, displaying a maximum stability in the case of the protonated 1,3-diaminopropane. For the bis(aza-15-crown-5) chemosensor **48a** the following binding constants were observed: K_{ass} = 4412 M^{-1} for n = 3; K_{ass} = 272 M^{-1} for n = 4; K_{ass} = 35 M^{-1} for n = 5; K_{ass} = 98 M^{-1} for n = 6. Compound **48b** showed a similar selectivity towards the guests.



48a n = 1
48b n = 2

Figure 30: Luminescent receptor **48** for bis-alkylammonium guests

König et al. combined both principles. They published luminescent crown ether amino acid (CEAA) dipeptides (**49b**), which revealed high affinity to ammonium ions and signalled the binding processes by an increase in their emission.[132] In contrast to Voyer´s system, the crown ether moieties are the central part of the CEAA enabling the synthesis of linear receptors. Both crown ether parts in the ditopic receptor bound independently to mono-ammonium guests with similar affinity than monomeric CEAAs. A bis-ammonium guest, such as lysine methyl ester, was cooperatively bound with a higher affinity (log K_{ass} = 4.3 for the phthalimide part and log K_{ass} = 4.7 for the phthalester moiety in methanol). The binding affinity increased more than 100-fold in comparison to a single receptor CEAA. The affinity of the bis-CEAA to bis-ammonium ions is distance dependent, which allowed distinguishing between isomeric small peptides containing a lysine residue in different positions. Peptides with *N*-terminal lysine showed the highest affinity to **49b**. The binding events of the crown ether groups can be monitored independently by changes of their specific emission properties.

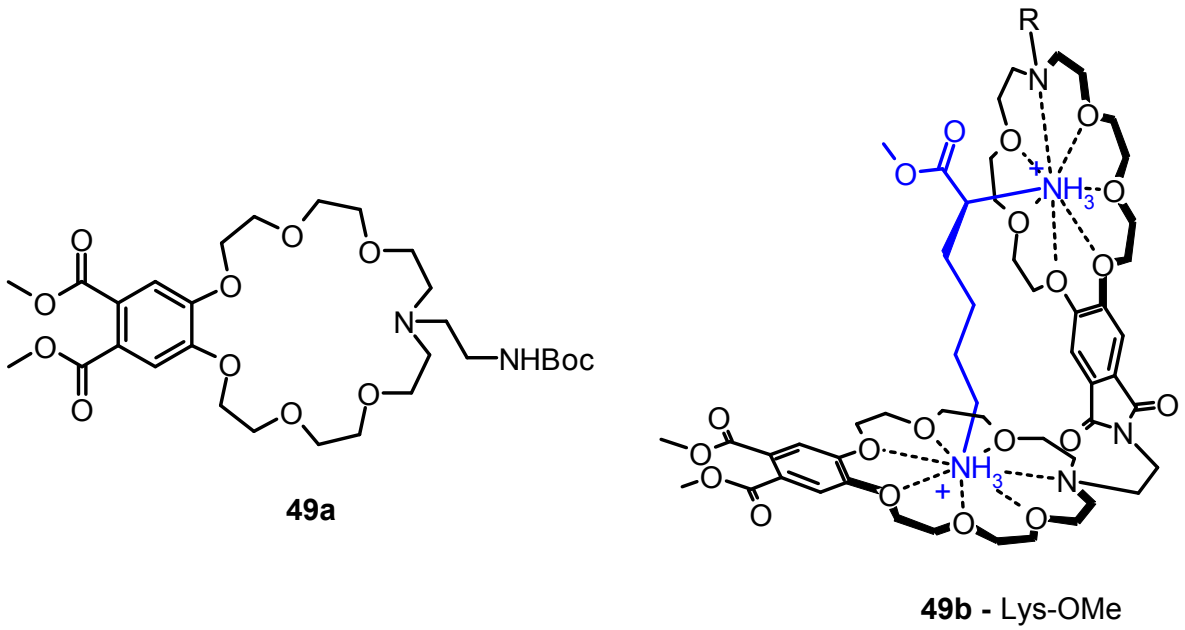


Figure 31: Luminescent CEAA (**49a**), a bis-CEAA receptor for amino acids (**49b**) and the proposed structure of lysine binding

The approach was extended to linear tris-CEAA receptors (**50**) for di-lysine peptides.[133] The additional chromophore leads to a stronger emission, which becomes visible by the naked eye, but the extension from bis- to tris-crown ethers does not lead to an increase of ammonium binding affinities as demonstrated by emission titration. Compared to **49b**, comparable binding constants for di-lysine-guests in methanol (log K_{ass} = 4.5) and in buffered water (log K_{ass} = 2.5) are reached with **50**. The flexible structure of the extended crown ethers and their peptidic guest molecules is a likely rational for the observation: the limited pre-organization of the extended receptors binding sites prohibits an additive or cooperative action of the intermolecular interactions, and illustrates the importance of well balanced entropy and enthalpy contributions in the design of synthetic receptors.

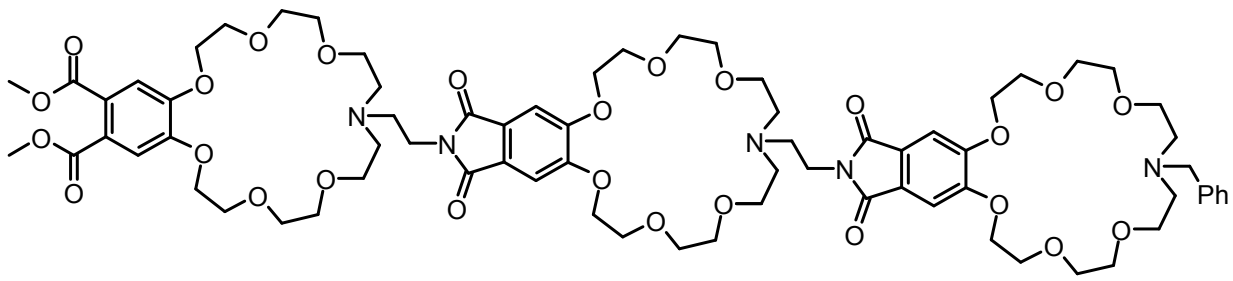


Figure 32: Luminescent CEAA tripeptide for binding small peptides

More unusual, but demonstrating the wide scope of ammonium ion recognition with crown ethers are systems which utilize guest self assembly for enhancement of binding strength. The assembly of the C_{60}–ammonium cation **51b** with the oligophenylenevinylene derivative bearing two crown ether moieties **51a** led to the cooperative formation of the 2:1 complex owing to intramolecular fullerene-fullerene interactions.[134] High stability constants in dichloromethane (log K_1 = 5.6 by luminescence titration and log K_2 = 6.5 by UV absorption) were reported, but due to the small spectral changes upon binding, the binding constants were obtained with high errors. The observation has been also evidenced by electrospray mass spectrometry. The cooperative recognition process could be shown by fluorescence quenching experiments: The stability of the supramolecular *syn*-complex is significantly higher than that of its corresponding *anti*-complex. The combination of several weak interactions like π–π-stacking and hydrophobic associations between the two C_{60} units was proposed to explain the stronger coordination and its ability to self-aggregate.

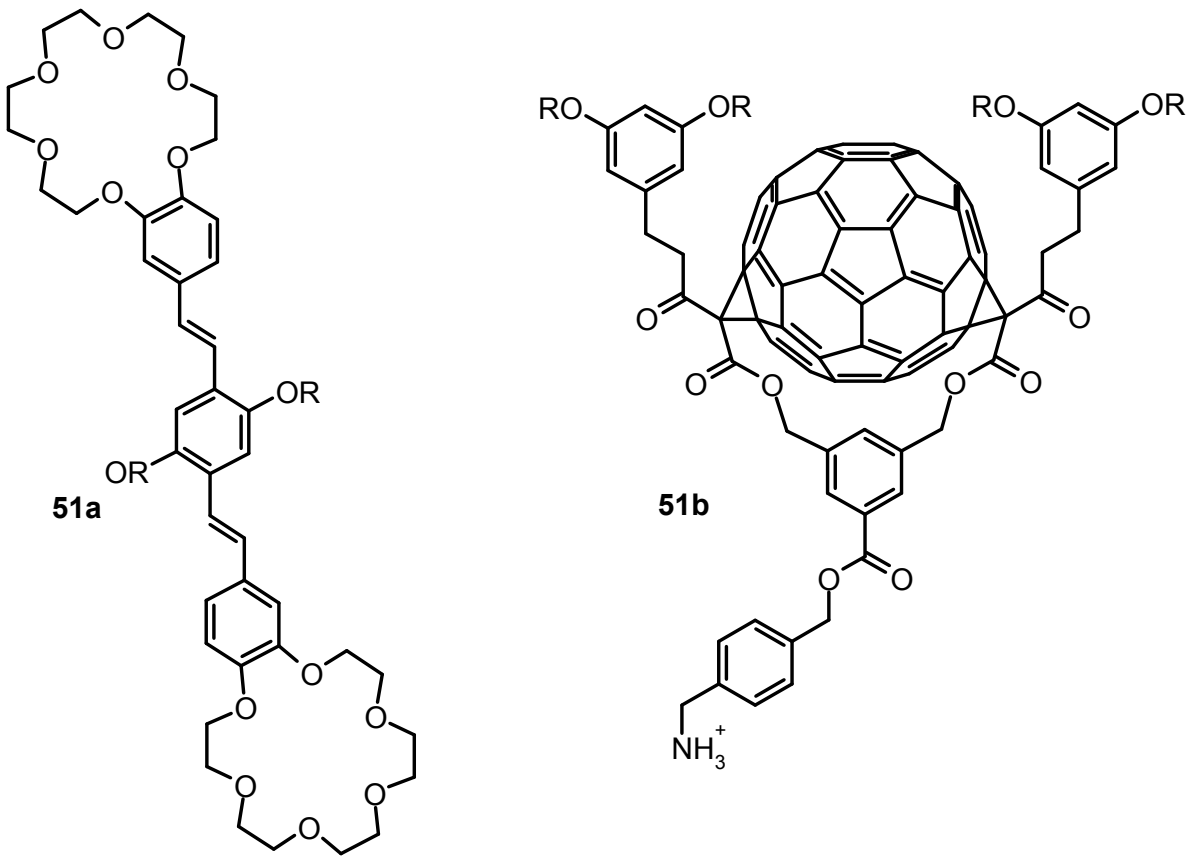


Figure 33: Bis crown ether **51a** self-assembles cooperatively with C_{60}-ammonium ion **51b**

With larger crown ethers (24-crown-8 and above) also secondary amines or pyridylium ions can be recognized. Such an approach for ditopic crown receptors with enhanced guest

selectivity was presented by Chen.[135] A triptycene-based macrotricyclic host **52** containing two dibenzo-[24]-crown-8 moieties selectively forms stable 1:1 or 1:2 complexes with different functional paraquat derivatives and secondary ammonium salts in a chemically controlled complexation process (K_{ass} ~ 10^3 -10^4 M^{-1} in acetonitrile/chloroform). These guest-dependent complexation modes have been confirmed by 2D-NMR experiments and X-ray crystallographic analysis. Alkyl substituted paraquat derivatives thread the lateral crown cavities of the host to form 1:1 complexes in chloroform/acetonitrile 1:1 (2 - 4*10^3 M^{-1}).[136] The host forms a 1:2 complex with two 9-anthracylmethylbenzylammonium salts (R = 9-antracenyl) in the same solvent (K_1 = 8.0*10^3 M^{-1} and K_2 = 1.2*10^3 M^{-1}), in which the two 9-anthracyl groups were selectively positioned outside the central cavity. The competing complexation of the host and these two different guests, the hexyl-substituted paraquat derivative and a dibenzylammonium salt, can be controlled by the addition of acid or base.

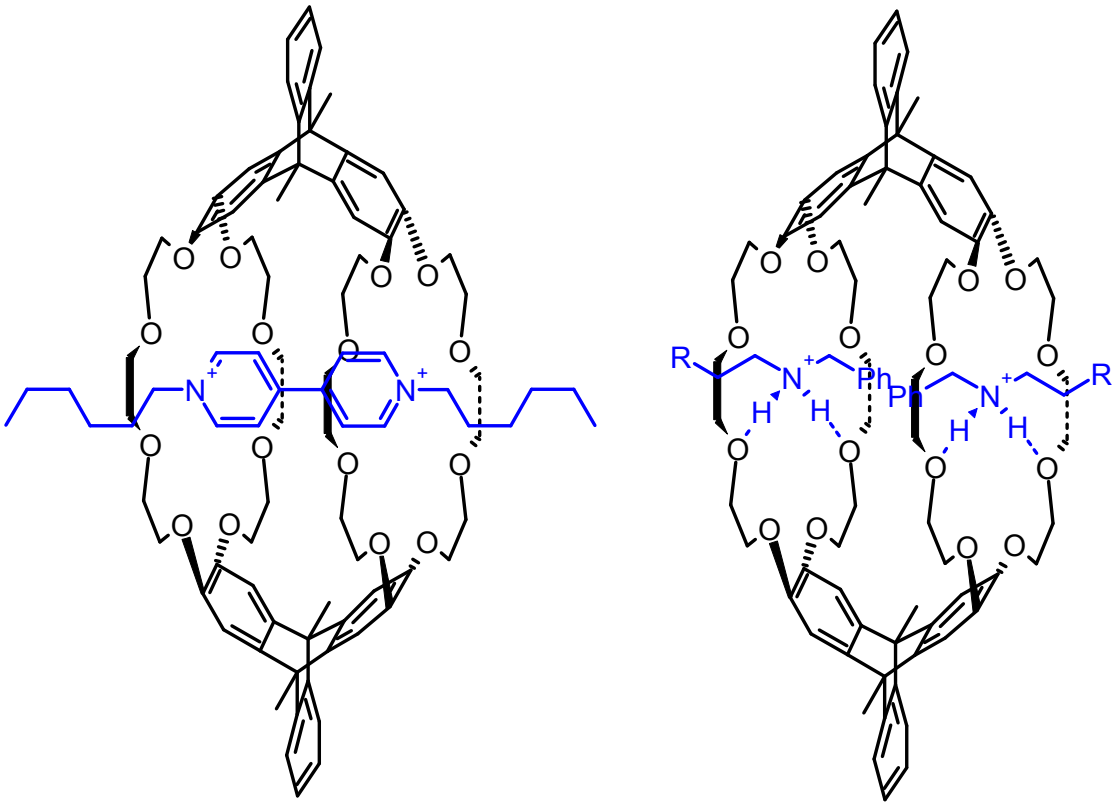


Figure 34: Triptycene-based macrotricyclic dibenzo-[24]-crown-8 ether host **52** and guests

Paraquat and its derivatives are widely used in crown ether rotaxanes and several recent examples on crown ether[137] or cryptand[138,139] complexes with paraquat were published. Such

complexes are not in the scope of this review and the interested reader is kindly referred to the cited literature.

I. 3.5. Crown Ether Ammonium Ion Receptors with Appended Binding Sites for Other Functionalities than Ammonium

Crown ether receptors with appended moieties for the binding of different functionalities in addition to the ammonium ion have been reported. The combination of the luminescent ammonium-binding crown ether (**49a**) with a pendant copper imido diacetic acid complex as imidazole-coordinating site led to receptor **53a**, which coordinates peptides bearing both functional groups with high affinity in buffered water.[140] Increase in emission intensity, visible with the naked eye, signals the guest binding: the response is triggered by the ammonium ion binding to the crown ether unit, which is in water only possible intramolecularily within the assembly. Compound **53** does not respond to the presence of an ammonium group, even in large excess. In the case of His-Lys-OMe a 1:1 complex with a molar binding constant of log K_{ass} = 4.2 is observed. The receptor was applied to the selective detection of small peptides containing *N*-terminal histidine or histidine (**81e**) among all other natural α-amino acids at physiological conditions.

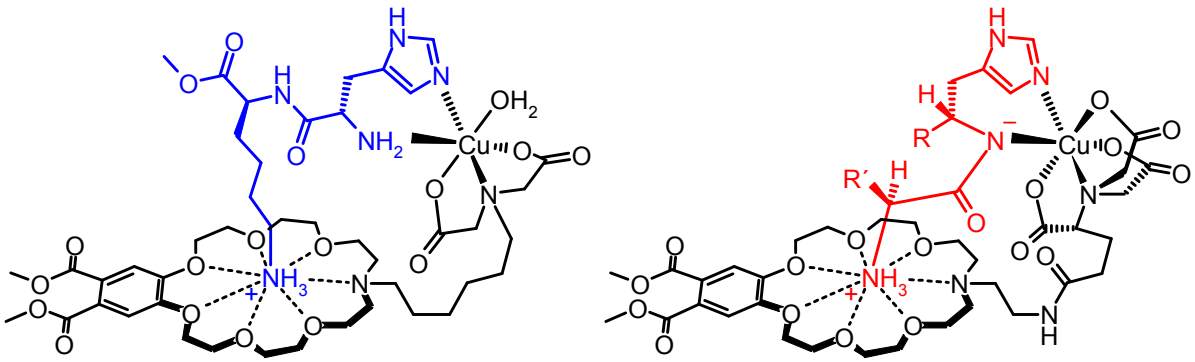
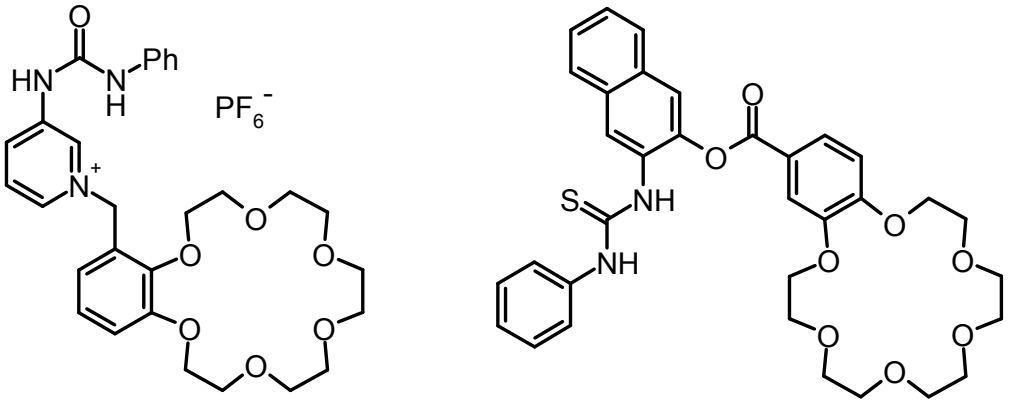


Figure 35: Copper imido diacetic acid azacrown receptor **53a** and the suggested His-Lys binding motif; a copper imido triacetic azacrown receptor **53b** and the target binding area (R = COO^-, $CONHCH_2COO^-$, $CONHCH_2COOCH_3$, $CONHCH_2CONHCH_2CONH_2$; R' = H, CH_3,CH_2-$CH(CH_3)_2$, $CH_2CH_2CONH_2$)

In succession, the combination of a copper(II)-NTA complex with the benzocrown ether led to a receptor (**53b**) that preferably binds to specific histidine-glycine peptide sequences under physiological conditions.[141] Nearly micromolar affinities were observed for Gly-Gly-His (log K_{ass} = 5.8) and Gly-His-Gly (log K_{ass} = 5.8) by emission titrations in HEPES-buffered (pH 7.5) aqueous solution. In tetrapeptides the recognition motif R´-xxx-HGG was identified, in which the *N*-terminal amino acid residue may vary (R´-xxx = Leu, Ala, Gly, Gln). Only the *N*-terminal amino group triggered an emission signal; the ammonium moiety of a lysine side chain did not.

Besides metal complexes, which will be discussed in detail in a later chapter, urea, thiourea and charged binding sites like quaternary ammonium ions or guanidines are often employed as second anchoring functionality for amino acids.

Receptor **54** binds to zwitterionic amino acids via a combination of urea-carboxylate and crown ether-ammonium hydrogen bondings, and thus efficiently transports them across a $CHCl_3$ liquid membrane.[142] The binding properties of **54** were also examined by solid-liquid and liquid-liquid extraction experiments. The amounts of amino acids extracted to the chloroform phase were determined by the ^{1}H-NMR. In comparison to similar compounds missing one of the functional groups, receptor **54** extracted efficiently amino acids with non-polar side chains such as Phe, Ile, Leu, and Trp into the $CHCl_3$. The overall transport efficiencies (Phe > Trp > Ile > Leu > Val >> Ala > Ser >> Asp, His) were consistent with the extraction results (Phe > Ile > Leu > Val > Ala >> Ser, Asp, His, Tyr). No preference of the aromatic amino acids over aliphatic ones was noticed in extraction and transport experiments; no binding constants were reported.



54 **55**

Figure 36: Urea (**54**) and thiourea (**55**) benzo crown ethers for transport and extraction of amino acids

A recent example by Costero et al. employed a comparable heteroditopic ligand (fig. 36) in the solid-liquid extraction of ω-amino acids into DMSO solutions. The prepared ligand contains thiourea or amide groups for anion recognition.[143] Compound **55** is an efficient solid-liquid extractant for lysine (**81c**) and 4-aminobutanoic, 5-aminopentanoic and 6-aminohexanoic acids, reaching the highest value for 4-aminobutanoic acid (GABA). The simultaneous complexation of the anionic and cationic moieties by the ligand gave rise to extraction values much higher than those obtained with the equimolar mixtures of the corresponding monotopic ligands. The introduction of a *para*-nitro group in the phenylthiourea made the extraction process much faster.

The molecular recognition of *S*-amino acids such as asparagine, glutamine, lysine (**81c**) and arginine (**81d**) with crown pyrylium ions **56a** to **56c** as receptors was examined by Moghimi et al.[144] Their receptors use a two point binding of the guest: Ion pairing for the two oppositely charged carboxylate anion and pyrylium cation, and hydrogen bonding between crown ethers' and the amino acid terminal NH's. The terminal NH_2 to COOH distance of *S*-asparagine is best matching with the receptors with crowns located in the *ortho*-position **56b** (K_{ass} = 1290 ± 60 M^{-1}) and **56c** (K_{ass} = 1740 ± 90 M^{-1}). The distance in *S*-asparagine and *S*-glutamine is not long enough for the interaction with **56a**. The binding properties were evaluated by fluorimetric titration in methanol.

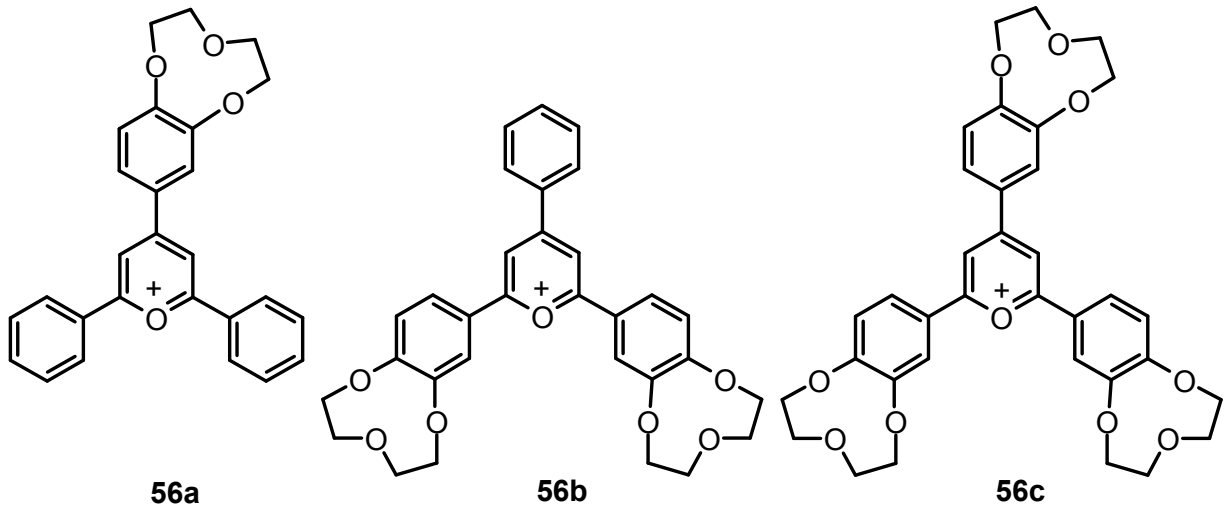
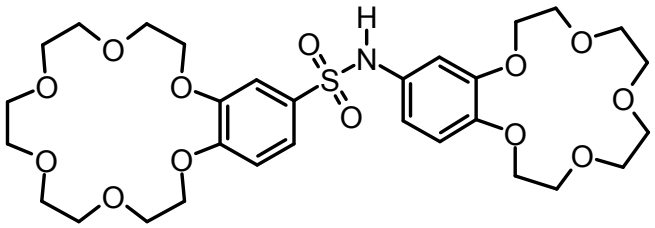


Figure 37: Crown pyryliums ion receptors **56** for amino acids

A different receptor type **57** for zwitterionic amino acids was provided by Barboiu et al.[145] Simultaneous complexation of the ammonium moiety of the amino acid by the benzo-18-

crown-6 cavity and of the sodium ion in the benzo-15-crown-5 cavity induces charge interactions of the carboxylate moiety with Na^+-15-crown-5 and π-π-stacking interactions between the aromatic ring of phenylalanine (**81a**) and the aromatic moieties of **57**. The membrane transport mechanism of phenylalanine (**81a**) through a bulk liquid membrane was achieved and monitored as a function of the co-transported alkali cation.



57

Figure 38: Ditopic sulphonamide bridged crown ether receptor **57**

Structurally related to Voyer´s example **47**, Schneider and Hossain[146] investigated crown ether **58** for peptide binding in water. Here, a peralkylated ammonium group interacts with the peptides carboxylate, while the primary ammonium ion is bound by the benzo crown ether. The bridging amine can be functionalized by a luminescent dansyl group as in **58b** to allow facile optical detection of the binding event and supplies additional hydrophobic interactions to aromatic peptide side chains. Several di- and tripeptides were tested with compound **58a**: Triglycine showed the highest binding affinity in water (K_{ass} = 200 M^{-1}) and methanol (K_{ass} = 13000 M^{-1}) as determined by NMR titration. Fluorescence titrations with **58b** revealed the effect of hydrophobic or π-stacking interactions of the dansyl group. Tripeptides bearing an amino acid with aromatic side chain functionality, such as Trp, showed a significant increased affinity (K_{ass} = 2150 M^{-1} for Gly-Trp-Gly) to **58b** in water compared to triglycine (K_{ass} = 210 M^{-1}).

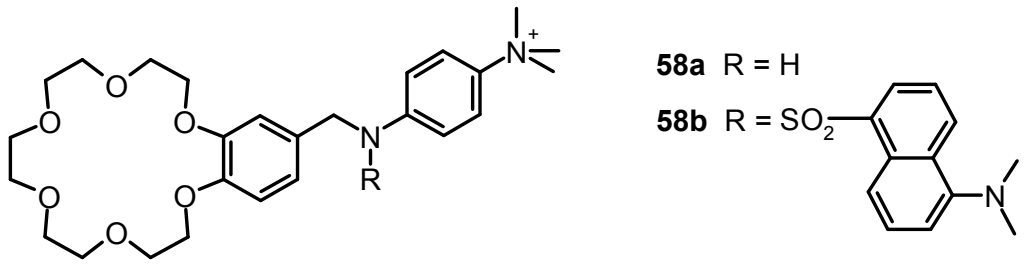


Figure 39: Luminescent peptide receptor **58**

Cooper and James prepared mono-aza-18-crown-6 ether **59** with boronic acid binding site.[147] The additional interaction of boronic acid has been used to create a photoinduced electron transfer (PET) sensory systems for saccharides. Binding studies were carried out in 33.2 % (w/w) ethanol–water buffer, showing selective fluorescent enhancement with *D*-glucosamine hydrochloride (log K_{ass} = 3.31) at pH 7.18. In this medium compound **59** showed no increase with *D*-glucose. For a fluorescent output both a diol and the ammonium group must be present in the guest. The increase in stability can be attributed to a cooperative binding by the boronic acid and azacrown ether.

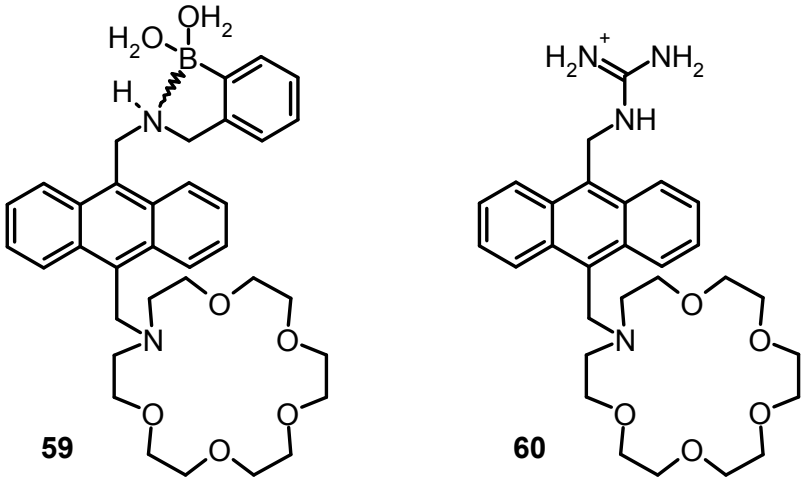


Figure 40: Luminescent receptor **59** for the detection of *D*-glucosamine hydrochloride in water/ethanol and luminescent receptor **60** for ω-amino acids

Guanidines are well known binders for oxoanions like carboxylates.[148] A molecule similar to **59** was introduced (fig. 40) for the recognition of amino acids by de Silva et al.:[149] Chemosensor **60** is capable of recognizing the distance between the two functional groups in methanol/water (3:2) at pH 9.5. Coordination of the carboxyl group to the guanidinium moiety of the receptor has a strong effect on the fluorescence output of the system. As in the former example, upon binding of the ammonium functionality in the crown ether the quenching by the PET of the nitrogen atom´s free electron pair disappears and an enhancement in the fluorescence of the anthracene is observed. 5-Aminopentanoic acid binds with K_{ass} = 84 M^{-1}, while 3-aminopropanoic acid with only K_{ass} = 17 M^{-1}. A limitation of the compound is its similar response to simple amines, e.g. propylamine (K_{ass} = 79 M^{-1}).

Suzuki et al. employed a similar approach for sensing amino acids in receptor **61**, which is based on tri-aza-18-crown-6.[150] The ammonium-ion binding crown ether is substituted by two guanidinium groups interacting with carboxylates and the luminescent anthracene moiety. Upon ammonium ion binding the quenching of the anthracene emission by PET is intercepted leading to an emission increase. The authors did not report binding constants, but describe glycine, lysine (**81c**) and GABA (4-aminobutyric acid) as preferred guests. The emission intensity increased upon addition of GABA to compound **61** in methanol/water 1:2 by a factor of 2.2.

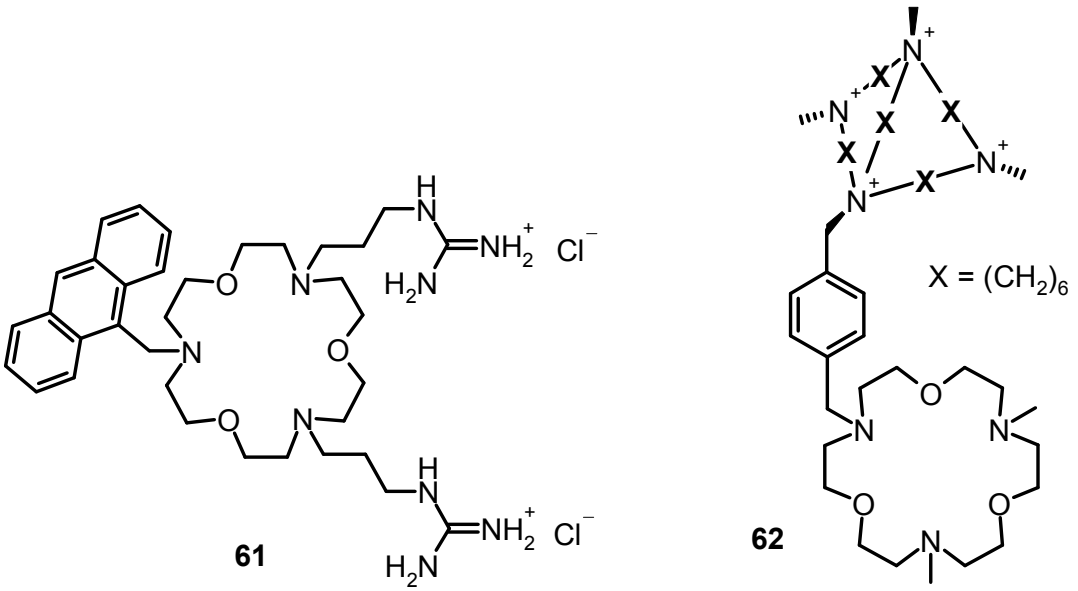


Figure 41: Guanidinium azacrown receptor **61** for simple amino acids and ditopic receptor **62** with crown ether and polyammonium macrocycle for GABA binding

A ditopic receptor **62** for the effective binding of zwitterionic GABA (4-aminobutyric acid) was investigated by Schmidtchen.[151] He combined triaza-18-crown-6 in with a positively charged polyammonium macrocycle (fig. 41) for the construction of the synthetic receptor.

The same group described synthetic receptor **63** with bicyclic guanidinium and aza-crown ether binding sites for amino acid zwitterions.[152] The chiral bicyclic guanidinium salt acts as strong anchor for the carboxylate and the triaza-crown ether binds the ammonium ion. The hydrophobic silyl ether provides additional interactions and facilitates the transfer of hydrophilic amino acid zwitterions into an organic phase. Quantification of the extraction process by radiometry revealed a 1:1 stoichiometry and suggests the zwitterion as the species undergoing phase transfer. Small hydrophilic (Ser, Gly), but no charged amino acids were

extracted. Some enantioselectivity was observed in the transfer of phenylalanine (**81a**, 40 % *ee*). The order of decreasing extractability was Phe > Leu > Trp > Gly, Ser in the case of **63**.

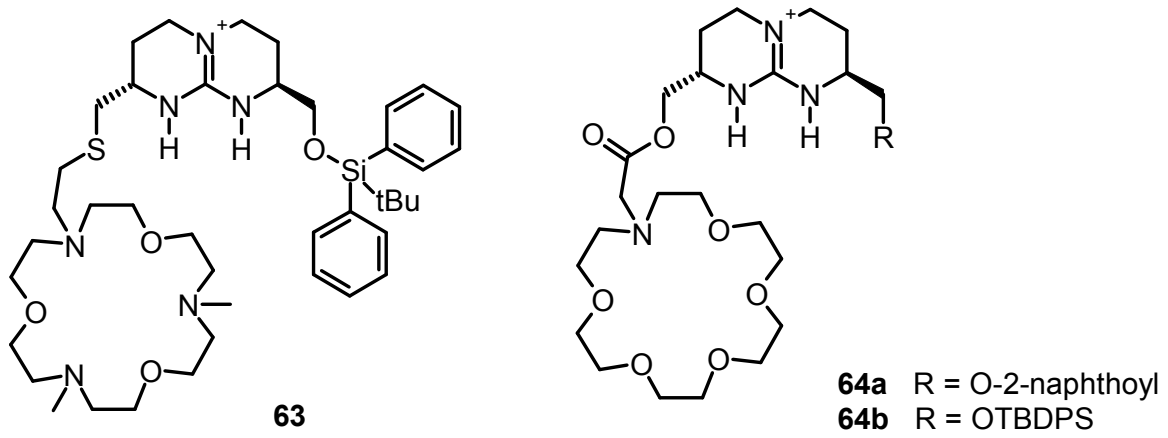


Figure 42: Chiral bicyclic guanidinium azacrown receptor **63** and similar receptor **64** for the enantioselective transport of simple amino acids into organic phases

Comparable artificial carriers based on this bicyclic chiral guanidinium scaffold attached to crown ethers (**64**) or lasalocid A (fig. 42) were able to reach up to 80 % enantiomeric excess in transport experiments separating mixtures of amino acid enantiomers under neutral conditions. Such chiral selectors for underivatized amino acids have been prepared, usually as the (*S*,*S*)-compounds, and evaluated by de Mendoza et al.[153] Crown ethers showed to be superior to lasalocid derivatives, amides were found to be better carriers than esters, though less enantioselective for the transport across the bulk model membranes. Receptor **64a** turned out to be the best "chiral selector", followed by **64b**.

CEAA **65** with appended guanidinium ions or quaternary ammonium side chains, as in **66**, were tested for amino acid recognition in aqueous methanol.[154] By following the binding events by fluorescence und UV-vis spectroscopy in methanol/water 9:1 (vol/vol) compound **65** showed selectivity for γ-aminobutyric acid (K_a = 1300 M^{-1}) over ε-aminohexanoic acid, β-alanine and lysine (**81c**) at pH = 6.5. Compound **66** revealed a pronounced selectivity for $(Gly)_3$ (K_a = 600 M^{-1}) over $(Gly)_2$, γ-aminobutyric acid and ε-aminohexanoic acid at pH 7.4. A 1:1 stoichiometry is always observed. Other amino acids were not bound by both receptors.

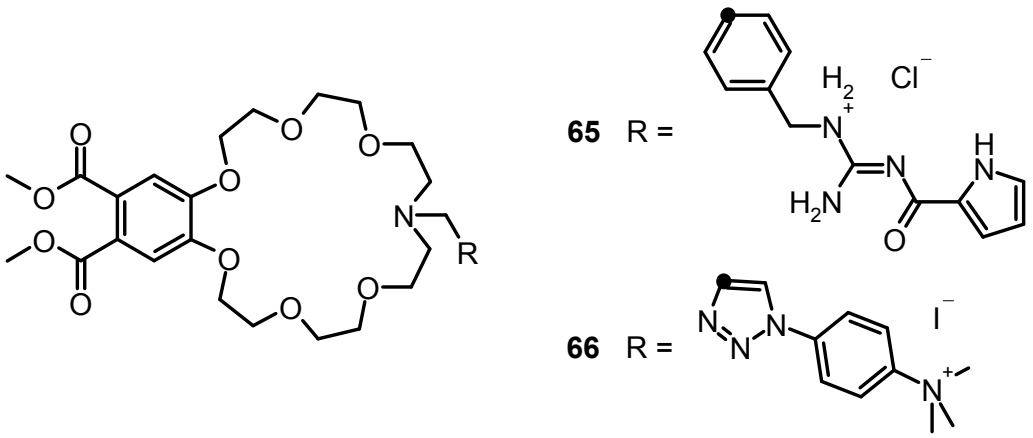


Figure 43: Receptors for zwitterionic species based on luminescent CEAAs

The last examples presented in this chapter combine crown ether ammonium recognition with moieties for coordination or inclusion of unpolar side chains. Extended π-systems like porphyrins, developing hydrophobic or stacking interactions, or carbohydrates and cyclodextrins, binding alkyl- and aryl chains by hydrophobic or van-der-Waals interactions, are discussed.

Cyclodextrins (**136**),[155] cyclic oligosaccharides of six (α), seven (β) or eight (γ) α-1→4 linked *D*-glucose units, can include non-polar guests like alkyl chains or aromatic moieties in their hydrophobic interior mainly by van-der-Waals and hydrophobic interactions. Entropic effects play an important role: The complex formation leads to the release of high-energy water molecules from the cavity of cyclodextrins and is therefore entropically favourable. The selectivity depends mainly on the steric fit, similar to the crown ethers.
Combinations of a diaza-18-crown-6-ether with α-cyclodextrin- (**67a**, **68**) and celobiosyl- (**67b**) residues bind efficiently *S*-arginine (**81d**), *S*-lysine (**81c**) and the anticancer agent busulfan.[156] The Job´s plot analyses indicate 1:1 stoichiometries in all the complexes. Complexation constants (K_{ass}) of ca. 4000 M^{-1} were estimated for [*S*-arginine/**68**], 5500 M^{-1} for [*S*-lysine/**68**], and 6000 M^{-1} for [*S*-arginine/**67b**] and 4500 M^{-1} for the [*S*-lysine/**67b**]. No significant differences between *S* and *R* series could be observed. Busulfan bound to all three ligands with the highest association constant of 1600 M^{-1} for **68**.[157] 2D-NMR results clearly established that a similar mode of complexation is involved for both studied amino acids and the anticancer agent: They are not embedded in the cyclodextrins cavity, but hydrogen bound across the azacrown macrocycle to the urea functions.

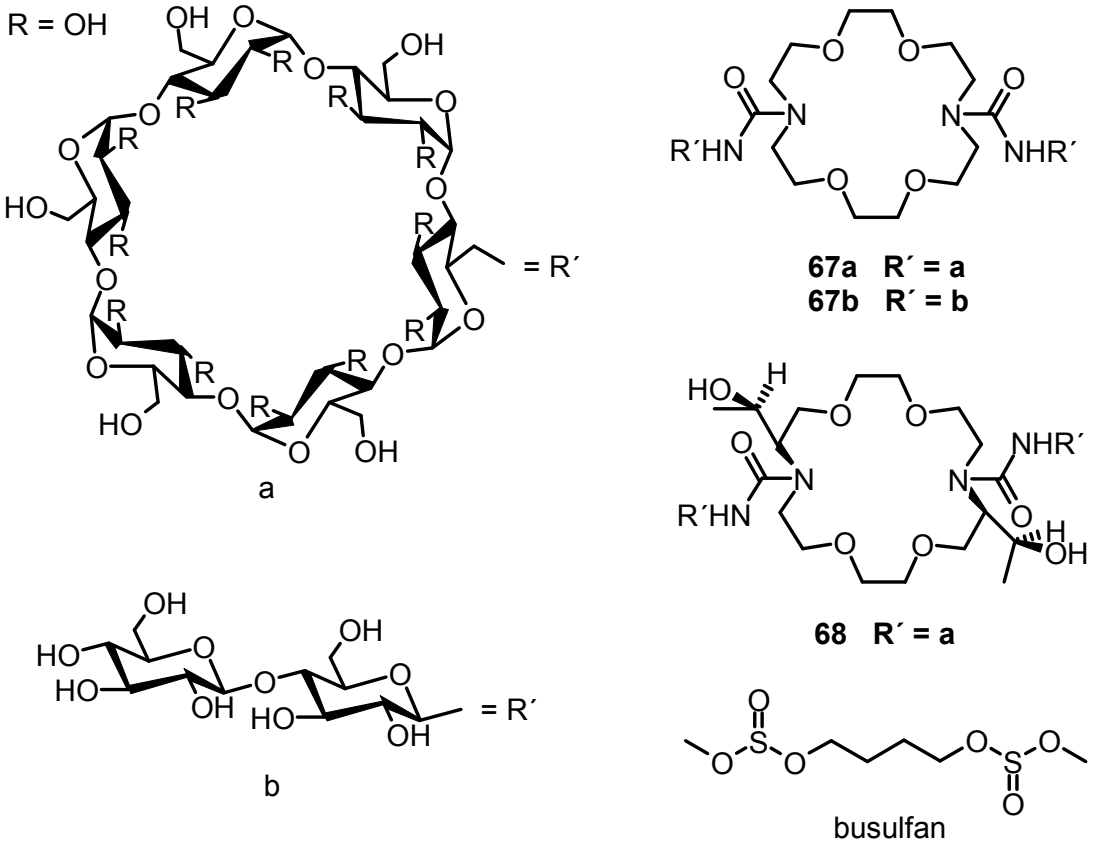
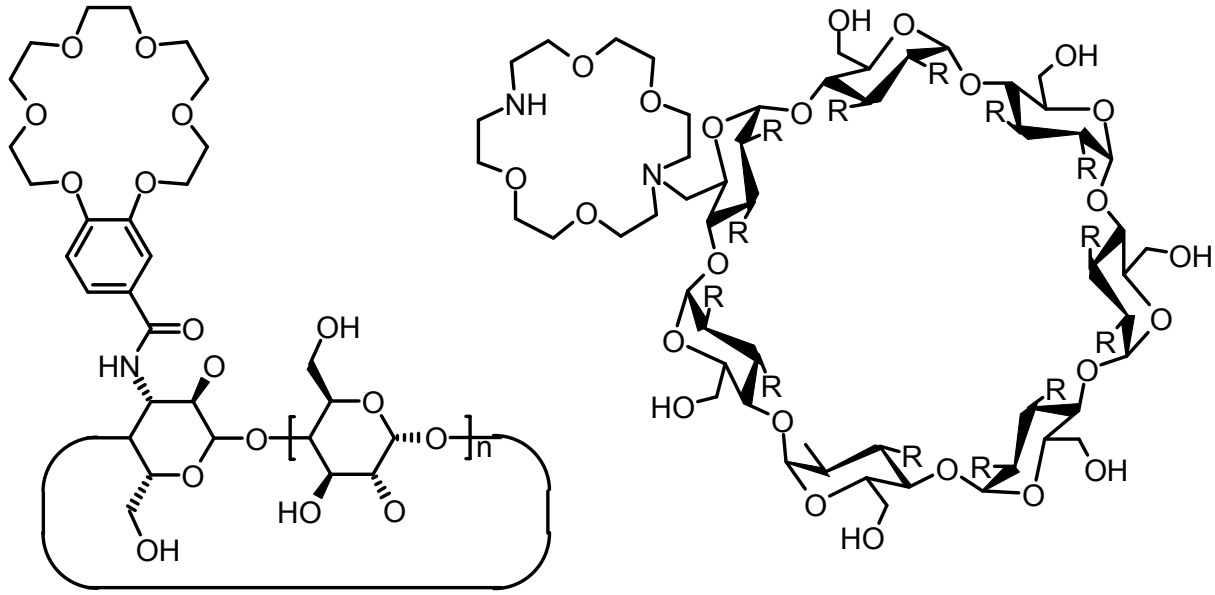


Figure 44: 1,10-Azacrown ethers with sugar podand arms and the anticancer agent busulfan

Another combination of crown ethers and sugars as ditopic receptors was described by Suzuki et al. who used a β-cyclodextrin derivative modified with benzo-18-crown-6 moiety for the recognition of tryptophan (**81b**) in zwitterionic form in water.[158] The molecular recognition ability of **69** was improved by the cooperation of hydrophobic binding by the cyclodextrin cavity and the ammonium cation binding by the benzocrown moiety (188 M^{-1} vs. 31 M^{-1} for single side interaction). 2D-ROESY experiments confirmed that the ammonium cation of Trp is located at the secondary hydroxy side of the cyclodextrin cavity and is recognized by the benzo-18-crown-6 moiety.



69 **70** R = OH

Figure 45: Benzo-18-crown-6 modified β-cyclodextrin **69** and β-cyclodextrin functionalized with diaza-18-crown-6 at primary face (**70**)

The association constant of ammonium ions with 18-crown-6 was reported to be 10 – 17 M^{-1} in water.[58] The β-cyclodextrin **70** functionalized with diaza-18-crown-6 at its primary face (fig. 45) showed a 7- to 10-fold enhanced binding affinity for aromatic ammonium ions in aqueous media compared to unmodified β-cyclodextrin.[159] Compared to **69**, this receptor reveals a binding constant in the same order of magnitude for the aromatic amine guest e.g. Trp. The point of attachment of the crown ether does not significantly alter the ammonium binding ability.

A crown-appended permethylated α-cyclodextrin azophenol **71a** showed a significant, distinguishable colour change, observable with the naked eye, for primary and secondary amines, but no change with tertiary amines, which is a similar analytic distinction as in the Hinsberg test.[160] The system was investigated by UV-vis spectrophotometry in chloroform. Association constants with primary amines were found to range from log K_{ass} = 4.2 to 4.8, for secondary amines from log K_{ass} = 2.0 to 2.3. The selective complexation is explained by H-bonding between the ammonium ion and oxygen atoms of the 18-crown-6.[161] The hydrophobic interaction between the cyclodextrin and the lipophilic tail of the amine in combination with the acidity of the host molecule (pK_a = 5.6) assist the binding.

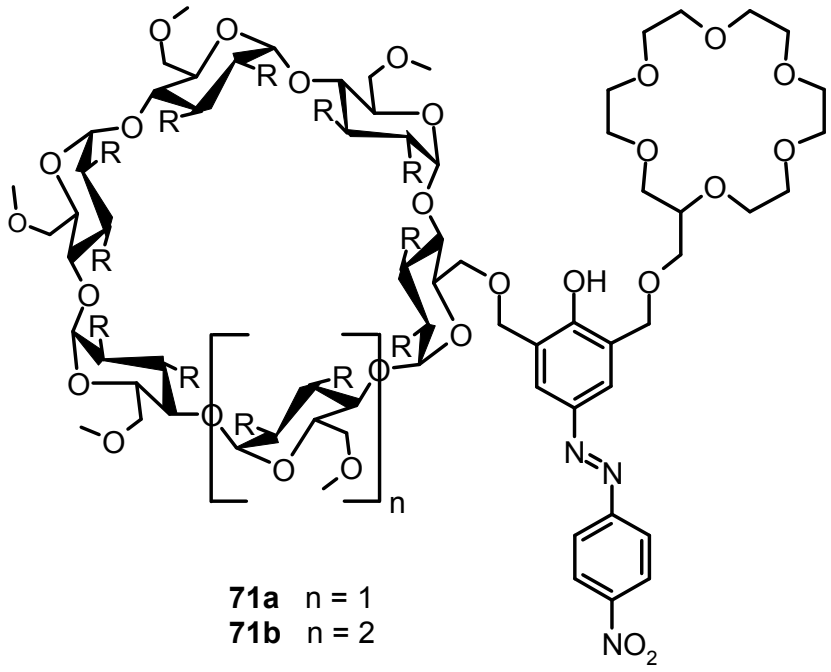


Figure 46: Receptors for colourimetric detection of primary and secondary ammonium ions

The studies were expanded by the related 18-crown-6 azophenol dye with permethylated β-cyclodextrin **71b.** [162] The binding of various amines was investigated by UV–vis spectrophotometry in chloroform. Again, addition of primary and secondary amines shifts the absorbance maximum differently, from 380 nm (yellow) to 580 nm (violet) and 530 nm (pink), respectively, but no change is observed with tertiary amines. The log K_{ass} values are, compared to compound **71a**, generally 5 to 10 % higher (4.25–4.95 for primary, 2.10–2.48 for secondary amines). The selectivity was calculated to be 60–720. Receptors which lack the crown ether moiety, changed from yellow (380 nm) to pink (500 nm) upon addition of amines, but with no selectivity and binding constants being one order of magnitude lower. NMR spectroscopy indicated the formation of 1:1 complexes and the inclusion of the alkyl chain in the cyclodextrin by a strong shift of the CH_2-protons. In a competition experiment, *n*-propylamine was added to the chloroform solution of **71b** containing 2000 equiv. of triethylamine. Already a small amount of the *n*-propylamine resulted in a marked increase in absorption intensity. In the reverse case no spectral changes were observed.

The formation of efficient H-bond interactions of the ammonium ion to the oxygen atoms of the crown ether and their number, the hydrophobic interaction between the cyclodextrins and the lipophilic tail of the amine and the acidity of the host molecule determine the selectivity and binding strengths of these ditopic receptors.

The following examples are crown ether – porphyrin conjugates. In these examples the ammonium ion binding takes place at the crown ether moiety. Ammonium ion binding using porphyrin based binding sites will be discussed later in this survey.

Schneider et al. described a water-soluble host compound with three pyridinium units and one spacer-connected benzocrown ether unit in the meso-positions of porphyrin and its Zn(II) or Cu(II) complexes.[163] They investigated the complexation constants of unprotected di-, tri- and tetrapeptides with the metal-free and the metalated hosts in water. Metalation led to small changes of the selectivities towards different peptides compared to the apo-derivative, with complexation constants in water of $10^5\,M^{-1}$ to $10^6\,M^{-1}$. One complex containing the tripeptide Gly-Gly-Phe was analyzed in detail by COSY, HSQC, HMBC, and NOESY NMR experiments clearly showing complexation of the ammonium ion in the crown and π-π-stacking interactions of the phenyl of Phe with the porphyrin. Peptides containing aromatic side chains were always bound better than the corresponding simple oligo-glycines. The titration curves showed isosbestic points, in line with the expected 1:1 complexes, which were supported by very good nonlinear least-squares fits to a 1:1 model.

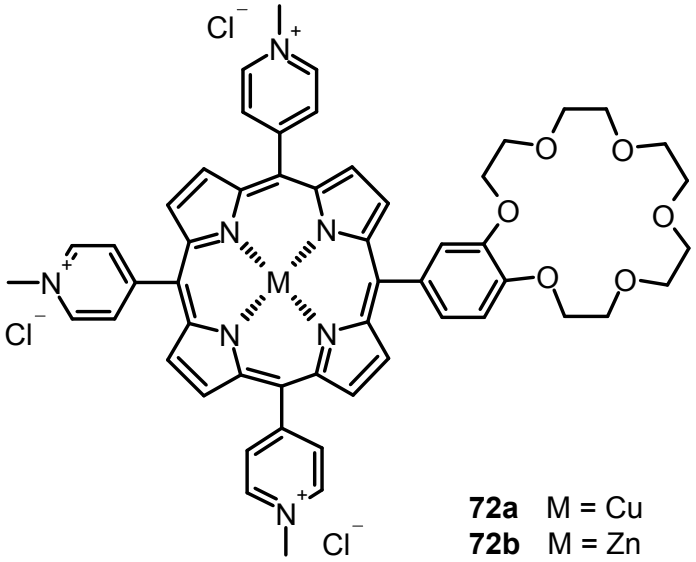
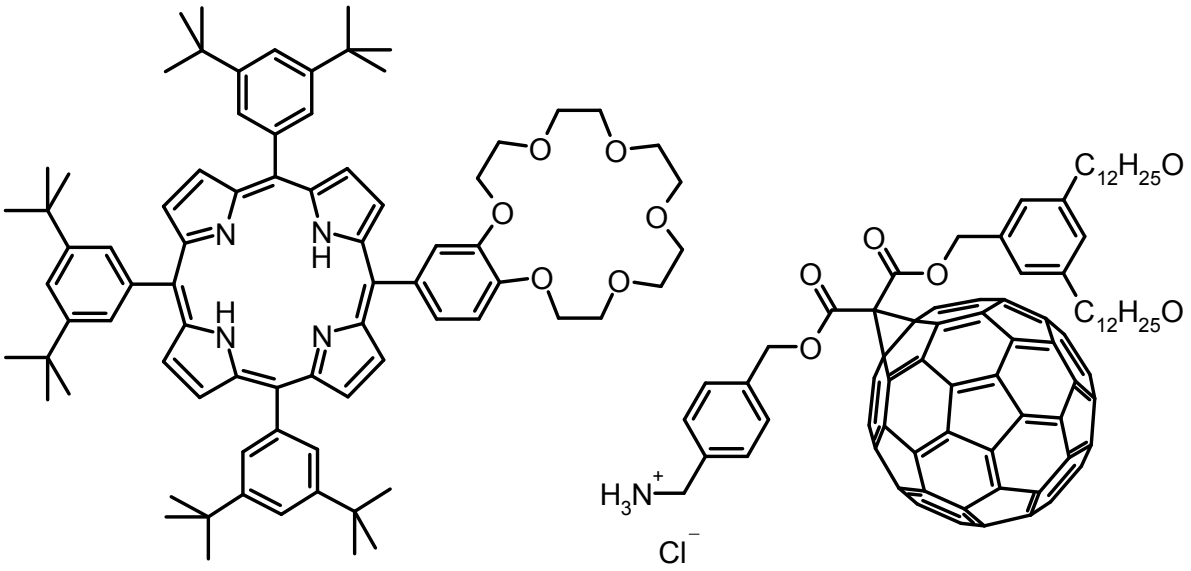


Figure 47: Porphyrin-crown-receptors **72**

Nierengarten et al. investigated the ability of a methanofullerene derivative with an ammonium subunit to form an aggregate with a porphyrin–crown ether conjugate by NMR, UV–vis, electrospray mass spectrometry and luminescence experiments.[164] In addition to the ammonium–crown ether recognition they found intramolecular stacking of the fullerene moiety to the porphyrin subunit. Due to this additional recognition element, the association

constant for the aggregate was increased by two orders of magnitude when compared to the K_{ass} values found for the complexation of **74** with the crown ether (2100 M^{-1} in $CDCl_3$). The value is consistent with association constants reported for associates resulting from ammonium–crown ether interactions.[165]



73 **74**

Figure 48: Porphyrin-crown ether conjugate **73** and fullerene-ammonium ion guest **74**

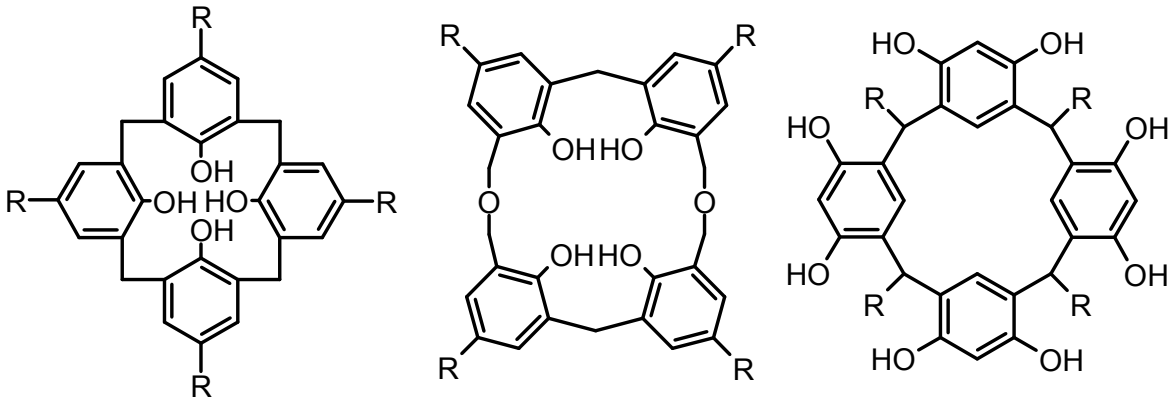
The broad variability of crown ethers allows manifold adaption to a specific tasks: A variety of crown ether receptors for cooperative recognition of ammonium moieties in diamines, for transport and effective enantioselective recognition of amino acids, as esters or in zwitterionic form have been described. Crown ethers have been widely used for the recognition of primary organoammonium compounds as found in amino acids, neurotransmitters like GABA and other biological important molecules like dopamine (**2**).

I. 4. Calixarenes, Resorcarenes and Cavitands

Calixarenes are versatile host molecules for ammonium ions with unique structure and complexation properties. In this chapter we would like to emphasize the approaches for ammonium ion recognition with calixarenes and related molecules. We will start our survey with simpler substitution patterns and proceed then to more complex substituted calixes: molecules for enantiodiscrimination, for colourimetric assays and capped calixarenes will be in the focus. Resorcarenes are the next discussion point, followed by deeper cavities, ditopic receptors, and finally capsules.

I. 4.1. Basic Examples with Simpler Substitution Pattern

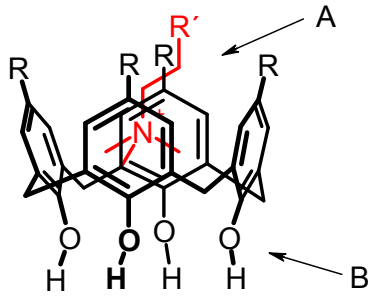
Calixarenes and resorcarenes (**75)** belong to the most versatile building blocks in supramolecular chemistry. Several books and reviews covering their synthesis, structural properties and applications have been published.[166] A variety of methods for the synthesis and functionalisation of the macrocycles has been developed.[167,168] Synthesis and application of resorcarenes and *O*-alkylated derivatives has been comprehensively summarized. [169] Calixarenes like **75a** resemble a vase like (chalice) shape, but are not completely rigid. They may form many conformational isomers by the rotation of the phenol units through the annulus, thus affording a large number of unique cavities with different size and shape. Homooxocalix[4]arenes (example **75b**) and their methyl esters are more recently studied examples.[170] Together with the structurally related resorcin[n]arenes (example **75c**) and calixpyrroles, calixarenes are used in a variety of applications, such as chromo- and fluorophores[171] for metal ion binding in solution,[172,173] anion complexation[174] and binding of neutral guests,[175] as potentiometric sensors[176] in ion selective electrodes[177] or as molecular switches.[178] The aromatic cavity of calixarenes is an excellent model for the investigation of cation–π-interactions.[8,179,180]



75a **75b** **75c**

Figure 49: Calix[4]arene (**75a**), homooxocalix[4]arene (**75b**) and resorc[4]arene (**75c**) compared (R = H, alkyl chain)

A calix[4]arene includes ammonium ions in its pre-organized cone cavity via electrostatic attraction between the positive charge of the guest and the electron rich faces of the aromatic rings ("cation-π-interaction").[181,182] The inclusion of alkyl ammonium ions in the cavity of calixarenes is therefore reflected in a high field shift of the host signals in the ^{1}H-NMR spectrum. Based on the magnitude of the shifts of the different host signals conclusions can be drawn on the preferred orientation of the guest in the cavity.[183]



75a + guest

Figure 50: Calix[4]arene and ammonium ion guest (R = H, alkyl, OAcyl etc.), possible binding sites; *A: coordination of cationic or neutral guests (cation-π-interaction), B: binding site for cationic guests (ion-dipole-interaction or H-bonding)*

Gutsche et al. reported the complexation of aliphatic amines by alkylcalix[4]arene with a binding strength in the order of 10^4 M^{-1} in acetonitrile.[184,185] The contribution of cation-π-interactions to the binding was demonstrated for several examples of complexes with

quaternary ammonium[186, 187] or tetralkylammonium[181, 188] salts in organic media. Proton transfer from OH-groups of the calixarene to the amine, followed by association and inclusion is a different binding situation: The guest is coordinated by a tripodal H-bonding.[185, 184, 189] The complexation behaviour seems to be mainly determined by the conformational mobility of the calix. Control of the conformational properties of these macrocycles is crucial for their applications in supramolecular chemistry.

Typical guests (fig. 51) in studies with calixarenes and resorcarenes utilizing the explained modes of interaction are the physiologically relevant quaternary ammonium compounds choline (**76**), acetylcholine (**3**), carnithine (**77a**) and acetyl-carnithine (**77b**), as well as the salts of the aromatic amines 2-phenethylamine (**78a**), dopamine (**2**), ephedrine (**79a**), norephedrine (**79b**), adrenalin (**80a**) and noradrenalin (**80b**).

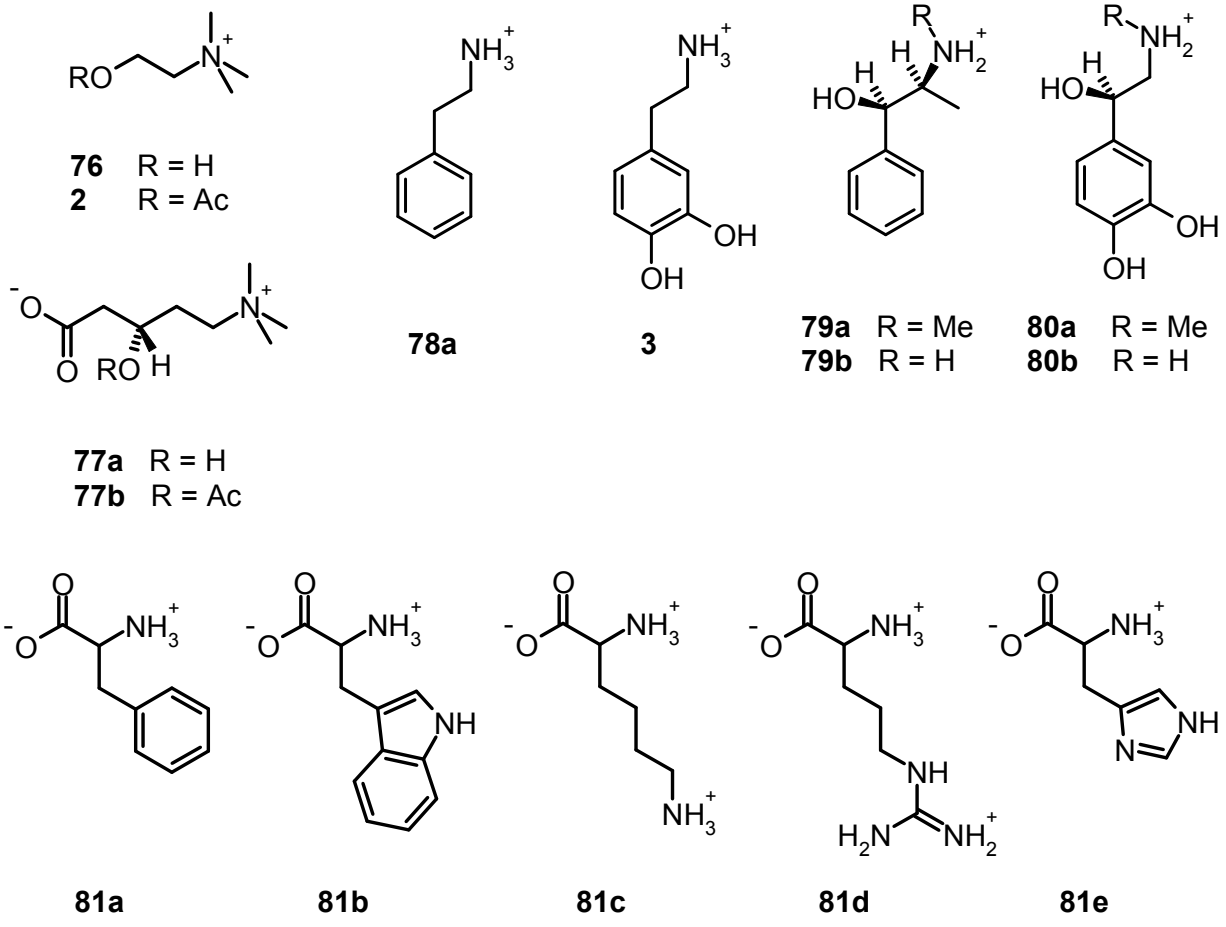
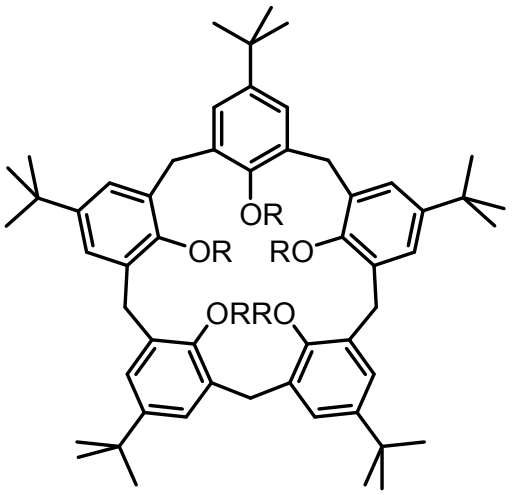


Figure 51: Typical guests for studies with calixarenes and related molecules

Additionally, amino acids and their derivatives are also bound by calixarenes, especially aromatic amino acids like phenylalanine (**81a**) or tryptophan (**81b**) or the basic representatives like lysine (**81c**), arginine (**81d**) and histidine (**81e**) and peptides containing

these residues. Similar to larger crown ethers (24-crown-8 and bigger) or cyclodextrins, calixarenes may also be threaded to form rotaxane like structures. A common guest for this is paraquat. The reader is referred to the literature covering this topic.[190] We discuss now some recent examples in ammonium ion recognition with the calixarene class of receptors and focus on the recognition of these ammonium targets e.g. *N*-terminal peptide recognition, preferably in water and/or under neutral conditions. The binding of metal ions is not covered and was already reviewed.[172] For detailed thermodynamic data we refer the readers to articles of Izatt et al.[89] and Namor et al.[191] Recognition of biochemical targets was recently covered comprehensively by Ludwig.[192] Biros and Rebek have summarized the application of water soluble resorcarenes for the recognition of ammonium ions in their recent review.[193]

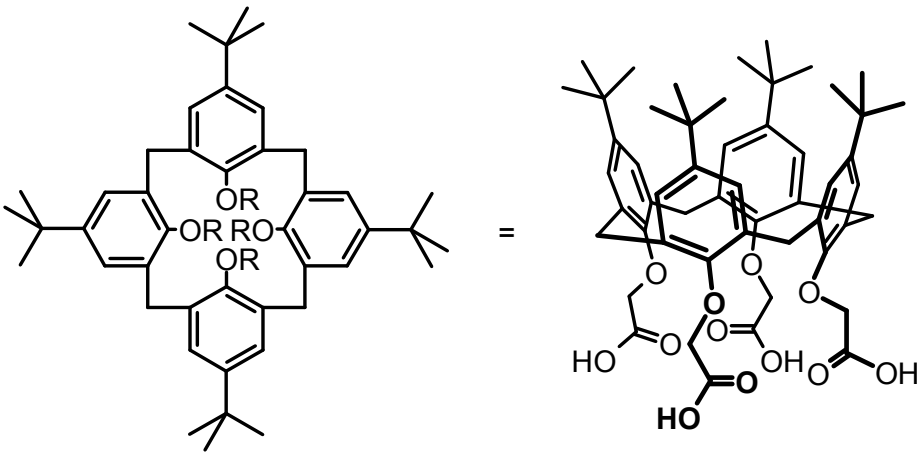
In the simplest case, only one side of the calixarene skeleton is substituted. For example, *p-tert*-butylcalix[5]arene **82** modified at the lower rim[194] was investigated in $CDCl_3/CD_3OD$ (9/1). The binding affinities of isomeric butylammonium picrate salts show high log K_{ass} values with the *n*-$BuNH_3^+$ ion ranging from 4.63 to 6.47, while other branched cations, such as *tert*-$BuNH_3^+$ give significantly lower values. The stability of the complexes generally decreased in the order: **82d** > **82a** > **82b** > **82c** for one given isomer, with the highest selectivity of calix[5]arenes **82a** and **82d** towards *n*-$BuNH_3^+$ ion. The presence of *tert*-butyl substituents on the upper rim is essential to force the molecule into a regular C_{5v} cone conformation and ensure selective inclusion of R-NH_3^+-ions. Receptors **82a** and **82d** formed 1:1 inclusion complexes only with Na-Ac-Lys-OMe hydrochloride and Lys-Gly-OMe dihydrochloride. In the latter the ε-butylenammonium group was recognised by the cavity and complexed in the presence of an unprotected α-ammonium group. The methyl ester hydrochlorides of the neurotransmitter γ-aminobutyric acid (GABA) and the related plasmin inhibitor ε-aminocaproic acid (ε-Ahx)[195] were also strongly included with degrees of complexation up to 80 %



82a R = $(CH_2)_3CH(CH_3)_2$
82b R = $(CH_2)_2OCH(CH_3)_2$
82c R = $CH_2COOCH(CH_3)_2$
82d R = $CH_2COOC(CH_3)_3$

Figure 52: Lower rim modified *p-tert*-butylcalix[5]arenes **82**

Similar to the unsubstituted calixarenes such examples are only poorly soluble in water and polar substituents are required to increase water solubility. Several examples of water soluble calixarenes bearing phosphonate,[196] amino acid[197] or neutral groups[198] at the upper rim have been reported already in the 1990´s. Arduini et al. reported the first example of a water soluble calix[4]arene (fig. 53) in the fixed cone conformation. It carries four carboxylate groups at the lower rim, but shows no inclusion of neutral molecules in water.[199]

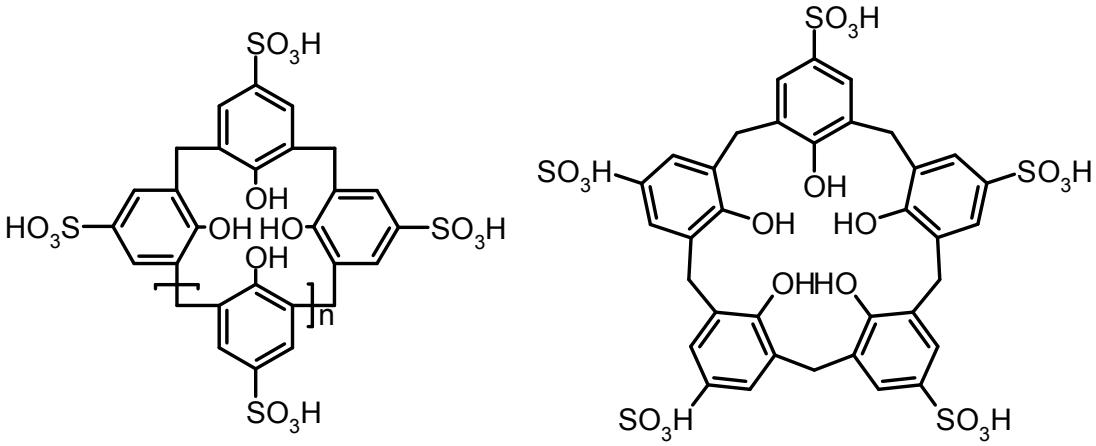


83 R = CH_2COOH

Figure 53: The first example of a water soluble calixarene

Sulfonated calix[n]arenes (**84,** n = 4, 6, 8)[186] are well water soluble. They complex trimethylanilinium cations (K_{ass} for n = 4 is 5600 M^{-1}) and adamantyltrimethylammonium cations (K_{ass} for n = 4 is 21000 M^{-1}) in water.[200] Studies by Gokel and Kaifer on the inclusion

of ferrocene derivatives in water showed that calix[6]arene hexasulphonate (**84b**) is a good receptor for the complexation of a bulky trimethylammonium ion with a association constant of K_{ass} = 10930 M^{-1}.[201]



84a n = 1
84b n = 3
84c n = 5

84d

Figure 54: Sulfonated water soluble calix[n]arenes that bind ammonium ions

Later, the investigated scope was expanded to the corresponding calix[5]arene (**84d**). The inclusion of tetramethylammonium and ditopic trimethylammonium cations was studied at neutral pH by ^{1}H-NMR and compared to the homologous tetrasulphonatocalix[4]arene (**84a**).[202] The more flexible host exhibits a more efficient and selective complexation of ditopic methylammonium ions compared to the more pre-organized calix[4]arene receptors (**84a**). This is a rare case of molecular recognition by induced fit enhancing affinity and selectivity.

Utilizing the outstanding complexation properties of calixarenes for quaternary ammonium ions, the binding of acetylcholine (**3**) has gained a lot of interest due to its biological importance as a neurotransmitter. It has been shown, that the cationic ammonium group of acetylcholine (**3**) binds to the aromatic cavity of calixarenes through cation-π-interactions (see also later examples of **75c**, **115c, 116, 117, 118** and **126a/c**).

Compound **84b** was used to sense the presence of acetylcholine (**3**) in neutral aqueous or water/methanol solution. The sulphonatocalix[6]arene binds acetylcholine (**3**) preferably over primary and secondary amines, which allows the use of the pyrene indicator **85** in a displacement assay (fig. 55). Upon displacement of the fluorescent pyrene cation by **3** the binding event is signalled by the increased fluorescence intensity of **85** in solution.[203]

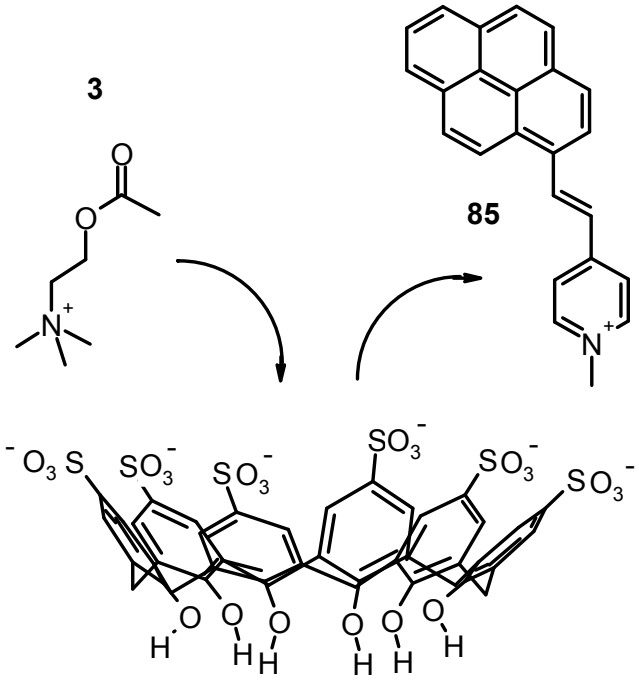


Figure 55: Displacement assay for acetylcholine (**3**) with a sulphonato-calix[6]arene (**84b**)

The affinity of the *p*-sulphonatocalix[n]arenes (**84**) (n = 4, 6, and 8) towards amino acids was also extensively investigated by ^{1}H-NMR,[204, 205, 206] microcalorimetry[206, 207] and HPLC-methods.[208]

The *p*-sulphonatocalix[4]arenes formed 1:1 complexes more strongly with basic amino acids with K_{ass} values for Arg and Lys of 1520 and 740 M^{-1}, respectively (phosphate buffer at pH 8), than with aliphatic or aromatic amino acids: Val, Leu, Phe, His, Trp, with K_{ass} values between 16 M^{-1} and 63 M^{-1} (phosphate buffer at pD 7.3).[205,209]

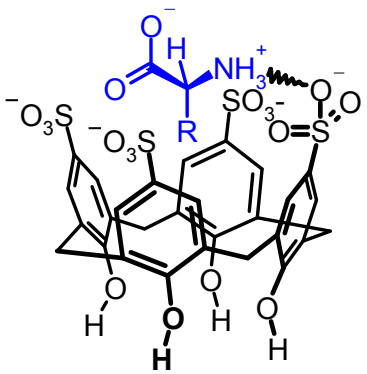
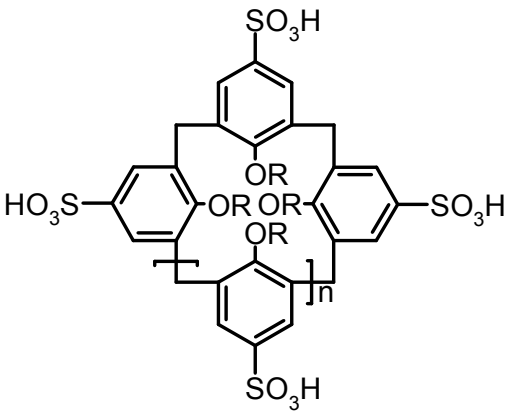


Figure 56: Amino acid inclusion in *p*-sulfonatocalix[4]arene (**84a**)

The basic amino acids arginine (**81d**) and lysine (**81c**) show strong electrostatic binding to calix[4]arene sulphonate at pH 5. For higher calixarenes, only weak interactions at the faces of the flattened macrocycles occur. This binding is in contrast to the inhibition of protein-protein interactions by the calixarenes where the calix[6]arene and calix[8]arene sulphonates show much stronger effects.[204]

Their application as glycosylaminoglycan (GAG) mimicry[210] was demonstrated by the binding thermodynamics towards certain di- and tripeptides bearing lysine (**81c**) or arginine residues in aqueous buffer at pH 8.0.[209] Due to their key role in these peptide sequences present in GAG recognition sites, arginine (**81d**) and lysine (**81c**) were also applied as guests in the titration microcalorimetry and NMR studies. The simple amino acids were bound with $K_{ass} = 10^3$ dm^3 mol^{-1}. With the corresponding dipeptides an increase in binding of 3- to 4-fold, with the tripeptide of 5- to 8-fold was observed in comparison to Arg or Lys, respectively. More interaction sites were involved in their binding. Mixed Arg-Lys-peptides bound sequence-independently comparably strong. The selectivity order (Arg > Lys > other amino acids) is kept in peptides and is governed by hydrophobic interactions between the calixarene cavity and the aliphatic or aromatic guest moiety. The apolar part of the peptide inserts into the cavity.

Ungaro et al. introduced sulfonate groups instead of the bulky *tert*-butyl groups in **83**,[211] resulting in more flexible hosts [n = 1; X = H (**83**) and SO_3H (**86a**); R = CH_2COO^-]. From compound **83** to **86a** a significant increase in log K_{ass} values for the binding of organic ammonium ions was observed: 1.7 and 3.3 for benzyl-NMe_3^+ or 1.7 and 3.4 for *p*-nitrobenzyl-NMe_3^+ respectively.[212] The inclusions were enthalpically driven and entropically unfavoured.



86a n = 1; R = CH_2COOH
86b n = 2; R = CH_2COOH
86c n = 3; R = CH_2COOH

Figure 57: Calixarene receptor family **86** with upper and lower rim functionalisation

Calix[5]arenepentasulphonates (**86b**) bind trimethylammonium ions in water (pD 7.3) with association constants between $4.0*10^3$ and $1.3*10^5$ M^{-1}. The alkylammonium group is completely immersed in the cavity.[202] The corresponding calix[6]arene (**86c**) binds a variety of amino acids in water. The highest binding affinities were found for aspartic acid, arginine (**81d**) and tryptophan (**81b**, K_{ass} = $4.1*10^3$ M^{-1}, $3.6*10^3$ M^{-1} and $2.5*10^3$ M^{-1}). Coleman et al. investigated a similar calix[6]arene with one carboxyl group at the lower rim in amino acid recognition in water.[213] The selectivity changed in favour of asparagine (log K_{ass} = 3.82 for **87a** and 3.61 for **87b**). These most stable complexes resulted from the double H-bonding, which is known from carboxylate dimers. Similar contributions could be observed for arginine (**81d**) and lysine (**81c**). Additional π-π-interactions stabilized the complexes with aromatic amino acids; the hydroxy or thiol groups in cysteine and serine showed no effect on the complex stability. In summary, the 1:1 complex stability follows the following order: acidic > aromatic ~ basic > aliphatic ~ polar amino acids. The more polar compound **86b** binds non-polar guests weaker.

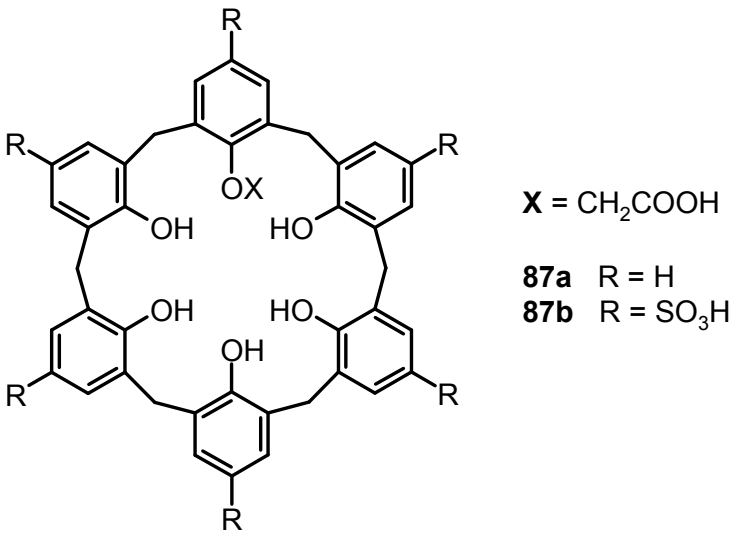
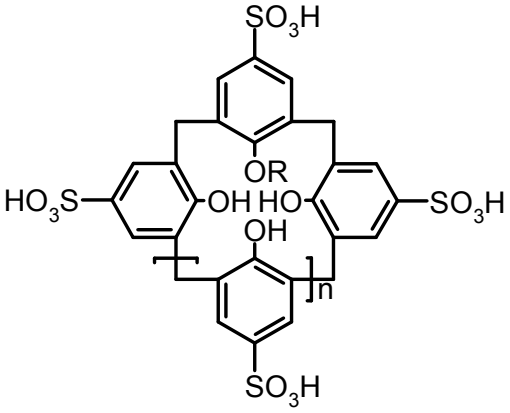


Figure 58: Calix[6]arenes **87** with one carboxylic acid functionality

Consequently, da Silva and Coleman studied complexing properties of *p*-sulphonatocalix[n]arenes (n = 4, 6, 8) mono-functionalized at a phenolic oxygen towards 11 amino acids by means of ^{1}H-NMR spectroscopy in unbuffered aqueous sodium hydroxide solution (pH 8.0) and compared them to the unsubstituted parent calixarenes.[214] In general, the receptors follow the trends discussed above: Arg and Lys, and sometimes His are bound more strongly than Gly, Ala, Leu, Pro, Phe and Trp. Receptors with acid functionality (**88a**,

89a and **90a**) often show higher binding values for the basic amino acids. Especially noteworthy is the enhanced complexing ability for aspartic acid with K_{ass} values ranging from 2200 (**88b**) to 2500 M^{-1} (**90b**) for the amide functionalisation, 2800 (**88a**) to 3200 M^{-1} (**90a**) for the acid functionality and, not surprisingly observing the highest values of 5600 M^{-1} (**88c**) to 5400 M^{-1} (**90c**) for the amine substitution pattern. Ser bound strongly to **88a** with K_{ass} = 3555 M^{-1} attributed to its additional hydrogen bonding site and the optimal fit.



88a n = 1; R = CH_2COOH
88b n = 1; R = CH_2CONH_2
88c n = 1; R = $CH_2CH_2NH_2$

89a n = 3; R = CH_2COOH
89b n = 3; R = CH_2CONH_2
89c n = 3; R = $CH_2CH_2NH_2$

90a n = 5; R = CH_2COOH
90b n = 5; R = CH_2CONH_2
90c n = 5; R = $CH_2CH_2NH_2$

Figure 59: Sulfonated calix[n]arenes with mono-substitution at the lower rim systematically studied on their response to amino acids

The formation of complexes between derivatized cyclotetrachromotropylene host (**91**) and Ala, Asp, and Lys in aqueous solution at pD 1.0 was also investigated.[215] For tetraalkylammonium ions the hosts reveals the same stability trend as has been reported for the 1:1 complexes of *p*-sulfonatocalix[4]arene (**84a**). The K_{ass} values, reaching $2.7*10^4$ M^{-1} for the complexation of Et_4N^+ in D_2O, are in the same order of magnitude as for **84a.** A similar behaviour is observed for amino acids. The basic representative lysine (**81c**) is bound best in a 1:1 complex with the host observing a K_{ass} value of $2.0*10^3$ M^{-1}. The binding values for aspartic acid and alanine were substantially smaller (250 M^{-1} and 70 M^{-1}, respectively).

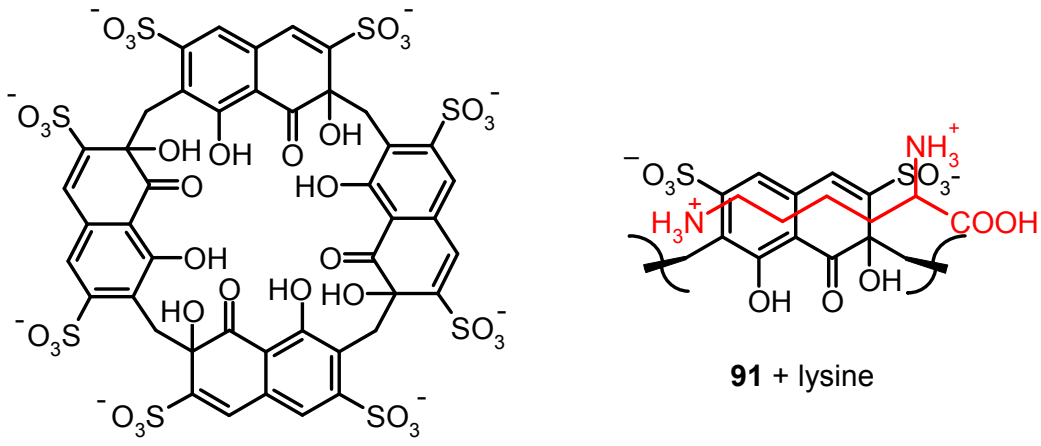


Figure 60: Cyclotetrachromotropylene host (**91**) and its binding to lysine (**81c**)

The non-covalent phosphate–ammonium interaction not only plays a key role in living systems for many critical molecular recognition processes, it can also inspire the design of water-soluble artificial receptors.

The influence of phosphonic acids groups instead of sulfonate groups at the upper rim of calix[4]arenes was investigated. Witt et al. were interested in the complexation properties of water-soluble calix[4]arenes based cavitands with (*1R,2S*)-(−)-ephedrine (**79a**), (*1R,2S*)-(−)-norephedrine (**79b**), (*R*)-(−)-noradrenalin hydrochloride (**80b**), and 2-phenylethylamine hydrochloride (**78a**) in phosphate buffer at pD 7.3.[216] The host molecules were intended mimicking the adrenergic receptor. The participation of the calixarene hydrophobic cavity was confirmed and the structural requirements for the binding of the ammonium ion guests were investigated. The host compounds were able to form 1:1 complexes with an association constant K_{ass} of up to 145 M^{-1} (2-phenylethylamine hydrochloride (**78**) – **92b**). The aggregate stoichiometry was confirmed by a Job´s plot analysis. For ammonium type guest stronger interaction is observed when phosphonic acids groups are attached at the upper rim (K_{ass} for **92b** > **92a**).

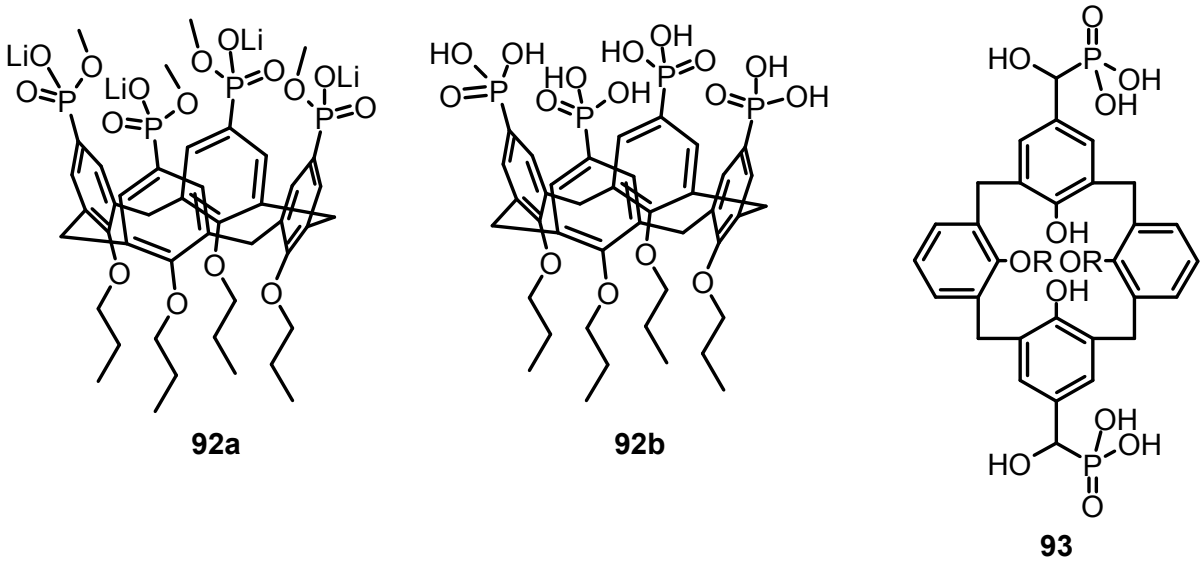


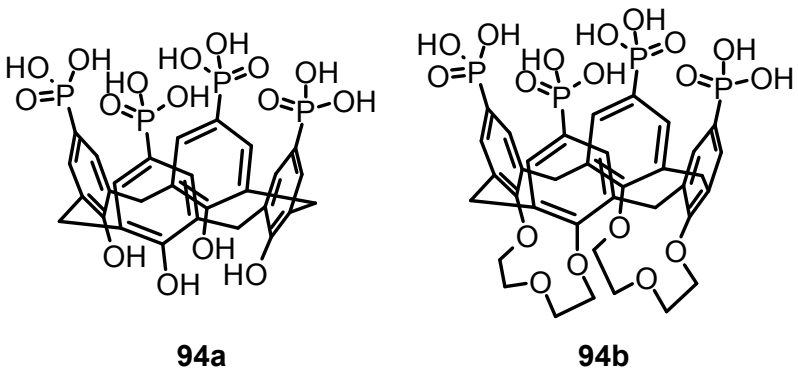
Figure 61: Calixarenes **92** and **93** with phosphonic acids groups

A similar receptor for amino acids was studied by Zielenkiewicz et al. (fig. 61). His group investigated the thermodynamics of distally substituted bis-(dihydroxyphosphoryl-hydroxymethyl)-calix[4]arene at the upper rim **93** in racemic form in the binding of several amino acids[217] and dipeptides[218] in methanol by isothermal titration calorimetry, NMR and UV–vis spectroscopy. Free amino acids as well as dipeptides gave strong 1:1 complexes. The complex stability correlates with the hydrophobicity of the amino acid residues and decreases with decreasing hydrophobicity: Ile > Leu > Val > Ala > Gly with log K_{ass} = 4.23 for Ile and 3.84 for Gly. Neutral aliphatic and aromatic amino acids are better bound than basic ones. The stability constants for dipeptides were in a similar range of 25000 - 45000 M^{-1}, enthalpy changes in the range of - 10.5 to - 5.9 kJ mol^{-1} and - 26.5 to - 25.3 kJ mol^{-1} in the estimated Gibbs free energy, respectively. The complexation phenomenon was found to be driven by electrostatic interactions between the protonated *N*-terminal amino group of the guest and the calixarene phosphoryl groups.

Water soluble calix[4]arenes with one, two or four dihydroxyphosphoryl groups at the lower rim can form salts with (*1S,2R*)-(+)-ephedrine and 2-phenylethylamine hydrochloride.[219] The salts of these inherently chiral calixarene phosphoric acids with the chiral amines are easily separated into diastereomeric forms.

Based on the results of the former studies, the studies with **92b** were extended towards amino acids derivatives, also in comparison to series of calix[4]arene phosphonic acids.[220] The

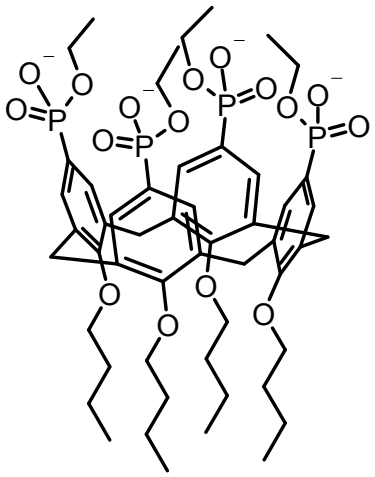
influence of the calixarenes conformation flexibility and its hydrophobic cavity shape dependent on the lower rim substitution pattern on the complexation process was monitored by ^{1}H-NMR spectroscopy in deuterated phosphate buffer at pD 7.3. Receptor **92b** did not show any remarkable selectivity towards the investigated amino acids methyl esters (K_{ass} = 10^2 M^{-1}). Only mixed 1:2 and 2:1 (host–guest) complexes were observed for compound **92b**. In contrast, compounds **94** showed selectivity for basic amino- acid methyl esters, i.e. Lys-OMe (K_{ass} (**94b**) = 170 M^{-1}, K_{ass} (**94a**) = 600 M^{-1}), Arg-OMe (K_{ass} (**94b**) = 120 M^{-1}, K_{ass} (**94a**) = 600 M^{-1}), and His-OMe (K_{ass} (**94b**) = 30 M^{-1}, K_{ass} (**94a**) = 200 M^{-1}) forming 1:1 complexes. More H-bonding sites aid the binding strength. Modification of the lower rim of the calix[4]arene skeleton by bridging ligands caused that complexation strength of the more rigid molecule **93b** to be lower but with preservation of the selectivity and specificity in its binding mode.



94a **94b**

Figure 62: Calix[4]arene tetraphosphonic acid (**94a**) and a double bridged analogue (**94b**)

Calixarene tetraphosphonate (**92c**) was described as specific receptor for basic amino acids, with preference for arginine (**81d**). Binding constants in methanol ranged from 7.9*10^2 M^{-1} for Ac-Lys-OMe (Lys, K_{ass} = 3*10^3 M^{-1}) to 1.9*10^4 M^{-1} for Ts-Arg-OMe (Arg, K_{ass} = 7.9*10^2 M^{-1}). Consequently, that host molecule was used in lipid monolayers for recognition of peptides and basic protein surfaces in buffered water[221] (HEPES), monitoring the binding events with the aid of a Langmuir film balance. Histone H1 and Cytochrome C were recognized in the range of 10^{-8} mol/L guest concentration.[218]

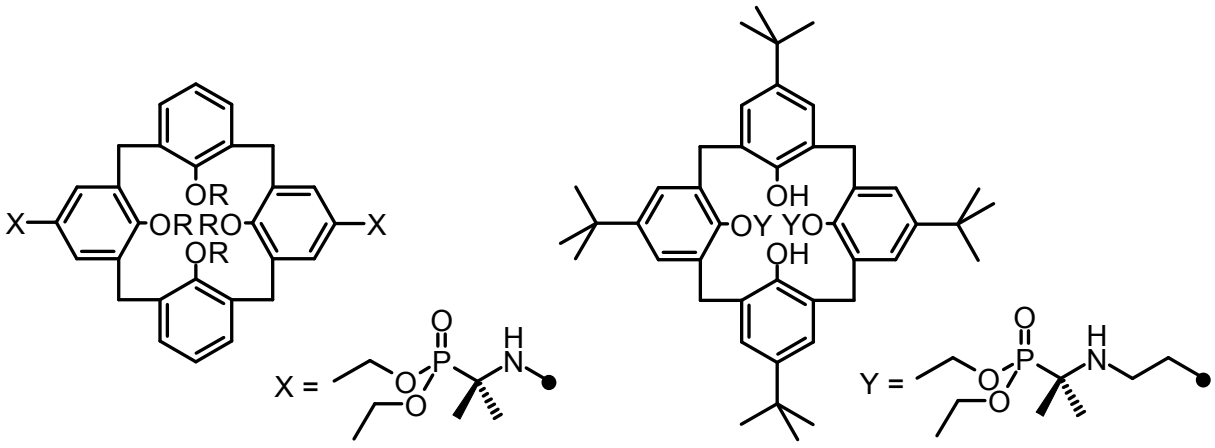


92c

Figure 63: Calix[4]arene tetraphosphonic acid ester (**92c**) for surface recognition experiments

Similar calix[4]arenes having α-aminophosphonic acid fragments at the upper or lower rim were described and their remarkable selectivity as carriers for zwitterionic aromatic amino acids in membrane transport was reported.[222]

By introduction of these H-donor and H-acceptor groups in the host skeleton, it was shown, that a calix[4]arene molecule binds hydrophilic amino acid zwitterions in its polar cavity: Two aminophosphonate groups at the lower rim lead to selective transport of His over Phe, Tyr and Trp, while upper rim modification changes the selectivity towards Phe. In the later case the substituents can participate in complexation and recognise the aromatic side chains of amino acids. The selectivity of membrane transport for phenylalanine (**81a**) was enhanced 40 times over tryptophan (**81b**) (fluxes ratio for **95a** - 7.3, for **95b** - 4.9).



94a **94b**

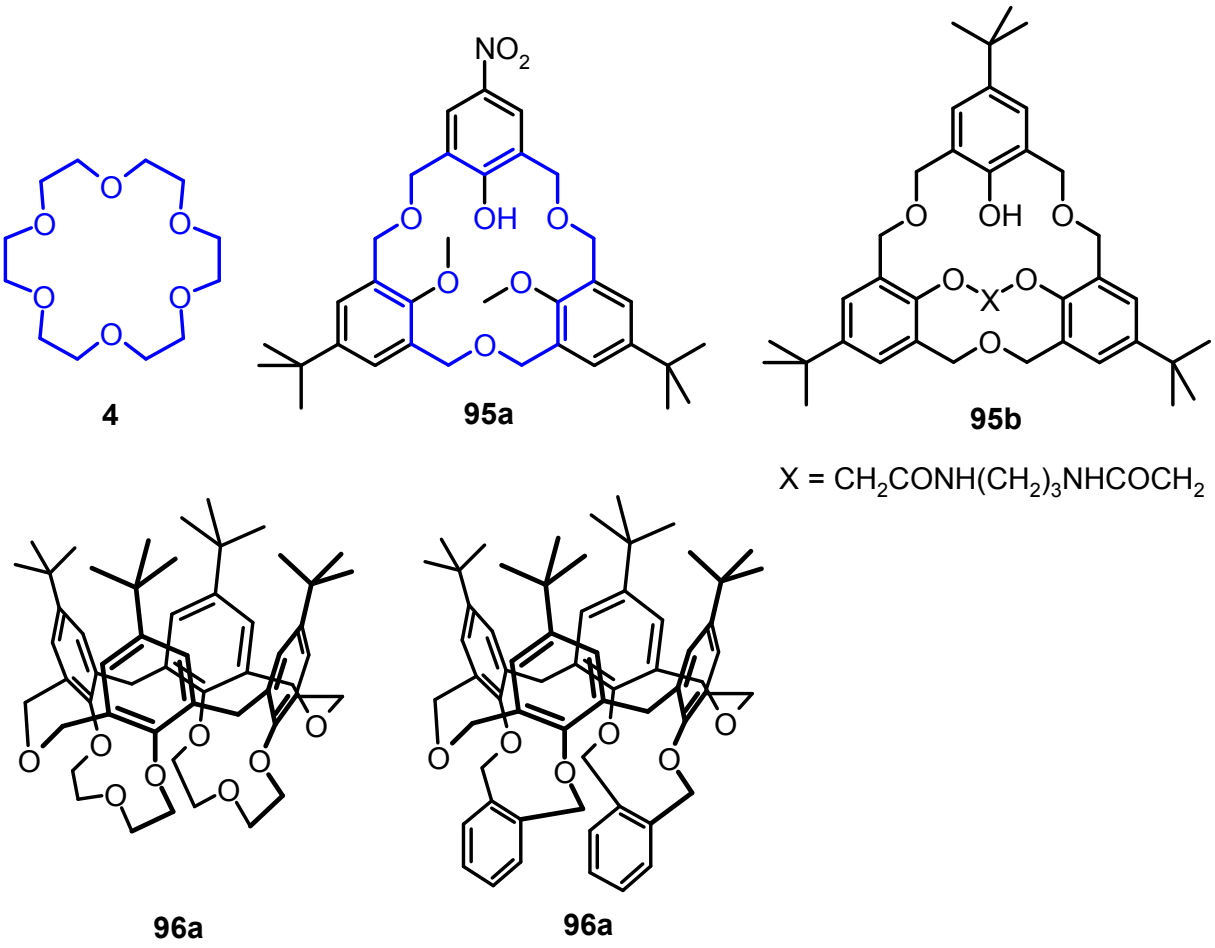
Figure 64: Calixarene receptors **95** with α-aminophosphonate groups

In addition, phosphorylated calixarenes have been used to bind uracils (K_{ass} up to $5.43*10^4$ M^{-1}) in aqueous solvent mixtures.[223,224] Together with the examples **92** and **94**, a whole series of phosphonate substituted calixarenes for amino acids binding has been reported, which proved to be more versatile than the *p*-sulfonatocalix[n]arenes and applicable at pH values being more closely to physiological conditions. The binding constants for amino acids in water are in the same order of magnitude for both functionalisations, where comparable. The preference for basic amino acids is evident.

I. 4.2. More Complex Calixarenes: Optical Readout, Enantiodiscrimination, Bridges and Caps

Calixarenes have also been modified to show special properties like optical readout by chromophoric groups, enabling quick and easy monitoring of guest binding, or groups supplying chirality for enantiodiscrimination. Also the cavity has been expanded or rigidified by bridges or even caps aiming for better binding properties. Often no sharp dividing line can be drawn between these concepts. We present now the current approaches, where we try to keep the direction, starting with optical readout systems, followed by calixarenes for chiral recognition and then go on to more complex systems ending with capped moieties with additional functionalities.

Bridging of calixarenes and resorcarenes with ethyleneglykol chains leads to calixcrowns and resorcarene crowns, or even calixcryptands.[225] Synthesis, structure and fundamental properties of such systems have been reviewed.[226] We will point out their application in ammonium ion recognition in comparison to other calixarenes with selected examples. Related systems carry ether bridges in the calixarene ring. Such homocalixarenes are structurally similar to crown ethers (**4**, see fig. 65) and can bind primary ammonium ions.[227]



4 **95a** **95b**

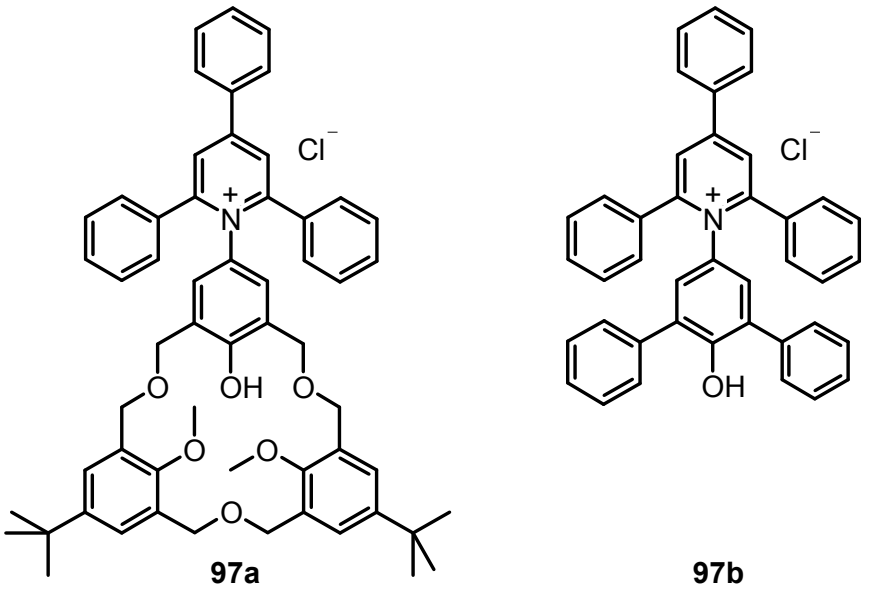
96a **96a**

Figure 65: A bridged homocalix[3]arene **95** and a distally bridged homocalix[4]crown **96**

Two of the basic examples have been presented by Chen et al. (**95**)[228] and Masci et al. (**96**)[229]. Compounds **95** show selectively binding ability towards linear primary alkylammonium ions from *n*-$BuNH_3^+$ up to *n*-hexyl-NH_3^+ with K_{ass} = 600 M^{-1} in $CDCl_3/CD_3CN$ 3:1 forming 1:1 complexes. Example **96a** binds the tetramethylammonium ion with K_{ass} = 280 M^{-1} in $CDCl_3$.

Homocalix[3]arene **97a**, reported by Tsubaki et al. consists, of an 18-membered ring and six oxygen atoms available for cation coordination.[230] In addition, the molecule contains a Reichhardt´s dye E_T1 (**97b**) type pyridinium phenolate moiety, which becomes deprotonated upon ammonium ion binding. The resulting betain structure shows long wavelength charge transfer absorption observable in the visible spectrum. Only compound **97a**, but not the dye E_T1 (**97b**) itself, showed a colour change upon addition of amines or some earth alkaline acetates. This confirms a binding process and excludes a simple deprotonation reaction as the origin of the colour change. Due to steric reasons primary amines are preferentially bound

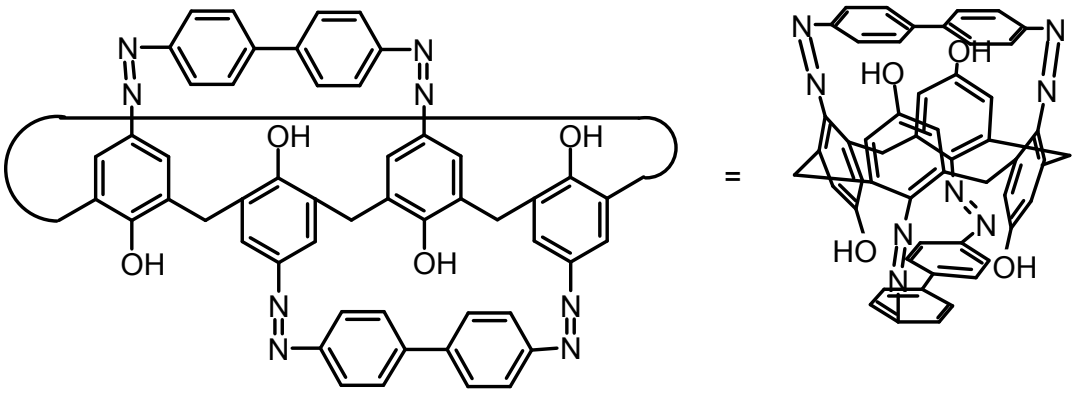
over secondary and tertiary amines. *N*-Butylamine showed a binding constant of 135 M^{-1} in DMSO.



97a **97b**

Figure 66: Homocalix[3]arene ammonium ion receptor **97a** and the Reichardt´s dye (**97b**) for colourimetric assays

Diazo-bridges in calix[4]arenes also allow distinguishing the binding of amines and diamines (or triamines) by colour changes, caused by host-guest proton transfer.[231] Bisazobiphenyl-bridged chromogenic calix[4]arenes **98** were employed as reagent for visual discrimination of aliphatic and aromatic amines.[232] Various amines were added to **98** in DMSO resulting in distinct colour changes. For instance, *tert*-butylamine induced bathochromic shift of the absorption of 84 nm, while added aromatic amines did not induce any colour change or shift in the absorption maxima. The yellow colour was restored upon acidification of a solution of the **98**-*tert*-butylamine complex. This indicated that the colour change could be attributed to the ionization of hydroxyl groups of **98**. Conductometric titration gave further evidence: upon guest addition the conductivity continuously increased until it reached a plateau at equimolar concentration of amine.

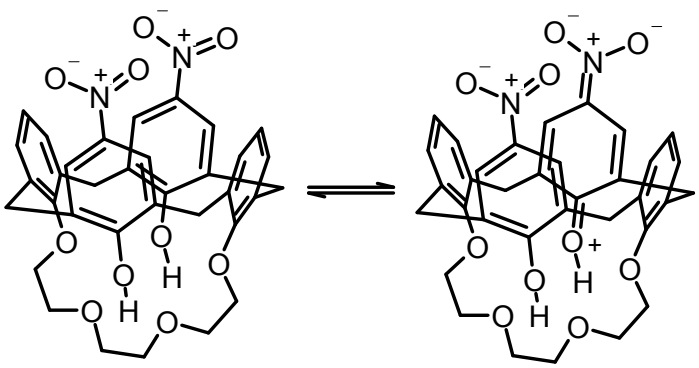


98

Figure 67: Chromogenic diazo-bridged calix[4]arene **98**

In an earlier publication, Arduini *et al*. introduced short diethylene glycol bridges into calix[4]arene. The resulting derivative was successfully used for the cation-π-complexation study of methylammonium and tetramethylammonium ions.[233] When a crown ether moiety bridges a calix[4]arene at the lower rim it prefers primary ammonium ions over the isomeric derivatives (*n*-butyl >> *tert*-butyl) for steric reasons;[234] a similar selectivity was observed if two parallel crown-3 moieties at the lower rim are introduced in *p*-phenylcalix[4]arene,[233] or in the same order (*n*- >> *s*- > *tert*-butylamines) if two carboxymethoxy groups at the lower rim of a calix[4]arene are bridged by a crown-3 group.[235]

The parent calix[4]arene was used by Huang to develop an amine receptor with optical readout. The dinitrated calix[4]arene is bridged by oligoethylenglycol chains of different length by alkylation of the phenolic hydroxyl groups of the non-substituted arenes.[236]



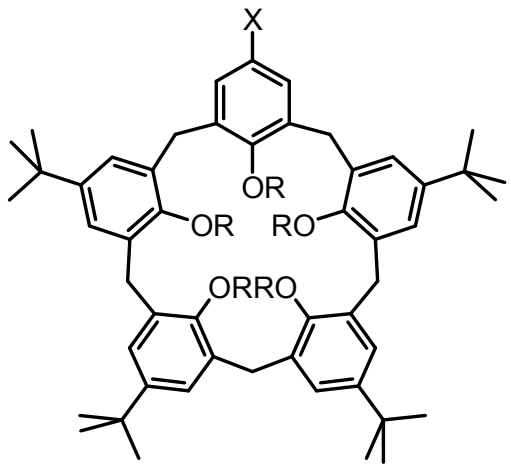
99

Figure 68: Calixarene receptor **99** by *Huang et al*.

As in the previous examples the binding of the amine by the resulting phenolate ion is crucial for the development of the colour. Because of two phenols being deprotonable per calixarene, it is not surprising that the authors identified a 1:2 receptor:amine stoichiometry. For this class of receptors a clear preference for binding of primary amines over branched, secondary and tertiary guests was observed. For the depicted receptor they found the best binding properties of *n*-butylamine (K = 326 M^{-1}) in chloroform.

Enantioselective analysis and separation of amino acids was addressed using chiral calixarene type macrocycles: A pseudo-C_2-symmetrical homooxacalix[3]arene discriminates between chiral amino acids[87] and chiral calix[4]crown ethers were used for the binding of alkylammonium ions.[237] Amino acid esters were separated in liquid membrane transport experiments with efficiency according to their guest hydrophobicity, with preference to *S*-Phe- and *S*-Trp- ester showing the highest flux.[238]

A calix[5]arene related to **82** attempting enantiodiscrimination was reported by Parisi et al.[239] Replacing the *tert*-butyl group (**100a**) by an urea functionality (**100b** and **100c**) on the upper rim improved the binding constants towards ammonium guests significantly.



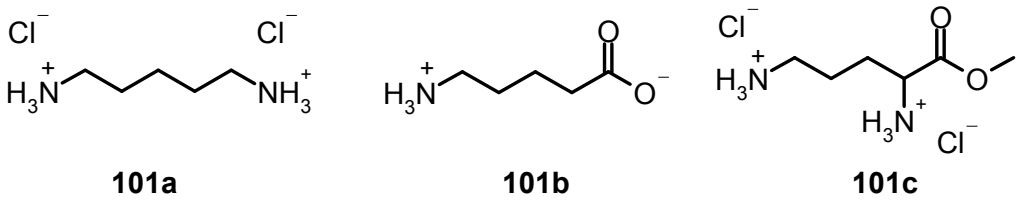
100a X = *tert*-Butyl
100b X = (*R*)-PhMeCHNHC(O)NH
100c X = (*S*)-PhMeCHNHC(O)NH

R = $(CH_2)_3CH(CH_3)_2$

Figure 69: Calixarenes **100** reported by *Parisi et al*.

The free rotation around the aromatic-*N*-(urea)-bond allows the urea unit to act as hydrogen bond acceptor to bind ammonium ions and as hydrogen bond donor for carboxylate binding. However, a comparison of the binding constants shows that carboxylate ions are bound more tightly. This is indicated by the difference between the binding of 1,5-diaminopentane dihydrochloride (DAP * 2 HCl, **101a**) and 5-amino pentanoic acid (APA, **101b**) (table 5). The

chirality of the receptors **100b** and **100c** did not lead to an enantiodifferentiation of chiral guest molecules.



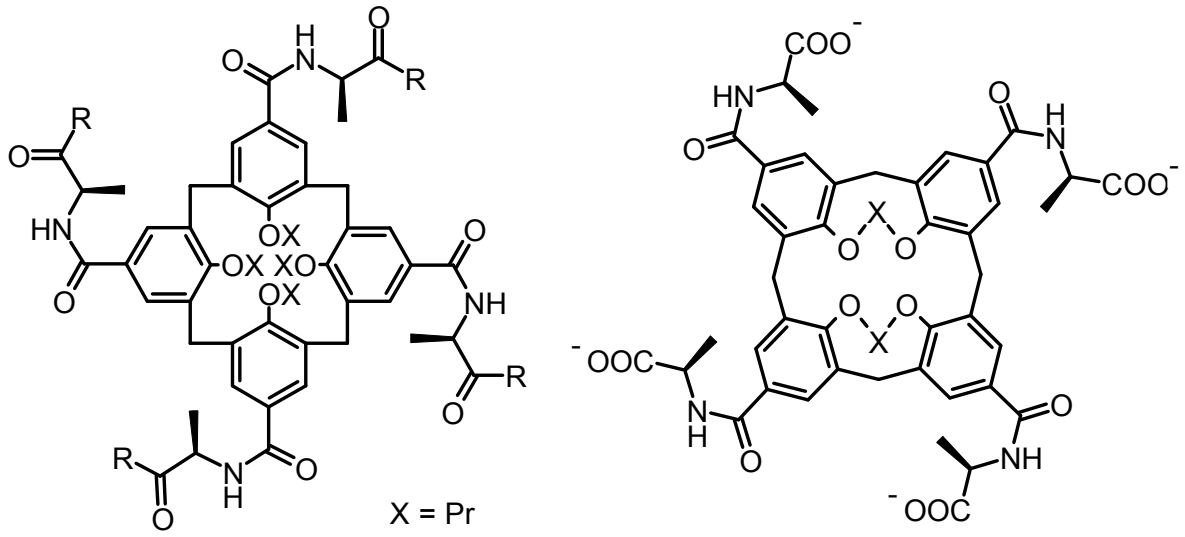
101a **101b** **101c**

Figure 70: Guest molecules for inclusion in calixarenes **100**: DAP * 2 HCl (**101a**), APA (**101b**) and Lys-OMe * 2 HCl (**101c**)

Receptor	101a	101b	101c
100a	300 M^{-1}	1070 M^{-1}	43 M^{-1}
100b	12820 M^{-1}	16140 M^{-1}	2240 M^{-1}
100c	11860 M^{-1}	16850 M^{-1}	2190 M^{-1}

Table 5: Binding constants of different guest molecules (**101**) with receptors **100** (NMR titration in $C_2D_2Cl_4/CD_3OD$ 2/1)

The inclusion properties of the chiral cone peptidocalix[4]arenes **102** with different conformation flexibility towards aliphatic and aromatic amino acids and their methyl esters were investigated in D_2O (pD 7.3, phosphate buffer).[240] The authors compared the recognition properties towards α-amino acids and aromatic quaternary ammonium cations of **102c** and the more rigid water soluble peptidocalix[4]arene **103** by ^{1}H-NMR titration experiments. The complexation occurred exclusively through the interaction of the calixarene cavity with the apolar groups of the guests.[241]



102a R = OMe
102b R = $NHNH_2$
102c R = OH

103

Figure 71: Different *N*-linked peptido-calixarenes open and with glycol chain bridges

Rigid receptor **103** with two di(ethylene glycol) units introduced in proximal positions at the lower rim of the calix[4]arene skeleton (fig. 71) was much more efficient than the flexible analogue in all complexation processes. The aromatic molecules were better bound than the aliphatic ones with the highest association constants values K_{ass} = 110 and 620 M^{-1} for *S*-Trp and *S*-Trp-OMe, respectively.[242] The order of log K_{ass} was decreasing in the order of higher hydrophilicity (log K_{ass} in brackets): *R*-Trp-OMe, *S*-Trp-OMe (2.8) > *R*-Phe-OMe, *S*-PhGly-OMe, *S*-Phe-OMe (2.6) > *S*-Leu-OMe (2.5) > *S*-Val-OMe (2.3) > *S*-Tyr-OMe (2.2) > *S*-Ala-OMe, *S*-Trp (2.0) > *S*-Phe (1.8) > *S*-Tyr, *S*-Leu (<1.3) > Ala, Val, Gly. A similar behaviour became obvious examining the pH dependence of the association constant between **103** and *S*-Phe-OMe. It decreased regularly from pH = 6.0 (K = 710 M^{-1}) to pH = 7.3 (K = 400 M^{-1}) and pH = 8.0 (K = 220 M^{-1}) corresponding to the decrease in the percentage of protonated guest species. The hydrazides of this '*N*-linked-peptido-calixarenes' were able to extract complementary amino acids and dipeptides such as acetyl-*R*-alanine and acetyl-*R*-alanyl-*R*-alanine.

Introduction of chirality by insertation of an amino acid in the ring of the calixarene moiety potentially enables enantiodiscrimination properties by formation of diastereomeric complexes with the racemic ammonium ion.[243]

For the visual discrimination between enantiomers Kubo et al. synthesised a receptor (**104**) which undergoes a colour change upon binding of chiral substrates.[244] Upon binding of the enantiomers two different bathochromic spectral shifts of the two chromophores attached to the binding cavity were observed, with significant optical response only for one enantiomer. The best strongest binding occurred with (*R*)-phenylalaninol salt in ethanol K_{ass} = 159 ± 16 dm³ mol^{-1}. The formation of a 1:1 complex was confirmed by mass spectroscopy. Other amino acids enantiomers, such as the ones of phenylglycine, were distinguishable with the system.

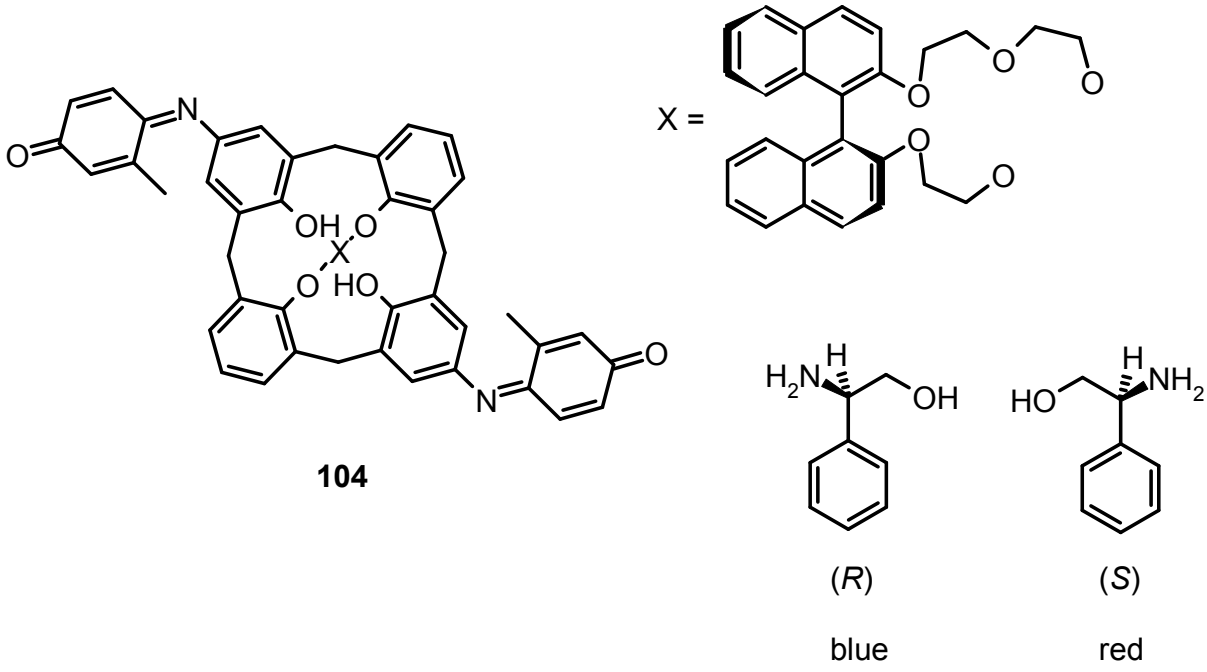
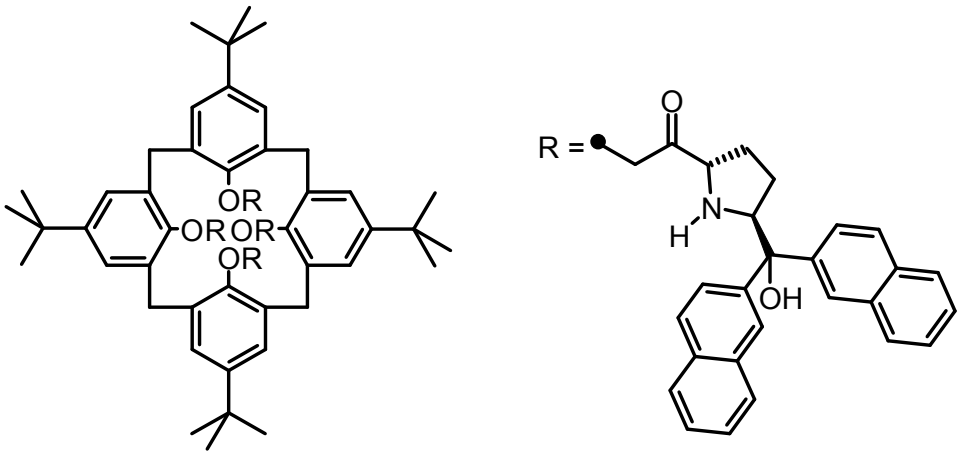


Figure 72: (S)-1,1´-Bi-2-naphthol calixarene derivative **104** published by *Kubo et al.*

Diamond et al. synthesized compound **105** to get to a sensor which discriminates enantiomers by hydrogen bonding interactions.[245] Without directly observable optical readout option, the fluorescence quenching of the receptor´s emission was investigated in chloroform (λ_{ex} 274 nm). Compound **105** shows some selectivity for (*R*)-1-phenylethylamine and discriminates between the enantiomers of phenylglycinol in methanol.



105

Figure 73: A chiral ammonium-ion receptor **105** based on the calix[4]arene skeleton

p-tert-Butylcalix[6]arenes were modified with chiral amino alcohols to achieve enantioselective binding of amino acids and amino alcohols.[246] The extraction properties of the two homochiral receptors **106a** and **106b** for some amino acid methyl esters and amino alcohols were studied by liquid–liquid extraction. The results show that these derivatives were excellent extractants for all the amino acids and amino alcohols, but only a weak or no chiral discrimination of the guests was found. Table 6 shows some selected results.

Receptor	106a	106b
S-Ala-OMe	91.4	84.3
R-Ala-OMe	89.1	89.6
S-Phe-OMe	90.3	87.2
R-Phe-OMe	90.7	82.5
R-Trp-OMe	87.5	85.4
S-Trp-OMe	93.2	89.8
R-phenylglycinol	92.3	83.5
S-phenylglycinol	72.5	87.6

Extraction for 1 h from water with $2.0*10^{-5}$ M ammonium picrate to CH_2Cl_2; 25 °C

Table 6: Extraction abilities in % of receptors **106a** and **106b**

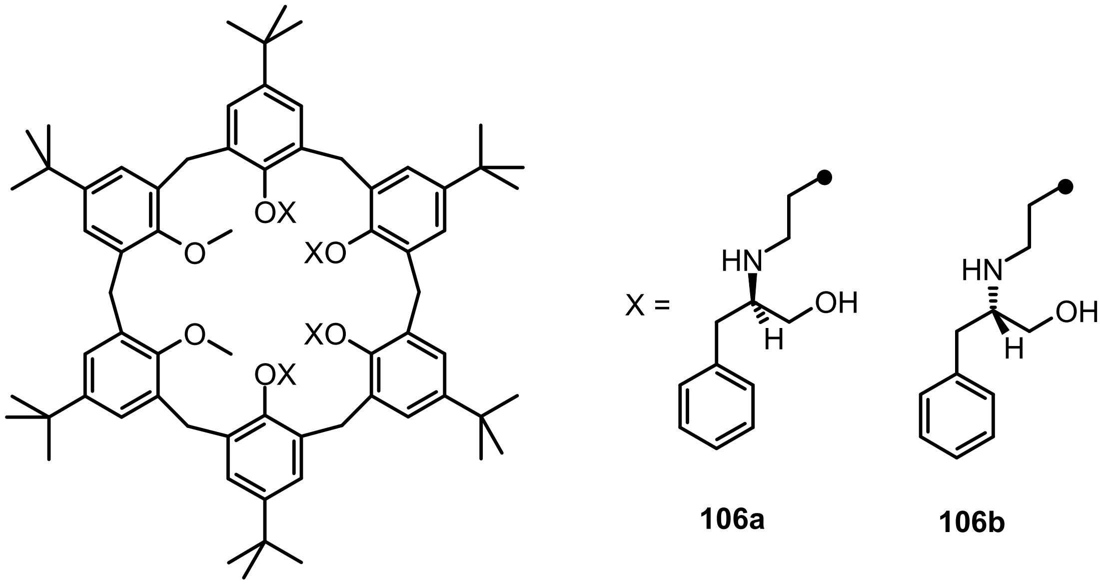


Figure 74: *R*-/*S*-phenylalaninol functionalised calix[6]arenes **106a** and **106b**

The inclusion of quaternary ammonium cations in the cavity of calixarenes with more enclosing substituents, has been studied extensively over the years in the gas phase, in solution and in the solid state.[247] The next step is to close the cavity from one side, to bridge or cap the moiety. Bridging of the upper rim of a calixarene may lead to altered selectivity and higher binding constants due to the pre-organized and fixed cavity.

A triply bridged capped C3-symmetric hexahomotrioxacalix[3]arene **107** exhibited high affinity (K_{ass} = 7.6*10^{4} M^{-1}) for the *n*-butylammonium ion.[248] The association constant of receptor **107** with the picrate salt was determined in CH_2Cl_2/THF (99:1, vol/vol) according to the Benesi-Hildebrand equation, which shows a very well-defined linear shape for a 1:1 interaction.

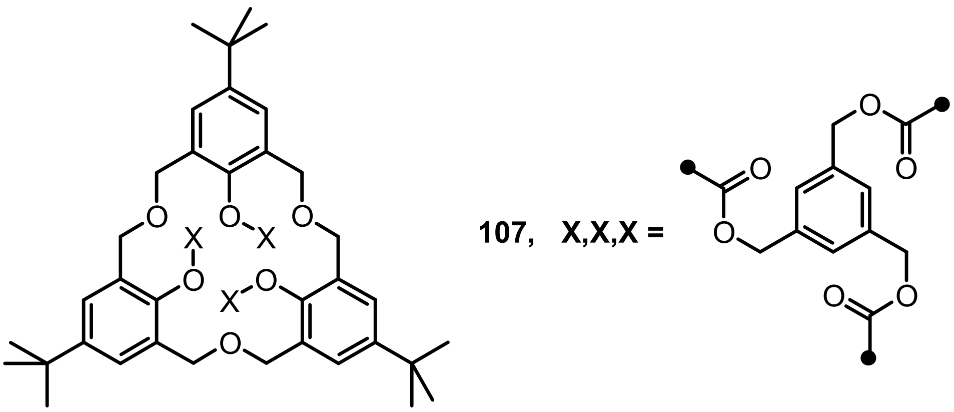


Figure 75: Capped homocalix[3]arene ammonium ion receptor **107**

A three point connected thioether bridge led to a rigid calix[6]arene moiety (**108**) exhibiting C_3 symmetry.[249] This pre-organization enabled better cation–π-interactions with the derivative **108** resulting in a 10- to 20-fold enhanced association constant for trimethylanilinium iodide (CD_2Cl_2, K_{ass} = 10^2 dm^3mol^{-1}) in comparison to the reference compound hexamethoxy-*tert*-butylcalix[6]arene.

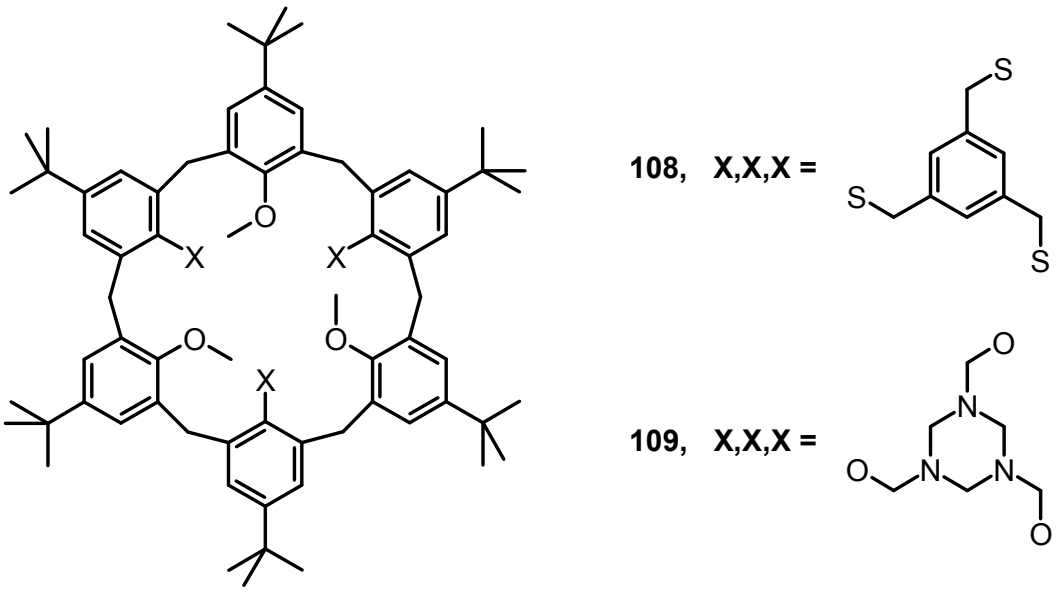


Figure 76: Two C_3-symmetric capped calix[6]arenes **108** and **109**

Rigidified in the cone conformation **109** (fig. 76) displayed an exceptionally high affinity for small ammonium ions forming *endo*-complexes.[250] Extraction and competitive binding experiments gave values that were, at this time, the highest ever obtained with a calixarene-type host. The best affinity was observed for ethylammonium picrate (K_{ass} = $3.3*10^4$ M^{-1}) with a more than 100-fold stronger association constant than butylammonium- and secondary ammonium ions. Quaternary ammonium ions were not complexed in chloroform. With the aid of X-ray diffraction the authors identified the origin of the strong inclusion as contributions of hydrogen bonding to both, the aza cap and one phenolic unit of the calixarene, and to cationic as well as to CH-π-interactions between the ammonium ion and the aromatic walls of the host compound.

A C_{3v}-symmetrical calix[6]cryptand with a *P*,*N*-crypto cap was prepared leading to a pre-organized well-defined hydrophobic cavity open at the large rim. The free base of **110a** is

able to complex cationic ammonium guests. ^{1}H-NMR studies showed that the methoxy substituents point towards the inside of the cavity.

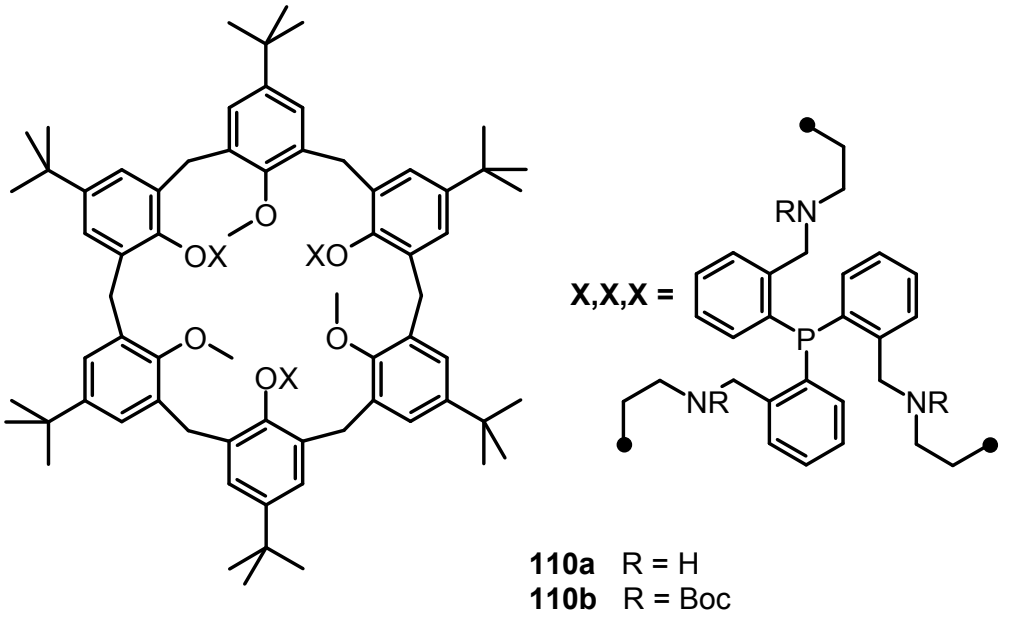


Figure 77: Phosphorous-containing rigidified calix[6]arene **110**

Reinaud et al. provided another example of synergistic combination of a polyaza and a calix[6]arene structure: Calix[6]tmpa **111**.[251] The compound behaved as a single proton sponge and appeared reluctant to undergo polyprotonation, unlike classical tris(2-pyridyl-methyl)amine (tmpa) derivatives.

Calix[6]tmpa **111** and its sodium and protonated species display conformational properties that differ from the properties previously observed for other calix[6]-azacryptands: The ^{1}H-NMR study indicated that the ligand, as well as its complexes, adopt a flattened cone conformation probably due to the high steric constrain from the tmpa cap.

The monoprotonated derivative behaved as a good receptor for amines, leading to inclusion complexes, and as a good host for ammonium ions. Interestingly, it strongly binds a sodium ion and a neutral guest molecule, such as a urea, an amide, or an alcohol, cooperatively. Since it preferentially includes cyclic ureas, amides, or alcohols rather than primary amines, the group showed with this the first example of a funnel complex binding an alkali-metal cation, comparable with related Zn^{2+} funnel complexes.[252] It displayed five fold selectivity in favour of propylammonium hydrochloride over the corresponding ethyl- and two fold selectivity over the butyl-guest in chloroform.

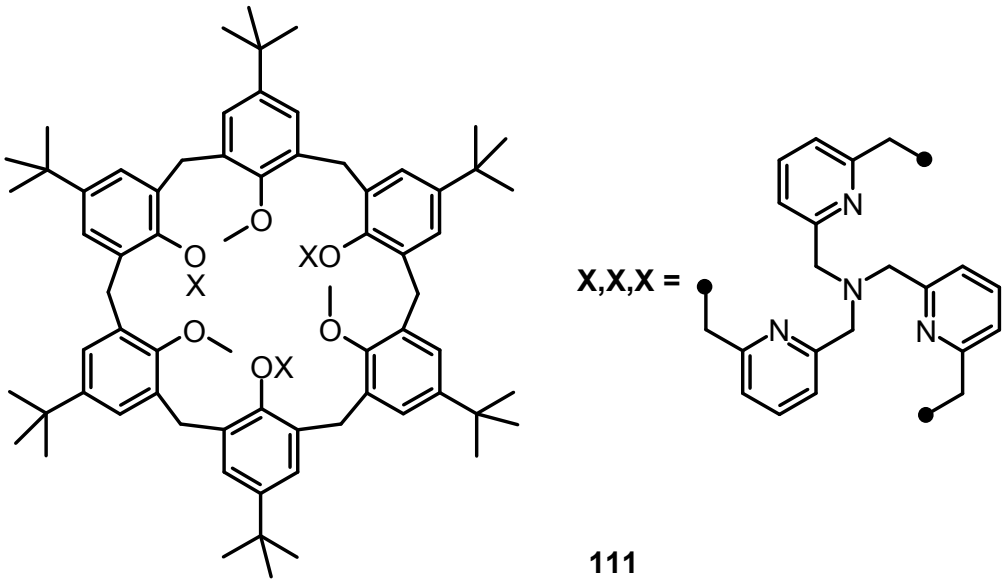


Figure 78: Calix[6]azacryptand **111**

Even larger structures, based on this trimethoxy-calix[6]arene scaffold triple-bridged with a cyclotriveratrylen or connected to dimers via alkyl bridges were applied for ammonium ion pair inclusion.[253]

The use of such ditopic receptors and capped calixarenes with enhanced strength by ion-pair recognition has been an emerging field. In succession of the presented examples a second generation of the hosts was introduced.[254] These heteroditopic receptors can bind ammonium ions or organic ion pair salts with a positive cooperativity.[255] The host-guest properties of receptors **112a** and **112b** toward the picrate and chloride salts of propylammonium ion were studied by ^{1}H-NMR spectroscopy and compared to **109**. No distinct binding constants were reported, but addition of 1 equiv. of $PrNH_3^+Pic^-$ to $CDCl_3$ solutions of **112a** or **112b** led to the quantitative formation of the corresponding endocomplexes [**112a**⊃$PrNH_3^+$],Pic^- and [**112b**⊃$PrNH_3^+$],Pic^-. With XCl, in comparison with [**109**⊃$PrNH_3^+$],Cl^-, a much larger amount of [**112b**⊃$PrNH_3^+$,Cl^-] was produced with less than 1 equivalent of $PrNH_3Cl$. This highlights that the simultaneous binding of the anion by the urea groups of the ditopic receptor **112b** enhances the endocomplexation of the ammonium ion indicating a much larger binding constant as observed with the first generation molecule **109**.

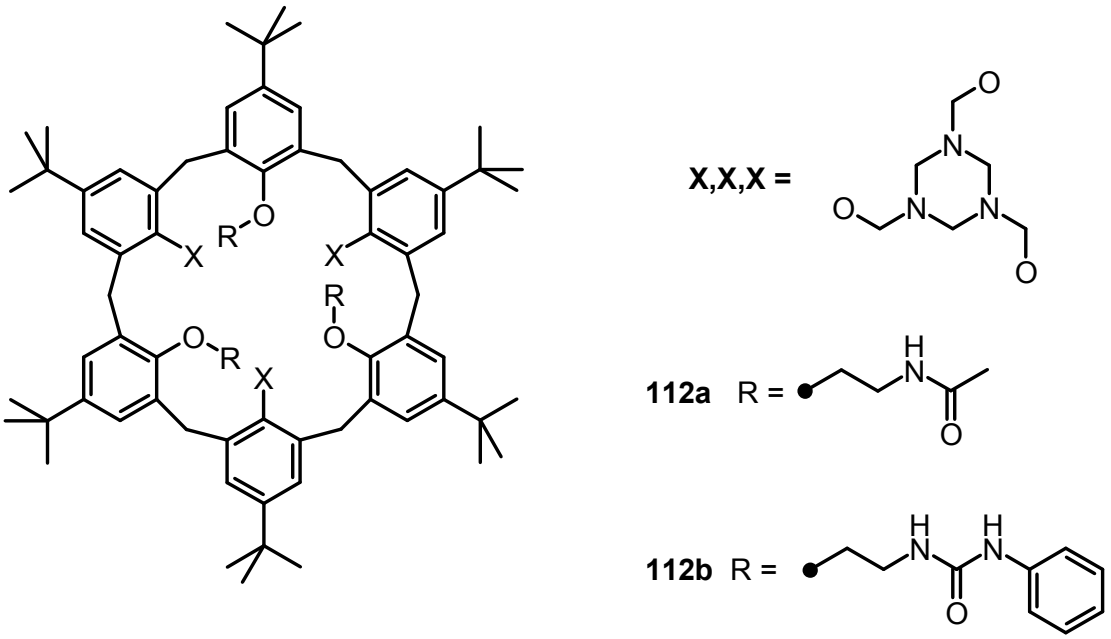


Figure 79: Further substituted calix[6]azacryptands **112**

I. 4.3. Resorcarenes and Deeper Cavities

Resorc[4]arene (**75c**) is a macrocycle with eight hydroxy groups at the upper rim, which form intramolecular H-bonds. Their interior is much smaller than that of cucurbituril. Resorcinarenes are versatile modules for modern studies of molecular recognition.[256] Resorcarene can, like calixarenes, include guest molecules in the bowl-shaped cavity (cation-π-interaction).

The monomeric resorcinarene (**75c**) and its simple derivatives show recognition properties, but their shallow curvatures cannot provide sufficient surface contacts for selecting between targets. Nevertheless, they bind ammonium ions, choline (**76**), acetylcholine (**3**), and carnitine (**77a**) in protic solvents.[257,258] Also larger guests like DABCO can therefore be included.[259] Significant interactions to the ammonium ion can also occur via hydrogen bonds to the phenolic OH-groups. In unsubstituted resorcarenes these are preferably formed intramolecularily, in which two neighbouring OH groups of the host are involved. For example, in dilute aqueous sodium hydroxide solution (pH 12 - 13) the tetraanionic structure, in which one hydroxyl group per aromatic moiety is deprotonated and stabilized by a strong intramolecular hydrogen bond, can bind tetralkylammonium ions in the 10^4 - 10^5 M^{-1} range.[260]

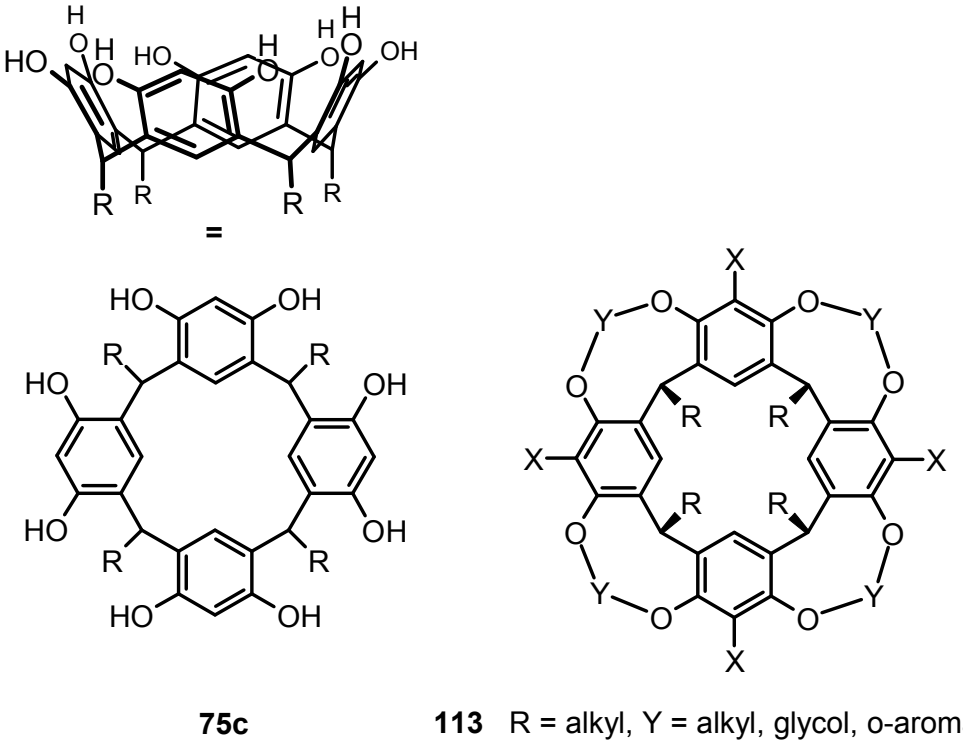


Figure 80: Resorc[4]arene (**75c**) and the cavitands (**113**)

Similar to *p*-sulfonatocalix[n]arenes (**84**) tetrasulphonatomethylcalix[4]resorcinarene forms complexes with amino acids in D_2O (pD 7.2, phosphate buffer).[261] The K_{ass} values for these complexes estimated from ^{1}H-NMR experiments decrease in the order Lys > Arg > Pro > Trp > Phe (with a maximum log K_{ass} of 3 for basic amino acids) and no interactions with Asp, Asn, Thr, Leu, Met were observed.

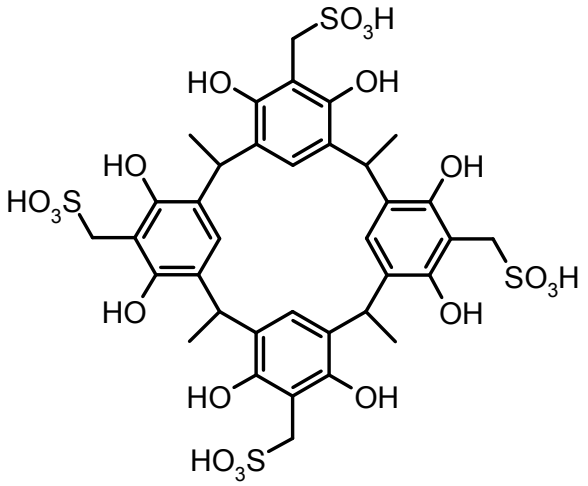
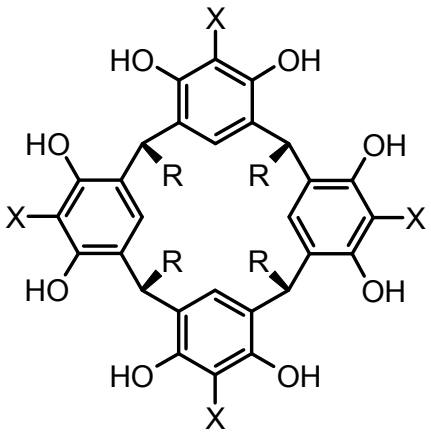


Figure 81: Tetrasulphonatomethylcalix[4]resorcinarene (**114**)

Just recently the complexation properties of pyrogallol[4]arenes (**115c**) towards quaternary ammonium salts were compared with two resorcin[4]arenes (**115a/b**).[262] The stability constants (*K*), standard free energy (ΔG_o), enthalpy (ΔH_o), and entropy changes (ΔS_o) for the complexation of pyrogallol[4]arenes with ammonium cations were determined in ethanol by isothermal titration calorimetry. The binding strengths were in the order of 10^3 - 10^4 M^{-1} and generally 2- to 7-fold higher compared to the corresponding simple resorcarenes. In the best example diethyldimethylammonium and triethylmethylammonium ions were included in **115c** with K_{ass} = 6900 M^{-1} and 7500 M^{-1}, respectively. The trends observed in the thermodynamic parameters for 1:1 and/or 1:2 host–guest complexations correspond to the systematic structural changes of the guest molecules. Molecular modelling calculations confirmed the results.



115a X = H, R = $CH_2CH(CH_3)_2$
115b X = CH_3, R = $CH_2CH(CH_3)_2$
115c X = OH, R = $CH_2CH(CH_3)_2$

116 X = OH, R = $(CH_2)_{10}CH_3$

Figure 82: Resorc[4]arenes (**115a/b**) and pyrogallo[4]arenes (**115c, 116**)

Similar pyrrogallol[4]arenes carrying long alkyl chains (**116**) were applied as amphiphilic receptors in an aqueous micelle system to study their interaction with dopamine (**2**) and acetylcholine (**3**) by NMR techniques.[263]

The inclusion of acetylcholine (**3**) by its quaternary ammonium ion in resorcarene (**75c**) via multiple cation-π-interactions was proved by a crystal structure.[257]

Not surprisingly, resorcarenes were also employed in a fluorescent displacement assay for acetylcholine (**3**). Similar to Shinkai´s study with *p*-sulfonatocalix[6]arene (**84b**) a tetracyanoresorc[4]arene (**117**) in comparison to the parent compound **75c** (R = Et) was used as complex with indicator **85**.[264] The binding constants observed for acetylcholine (**3**) were 2 to 2.5-fold higher for the tetracyanoresorc[4]arene (**117**). This was attributed to the larger contact area and a more suitable pK_a value of the resorcarene in consequence of the strong

electron withdrawing effect of the cyano groups. With rising pH acetylcholine (**3**) was bound more strongly by the receptors, with a K_{ass} of up to 10^6 in phosphate buffer at pH 8.

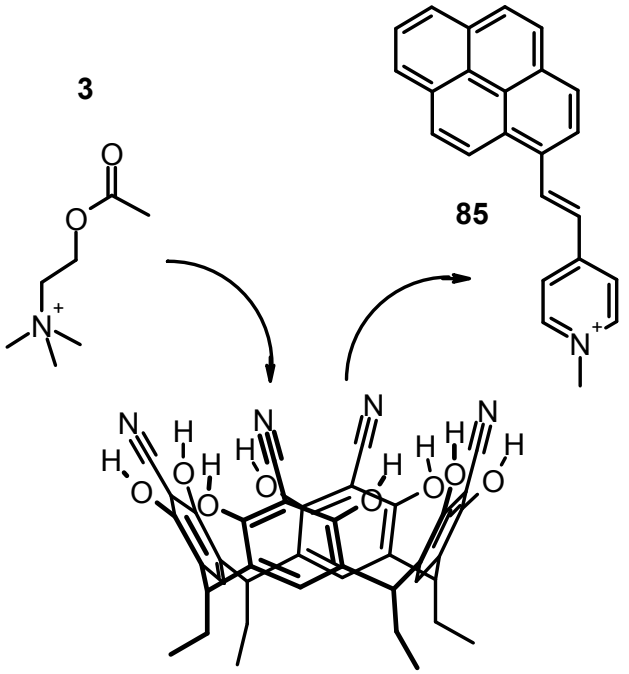
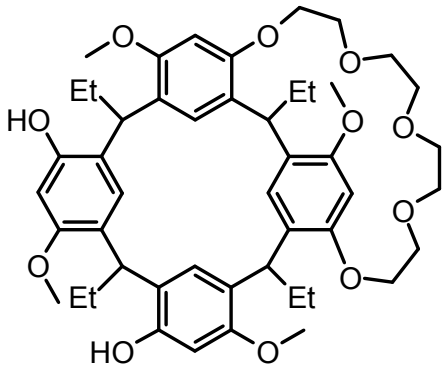


Figure 83: Displacement assay for acetylcholine (**3**) with tetracyanoresorc[4]arene (**117**)

A mono-bridged resorcinarene host for acetylcholine (**3**) was reported with tetramethoxy resorcinarene mono-crown-5 (**118**).[265] The dual nature of the cavity formed between the crown bridge at the one end and the two hydroxyl groups at the other offers a better fit to acetylcholine (**3**) compared to the smaller tetramethylammonium cation. Acetylcholine (**3**) is able to interact with both the crown ether moiety and the free hydroxyl groups of receptor **118** simultaneously: the quaternary trimethylammonium group binds to the crown moiety through cation–*O* and cation-π-interactions, whereas, hydrogen bonding interactions prevail between the acetate group and the hydroxyl part of the cavity. The binding of acetylcholine (**3**) to **118** was investigated by ^{1}H-NMR titration technique in $CDCl_3$ observing a 1:1 host–guest complex formation. The titration data provided the stability constant of 150 M^{-1}, which is 10 - 10^3 orders smaller compared to the values found with acetylcholine complexes of resorcinarenes (**75c** and **117**), pyrogallolarenes (**115c** and **116**) or deep-cavitands (**126a/c**).

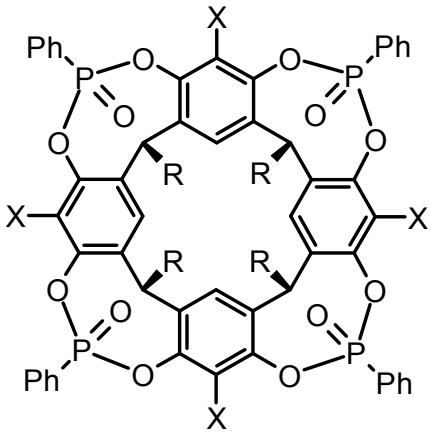


118

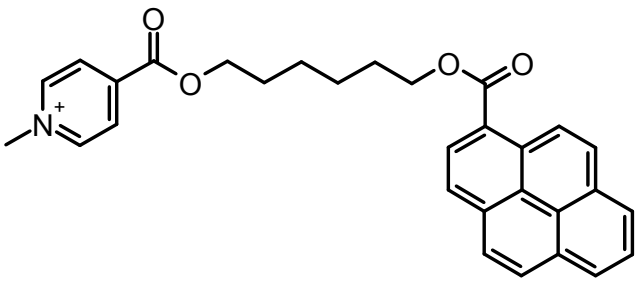
Figure 84: Tetramethoxy resorcinarene mono-crown-5 (**118**)

Following such a bridging approach, even deeper cavities (**113**) can be formed based on the structurally related resorcarenes like **75c** (fig. 80). By covalent bridging of the OH groups of two neighbouring aromatic subunits by aromatic moieties a resorcarene can be rigidified further and the cavity formed can enclose guest molecules entirely.

One way achieving this are phosphonate-cavitands.[266] Following a similar principle as in the acetylcholine (**3**) displacement assays (**84b** or **117** + **85**) mentioned above Prodi et al. reported a suitable protocol for the reversible complexation of methylammonium and methylpyridinium salts by the phosphonate cavitand **119**.[267] The K_{ass} values measured for the *N*-methyl complexes exceeded 10^7 M^{-1} in dichloromethane. As displaceable guest they used compound **120**, consisting of a methylpyridinium unit as recognition moiety connected to a pyrene probe via a diester. In this molecule the cation–dipole interactions and CH_3–π-interactions of the acidic $+N–CH_3$ group with the π-basic cavity could be assisted in a synergistic manner by two simultaneous hydrogen bonds to the phosphonate groups. In the case of protonated secondary amines like *N*-methyl-butylamine, a $K_{ass} = 7.8*10^6$ M^{-1} was determined for **119a**.



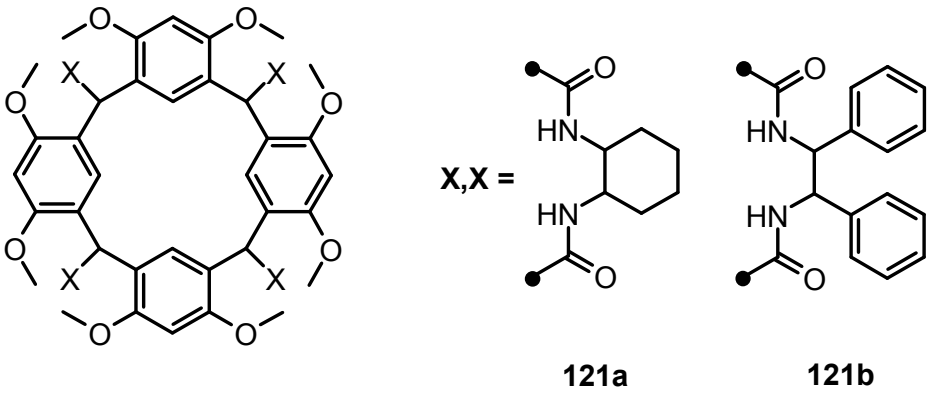
119a R = Pr, X = Me
119b R = $C_{11}H_{23}$, X = H



120

Figure 85: Components of a resorcarene based displacement assay for ammonium ions

As a different approach of cavity deepening, Botta, Speranza and colleagues presented both enantiomers of the two chiral basket resorcin[4]arenes **121a** and **121b** rigidified and double spanned with 1,2-diaminocyclohexane and 1,2-diphenylethylenediamine bridges, respectively, in a flattened cone conformation.[268] Binding constants were not published, but in several ESI-experiments the proton- bonded diastereomeric complexes with amino acid guests exhibited a pronounced selectivity towards the enantiomers of tyrosine methyl ester and amphetamine. An additional kinetic study on the base-induced displacement of the guest revealed that the *S*-Tyr-OMe and *R*-amphetamine enantiomer was faster displaced from the heterochiral complex than from the homochiral one.



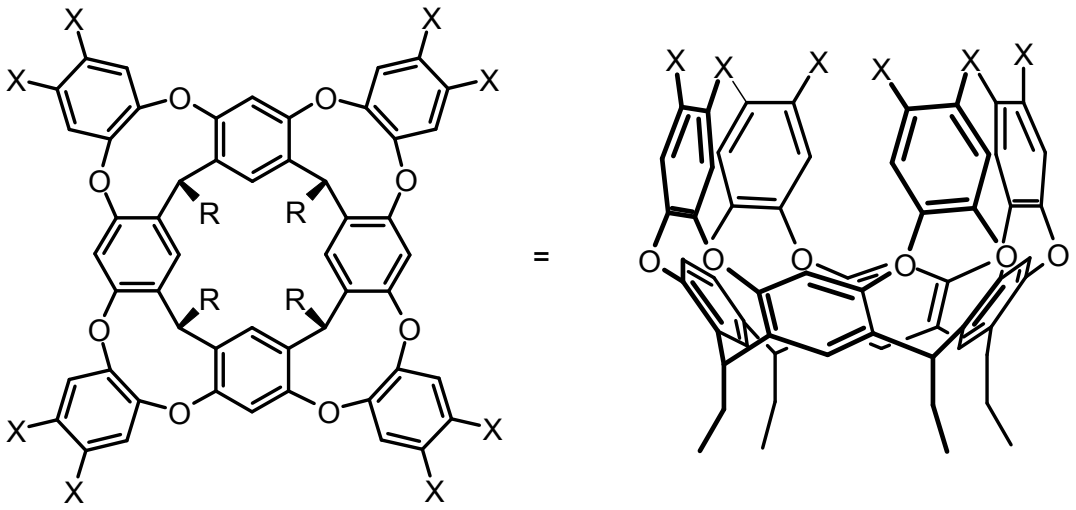
121a **121b**

Figure 86: Chiral basket resorcin[4]arenas **121**

Cavitands [269] and carcerands [270] are additional examples of resorc[4]arene based supramolecular host systems. Ideally, a synthetic receptor should provide a congruent surface

and chemical complementarity to the target molecule. Cavitands (**113**) with (hetero-) arene linker between the resorcin[n]arene oxygen atoms, thus adding three or four walls to the resorcarene skeleton, form a larger and deeper cavity than the according alkyl or glycol chain bridged homologues.[193, 271, 272] Besides the cavitand's space this increases also the curvature. Non-functionalized resorc[4]arenes are dominated by hydrogen bonding as driving force for complex formation and aggregation. For the latter cases the resorcinol hydroxyl groups are functionalized and, therefore, π-interaction and electron donation become more important in their binding processes. Bigger guests can be included, more surface capacitating cation-π-interaction is available and a stronger solvent shielding effect can be achieved. Thus, their binding properties and selectivities can be enhanced.[272]

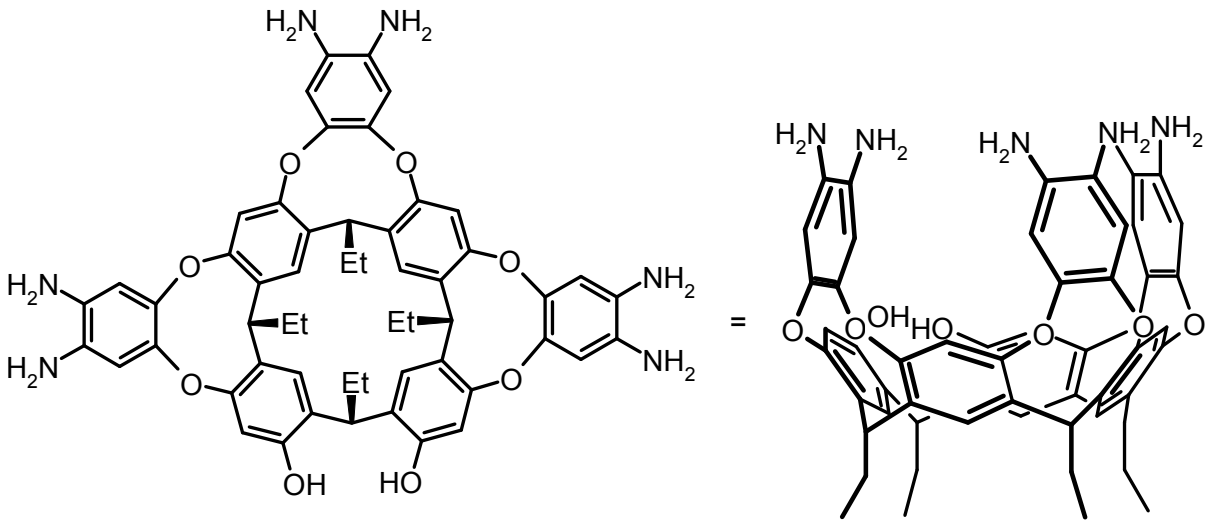
Two examples for this were recently studied by Rebek et al. as a different concept of the molecular recognition of choline (**76**) and carnitine (**77a**). They enhanced the affinity and the selectivity by a better complementarity of size and shape instead of optimizing charge/charge attractions.[273] Specific cation-π attractions between the positive charge of the guest and the electron-rich aromatic surfaces of the host result in the formation of complexes with highly kinetic and thermodynamic stability. *R*-Carnitine (**77a**) is complexed with an association constant of 15000 ± 3000 M^{-1} reflecting its carboxyl and hydroxyl functions being well-positioned for hydrogen bonding to the amino groups at the rim of the host. Choline hydrochloride (**76**) with 12000 ± 2400 M^{-1}, but also tetramethylammonium chloride in DMSO with 22000 ± 4000 M^{-1} are bound tightly by **119a**. The molecule can be seen as a further development of the calixarene tetrasulphonate of Shinkai et al., which also offered a very good affinity for choline (**76**) in water (log K_{ass} = 4.7), but was less selective.



122a R = Et, X = NO_2
122b R = Et, X = NH_2
122c R = $C_{11}H_{23}$, X = NHC(O)Alk

Figure 87: Resorcarenes with deeper cavitand structure (**122**)

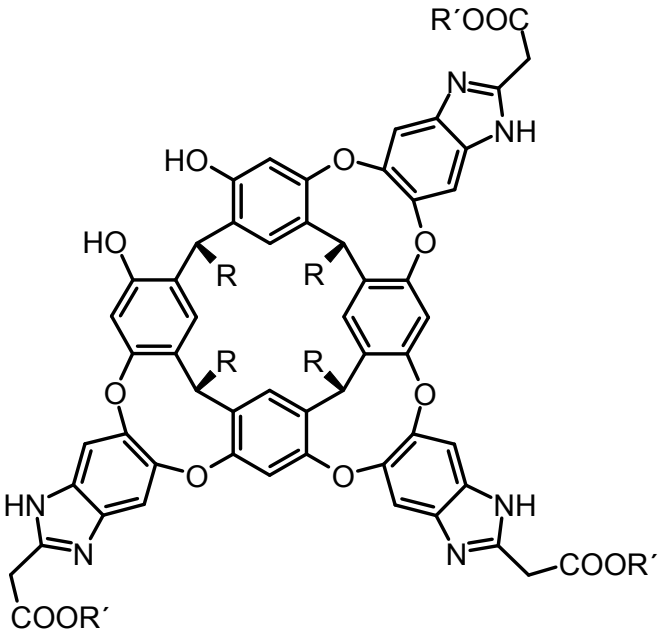
A comparable receptor molecule **123** in a vase-like conformation was employed as supramolecular fluorescent sensor system for choline (**76**). The selectivity of the hybrid cavitand resorcin[4]arene receptor is explained by its enforced scoop-shaped cavity and multiple cation-π-interactions. Deprotonation in alkaline aqueous media afforded a negatively charged receptor which interacted more strongly by means of charge-charge attraction. NMR titration gave the stability constant of **123** in DMSO for the tetramethylammonium chloride complex with $0.1*10^2$ M^{-1}. The tetraethylammonium chloride was bound with a similar affinity, the larger tetrapropylammonium chloride showed a sharp decrease in affinity. Choline (**76**) chloride was bound in pure DMSO with a K_{ass} of 80 M^{-1}. In alkaline media (0.01 M KOH/DMSO) the stability constants for the complexes of tetramethylammonium chloride and choline (**76**) hydrochloride were determined as $0.2*10^3$ and $0.1*10^3$ M^{-1}, respectively. In dipolar aprotic solvents such as DMSO, the ammonium salt is recognized as a close contact ion pair. Consequently, the chloride may also interact with the receptor.[274] In protic solvents, such as methanol, **123** is a neutral species capable of forming thermodynamically stable complexes exclusively by cation-π- and CH-π-interactions with ammonium cations which are complementary in size and shape.



123

Figure 88: Resorcarene with partially open deeper cavitand structure (**123**)

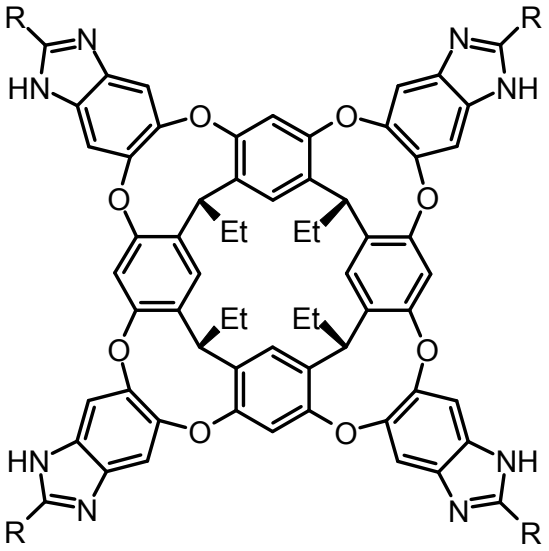
Rebek et al. presented a similar water-stabilized, deep cavitand recognizing various amines and ammonium guests of different shapes. The absence of a fourth wall allows the binding of bulky ammonium groups.[275] In D_2O saturated chloroform **124a** most strongly includes 1-aminoadamantan (K_{ass} = 1*10^3 M^{-1}) and carnitine (**77a**, K_{ass} = 2*10^3 M^{-1}) as measured by NMR titration methodology. Choline (**76**, K_{ass} = 4*10^2 M^{-1}) and carnitine (**77a**), which are poorly soluble in water-saturated chloroform, were taken up forming 1:1 complexes, but acetylcholine (**3**) was not. Such guests with small hydrophobic regions are accommodated with the trimethylammonium group positioned deep inside the cavity. The hydroxyl and carboxylate functions can then provide hydrogen bonding interactions with the groups at the rim. The ester group of acetylcholine (**3**) appears unable to reach such binding sites. Cavitand **125** exists as dimer or larger, kinetically unstable aggregates. With an excess of 1-adamantanol the aggregates break up and providing a sharp NMR-spectrum of a 1:1 complex. Other guests are not included or to disassemble the aggregates.



124a R = $CH_{10}CH_3$, R´ = Et
124b R = Et, R´ = Et
125 R = Et, R´ = Na

Figure 89: Water-stabilized deep cavitands with partially structure (**124, 125**)

Molecules of the cavitand family **126** are all effective phase transfer catalysts which transport a hydrophobic guest, for example with adamantyl residue, from dichloromethane into water. If the reaction product is water soluble it is easily released.[276] **126a** forms stable 1:1 complexes with a variety of guests in water: (*S*)-nicotinium, chinuclidinium (both with $K_{ass} > 10^4$ M^{-1}), *R*-carnitine (**77**, $1.5*10^2$ M^{-1}), choline (**76**, $2.6*10^4$ M^{-1}) and acetylcholine (**3**, $1.5*10^4$ M^{-1}).[277] Compound **126b** shows a folded vase conformation in water and encloses cyclohexane and cycloheptane effectively ($K_{ass} > 10^4$ M^{-1}).[278] Cavitand **126c** can distinguish between several substituted adamantyl residues.[279]



126a R = CH_2COO^-
126b R = p-$CH_2C_6H_4COO^-$
126c R = $CH_2NH_3^+$

Figure 90: Charged cavitands **126** for tetralkylammoniums

Later, the studies of **126c** with choline (**76**), acetylcholine (**3**) and carnithine (**77a**) were extended. Binding mode and properties of these guest complexes were studied by NMR and calorimetry in water at pH 7.8.[280] It was found, that **126c** binds preferably choline (**76**, $2.6*10^4$ M^{-1}) over acetylcholine (**3**, $1.5*10^4$ M^{-1}). The binding of carnithine is in comparison negligible small ($1.5*10^2$ M^{-1}). The guest is inserted with its tetramethylammonium substituent deep in the cavity; the other end is pointing to the carboxylic acid groups at the upper rim of the host.

I. 4.4. Larger Structures, Capsules and Ditopic Binders

Enhancing the binding strength and the selectivity can also be achieved by adding more binding sites. Comparable to a hemicarcerand,[44,281] two calixes can be connected by a suitable spacer to obtain in this way a ditopic binder for ammonium ions. Using only one connection point, makes the molecule sufficiently flexible to bind a bis-ammonium guest. Some recent examples of calixarenes following this concept were published.
The binding abilities of a head-to-head linked bis-calix[4]arene-bis(crown-3) fixed in the rigid *cone* conformation with bridges of different nature and length was described (fig. 91, **127**).[282]

Tetraalkylammonium and *N*-methylpyridinium cations different in size and shape were investigated by ^{1}H-NMR spectroscopy in $CDCl_3$ solution and in the more polar $CDCl_3/CD_3CN$ solvent mixture. As a result a substantial decrease in the K_{ass} values was observed: association constants were generally almost an order of magnitude lower for all guests, due to CD_3CN competing for the binding sites of the host. The double calixarenes have been found to exhibit efficiencies much higher than that of the corresponding reference cavitand calix[4]arene-bis(crown-3). The bridge present in these double calix[4]arenes dictated the orientation and distance between the two rigid caps and thus determines the efficiency and selectivity of binding. The two rigid caps could adapt in response to a potential guest and possibly cooperate in binding by forming a capsule.

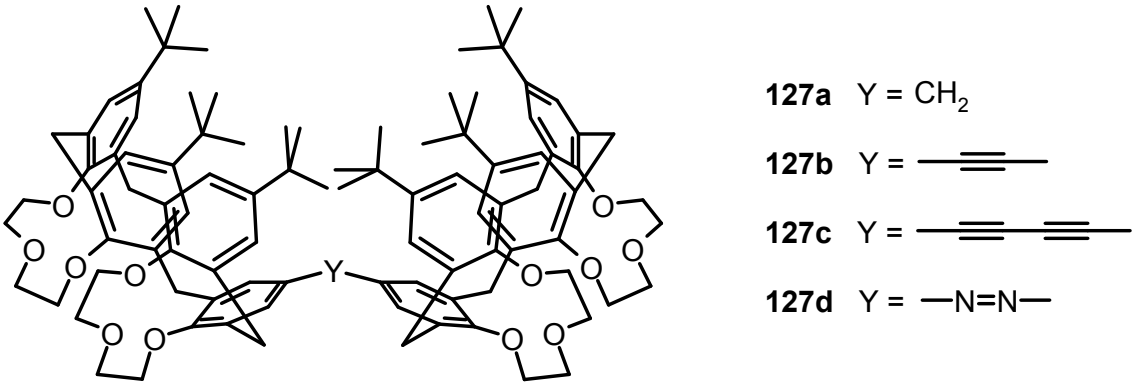


Figure 91: Ditopic calix[4]arene receptor **127** capped with glycol chains

Another ditopic receptor was presented by Parisi´s group.[283] It was developed for binding of linear, long-chain α,ω-alkanediyldiammonium dichloride salts, combining the cooperative action of two converging calix[5]arene cavities in the encapsulation of the dication with the ability of the two ureido functions to bind the relevant counter anions. Binding properties as well as the host-guest architectures, were investigated by a combination of ^{1}H-NMR spectroscopy in $(CDCl_2)_2/CD_3OD$ (2:1 vol/vol) and electrospray mass spectrometry (ESI-MS). Addition of the guest salts to a solution of **128** caused the formation of very strong inclusion complexes, whose host–guest stoichiometries (1:1 and/or 2:1) and geometries were dependent on the length of the diammonium ion and the [host]/[guest] ratio. The use of non-protic solvents showed a beneficial effect of the ureido functions by loosening the ion-paired salt and the association of the anion by formation of six-membered chelate rings with halide or picrate anions and eight-membered chelate rings with carboxylate anions. Table 7 shows the binding constants for long chain diammonium ions:

$H_3N^+(CH_2)_nNH_3^+ * 2\ Cl^-$	K_{ass} [M^{-1}]
n = 8	212
n = 10	163
n = 12	2400
n = 16	2600

NMR titration in $CDCl_3$/DMSO 3:2; 1:1 complexes; errors < 15 %

Table 7: Binding constants of different guests with the ditopic receptor **128**

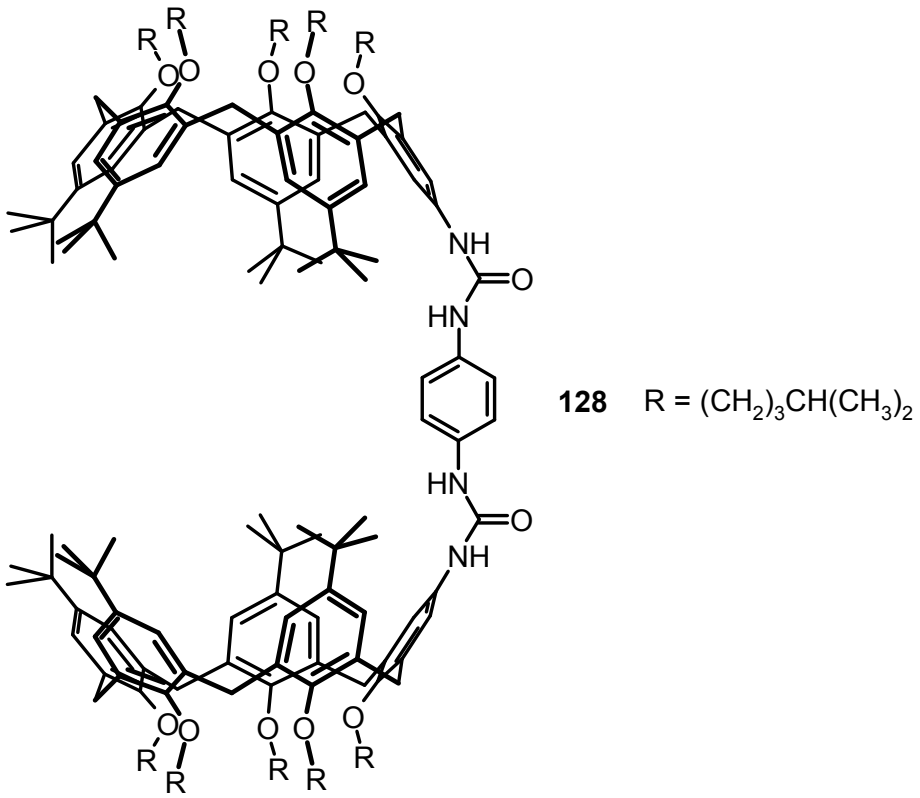
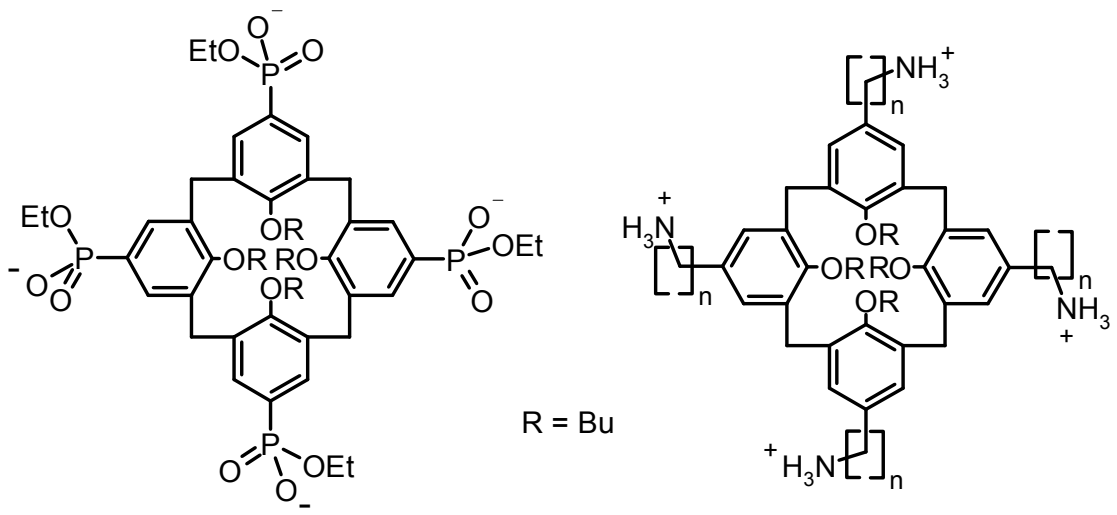


Figure 92: A calix[5]arene dimer for diammonium salt recognition

Biological molecules often possess ionic moieties as well as functional groups capable of forming hydrogen bonding interactions within the same molecule. It is quite appealing to consider ditopic cavities as binding sites based on this principle. Even larger structures can be assembled by complementary recognition of receptor parts to each other[284] - a more specialized case of recognition involving self assembly.[285]

In the following example the authors used the receptor structure **92c**, and appropriate ammonium counterparts, for example **129a**, to form supramolecular assemblies.[286] Evaluated

by NMR titration, the stability constants of the assembly **92c** and **129a** revealed a K_{ass} of (7.0 ± 2.5)*10^5 M^{-1} in methanol, and for **92c** with **129b** a K_{ass} of (1.0 ± 0.4)*10^4 M^{-1} in methanol/water 4:1.



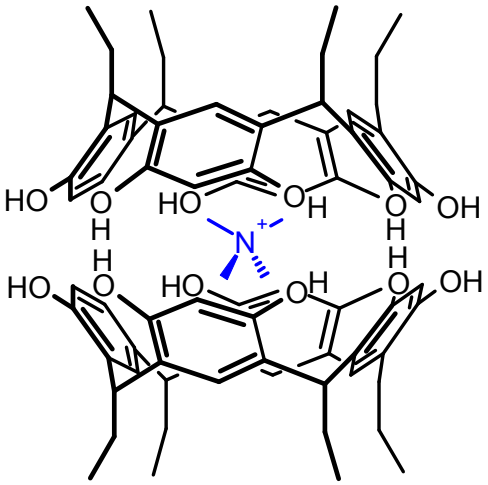
92c

129a n = 0
129b n = 1

Figure 93: Calixarene parts **92c** and **129** for the formation molecular capsules

Zadmard et al. studied these capsules formed in polar solvents by two cone calix[n]arenes more deeply (n = 4 and 6). The first was substituted at the upper rim by phosphonic ethyl ester lithium salt groups (**92c** and its calix[6]arene analogue), while the second consisted of ammonium cations (**129a** and its calix[6]arene analogue).[287] Inclusion of Phe, aniline, tetramethylammonium salts and other organic molecules into the capsule cavity in methanol was investigated.[288] Since the capsules were far more stable than the complex with the guest molecule, *10^5 vs. *10^3 M^{-1} in methanol-d4, the authors claimed that a guest molecule was included inside the anionic half-sphere after opening the capsule.

Resorcarene can also form dimers by a self-assembling process, in which the cavity is filled.[289] For instance, the tetramethylammonium cation can be included. This was nicely evidenced by mass spectroscopy and several crystal structures of smaller tetraalkylammonium cations with unsubstituted resorcarenes like **75c** with different alkyl chain length. Competitive mass spectrometric studies clearly indicated preference of the tetramethyl ammonium cation over tetraethyl ammonium cation and especially tetrabutylammonium cation. The two resorcarene units are held together mediated by hydrogen-bonded networks via solvent molecules of methanol and water.[290]

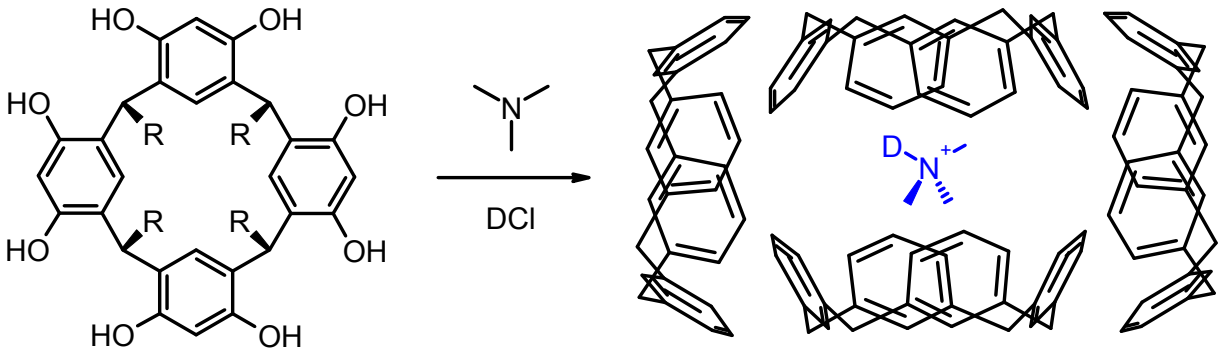


$NMe_4^+@[\mathbf{75c}]_2$

Figure 94: Encapsulation of a quaternary ammonium cation by two resorc[4]arene molecules ($NMe_4^+@[\mathbf{75c}]_2*Cl^-*6MeOH*H_2O$; solvent molecules and counterions are avoided for clarity)

A tetralkylammonium ion (R = propyl to hexyl), together with one to three chloroform molecules can also be complexed and included in a capsule surrounded by six resorcarenes stabilized by H-bonds.[291]

Expanding the studies, Cohen et al. demonstrated a pH dependent inclusion of quaternary ammonium salts in a hexameric structure like **130** in $CDCl_3$ by NMR studies.[292]



130 R = $(CH_2)_{10}CH_3$

Figure 95: Encapsulation of a quaternary ammonium cation by six resorc[4]arene molecules ($NMe_3D^+@[\mathbf{130}]_6*Cl^-$; solvent molecules, substituents and counterions are omitted for clarity; the last two resorcarene calixes are arranged behind and in front of the scheme´s plane)

These selected, recent examples are thought to complement this chapter and to give a good impression of the possibilities for ammonium recognition with calixarenes and resorcarenes utilizing self-assembly. A representation of all possibilities is beyond the scope of this review. The reader is referred to the large body of actual publications.[293] Larger capsules for the inclusion of a variety of guests were recently published by the Rebek group.[294]

The advantages of calixarenes as basic building blocks in supramolecular chemistry in comparison with other synthetic macrocycles is obvious: Good accessibility, the possibility of tuning shape and size of the inner cavity as well as the opportunity to introduce various functional groups and their further transformations enables to adress nearly any ammonium ion guest selectivity. Calixarenes are often used for synthesis of more complicated and elaborated structures, to enclose or strongly complex larger guests with high selectivities and outstanding binding strengths.

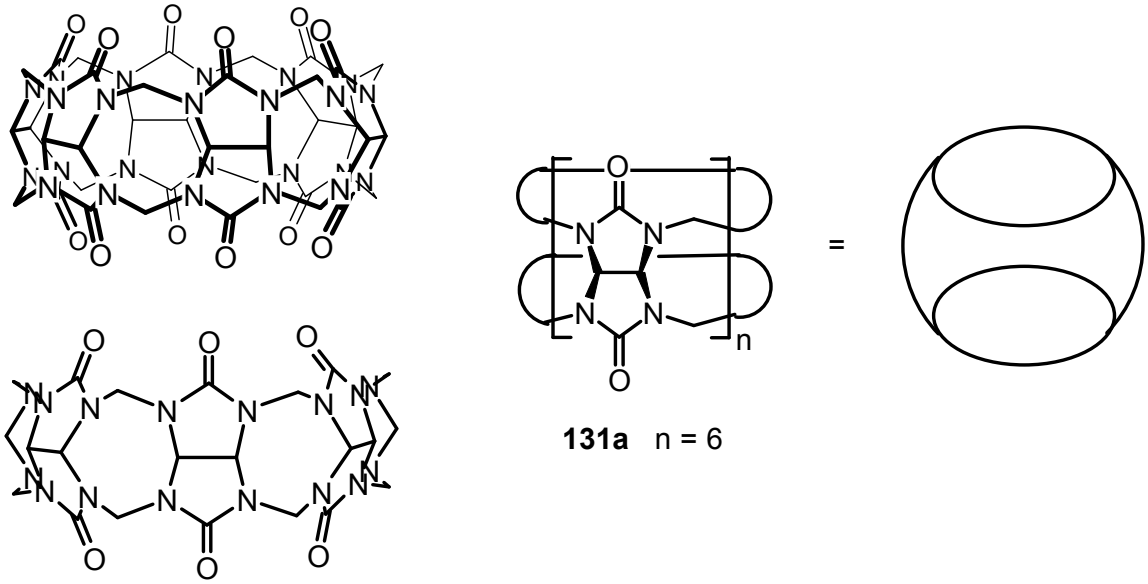
Calixarenes often achieve selectivities in cation binding which are superior to crown ethers due to the guest inclusion being controlled by steric reasons and various interactive forces of host and guest. Some calixarene-based artificial receptors show remarkable selectivities for amine isomer recognition. Especially noteworthy is their ability to complex strongly with quaternary ammonium ions outperforms nearly every other receptor class, except the cucurbiturils (see next chapter). This was applied with benefit in assays for such important biomolecules as acetylcholine (**3**).

A considerable number of synthetic receptors based on a calixarene framework for amino acids derivatives has been designed and studied in organic media but only a few examples have been reported in aqueous solution. Calixarenes are able to select precisely basic or aromatic amino acids in aqueous solution. Because of this property, they can be applied even as enzyme mimetics.

I. 5. Cucurbiturils and Related Structures

Behrend´s polymer was reported over a century ago as a by-product of aminal type polymers,[295] but the structure of the material was fully characterised only in 1981. Because of the resemblance of the barrel-shaped molecule to a pumpkin, the investigators gave the macrocyclic methylene-bridged glyconuril oligomers the name cucurbiturils, derived from the Latin name of the plant family (cucurbitacae). All have a hydrophobic cavity and two identical carbonyl-laced portals ("occuli") in common and are readily prepared by the condensation of glyconuril with formaldehyde.

Cucurbit[6]uril (CB[6], **131**), a macrocycle comprising six glycoluril units connected by 12 methylene bridges, is the oldest and most popular member of the host family cucurbit[n]uril (CB[n], n = 5 – 11).[296]



131a n = 6

Figure 96: Structure and schematic of cucurbit[6]uril (CB[6], **131a**)

Crystalline complexes incorporating various metal salts and some dyes were observed and therefore cucurbiturils were applied as receptors by Mock and Shih.[297] Alkylammonium ions were the first organic guests to be reported for CB[6] (**131a**).[298] Mock,[299] Buschmann and co-workers [300] and Kim et al. [301] further investigated the molecular-recognition properties. Cucurbiturils bind their guests by hydrogen-bonding or ion-dipole interactions in combination with the hydrophobic effect of the cavity. The rigidity of the structure enables selective recognition of hydrophobic residues or cations. The selectivity strongly depends on the inner size of the cavity and possible guest orientations therein, as in cyclodextrins and calixarenes:

para-Methylbenzylamine is bound, while the *ortho*- and *meta*-isomers are not.[302] Isaacs et al. published a crystal structure of the cucurbit[6]uril *p*-xylylenediammonium inclusion complex. The ammonium cations are symmetrically located in the centre of a ring formed by the carbonyl oxygens. The benzene ring is rotationally disordered in the cavity between two orientations.[303]

The upper and the lower regions of the cucurbituril - the occuli - bear at least six urea carbonyl groups, representing an area of negative charge accumulation, coordinating to cationic species like alkanediamines. The high specificity for ammonium ions is explained mainly by this electrostatic ion-dipole attraction assisted by hydrogen bonding. Proper alignment of the bound ammonium ions with the host carbonyl dipoles is critical: In the homologous series of *n*-alkane amines a clear trend in stability of the complexes was observed, reaching the maximum for *n*-butylamine: n = 1 < 2 < 3 < 4 > 5 > 6 > 7. α,ω-Alkanediammonium ions (H_3N^+-$(CH_2)_n$-NH_3^+) are bound by CB[6] (**131a**) with a preference for alkyl chain length of n = 5 or 6. Substituents fitting the size of the cavity are bound with the highest strength and affinity; longer chains protrude into the second oculus of the cucurbituril, interfering with the carbonyl dipoles and their solvation sphere. [304]

In contrast to the moderate to good water soluble related host molecules with a comparable cavity size, the cyclodextrins (**136**),[35,155,305] the poor solubility of CB[6] (**131a**) in common solvents and water makes it difficult to study its host–guest chemistry in solution.

During the 1990s it was discovered that it becomes readily soluble in aqueous solutions containing alkali or alkaline earth metal ions. Since then, such aqueous solutions have often been employed for studies on complexation properties of CB[6] (**131a**).[306] Mock and Shih examined its binding affinity towards a variety of aliphatic ammonium ions in 50 % (vol/vol) aqueous formic acid, observing binding constants (*K*) around 10^3 – 10^4 M^{-1} for *n*-alkylammonium ions and 10^4 – 10^5 M^{-1} for α,ω-alkanediammonium ions, as determined by NMR and/or UV spectroscopy.[304] Generally, typical binding constants for ammonium guests, e.g. simple amines, diamines and aromatic amines range from 10^1 to 10^7 M^{-1} in H_2O/HCOOH mixture.[307] In aqueous salt solutions, for example 50 mM sodium chloride solution, even higher values for α,ω-alkanediammonium ions (up to $1.5*10^9$ M^{-1} for H_3N^+-$(CH_2)_5$-NH_3^+, cadaverin) are reported.[308]

Not only simple amines, but also many amino acids and amino alcohols have been employed as guests. Buschmann and co-workers first studied the complex formation between cucurbituril and some aliphatic amino acids by means of calorimetric titrations in aqueous

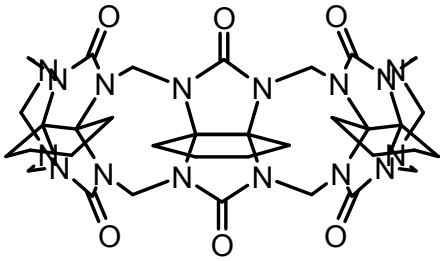
formic acid (50 % vol/vol) or aqueous solution for comparison of the interaction of cucurbituril with some aliphatic amino alcohols and aliphatic amino compounds: The complex formation of amino acids was found to be favoured by enthalpic and entropic contributions. The situation changes completely in the case of amino alcohols. Reaction enthalpies and entropies are influenced by the number of methylene groups. The amino alcohol 3-aminopropanol formed the most stable complex. With an increasing number of methylene groups the stability of the complexes decreased, which is attributed to entropic factors.[309]

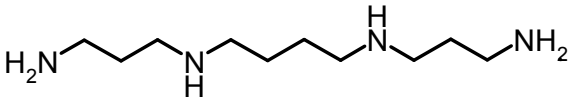
Paraquat and its derivatives are typical guests for cucurbit[n]urils.[310] Amino azabenzenes are bound with binding strengths in the range of 10^3 - 10^6 M^{-1}.[311] Many homologues from cucurbit[5]uril to cucurbit[10]uril, as well as derivatives, congeners and analogues are available, even exceeding the cavity size span of the cyclodextrin family. Their chemistry has been discussed in several books[312] and reviews.[308,313,314,315] In the following, some recent examples in view of molecular recognition of ammonium ions will be discussed.

Various cucurbit[n]uril derivatives have been synthesized by introducing alkyl groups at the equator of the molecules to improve their solubility in water and other commonly used organic solvents.[316] Different reactive functional groups have been introduced directly onto the surface of the cucurbit[n]urils to improve solubility and for further modification.[301, 317, 318]

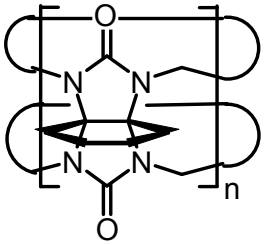
Such a water soluble example was reported with cyclohexanocucurbit[6]uril (CB*[6], **132**). Complexation properties with various organic mono- and diammonium ions were studied by isothermal titration calorimetry and ^{1}H-NMR spectroscopy.[319] X-ray crystal structures of α,ω-alkanediammonium ions (H_3N^+-$(CH_2)_n$-NH_3^+, n = 4 – 8) and spermine (**133**) complexes with **132** revealed the aliphatic chains of the guest molecules in an extended or partially bent conformation included in the cavity, depending on their length. The hexamethylene chain conformation is twisted to allow strong ion–dipole interactions between both ammonium groups and the carbonyl groups at the portals. This is also increasing the hydrophobic interactions between the alkyl part of the guest and the inner wall of the host, which results in the largest enthalpic gain and a preference for this guest among all α,ω-alkanediammonium ions. The selectivities match with those of **131a.** The cavity dimensions are essentially the same as in CB[6] (**131a**). The binding affinities of CB*[6] (**132**) towards *n*-alkylammonium ions (10^4 – 10^8 M^{-1}) and α,ω-alkanediammonium ions (10^7 – 10^{10} M^{-1}) in water are 3 – 5 and 2 – 3 orders of magnitude higher than those of CB[6] in 50 % formic acid[298,304] and in 0.05 M

NaCl solution,[308] respectively. This was attributed mainly to the larger enthalpic gain upon complex formation in the absence of interfering ions, such as protons and Na^+. In particular, the binding constant of spermine to CB*[6] was measured to be $3.4*10^{12}$ M^{-1}, which is the highest binding constant ever reported for CB[6] or its derivatives.





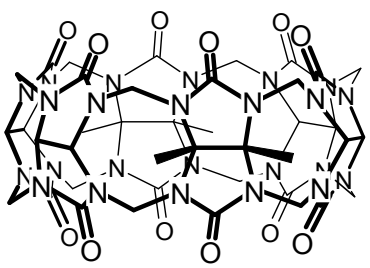
133

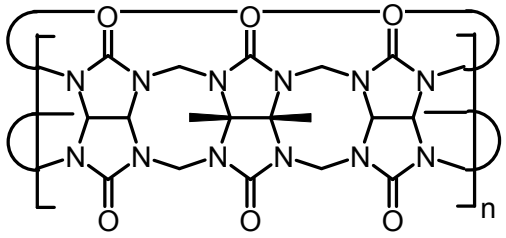


132 n = 6

Figure 97: Cyclohexanocucurbit[6]uril (CB*[6], **132**) and the guest molecule spermine (**133**)

Cucurbit[n]urils bind amino acids strongly. A crystal structure of the inclusion complex of *S*-glutamate (*S*-Glu) in α,α,δ,δ-tetramethylcucurbit[6]uril (**134**) captured by a host in a 1:1 host:guest ratio gives more insight.[320] The protonated amino moiety is located at the portal of the host, the side chain carboxyl anion moiety is included in the cavity of **134**. A combination of hydrogen binding and ion–dipole interaction of the ammonium group and the portal carbonyls of the host were seen as the driving forces for the complex formation. In addition, the carboxyl moiety of the amino acid located at the portal of the host could interact with the portal carbonyl of the host through hydrogen bonding.

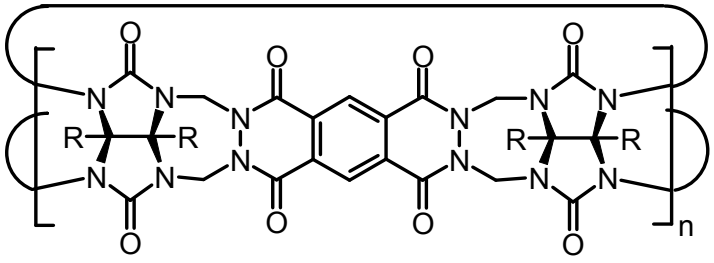




134 n = 2

Figure 98: α,α,δ,δ-Tetramethylcucurbit[6]uril (**134**)

Unsubstituted cucurbiturils are not fluorescent. Issacs and co-workers described the incorporation of a fluorescent (bis)-phthalhydrazide in cucurbit[6]uril, which made the system accessible to monitoring via fluorescence spectroscopy.[318] This analogue (**135**) shows good molecular recognition properties for a variety of guests in aqueous sodium acetate buffer at pH 4.74: Association constants for α,ω-alkanediammonium ions (H_3N^+-$(CH_2)_n$-NH_3^+, n = 6 to 12) increase with the length of the alkane chain. The maximum binding strength was observed for n = 10 and 11 with $2.3*10^4$ M^{-1}. Aromatic ammonium targets were complexed even stronger due to the additional π-π-interactions. The best examples were benzidine with $4.6*10^6$ M^{-1}, nile red[321] with $8.2*10^6$ M^{-1} and the similar dye nile blue chloride with an association constant of $1.1*10^6$ M^{-1}. The authors argue, that increasing the surface area for π-π-interactions by increasing the size of the π-system of the guest as well as increasing the co-planarity of the guest molecule significantly increases the association constant. Biologically relevant guests such as amino acids and nucleobases were bound in the cavity of **135** with K_{ass} values ranging from 10^3 to 10^6 M^{-1}. In this line good affinities to aromatic amino acids as a result of π-π-stacking and ion-dipole interactions were observed: For *S*-phenylalanine (**81a**), *S*-tyrosine and *S*-tryptophan (**81b**) association constants of $4.2*10^4$, $5.7*10^4$ and $3.2*10^6$ M^{-1}, respectively. Due to the larger size of the indole ring compared to that of the monocyclic systems, tryptophan (**81b**) was bound more tightly.



135 n = 2; R = COOH

Figure 99: Structure of the cucurbituril-phthalhydrazide analogue **135**

A dual-response colourimetric sensor array based on supramolecular host–guest complexation in cyclodextrins (α-, β- and γ-cyclodextrin, **136**) and cucurbit[n]urils (CB, n = 5 - 8, **131**) was used for the identification of amines in water.[322] The displacement of coloured or fluorescent dyes like methylene blue (**137a**), pyronine (**137b**) and acridine orange (**137c**) led to discrimination among primary, secondary, tertiary, aliphatic, aromatic, linear and branched amines by colour change or fluorescence increase. The combination of the images obtained from visible and UV light identified each of the 14 analytes. The selectivity of the sensor

array is based on the analytes interaction with the host–guest complex, which involves the combination of a large number of parameters, including hydrophilicity–hydrophobicity, coulombic effects, dipolar interactions and hydrogen bonds.

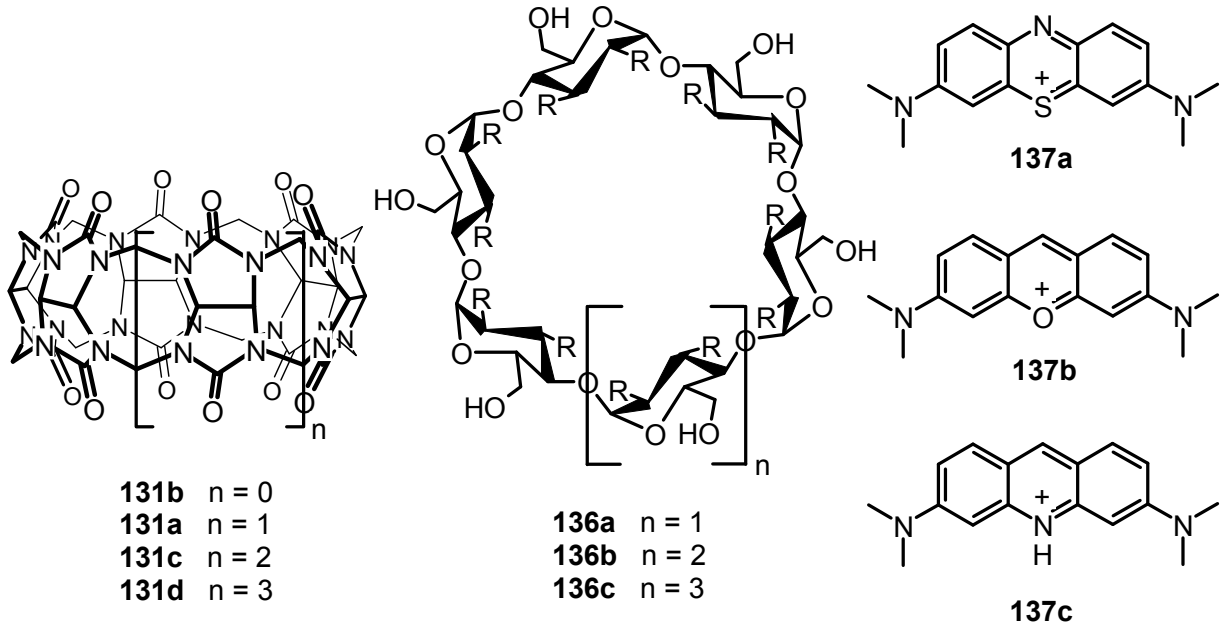


Figure 100: Organic cavities for the displacement assay for amine differentiation

Nau and co-workers introduced a general supramolecular assay principle in which amino acid decarboxylase activity can be continuously monitored by measuring changes in fluorescence, which result from the competition of the enzymatic product and the dye for forming a complex with a cucurbit[n]uril macrocycle.[323]

The combination of cucurbit[6]uril (**131a**) and the 3-amino-9-ethylcarbazole dye **138a** leads to a suitable displacement assay (fig. 101) for monitoring the enzymatic activity of lysine decarboxylase in aqueous buffer at pH 7.[324] Due to a complexation-induced pK_a shift, a large dual fluorescence response (100-fold increase at 375 nm and 9-fold decrease at 458 nm) accompanied by a colour change upon supramolecular encapsulation in cucurbit[6]uril (**131a**) is observed. The enzymatic decarboxylation of lysine (**81c**) converts the amino acid *S*-lysine (**81c**) into cadaverin (**139a**), which competes very efficiently (K_{ass} = 9.5*10^9 M^{-1} in 10 mM NH_4OAc buffer) and so fully reverts the fluorescence changes originally caused by the addition of the macrocycle. The binding constant of the substrate lysine (**81c**) is too low to displace the more strongly bound fluorescent dye (K_{ass} = 2.22*10^7 M^{-1}) and causes no effect.

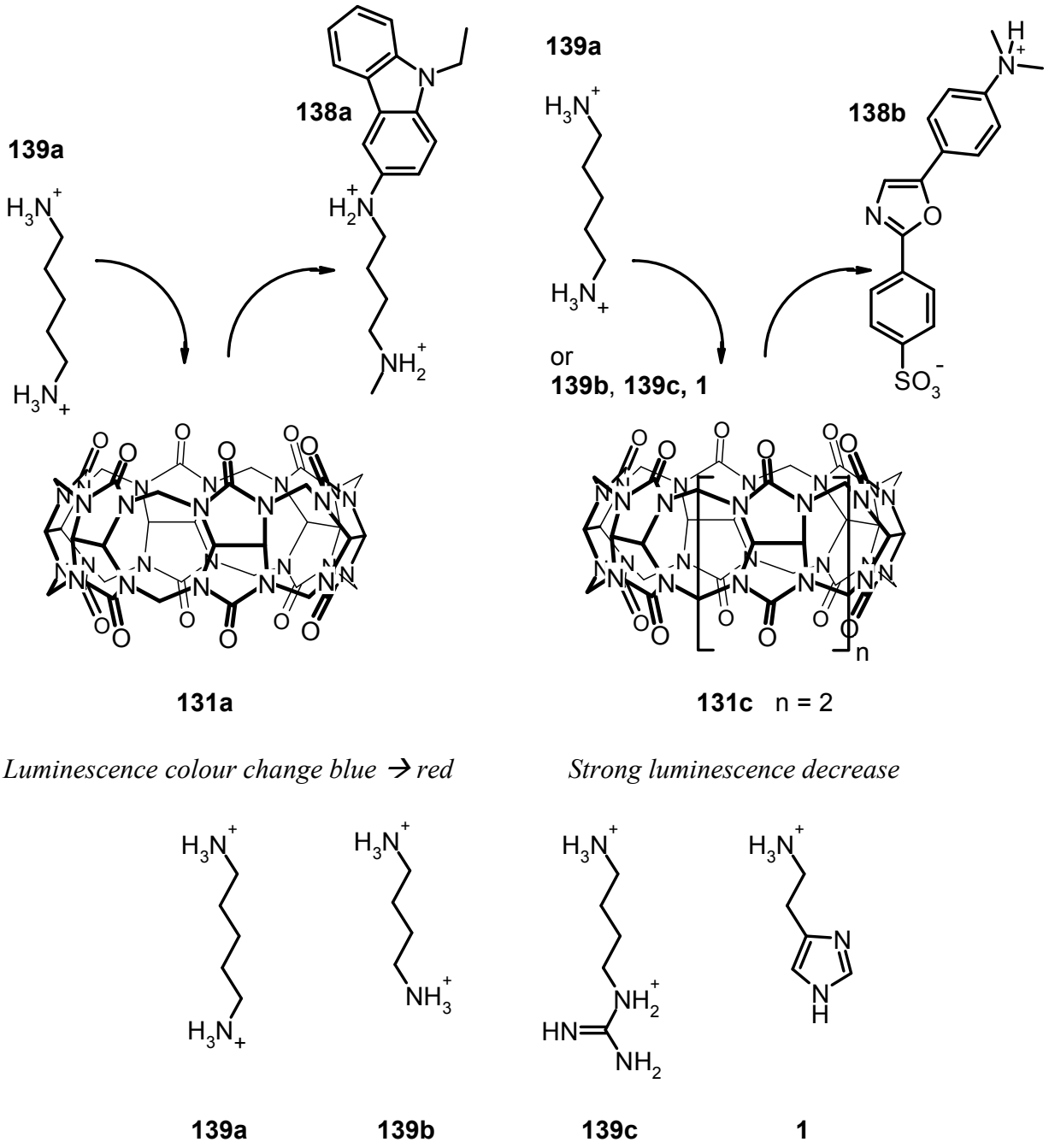


Figure 101: Displacement assay methodology for diammonium- and related guests involving cucurbiturils and some guests

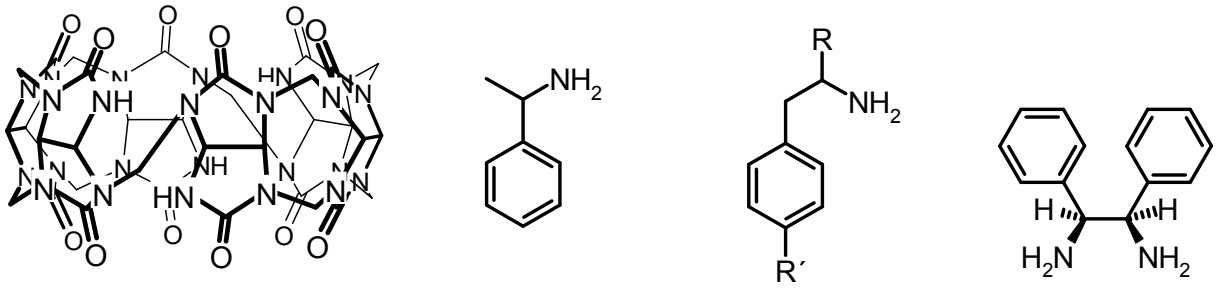
This principle was employed in a similar manner with cucurbit[7]uril (**131c**) and the fluorescent dye Dapoxyl (**138b**). It forms a strong inclusion complex with **131c** (K_{ass} = (2.0 ± 0.2)*10^4 M^{-1}) in ammonium acetate buffer at pH 6, which shows up to 200 times higher emission intensity (λ_{em} = 380 nm) than the free dye.[325] Addition of amino acids has little effect on the fluorescence intensity of the CB[7]-Dapoxyl reporter pair. Addition of low-micromolar concentrations of amines lead to a steep decrease in fluorescence as a result

of competitive binding. This allows real-time monitoring of enzymatic activity by a switch-off fluorescence response in 10 mM NH_4OAc buffer at pH 6.0.
As demonstrated by simple titration experiments, the substrates lysine (**81c**), arginine (**81d**), histidine (**81e**) and ornithine have low affinity to **131c**, and cannot interfere with the formation of the strongly fluorescent complex ($K_{ass} < 10^3$ M^{-1}). Decarboxylation produces the corresponding amines cadaverin (**139a**), agmatine (**139c**), histamine (**1**) or putrescine (**139b**), so increases the net positive charge and thereby the affinity of the competitor by removal of the carboxylate group. These guests exist in their ammonium ion forms near neutral pH and thus have a very high affinity for **131c** ($K_{ass} < 4.3*10^4$ M^{-1}). This tandem assay principle has millimolar sensitivity.

The versatile approach was extended to aromatic guests and applied for enantiodiscrimination, respectively resolution.[326] Similar observations were published: The amino acids histidine (**81e**), tyrosine and tryptophan (**81b**) are binding to the reporter pair **131c/138b** with approx. 1000 M^{-1}, the diamines in contrast with 10^4 to 10^6 M^{-1} affinity in 10 mM NH_4OAc buffer solutions (pH 6.0) and, therefore, displace the dye from the complex.
Time-dependent fluorescence response monitoring of *S*-lysine decarboxylation with varying *R*-lysine enantiomeric excess allowed accurate determination of optical purity of the amino acid over a wide range of *ee* (64 – 99.98 %) by different kinetic fluorescence decay traces with 2.4 nmol limiting sensitivity. Only the *S*-enantiomer is accepted by the enzyme as a substrate and is converted to the product that is responsible for the observed fluorescence signal. No response and no conversion by the enzyme are observed with the *R*-enantiomer.

Recently, Isaacs et al. demonstrated the chiral recognition of some amino acids inside a novel chiral cucurbituril: nor-seco-cucurbituril (±)-bis-ns-CB[6] (**140**), which demonstrates enantio- and diastereoselective recognition inside its cavity. [327]
The K_{ass} values for **140** towards diammonium guests were measured by UV-vis spectroscopic titration and ^{1}H-NMR spectroscopy competition experiments in water finding association values mainly in the range of 10^3 to 10^4 M^{-1}. The affinity of (±)-bis-ns-CB[6] toward 1,6-diaminohexane in its protonated form was even higher ($1.3*10^5$ M^{-1}). Conversely, this affinity is 3400-fold lower than found with CB[6] (**131a**), which presumably arises from differences in the strength of ion–dipole interactions, the degree of aqueous solvation of the C=O portals, or both.

Host **140** undergoes diastereoselective complexation (up to 88:12) with chiral amines including amino acids and amino alcohols as well as meso-diamine **78c**. In the ^{1}H-NMR spectra recorded for a mixture of (±)-bis-ns-CB[6] and excess of the guest (-)-**78b**, a 72:28 ratio of the diastereomer was found. Toward amino acids **81f** (77:23) and **81a** (88:12) and amino alcohol **81g** (76:24) minimal higher values were observed. Interestingly, (±)-bis-ns-CB[6] is even able to distinguish between the enantiotopic groups of meso-compound **78c** (74:26).



140 n = 6 **78b** **81a** R = COOH, R´ = H **78c**
81f R = COOH, R´ = NH_2
81g R = CH_2OH, R´= H

Figure 102: nor-seco-Cucurbituril (±)-bis-ns-CB[6] (**140**) and guest molecules

A combination of achiral host cucurbiturils and chiral inductor can also serve as a supramolecular chiral host (fig. 103). A chiral guest added to the solution of cucurbit[6]uril-based complexes with enantiopure amines can replace one of the originally bound amines achieving an enantiodifferentiation by accommodating two different chiral guests inside a self-assembled achiral capsule. In this way significant enantiomeric and diastereomeric discrimination by incorporating a strong chiral binder is possible.[328] Comprehensive studies on the chiral recognition of guests were performed: Dissolving cucurbit[6]uril (CB[6]) in an aqueous solution of an enantiopure organic amine, such as (*R*)- or (*S*)-2-methylpiperazine (MP) or (*R*,*R*)- or (*S*,*S*)-*trans*-1,2-diaminocyclohexane (DC), led to the formation of the respective enantiopure complex, i.e., (*R*;*R*)- or (*S*;*S*)-[CB-[6]*2MP]$^{4+}$ (**141b**) or (*R*,*R*;*R*,*R*)- or (*S*,*S*;*S*,*S*)-[CB[6]*2DC]$^{4+}$ (**141a)**. (*S*)-2-Methylbutylamine could be discriminated by this assemblies with up to 95 % *ee* by formation of diastereomeric (*S*;*R*)- and (*S*;*S*)-[CB[6]*MP*MB]$^{3+}$ ternary complexes. (*S*)-MB controls the degree of chiral supramolecular assembling of (*R*)-MP or (*S*)-MP with cucurbit[6]uril:

$$[CB[6]*2((R)\text{-}MP)]^{4+} + (S)\text{-}MB^{+} \rightarrow [CB[6]*(R)\text{-}MP*(S)\text{-}MB)]^{3+} + (R)\text{-}MP^{2+}$$

with a K_{ass} of 15000 ± 3000 M^{-1} for this process

$$[CB[6]*2((S)\text{-}MP)]^{4+} + (S)\text{-}MB^{+} \rightarrow [CB[6]*(S)\text{-}MP*(S)\text{-}MB)]^{3+} + (S)\text{-}MP^{2+}$$

with a K_{ass} of 800 ± 100 M^{-1} for this process

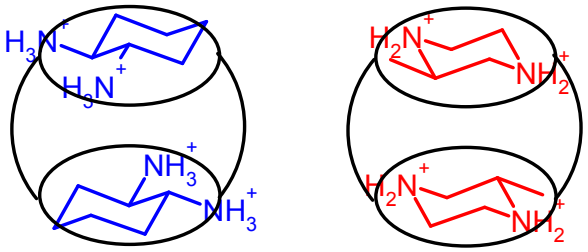


Figure 103: The cucurbit[6]uril based complexes **141** for chiral discrimination

The authors also found cucurbit[7]uril (**131c**) binding the diastereomeric dipeptide *S*-Phe-*S*-Leu-NH_3^+ up to eight times tighter than *S*-Phe-*R*-Leu-NH_3^+ with its larger cavity. The discrimination of dipeptides was not possible with the previously discussed system.

The cavity size of cucurbit[7]uril enables the molecule to bind ferrocenyl and adamantyl substituted amines strongly as 1:1 complexes: Rimantadin, an amino adamantyl derivative, which is used as an anti viral drug, is included in aqueous buffer at pD 4.74 with an association constant of around $4.2*10^{12}$ M^{-1}.[329]

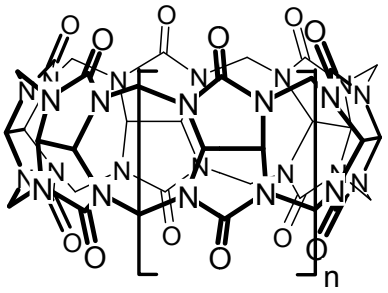
The molecular host cucurbit[7]uril (**131c**) forms an extremely stable inclusion complex with the dicationic ferrocene derivative bis(tri-methylammoniomethyl)ferrocene (**142c**) in aqueous solution.[330] The equilibrium association constant for this host-guest pair is $3*10^{15}$ M^{-1}, equivalent to that exhibited by the avidin–biotin pair.
The large association strength has been determined from serial competitive ITC binding studies (table 8). Two different series, also giving K_{ass} values for other interesting ammonium guests, were pursued. All amines were protonated under the conditions of the study.

Guest	Competitor	K_{ass}/M^{-1}
S-Phe (**81a**)	None	$(1.8\pm0.2)*10^{6}$
1,6-Hexanediamine	*S*-Phe (**81a**)	$(2.1\pm0.4)*10^{9}$
Aminocyclohexane	1,6-Hexanediamine	$(1.3\pm0.4)*10^{11}$
142c	Aminocyclohexane	$(3.0\pm1.0)*10^{15}$
Cyclopentanone	None	$(4.2\pm0.3)*10^{5}$
Spermine (**133**)	Cyclopentanone	$(4.8\pm0.6)*10^{8}$
N,N´-bis(aminoethyl)-1,6-Hexanediamine	Spermine (**133**)	$(1.7\pm0.4)*10^{11}$
142c	N,N´-bis(aminoethyl)-1,6-Hexanediamine	$(3.3\pm1.0)*10^{15}$

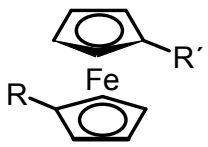
Table 8: Two series of binding constants for different guests to CB[7] (**131c**)

The values for **142a** and **142b** are $(3.2 \pm 0.5)*10^{9}$ M^{-1} and $(4.1\pm1.0)*10^{12}$ M^{-1}, respectively. A significant loss in the complex stability by a factor of 1400 in the K_{ass} value is observed upon oxidation of the ferrocene centre of **142c**, enabling a switching process of complexation/decomplexation dependent on the competitor.

The extremely large affinities of the complexes surveyed are due to a large enthalpic gain, originating from the tight fit of the ferrocene core to the rigid CB[7] cavity achieving optimal van der Waals contacts, critically assisted by the entropic gain arising from the extensive host desolvation, and largely uncompensated by losses in configurational entropy. The crystal structure of the complex shows the complete inclusion of the ferrocenyl residue in the CB[7] cavity and the almost ideal positioning of each of the trimethylammonium groups maximizing ion–dipole interactions with the carbonyl rims on each of the host portals. The ferrocene core of the guest fills 55 % of the host cavity volume, approximately equal to the optimal filling fraction proposed.[331]



131c n = 2



142a R = CH_2OH, R´ = H
142b R = $CH_2N^+(CH_3)_3$, R´ = H
142c R = R´ = $CH_2N^+(CH_3)_3$

Figure 104: Cucurbit[7]uril (**131c**) and its ferrocene guests (**142**) opposed

Quaternary cations like NMe_4^+, NEt_4^+, PMe_4^+, and PEt_4^+ are encapsulated within the cavity of CB[7] (**131c**), with K_{ass} = (1.2 ± 0.4)*10^5, (1.0 ± 0.2)*10^6, (2.2 ± 0.4)*10^6, and (1.3 ± 0.3)*10^5 M^{-1}, respectively.[332]

Consistent with these values, acetylcholine (**3**) and other cationic cholines ($R_3NCH_2CH_2OR'^+$), their phosphonium analogues ($R_3PCH_2CH_2OR'^+$) (R_3 = Me_3, Et_3, or Me_2Bz, or R_3N = quinuclidinium, and R´ = H, $COCH_3$, $CO(CH_2)_2CH_3$, or PO_3H) and (±)-carnithine (**77a**) form stable 1:1 host-guest complexes with cucurbit[7]uril (**131c**) in aqueous solution (K_{ass} in the order of magnitude 10^5 - 10^6 M^{-1}).[333] The complexation behaviour has been investigated using ^1H- and ^{31}P-NMR spectroscopy, and ESI mass spectrometry. This study is one rare example, where molecular recognition of cholines in aqueous solution is achieved with a neutral host without aromatic walls for cation–π interactions. The acetyl-substituent is included in the cavity and the quaternary ammonium ion is coordinated by the carbonyl functions of **131c**. In the case of phosphonium groups, these substituents are generally included in the cavity additionally stabilized by van der Waals contacts. The acetyl substituent sits on the outside of the cavity (fig. 105).

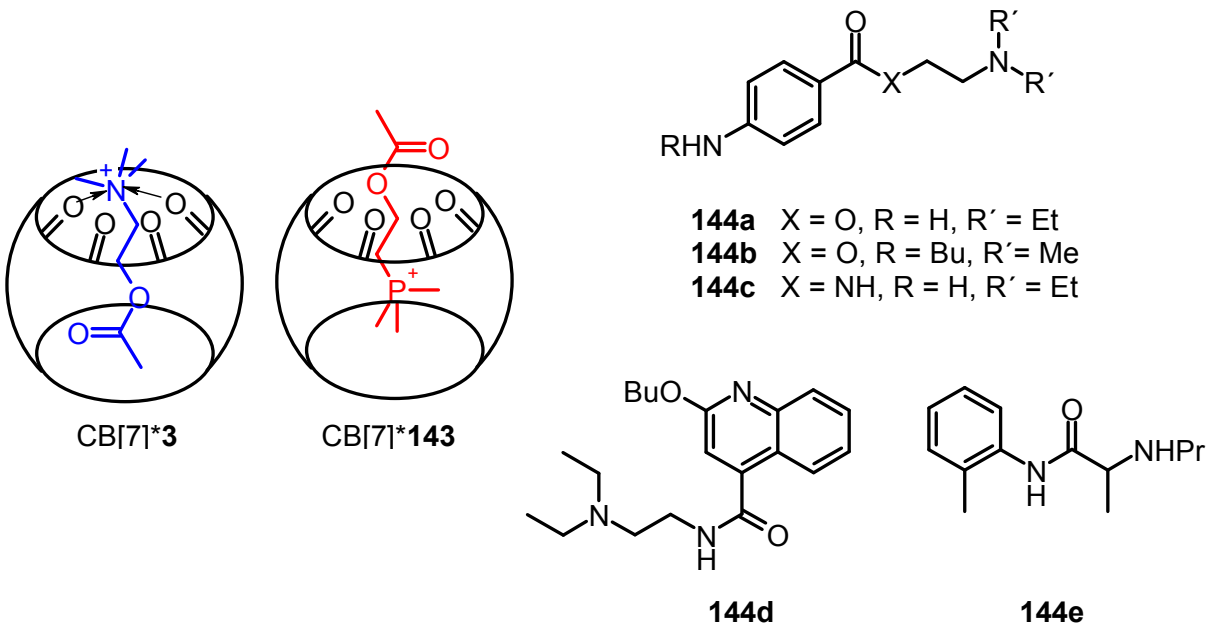


Figure 105: Cucurbit[7]uril (**131c**) guest inclusion and representative guests

The cucurbit[7]uril (**131c**) host molecule forms also very stable host–guest complexes with the local anaesthetics procaine (**144a**, K_{ass} = (3.5 ± 0.7)*10^4 dm^3mol^{-1}), tetracaine (**144b**, K_{ass} = (1.5 ± 0.4)*10^4 dm^3mol^{-1}), procainamide (**144c**, K_{ass} = (7.8 ± 1.6)*10^4 dm^3mol^{-1}), dibucaine (**144d**, K_{ass} = (1.8 ± 0.4)*10^5 dm^3mol^{-1}) and prilocaine (**144e**, K_{ass} = (2.6 ± 0.6)*10^4 dm^3mol^{-1}) in aqueous solution (pD = 4.75) as observed in NMR studies.[334] The stability constants are 2–3 orders of magnitude higher than the values reported for binding by the comparably sized β-cyclodextrin (**136b**) host molecule. The protonated forms are bound more strongly in acidic solution. Upon protonation the cucurbit[7]uril sits around the aromatic unit of **144a-144c**, in the deprotonated case it includes the alkylated amine centre.

Similarly, "bolaform" guests with two cationic end groups, such as succinylcholine chloride (**145**) and α,ω-bis(trialkylammonium)alkane dications (or their phosphonium analogues) form strong host-guest complexes and [2]pseudorotaxanes with cucurbit-[7]uril.[335] An analogous dimeric guest series to the amines discussed before containing NMe_3^+, NEt_3^+, quinuclidinium (**146g**), PMe_3^+ and PEt_3^+ end groups, was studied in aqueous solution by ^{1}H- and ^{31}P-NMR spectroscopy, as well as ESI mass spectrometry.[336]

The formation of 1:1 aggregates is assigned to a [2]pseudorotaxane structure with the NMe_3^+ and NEt_3^+ end groups outside the cavity near the carbonyl oxygens on the portal and the guest molecule located in the hydrophobic cavity. The 1:1 host-guest stability constants range from 8*10^6 (guest **145**) to 3*10^{10} M^{-1} (guest **146b**) and are dependent on the nature of the end

group and the length and hydrophobicity of the central linker. The stability constants for the 1:1 complexes with guests with the same decamethylene linker follows the order **146c** > **146e** > **146f** > **146d**, indicating that for threads with the same alkyl chain length, the stability constant is related to charge diffusion on the peralkylonium end group. Changing the end groups from NMe_3^+ to NEt_3^+ (**146c** to **146d**) or PMe_3^+ to PEt_3^+ (**146e** to **146f**) results in a lowering of K_{ass} value by one order of magnitude as the less polar triethyl-substituted groups have weaker ion-dipole interactions with the polar portals of CB[7] than the methyl analogues. With the exceptions of the shorter $[(CH_3)_3N(CH_2)_nN(CH_3)_3]^{2+}$ (n = 6, 8) dications, the addition of a second CB[7] results in the translocation of the first CB[7], such that the hydrophobic $-NR_3^+$ and $-PR_3^+$ end groups (R = Me or Et) are encapsulated in the cavities, while the central linker extends through the CB[7] portals. The magnitude of the stability constants for the 2:1 complexes closely follows the trend observed previously for CB[7] binding with the NR_4^+ and PR_4^+ cations.

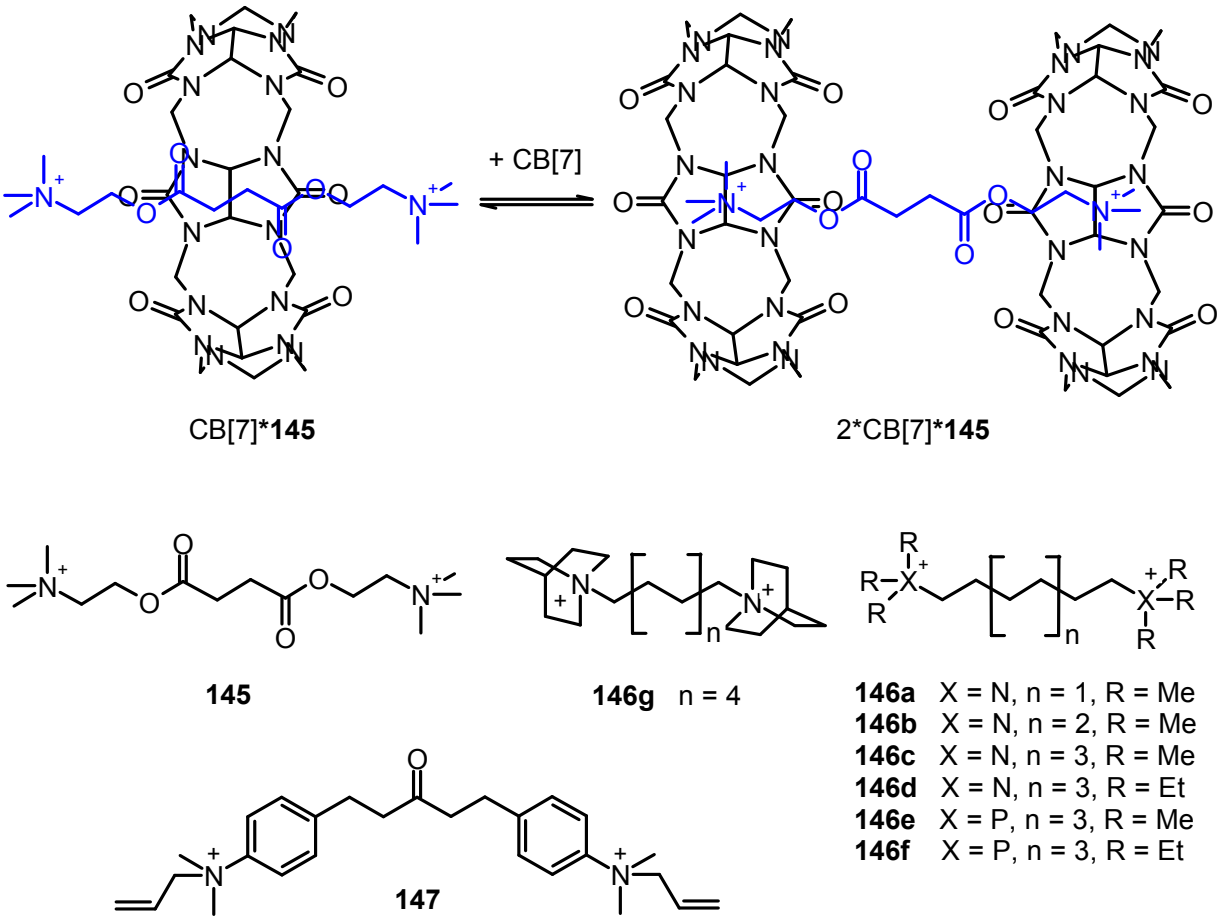


Figure 106: Cucurbit[7]uril (**131c**) binding to succinylcholine (**145**) and different bis-ammonium and bis-phosphonium guests

The vast majority of host-guest complexes of CB[7] (**131c**) with cationic guests, such as paraquat,[337] assemble with the cationic part of the guest located outside of the cavity, adjacent to the oxygens of the portal carbonyls. The remaining hydrophobic region of the guest is positioned inside the cavity.

Mohanty and co-workers have found that the fluorescent dye thioflavin T, used extensively to probe the presence of amyloid fibrils, forms 1:1 and 2:1 host-guest complexes with cucurbit[7]uril (**131c**), with binding constants in the order of magnitude of 10^5 and 10^3 M^{-1}, respectively.[338]

Enlarging the host by one glyconuril unit to cucurbit[8]uril (**131d**) a cavity comparable in size with γ-cyclodextrin (**136c**) results, which is in the position to capture and include even other macrocycles like cyclene (**6**) or cyclam (**7**) and their complexes with transition metals.[339]

Kim and co-workers report that **131d** can bind to aromatic guests, such as tryptophan (**81b**), tyrosine, and dopamine (**2**) as observed by the resulting changes in visible colour and in their NMR spectra.[314,340]

In the crystal structures of the inclusion complexes of *S*-tyrosine (*S*-Tyr), *S*-histidine (**81e**, *S*-His), *S*-leucine (*S*-Leu) in cucurbit[8]uril (**131d**) a 1:2 host:guest ratio was found.[320] It is common, that the ammonium moiety is always located at the portal of the host, coordinated by hydrogen bonding and ion–dipole interaction with the carbonyl groups of the host. The host can include not only the stacked aromatic moieties, but also the alkyl moieties of the amino acids.

Consistent with these observations, cucurbit[8]uril (**131d**) is known to form 1:1:1 heteroternary complexes with paraquat (**148**) and a second aromatic guest: Urbach et al. describe the molecular recognition of amino acids by cucurbit[8]uril and its complex with 1,1′-dimethyl-4,4′-bipyridinium (paraquat, **148**). A comprehensive examination of the 20 genetically encoded amino acids was carried out by ^{1}H-NMR spectroscopy and isothermal titration calorimetry in aqueous solution.[341] The amino acid : host stoichiometry is controlled by the presence (1:1) or absence (2:1) of paraquat (**148**). Both **131d** and the complex **149** bind measurably to only tryptophan (**81b**), phenylalanine (**81a**) and tyrosine. For the 1:1 complexes with the cucurbit[8]uril-paraquat-assembly (**149**) a selectivity of Trp (**81b**, K_{ass} = $4.3*10^4$ M^{-1}) with 8-fold and 19-fold specificity over Phe (**81a**, K_{ass} = $5.3*10^3$ M^{-1}) and Tyr (K_{ass} = $2.2*10^3$ M^{-1}), respectively, can be found. The binding strengths for the 2:1 complexes of cucurbit[8]uril reach 10^8 M^{-2} (Trp, K_{ass} = $6.9*10^7$ M^{-2} and Phe, K_{ass} = $1.1*10^8$ M^{-2}).

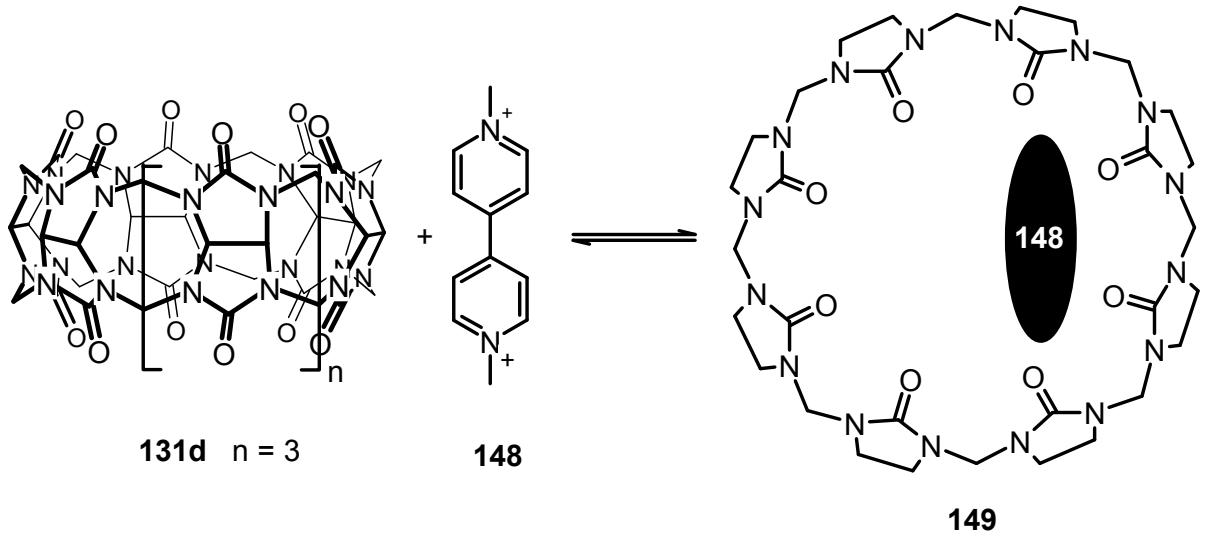


Figure 107: Paraquat-cucurbit[8]uril complex **149**

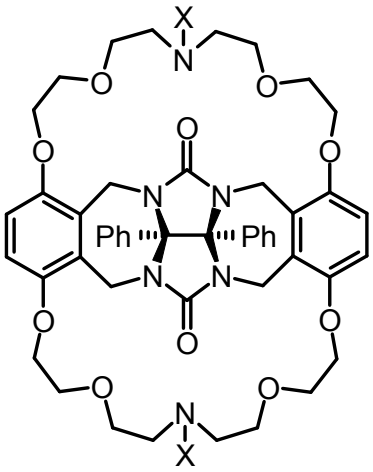
The interaction of the host system with tryptophan (**81b**) was investigated more deeply by using a combination of isothermal titration calorimetry, mass spectrometry, and UV-visible, fluorescence, and ^{1}H-NMR spectroscopy methods,[342] finding that the selectivity is mediated by the electrostatic charge in aqueous solution.

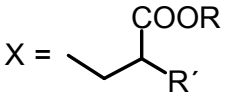
The ITC data showed that **149** binds Trp guests with ammonium group like Trp-OMe and tryptamine ($K_{ass} \sim 5*10^4$ M^{-1}) with approximately 20-fold selectivity over guests lacking this functionality, like *N*-acetyl-Trp ($K_{ass} = 2 - 3*10^3$ M^{-1}). For the binding of Trp (**81b**) and its derivatives a 1:1 binding stoichiometry was observed in all experiments. *N*-Terminal tryptophan residues are bound with higher affinity than *C*-terminal or internal tryptophan residues. The complex binds Trp-Gly-Gly with high affinity ($K_{ass} = 1.3*10^5$ M^{-1}, log K_{ass} = 5.1), with 6-fold specificity over Gly-Trp-Gly (log K_{ass} = 4.3), and with 40-fold specificity over Gly-Gly-Trp (log K_{ass} = 3.5).

In succession cucurbit[8]uril (**131d**) was reported to be a remarkably synthetic host for selective recognition and non-covalent dimerization of *N*-terminal aromatic peptides in aqueous solution.[343] Cucurbiturils are known to recognise *N*-terminal tryptophan over internal and *C*-terminal sequence isomers. Tripeptides of the sequence X-Gly-Gly, Gly-X-Gly, and Gly-Gly-X with X being Trp, Phe, Tyr and His were studied. Compound **131d** selectively binds and dimerizes Trp-Gly-Gly and Phe-Gly-Gly with high affinity (ternary complex association constant in the range of 10^9 - 10^{11} M^{-1}), the binding constants for the other 10 peptides were too small to be measured by ITC. Both peptides are bound in a stepwise manner,

the later one with positive cooperativity. The crystal structures revealed the structural basis for selective recognition as the inclusion of the hydrophobic aromatic side chain and chelation of the proximal *N*-terminal ammonium group by carbonyl oxygens on the cucurbituril. In view of application the authors pointed out the potential study of dimer-mediated biochemical processes and the use for the separation of peptides and proteins.

Nolte and Escuder published a series of cucurbituril related molecules, amino acid appended diphenylglycouril-based chiral molecular receptors (**150**). [344] The binding of several biologically relevant guests with aromatic moieties was studied with UV–vis spectroscopy in competition experiments with 4-(4-nitrophenylazo)resorcinol ("Magneson") and 2-(4-hydroxyphenylazo)benzoic acid (HABA) in water at pH 8 and 4.5, respectively. Compound **150b** forms thin tubules in chloroform and vesicles in water, with the possibility of surrounding the guest. Aggregates of the chiral host **150b** bind catecholamines and aromatic amino acids in water and are able to discriminate between their enantiomers. The calculated binding constants were moderate to high and a remarkable enantioselectivity for the corresponding enantiomers of *R*-tyrosine ($1.6*10^4$ M^{-1} vs. $2*10^3$ M^{-1}), *S*-phenylalanine (**81a**, $2.6*10^4$ M^{-1} vs. $1.2*10^4$ M^{-1}) and *R*-tryptophan (**81b**, $5.6*10^4$ M^{-1} vs. $1.7*10^4$ M^{-1}) was observed.





150a R = H, R´= NHBoc, n = 1
150b R = H, R´= NHBoc, n = 4
150c R = $C_{16}H_{33}NH$, R´= NH_3^+, n = 1
150d R = $C_{16}H_{33}NH$, R´= NH_3^+, n = 4

Figure 108: Gluconuril-based ammonium receptors **150**

The rigid structure and capability of forming stable complexes with a wide range of molecules and ions, mediated by ammonium ion coordination in combination with inclusion of the side chains make cucurbit[n]urils very attractive not only as a synthetic receptor. We are not covering self-assembly systems in this review, but it has to be mentioned that nearly as many papers as published for molecular recognition with cucurbit[n]urils are found using the

macrocycles as a building block for the construction of supramolecular architectures, often relying on the interaction with an ammonium species. The interested reader is kindly referred to the large body of recent literature.[345]

In summary, cucurbiturils and their derivatives are valuable and versatile hosts for ammonium and diammonium guests, as well as amino acids and peptides, reaching the highest binding constants of all presented receptor families in highly competitive aqueous media (up to 10^{10} to 10^{12} M^{-1}). Generally ammonium guests are coordinated by the carbonyl groups of the moieties by electrostatic ion-dipole attraction assisted by hydrogen bonding. The unpolar part of the guest is included in the cavity. The binding is governed by hydrophobic effects and van der Waals contacts. The entropic gain upon binding additionally supports the high association constants found with cucurbiturils. Similar facts meet quaternary ammonium species, which are bound by the same interactions. Notably, cucurbit[n]urils are one example, where these guests are not bound by cation-π-interactions. Here, the area of negative charge accumulation represented by the carbonyl groups, coordinates to cationic species strongly. For a more comprehensive discussion of the binding properties of the cucurbit[n]uril family we refer the reader to the recent review article by Issacs et al.[315], thermodynamic aspects of the binding process are discussed in detail in recent overviews.[312,313]

I. 6. Molecular Clefts, Tweezers, Trigonal Ligands, Phosphonates and Cyclophane Structures as Receptors for Ammonium Ions

Macrocycles and cyclic structures like calixarenes, cyclodextrins or cucurbiturils with polar functionalities organized in a circular manner are the dominant molecular architecture for molecular recognition with ammonium guests. They were discussed in the chapters before.

However, many suitable synthetic receptors fall in a second category: Non-cyclic compounds, with more open structures. These hosts have pockets or cavities into which a guest can fit but is not completely encapsulated. These clefts, clips and tweezers are discussed in the following chapter together with tripods and suitably functionalized cyclophanes. In the topic of ammonium ion recognition it is difficult to draw a dividing line, as both concepts – clefts and cyclophanes - are supporting the understanding of each other, function similarly or were developed in parallel for similar purposes. We will first discuss clefts, clips and tweezers, then tripods and related systems and finally cyclophanes with ionic functionalities.

Vitally important biochemical processes involving the ammonium ion rest upon the specific interactions supported by negatively charged substituents such as carbonates, sulphates, or phosphates. As demonstrated with several examples before, these charged groups contribute significantly to the binding of substrate. For clefts, tweezers and cyclophane structures such substituents are of basic importance to complement the ammonium ion by ionic and hydrogen bond interactions. In the cavities the guests can be bound utilising non covalent bonding interactions like hydrophobic forces, van der Waals or dispersion forces, π-stacking, hydrogen bonding, as well as metal coordination and electrostatic effects.

Clefts (fig. 109) have a certain degree of flexibility, provided that the open cavity is big enough and the geometry is optimal to accommodate the desired guest molecule. Clefts organize polar functionality with hydrogen bonding or ionic coordination capabilities at precise distances and orientations. This conformational fixing is achieved via covalent and non-covalent restraints. Generally**,** acylic clefts, clips and tweezers must position functionality through a rigid molecular scaffold, often of concave shape, to focus functionality inwards, to assure the functionality spending most of its time in the desired conformation and to prevent the collapse of the binding pocket. As in macrocycles, proper pre-organization can significantly augment binding strengths.

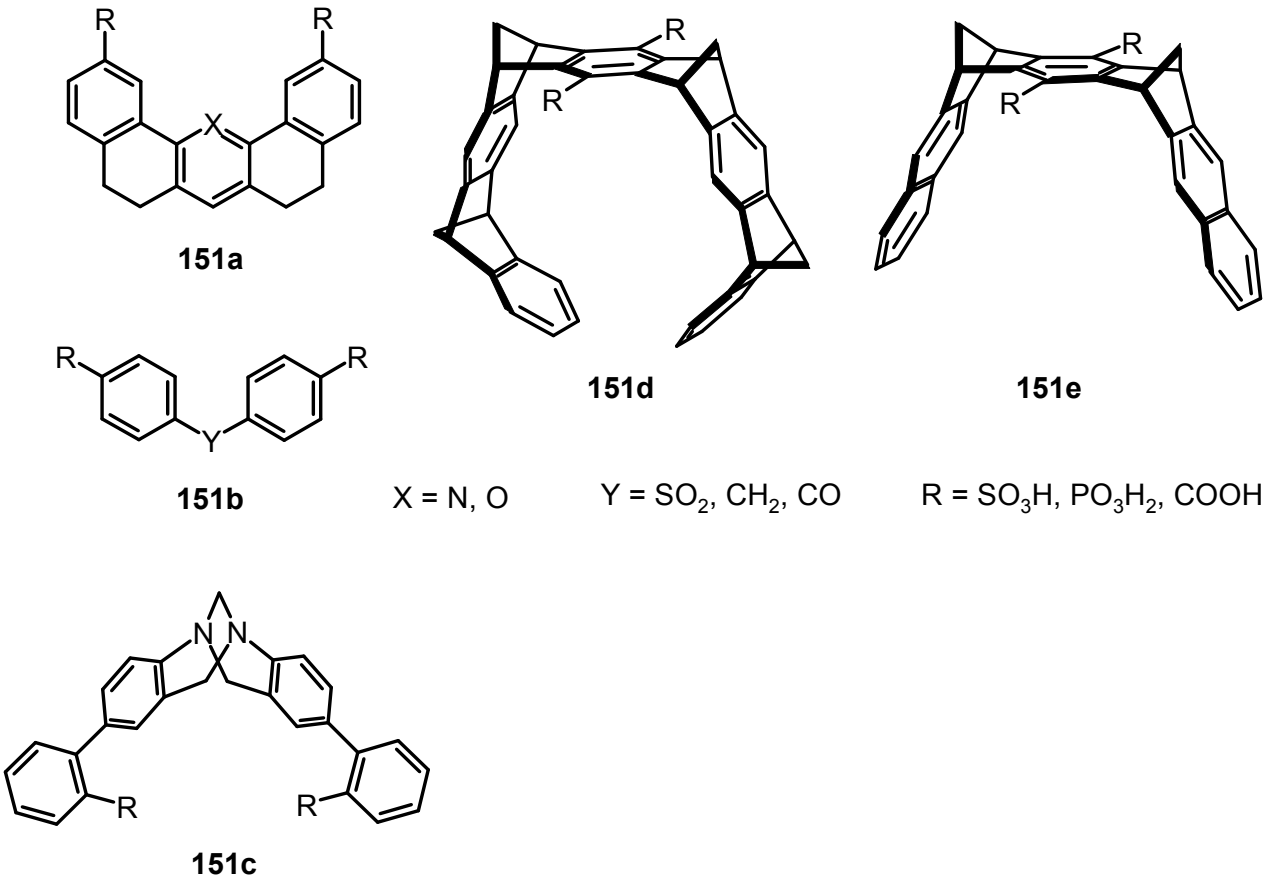


Figure 109: Examples of clefts (**151a**), tweezers (**151b, 151c, 151d**) and clips (**151e**)

Molecular tweezers (fig. 109) are different examples of molecular clefts. Molecular tweezers or molecular clips can be understood as non-cyclic macrocyclic molecular complexes with open cavities bearing two "arms" that bind the guest molecule between them.[346] For ammonium ion recognition they divide into two different subtypes: Either they are characterized by convergent functional groups directed toward each other, mounted on and separated by a rigid backbone with a certain degree of freedom - the space between the functional groups provides the cleft into which a guest can bind -, or the cavity of this kind of receptors is made up of two so-called sidewalls connected to each other by a central spacer unit, which can be either flexible or rigid. The second type contains two aromatic surfaces which 'pinch' aryl or more seldom an unpolar guest between them and uses an additional ionic functionality to complement the ammonium part. Molecules like Kagan´s ether or Tröger´s base (see **151c**) are employed in many examples to give the tweezer a bent shape. Synthesis and properties of such often chiral molecular clefts and tweezers have been reviewed.[347]

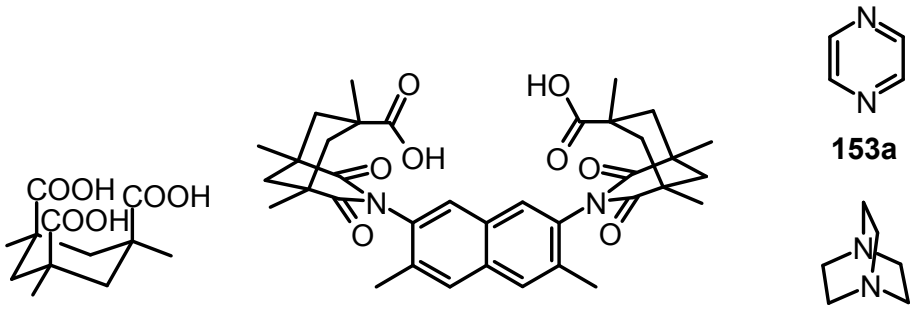
Tweezers and similar molecules „wrapping around“ their targets, namely cyclophanes and cavitands, benefit to an large extent of selective coordination and inclusion from charged

groups. Quaternary ammonium ions can be additionally coordinated by the cation-π-interaction to the aromatic surfaces.

Molecular tweezers were originally developed by Whitlock[348] and Zimmerman.[349] These formed sandwich complexes with aromatic guests by π-π-interaction. Hydrophobic interaction also played a significant role in their tight binding to aromatic (bis-phenol)carboxylates in water. The tweezers constructed by Zimmerman were more rigid and showed high association constants with guests such as polynitroaromatics and 9-alkylated adenines in chloroform.
Further contributions and examples representing the different types of such molecules with open cavities were published by the groups of Vögtle,[350] Rebek,[351] Nolte,[352] Harmata,[353,363] Chen,[354] Klärner[346, 355] and Schrader (see discussed example, fig. 112). Cations and some alkyl- or a variety of aromatic guests, especially electron deficient aromatic systems[355,356, 357] can be coordinated by dispersive forces like π-π, CH-π- und cation-π-interaction. Polar functionality introduced enables to bind guests by additional interactions, for example 1,3-dihydroxybenzene[352] by H-bonding or nucleosides [358] by ionic interactions. Similarly, ammonium ions, diamines[359] or chiral guests[360] can be recognized by appropriate functional groups arranged on these scaffolds.
Clips, tweezers,[346, 361] related V-shaped molecules[362] and their chiral analogs (e.g. fig. 109, **151c**)[363,364] have been reviewed. In the following we will discuss recent examples based on these backbones for inclusion of quaternary ammonium compounds, or, suitably substituted, for ammonium ion recognition.

I. 6.1. Clefts for Different Ammonium Targets

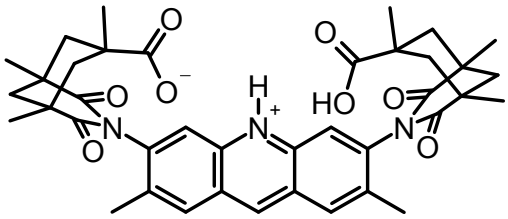
The ability to bind the guest by π-π interactions and the hydrophobic effect is extended by the possibility of hydrogen bonding to the guest molecule with a receptor family developed by Rebek et al. on the basis of Kemp's triacid (**152a**). Due to the convergent carboxyl groups on the cyclohexane ring, condensation of the acid with aromatic amines – one to three aromatic rings are arranged in a linear manner - yields receptors like **152b**, in which two carboxyl groups are pre-orientated in a convergent, optimal arrangement for the substrate binding. The rotation around the C-N bond can be prevented by a methyl group in *ortho*-position to the aromatic amine.



152a **152b** **153a** **153b**

Figure 110: Kemp´s triacid (**152a**), on example of Rebek´s receptors (**152b**) and guests

The largest receptor binds diamines, such as pyrazine (**153a**) or DABCO (**153b**), in chloroform by salt formation. Dicarboxylic acids are linked by hydrogen bonds, similar to those which are found in carbonic acid dimers. When binding amino acids a carboxyl group of the receptor coordinates to the carboxyl group of the substrate. In addition, salt formation occurs between the other carboxyl group of the receptor and the amino group of the guest. [365] Receptor **154** is able to complex ammonium ions with its carboxylate group; the pyridinium cation binds in addition. The extended π-system allows for π-stacking. [366]

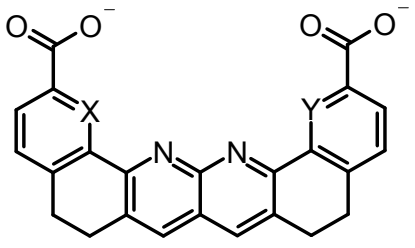


154

Figure 111: Amino acid receptor (**154**) by *Rebek et al.*

The authors identified a binding preference for phenylalanine (**81a**), tyrosine and tryptophan (**81b**) by extraction experiments (water/chloroform) with unprotected amino acids. Leucine, isoleucine and valine were, however, not transported into the organic phase. Thus, the π-stacking interaction seems to result in a decisive contribution to the complex stabilization here. Phenylglycine, due to its geometry is also not in the position to participate in π-stacking in addition to the molecular bonds of the charged parts. The mode of binding and the interactions were investigated in detail by a theoretical study verifying the results and considerations.[367]

Because of the frequent use of the side chains of basic amino acids (Lys, Arg, His) for biological processes the molecular recognition of these amino acids by synthetic receptor molecules is of special interest.[368,369] Bell et al. presented three receptors for guanidinium and ammonium guests.[370] These highly pre-organized clefts, bearing two carboxylate groups on a hexagonal lattice design with defined planar arrays of hydrogen-bonding groups, differ in the number of nitrogen atoms contained in their cavity. Complexation studies were conducted in methanol by ^{1}H-NMR titration for several guanidinium and ammonium ion guests. Compound **155a** bound most guests very strongly (K_{ass} > 100 000 M^{-1}) and was selective for arginine (**81d**) more than 3-fold versus lysine (**81c**,K_{ass} = 29 000 M^{-1}). Surprisingly, the affinity for *N*-acetyl-*S*-lysine and propylammonium chloride was also found to be very high (K_{ass} = 10^5 M^{-1}). Interesting for ammonium ion recognition is receptor **155b,** which bound lysine (**81c**) better than **155a.** In general, it tends to have higher affinity towards alkylammonium guests than to alkylguanidinium salts. It displayed a preference for binding primary alkylammonium guests, including *S*-lysine (**81c**), *N*-acetyl-*S*-lysine, 6-aminocaproic acid and 1-propylamine (K_{ass} = 10^5 M^{-1}). Among guanidinium guests, only arginine (**81d**) bound with very high affinity to **155b**. The complex of **155b** with *N*-methylguanidinium had a significantly lower stability (K_{ass} = 3900 M^{-1}). This selectivity was explained in terms of energies of cavity solvation: The larger cavity of **155a** is more highly solvated prior to binding than the smaller cavity of **155b**. The compact ammonium ion with higher charge density was expected to form stronger attractive electrostatic interactions. In contrast, the alkylguanidinium ion was able to form more H-bonds with the planar receptor **155a**.

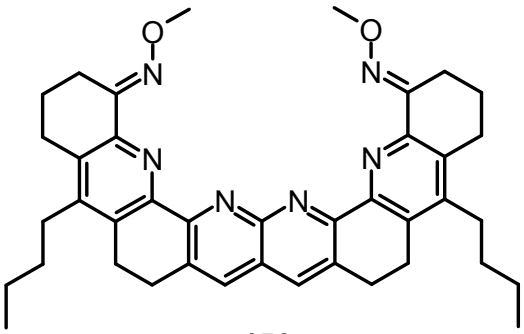


155a X = Y = N
155b X = N, Y = CH
155c X = Y = CH

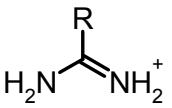
Figure 112: Hexagonal lattice designed hosts by *Bell et. al.*

The amidinium ion is closely related to the ammonium and the guanidinium ion. The amidinium functionality plays an important role in drugs targeting binding pockets for the

arginine side chain. In contrast to the spherical ammonium ion, the amidinium group has to be surrounded in a half-moon-like array with at least four hydrogen bond acceptors, which are ideally pre-oriented for maximum electrostatic as well as hydrogen bond interactions for good binding. This was demonstrated by Bell et al., who developed a concave, highly pre-organized receptor molecule based on annulated pyridines (**156**) which binds benzamidine (**157**, R = Ph) very efficiently in 10 % methanolic dichloromethane ($K_{ass} \sim 10^7$ M^{-1}).[371]



156



R = alkyl, aryl

157

Figure 113: Bell´s amidinium receptor (**156**) and the amidinium ion (**157**)

The efforts of the group complementing ureas, amines and guanidines by the hexagonal lattice design receptors have been nicely summarized in an overview.[372]

I. 6.2. Clips and Tweezers

The interaction of carboxylates with a variety of functional groups, receptors for amino acids and nucleotides was explained in detail in the literature,[373] detailed binding data for oxoanions to ammonium- and guanidinium groups were published.[374]

Sulfonate groups were widely used with success for the recognition of ammonium ions in calixarenes (see chapter 4), but are of low importance for ammonium recognition with tweezers and clefts. The ammonium – phosphonate binding is by far more widely used as interaction.

The P=O double bond system features strong hydrogen bond acceptor property and weak Brønsted basicity in combination with a high dipole moment. Additional cooperative hydrogen bonds render even simple bisphosphonates highly selective.[375]

Many biologically important classes of organic cations like mono- and disaccharides, amino alcohols, arginine derivatives and guanidines are bound in polar media.

Especially phosphonic acids (fig. 114), phosphonates and their mono esters are employed for cation recognition. Simple representatives like benzyl phosphonic (**158a**), *meta*- and *para*-xylene diphosphonic (**158b/c**) and mesitylene triphosphonic acid (**158d**) showed their ability to complex selectively potassium and ammonium cations.[376] Ammonium ions were bound 2 to 3 times better than potassium in capillary electrophoresis experiments in protic media.

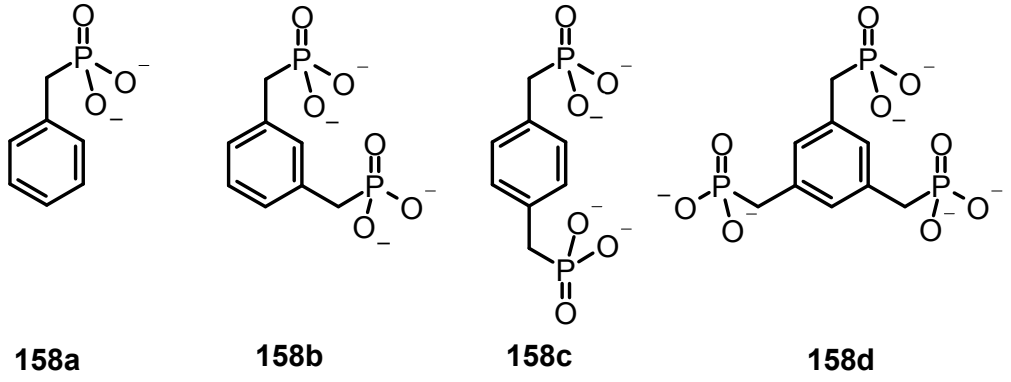


Figure 114: Aromatic phosphonic acids

Already 1996 Schrader presented a new class of artificial receptor for alkylammonium ions, xylene bisphosphonates like **159**.[377] The host molecules, designed to imitate the natural adrenergetic receptor,[378] are selective for 1,2- and 1,3-amino alcohols. In their 1:1 chelate-binding mode an almost ideal array of short, linear hydrogen bonds with the ammonium ion is created pointing to one of the phosphonate moieties. Formation of an additional cooperative hydrogen bond between the second phosphonate anion and the hydroxyl groups provides maximum electrostatic and hydrogen-bond interactions. Biologically important amino alcohols such as glucosamine, 1-aminosorbitol, ephedrine, and the β-blocker propranolol were bound in DMSO with K_{ass} values between 60 000 and 130 000 M^{-1}. Secondary amines are complexed at least as strong as primary amines; amino alcohols were bound much stronger than their simple amine counterparts. The association constants for some of the amino alcohols with 60 000M^{-1} is five times higher than the average estimate for simple amines of 12 000 M^{-1}. In addition, adrenaline model compounds were recognised by phosphonates which allow lateral recognition of the substrate by extended aromatic ester groups by π-π-interactions (**160a** and **160b**).[379] Only a moderate binding of adrenaline to **159** was observed and rationalised by intermolecular competition of the catechol OH groups.

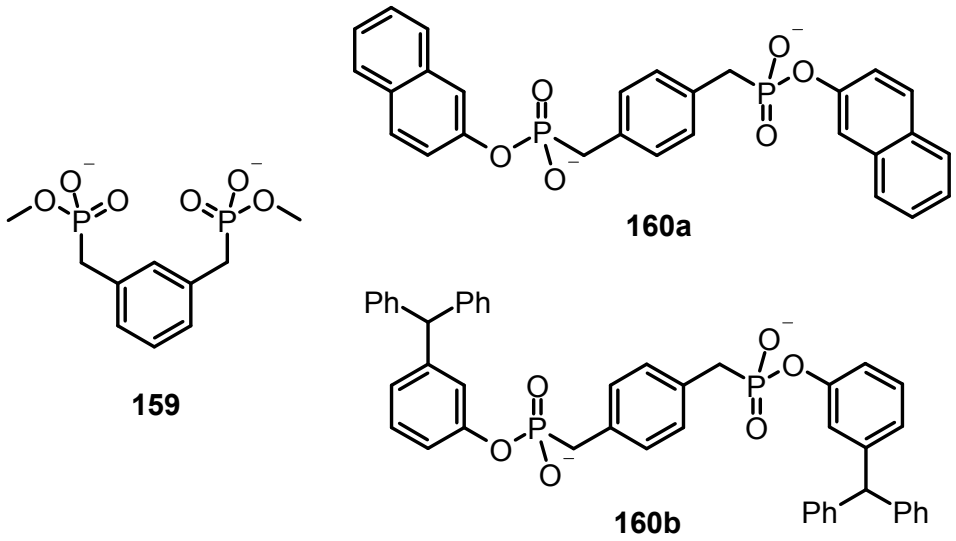


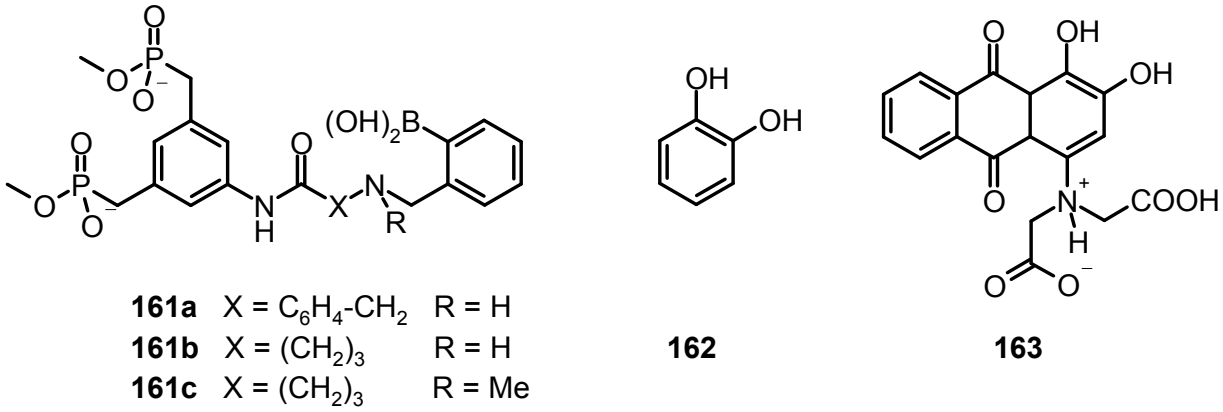
Figure 115: Xylene phosphonates **159** and **160a/b** for recognition of amines and amino alcohols

The recognition with *para*-xylene-bisphosphonates was shown with several examples of ammonium [380] - and guanidinium[369, 381] -cations by Schrader et al. Similarly, the group demonstrated the recognition of the amidinium ion with the simple *m*-xylene bisphosphonate **159**.

A bifurcated hydrogen bond complex is typical for the classical amidinium binding pattern with carboxylates or phosphonates[382] with values for association constant usually in the range of $K_{ass} \sim 10^3$ M^{-1} in solvents like DMSO.[383] This binding constant could be also observed for the 2:1 complex with **159**. Interestingly, when a 1:1 stoichiometry is ensured by performing dilution experiments with a surplus of **157** with respect to the amidinium ion, a far stronger coordination is observed in DMSO: Each amino group is bound by a phosphonate moiety of the tweezer ligand. All association constants lie two orders of magnitude higher than the classical amidinium-phosph(on)ate complexes (10^5 M^{-1} vs. 10^3 M^{-1}). The association constants for various substituted benzamidines correlate with the electronic character of the substituents. The electron rich *p*-methoxybenzamidine is bound with $K_{ass} = 7.6*10^4$ M^{-1}, acetamidine and benzamidine with $\sim 10^5$ M^{-1}, and the electron deficient *m*-nitrobenzamidine even with $K_{ass} = 2.5*10^5$ M^{-1}.

Combination of a boronic ester as recognition motif with the xylene bisphosphonate unit **159** and an appropriate spacer permitted recognition of neurotransmitters.[384] For noradrenalin (**80b**) in 100 mM phosphate at pH 7.0 a strong association was found (K_{ass} = 190, 340 and 690 M^{-1} for **161a**, **161b** and **161c**, respectively). It was possible to evaluate the association

constants for a number of catecholamines such as adrenalin (**80a**) and noradrenalin (**80b**) highlighting the importance of both the aminoalcohol and catechol motifs within the guest. Receptor **161c** as the best example bound adrenaline (**80a**, K_{ass} = 550 M^{-1}), 3,4-dihydroxyphenethylamine (K_{ass} = 590 M^{-1}), dopamine (**2**, K_{ass} = 630 M^{-1}) and noradrenalin (**80b**, K_{ass} = 690 M^{-1}) with about 2-fold selectivity over catechol (**162**, K_{ass} = 350 M^{-1}). The receptor was then developed into a colour sensor by employing the coloured dye alizarin complexone in an indicator displacement assay. On binding to the receptors, the colour of the dye changed from deep red to orange, permitting an association constant of K_{ass} = 1700 M^{-1} according to ^{1}H-NMR titrations. Upon addition of catecholamines, displacement of the indicator and recovery of the original colour were observed. Binding constants similar to those obtained by NMR spectroscopy were obtained by UV spectroscopy in water. Finally a calibration curve for the receptor-indicator complex in the presence of varying concentrations of nor-adrenalin was constructed, which allowed an exact quantitative determination of the concentration of catecholamines even in complex mixtures and urine samples. Changing from water to a 3:1 mixture of methanol/water (HEPES buffer, pH 7.0) the K_{ass} value for alizarin complexone increased to 7000 M^{-1}. A rise in noradrenalin binding could not be confirmed. All catecholamines were bound in the range of 300–400 M^{-1}, catechol somewhat less tightly with 200 M^{-1} and simple amines such as phenylethylamine were not bound at all. Adrenalin was bound 2 – 3 times stronger than catechol.



161a X = C_6H_4-CH_2 R = H
161b X = $(CH_2)_3$ R = H
161c X = $(CH_2)_3$ R = Me

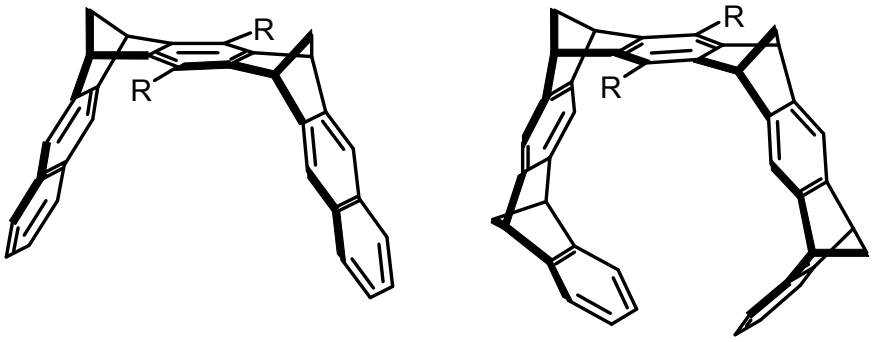
162

163

Figure 116: Bisphosphonate recognition motif **161** for a colourimetric assay with alizarin complexone (**163**) for catechols (**162**)

Klärner and Schrader introduced tweezers and clips based on an electron-rich torus-shaped cavity adorned with two peripheral anionic phosphonate and phosphate groups capable of ammonium ion and amino acid recognition in water. These molecular tweezers were

synthesized via repetitive Diels-Alder reactions and combine the binding properties of an unpolar aromatic cavity with the bisphosphonates. In addition, the bisphosphonate units lead to the desired solubility in polar protic solvents such as methanol and water. In water, the π-π and cation-π interaction are coupled with the hydrophobic effect, and are therefore much more pronounced than in aprotic solvents and provide higher binding constants. The phosphonates are fully deprotonated due to their pK_a value of 1.8 in neutral aqueous solution. Upon inclusion of a guest in the cavity they can grab it like a pair of pincers, build ionic hydrogen bonds to the ammonium ion and support the binding.



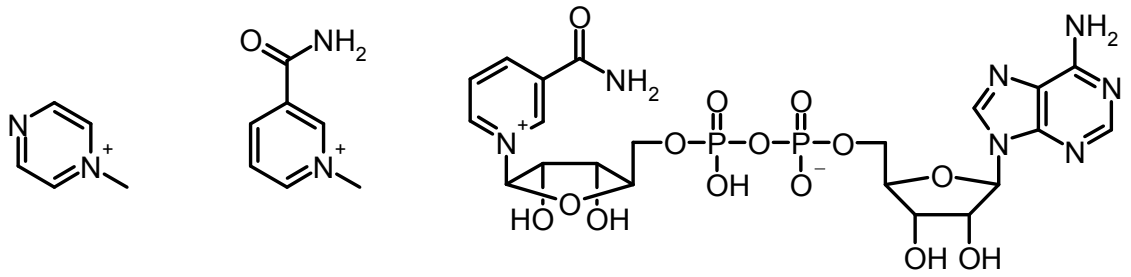
164a R= O-P(O)(CH_3)OH
164b R= O-P(O)(CH_3)O^-·N^+(n-Bu)$_4$
164c R= OP(O)(OLi)$_2$

165a R= O-P(O)(CH_3)O^-·Li^+

Figure 117: Bisphosphonate/phosphate clip **164** and bisphosphonate cleft **165**

The phosphonate substituted clip **164b**[385] binds selectively *N*-alkylpyridinium salts such as *N*-methylnicotinamide iodide (**166b,** NMNA) and NAD^+ (**166c**) in methanol and in aqueous solution. Further studies pointed to a significant contribution of the hydrophobic effect to the host-guest interaction in aqueous solution.[386] The binding constants in water are significantly higher, than those observed in methanol: for example **166a** bound with K_{ass} = 9400 or 600 M^{-1} and **166b** with K_{ass} = 68000 or 16700 M^{-1} in water or methanol, respectively.

In the complex with NAD^+ (**166c,** K_{ass} = 6500 M^{-1}**)**, one of the most important redox coenzymes in nature, a dynamic equilibrium is observed in aqueous solution. The protons of the subunits, the nicotinamide as well as the adenine moiety, are shifted upfield in the ^{1}H-NMR spectrum indicating that at either the nicotinamide or the adenine subunit are included inside the cavity, equilibrating rapidly on the NMR time scale. A Monte Carlo conformer search, leading to the energy-minimized double-sandwich structures supported the experimental finding.



166a **166b** **166c**

Figure 118: *N*-Methyl-pyrazine **166a**, *N*-methylnicotinamide iodide (**166b**) and NAD^+ (**166c**)

Water-soluble molecular clips substituted with phosphate groups (**164c**, fig. 117) were also investigated on their binding properties. Despite the similarity between the phosphonate and phosphate functional groups, the supramolecular properties of both clips are different from each other. The phosphate clip lithium salt **164c** shows self-aggregation in water solution while there is no evidence of this phenomenon for the phosphonate clip dilithium salt **164a**.[387] Additionally, the binding properties of these clips in phosphate buffer solution (pH = 7.2), change dramatically from one clip to another (table 9) as well as with the pH values of the solution. For the most guest molecules the phosphate clip **164c** shows association constants between 2 and 10 times larger than those of the phosphonate clip **164a**.

Guest	**Phosphonate clip 164a** K_{ass} **[M^{-1}]**	**Phosphate clip 164c** K_{ass} **[M^{-1}]**
Nicotinamide mononucleotide	550	1120
Adenosine	1115	1400
Cytidine	1070	9685
N-methylnicotinamide iodide (**166b**)	11270	35000
Caffeine	9550	42700
NAD^+ (**166c**)	4200	5630

Table 9: Comparison of association constants (M^{-1}) of biological relevant molecules with the phosphonate and phosphate clips in phosphate buffered aqueous solution (pH = 7.2)

N-Alkylated pyridinium salts are also strongly bound in the tweezer **165a**. Only *para*-substituted compounds are strongly bound, other substitution patterns do not lead to an effective inclusion in the downward shielded cavity.

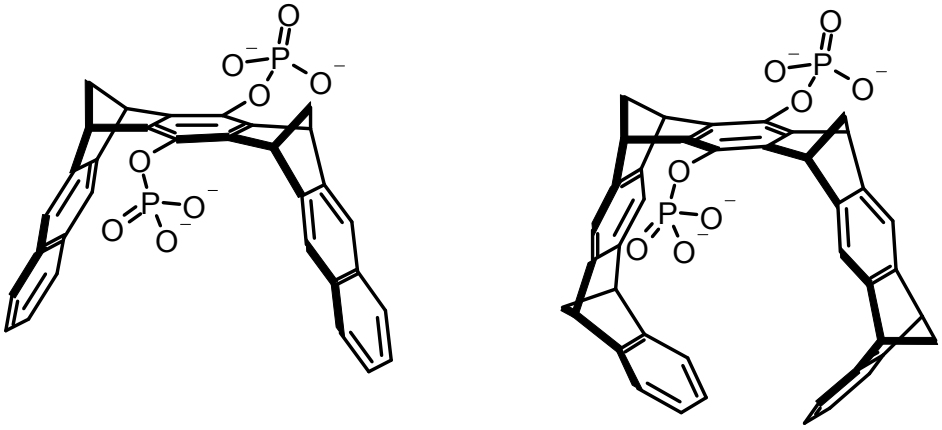
In contrast to the bisphosphonate clip, the bisphosphonate tweezer also binds primary and secondary ammonium cations. The binding correlated with the steric requirements of substituents. The bulkier the substituents are, the lower is the binding constant. Primary ammonium cations (K_{ass} up to 800 - 900 M^{-1} in aqueous solution) are bound more strongly than their secondary analogous. Dopamine (**2**) is bound with millimolar strength in water. Interestingly, the basic amino acids arginine (**81d**) and lysine (**81c**) are significantly better bound (up to 23000 M^{-1} for Ts-Lys-OMe in aqueous phosphate buffer) compared to 900 M^{-1} for simple amines.

The molecular cleft (**165a**) displayed comparable and also exceptionally high affinity for lysine (**81c**, K_{ass} = 5000 M^{-1} in neutral phosphate buffer).[388] Selectivity for arginine (**81d**) and lysine (**81c**) is achieved by threading the whole amino acid side chain through the cavity and subsequent locking by formation of a phosphonate-ammonium/guanidinium salt bridge, reflecting a pseudorotaxane-like geometry. So the aggregate can be stabilized by strong electrostatic and dispersive interactions, supported by the hydrophobic effect.

The basic amino acids were effectively bound also in peptidic environment as found in small basic signalling peptides (Lys or Arg rich). These experiments showed that any other amino acids are not bound. When two lysine residues separated by other amino acids are present in the peptide, both can be individually bound by one bisphosphonate tweezer in a 2:1-complex. With two lysine residues close together, the formation of a cluster with the bisphosphonates was preferred in a water / methanol mixture. In this case it is apparently more favourable to build hydrogen bonds from the ammonium cations to the bisphosphonates, rather than trapping the lysine side chains in the cavity. This artificial lysine binder was roughly one order of magnitude superior to all other receptor molecules that have been designed for this purpose. Only Bells molecule (**155c**) was later identified as a selective lysine binder ($K_{ass} > 10^5$ M^{-1} in methanol). The binding mode and strength seem to be largely governed by steric effects: Bulky substituents close to the ammonium functionality prevent an effective inclusion, while a slim ethylammonium environment allows complete insertion into the host interior.

The two corresponding water-soluble host molecules with phosphate substituents designed for cofactor and amino acid recognition are able to inhibit the enzymatic activity of alcohol dehydrogenase (ADH) in vitro.[389] As mentioned before, clip **164c** binds strongly to NAD^+ (**166c**), tweezer **165a** shows high affinity to lysine (Ac-Lys-OMe, K_{ass} = 5000 M^{-1}) in aqueous buffer. Clip **164c** pulls out NAD^+ (**166c**) from the Rossman fold and thereby depletes the cofactor level below a critical threshold. An excess of this molecule led to irreversible

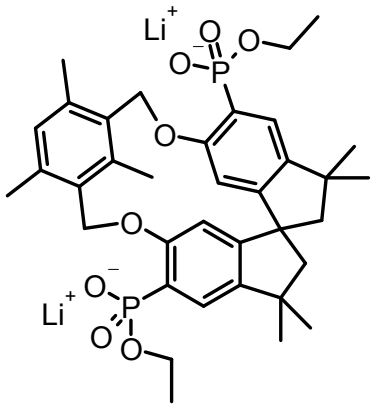
denaturation. Tweezer **165b** with its high lysine preference decorates the whole enzyme surface, especially the cofactor entrance site. While the absolute enzymatic activity was not influenced at all, 0.6 equiv of tweezer were sufficient for a total enzyme shut down. Addition of lysine (**81c**) could switch on the enzyme function again in total reversible manner. Lineweaver-Burk plots indicated a competitive mechanism for the clip, with respect to both substrate and cofactor, while the tweezer clearly follows a non-competitive mechanism.



164c **165b**

Figure 119: Bisphosphate cavitands

In 2000 a macrocyclic receptor molecule, which binds arginine (**81d**) and lysine (**81c**) in a stereoselective fashion was reported.[390] The chiral bisphosphonate **167** binds ammonium and guanidinium by hydrogen and salt bridges. The mechanism of enantioselective recognition relies on two simultaneous cation-phosphonate interactions. After docking the amino acid comes into van der Waals-contact of the chiral surface of the chiral bridging unit in **167** and one enantiomer is bound preferentially. The overall binding constants were in the range of 10^4 M^{-1} only in DMSO.



167

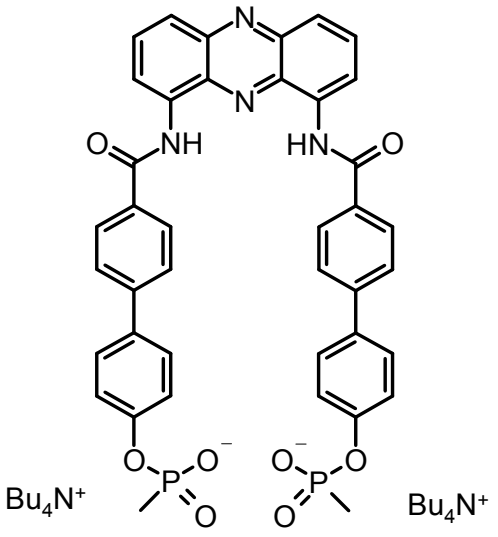
Figure 120: Bisphosphonate **167** of Schrader and Finocchiaro

In examining the binding properties by NMR titration in DMSO, the authors found that for short diammonium guests like *S*-histidine (**81e**), and *S*-ornithine (both as dihydrochlorides) a 1:2 (receptor: guest) stoichiometry is present, but there is no chiral discrimination. However, the complexes for lysine (**81c**, $K_{ass} = 2.1*10^4$ M^{-1}) and arginine (**81d**, $K_{ass} = 9.4*10^3$ M^{-1}) have a 1:1 molar ratio and a distinction between the enantiomers is possible. The distance between the two ammonium groups in a guest molecule must be obviously large enough to bind to both phosphonates of the receptor. The enantiomeric excess was determined to be 17 % for arginine (**81d**) and 33 % for lysine (**81c**).

An artificial receptor molecule (**168**) with high noradrenalin specificity uses highly pre-organized stiff elements and connections (fig. 121) for more favourable complexation entropy and improved desolvation of the included guest.[391]

NMR titrations with neurotransmitters and related guests in d_4-methanol revealed low micromolar affinity to *rac*-adrenaline (**80a**, 260 M^{-1}), dopamine (**2**, 340 M^{-1}) and aromatic amino acid esters (~ 200 M^{-1}). Other amino acids, catechol (**162**) and phenylethylamine (**78a**) gave no response. Job´s plot analysis confirmed 1:1 complex stoichiometry. The rigid phenazine moiety in receptor **168** strongly improves the affinity for the desired guest (K_{ass} = 1800 M^{-1}). The effective 1:1 complex formation between (**168**) and noradrenalin (**80b**) could also be monitored by ESI-MS, producing clean mass spectra with host and aggregate ion peaks, exclusively.

Due to the highly amphiphilic structure of **168**, the receptor molecule was incorporated in a stearic acid monolayer at the air/water interface. In the Langmuir film balance, substantial shifts are produced upon subinjection of the various analytes into the aqueous subphase (10^{-4} M) reflecting the interaction with the embedded receptor molecule (no effects are produced with stearic acid alone). By far the largest shift is obtained from noradrenalin (**80b**), followed by much smaller shifts from adrenaline (**80a**) and dopamine (**2**).



168

Figure 121: Tweezer **168** for noradrenalin (**80b**)

I. 6.3. Tripodal Receptors

Tripodal ligands (see fig. 122) are C3-symmetrical molecules related to tweezers, with three side chains on a rigid platform. Several of these artificial receptors have C3v symmetry.[392] In ammonium ion recognition with tripods, the flexible arms form three hydrogen bonds to acidic protons of the guest amine RNH_3^+.

The binding can benefit from this additional coordination site. Even more, recognition of biologically important guests often necessitates building a receptor that can make multiple non-covalent contacts. This concept was nicely demonstrated with receptor **169** utilizing the threefold ammonium sulfonate/sulphate contact to recognize heparin (**170**)[393] and bind it strongly with $K_{ass} = 1.4*10^8\ M^{-1}$ in 10 mM HEPES buffer.[394]

Such a three-point coordinating cavity can better exclude solvent influences and enables recognition in strong competitive solvent mixtures. For example colourimetric discrimination between certain ω-aminoacids (H_3N^+-$(CH_2)_{n-1}COOH$) was achieved by the use of a chromogenic tripodal receptor functionalized with stilbazolium dyes (**171**) in mixed DMSO–water 90:10 vol/vol solutions.[395] UV-experiments revealed a preference for n = 4 - 6 (λ = 560 nm).

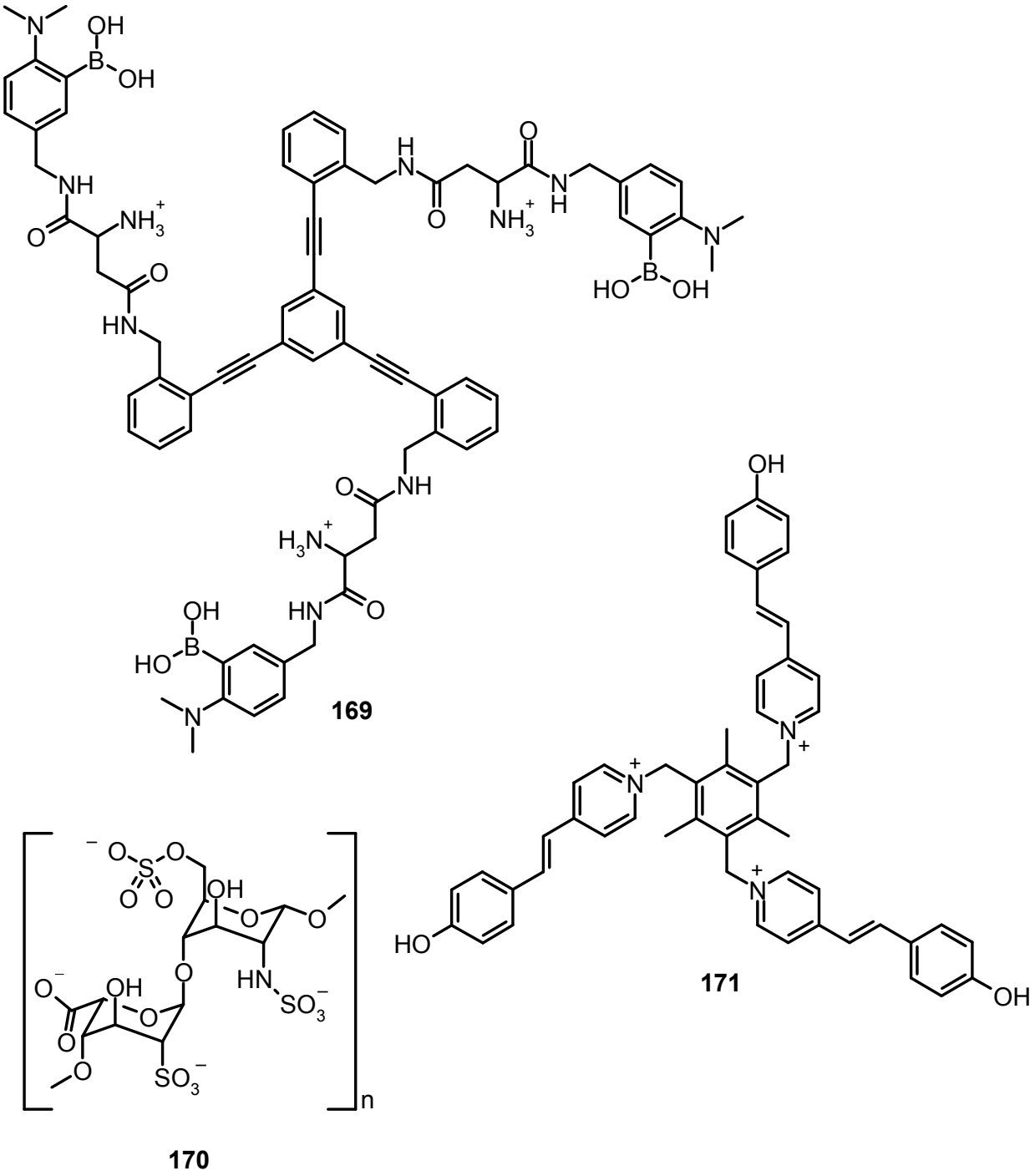
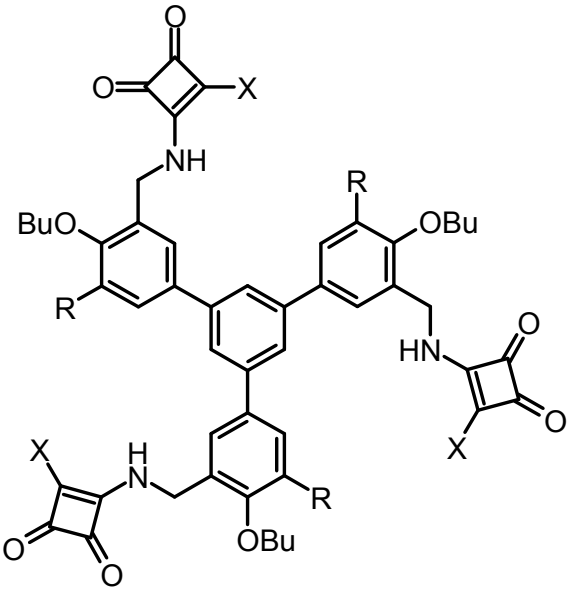


Figure 122: Different tripods and heparin (**170**)

Quaternary ammonium ions can be coordinated entirely utilizing for example an additional cation-π-interaction with the third arm.

The group of Ballester introduced squaramido rings as binding units in abiotic tripodal receptors, thus utilizing multiple O to C-H interactions.[396] This led to efficient receptors for tetraalkylammonium compounds like choline (**76**), acetylcholine (**3**) and related ammonium

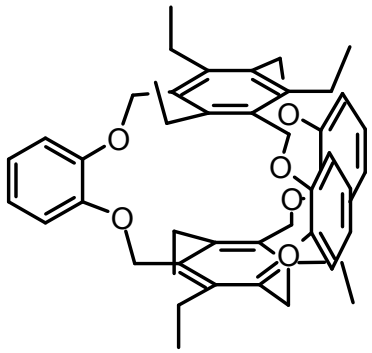
salts. Association constants in the range 10^3 to 10^4 M^{-1} were elucidated by a ^{1}H-NMR titration using a 1:1 model (**172e** vs. choline (**76**) hydroiodide in $CDCl_3$: K_{ass} = 14509 ± 1403 M^{-1}). The formation of intracavity complexes was supported by intermolecular cross peaks in 2D-ROESY experiments. Complexation studies carried out in 10 % MeOD-*d*4/$CDCl_3$ mixtures gave association constants that were roughly 20 - 25 times weaker than in $CDCl_3$ alone, but the formation of the corresponding complexes was still evident.



172a R = H, X = Et_2N
172b R = Pr, X = BnNH
172c R = Pr, X = $HO(CH_2)_5NH$
172d R = Pr, X = p-$Me_2NC_4H_6NH$
172e R = Pr, X = p-$Me_2NC_4H_6$
172f R = H, X = OEt
172g R = Pr, X = OEt

Figure 123: Squaramide based receptors **172**

The interaction with aromatic π-electron clouds plays an important role in the interaction of the synthetic NH_4^+ receptor (**173**) by Kim et al.[77] The cage like molecule binds ammonium ions also by multiple hydrogen-bond-, and in addition by cation-π-interactions.

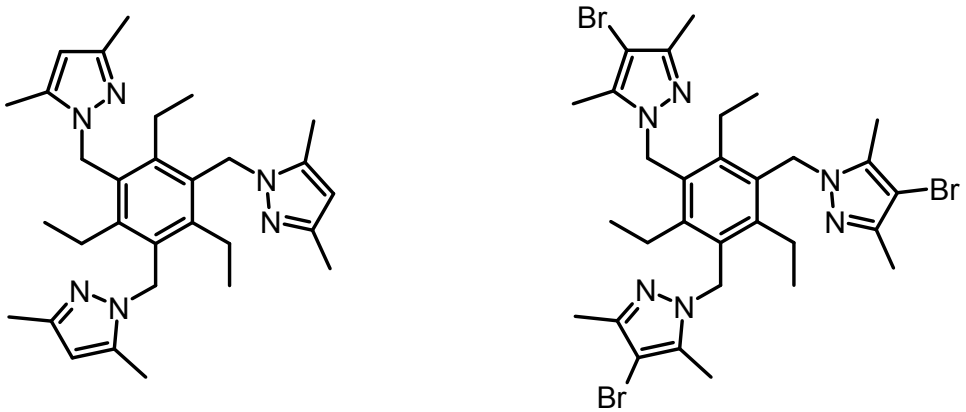


173

Figure 124: Cage like NH_4^+ receptor **173** of Kim et al.

The cavity has been calculated to be optimal for ammonium ions, but too large for lithium- and sodium ions. When used in ion selective electrodes **173** showed a slightly higher detection limit ($3.2*10^{-6}$ M) as the natural ammonium sensor nonactin ($1.5*10^{-6}$ M) and an increased ammonium / potassium selectivity coefficient of log K $(NH_4^+)/(K^+)$ = -0.97 (Nonactin: log K $(NH_4^+)/(K^+)$ = -0.88). The binding constant of the ammonium ion determined by extraction experiments[128] was $3.3*10^7$ M^{-1}.

Chin and co-workers synthesized 1,3,5-tri(3,5-dimethylpyrazol-1-ylmethyl)-2,4,6-triethylbenzene in which the three pyrazole groups provide hydrogen-bonding sites. [397] In comparison to **173**, receptor **174a** shows an increased ammonium selectivity (log K $(NH_4^+)/(K^+)$ = -2.6), but the binding constant, determined by extraction experiments,[128] was lower (K_{ass} = $1.4*10^6$ M^{-1}). An ion selective electrode (ISE) incorporating this molecule showed improvement in ammonium ion selectivity over potassium ion as compared to nonactin (log K $(NH_4^+)/(K^+)$ = -2.6), again illustrating the importance of hydrogen bonding and symmetry. This ionophore is pre-organized into the required tetrahedral geometry for complexing ammonium ions through hydrogen bonding involving the imine nitrogen atoms. The ethyl and methyl groups provide steric interactions to force the receptor into the desired geometry and to block the ligands from binding potassium ions. Despite its high selectivity for ammonium, the limit of detection for this ionophore is two orders of magnitude higher than for nonactin, and therefore, it is not sufficiently sensitive for some applications.



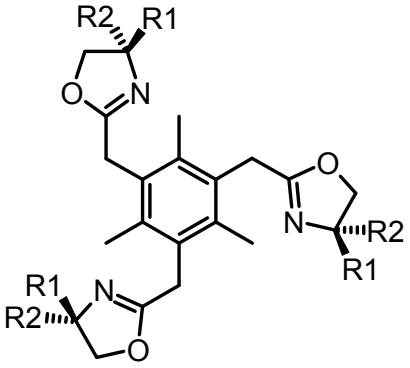
174a **174b**

Figure 125: Ammonium receptors **174** of *Chin et al.*

To lower the binding of water and thus increase the sensitivity of the receptor, electron withdrawing groups - bromine atoms – were placed on the pyrazole rings of the receptor (**174b**).[398] Indeed this led to a far lower detection limit ($2.5*10^{-5}$ M) for ammonium ions in an

ISE, comparable to nonactin ($2.2*10^{-5}$ M). The ammonium versus potassium selectivity of this receptor was strongly enhanced compared to the natural heterocycle (log $K_{NH4+/K+}$ = -2.3, nonactin log $K_{NH4+/K+}$ = -1.3).

The further development of this structural motif, carried out by Ahn et al., led to an exchange of the low-alkaline pyrazole (pK_a ≈ 2.5) against the 2-oxazoline with slightly higher basicity (pK_a ≈ 5).[399]



175a R1=R2=H
175b R1=Me, R2=H
175c R1=H, R2=^{i}Pr
175d R1=R2=Me

176 R1=H, R2=Ph

Figure 126: 2-Oxazolin-based ammonium receptors **175a-d** and **176** by *Ahn et al*.

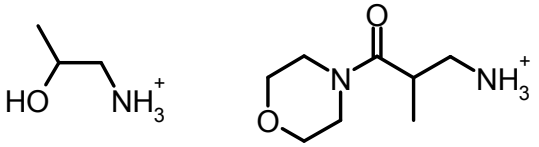
The binding constants of the molecules **175a** to **175d** towards ammonium and potassium ions were investigated by picrate extraction experiments[128] and were compared to the natural ammonium binder nonactin (table 10).

	175a	**175b**	**175c**	**175d**	***Nonactin***
K_{ass} (NH_4^+) [M^{-1}]	$5.1*10^6$	$2.5*10^7$	$9.4*10^6$	$3.9*10^6$	$2.0*10^8$
K_{ass} (K^+) [M^{-1}]	$3.0*10^4$	$5.7*10^4$	$2.4*10^4$	$5.7*10^4$	$6.7*10^7$
$K_{ass}(NH_4^+)/K_{ass}(K^+)$	173	437	393	68	3

Table 10: Binding constants and selectivity constants of the receptors **175a-d**

Due to these structural changes, the authors have succeeded in further improving the binding constants (K_{ass} (**174**, NH_4^+) = $1.4*10^6$, K_{ass} (**175b**, NH_4^+) = $2.5*10^7$) and enhancing the NH_4^+ / K^+ selectivity of 398 to 437. Another advantage of oxazoline- compared to the pyrazole substituents is the possibility to introduce chirality into the receptor. Ahn et al. have studied the binding of enantiomerically pure **176** towards a variety of guest molecules.[400] An increase

in discrimination of the enantiomers of racemic molecules is represented in the presence of a hydrogen bridge acceptor in γ- or β- position to the ammonium ion. The authors rationalized this to the existence of a "bifurcated" H-bridge, which restricts the free rotation of the β-substituent. By ITC titration experiments in acetonitrile, the binding constants for the *R*- and *S*-enantiomers of **177a** were found to be $3.0*10^4$ M^{-1} or $9.2*10^3$ M^{-1}, respectively. The enantioselectivity of the extraction is 63:37 in favour of the *R*-enantiomer. The best selectivity found for **177b** was 83:17, but only an extraction of < 5 % was possible due to the increased water solubility of **177b**.



177a **177b**

Figure 127: Racemic guest molecules **177**

Theoretical studies indicated such trisoxazolines being an alternative to azacrowns for binding and sensing of ammonium and alkylammonium ions.[400, 401] The importance of C3 symmetry in chiral recognition has been pointed out.[392b] Including Kubik´s cyclo-hexapeptide (*vide infra*, **233**) and the example **176** from Ahn et al. presented before, there are only a few examples of enantioselective receptors for chiral ammonium ions with C3 symmetry.[392a, 402]

This receptor type is built by coupling the chiral binding arms to the achiral backbone in such a way that they can organize themselves around a potential guest in a predetermined arrangement. To obtain sufficient stereoinduction, the chiral elements and the donor groups have to be arranged closely to each other. An alternative design of three-armed, C3-symmetric receptors for enantiomeric discrimination is the use of chiral scaffolds to which achiral binding arms can be coupled. Here, the scaffold not only serves as a spacer but also pre-organizes the conformation of the binding arms, thus leading to an enantioselective discrimination of chiral guests.

Just recently Schnopp and Haberbauer presented C3-symmetric, imidazole-containing, macrocyclic peptides with different binding arms binding α-chiral primary organoammonium ions with up to 30.000 M^{-1}.[403] The binding constants and the selectivity ratios were estimated by standard ^{1}H-NMR titration techniques in $CDCl_3$. The chirality of the backbone[404] and the selection of adequate receptor arms make these systems highly selective enantiodiscriminators. The receptors **178b** and **178c** showed opposite selectivities toward those organoammonium

ions bound most strongly. With the isoquinoline receptor **178c**, it was possible to generate a C3-symmetric receptor with a good selectivity ratio of 87:13 for (*R*)-PEA (**20b**). The obtained binding constants were 4500 M^{-1} for (*S*)-PEA and 30,000 M^{-1} for (*R*)-PEA (**20b**).

The titrations of (*R*)-PAM (**179a**) und (*S*)-PAM with **178b** resulted in values for K_{ass} of 16.000 M^{-1} and 1900 M^{-1}, respectively, thus reaching the high selectivity ratio of 90:10.[405] A possible explanation for the enantioselectivity was deduced from the conformation of the complexes: They calculated the molecular structures of the energetically preferred conformers of **178c***(*R*)-PEA and **178c***(*S*)-PEA using density functional theory (DFT) reproducing their observations in the theoretical model finding an less favoured conformation and higher steric repulsion for the complex with (*S*)-PEA.

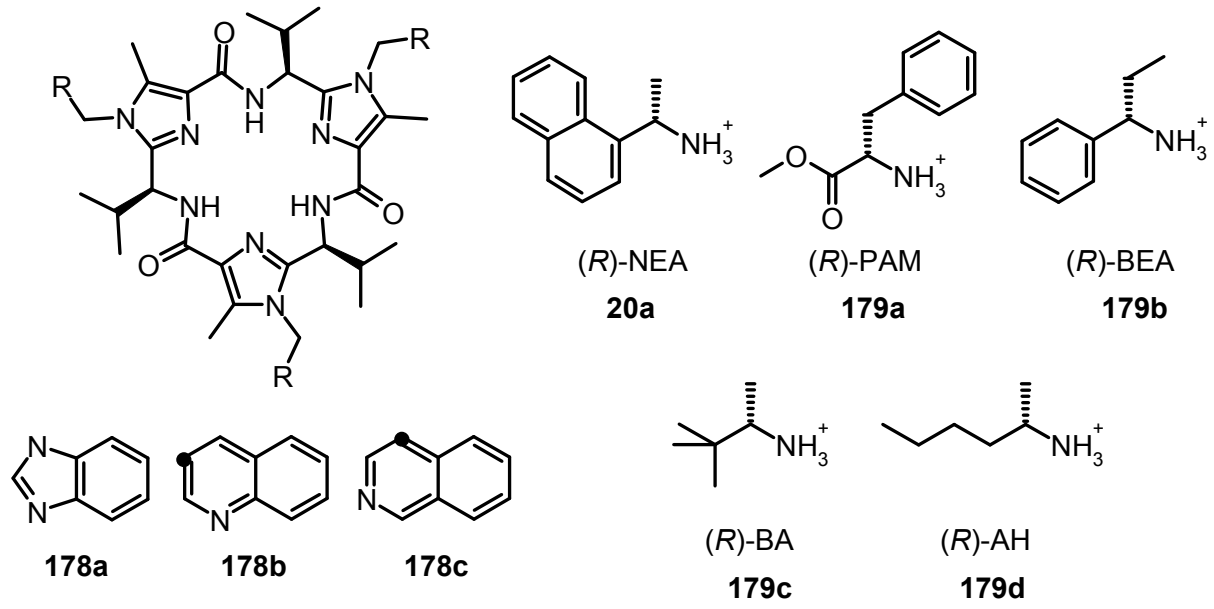
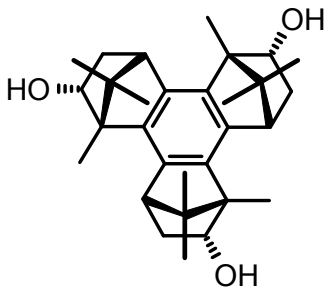


Figure 128: Tripods based on a imidazole containing macrocycle (**178**) and the guest molecules employed in the study (**20a, 179a-d)**

The enantiopure C3-symmetric *syn*-benzotriborneol **180** revealed the capability to act as host for ammonium ions, and in particular, the efficient chiral recognition of the two enantiomers of (1-phenylethyl)ammonium chloride.[406] The rigid C3-symmetric structure of triol **180** bearing three hydroxy groups on the concave side of the molecule, led to two fold better complexation capabilities of the triol *syn*-**180** with (–)-(1-phenylethyl)ammonium chloride ($K_{ass\ 1:1}$ = 230 M^{-1}, $K_{ass\ 1:2}$ = 2380 M^{-1}) with respect to the (+)-enantiomer ($K_{ass\ 1:1}$ = 120 M^{-1}, $K_{ass\ 1:2}$ = 1220 M^{-1}). The complexes were characterized in deuteriochloroform by means of ^{1}H-NMR titrations. The Job´s plots showed the clear formation of the 1:2 complex between the triol and the ammonium salt. The NMR titration experiments clearly showed that two

different processes take place. The process that takes place at low concentrations is the complexation of the first ion pair, and the process at high concentrations is the binding of a second ion pair for the reformation of the dimer present in solution.



180

Figure 129: Ammonium ion receptor **180**

I. 6.4. Cyclophane Structures for Binding Ammonium Ions

Cyclophanes are well pre-organized macrocycles with several aromatic subunits,[179] which usually have a large hydrophobic cavity capable of inclusion of neutral or positively charged guest molecules. Their binding properties and their solubility can be varied within a wide scope by introducing appropriate substituents.

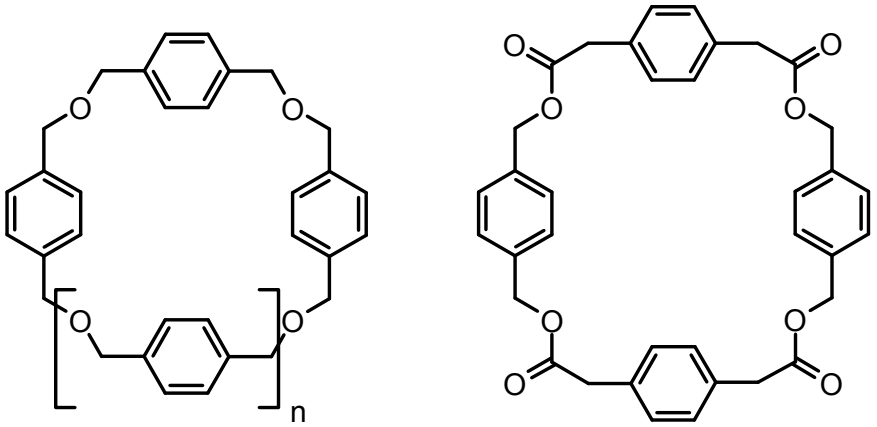
Neutral aromatic guest molecules bind to cyclophanes over dispersive and π-π interactions. In the complexation of organic cations the cation-π interaction gives crucial contributions. Dougherty and co-workers[407] and the group of H. J. Schneider[408, 409] proved cyclophane hosts to be suitable for recognition of quaternary ammonium salts: The positive charge of the guest interacts with attractive cation-π-interactions provided by the electron-rich surfaces of their aromatic rings. This fact was also verified by a theoretical study.[410] Such a charge-assisted NH-π interaction was confirmed just recently.[411]

Quaternary ammonium guests like acetylcholine (**3**) and tetramethylammonium salts (TMA) are strongly bound mainly by cation-π-interaction.[181,412] Paraquat and its derivatives are also strongly included also assisted by π-π-interaction.[139,413]

Of equal importance to the properties of these cavities are their peripheral solubilising groups. Especially water-soluble derivatives have a great importance in the host-guest chemistry of cyclophanes. Water soluble cyclophanes are a long known receptors class providing

hydrophobic cavities of definite shape and size for forming inclusion complexes with various organic compounds in aqueous solution.[414] The hydrophobic effect critically assists the coordination to ammonium compounds via strong inclusion of the nonpolar part of the guest in the cavity[408,415] and plays an important role in the complex formation in general, i.e. the release of guest molecules from the solvation shell around host and guest.[416] In addition, competitive interactions of the H-bond-donor water are reduced by the apolar shielding area. The synthesis[417] and interactions[418] of cyclophanes with typical guest molecules are the topic of several articles.

A series of oxa[3.n]paracyclophanes was investigated upon their binding properties towards quaternary ammonium ions, namely tetramethylammonium and acetylcholine (**3**) with different counterions in $CDCl_3$ by ^{1}H-NMR titrations.[419]
Association of **181a** with tetramethylammonium picrate (K_{ass} = 460 M^{-1}) was compared to the parent tetraester **182**, the corresponding cyclophanic tetraamine, the open-chain counterpart of **181a**, and its cyclo-oligomers from pentamer (**181b**) to octamer (**181e**). Binding enhancements ranging from 15-fold (with respect to the tetraester and the tetraamine) to over 80-fold (with respect to the open-chain tetraether) were observed. With the appropriate choice of the anion, i.e., with a poorly inhibiting counterion ($Me_2SnCl_3^-$), the association constant for tetramethylammonium is raised to the order of 10^3 M^{-1}, with a binding increase of over 400-fold with respect to the tetraester. Acetylcholine (**3**) was bound by **181a** with 440 M^{-1} (counterion $Me_2SnCl_3^-$) or 360 M^{-1} (picrate salt).



181a n = 1
181b n = 2
181c n = 3
181d n = 4
181e n = 5

182

Figure 130: Tetraoxa[3.3.3.3]paracyclophanes **181** and a cyclophanic tetraester (**182**)

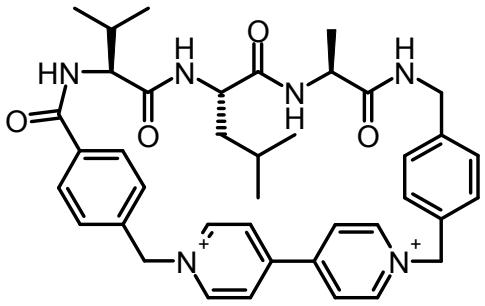
Many attempts have been made to create synthetic receptor molecules for catecholamines. Most of these are monotopic: for example dopamine selectivity has been achieved with a pyrazol-containing podand,[420] a homocalix[3]arene triether,[421] or with a sol – gel process.[422] just to name a few.

Boronic acids have been used in ditopic receptors for molecular recognition of the catechol ring, as shown in the example above (**161**), the systems of Glass et al. (*wide infra*, **247**) and with related systems in literature.[423] In an alternative design the catechol has been bound by a symmetric hydrophobic cavity with peripheral carboxylate groups for dopamine (**2**) recognition.[424]

A cationic chiral cyclophane was synthesized and studied as a host for chiral and racemic π-donor molecules. The cyclophane host **183** has a rigid binding cavity flanked by (*S*)-(valine-leucine-alanine) and N,N′-dibenzyl-4,4′-bipyridinium subunits, which allow for hydrogen-bonding and π-stacking interactions with included aromatic guest molecules.[425]

^{1}H-NMR binding titrations were performed with several different pharmaceutically interesting guest molecules including β-blockers, NSAIDs, and amino acids and amino acid derivatives. The host guest complexation constants were generally small for neutral and cationic guests (0 - 39 M^{-1} at 20 °C in water/acetone mixtures). However, an enantioselectivity ratio of 13 was found for dopamine (**2**), a strongly π-donating cationic guest. (*R*)-Dopamine showed the strongest association in 1:1 water/acetone (39 M^{-1}).

Two-dimensional NOESY ^{1}H-NMR spectra confirm that (*R*)-dopamine binds inside the cavity of the host and that there is no measurable interaction of the cavity with (*S*)-dopamine under the same conditions.



183

Figure 131: Peptidic bridged paraquat-cyclophane

All these artificial host molecules, are not biomimetic and not selective for catechol-aminoalcohols. Schrader et al. studied the natural surroundings of such guest and published several approaches based on the imitation of the natural receptors.

In order to imitate the natural binding site, an artificial biomimetic adrenaline host should be able to provide - at least after an induced-fit process - a microenvironment with a shape complementary to the geometrical form of its guest. A high number of van der Waals contacts would help desolvation in water and lead to a strong hydrophobic attraction.

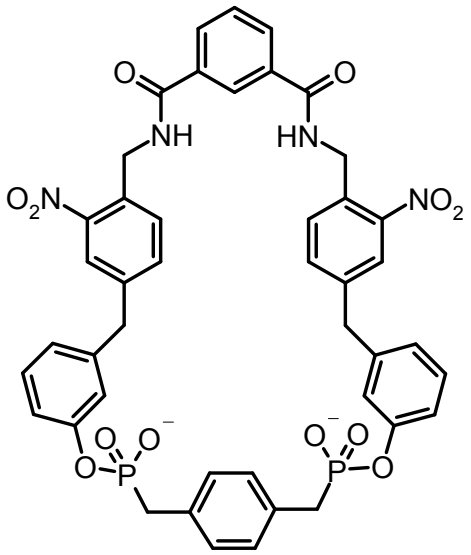
A shape-selective adrenaline-inspired host was presented.[426] A number of closely related biogenic amines and amino alcohols were examined in a 1:1 mixture of water and methanol by NMR to check the selectivity of the new host molecule.

Adrenaline (**80a**, K_{ass} = 153 M^{-1}), noradrenalin (**80b**, K_{ass} = 215 M^{-1}) and dopamine (**2**, K_{ass} = 246 M^{-1}) were stronger bound than 2-phenylethylamine (**78a**, 102 M^{-1}) and ethanolamine (54 M^{-1}). The binding constant for dopamine (**2**) in water is three orders of magnitude lower than that of the natural example (10^5 M^{-1})

The small K_{ass} value of ethanolamine, which is half an order of magnitude below that of noradrenalin (**80b**), shows the receptor molecule clearly recognizes the hormones catechol ring. This is supported by the decrease in binding energy when the phenolic hydroxyl groups are deleted from the guest structure (**78a**, 2-phenylethylamine).

All the effects discussed above confirm that macrocyclic host **184** recognizes adrenaline derivatives in mixtures of water and methanol (1:1) by multiple non-covalent interactions including electrostatic attraction, hydrogen bonds, π-stacking, and hydrophobic forces.

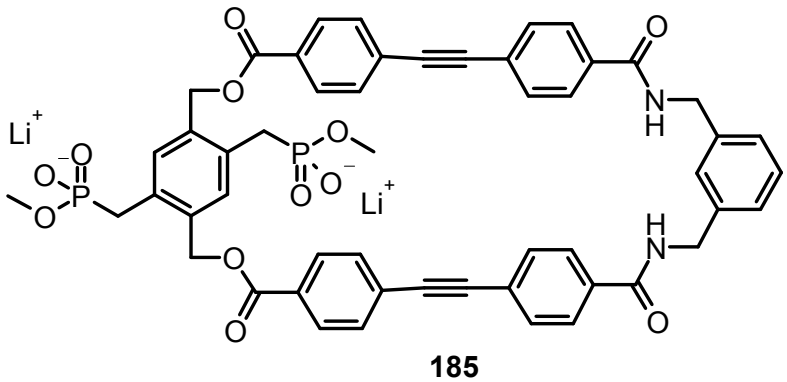
The nitro-arene groups in the macrocyclic receptor molecule can undergo double π-stacking interactions with the catechol ring of adrenaline without producing any significant ring strain in the receptor molecule, while the isophthalic amide group is ideally pre-oriented to form hydrogen bonds to the phenolic OH groups



184

Figure 132: Shape-selective noradrenalin host

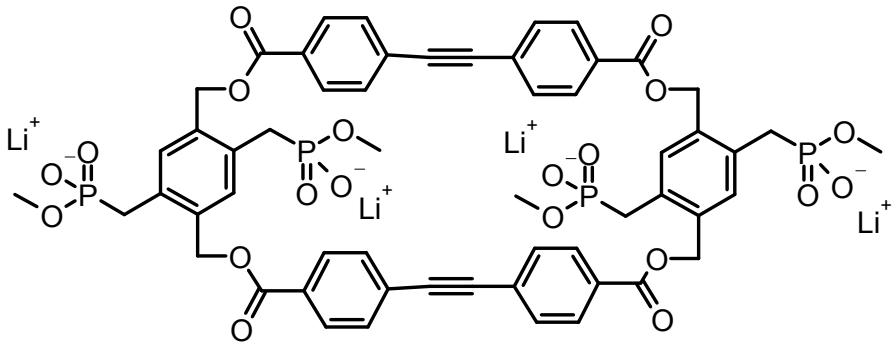
Schrader et al. introduced a similar system **185** for the detection of adrenaline and related biologically important amines.[427] Various amines, such as ethanol amine and propranolol bind to the receptor in methanol with low selectivity. The values of the binding affinities vary between 700 and 1600 M^{-1}. However, the insertion of **185** in a mono-layer of stearic acid at the air-water interface leads to selective noradrenalin (**80b**) binding (10^5 M^{-1}). The binding is monitored by changes in the pressure dependent surface area diagrams with the Langmuir film balance. The drastic change in comparison to solution is explained by the forced inclusion of the guests in the cavity of the receptor on the surface and the formation of new hydrogen bonds between the NH of **185** and the phenolic oxygen of the noradrenalin. Other catecholamines do not show this effect.



185

Figure 133: Receptor **185** for binding of noradrenalin on surface layers from *Schrader et al.*

A slight variation of the receptor, introducing a second bisphosphonate moiety resulted in high affinity towards catecholamines in water, especially for structures with extended aromatic π-faces as found in many β-blockers (up to $7*10^3$ M^{-1} for each single complexation step or $5*10^7$ M^{-2} for both steps). Job´s plot analyses showed a 2:1-stoichiometry, NMR titrations revealed no cooperativity in any case. For ease of comparison the authors always use the 1:1 association constants for each single binding step and varied the solvent polarity from pure methanol over methanol/water (1:1) to pure water. Here, the recognition profited from the amphiphilic structural design [428] and the extensive self-association by the aromatic π-planes even more. Affinity and selectivity towards adrenergic receptor substrates was greatly enhanced if the receptor molecule **186** was transferred from water into a lipid monolayer. Above the critical micelle concentration of $3*10^4$ M, the host formed micelles that produce a favourable microenvironment for hydrophobic attraction of the ammonium alcohol by the phosphonate anions, combined with hydrophobic contributions between the aromatic moieties. Ionic hydrogen bonds with the polar OH or NH groups of the guest enforced the non-covalent interactions, and finally led to increased specificity. Especially β-blockers with minute structural changes can be easily distinguished from each other. A remarkable dependence of the 1:1 binding constant was revealed for noradrenalin. The binding amounts to 4000 M^{-1} in MeOD, it drops to ~700 M^{-1} in MeOD/D_2O (1:1). However, in water an increase to 1200 M^{-1} is observed.



186

Figure 134: Tetraphosphonate receptor for binding of noradrenalin

For further and more detailed discussion of the interesting topic of recognition of catecholamines with artificial receptors in aqueous solution, we refer to a recent overview.[429] Bell's receptors **155** (fig. 112) can bind free arginine (**81d**) with a K_{ass} value of 900 M^{-1}, another binds lysine derivatives with a millimolar binding strength. The tetrasulphonate calixarene hosts (**84**) reach 1500 M^{-1} in borate buffer (see chapter 4); in calixarenes **92**, the

phosphonate groups give a main contribution to binding and selectivity. Following these examples and the survey of molecules given above, this shows that by adding more phosphonate groups to a rigid scaffold binding strength and selectivity are increased. Indeed, by virtually "dimerising" clefts, cyclic moieties like cyclophanes result, which have suitable cavities and substitution patterns for a selective artificial ammonium ion receptor. These molecules bind strongly to bis-ammonium guests in even more polar solvents.

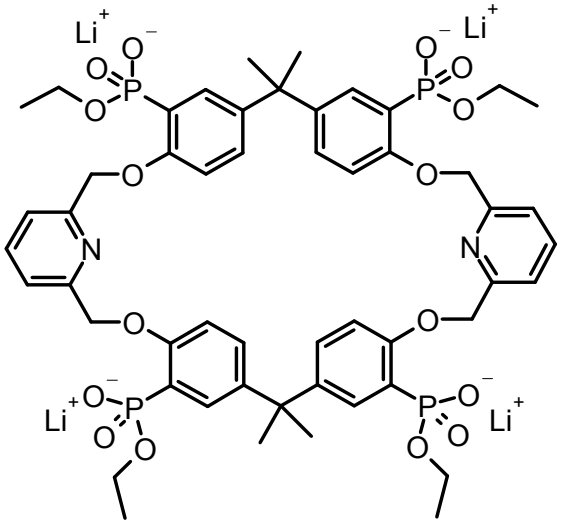
The further development of receptor **167** led to the tetraphosphonate (**187**).[430] By doubling the number of phosphonate groups binding increases, so that the receptor can be used in water. X-ray analysis and molecular modelling revealed that the host adopts a favourable open conformation.[431] Typical stoichiometries with diammonium amino acids are 1:2; only lysine (**81c**) forms a 1:1 complex. Table 11 summarises the results:

Amino acid (dihydrochlorides)	K_{ass} **[M^{-1}] (methanol)**	K_{ass} **[M^{-1}] (water)**	**Receptor:Guest Stoichiometry**
His	29000	650	1:2
Orn	9500	221	1:2
Arg	8800	165	1:2
Lys	21000	1200	1:1

Table 11: Binding constants for the complexes of **187** with different amino acids

In methanol all amino acids are bound strongly in a double chelate binding mode. The exceptionally good binding of histidine (**81e**) is explained by a chelate complex, which is including both imidazole nitrogen atoms in addition to the amino acids ammonium functionality. From methanol to water, the stoichiometry of all complexes is retained, but a 20- to 50-fold drop is observed in the association constants of the four investigated amino acids attributed to the competition of the water molecules. Lysine (**81c**) is complexed 5 - 7 times more strongly than ornithine and arginine (**81d**) and even twice as strongly as histidine (**81e**). The contribution of hydrogen bonds in water is negligible, while electrostatic interactions represent the major attractive force. It is known, that in this respect the hard ammonium ion with its high charge density is superior to the softer guanidinium and also the imidazolium ion, where the positive charge is delocalized across several atoms.[432] The electrostatic attraction exerted by the second ammonium functionality of lysine (**81c**) is stronger than that of arginine's guanidinium ion and even histidine's imidazolium ion. In

addition, lysine (**81c**) is in the position to undergo a four-point interaction in its complex with **187** being stronger than the two-point interaction in the related assemblies with ornithine and arginine (**81d**).



187

Figure 135: Tetraphosphonate **187** of *Schrader* and *Finocchiaro*

We discussed charged clefts. A similar class, quite related to the hosts presented in this chapter are cavitands or macrocycle bearing phosphate and phosphonate groups. The negative charged phosphor derivatives are closely comparable to the carboxylate residues mentioned. In combination with cavitands structures and/or molecular clefts e.g. tweezer backbones they are employed with huge benefit for ammonium ion recognition.

Extensive hydrophobic interactions with a self-associated or self-organized microenvironment and utilising a combination of van der Waals interactions and substantial electrostatic contributions for locking of the guest are responsible for the observed high efficiency and specificity found in clefts and cavitands. Often electrostatic interactions contribute most to the stabilisation energy in the complexes. In larger cavities the loss of one hydrogen bond can be overcompensated by e.g. hydrophobic interactions. Optimized host structures implementing elements of much higher rigidity can achieve more effective pre-organization and desolvation. In summary, C_{3v}-symmetric tripods, tweezer ligands and pre-organized molecular clefts reach selectivities and affinities in ammonium ion binding which compete with naturally occurring recognition motifs like nonactin or valinomycin.[433]

I. 7. Porphyrins and Other Metal Complexes

In this part of the review we will discuss ammonium ion recognition involving metal complexes. Metal complexes are important binding sites for amines, but have even more extensively been used for amino acid recognition. In fact, the following examples typically involve simultaneous binding of ammonium and carboxylate ions. We add the discussion of amino acid zwitterion binding by metal complexes to supplement our survey, although the ammonium ion recognition is only part of the binding process.

I. 7.1. Porphyrins

Porphyrins and their metal complexes play a fundamental role in a variety of biological processes, for example the chlorophylls as photoreaction centres in photosynthesis, haemoglobin as oxygen carrier in blood and myoglobin as oxygen storage in the muscles, cytochromes in electron-transfer processes in respiration or as important prosthetic groups and coenzymes as found in vitamin B12.[434] They have been employed as electroactive materials for molecular electronics,[435] effective photosensitizers[436] for photodynamic therapy or as supramolecular building blocks for energy conversion devices[437] and dye sensitized solar cells.[438] Synthesis and properties of porphyrins and related compounds, such as porphycenes or texaphyrins, have been extensively reviews in several books and articles.[439,440,434]
Porphyrins have been widely used for the recognition of various guest molecules.[441] Two reviews on their general properties and recognition scope were published.[442] Articles on the related porphyrinoid,[443] and chiral multifunctional porphyrins[444] have been reviewed. We will focus in the following on examples of porphyrin based receptors for amines or ammonium ion recognition.

Zinc porphyrin receptors bearing 12 ester groups in the meso phenyl groups[445] and the water soluble corresponding potassium carboxylates[446] are selective receptors for amines amino acid esters and oligopeptides as demonstrated by UV-vis experiments in dichloromethane and buffered aqueous medium. Using small substituents as in **188a** or the unsubstituted parent compound, butylammonium chloride or phenethylamine hydrochloride (up to 52700 M^{-1} in dichloromethane) bind with highest affinity. The ester functional groups of **188a** assisted the binding of aromatic *R*-amino esters (K_{ass} = 8 000 - 23000 M^{-1}) in this medium and inhibited the binding of bulky aliphatic *R*-amino esters (K_{ass} of 460 M^{-1} for Leu-OMe). This indicated

that CH-π-type interactions and steric repulsions controlled the selectivity. The corresponding salts **189** showed a good selectivity for binding of hydrophobic guests: **189c** binds Trp-OMe or pyridine in water with binding constants of 7000 - 8000 M^{-1}. These anionic zinc porphyrins bind histamine (**1**) and a histidine-containing oligopeptides even more tightly. The highest binding strength for histamine was found for **189a, 189b** and **189c** in pH 8 buffer with binding constants of 157000, 31000, and 18200 M^{-1}, respectively. Coordination of the imidazole to the zinc centre and a significant electrostatic interaction between the ammonium group of histamine and the carboxylate groups of receptor stabilises these complexes. In the series of amino acid esters, receptor **189a** coordinated best to the cationic Arg-OMe, with an enthalpically driven binding of 11000 M^{-1}. Strong dependence of the binding affinity on ionic strength and pH revealed that electrostatic interactions between charged functional groups are an important driving force for recognition of hydrophilic guest molecules in water. Comparisons of binding affinity between hydrophilic receptor **189a** and hydrophobic receptor **189c** revealed that the hydrophobic binding pocket of **189c** enhanced the affinity in water towards hydrophobic guests. A lower affinity of the receptors in methanol-water than in water indicated that water plays a significant role in binding energetics.

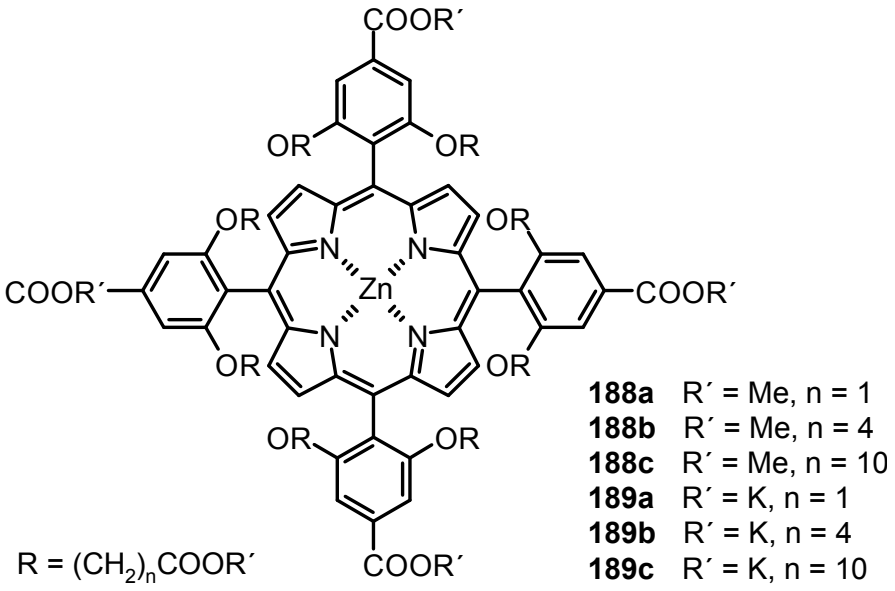
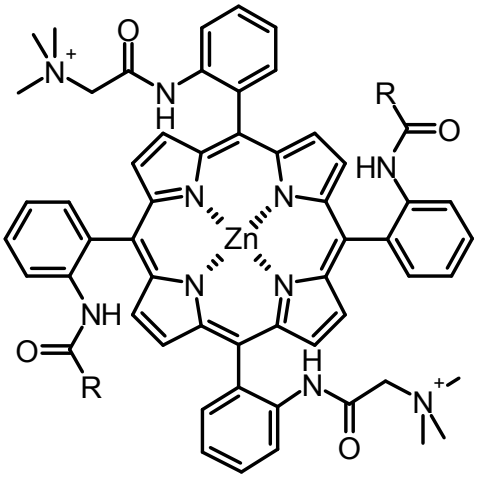


Figure 136: Zinc-Porphyrin ammonium-ion receptors **188** and **189** of *Mizutani et al.*

Imai et al. employed also highly charged water-soluble zinc porphyrins (fig. 137). Bearing an ammonium group and a phenyl or tertiary butyl group above each porphyrin plane they recognise amino carboxylates in aqueous solution.[447] Binding constants were determined spectrophotometrically in aqueous carbonate buffer at pH = 10.4 and revealed the maximum

binding strength for *rac*-tryptophan (**81b**) being 1000 M^{-1} for **190a** and 830 M^{-1} for **190b**. The authors suggest a three point recognition for amino carboxylates by cooperative coordinative, Coulomb, and hydrophobic interactions.



190a R = Ph
190b R = ^{t}Bu

Figure 137: Zinc porphyrin receptor **190**

The binding of amino acids to water-soluble zinc porphyrins in basic aqueous solution was spectrophotometrically analyzed with similar receptors (**191**).[448] The amino acids were bound to the porphyrins through the coordination of the N atom with the central zinc ion. Additional stabilisation of the aggregate comes from Coulomb interactions between the $–COO^-$ anion of the amino acids and the $–N^+(CH_3)_3$ cation of the porphyrin substituents, and the hydrophobic interactions between the porphyrin plane and the hydrophobic substituents of the amino acids. In the study, the binding of amino acids (10^2 M^{-1}) is apparently stronger than that of aminoethanol (10 M^{-1}), due to additively cooperated Coulomb interaction between the cation substituent(s) of porphyrins and the carboxylate anion of amino acids. This explanation is supported by the fact that the K_{ass} values increase as the number of possible Coulomb interactions increases: the K_{ass} values for amino acids for **191a** and **191b** are approximately two times larger than those for **191c**, and the binding of *S*-Asp ($K_{ass,191a}$ = 780 M^{-1} and $K_{ass,191b}$ = 770 M^{-1}) and *S*-Glu ($K_{ass,191a}$ = 390 M^{-1} and $K_{ass,2}$ = 540 M^{-1}) is enhanced compared to that of Gly ($K_{ass,1}$ = 110 M^{-1} and $K_{ass,191b}$ = 150 M^{-1}). Coordination of the aromatic amino acids Phe ($K_{ass,191a}$ = 320 M^{-1} and $K_{ass,191b}$ = 180 M^{-1}) and Trp ($K_{ass,191a}$ = 1300 M^{-1} and $K_{ass,191b}$ = 770 M^{-1}) is strengthened by hydrophobic interactions between the phenyl or indole group of

the amino acids and the porphyrin plane, as also supported by observations of the according peak shifts by ^{1}H-NMR in Na_2CO_3 buffered D_2O.

The coulomb interactions between dipeptides and porphyrins are comparable to those between amino acids and porphyrins. The K_{ass} values of Gly-*S*-Phe ($K_{ass,191a}$ = 200 M^{-1} and $K_{ass,191b}$ = 340 M^{-1} and $K_{ass,191c}$ = 240 M^{-1}) and Gly-*S*-Trp ($K_{ass,191a}$ = 770 M^{-1} and $K_{ass,191b}$ = 1100 M^{-1} and $K_{ass,191c}$ = 780 M^{-1}) are larger than those of Gly-Gly (K_{ass} ~ 100 M^{-1}), indicating that the interactions between these dipeptides and the porphyrins are similar to those between *S*-Phe and *S*-Trp and porphyrins.

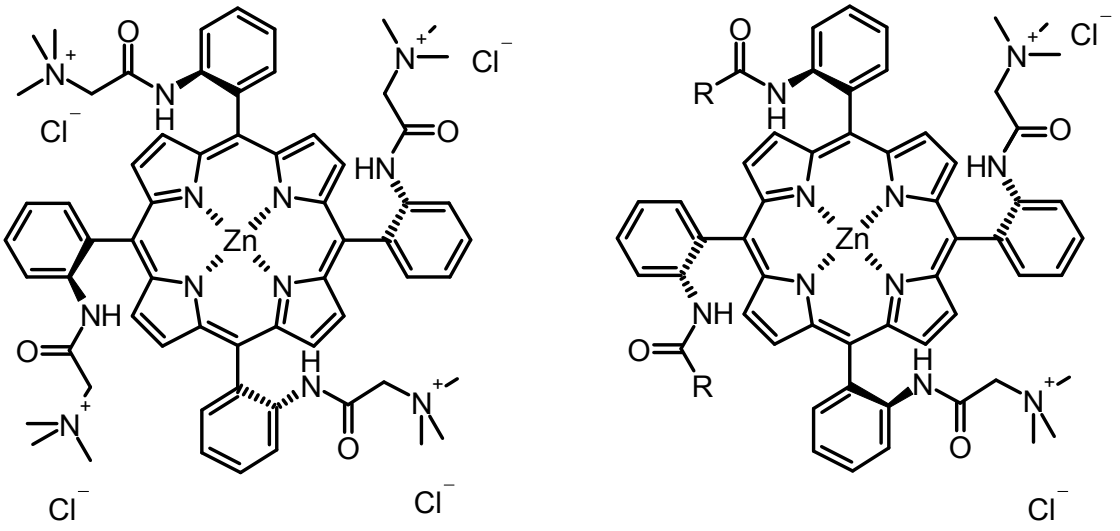


Figure 138: Zinc porphyrin receptors **191** capable of amino acid binding

The molecular recognition of amino acid esters in $CHCl_3$ was investigated by UV-vis titration with *S*-tyrosine-[449] and *S*-threonine[450] substituted chiral zinc porphyrins (**192**). The association constants of the molecular recognition reactions were all $K_R > K_S$ and followed the order of *K*(Phe-OMe) > *K*(Leu-OMe) > *K*(Val-OMe) > *K*(Ala-OMe) in host **192a** and *K*(Thr-OMe) > *K*(Leu-OMe) > *K*(Val-OMe) > *K*(Ala-OMe) > *K*(Phe-OMe) in host **192b**. All results are summarized in table 12.

Guest	K_{ass} [M^{-1}], 192a	K_R/K_S	K_{ass} [M^{-1}], 192b	K_R/K_S
S-Ala-OMe	320	1.4	155.2±12	3.1
R-Ala-OMe	450		488.6±20	
S-Val-OMe	621	1.2	175.2±10	2.9
R-Val-OMe	713		502.2±15	
S-Leu-OMe	1030	1.2	179.8±13	4.9
R-Leu-OMe	1290		881.5±22	
S-Phe-OMe	679	2.2	420.7±10	1.1
R-Phe-OMe	1490		442.3±10	
S-Thr-OMe	n.d.	n.d.	537.6±15	2.6
R-Thr-OMe	n.d.		1391.3±25	

Table 12: Binding constants and enantiomeric distinction factors of chiral porphyrin-amino-acid dipeptide receptors in chloroform at 20°C

A significant contribution of π-π-interaction can be observed for the binding of phenylalanine (**81a**) to receptor **192a**, as also evident by comparison to the second system with threonine side chain (**192b**). Here the binding constant for the aromatic amino acid is the lowest in the series.

Circular dichroism spectra were used to explain chiral molecular recognition. It was found that chiral recognition arose mainly from the chiral matching between host and guest. The enthalpy-entropy compensation relationship revealed a significant conformational change during the process of chiral recognition. The induced CD spectra of the complexes exhibited characteristic Cotton effects. The authors proposed that the induced CD spectrum was caused by the coupling between the electric transition moment (the π-π*-transition) of the carbonyl group in Boc-*S*-Tyr side chain and that of the porphyrin. The molecular recognition process of this host-guest system was confirmed by quantum chemical methods. The result was a structure where the *R*-enantiomer was more tightly bound with a better sterical fit to the host than its enantiomer. By comparison the minimal energy conformation, it was evident that host-*R*-AlaOCH$_3$ has lower energy than host-*S*-Ala-OCH$_3$, indicating that the former was more stable than the latter.

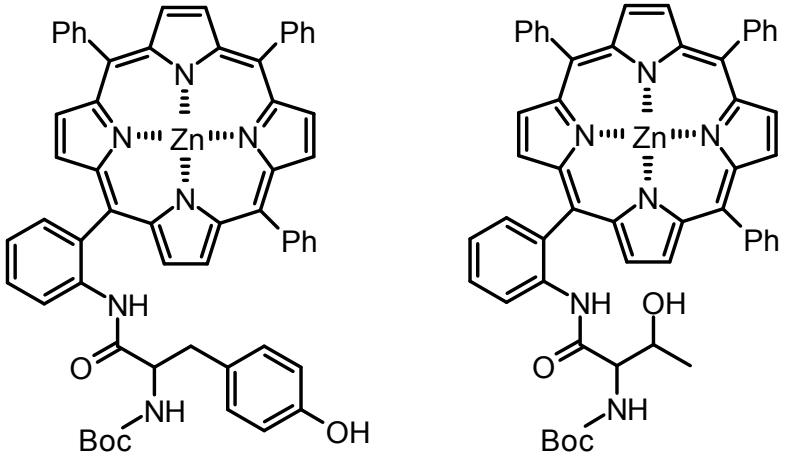


Figure 139: Zinc-porphyrins with amino acid side chains for stereoinduction

Porphyrin dimer- or tweezer-systems have been successfully used to determine the stereochemistry of chiral amines,[451,452] alcohols,[453] and carboxylic acids.[454]
The principle advantage of the porphyrin tweezer system resides with the non-covalent binding of the chiral guest and the stereoinduction by the two asymmetrically linked metal-coordination centres.

Crossley and his co-workers have reported a bis-zinc(II)-bis-porphyrin Tröger's base analogue (**193**) as a host molecule for diamines[455] and chiral recognition of histidine and lysine esters.[456] The X-ray crystal structure of the analogous palladium bis(tetraphenylporphyrinato)-complex reveals a concave chiral cavity with two metal ion binding sites suitable for ditopic interactions with guest molecules.
Several α,ω-diamines (H_2N-$(CH_2)_n$-NH_2) are strongly coordinated with a certain preference for n = 2 – 4 and $K_{ass} \sim 2*10^8$ M^{-1} as measured by spectrophotometric titrations in toluene. With increasing chain length the affinity starts to decrease with $K_{ass} \sim 6.1*10^7$ M^{-1} and $K_{ass} \sim 3.7*10^7$ M^{-1} for 1,5-diaminopentane and 1,6-diaminohexane, respectively. Mono-amines, such as hexylamine are less strongly bound ($K_{ass} \sim 5.1*10^4$ M^{-1}).
The tweezer can be resolved on small scale by chromatography on silica - *S*-histidine benzyl ester support.[457] Resolution of the bisporphyrin Tröger's base analogue **193** affords homochiral clefts that tightly bind histidine esters with 80 – 86 % *ee* and lysine benzyl ester with 48 % *ee*. The histidine esters are bound in fixed conformations that can be readily detected by ^{1}H-NMR spectroscopy as a result of the large dispersion of proton resonances by

the ring currents of the two porphyrins. The binding constants are in the same order of magnitude as observed previously for diamines.

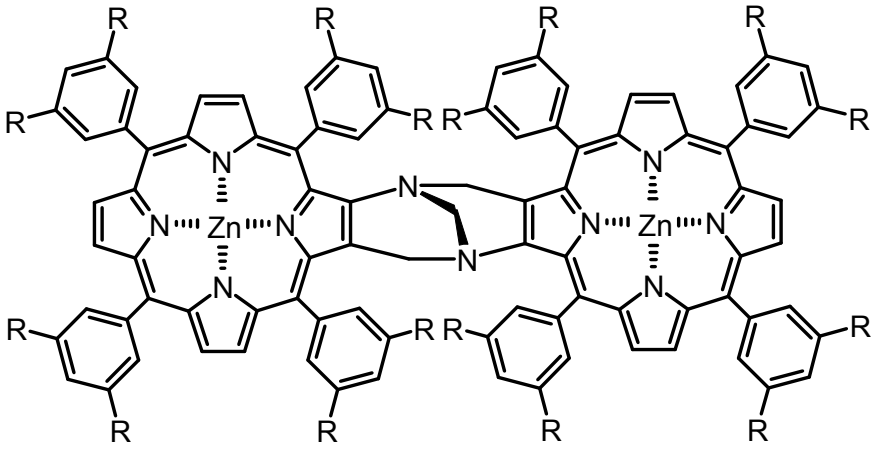


Figure 140: Bis-zinc-bis-porphyrin based on Tröger´s base **193**

A zinc porphyrin dimer (**194**) linked by chiral 1,1´-binaphthyl derivative shows a size specific interaction with α,ω-diamines (H_2N-$(CH_2)_n$-NH_2):[458] The zinc complex binds α,ω-diamines $H_2N(CH_2)_nNH_2$ (n = 6, 8, 10, 12; K_{ass} = 5*10^5 – 2*10^6 M^{-1} in CH_2Cl_2) with preference for n = 6 and 8. Shorter guests like ethylenediamine or monoamines like *n*-butylamine gave binding constants (K_{ass} ~ 3*10^3 M^{-1}) comparable to the coordination of alkylamine guests to the according zinc porphyrin monomer (K_{ass} = 2.2*10^3 M^{-1}). These complexes gave characteristic CD spectra due to the exciton coupling of the two zinc porphyrins. Their intensity depends on the length of diamine. The CD spectrum in the complex reflects angle and flexibility of the chiral twist between two zinc porphyrin units.

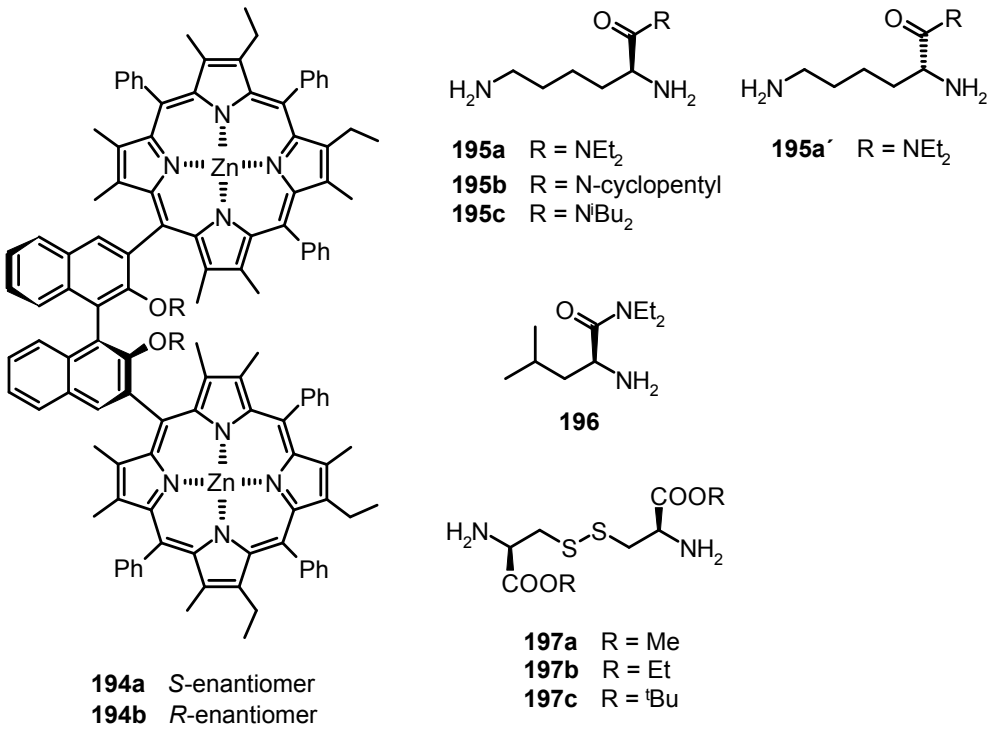


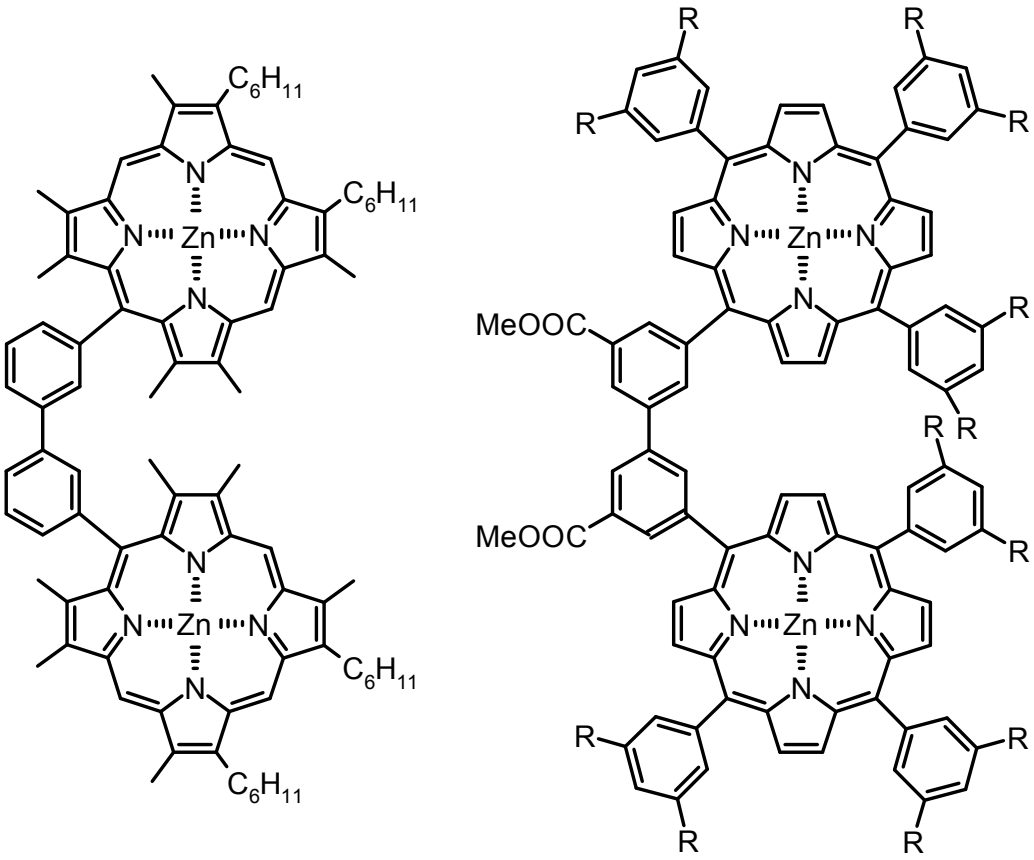
Figure 141: BINAP-zinc-porphyrin derivative **194** and it´s guests

The chiral zinc porphyrin dimer linked by (*R*)-2,2′-dimethoxy-1,1′-binaphthyl (**194**) not only tightly binds diamines via a zinc–nitrogen coordinated ditopic interaction, it displays a prominent enantioselectivity for several lysine derivatives (table 13).[459] The enantioselectivity obtained is one of the best for chiral zinc-porphyrin recognition systems. In particular, the *R/S*-selectivity is determined to be 11 to 12 for lysine derivatives, as also demonstrated by CD-spectroscopy.

Host	***Guest***	K_{ass} [M^{-1}]	K_R/K_S
(S)-194	**195a**	160000	12
(R)-194	**195a**	13000	
(S)-194	**195a´**	14000	11
(R)-194	**195a´**	150000	
(S)-194	**195b**	120000	8.6
(R)-194	**195b**	14000	
(S)-194	**195c**	120000	11
(R)-194	**195c**	11000	
(S)-194	**196***	1200	1.2
(R)-194	**196***	980	

Table 13: Binding constants and enantiomeric distinction factors for chiral porphyrin-dimers **194** in dichloromethane; * = for the 1:1 complex formation

Two different achiral hosts were investigated for their binding properties to the same guests in the course of the study. Titration in dichloromethane monitored by UV-vis titration demonstrated a 1:1 complexation between the zinc-porphyrin-dimers and the amino acid derivatives **195** and **197**. Compared to **198a** (K_{ass} for **195** = 1 - 8*10^5 M^{-1}, K_{ass} for **197** = 1 - 4*10^5 M^{-1}), the zinc porphyrin dimer **198b** has higher affinity for cysteine derivatives. The binding constants of **198b** for **197a** and **197b** were determined to be 1.7 and 2.4*10^6 M^{-1}, respectively. The length of both amine-guests almost fits the Zn-to-Zn distance, leading to the strongest binding, consistent with the former study of **194** versus diamines. The other values range from 3 to 5*10^5 M^{-1}. The achiral zinc porphyrin dimers linked by a biphenyl unit exhibit a significantly induced CD in the Soret region in the presence of chiral diamines such as lysine amides and cysteine diesters, indicating that the chirality of the amino acid derivatives can be monitored by complexation to the achiral zinc porphyrin dimer.



198a **198b** R = ^{t}Bu

Figure 142: Bisaryl-linked-zinc-porphyrin receptors

Kubo et al. presented a bis-porphyrinic system coupled with biphenyl-20-crown-6 as an allosteric spacer.[460] The biphenyl unit is connected by a rigid spacer to the two porphyrins and

bridged with the crown-ether. The porphyrin centre-to-centre distance can be switched by Ba^{2+} ion complexation in the crown-ether cavity. In its concave conformer, **199** can bind a diamine guest, such as 1,4-bis(3-aminopropyl)piperazine (**200a**). UV-vis titration in CH_2Cl_2/CH_3CN 9:1 confirmed a 1:1 complex formation and a binding constant (K_{ass}) of $7.9*10^5$ M^{-1}. In addition, the chiral bis-amino guest Tröger's base **200b** was used to probe an anti-cooperative binding event. Due to the axial chirality, **199** existed as two chiral atropisomers that rapidly interconvert at room temperature as monitored by CD measurement. The binding of the chiral base transferred its chirality to the host upon complexation.

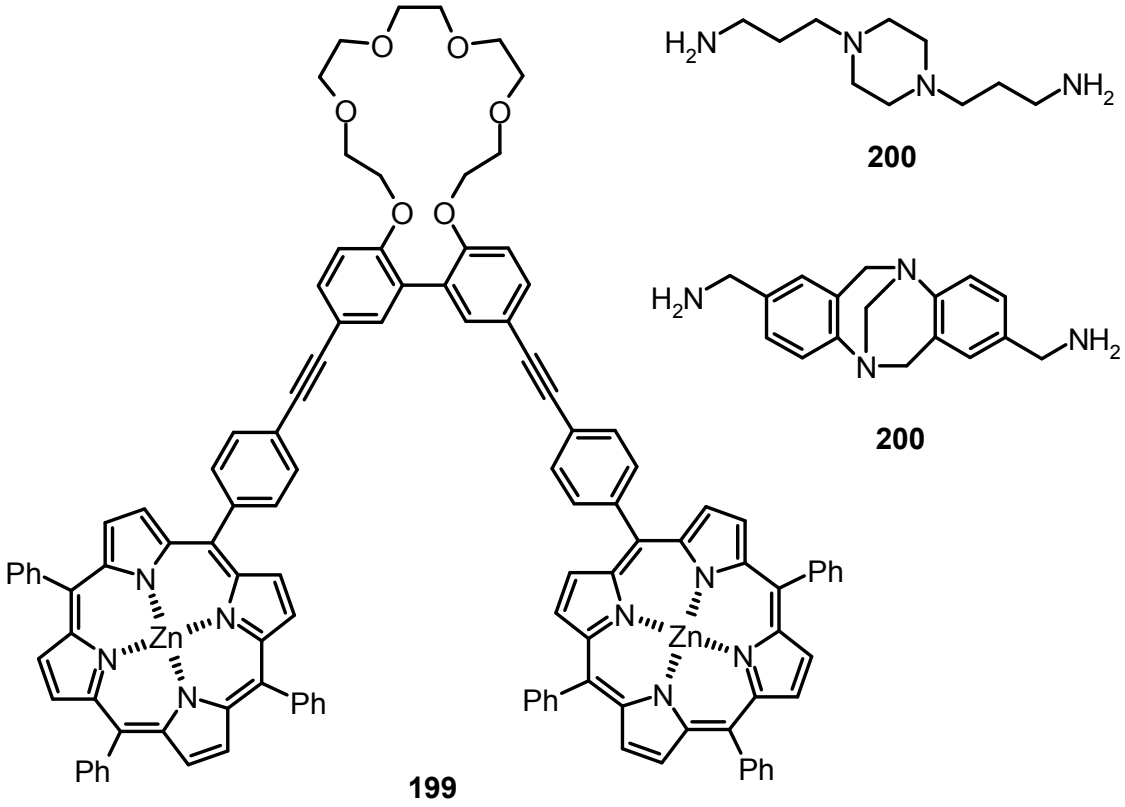
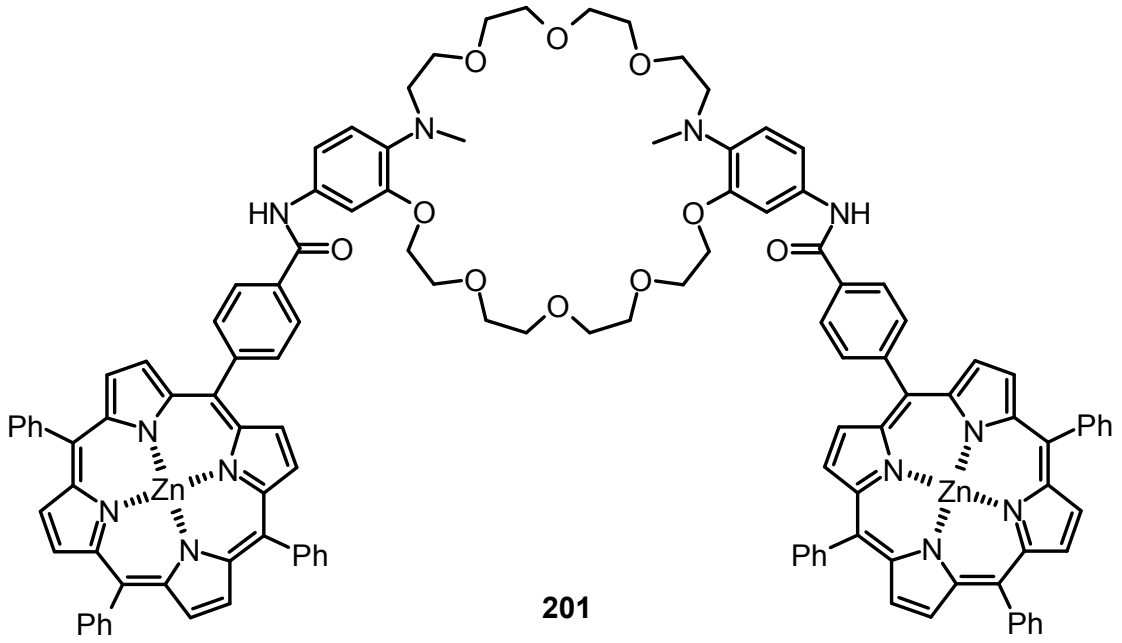


Figure 143: Bis-zinc-porphyrin **199** for diamine recognition and guests

Another example from the same group also demonstrated this for the chiral induction via a crown-ether bis-zinc-porphyrin combination (**201)**. Upon complexation of a chiral sodium carboxylate by the flexible dibenzo-30-crown-10 ether, the topology was changed into a tweezers-like structure[461] and gave a ditopic chiral guest binding site. Circular dichroism (CD) spectroscopy revealed a chiral screw conformation, which interacted with various chiral diamines, for example *N*,*N*-dimethylcyclohexane-1,2-diamine.



201

Figure 144: Bis-zinc-porphyrin crown ether **201**

This chiral induction by a ditopic bound guest was employed to determine the absolute configurations of diamines, amino acids and amino alcohols by exciton-coupled circular dichroism (ECCD).

The achiral chromophoric host porphyrin tweezer **202a**[451] or its electron deficient fluorinated analogue **202b**[462] both bind to an acyclic chiral diamine through nitrogen/zinc coordination to form a macrocyclic host-guest complex with a CD spectrum, which reflects the absolute configuration of the diamine. The exhibited exciton-coupled bisignate CD spectra reveal predictable signs based on the substituents on the chiral centre. The absolute stereochemical determination of both threo and erythro systems without the need for chemical derivatization is thus possible.

This method can be extended to amino acids and amino alcohols after simple chemical modifications. With the fluorinated system **202b** the absolute configurations of erythro and threo diols could be also effectively determined. Binding of diols to the porphyrin tweezer system is greatly enhanced by increasing the Lewis acidity of the metalloporphyrin by the strong electron withdrawing effect of the fluorine substituents.

The binding constants to amino- and hydroxy-functionalities were determined for the according monoesters (**203**) by UV-vis titration. For isopropanol as guest K_{ass} = 2140 and 50 M^{-1} and for isopropylamine K_{ass} = 473000 and 11400 M^{-1} are observed for the fluorinated porphyrin **203b** and the triphenyl substituted compound **203a** respectively.

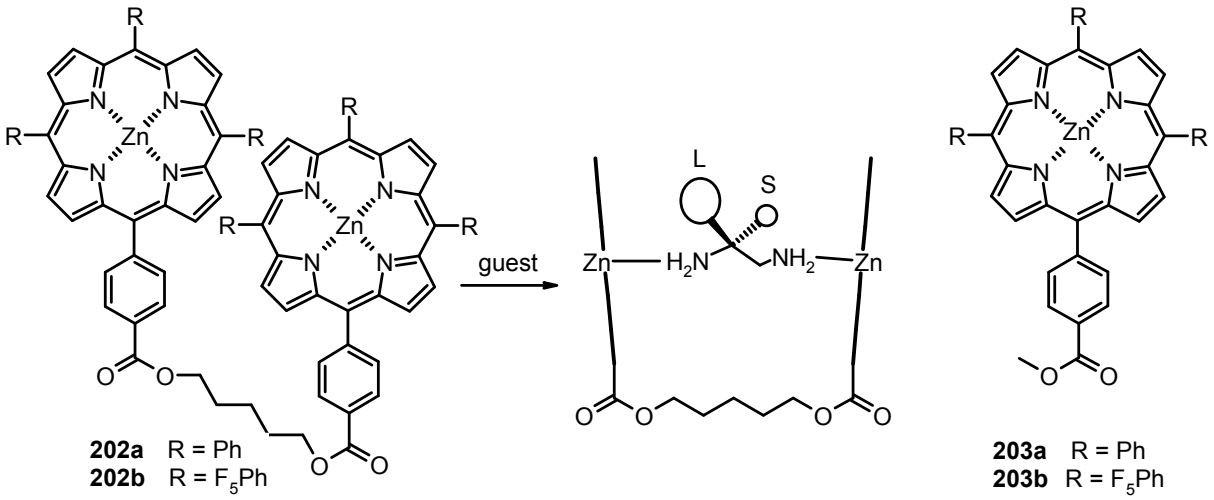


Figure 145: Bis-zinc-porphyrin **202** for stereodiscrimination (L = large substituent; S = small substituent)

A [3]rotaxane and its copper complex (**204**) have been presented as new, innovative binding concept.[463] The properties of the system were investigated by UV-spectroscopy in toluene. The complexes were also investigated and assigned by NMR-DOSY experiments. In these two states of the [3]rotaxane, free and complexed with copper, the two zinc(II) porphyrins attached to the rings can bind different ditopic guests bearing pyridyl groups or amines as terminal functions.

Removal of the two Cu(I) cations releases the two rings which are now free to move along and around the thread. The metal-free [3]rotaxane is a new type of receptor by which guests of very different sizes can be trapped between the two mobile porphyrins since they can move over an 80 Å plane-to-plane distance on the thread. It is both a strong and highly adaptable receptor with high stability constants for the host/guest complexes, log K_{ass} being in the range of 6.3 to 7.5 for guests between 2.8 and 18 Å.

In the copper-complexed [3]rotaxane, the rings are fixed by coordinative bonds to the rod and the distance between the porphyrins is therefore controlled to a certain extent, leading to destabilization of the host/guest complex with long guests, due to distortions on both the guest and the porphyrin rings. The copper-complexed [3]rotaxane is a good receptor for small guests with preference for **205c** (log K_{ass} = 7.5) due to an entropic gain for this pre-organized molecule compared to the free [3]rotaxane.

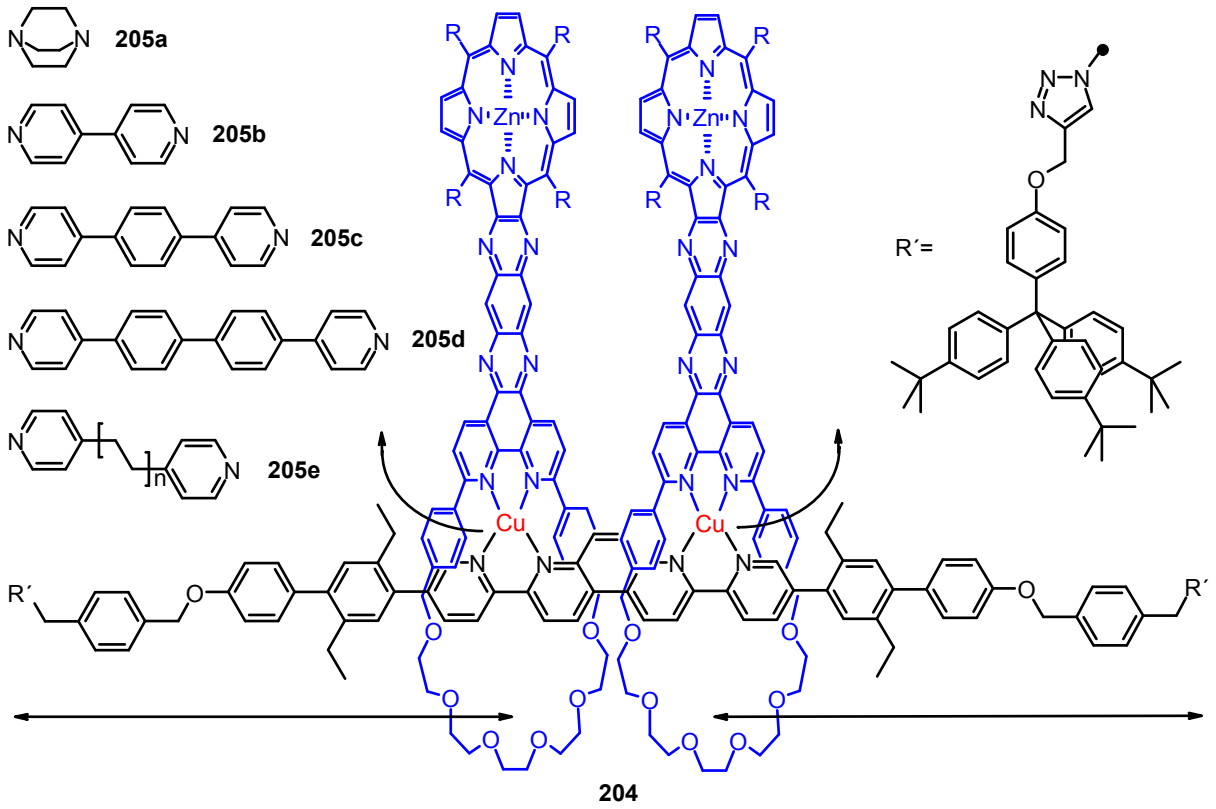


Figure 146: Bis-zinc-porphyrin[3]rotaxane and its copper complex and guests

I. 7.2. Other Metal Complex Centres

Many other coordinatively unsaturated metal complexes, due to their strong complexing ability, can be employed as suitable potential binding sites for synthetic receptors, especially for molecular recognition in protic solvents. [464] Non-covalent forces are weakened in this medium with high dielectric constant, since a large number of solvent molecules interfere. The selection of the ligands is defined by the ability of their corresponding transition metal complexes to reversibly and tightly bind Lewis basic guest molecules in competing solvents, such as water. Amino acids are strongly bound by their side chains or in a bidentate complex bridging the metal. Complexes of cyclene, cyclam, and related structures are widely used. The recognition with aza macrocycle complexes was recently reviewed.[465]

Amino acids can be targeted by cooperative chelation between the carboxylate and the amine: The coordination of metal ions through the amino and carboxyl groups gives five-membered metallocycles.[466] Bipyridines (bpy) or nitrilotriacetic acid (NTA) are widely used ligands. A typical example are [Cu(NTA)]-complexes, which coordinate amino acids. [467] Binding

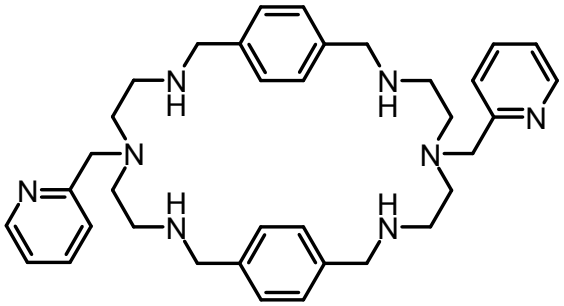
affinities have been determined for a variety of amino acids in aqueous medium (table 14). The coordination of His to [Cu(NTA)] is a special case, containing mixtures of species in which His is coordinated either as an anion or in its zwitterionic form.[468]

Amino acid	**Log K_{ass}**
Gly	5.44
Ala	5.42
Phe	4.99
Leu	5.35
Val	5.10
β-Ala	4.56
His	4.16 (monodentate) 5.73 (bidentate)

Standard deviation < 0.01; at 25 °C

Table 14: Binding constants of amino acid guests to Cu[NTA]

Bis-dien bis-copper complexes of ligand **206** bind imidazole as bridging ligand between two Cu(II) ions with the simultaneous extrusion of a proton as demonstrated by Fabbrizzi et al.[469] A binding constant of log K_{ass} = 4.7 was derived by pH titration. For histamine a binding of log K_{ass} = 4.3 and for *S*-His of log K_{ass} = 5.5 was obtained. The 1:1 complex stoichiometry was verified by spectrophotometrical titrations. Later the same group reported a luminescent sensor for histidine (**81e**) based on a tridentate Zn(II)-tren complex.[470]

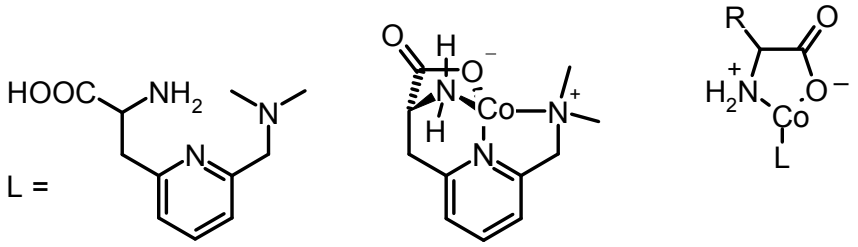


206

Figure 147: Dien-bipyridyl ligand **206** for coordination of two metal atoms

The dichloro-cobalt-complex **207** was reacted with glycine, *S*-alanine, *R*-alanine, *S*-phenylalanine (**81a**), *R*-phenylalanine (**81a**), *S*-tryptophan (**81b**), and *R*-tryptophan (**81b**).[471] Alanine forms a five-membered ring upon chelation to the metal complex. Deuteration experiments monitored by NMR showed that α-hydrogens of the three coordinated *R*-amino acids exchanged rapidly with little or no observable epimerization. In contrast, the α-hydrogens of the three *S*-amino acids exchanged slowly with concomitant epimerization. It was not possible to fully deuterate the *S*-amino acid complexes due to competing decomposition reactions. Thus, the *R*-enantiomer of the receptor binds the *R*-enantiomers of the amino acids more tightly and converts the *S*-enantiomers to the *R*-enantiomers.

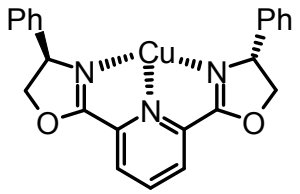
The X-ray crystallographic and ^{1}H-NMR data underlined that coordination of alanine takes place with unprecedented regiospecicity and stereospecicity. The regiospecicity is apparently controlled by electrostatic effects while the stereospecicity is controlled by steric effects in a highly predictive manner. This approach thus provides detailed structural insights into general separation of bidentate α-H-amino acids into *R*- and *S*- forms with a single chiral metal complex.



207

Figure 148: The ligand and corresponding tetradentate Co-complex **207** serving as enantio-selective receptor for amino acids

Bis(oxazolines) are widely employed in asymmetric catalysis, for example in cyclo-propanations. Besides this they are also valuable receptor moieties:[472] The enantioselective recognition of amino acids has been studied with C2-symmetric chiral pyridine bis(oxazoline)–copper(II) complexes **208** at physiological pH by UV–vis titration and revealed a strong binding with a submillimolar dissociation constant in aqueous solution. Moderate selectivity of up to 2:1 between *R*- and *S*-amino acids was achieved with best affinities of the *R*-host to *R*-amino acids.



208

Figure 149: Bis(oxazoline)–copper(II) **208** for the recognition of amino acids in aqueous solution

Zinc–salophen complexes have also attracted much attention as receptors. Their well known capability to accept one axially coordinated donor species, along with their photophysical properties,[473] make them suitable candidates for the development of amine receptors.[474]

Zinc–salophen compounds incorporating 2,3-diaminonaphthalene (**209a**) and 9,10-diaminophenantrene (**209b**) moieties show unprecedented selectivities of quinuclidine (**210d**) *vs*. triethylamine (**210b**) higher than 10^5 as investigated by UV-Vis and fluorescence spectroscopy in chloroform solution.[475] The binding to the zinc–salophen compounds to tertiary amines is subjected to the influence of steric effects. The binding constants for quinuclidine (**210d**) were all larger than 10^6 M^{-1}, for triethylamine (**210b**) values of ~ 50 M^{-1} and smaller were recorded. Dimethylethylamine (**210c**) bound with 1500 to 1900 M^{-1}, where else diisopropylethylamine (**210a**) gave a negligible response. The axial coordination of tertiary amines is in general stronger for zinc–salophen compounds than for zinc–porphyrins. X-ray diffraction analyses showed that in the solid state compound **209a** is dimeric, but its 1:1 quinuclidine complex is monomeric. Strong indications were obtained that both free receptors and their amine adducts are monomeric in dilute chloroform solution.

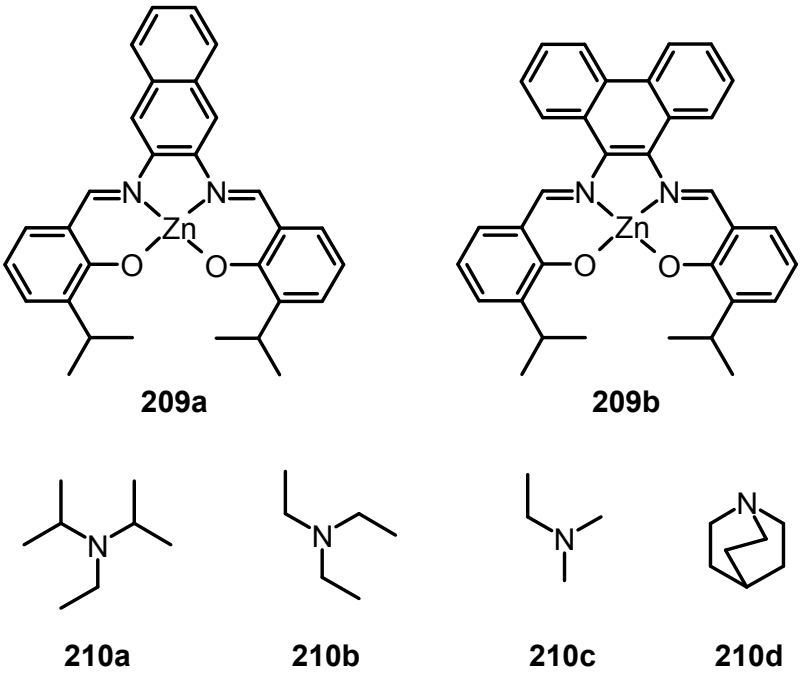


Figure 150: Zinc-salen-complexes **209** for the recognition tertiary amines

A "ditopic binder" recognizing ammonium ions with its side chains in water was described with a water soluble zinc-salophen complex **211**.[476] Its binding to carboxylate anions in water is very strong (K_{ass} > 10^6 M^{-1}). Amino acids are bound with associations constants ranging from K_{ass} = 3800 M^{-1} for glycine to K_{ass} < 5 M^{-1} for tryptophan (**81b**) as investigated by UV-vis spectrophotometric titrations. The general trend shows a gradual decrease in binding strength with increasing steric hindrance. The K_S/K_R ratio of 9.6 observed for phenylalanine (**81a**, 2500 M^{-1} and 260 M^{-1}, respectively) is among the highest values found for the chiral recognition of amino acids in water.[477] These findings led to the conclusion that amino acids are bound via zinc-carboxylate coordination and hydrogen bonding between the ammonium group and two oxygen atoms of one of the *D*-glucose moieties. This was supported by structures of the 1-glycine complex calculated at the semiempirical level (PM3).

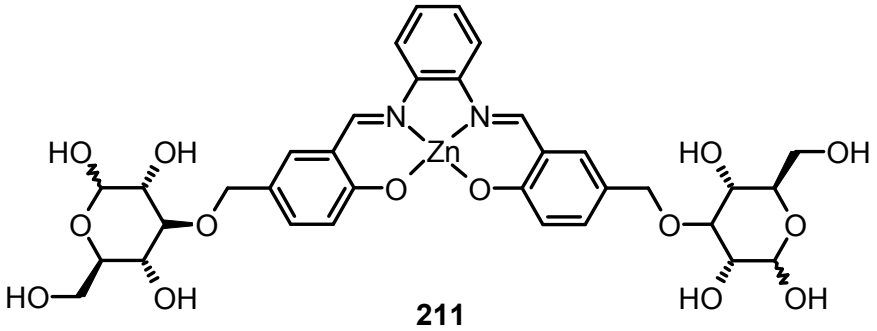
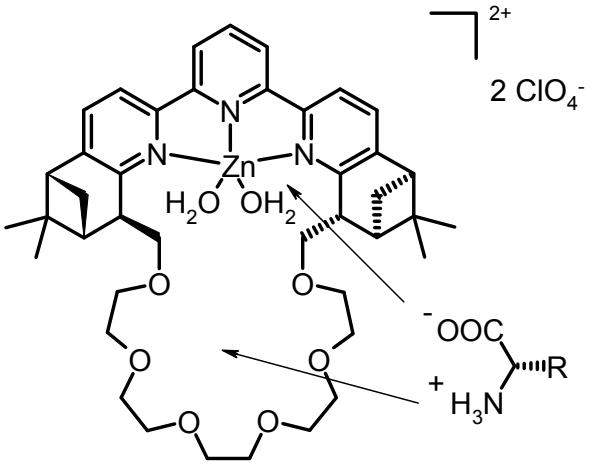


Figure 151: Zinc-salen-complex **211** for the recognition of amino acids in aqueous solution

A new fluorescence macrocyclic receptor **212** based on the Zn(II)-complex of a C2-terpyridine and a crown ether has been developed for molecular recognition of zwitterionic amino acids in water/DMF solution with strong bindings towards *S*-aspartate (K_{ass} = 4.5*10^4 M^{-1}) and *R*-cysteine (K_{ass} = 2.5*10^4 M^{-1}). [478] The Zn(II)-tpy subunit coordinates with the carboxylate group of the zwitterionic amino acids, and functions as a chromophore (λ_{max} = 348 nm) for the fluorescence sensing in aqueous solutions. The crown ether subunit binds the ammonium group of the zwitterionic amino acids. Without the crown ether subunit the binding towards *S*-aspartate was about 90 times smaller, no significant change in fluorescence was observed for other amino acids. Results of MM2 calculations gave the same picture. The binding properties of receptor **212** towards different amino acids were studied using UV and fluorimetric titration methods. In all cases a 1:1 stoichiometry was observed, the equilibrium binding constant K_{ass} was estimated using the Benesi-Hildebrand equation. The binding affinity of receptor **212** towards amino acids is highly dependent on the coordinating abilities of the side-chain chelating groups towards the Zn(II) metal (carboxylate > thiol >> amide > hydroxyl-ammonium). *S*-Aspartate and *R*-cysteine showed the highest level of affinity towards receptor **212**, which is about 4 – 14 times higher than *S*-asparagine and *S*-serine. *S*-Aspartate exhibited a much stronger binding (18 to 79 times) than the amino acids bearing an alkyl or aryl side-chain, and about 180 times higher than the cationic substrate
(*S*-ornithine). The rigid C2-symmetric chiral groups the Zn(II)-tpy subunit lead to enantio-selectivity towards *R*-amino acids with K_R/K_S up to 3.0 for phenylglycine.



212

Figure 152: Zn(II)-complex of a C2-terpyridine crown ether

Indicator displacement assays are a popular method for converting synthetic receptor into optical chemosensors. Recognition of amino acids is only one substance class, which can be targeted by such colourimetric, fluorescent, and metal containing assays. Many examples along with their biological counterparts were highlighted. [479]

Anslyn et. al. targeted the neurotransmitters aspartate and glutamate in a pyrocatechol violet displacement assay (fig. 153) in a water/methanol mixture (1:1; buffered with 10 mM HEPES at pH 7.4).[480] The zinc complex was perfectly stable under these conditions. The highest affinity was found for aspartate (K_{ass} = 1.5*10^5 M^{-1}) with a seven fold stronger recognition over succinate, or glutamate, and a factor of near 15 over the hydrophobic amino acids. The affinity of **213** is dominated by the interaction with Zn(II). In the case of aspartate the appended guanidinium groups also contributed to the binding. In addition, it was also observed that the use of metals in receptors can lead to larger colour changes in indicator displacement assays. A shift in absorbance of the bound indicator that cannot be achieved with receptors that simply rely on hydrogen bonding and ion pairing for perturbing the ionization state was given as reason for this observation.

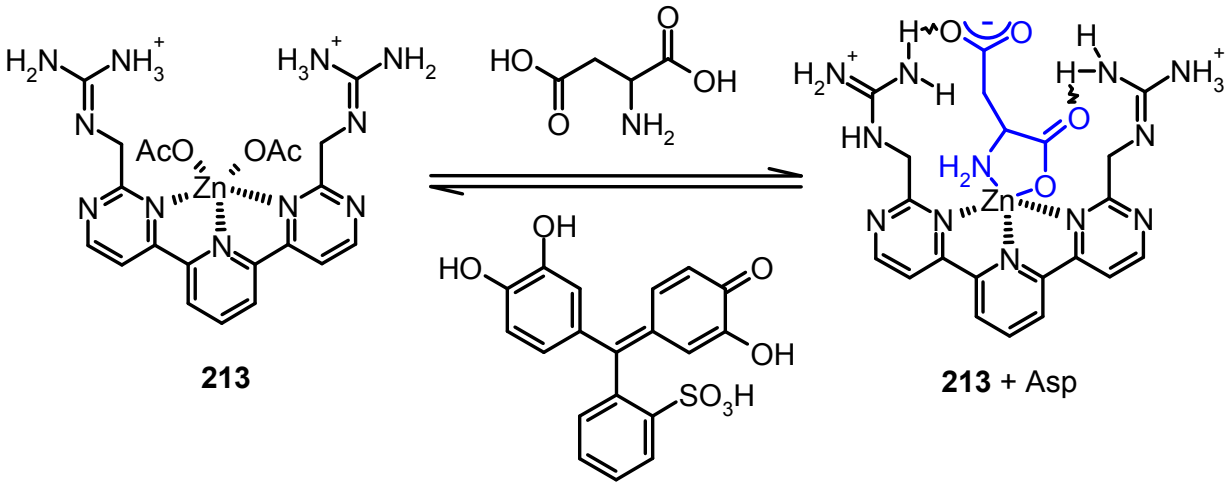
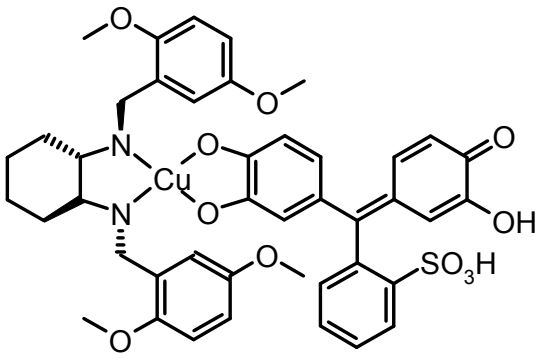


Figure 153: Displacement assay and receptor for aspartate over glutamate

They also reported on a comparable colourimetric technique for *ee* determination of non-derivatized *R*-amino acid samples in H_2O/MeOH solutions based on a displacement assay with pyrocatechol violet. This time a copper complex was used for the competitive metal coordination. [481] The ability of (*S*,*S*)-**214** to enantioselectively differentiate four of the hydrophobic *R*-amino acids was shown by UV-vis spectroscopy. Titration of *R*-amino acids into (*S*,*S*)-**214** resulted in a decrease of the Cu(II) absorbance. These experiments were carried

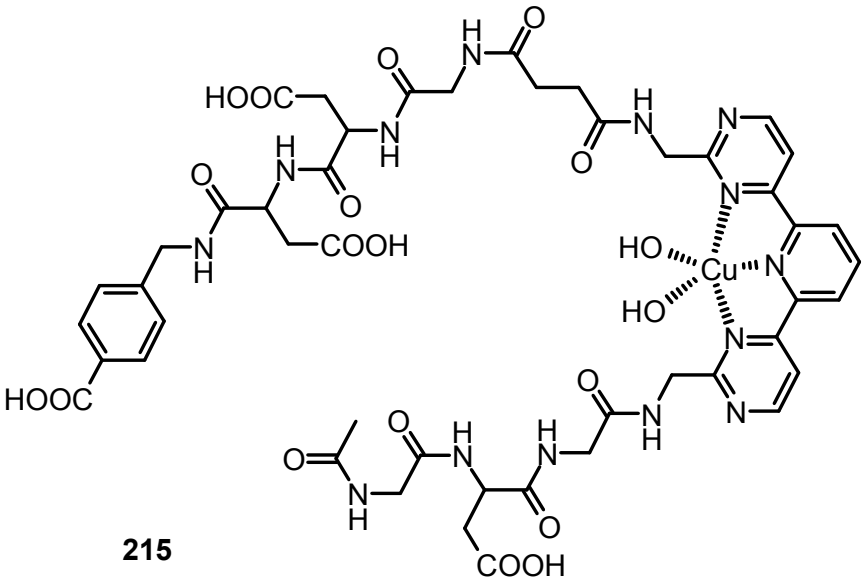
out in the presence of a 10-fold excess of ligand (*S*,*S*)-**214** to discourage dissociation of (*S*,*S*)-**214**, avoiding the creation of 2:1 complexes. Valine and tryptophan (**81b**) gave the best values for their 1:1 complexes. *R*-Val and *S*-Val bound with association constants of $5.2*10^5$ M^{-1} and $2.0*10^5$ M^{-1}, resulting in an enantioselectivity $K_R/K_S = 2.6$, for *R*-Trp and *S*-Trp values of $1.1*10^6$ M^{-1} and $5.0*10^5$ M^{-1}, giving an discrimination of $K_R/K_S = 2.2$ were found. Overall, the data showed a consistent preference for *R*-amino acids by about a factor of 2 to 2.6.



214

Figure 154: Chiral complex **214** for a colourimetric displacement assay for amino acids

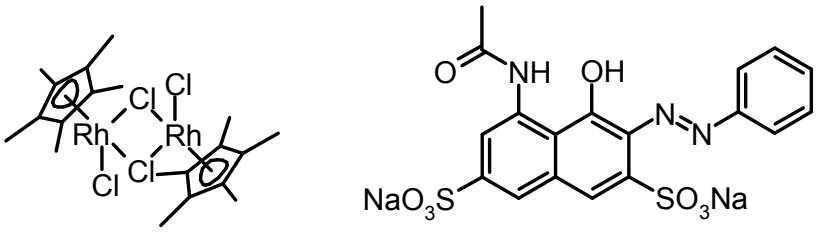
The insertion of strong coordination centres into peptides enables the construction of selective molecular receptors with complementary frameworks suitable for differentiation of amino acids and small peptides. A metal-centred receptor **215** consisting of a rigid backbone region and variable tripeptide arms[482] for the recognition of tripeptides was reported. The receptor is selective by cooperative interactions of the peptidic arms for xxx-*S*-Lys-*S*-Lys, with xxx = *S*-His, *R*-Cys, and *S*-Met, giving association constants near 10^6 M^{-1}. The binding studies in a water/methanol solution (1:1; buffered with 100 mM HEPES at pH 7.4) by UV-vis titration indicated by the association constants of the protected peptides, that amino acids bound through their amino terminus. *N*-Terminal metal-chelating amino acids appended to basic amino acids bound with enhanced affinities via metal-chelating and ion pairing. *N*-Terminal His with two appended Lys showed the maximum binding with a value of 10^6 M^{-1}. The increase in affinity by a factor of near 10 - 30 over *R*-Cys-*S*-Lys-*S*-Lys and *S*-Met-*S*-Lys-*S*-Lys with $K_{ass} = 3.0*10^5$ and 10^5 M^{-1}, respectively, was contributed to the ion-pairing interactions possible with the guest peptide residues. In contrast, the His-, Cys-, and Met-Gly-Gly analogues dropped in affinities by approximately 100-fold.



215

Figure 155: Metal complex receptor **215** with tripeptide side arms

Recognition of amino acids[483] and peptides[484] is performed by a displacement assay with the rhodium sandwich complex **216** and an azo dye like **217**. The aggregate formed distinguishes peptides with His and Met residues in position 1 or 2 at the *N*-terminus from other peptide sequences. The association constant of His-Ala, His-Gly-Gly, Leu-His-Leu or Gly-Met-Gly with **216** is with values around 10^{10} M^{-1} three orders of magnitude higher than the binding strength of the dye **217** to it. Peptides like Val-Phe or Lys-Tyr are so weakly competing with the dye that recognition of the above mentioned peptide in aqueous solution is possible even in the presence of a 100-fold excess of them. With this system a colourimetric assay for the 20 natural amino acids in water was developed.[484]

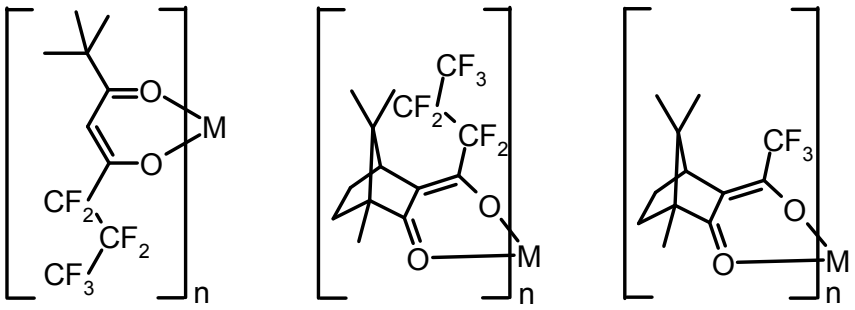


216 **217**

Figure 156: A sandwich complex **216** and its displaceable dye **217**

A recent example uses lanthanide complexes as receptors for the recognition of unprotected amino acids. Lipophilic lanthanide complexes of fluorinated diketonate ligands **218** to **220**

were demonstrated by extraction experiments to bind unprotected phenylalanine (**81a**), leucine, and other amino acids under neutral conditions.[485] All tris(diketonates) formed 1:1 complexes with amino acids. The observations were verified by NMR and CD spectroscopic studies, which also suggested that the metal complexes bound the amino acid guests at two points. Their extraction, transport, and chiral recognition behaviours were significantly controlled by a combination of central lanthanide cation and coordinating ligand: The chiral ytterbium complex **219d** offered good enantioselectivity in the extraction of unprotected amino acids (Ph-Gly; 49 % *ee*), and the related praseodymium complex **219a** provided efficient membrane transport (Phe; 62 %). For receptors **218** the order of extraction from DCM to water was determined as Phe > Trp > Leu > Ph-Gly with a maximum value of 52 %. **219b** and **219b** extracted Ph-Gly, Phe and Trp to up to 62 % under the same conditions. Especially complex **219b** exhibited excellent extraction ability for amino acids due to the effect of the electronegative fluorinated moieties[486] of the ligand increasing Lewis acidity of the lanthanide tris(diketonate). This led to strong coordination of the carboxylate anion of the amino acid guest. In addition, fluorinated ligands enhanced the solubility of lanthanide tris(diketonates) and of their ternary complexes with amino acids in the organic media.[487]



218a M = Pr
218b M = Eu
218c M = Dy
218d M = Yb

219a M = Pr
219b M = Eu
219c M = Er
219d M = Yb

220a M = Eu
220b M = Er

n = 3

Figure 157: Lanthanide complexes **218** – **220** for amino acid recognition

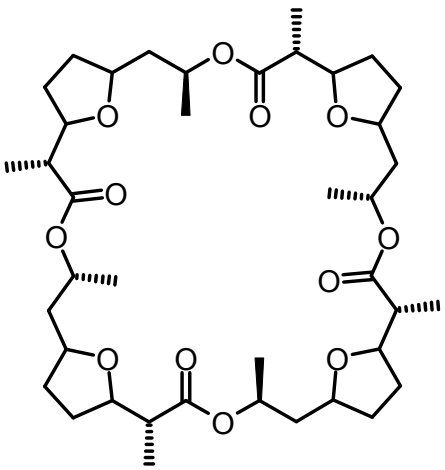
Metal complexes of porphyrins, bisoxazolines, tripyridines, salens and many other ligands are valuable binding sites for amines and amino acids. By coordinative bonds they are able to form stable aggregates even in highly competitive media, such as water. Thus, they enable the recognition of targets like amino acids and peptides in this challenging surrounding. Bidentate coordination of the guest allows enantiodiscrimination.

I. 8. Other Concepts: Natural Ionophores, (Cyclo)peptidic hosts, Covalent Bond Formation and more

A variety of less frequently applied concepts for ammonium ion binding are present in the literature, which cannot be allocated to one of the former chapters: Natural ionophores, their derivatives and related molecules, peptidic- and cyclopeptidic structures and reactive groups. We discuss these concepts with selected examples in the following.

I. 8.1. Natural Ionophores

The best known naturally occurring macrocycles showing ammonium ion affinity are the nonactins (**221**), valinomycin (**222**)[433] or the natural antibiotic vancomycin (**223**)[488]. Vancomycin (**223**) recognizes the Lys-*R*-Ala-*R*-Ala sequence and inhibits linking of these building blocks of the bacterias cell walls, thus causing cell death by osmotic overpressure.[2] For a long time it has been used as a reserve antibiotic, a so called "last line of defence", because no little resistance was observed,[488] which is no longer the case.



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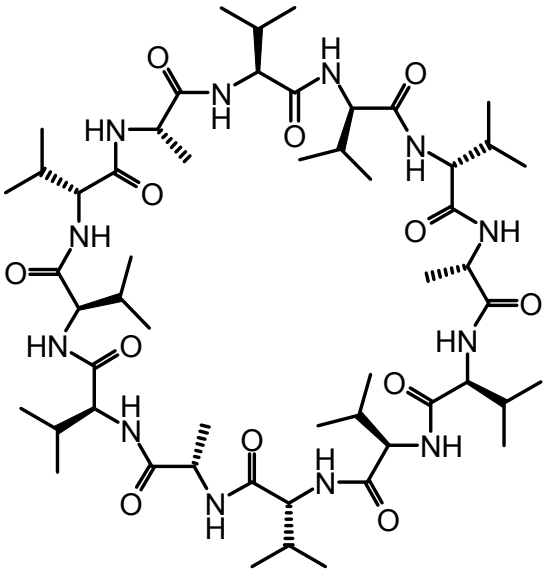

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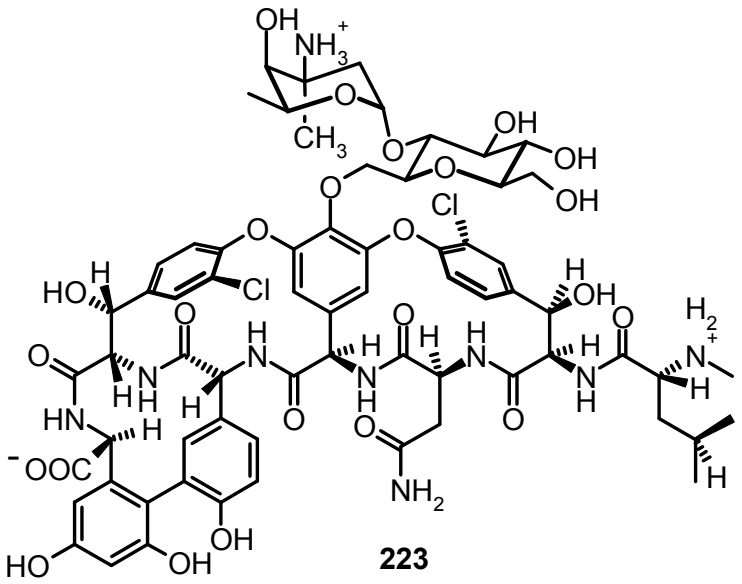


223

Figure 158: Nonactin (**221**), valinomycin (**222**) and vancomycin (**223**)

Valinomycin (**222**) is a cyclodeca-depsipeptide consisting of *S*-valin, *R*-valin, *S*-lactat and *R*-hydroxyisovalerat with the repetitive structure (*S*-Lac-*S*-Val-*R*-Hiv-*R*-Val-)$_3$, forming a ring of 36 atoms, with alternating amide and ester bonds. Similar to the interaction of crown ethers with cations valinomycin guest binding is based on ion-dipole interactions between the oxygen atoms positioned along the ring and the guest.[489] The molecule is pre-organized through hydrogen bonding of its amide carbonyl groups to form a pocket with six ester carbonyl oxygens available for electrostatic stabilization of potassium ions through octahedral complexation.[490] Ammonium ions are bound in the same way. The selective transport of potassium ions by valinomycin through the cell membrane causes cells death by breakdown of the membrane potential.[491] The binding strength for potassium ions in aqueous media is 10^6 M^{-1}.[492]

Investigations of the ammonium ion complex of valinomycin in methanol by capillary electrophoresis gave a apparent stability constant of log K_{NH4+} of 1.52 ± 0.22,[433,493] which is in good agreement with the earlier determined value of log K_{ass} = 1.67 obtained from spectro-photometric measurements.[494] In comparison to the ammonium ion binding ability of 18-crown-6 (**4**) in the same solvent obtained by conductivity measurements (log K_{ass} = 4.1),[495] the value is two orders of magnitude lower.

The binding properties and association constants (K_{ass}) of synthetic crown ethers with different cavity size and substituents and the natural ionophores valinomycin and nonactin versus deferriferrioxamine B, $CH_3(CH_2)_4NH_3^+$, NH_4^+, K^+, and Mg^{2+} in water saturated chloroform were reported (table 15).[496]

Host	Log K_{ass} (guest perchlorate salt)		
	potassium	*ammonium*	*n-butylammonium*
*Cis-dicyclohexano -18-crown-6**	8.23	7.69	6.16
*Valinomycin (**222**)*	8.99	7.15	4.20
*Nonactin (**221**)*	7.18	7.66	5.19

Table 15: Binding values of natural ionophores compared to a 18-crown-6-derivative in chloroform; * = reference[497]

These values were later confirmed by a mass spectrometric study.[498] Evaluation of the cation complexation by ^{1}H- or ^{13}C-NMR methods, in solution or solid-state, has been reported for all ionophores: valinomycin,[499] nonactin and tetranactin,[500] and cereulide.[501] Potassium ions are a major interferant in ammonium ion detection because potassium has a size similar to that of ammonium (1.33 Å).[68]

In contrast to valinomycin, nonactin is selective for ammonium ions over potassium ions. It exceeds crown ethers in selectivity and shows excellent selectivity in NH_4^+ transport in relation to K^+ ($NH_4^+/K^+ \sim 14$).[75] In ion transfer reactions of the ammonium, potassium, and sodium ions with the ionophores dibenzo-18-crown-6, nonactin (**221**), and valinomycin (**222**) investigated at the water/1,2-dichloroethane interface, nonactin was found to be the most selective towards the ammonium ion, with a calculated association constant of 14.1.[502] Therefore, it is widely employed in ion selective electrodes, were it is superior to many artificial ionophores [log $K_{NH4+,\ K+} = -1.0$, log $K_{NH4+,\ Na+} = -2.6$][75] and exhibits a detection limit for ammonium ions of 10^{-6}M.[77] Often it serves as a reference compound for development of new ionophores for ISE.

Nonactin (**221**) is a naturally occurring ionophore, a highly symmetric meso compound with flexible conformation, when no ion is present.[503] The unbound conformation is relaxed and almost planar, possesses strong intramolecular non-bonding dipoles and lacks hydrogen bonding interactions.[504] It adopts a puckered conformation if bound to ammonium, pre-organized with the ion bound, leading to a good overlap of the oxygens to stabilize the charged ammonium hydrogens.[505,506]

Monensin esters (fig. 159) are sodium ionophores, but synthetic analogs bind primary ammonium ions selectively and offer chiral recognition ability comparable to that of Cram's binaphthyl crown ether. As demonstrated by experiments in an ion selective electrode in buffered water and by NMR-studies in chloroform, enantioselective complexation can be found for chiral phenethylamine and naphthylamine salts, as well as for some amino acid esters. (*R*)-1-(l-Naphthyl)ethylammonium acetate is bound with three fold selectivity over the corresponding *S*-enantiomer by (*S*)-**224b**.[507]

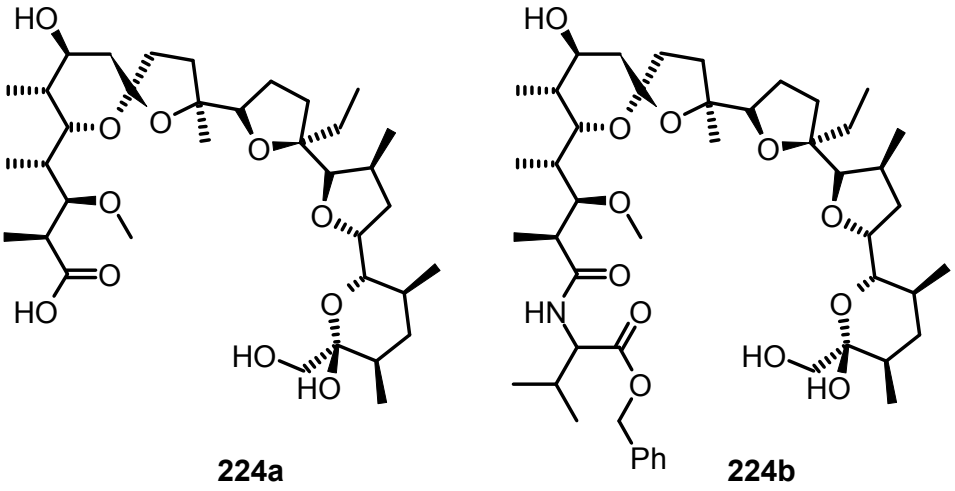
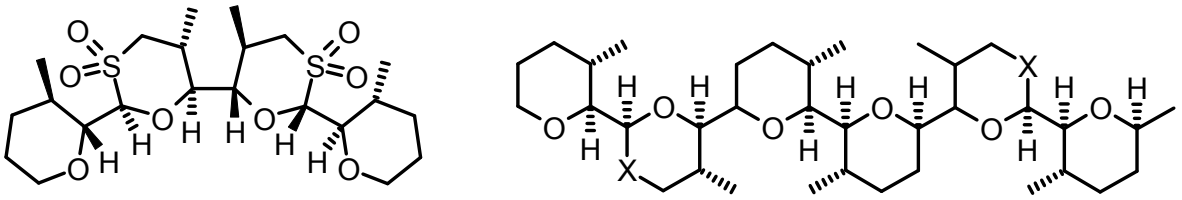


Figure 159: Monesin (**224a**) and a chiral analogue for enantiodiscrimination of ammonium guests (**224b**)

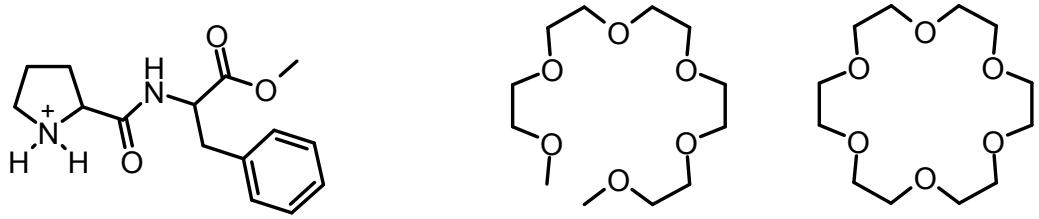
A variety of natural polycyclic antibiotics bear a structural resemblance to podands and they reveal often stunning selectivities and binding properties. Podands form complexes of lower stability than their corresponding macrocyclic counterparts. In the case of pentaglyme dimethylether (**225**) versus 18-crown-6 (**4**), the macrocyclic ether binds the *tert*-butylammonium ion 10^4 times more tightly.[52] The enormous difference in binding results from the macrocyclic effect. In structures like the monesins (**224a**, fig. 159) and lasalocid (**228**, fig. 161) these is overcome by the pre-organizing effect of the furan and pyran rings leading to a half-moon like array, as well as the possibility to build manifold contacts to the guest.

This effect can be nicely seen in the artificial systems presented by Still et al. Chiral podand analogs (**226b**) of 18-crown-6, conformationally locked, reveal ionophoric properties closely related to the macrocycle. These host molecules have a cation-binding site with six oxygens arranged in the geometry as found in the crystal structure of potassium 18-crown-6.[508]

The conformationally homogeneous podand receptor (**226a**) even binds proline-derived dipeptidic substrates (**227**) enantioselectively and diastereoselectively.[509] A closely related enantiomerically pure, C2-symmetric tetracyclic podand forms well-defined complexes with chiral ammonium salts. With derivatives of α-phenethylammonium hexafluorophosphate as guests, binding enantioselectivity up to 60 % *ee* is achieved.[85]



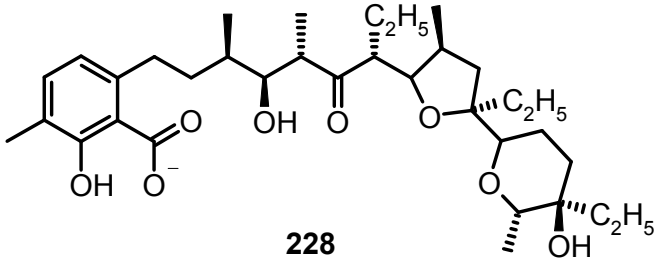
226a **226b,** X = O, SO_2



227 **225** **4**

Figure 160: Chiral podands (**226**) compared to pentaglyme-dimethylether (**225**) and 18-crown-6 (**4**)

Also featuring the coordination by OH groups, but also by ether oxygens, lasalocid A (**228**), can compete with the macrocycles. It is a widely employed ionophore antibiotic, which can effectively complex ammonium ions like crown ethers. In medicine it is directly employed as its sodium salt. The mechanism of lasalocid activity is clearly attributed to its ionophoric properties. Especially the influx of Na^+ in the cell of Gram-positive and anaerobic bacteria causes swelling, vacuolization and finally cell death.[510]



228

Figure 161: Lasalocid A (**228**)

Lasalocid can form strong complexes with biogenic amines such as dopamine (**2**), norepinephrine, 2-amino-heptane, as well as tyramine and transport them across biological membranes.[511] The crystal structure of a protonated amine with lasalocid shows all protons of NH_3^+ hydrogen bonded. The complex is also stabilized by some intramolecular hydrogen bonds.[512] In the gas, liquid and solid states lasalocid forms this very stable 1:1 complex with allylamine with its structure being comparable in all states. Due to these interactions the outside of the complex is hydrophobic enabling ammonium transport across the biological membranes.

Sessler et al. published sapphyrin ± lasalocid conjugates (**230**) which feature binding sites for both carboxylate anion complexation and ammonium group recognition as efficient and selective carriers for aromatic amino acids.[513] In through-membrane model transport experiments carrier **229** showed selectivity for phenylalanine (**81a**) over tryptophan (**81b**). Tyrosine is not transported to any significant extent. In general *S*-amino acids were transported with greater efficiency than the corresponding *R*-enantiomers by this particular carrier. The high level of amino acid carrier capability displayed by receptor **229** in dichloromethane solutions correlates well with the results of equilibrium binding studies carried out using visible-spectroscopic titrations.

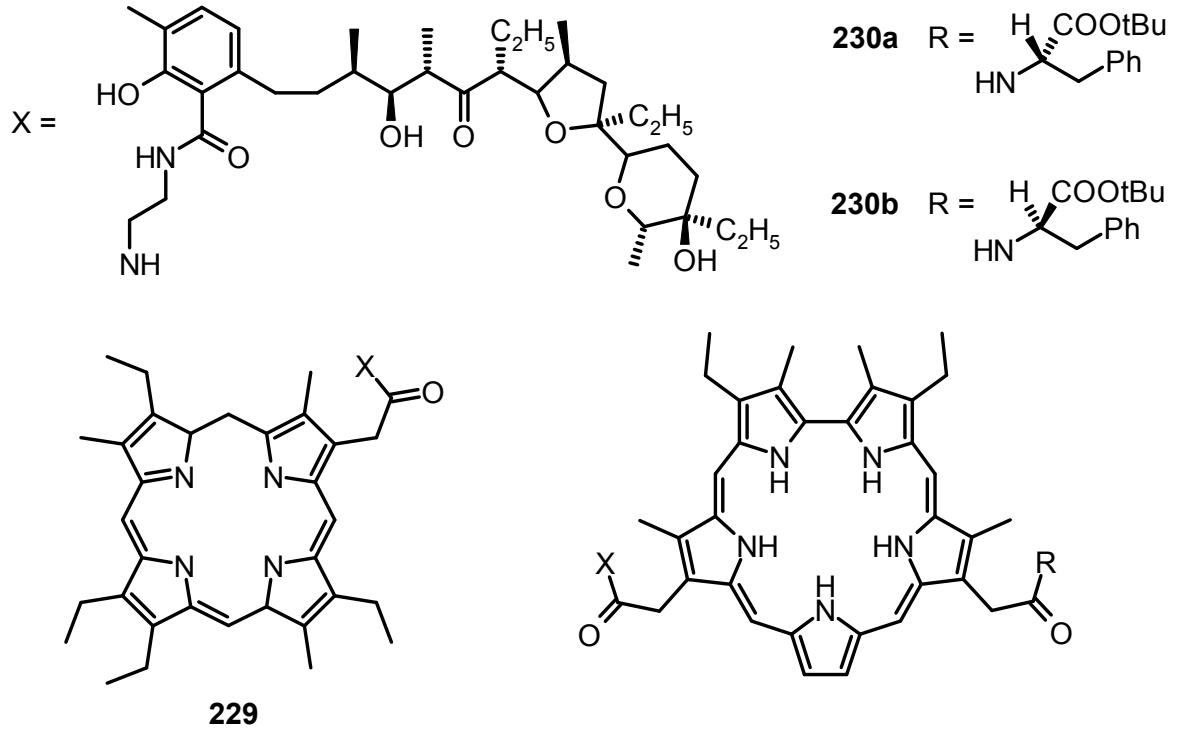
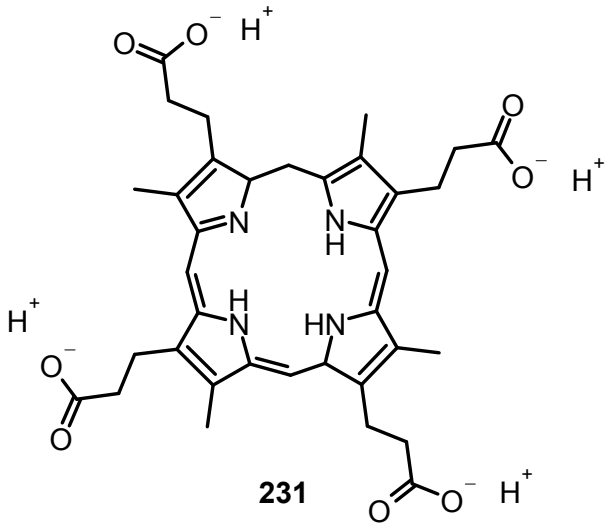


Figure 162: Lasalocid derivatives (**230**) of *Sessler et al.*

In comparison two second-generation sapphyrin ± lasalocid conjugates **230** were reported as carriers for the transport of Phe, Trp, and Tyr. A clear difference was observed between the free acid and the ester of **230**. The former did not affect amino acid transport, which was explained by receptor inactivation by self-assembly. Depending on the chirality of the phenylalanine appendage (**230a** or **230b**) used, either *S*- or *R*-enantiomers of amino acid substrates were transported faster.

Coporphyrin I (CP, **231**) was employed as a host molecule.[514] As a tetraanion it binds electrostatically to the terminal ammonium groups of diammonium cations and interacts simultaneously with the hydrocarbon chain by its hydrophobic π-plane. Aliphatic diamines [$H_2N(CH_2)_nNH_2$, n = 2 - 8] were studied by spectrophotometry, fluorimetry and ^{1}H-NMR spectroscopy in the pH range 7–10 and ionic strengths 0.01 – 0.1 M in water. The dominant factor for binding was assigned to the ion-pair interaction. Diprotonated diammonium cations induced dimerization of CP by forming 1:1 complexes with CP, which undergo much stronger self-aggregation than free CP tetraanions. Increasing numbers of methylene units connecting the ammonium groups, lead to an increase of the binding constants for the complex formation with monomeric CP (K_L), but the dimerization constants of the resulting complexes decreased. Even at I = 0.1 M the association is still fairly strong with log K_{ass} = 3. In the series of $H_3N^+(CH_2)_nNH_3^+$ cations, the log K_{ass} decreases with the increasing length of the guest by 0.1 – 0.3 units per methylene group.



231

Figure 163: The Coporphyrin I tetraanion **(231)**

I. 8.2. Peptidic- and Cyclopeptidic Ammonium Hosts

Cyclic peptides are known to bind and transport metal cations in biological systems.[515] Their ease of synthesis and potential for flexible sequence modification make them good candidates or new ionophores.[516]

Cyclic peptides platform structures with convergently oriented groups for ion recognition were highlighted.[517] A review about peptide cyclisation and cyclopeptides just recently appeared.[518] Many examples for the synthesis of cyclic and bicyclic peptides can be found in the literature.[519] Kubik et al. published a comprehensive review about cyclopeptides as macrocyclic hosts.[520] Several cyclic peptide systems have been synthesized for ammonium complexation. We would like to present some representative, recent examples.

The RGD sequence is a key recognition element found in many proteins that interact with integrins on cell surfaces.[521] The combination of an integrin-binding RGD-cyclopeptide with a hexadecalysine DNA binding domain leads to peptidic minivectors for efficient gene transport.[522] The recognition of the ammonium residues by the DNA is crucial for this process.

The two tetrapeptide sequences Trp-Aib-Gly-Leu-NH-Ar (Aib: α-aminoisobutyric acid, 2-amino-2-methylpropanoic acid, Ar = phenyl or 3,5-dimethylphenyl) bind ammonium ions with their aromatic moieties. The turn structure induced by the amino acid sequence leads to a sandwich complex of the guest between both π-systems as confirmed by 2D-NMR ROESY experiments.[523] The peptide **232** bound several quaternary ammonium salts in $CDCl_3$ with the highest binding constants for benzyltrimethylammonium chloride and *N*-butylpyridinium chloride with an association constant of 580 M^{-1} and 1000 M^{-1}, respectively.

The chiral recognition of guest compounds by the tetrapeptides (X-Trp-Aib-Gly-Leu-NH-Ar) was also observed. The binding constants and the enantioselectivities of *N*-terminal free peptides were larger than those of peptides, which have a benzyloxycarbonyl group at the *N*-terminus.[524]

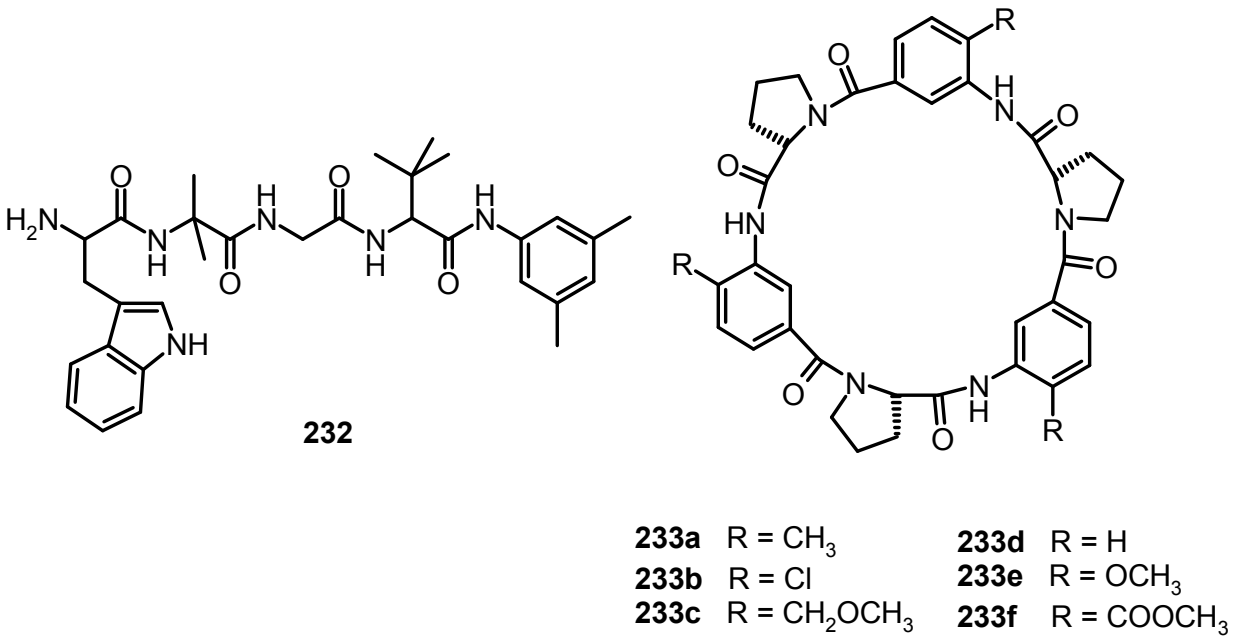


Figure 164: Linear and cyclic peptides for ammonium ion recognition

Kubik et. al constructed a cyclic peptide composed of *S*-proline and three amino benzoic acids in an alternating sequence that was able to bind ammonium ions with stability constants between 11000 and 42000 M^{-1} in chloroform. The series of cyclic hexapeptides contains different 4-substituted 3-aminobenzoic acid units (R = CH_3, Cl, CH_2OCH_3, OCH_3, $COOCH_3$).[525] The authors demonstrated that cyclic peptides **233** bind a variety of ammonium iodide salts with positive cooperativity in $CDCl_3$. The cation complex stabilities depend on the substituents and can cover a wide range from K_{ass} = 140 M^{-1} for R = CH_3 to K_{ass} = 10800 M^{-1} for R = $COOCH_3$ (K_{ass} = 1260 M^{-1} for R = H) with *n*-butyltrimethylammonium picrate for example. The peptide was found to adopt a conformation analogous to the cone conformation of a calixarene. Cations were bound by cation-π interactions, while the iodide counter ion coordinates *via* peptidic NH hydrogen bonds.

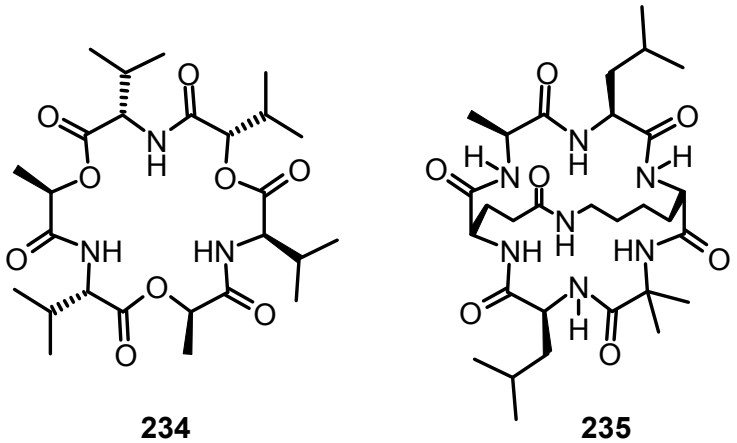
In a second study it was shown, that these cyclic peptides show enantiodiscrimination properties.[526] The best two examples, **233e** and **233f**, distinguish the two enantiomers of *N*,*N*,*N*-trimethyl-1-phenylethyl ammonium picrate in 0.1 % DMSO–$CDCl_3$ with K_R/K_S = 1.5. NMR titrations revealed binding constants (K_{ass}) with the quaternary ammonium ion of 1550 M^{-1} for the *R*- and 1030 M^{-1} for the *S*-enantiomer binding to **233e** or 4550 M^{-1} for the *R*-, and 3050 M^{-1} for the *S*-enantiomer binding to **233f** in 1:1 complex formations.

The corresponding cyclic tetrapeptides composed of alternating *S*-proline and 3-aminobenzoic acid subunits each, possesses a significantly smaller cation affinity than the hexapeptides.[527]

Derivatives with suitable substituents on the aromatic subunits can be used as tweezer-type receptors.

As illustrated by the discussed examples and demonstrated by several further publications,[528] cyclic peptides, depsipeptides and many natural ionophores bind selectively ammonium cations. Therefore such structures can be utilized for the electrochemical analyses of such ions. The group of McGimpsey presented two approaches using cyclopeptides for ammonium ion detection in an ion selective electrode.

A cyclic depsipeptide **234**, consisting of alternating amide and ester groups which is in effect half of the valinomycin structure, was employed as ammonium ionophore. Unlike valinomycin, this depsipeptide is too rigid to fold upon itself and therefore provides a cavity appropriately sized for ammonium ions, but not the octahedral binding geometry required by potassium ions (ionic radii: 1.43 and 1.33 Å, respectively).[529] ISE sensors with this ionophore exhibited similar selectivity for ammonium over potassium and sodium ions compared to nonactin-based sensors (**221**).[75] The ion selectivity follows the order of $NH_4^+ > K^+ > Na^+$, Ca^{2+}, Mg^{2+}, Li^+. The energy minimized structures showed the ammonium cation located within the pocket and able to hydrogen bond with at least five of the carbonyl groups. In contrast, the potassium cation adopts a position that is shifted to one side well above the plane of the disk-like structure of **234** reflecting an unfavourable binding site for potassium.



234 **235**

Figure 165: Cyclic and bicyclic depsipeptides for ammonium ion recognition

These cyclic peptides are still too flexible to bind substrates in a well-defined cavity,[530] leading to lowered selectivity as sensor components. The addition of a second ring yielding bicyclic peptides was thought to increase cation binding selectivity by increasing rigidity.

The bicyclic peptide **235**, cyclo (*S*-Glu1 – *R*-Leu2 –Aib3 – *S*-Lys4 – *R*-Leu5 – *R*-Ala6)-cyclo-(1γ - 4ε) was introduced,[531] to provide an ammonium ion complexation site in a tetrahedral geometry. The bicyclic ammonium ionophore **235** was designed for optimal size-fit/pre-organization, binding geometry and ISE membrane compatibility. A semi-rigid framework with a cavity appropriately sized for ammonium ions (ionic radius 1.43 Å) is necessary to impart high selectivity over interfering cations of other sizes.[74]

The bicyclic molecule provides hydrogen bonding opportunities for the ammonium ion, primarily through the amide carbonyl groups, but also potentially through the amide nitrogen atoms. NMR measurements in $CDCl_3/CD_3OD$ (1:1) indicate that four of the carbonyl groups are oriented towards the internal side of the cavity thus donating electron density upon complexation of ammonium ions. The compound shows higher selectivity for ammonium over potassium and sodium ions as determined by the downfield shifts in carbonyl ^{13}C-NMR signals upon complexation.

I. 8.3. Miscellaneous Concepts

Cyclodextrins were one of the first molecular receptors described to bind organic molecules and are widely used for inclusion of unpolar guests; in some cases they have been used for the recognition of quaternary ammonium ions.[35,532] Just recently an extensive thermodynamic study on the inclusion of quaternary ammonium surfactants appeared.[533]

The formation of inclusion complexes between α-cyclodextrin (**136a**) and the local anesthetic 2-(diethylamino)ethyl-*p*-amino-benzoate (novocaine, **236**) was investigated in aqueous solution using steady-state fluorescence-, UV-vis spectroscopy and electrical conductivity measurements.[534] In addition, both the nitrosation reaction of the primary amine group in a mild acid medium and the hydrolysis of the ester function in an alkaline medium have been studied. The inclusion complex formation between neutral or protonated novocaine and **136a** with a 1:1 stoichiometry was observed. However, the binding constants depend on the nature of guest and host: high affinities with an inclusion constant K_{ass} = 1500 mol^{-1} dm^3 are observed under conditions where the novocaine and the cyclodextrin are neutral molecules.

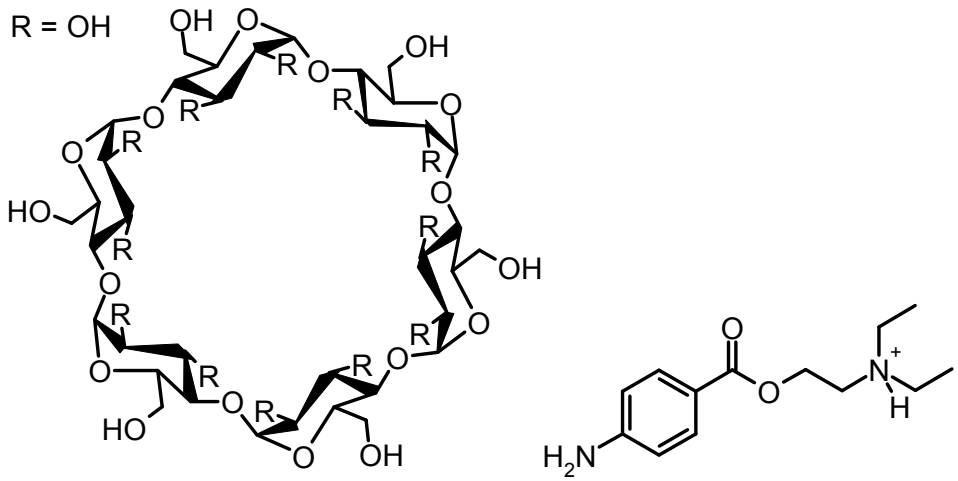
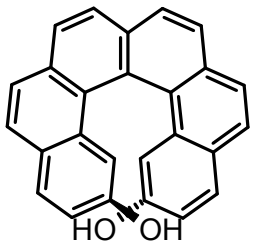


Figure 166: α-Cyclodextrin (**136a**) and novocaine **(236)**

The results obtained in this study evidenced that van der Waals interactions and hydrophobic interactions constitute the major driving forces for cyclodextrin complexation provided that the size and the conformation of the guest are complementary to the host cavity.

A completely different molecule has been shown to interact with various chiral amines and amino alcohols in organic solvents: the fluorescent helical diol **237**, reported by Reetz and Sostmann.[535] The authors suggest that the hydroxy moieties of **237** form hydrogen bonds with the amino group of the analyt, and no proton transfer is involved. Chiral discrimination was detected by differences in the fluorescence quenching observed upon binding to an amine. This chemosensor binds amines with modest stability constants.

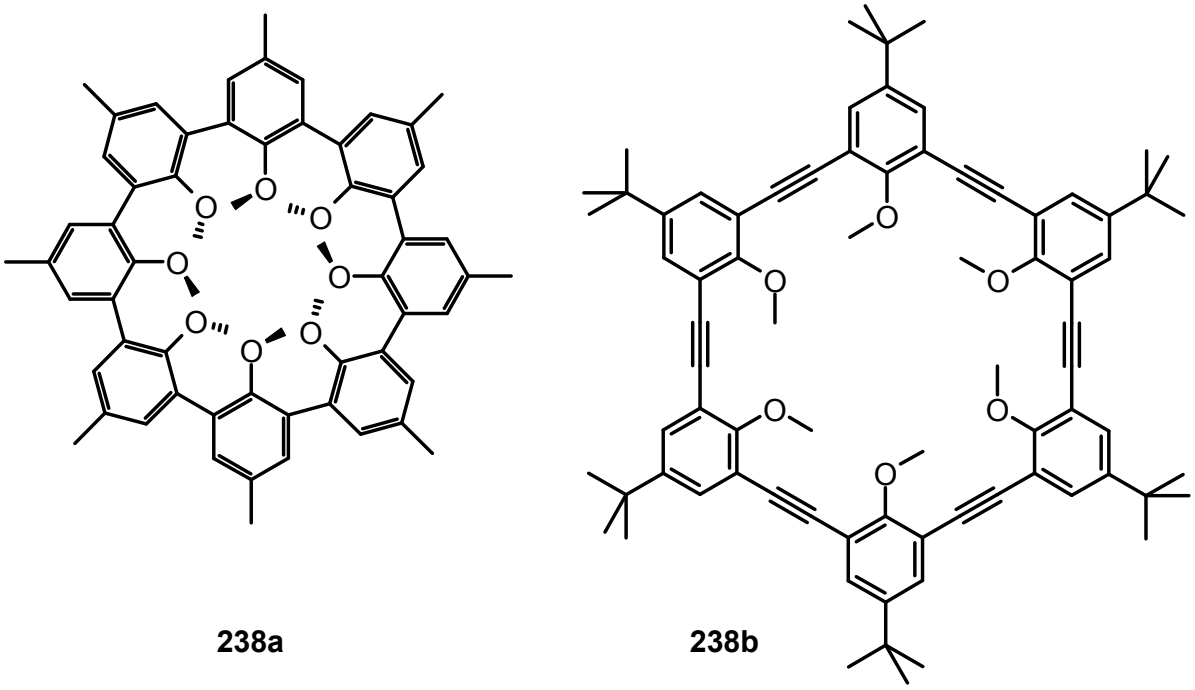


237

Figure 167: Helical diol receptor **237** by Reetz and Sostmann

Oda et al. further developed Cram´s spherands **238a**[13, 536] to a better ammonium binder. They presented a cyclophane (cyclic[6]metaphenylacetylene)[537] with six methoxy groups inside the cavity spacered by acetylene units (**238b**) in a nearly planar carbon framework as observed in

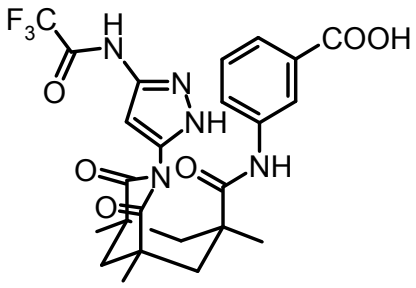
the molecules crystal structure. The six methoxy groups' point up and down alternately. The cavity size is appreciably larger than the size of a caesium ion (3.4 Å). No measurable complexation with alkali metal ions upon solvent extraction experiments (chloroform/aq. picrate salts) was found. Compound **238b** exhibits good ionophoric selectivity for the ammonium ion in spite of its smaller size (2.86 Å) compared with a caesium ion. A plot by Shono's method shows a straight line with a slope of approximately unity suggesting the formation of a 1+1 complex between **238b** and the ammonium ion in solution. The association constant obtained for the ammonium ion (log K_{ass} = 7.84) is smaller than that 18-crown-6 (log K_{ass} = 9.38), but larger than cram´s cavitand **238a** (log K_{ass} = 6.59).



238a **238b**

Figure 168: Ammonium binding spherand by *Cram et al.* **(238a)** and the cyclic[6]metaphenylacetylene **238b** in comparison

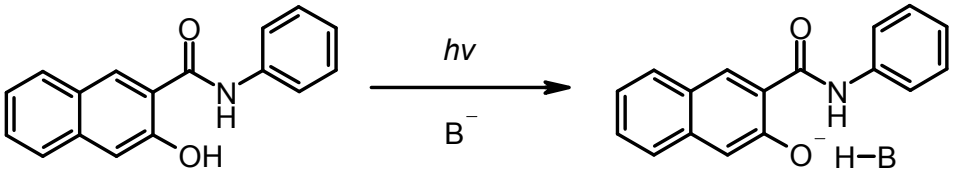
An interesting example was published by Schrader. Based on the Kemp´s triacid they introduced a building block **239** for combined backbone and functional group recognition in peptides.[538] One molecule binds the ammonium ion side chain, as demonstrated with Ac-Orn-Ala-OMe (K_{ass} = 2400 M^{-1}). A control experiment with *n*-propylammonium acetate gave a value of 490 M^{-1} for the salt bridge alone. Ornitihine is bound with a 9:1 selectivity compared to all other amino acids employed in the dipeptides in the study. All binding values were obtained by NMR titrations in chloroform; Job´s plot analyses confirmed a 1:1 stoichiometry.



239

Figure 169: Receptor for peptide backbone and ammonium binding **(239)**

The demethylated naphthol by Lambert et al. is binding by coordination via H-bonds, also over the amide nitrogen.[539] The authors chose a variation of the molecule[540] of Jiang et al., which was able to bind a variety of anions (**240**). This group used the commercially available dye naphthol AS-BI, which was developed for the cytochemical detection of alkaline phosphatase.[541] Aliphatic amines are detected through binding with 7-bromo-3-hydroxy-2-naphth-o-hydroxyanilide and the fluorescence of the resulting complex.



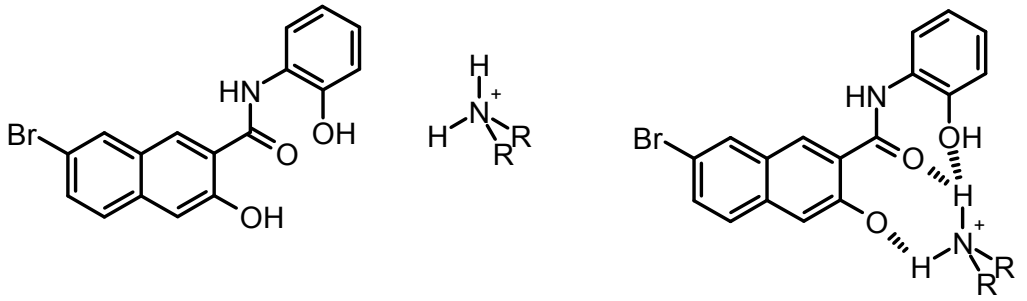
240 **240a**

Figure 170: Anion sensor principle with 3-hydroxy-2-naphthanilide of Jiang et al.

The demethylated derivative 7-bromo-3-hydroxy-2-naphth-*o*-hydroxyanilide (**241,** fig. 171, colourless in the ground state, λ_{max} = 335 nm), emits upon excited-state complexation at 525 nm. Proton transfer is enabled by the enhanced acidity of the naphtholic OH upon photoexcitation. Recognition of the amine by the chemosensor **241** therefore occurs via proton transfer of the naphtholic proton to the amine and is facilitated by the presence of the phenol group. Amine basicity is the primary parameter of the detection, so poorly basic aromatic and conjugated amines such as pyridine and aniline are not detected, but almost all aliphatic amines. Hydrogen bonding within the complex allows further differentiation of aliphatic amines in the following order of binding strength: Diamines > secondary amines > primary amines > tertiary amines > aromatic amines, heterocycles. Table 16 gives an overview on the binding strengths:

Amine	K_{eq} (241) [M^{-1}]	Diamine	K_{eq} (241) [M^{-1}]
1-Propylamine	*80000*	1,2-Diaminoethane, 1,4-Diaminobutane	*160000*
1-Butylamine	*92000*		
Benzylamine	*7000*	1,3-Diaminopropane, piperidine	*180000*
Histamine	*35000*		
Diethylamine, Diisopropylamine	*150000*	1,5-Diaminopentane, 1,7-Diaminoheptane	*290000*
4-(Dimethylamino)-pyridine	*6900*	1,8-Diaminooctane	*310000*
Triethylamine	*28000*		

Table 16: Binding constants for **241** in acetonitrile



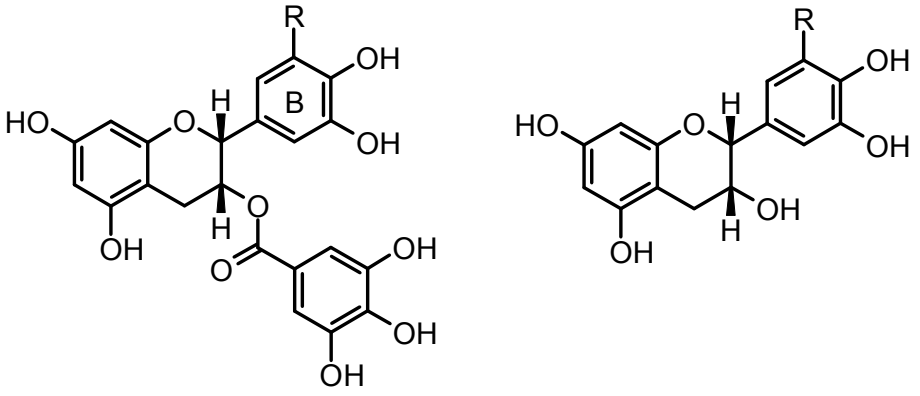
241 **241a**

Figure 171: 7-Bromo-3-hydroxy-2-naphth-*o*-hydroxyanilide (**241**) and its amine binding

Although non-covalent interactions are generally weak compared to covalent bonds, biomolecules achieve strong intermolecular binding forces by using several non-covalent interactions simultaneously. In a similar fashion, naturally occurring gallate-type catechins[542] stabilize complexes with quaternary ammonium ions by using dual non-covalent interactions.[543]

Binding studies between the major catechins of green tea (fig. 172) and tetramethylammonium chloride (TMAC)[212] or benzyltrimethylammonium chloride (BMAC) were carried out by means of standard ^{1}H-NMR titration experiments in acetonitrile-d3/chloroform-d (1:1). The gallate-type catechins (for example **242**) had much higher binding ability (1300 - 2300 M^{-1}) than the non-gallate-type catechins (200 – 400 M^{-1},

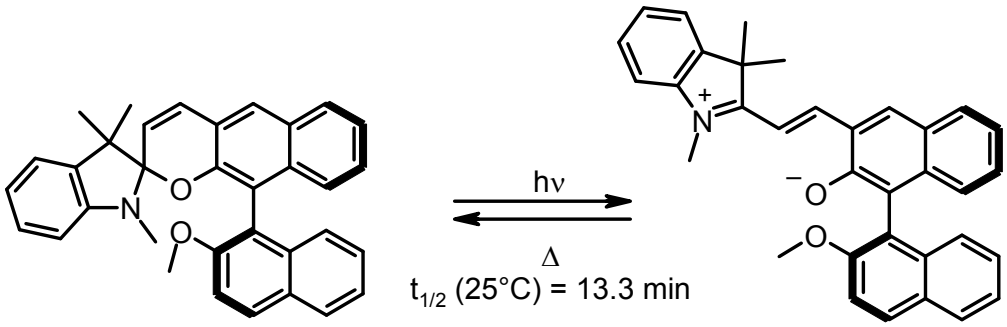
for example **243**). This was attributed to the 'biting effect' by the galloyl group and the B-ring. Compound **242** has the best binding ability of K_{ass} = 2300 M^{-1} towards BMAC.



242 **243**

Figure 172: Naturally occurring catechins with affinity to quaternary ammonium ions

Fuji et al. published a system for optical distinction of enantiomers of amino acids.[544] The authors used the thermo- and photochrome, colourless spiropyrane **244**. By treatment with UV light the coloured merocyanine is formed: The zwitterionic species **244a** binds to amino acids by ionic and hydrogen-bond interactions. This complex formation in turn stabilizes the coloured merocyanine state and so the bleaching observed under dark conditions is slowed down.



244 **244a**

Figure 173: Spiropyrane- (**244**) and merocyanine form (**244a**) of the amino acid receptors of *Fuji et al*.

Due to the binaphthyl system diastereomeric complexes arise with chiral amino acids, which are distinguished by their decolouration rates (table 17). The best stabilization of **244a** was achieved with ammonium acetate (t ½ = 122 min).

Guest	$t_{1/2}$ (*R*, *S*) [min]
None	13.3
Alanine	24.1, 23.4
Valine	32.5, 28.1
Tryptophan	20.2, 17.0
Phenylalanine	30.4, 26.8
Ammonium acetate	122

Table 17: Dependency of the decolouring rate of **244a** in the presence of different *R*- and *S*-amino acids and ammonium acetate

I. 8.4. Recognition by Covalent Bond Formation

The ammonium ion is always in the equilibrium with it´s corresponding amine. Thus, the possibility of nucleophilic attack can be used for recognition, simply binding the guest as imine or aminal. Such concepts are now presented in the last part of this review.

A covalent approach for the detection of ammonium ions was applied by Glass et al. Their coumarin derivative **245** forms iminium salts with ammonium ions (**245a**).[545] The iminium building can be monitored by UV spectroscopy using the caused redshift of the long wavelength absorption band of approximately 440 nm to approximately 480 nm, as well as by a substantial (up to 45-fold) increase in the fluorescence intensity. As the main reason for the spectroscopic changes, the authors considered, the electronic effects caused by the formation of a hydrogen bond between the iminium hydrogen and the lactones carbonyl oxygen. The measurements were conducted under physiological conditions. Similar receptors based on hydrogen bond interaction show usually no affinity under these conditions. So, the equilibrium constants, e.g. for lysine (**81c**) K_{eq} = 6.5 M^{-1} for the retention of amino acids are certainly noteworthy.

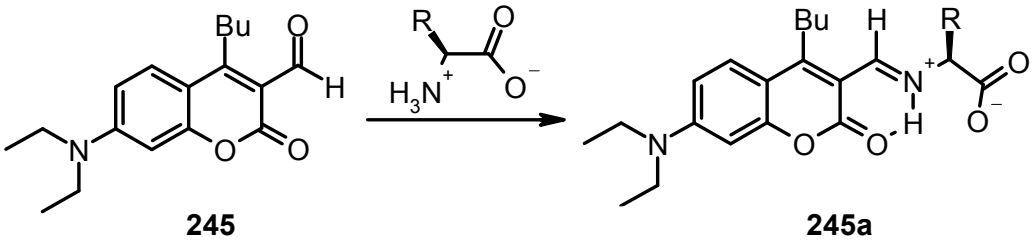


Figure 174: Coumarin aldehyde (**245**) and its iminium species with amino acid bound (**245a**) by *Glass et al*.

Later the group published a dopamine (**2**) receptor based on the same principle: A boronic acid-containing coumarin aldehyde was designed (**246**). [546] The sensor binds to catecholamines such as dopamine (**2**) and norepinephrine by forming an iminium ion with the amine as well as a boronate ester with the catechol. It acts as an effective colourimetric sensor for dopamine (**2**, K_{ass} = 3400 M^{-1}, $\Delta\lambda_{max}$ = 30 nm) and norepinephrine (K_{ass} = 6500 M^{-1}, $\Delta\lambda_{max}$ = 24 nm) with excellent selectively over epinephrine (K_{ass} = 5000 M^{-1}, $\Delta\lambda_{max}$ = 0 nm), amino acids, and glucose (K_{ass} = 5 - 7 M^{-1}). The sensor responds differentially to catechol amines over simple amines, giving a fluorescence decrease in response to catechol-containing compounds (40 – 60 % decrease) and a fluorescence increase with other amines (up to 50-fold for tyramine). The fluorescence quenching effect was found to be directly related to the catechol group. The electron-rich catechol is likely acting as a photoinduced electron transfer (PET) quencher of the coumarin under these conditions.

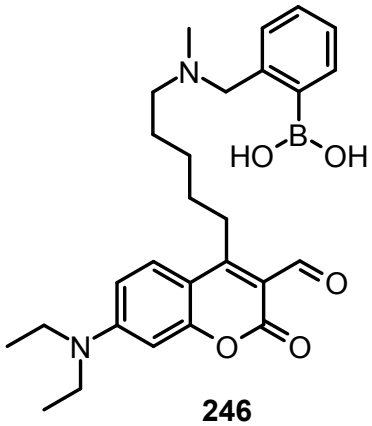
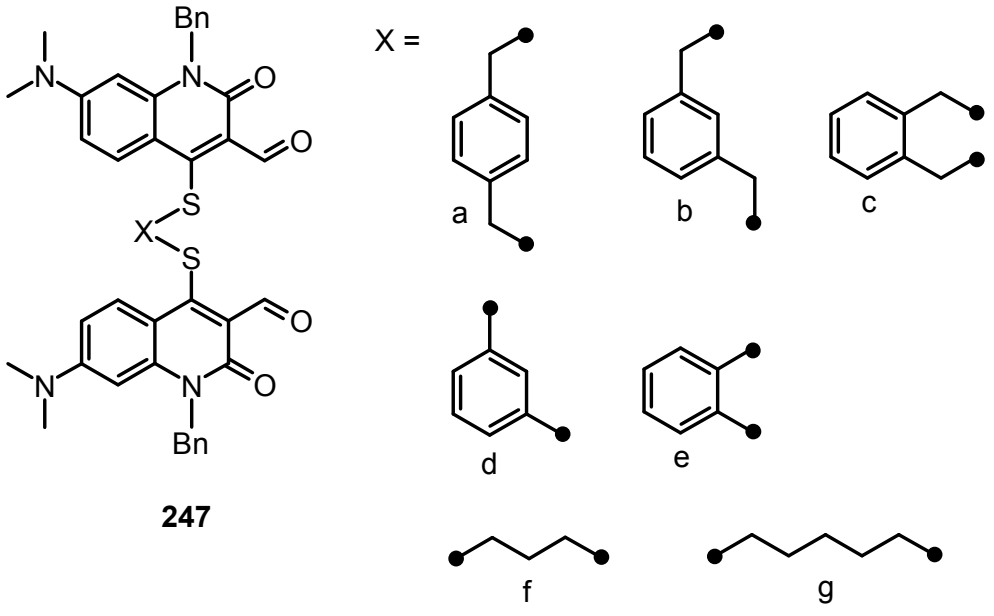


Figure 175: Coumarin aldehyde appended with boronic acid

Other valuable binders for dopamine (**2**) have of course been published: Cyclophanes have been quite useful for selective dopamine recognition,[547] including a recent example that

displays shape-selective recognition with only non-covalent interactions.[548] For more examples the reader is kindly referred to chapter six of this review.

A series of ditopic receptors (**247**) for diamines using dimers of a quinolone aldehyde chromophore was explored by a combination of NMR, absorption and fluorescence spectroscopy.[549] It was shown that the dimeric sensors bound the diamine guests by formation of a bis-iminium ion, which produced large changes in the fluorescence of the quinolone core. Spectroscopic analysis was carried out in a 1:1 methanol–buffer system. The absorption spectra showed trends similar to those observed with the coumarin analogs in which a large red shift in absorption maximum was observed upon addition of diamines to the sensors. Diaminopropane was the best guest for all systems, with the highest binding to **247g** with a binding constant of 6700 M^{-1} being 3- to 4-fold stronger in favour over diamino-butane/pentane and 2.5-fold over ornithine/lysine (**81c**) with a maximum fluorescence increase at saturation (I_{sat}/I_0) of 6.6-fold. It bound lysine (**81c**) with 2800 M^{-1} and a fluorescence increase of 30-fold. The second best binder was **247d**. A shift in absorbance up to 28 nm was observed, consistent with a shift from aldehyde to iminium ion forms. The red shift in absorption has been attributed to the hydrogen bond between the formed iminium ion and the carbonyl group of the chromophore. In fluorescence mode, by exciting the chromophore at 495 nm, a large increase in fluorescence was observed upon titration with the diamine: Up to 160-fold better binding for diamines compared to butylamine. The mode of binding and the 1:1 stoichiometry were confirmed by NMR experiments in chloroform.



247

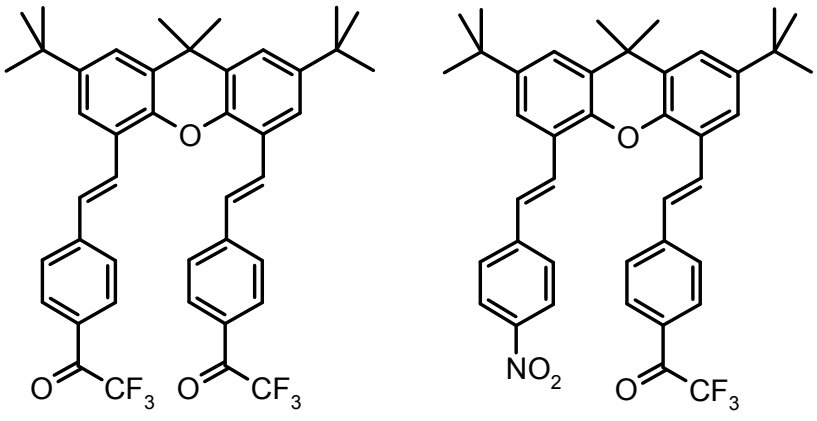
Figure 176: Quinolone aldehyde dimers by *Glass et al*.

Reversible covalent binding of an amino, e.g. forming a hemiaminal, has been realized in two chemosensor dyes with either one or two trifluoroacetophenone recognition moieties (fig. 177). As amines 1-propylamine, diethylamine, triethylamine, and aliphatic diamines of different chain length were used.[550] Their conversion into a hemiaminal or a zwitterion leads to a change in the electron delocalisation within the dye molecule and subsequently to a shift in absorbance to shorter wavelengths. Comparing the interaction of **248a** and **248b** with amines in homogenous solution it was found, that for their reaction with diamines the K_{eq} values are significantly increased. The highest values were observed for 1,2-diaminoethane and the lowest for 1,4-diaminobutane. Table 18 compares the results:

amine	K_{eq} (249a) [M^{-1}]	K_{eq} (249b) [M^{-1}]
1-propylamine	195	210
1,2-diaminoethane	30000	5000
1,3-diaminopropane	26000	3500
1,4-diaminobutane	13000	700

Table 18: Binding constants of amines to compounds **248a** and **248b** in ethyl acetate

The response and sensitivity towards monoamines was comparable, because only one functional group in **248a** can react with amines. The dyes embedded in thin layers of plasticized PVC (fig. 178) showed clear changes in absorbance on exposure to aliphatic amines.



248a **248b**

Figure 177: Chromogenic ammonium ion receptors with trifluoroacetophenone recognition motifs

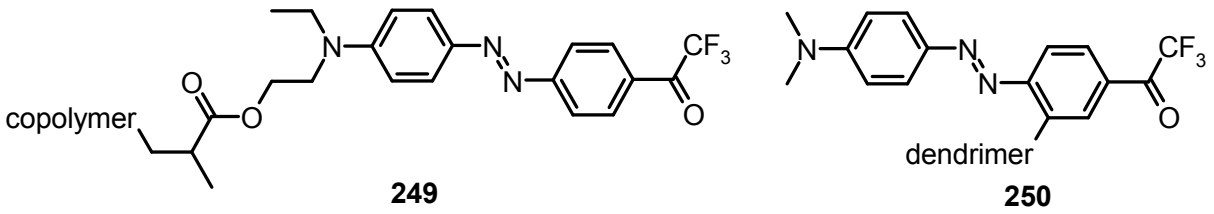


Figure 178: Chromogenic ammonium ion receptor with trifluoroacetophenone recognition motif bound on different matrices

Similarly, the chromogenic functional dye **249** shows a significant colour change in the presence of amines in organic solvents with high sensitivity.[551] The cross-linked polymer sensor membranes allow a fast and reversible chemical reaction with solutions of primary aliphatic amines in most organic solvents. The equilibrium constants varied, depending on the solvent and analyt molecule, the sensor layers typically exhibited equilibrium constants of 100 M^{-1} for *n*-butylamine in chloroform, 1300 M^{-1} for 1,4-diaminobutane and 20,000 M^{-1} for tris-(2-aminoethyl)amine in toluene. A change in selectivity due to the size or polarity of the analyt could not be observed. The reaction rate of the membranes with secondary and tertiary amines as well as with alcohols is slower than the rate with primary aliphatic amines, which gave the opportunity to distinguish ammonium guests by structure.

Similarly, Zimmerman et al. have prepared receptors for diamines by incorporating trifluoromethyl ketones into a dendrimer (**250**) with success.[552] Such receptors showed for example selectivity for α,ω-diamines (H_3N^+-$(CH_2)_n$-NH_3^+) versus aromatic and cycloaliphatic diamine, amines, amino alcohols and diols. Complexation studies in THF by visible spectroscopy and NMR afforded an apparent association constant (K_{ass}) of $2.7*10^4$ M^{-1} for n = 3 that was ca. 200-fold higher than that for *n*-butylamine (140 M^{-1}). The association constant for n = 4 was even 10 – 20 % higher. Longer and shorter diamines bound less strongly.[553]

I. 9. Conclusions

We have presented various approaches for the detection and binding of ammonium ions and amino acids spanning from metal-complexing agents or reactive molecules via different inclusion compounds to weakly coordinating systems, such as crown ethers. A large number of molecular receptors of varying sizes, shapes and functionalities have been discussed in their interaction with the guests.

The synthetic hosts require complementarity to the ammonium guests in size, shape, and molecular interactions.[554] Typical interactions observed in the complexes of primary and secondary ammonium cations are ionic and dipolar interactions, dispersive forces like van der Waals or hydrogen bonds. Cation-π- and ionic-interactions, often assisted by the hydrophobic effect and dispersive forces determine the binding of quaternary ammonium ions.

Binding an organic ammonium ion in solution three considerations have to be taken into account:

An organic ammonium ion exists of course never as sole cation, an anion is always associated with it. Depending on the polarity and hydrogen donor/acceptor abilities of the solvent, the association is differently strong.[555] The strength of the electrostatic interaction in solution, despite the solvation[556] of host and guest, influences the binding to an artificial receptor. Strongly coordinating counterions like chloride generally lead to weaker binding constants upon recognition of the associated cation as if large, soft and weakly coordinating counterions like iodide (tetrafluoroborate, hexafluorophosphate or perchlorate) are employed.[557]

The binding of primary, secondary and tertiary ammonium ions to the most receptor structures relies on H-bonding to a large extent. The complex stability depends on the number of H-bonds possible between host and guest,[558] but also on the acidity of the ammonium ion. The more acidic an ammonium ion actually is, the stronger are the H-bonds with a particular donor site. For instance, primary, secondary and tertiary ammonium ions possess a pK_b-value between three and four and therefore stabilize a complex to a larger extent, then an anilinium ion with a pK_b-value of nine to ten.

The third fact of importance is the steric bulk present in the guest (and the host). The better an ammonium ion can be placed in the recognition motif and the less interference is present in the complex, the stronger the association (assuming no additional coordination of the substituents can take place).

Crown ethers are one of the most versatile classes of synthetic receptors for the recognition of ammonium ions. Crown ethers recognize ammonium-ions typically by hydrogen-bond interactions. Therefore only ammonium ions of primary and secondary amines are typical guests and quaternary ammonium ions are not bound. The crown-ether ammonium ion recognition motif has been extended to multitopic receptors allowing an analytical discrimination of diamines of different length and combined with anion recognition for the binding of amino acids. Many examples for transport and effective enantioselective recognition of amino acids, as esters or in zwitterionic form have been described. Crown ether amino acid building blocks for synthetic receptor were developed by Voyer[127] (fig. 29) and König[132,133] (fig. 31/32). Such systems allow the easy assembly of larger structures like membrane channel mimics, which are of fundamental interest for medicine and biochemistry.[559,560]

Substituted calixarenes can bind primary and secondary ammonium ions by ion-ion-, ion-dipole- and H-bond-interactions, and quaternary ammonium guests by ion-ion-, cation-π- and hydrophobic interactions. The molecular geometry of calixarenes is adjustable via their conformation, allowing a fine tuning of their selectivity for shape and size of the guest. This is not possible to the same extend by crown ethers. In addition, calixarenes often achieve binding selectivities exceeding to ones achieved with crown ethers due to the guest inclusion being controlled by steric reasons and various interactive forces of host and guest. Therefore, they can show remarkable selectivities in the discrimination of ammonium ion isomers. Especially noteworthy is their ability to complex strongly with quaternary ammonium ions.

Molecular tweezers and clips (fig. 109) serve as selective receptors for electron-deficient aromatic and aliphatic substrates. Cavity or cleft affect the thermodynamic stability and the binding kinetics; addition of side arms may enhance lipophilicity (long alkyl chains) or encourage interaction with some external entity, which makes these systems especially interesting for ammonium binding. Assisted by the hydrophobic effect of the cavity, van der Waals interactions and substantial electrostatic contributions for locking of the guest are responsible for the observed high efficiency and specificity found in clefts and cavitands. Water-soluble clips form stable complexes with *N*-alkylpyridinium, phenethylammonium ions, catechols and basic amino acids, which are often more stable in aqueous solution than in methanol due to a positive contribution of the hydrophobic effect to the receptor-substrate binding processes. C_{3v}-symmetric tripods, tweezer ligands and pre-organized molecular

clefts reach ammonium ion binding selectivities that compete with naturally occurring recognition motifs like nonactin or valinomycin.[433]
Cucurbiturils often reveal remarkably high affinity for alkanediammonium ions, size, shape, and functional group selectivity as a consequence of ion-dipole and hydrophobic interactions reaching the highest binding constants of all presented receptor families in aqueous media (up to 10^{10} to 10^{12} M^{-1}). Generally ammonium guests are coordinated by the carbonyl groups of the moieties by electrostatic ion-dipole attraction assisted by hydrogen bonding. The unpolar part of the guest is included in the cavity. The binding is governed by hydrophobic effects and van der Waals contacts. The entropic gain upon binding additionally supports the high association constants found with cucurbiturils. Together with cyclodextrins a wide range of host cavities for ammonium ions with different shape, solubility, and chemical functionality is available.

Lewis-acidic metal centres in combination with carboxylate, trimethylammonium or H-bond donors bind guests with a high degree of selectivity and affinity. Amines and amino acids are preferred guests. Ionic interactions in combination with hydrogen bonds and the hydrophobic effect are the main stabilizing contributions for their complex stabilisation. The strong coordination of the metal centre allows guest binding even in competitive media like water.
Especially porphyrins provide a useful framework for artificial receptors. The conjugated system facilitates the detection of interactions by UV-vis, fluorescence or circular dichroism measurements. It also provides a planar structure for the design of well-defined binding pockets with recognition groups attached in several distinct positions. The types of interactions utilized in these receptors include hydrophobic interaction, hydrogen bonding and in most cases coordinative bonds, taking advantage of the Lewis acidity of a metal, typically zinc.[561] Dimer structures based on metal-porphyrins allow for the enantiodiscrimination of diamines, amino acids, peptides and amino alcohols.

The rules how synthetic receptors interact with ammonium ion guests become clearer, which paves the way for a rational design of biomimetic devices, non-covalent synthesis and responsive host-guest systems. The study of synthetic ammonium ion receptors has certainly contributed to a better understanding of intermolecular interactions in various fields including drug design, DNA processing, enzyme interactions or approaches for the inhibition of protein–protein interactions.[562] Applications of ammonium ion recognition may be envisaged

in many areas: Drug design, photo switching, separation, or motion and transport,[563] self-assembly in solution, and in the solid state.

I. 10. References and Notes

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II. Modular Synthesis of Di- and Tripeptides of Luminescent Crown Ether Amino Acids[i]

In this chapter we deal with the fundamental properties of the crown ether amino acids (CEAAs) and describe a general synthesis for these moieties. Different examples are prepared, of which we make use for preparation of the receptors in most of the later chapters.

The luminescent benzo crown ether aminocarboxylic acids with ammonium ion affinity were converted into linear bis- and tris-benzo crown ether amides using standard peptide coupling protocols. The affinities of the new crown ethers to ammonium ions and di- and tetrapeptides bearing ammonium ion moieties were determined by emission titration in methanol and buffered water.

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II. 1. Introduction

Many biologically important molecules have ammonium ions as functional groups. Typical examples are neurotransmitters,[1,2] growth factors,[3] histamine[2,4] or peptides.[5] The development of synthetic receptors that allow the specific recognition of organic ammonium ions is therefore an area of ongoing interest and results have potential applications in medicinal diagnostics or chemosensors. Typical artificial binding sites for ammonium ions are crown ethers,[6] calixarenes,[7] phosphonate-[8] or oxazoline-based[9] receptors and different metal complexes; most examples have been reported for crown ether systems.[10] However, for many cases the binding strengths of a single ammonium ion – receptor site interactions is too weak in an aqueous environment to give stable and defined aggregates. The combination of several binding sites in one synthetic receptor can improve the situation, if their individual contributions are additive or even show positive cooperativity.

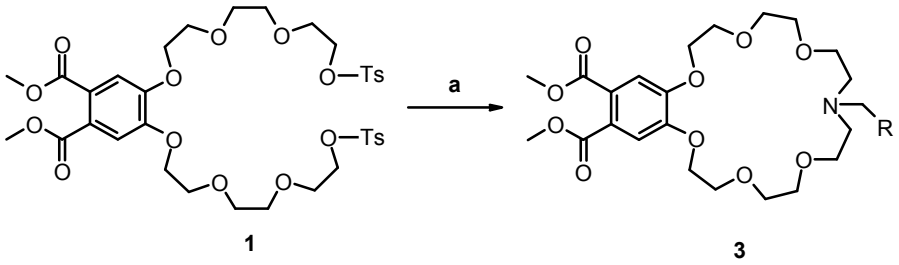
Several examples of oligovalent artificial cation receptors based on crown ethers have been reported: Bis- and tris-crown ethers as host compounds for alkaline metal ions have been published by Huang,[11] and a carrier molecule composed of three crown ethers was used to mediate the transport of mercury ions through a chloroform phase.[12] Nolte[13] polymerized an isocyanide derivative of benzo-18-crown-6 and applied the polymer to liposome mediated copper-ion transport. Oligo- or poly[14] crown ethers have been used as chemical model systems for ion channels. A poly(crown) channel was constructed using the benzo-21-crown-7 derivative of phenylalanine and leucine.[15] Incorporation of the resulting helical peptide into a lipid bilayer leads to proton and sodium ion transport through a columnar crown ether stack. Gokel[16] developed a family of tris-macrocycles that permits the transport of sodium ions through membranes, using crown ethers, connected by hydrophobic alkane spacers, as channel head groups and cation entry portals.

For ammonium ion binding, only ditopic receptors have been reported so far. Phenolphtalein bis-crown ethers have been used for the binding of diamines and dipeptides with a *C*-terminal lysine.[17] Two crown ether amino acids, which were incorporated into an oligo alanine peptide were used by Voyer[18] to bind α,ω-diammonium alkanes. We have recently reported the synthesis and dimerization of ammonium ion binding phthalimide crown ethers.[19] We now extend the approach and present here a modular synthetic route to functionalized, luminescent bis- and tris-crown ethers with ammonium ion affinity.

II. 2. Results and Discussion

II. 2.1. Syntheses of Building Blocks and Receptors

The twofold ring closing substitution reaction of di-tosylate **1**[19] with primary amines **2** under basic conditions gave crown ether amino acids **3** in good yields (Scheme 1, Table 1). The presence of potassium ions favours the ring closing reaction by its template effect. No by-products from intermolecular reactions were observed under the experimental conditions (c = $6*10^{-2}$ mol/L). Alkyne or azide functional groups (entries **g, h**) are tolerated and the resulting products are suitable substrates for Huisgen cycloaddition reactions. The reaction tolerates Cbz- or Boc-protected amines (entries **a-d**).

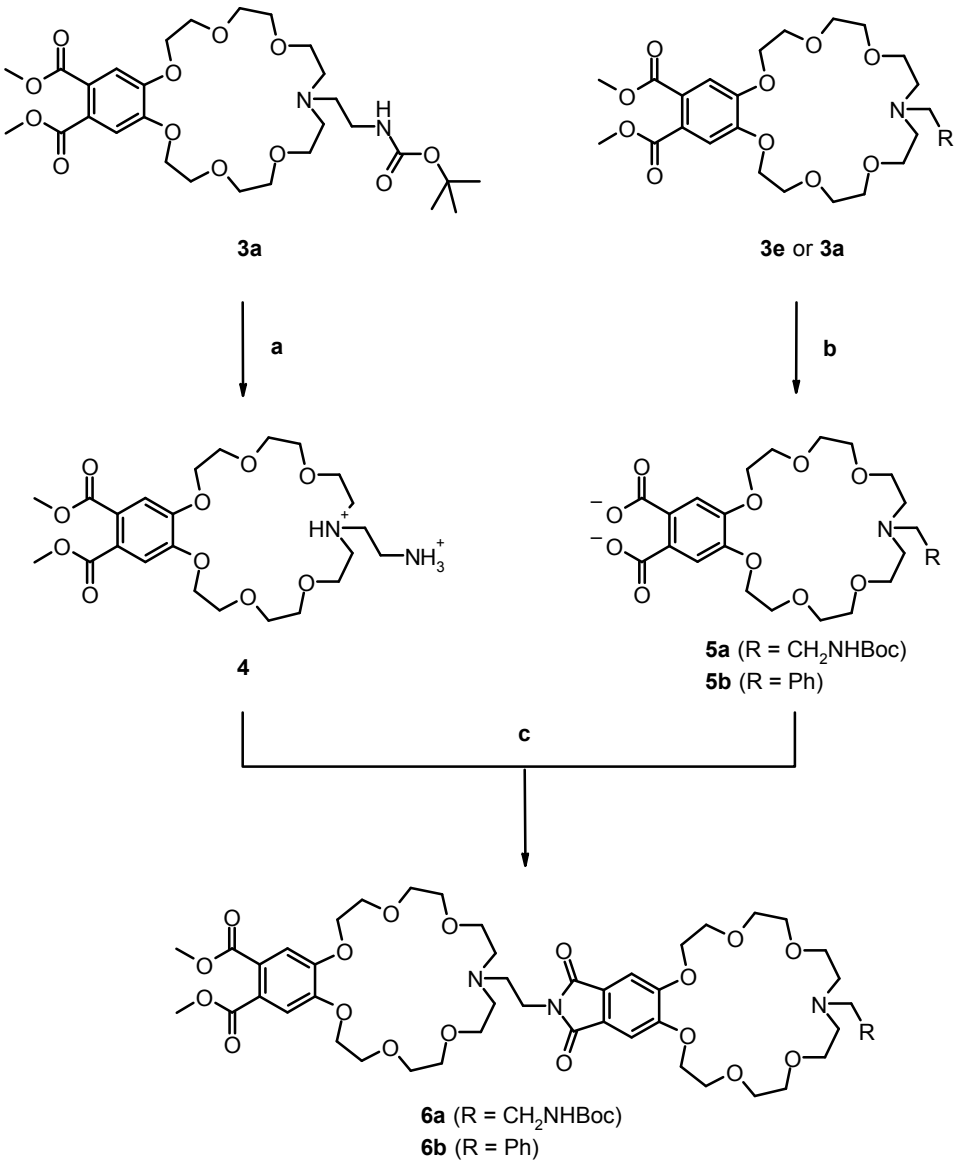


Scheme 1: Synthesis of a luminescent benzo azacrown ethers **3**; *conditions: a) H_2NCH_2R (**2**, see table 1), K_2CO_3, KI, MeCN, H_2O, 80 °C.*

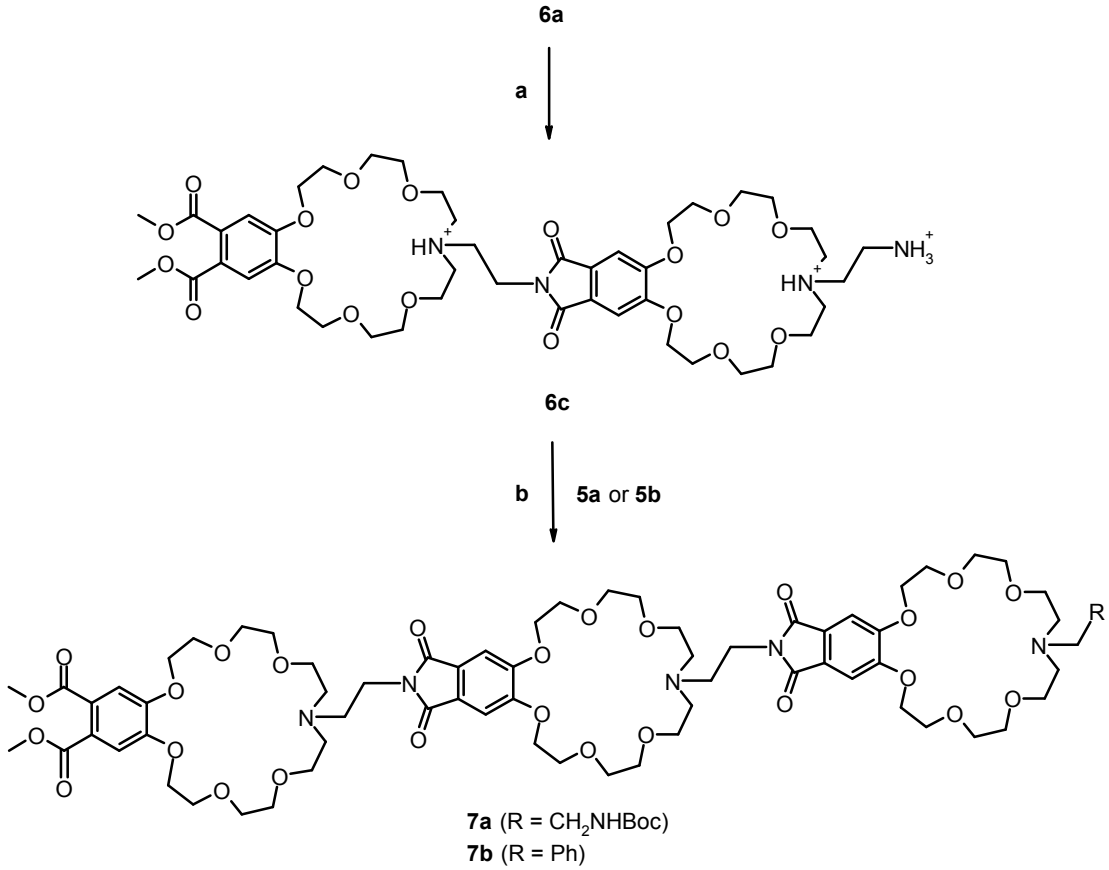
Entry	R	Yield of 3 [%]
a[19]	NHBoc	79
b	NHCbz	68
c	NHBoc	72
d	NHBoc	63
e		86
f	NHBoc	76
g[a]	H	71
h[a]	N_3	69
i	O O	78

Table 1: Synthesis of compounds **3a-i**; [a] In the dark

The Boc-deprotection of compound **3a** and the ester hydrolysis of compound **3a** or **3e** allows the preparation of crown ether amino acid dipeptides **6**[20] in good yield using standard peptide coupling conditions (Scheme 2). A further extension to compounds **7**, bearing three crown ether units, is possible by removal of the Boc-protecting group of **6a** yielding amine **6c,** and coupling with **5a** or **5e**, again, using standard peptide coupling conditions (Scheme 3). The purification of tripeptides **7** requires repeated column chromatography to remove by-products and reagents.



Scheme 2: Preparation of crown ether amino acid dipeptides **6a** and **6b**; *conditions a) HCl in Et_2O, DCM, 95 %, b) NaOH 1M, MeOH, H_2O, 40 °C, quant., c) EDC, HOBt, DIPEA, DMF, $CHCl_3$, 70 °C, 79 % (**6a**);*[19] *76 % (**6b**); counterions are omitted for clarity.*



Scheme 3: Synthesis of crown ether amino acid tripeptides **7a** and **7b**; *conditions: a) HCl in Et_2O, DCM, 92 %, b) EDC, HOBt, DIPEA, DMF, $CHCl_3$, 70 °C, 35 % (**7a**); 37 % (**7b**); counterions are omitted for clarity.*

II. 2.2. Binding Studies

II. 2.2.1. Photophysical Properties

Compounds **3** show absorption maxima in methanol at 220 nm and 270 nm, and emit upon excitation at 390 nm with a quantum yield of about $\phi = 0.1$.[21] The absorption and emission properties are only marginally affected by the nature of the substituent R. Upon addition of KSCN or *n*-butylammonium chloride the emission intensity increases significantly and binding affinities were derived from emission titration experiments. Table 2 summarizes the results. The affinity for potassium ions is typically one order of magnitude larger than the ammonium ion binding, which is in accordance with earlier reports.[19,22] The cation affinity for all crown ethers **3** is very similar in methanol solution. In buffered aqueous solution (50 mM

HEPES, pH 7.5; c = $2*10^{-5}$ mol/L) no change in the emission intensity was observed, even if a large excess of KSCN or *n*-butylammonium chloride was used.

Compound	*n*-Butylammonium chloride		KSCN	
	log *K*	I_∞/I_0	log *K*	I_∞/I_0
3a	2.8 ± 0.3	1.7	3.9 ± 0.4	3.5
3b	2.8 ± 0.2	1.7	3.8 ± 0.3	3.5
3e	2.8 ± 0.1	1.9	3.9 ± 0.3	3.6
3d	2.7 ± 0.2	1.8	3.8 ± 0.4	3.4
3f	2.7 ± 0.3	1.7	3.6 ± 0.3	3.4
3g	2.6 ± 0.2	1.6	---	---
3i	3.2 ± 0.3	2.1	4.0 ± 0.4	3.7

Table 2: Binding affinities and emission intensity changes of compounds **3** and *n*-butylammonium chloride or KSCN in methanol

Upon the addition of the second crown ether moiety, as in compound **6**, the phthalic amide fluorophore is created and a new absorption band at 247 nm appears (figure 1). The tris-crown ether compounds **7** bear two phthalimide chromophores, which results in a significant increase of the UV absorption intensity at 247 nm if compared to **6**. Excitation of the fluorophores at 309 nm stimulates emission at 386 nm with a shoulder at 488 nm.

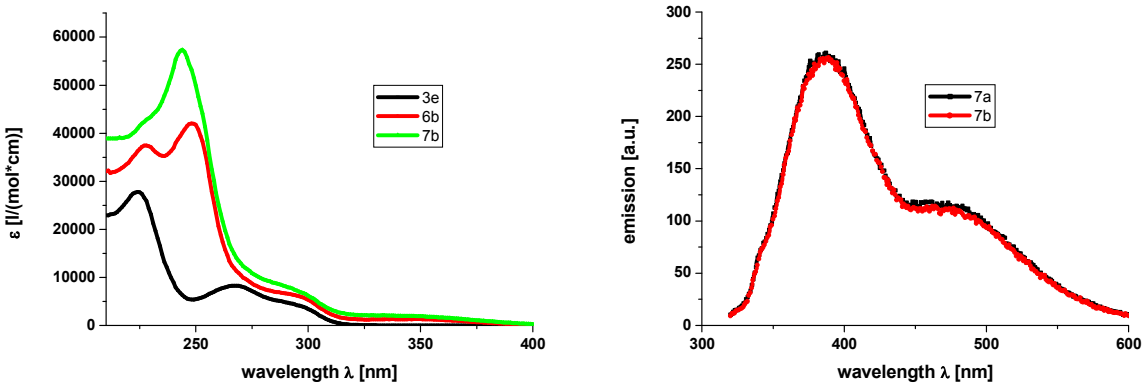


Figure 1: Absorption spectra of compounds **3e**, **6b** and **7b** (in methanol, left) and emission spectra of compounds **7a** and **7b** (methanol, c = $2*10^{-5}$ mol/L, excitation at 309 nm, right)

II. 2.2.2. Evaluation of the Binding Strength

The binding properties of bis-crown ether **6b** to *n*-butylammonium chloride and lysine methyl ester hydrochloride were evaluated by fluorescence titration in methanol and buffered aqueous solution (50 mM HEPES, pH 7.5). The different optical properties of the phthalic ester and the phthalimide moiety of **6b** allow the individual monitoring of binding to the two crown ether cation binding sites. Ammonium ions bind to both crown ethers in methanol with a stoichiometry of 1:1 and similar affinity in the order of log K = 2. In aqueous solution no interaction between **6b** and ammonium ions can be detected. The affinity of lysine methylester hydrochloride reaches log K ~ 4 in methanol, due to the simultaneous interaction of both ammonium ions with the bis-crown compound. In buffered aqueous solution the interaction of Lys-HCl – **6b** is detectable via the phthalic ester emission change, but drops to log K < 2. The emission of the phthalimide moiety is quenched in aqueous solution.

Solvent	**Guest**	**Phthalic ester moiety of 6b**		**Phthalimide moiety of 6b**	
		log *K*	**I_∞/I_0**	**log *K***	**I_∞/I_0**
Methanol	*n*-Butyl-ammonium chloride	2.2 ± 0.4	1.3	2.6 ± 0.3	4.7
	Lysine methylester hydrochloride	4.3 ± 0.3	1.6	4.6 ± 0.2	8.1
H_2O, HEPES (50 mM, pH 7.5, 2 % MeOH)	Lysine methylester hydrochloride	1.8 ± 0.2	1.7	---	---

Table 3: Binding affinity and emission enhancement of compound **6b** in the presence of *n*-butylammonium chloride and lysine methyl ester hydrochloride in methanol and buffered aqueous solution

Next, the trimeric crown ethers **7** were investigated for ammonium ion binding. As expected, the affinity for ammonium ions in methanol solution of the crown ether moieties remain unchanged if compared to **6**. Lysyllysine methyl ester trihydrochloride was chosen as a guest with three ammonium groups to determine the binding to compound **7**. Although the aggregate of **7** and lysyllysine is potentially stabilized by three simultaneous ammonium ions

– crown ether interactions, the determined affinity constants in methanol and buffered water only slightly exceed the values for the interaction of bis-crown ether **6** with a diammonium compound. The differences of lysyllysine binding affinities of **7a** and **7b** are within the error margins of the measurements. For both interactions a stoichiometry of 1:1 is indicated by the Job´s plot analysis.

Solvent	Compound	Guest	Phthalic ester		Phthalimide	
			log *K*	I_∞/I_0	Log *K*	I_∞/I_0
Methanol	**7a**	*n*-Butylammonium chloride	2.2 ± 0.4	1.2	2.1 ± 0.2	4.5
Methanol	**7a**	Lysyllysine methylester hydrochloride	4.6 ± 0.5	2.6	4.4 ± 0.1	6.8
H_2O, HEPES (50 mM, pH 7.5, 2 % MeOH)	**7a**	Lysyllysine methylester hydrochloride	2.6 ± 0.3	1.4	---	---
Methanol	**7b**	*n*-Butylammonium chloride	2.0 ± 0.3	1.3	1.9 ± 0.2	4.6
Methanol	**7b**	Lysyllysine methylester hydrochloride	4.2 ± 0.3	2.0	4.3 ± 0.1	6.8
H_2O, HEPES (50 mM, pH 7.5, 2 % MeOH)	**7b**	Lysyllysine methylester hydrochloride	2.8 ± 0.3	1.4	---	---

Table 4: Affinity constants and emission enhancement of compounds **7** in the presence of *n*-butylammonium chloride and lysyllysine methylester in protic solvents

Although the extension of the ammonium ion receptor by one crown ether from **6** to **7** had little impact on the ammonium binding affinity, the additional chromophore leads to a stronger emission in the presence of ammonium ions, which becomes visible by the naked eye.

II. 2.2.3. Binding Studies with Isomeric Tetrapeptides – Sequence Selectivity

The ability of compounds **7** to bind ammonium ions within small peptides was investigated using isomeric tetrapeptides bearing two lysines (K) and two glycines (G) in their sequence. H-K-K-G-G-NH_2 carrying two lysines at the *N*-terminus has the same ammonium ion pattern as H-K-K-OMe. In H-K-G-K-G-NH_2 one glycine and in H-K-G-G-K-NH_2 two glycines separate the lysines. All peptides were prepared on Rink amide resin (see experimental part) and their binding affinities as trihydrotriflates to compounds **7** were determined by titration in methanol and buffered water (see experimental part). The derived affinities are, within the errors of the experiments, identical to the values obtained for H-K-K-OMe.

Guest	Compound	Phthalic ester		Phthalimide	
		log *K*	I_∞/I_0	log *K*	I_∞/I_0
H-K-K-G-G-NH_2	**7a**	4.8 ± 0.2	2.2	4.6 ± 0.2	5.4
H-K-G-K-G-NH_2	**7a**	4.6 ± 0.5	1.8	4.6 ± 0.2	5.3
H-K-G-G-K-NH_2	**7a**	4.4 ± 0.4	2.0	4.5 ± 0.2	7.1
H-K-K-G-G-NH_2	**7b**	4.6 ± 0.5	1.9	4.5 ± 0.5	3.8
H-K-G-K-G-NH_2	**7b**	4.7 ± 0.3	2.1	4.6 ± 0.2	7.4
H-K-G-G-K-NH_2	**7b**	4.6 ± 0.4	2.0	4.6 ± 0.2	6.9

Table 5: Affinity constants and emission enhancement of compounds **7a** and **7b** in the presence of Lys-tetrapeptides in methanol

Guest	Phthalic ester part of 7a		Phthalic ester part of 7b	
	log *K*	I_∞/I_0	log *K*	I_∞/I_0
H-K-K-G-G-NH_2	2.4 ± 0.2	1.6	2.7 ± 0.3	1.6
H-K-G-K-G-NH_2	2.8 ± 0.4	2.0	2.9 ± 0.4	1.9
H-K-G-G-K-NH_2	2.7 ± 0.2	1.9	2.7 ± 0.3	1.7

Table 6: Affinity constants and emission enhancement of compound **7a** and **7b** versus sequential different Lys-tetrapeptides in HEPES buffered aqueous media.

II. 3. Summary and Conclusions

In summary, luminescent aza benzo crown ethers and crown ether amino acids are available by an efficient ring closing reaction of bis-tosylates and amines. Using standard peptide coupling procedures, linear bis- and tris-crown ether peptides were obtained. Emission titrations in methanol or buffered aqueous solution revealed affinities of ammonium ions to the benzo crown ether moieties of approx. log K = 2 in methanol, but no affinity in buffered water. The interaction of bis-crown ethers **6** and tris-crown ethers **7** with bis- or tris-ammonium ions, respectively, is of similar strengths in methanol (log K ~ 4.5) and in buffered water (log K ~ 2.5). This shows that the extension of bis- to tris-crown ethers does not lead to a significant increase of binding affinities. The flexible structure of the extended crown ethers and their peptidic guest molecules is a likely rational for the observation: The limited preorganisation of the extended receptors binding sites prohibits an additive or cooperative action of the intermolecular interactions, and illustrates the importance of well balanced entropy and enthalpy contributions in the design of synthetic receptors.

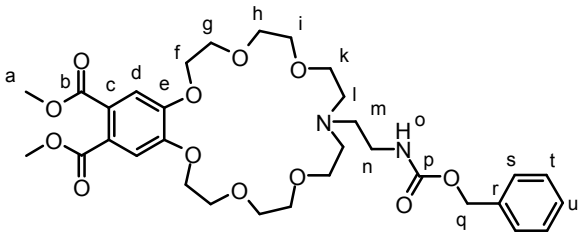
II. 4. Experimental

Compounds **1**[19], **3a**[19], **4**[19], **6a**[19] **and H-Lys-Lys-OMe**[23] were synthesized in solution according to published procedures.

II. 4.1. Synthesis of Crown Ether Amino Acids (CEAAs)

General method for the preparation of aza-benzo-21-crown-7-ethers: Compound **1** (2.00 g, 2.5 mmol)[19] was dissolved in 40 mL of acetonitrile and 0.3 mL of H_2O. The corresponding amine or mono-Boc-protected diamine (2.5 mmol), 610 mg KI (3.6 mmol) and 3.45 g K_2CO_3 (25 mmol) were added successively and the mixture was refluxed overnight. After cooling to room temperature, the mixture was filtered over celite and the solid residue washed with acetonitrile and dichloromethane. The solvent was evaporated and the crude product was purified by column chromatography. If not stated differently, silica gel and ethyl acetate / ethanol 3:1 as eluent was used.

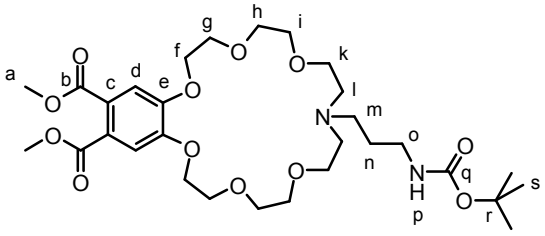
14-(2-Phenyloxycarbonylamino-ethyl)-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosen-2,3-dicarbonic acid dimethyl ester ***(3b)***



2-Phenyloxycarbonylamino-ethylamine (240 mg) was used. Column chromatography was performed on silica gel with ethyl acetate / ethanol 3:1. (R_f = 0.17, ethyl acetate / ethanol 3:1). A yellowish solid was obtained (1.10 g, 1.70 mmol, 68 %).

M.p. (uncorrected) = 63 – 65°C; - **^{1}H- NMR** (400 MHz, $CDCl_3$): δ [ppm] = 2.71 (bs, 2 H, m), 2.77 (bs, 4 H, l), 3.42 (bs, 2 H, n), 3.57 – 3.60 (m, 4 H, i), 3.61 – 3.66 (m, 4 H, f), 3.68 – 3.72 (m, 4 H, h), 3.86 (s, 6 H, a), 4.17 – 4.20 (m, 4 H, g), 5.08 (s, 2 H, q), 6.06 (bs, 1 H, o), 7.19 (s, 2 H, d), 7.22 – 7.35 (m, 5 H, s, t, u); - **^{13}C- NMR** (100 MHz, $CDCl_3$): δ [ppm] = 51.5 (+, 2 C, a), 52.8 (-, 1 C, m), 53.6 (-, 2 C, l), 65.4 (-, 2 C, q), 67.9 (-, 1 C, n), 68.3 (-, 4 C, g), 68.4 (-, 4 C, f), 69.6 (-, 2 C, h), 70.0 (-, 2 C, i), 112.5 (+, 2 C, d), 124.4 (C_{quat}, 2 C, c), 127.0 (+, 2 C, s), 127.1 (+, 1 C, u), 127.4 (+, 2 C, t), 135.8 (C_{quat}, 1 C, r), 149.5 (C_{quat}, 2 C, e), 155.6 (C_{quat}, 2 C, p), 166.8 (C_{quat}, 2 C, b); - **IR** (KBr): ν [cm^{-1}] = 2965 (m), 2940 (m), 2863 (m), 1723 (s), 1587 (m), 1519 (m), 1436 (m), 1351 (m), 1287 (s), 1251 (s), 1189 (m), 1129 (m), 1056 (m), 978 (m), 948 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol/l NH_4OAc): m/z (%) = 649.3 (100, MH^+); - **UV** (MeOH): λ (ε) = 268 (6700), 225 (19600), 203 (18200); - **MF:** $C_{32}H_{44}N_2O_{12}$ – **FW:** 648.71 g/mol;

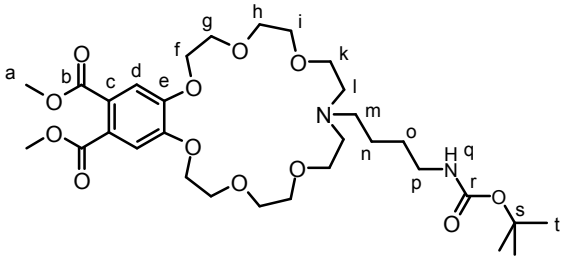
14-[3-(^{tert}*Butyloxycarbonylamino)-propyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester* ***(3c)***



3-(*tert*Butyloxycarbonylamino)-propylamine (435 mg) was reacted according to the general procedure to yield product **3c** (910 mg, 58 %) as a clear, yellow oil, which solidifies as a glass after some days (R_f = 0.08).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.33 (s, 9 H, s), 1.57 (m, 2 H, n), 2.51 (m, 2 H, m), 2.65 (bs, 4 H, l), 3.12 (m, 2 H, o), 3.55 – 3.57 (m, 4 H, k), 3.61 – 3.65 (m, 4 H, i), 3.70 – 3.76 (m, 4 H, h), 3.81 (s, 6 H, a), 3.84 – 3.90 (m, 4 H, g), 4.12 – 4.19 (m, 4 H, f), 5.43 (bs, 1 H, p), 7.15 (s, 2 H, d); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 26.6 (-, 1 C, o), 28.5 (+, 3 C, r), 38.3 (-, 1 C, n), 52.5 (+, 2 C, a), 53.2 (-, 1 C, m), 53.9 (-, 2 C, l), 68.9 (-, 2 C, k), 69.4 (-, 4 C, 6, g), 70.3 (-, 2 C, i), 70.7 (-, 4 C, h), 78.8 (C_{quat}, 1 C, r), 114.0 (+, 2 C, d), 125.7 (C_{quat}, 2 C, c), 150.4 (C_{quat}, 2 C, e), 156.3 (C_{quat}, 1 C, q), 167.7 (C_{quat}, 2 C, b); - **IR** (KBr): ν [cm^{-1}] = 3393 (bm), 2939 (m), 2874 (m), 1708 (m), 1593 (m), 1517 (m), 1435 (m), 1352 (m), 1286 (m), 1250 (m), 1186 (m), 1126 (m), 1050 (m), 978 (m), 940 (m), 785 (m); - **UV** (MeOH): λ (ε) = 267 (7700), 224 (24700); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 629.6 (100, MH^+); - **$C_{30}H_{48}N_2O_{12}$**: calc. C 57.3, H 7.7, N 4.5, found. C 57.1, H 7.5, N 4.2.; - **MF:** $C_{30}H_{48}N_2O_{12}$ – **FW:** 628.72 g/mol;

*14-[4-(*tert*Butyloxycarbonylamino)-butyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**3d**)*

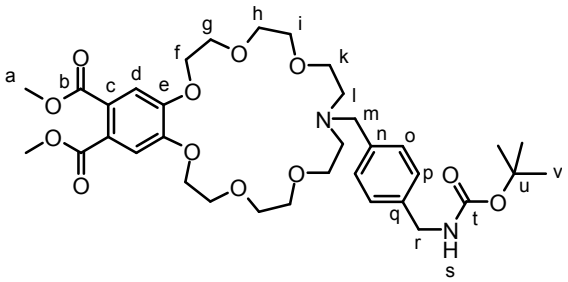


4-(*tert*Butyloxycarbonylamino)-butylamine (470 mg) was used as reactant in the procedure. A clear, yellow oil (847 mg, 53 %) is obtained, which solidifies as a glass after prolonged standing (R_f = 0.05).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.40 (s, 9 H, r), 1.44 (bs, 4 H, o, p), 2.50 (m, 2 H, m), 2.74 (m, 4 H, l), 3.06 (m, 2 H, n), 3.56 – 3.58 (m, 4 H, k), 3.61 – 3.63 (m, 4 H, i), 3.72 – 3.74 (m, 4 H, h), 3.84 (s, 6 H, a), 3.88 – 3.89 (m, 4 H, g), 4.18 – 4.19 (m, 4 H, f), 4.90 (bs, 1 H, q), 7.17 (s, 2 H, d); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 24.1 (-, 1 C, o), 27.7 (-, 1 C,

p), 28.4 (+, 3 C, t), 40.3 (-, 1 C, n), 52.5 (+, 2 C, a), 53.8 (-, 1 C, m), 55.0 (-, 2 C, l), 69.3 (-, 2 C, k), 69.5 (-, 2 C, f), 69.5 (-, 2 C, g), 70.6 (-, 4 C, i), 71.1 (-, 4 C, h), 78.8 (C_{quat}, 1 C, s), 113.7 (+, 2 C, d), 125.4 (C_{quat}, 2 C, c), 150.5 (C_{quat}, 2 C, e), 156.0 (C_{quat}, 1 C, r), 167.7 (C_{quat}, 2 C, b); - **IR** (KBr): ν [cm^{-1}] = 3375 (bm), 2936 (m), 2870 (m), 1715 (m), 1589 (m), 1519 (m), 1436 (m), 1353 (m), 1284 (m), 1185 (m), 1126 (m), 1053 (m), 979 (m), 945 (m), 785 (m); - **UV** (MeOH): λ (ε) = 267 (8000), 224 (25200); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 643.6 (100, MH^+); - **$C_{31}H_{50}N_2O_{12}$**: calc. C 57.9, H 7.8, N 4.4, found. C 57.7, H 7.6, N 4.2.; - **MF:** $C_{31}H_{50}N_2O_{12}$ – **FW:** 642.75 g/mol;

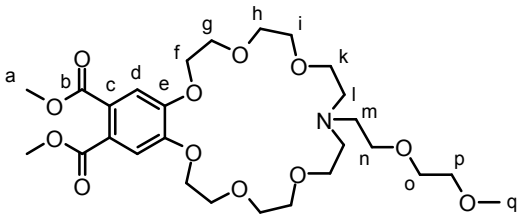
*14-[(4-(N-(^tertButyloxycarbonyl-aminomethyl)phenyl)methyl-amino]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**3f**)*



4-(*N*-(^tertButyloxycarbonyl-aminomethyl)phenyl)methyl-amine (590 mg) was employed to yield product **3f** (1.26 g, 1.83 mmol, 73 %) as a yellow glass (R_f = 0.16).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.38 (s, 9 H, v), 2.70 (m, 4 H, l), 3.63 (m, 8 H, 9, k), 3.76 (m, 4 H, i), 3.80 (m, 2 H, m), 3.82 (s, 6 H, a), 3.91 (m, 4 H, h), 4.20 (m, 6 H, g, r), 4.92 (bs, 1 H, s), 7.07 (m, 2 H, o), 7.22 (m, 4 H, 4, p); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 28.4 (+, 3 C, v), 44.1 (-, 1 C, r), 52.7 (+, 2 C, a), 58.0 (-, 1 C, m), 67.8 (-, 2 C, l), 68.9 (-, 4 C, 9, k), 69.5 (-, 2 C, h), 69.7 (-, 4 C, f, g), 79.4 (C_{quat}, 1 C, u), 115.3 (+, 2 C, d), 127.3 (C_{quat}, 4 C, o, p), 130.1 (C_{quat}, 2 C, n, q), 150.1 (C_{quat}, 2 C, e), 156.1 (C_{quat}, 1 C, t), 168.2 (C_{quat}, 1 C, b); - **IR** (KBr): ν [cm^{-1}] = 3430 (bm), 2930 (m), 2870 (m), 1714 (m), 1601 (m), 1518 (m), 1436 (m), 1351 (m), 1285 (m), 1177 (m), 1118 (m), 1049 (m), 980 (m), 941 (m), 795 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 691.3 (100, MH^+); - **UV** (MeOH): λ (ε) = 268 (7600), 221 (41400); - **HRMS** (EI-MS 70 eV): calc. for $C_{35}H_{50}N_2O_{12}$: 690.3364, found: 690.3362.; - **MF:** $C_{35}H_{50}N_2O_{12}$ – **FW:** 690.79 g/mol;

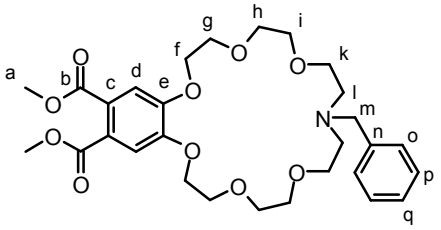
*14-[2-(2-Methoxy-ethoxy)-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethyl ester (**3i**)*



2-(2-Methoxy-ethoxy)-ethylamine (300 mg) was reacted in the preparation under nitrogen to yield the product **3i** (1.17 g, 1.95 mmol, 78 %) as a thick yellow oil, which solidifies as glass after prolonged standing (R_f = 0.27). Column chromatography was performed with chloroform and methanol in 8:1 ratio.

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 3.16 – 3.29 (m, 4 H, l), 3.25 (s, 3 H, q), 3.40 (m, 2 H, n), 3.48 (m, 2 H, m), 3.63 (m, 4 H, k), 3.67 (m, 4 H, f), 3.63 (m, 6 H, i, p), 3.63 (m, 2 H, o), 3.81 (s, 6 H, a), 3.85 (m, 4 H, h), 4.16 (m, 4 H, g), 7.14 (bs, 2 H, d); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 52.6 (+, 2 C, a), 54.2 (-, 1 C, n), 54.6 (-, 1 C, m), 58.9 (+, 1 C, q), 59.3 (-, 2 C, l), 66.9 (-, 6 C, l, o), 68.9 (-, 4 C, f, g), 69.4 (-, 2 C, k), 70.4 (-, 2 C, i), 70.7 (-, 2 C, h), 71.6 (-, 1 C, p), 113.3 (+, 2 C, d), 125.4 (C_{quat}, 4 C, c), 150.1 (C_{quat}, 1 C, e), 167.6 (C_{quat}, 1 C, b); - **IR** (KBr): ν [cm^{-1}] = 3444 (bm), 2930 (m), 2875 (m), 1718 (m), 1597 (m), 1518 (m), 1435 (m), 1349 (m), 1284 (s), 1191 (s), 1119 (s), 980 (m), 975 (m), 945 (m), 886 (m), 842 (m), 781 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 574.4 (100, MH^+); - **UV** (MeOH): λ (ε) = 267 (7500), 223 (38200); - **HRMS** (EI-MS 70 eV): calc. for $C_{27}H_{43}NO_{12}{}^{*+}$: 574.2864, found: 574.2851.; - **MF:** $C_{27}H_{43}NO_{12}$ – **FW:** 573.64 g/mol;

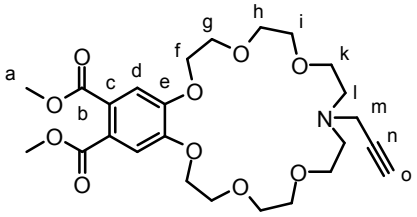
*14-Benzyl-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosen-2,3-dicarbonic acid dimethyl ester (**3e**)*



Benzyl amine (270 mg) was used. Column chromatography was performed on silica gel with ethyl acetate / ethanol 3:1 → 5:2. (R_f = 0.14, ethyl acetate / ethanol 3:1). A yellowish solid was obtained (1.20 g, 2.14 mmol, 86 %).

M.p. (uncorrected) = 57 – 59°C; - **^{1}H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 2.77 (bs, 4 H, l), 3.57 – 3.61 (m, 4 H, k), 3.63 – 3.65 (m, 4 H, i), 3.69 (m, 2 H, m), 3.75 – 3.78 (m, 4 H, f), 3.86 (s, 6 H, a), 3.91 – 3.94 (m, 4 H, h), 4.19 – 4.22 (m, 4 H, g), 7.19 (s, 2 H, d), 7.20 – 7.31 (m, 5 H, o, p, q); - **^{13}C- NMR** (75 MHz, $CDCl_3$): δ [ppm] = 52.6 (+, 2 C, a), 53.7 (-, 2 C, l), 59.5 (-, 1 C, m), 69.4 (-, 2 C, k), 69.5 (-, 4 C, f, g), 70.6 (-, 2 C, h), 71.0 (-, 2 C, i), 113.8 (+, 2 C, d), 125.5 (C_{quat}, 3 C, n, c), 127.0 (+, 1 C, q), 128.2 (+, 2 C, o), 129.1 (+, 2 C, p), 150.5 (C_{quat}, 2 C, e), 167.8 (C_{quat}, 2 C, b); - **IR** (KBr): ν [cm^{-1}] = 2968 (m), 2870 (m), 1719 (s), 1598 (m), 1518 (m), 1436 (m), 1350 (m), 1284 (s), 1192 (m), 1121 (m), 1054 (m), 979 (m), 946 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol/l NH_4OAc): m/z (%) = 562 (100, MH^+); - **UV** (MeOH): λ (ε) = 268 (6200), 224 (20800), 202 (14100); - **HRMS** (PI-LSIMS FAB, MeOH/glycerine): calc. for $C_{29}H_{39}NO_{10}*H^+$: 561.2576, found: 561.2571.; - **MF:** $C_{29}H_{39}NO_{10}$ – **FW:** 561.63 g/mol;

*14-Propargyl-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**3g**)*

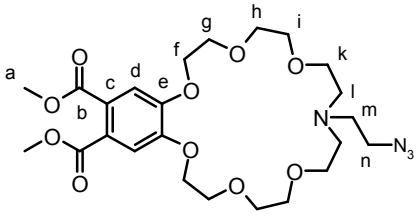


Propargylamine (140 mg) was allowed to react according to the general procedure. The reaction was performed under nitrogen atmosphere and protection from light. The product **3g** was obtained as a clear, yellow oil (910 mg, 71 %, R_f = 0.17).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 2.09 (m, 1 H, o), 2.75 (m, 4 H, l), 3.45 (m, 2 H, m), 3.55 – 3.58 (m, 4 H, k), 3.61 – 3.65 (m, 4 H, i), 3.68 – 3.74 (m, 4 H, h), 3.80 (s, 6 H, a), 3.84 – 3.88 (m, 4 H, g), 4.12 – 4.17 (m, 4 H, f), 7.12 (s, 2 H, d); **- ^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 31.1 (+, 1 C, o), 43.3 (-, 1 C, m), 52.5 (+, 2 C, a), 53.3 (-, 1 C, l), 69.4 (-, 2 C, k), 69.5 (-, 2 C, f), 70.6 (-, 4 C, 9, g), 71.2 (-, 4 C, h), 73.0 (C_{quat}, 1 C, n), 113.5 (+, 2 C, d), 125.3 (C_{quat}, 2 C, c), 150.5 (C_{quat}, 2 C, e), 167.8 (C_{quat}, 2 C, b); - **IR** (KBr): ν [cm^{-1}] = 3310 (s), 2976

(m), 2880 (m), 1721 (s), 1599 (m), 1516 (m), 1438 (m), 1348 (m), 1282 (s), 1194 (m), 1123 (m), 1053 (m), 981 (m), 943 (m); - **UV** (MeOH): λ (ε) = 267 (7400), 224 (26800); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 510.3 (100, MH^+); - **$C_{25}H_{35}NO_{10}$**: calc. C 58.9, H 6.9, N 2.8, found. C 58.6, H 6.6, N 2.6.; - **MF:** $C_{25}H_{35}NO_{10}$ – **FW:** 509.56 g/mol;

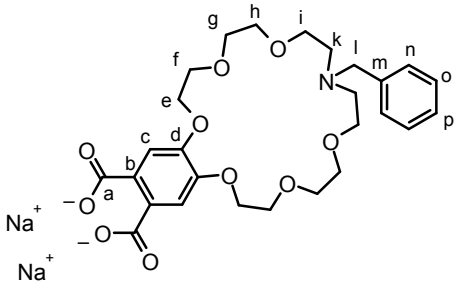
*14-[2-Azido-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**3h**)*



2-Azido-ethyl-amine hydrobromide (250 mg) was added as amine component and the reaction was carried out in nitrogen atmosphere. Column chromatography was performed with chloroform and methanol in a 10:1 ratio. A clear, yellow glass (934 mg, 69 %) was obtained (R_f = 0.42).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 2.76 (t, 2 H, *J* = 6.8 Hz, m), 2.80 (m, 4 H, l), 3.26 (t, 2 H, *J* = 6.8 Hz, n), 3.59 (t, 4 H, *J* = 5.6 Hz, k), 3.66 – 3.69 (m, 4 H, i), 3.72 – 3.74 (m, 4 H, h), 3.86 (s, 6 H, a), 3.91 (t, 4 H, *J* = 5.6 Hz, g), 4.20 (t, 4 H, *J* = 5.6 Hz, 6), 7.19 (s, 2 H, d); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 49.4 (-, 1 C, n), 52.6 (+, 2 C, a), 54.5 (-, 2 C, l), 54.7 (-, 1 C, m), 69.3 (-, 2 C, k), 69.5 (-, 2 C, f), 70.0 (-, 2 C, g), 70.7 (-, 4 C, i), 71.2 (-, 4 C, h), 113.6 (+, 2 C, d), 125.3 (C_{quat}, 2 C, c), 150.5 (C_{quat}, 2 C, e), 167.8 (C_{quat}, 2 C, b); - **IR** (KBr): ν [cm^{-1}] = 2971 (m), 2876 (m), 1719 (s), 1596 (m), 1521 (m), 1438 (m), 1348 (m), 1282 (s), 1195 (m), 1123 (m), 1049 (m), 983 (m), 942 (m); - **UV** (MeOH): λ (ε) = 267 (7800), 224 (27300); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 541.4 (100, MH^+), 513.4 (11, $(M-N_2)H^+$); - $C_{24}H_{36}N_4O_{10}$: calc. C 53.3, H 6.7, N 10.4, found. C 52.8, H 6.3, N 9.9.; - **MF:** $C_{24}H_{36}N_4O_{10}$ – **FW:** 540.58 g/mol;

*14-Benzyl-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosen-2,3-dicarboxylic acid sodium salt (**5b**)*

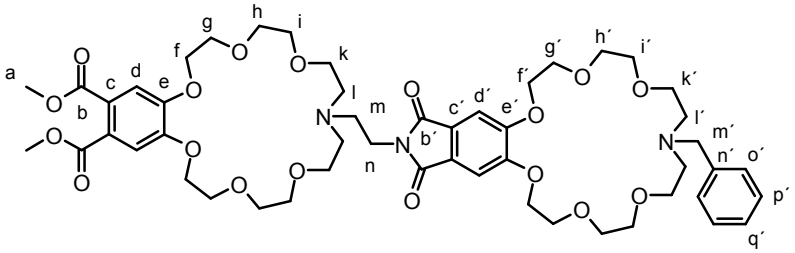


Compound **7** (1.53 g, 2.50 mmol) in 25 mL of THF was stirred for two days with 5.0 mL of a 1 N aqueous sodium hydroxide solution (5 mmol) in water. The solvent was evaporated and the residue was lyophilised to yield 1.57 g of a colourless solid (2.45 mmol, 99 %).

M.p. (uncorrected) = 156 – 159°C; - **^{1}H-NMR** (300 MHz, MeOD): δ [ppm] = 2.75 (m, 4 H, k), 3.62 – 3.65 (m, 8 H, i, h), 3.68 – 3.76 (m, 6 H, e, l), 3.91 (m, 4 H, g), 4.23 (m, 4 H, f), 7.22 (s, 2 H, c), 7.29 – 7.35 (m, 5 H, n, o, p); - **^{13}C-NMR** (75 MHz, MeOD): δ [ppm] = 54.0 (-, 2 C, k), 61.1 (-, 1 C, l), 68.8 (-, 2 C, i), 69.9 (-, 2 C, f), 70.2 (-, 2 C, e), 70.5 (-, 2 C, g), 70.8 (-, 2 C, h), 113.8 (+, 2 C, c), 128.4 (+, 1 C, p), 129.4 (+, 2 C, n), 130.8 (+, 2 C, o), 134.4 (C_{quat}, 2 C, b), 139.2 (C_{quat}, 1 C, m), 148.4 (C_{quat}, 2 C, d), 177.5 (C_{quat}, 2 C, a); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 534.3 (100, $(M + 3\ H^+)^+$), 556.2 (16, $(MNa^+ + 2H^+)^+$), 578.3 (4, $(MH^+ + 2\ Na^+)^{2+}$); - **HRMS** (PI-LSIMS FAB, MeOH/glycerine): calc. for $C_{27}H_{35}NO_{10}{*}H^+$: 534.2339, found: 534.2338; - **MF:** $C_{27}H_{35}NO_{10}Na_2$ – **FW:** 529.56 g/mol;

II. 4.2. Synthesis of Crown Ether Amino Acid Di- and Tripeptides

*Compound **6b***

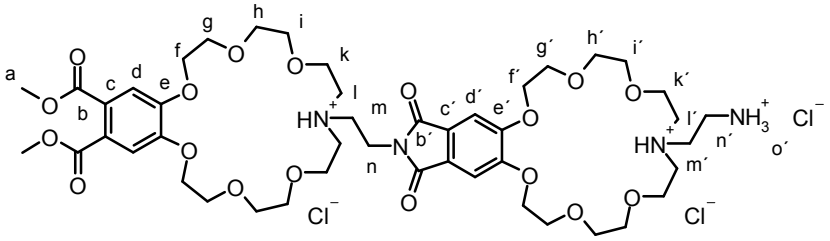


A solution of **4** (144 mg, 0.25 mmol) in chloroform (10 mL) containing HOBt (*N*-hydroxybenzotriazole) (68 mg, 0.5 mmol) and EDC (1-ethyl-3-(3-dimethylaminopropyl)

carbodiimide) (79 mg, 0.5 mmol) was stirred for 15 minutes at 0 °C. A mixture of **5b** (141 mg, 0.25 mmol) and DIPEA (*N,N*-diisopropylethylamine) (104 mg, 0.80 mmol) in chloroform (10 mL) was added drop wise under cooling. The mixture was stirred 3 h at ambient temperature and then refluxed over night. After cooling, the suspension was filtered over celite and the filter cake was washed with chloroform. The solvent was removed under reduced pressure and the residue was purified by column chromatography (chloroform / methanol 6:1) to give a yellow glass (193 mg, 76 %, R_f ($CHCl_3$ / MeOH 6:1) = 0.44).

^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 2.76 – 2.84 (m, 10 H, l, m, l´), 3.55 (t, 4 H, *J* = 5.30 Hz, k´), 3.59 – 3.64 (m, 8 H, i, k), 3.65 – 3.75 (m, 10 H, h, n, i´), 3.78 (t, 4 H, *J* = 4.80 Hz, h´), 3.86 (s, 6 H, a), 3.90 (t, 4 H, *J* = 4.56 Hz, g), 3.95 (t, 4 H, *J* = 4.56 Hz, g´), 4.19 (t, 4 H, *J* = 4.56 Hz, f), 4.23 (t, 4 H, *J* = 4.56 Hz, f´), 7.17 (s, 4 H, d), 7.21 – 7.28 (m, 5 H, o´, p´, q´), 7.33 (bs, 2 H, d´); - **^{13}C-NMR** (150 MHz, $CDCl_3$): δ [ppm] = 36.1 (-, 1 C, n), 52.5 (+, 2 C, a), 52.9 (-, 1 C, m), 53.8 (-, 2 C, l´), 54.3 (-, 2 C, l), 59.8 (-, 1 C, m´), 69.3 (-, 2 C, f), 69.4 (-, 2 C, g´), 69.5 (-, 2 C, g), 69.6 (-, 2 C, f´), 70.2 (-, 2 C, k), 70.7 (-, 6 C, i, i´, k´), 71.1 (-, 2 C, h), 71.2 (-, 2 C, h´), 107.0 (+, 2 C, d´), 113.7 (+, 2 C, d), 125.3 (C_{quat}, 2 C, c´), 125.6 (C_{quat}, 2 C, c), 126.9 (C_{quat}, 1 C, n´), 128.2 (+, 3 C, p´, q´), 128.9 (+, 2 C, o´), 150.5 (C_{quat}, 2 C, e), 153.4 (C_{quat}, 2 C, e´), 167.8 (C_{quat}, 2 C, b´), 168.4 (C_{quat}, 2 C, b); - **IR** (NaCl): ν [cm^{-1}] = 3400 (bw), 2930 (m), 2880 (m), 1707 (s), 1599 (m), 1510 (m), 1436 (m), 1393 (m), 1352 (m), 1291 (s), 1196 (m), 1129 (s), 1056 (m), 980 (w), 945 (w); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 506.8 (100, $(M+2\ H^+)^{2+}$), 1012.6 (8, MH^+); UV (MeOH): λ (ε) = 340 (1200), 248 (43100), 227 (37400); - **HRMS** (PI-LSIMS FAB, MeOH/glycerine): calc. for $C_{51}H_{68}N_3O_{18}*H^+$: 1011.4576, found: 1011.4558.; - **MF:** $C_{51}H_{69}N_3O_{18}$ – **FW:** 1012.13 g/mol;

Hydrochloride of compound ***6c***

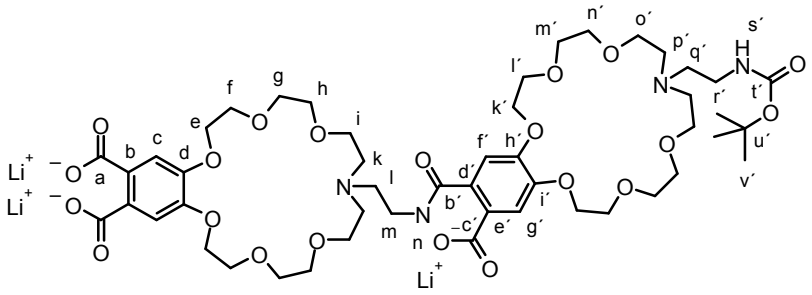


To a solution of the dimer CEAA (**6a**) (319 mg, 0.3 mmol) in DCM (10 mL) was slowly added 6 mL of HCl saturated diethylether. After 3 h of stirring at room temperature diethylether (50 mL) was added and the solvent was decanted off the formed precipitate. The

sticky solid was taken up in a little of DCM, precipitated with an excess of diethylether and the solvent was decanted off again. The residue was suspended in diethylether, allowed to settle completely and the solvent was decanted off. The compound was dried under vacuum yielding a yellow, hygroscopic solid (321 mg, 0.29 mmol, 97 %).

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.26 – 1.50 (m, 2 H), 3.10 – 4.80 (m, 64 H), 7.22 – 7.24 (m, 4 H), 8.52 (bs, 3 H); - **^{13}C-NMR** (100 MHz, $CDCl_3$): δ [ppm] = 50.8 (+), 52.5 (+), 68.8 (-), 69.3 (-), 69.6 (-), 70.6 (-), 72.7 (-), 107.3 (+), 113.7 (+), 125.3 (C_{quat}), 125.6 (C_{quat}), 150.3 (C_{quat}), 152.9 (C_{quat}), 167.7 (C_{quat}), 167.9 (C_{quat}), further signals were not detectable; - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 483.4 (100, $(M+2\ H^+)^{2+}$), 965.5 (5, MH^+); - **HRMS** (PI-LSIMS FAB, MeOH/glycerine): calc. for $C_{46}H_{68}N_4O_{18}$*H^+: 965.4607, found: 965.4590.; - **MF:** $C_{46}H_{73}N_4O_{18}Cl_3$ – **FW:** 1076.46 g/mol;

*Lithium salt of compound **6d***

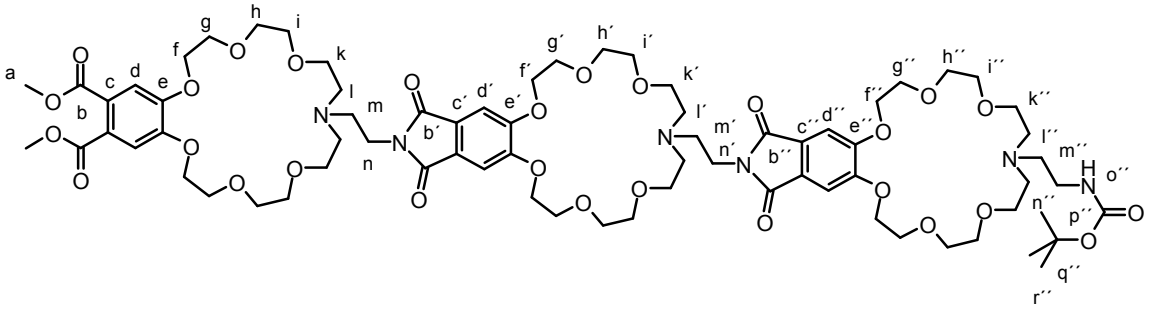


Compound **6a** (320 mg, 0.3 mmol) was dissolved in THF (6 mL) and mixed with an aqueous solution of lithium hydroxide (0.45 mL, 0.9 mmol, 2 M). After three days of stirring at ambient temperature the solvent was removed under reduced pressure and the product was lyophilised to give **6d** (317 mg, 0.297 mmol, 99 %) as a pale yellow solid.

M.p. (uncorrected) = 151 °C (decomp.); - **^{1}H-NMR** (400 MHz, CD_3OD): δ [ppm] = 1.42 (s, 9 H, i´´), 2.60 (t, 2 H, *J* = 6.4 Hz, d´´), 2.72 – 2.84 (m, 8 H, k, m, c´´), 3.10 (t, 2 H, *J* = 6.4 Hz, e´´), 3.34 (bs, 1 H), 3.42 (t, 2 H, *J* = 6.4 Hz, n), 3.56 – 3.68 (m, 20 H, g, h, i, l, b´´), 3.71 – 3.75 (m, 4 H, m´), 3.80 – 3.88 (m, 8 H, f, l´), 4.14 – 4.20 (m, 8 H, e, k´), 7.10 (s, 1 H, g´), 7.13 (s, 2 H, c), 7.29 (s, 1 H, f´); - **^{13}C-NMR** (100 MHz, CD_3OD): δ [ppm] = 28.9 (+, 3 C, i´´), 39.3 (-, 1 C, n), 39.6 (-, 1 C, e´´), 54.9 (-, 1 C, m), 55.3 (-, 1 C, k), 55.5 (-, 2 C, c´´), 70.2 (-, 2 C, e), 70.5 (-, 2 C, k´), 70.7 (-, 2 C, i), 70.9 (-, 2 C, b´´), 71.6 (-, 2 C, f), 71.7 (-, 2 C, l´´), 71.7 (-, 2 C, l), 71.9 (-, 2 C, g), 72.0 (-, 2 C, h), 72.0 (-, 2 C, m´), 80.0 (C_{quat}, 1 C, i´´), 114.8 (+, 1 C, g´), 114.9 (+, 1 C, c), 115.6 (+, 2 C, f´), 129.7 (C_{quat}, 1 C, d´), 133.6 (C_{quat}, 1 C, b), 133.7

(C_{quat}, 1 C, e´), 149.5 (C_{quat}, 2 C, d), 150.0 (C_{quat}, 1 C, h´), 150.7 (C_{quat}, 1 C, i´), 172.5 (C_{quat}, 1 C, b´), 175.3 (C_{quat}, 1 C, c´), 177.7 (C_{quat}, 2 C, a); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 1055.6 (4, MLi^+), 528.5 (100, $(M^{2-} + NH_4^+ + 3\ H^+)^{2+}$); - **MF:** $C_{49}H_{73}N_4O_{21}Li_3$ – **FW:** 1074.96 g/mol;

Compound ***7a***



A solution of **5a** (132 mg, 0.24 mmol) in chloroform (10 mL) containing HOBt (*N*-hydroxybenzotriazole) (55 mg, 0.40 mmol) and EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) (63 mg, 0.40 mmol) was stirred in nitrogen atmosphere for 30 minutes at 0 °C. A mixture of **6c** (215 mg, 0.2 mmol) and DIPEA (*N,N*-diisopropylethylamine) (156 mg, 1.20 mmol) in chloroform (10 mL) was added drop wise under cooling. The mixture was stirred 2 h under nitrogen at ambient temperature and refluxed over night. After cooling to room temperature the solution was washed with brine (3 x 10 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude product was purified by repeated column chromatography (chloroform / methanol, 6:1; 8:1 → 5:1, 6:1 → 5:1) yielding a yellow glass (106 mg, 35 %) [R_f ($CHCl_3$ / MeOH 6:1) = 0.4].

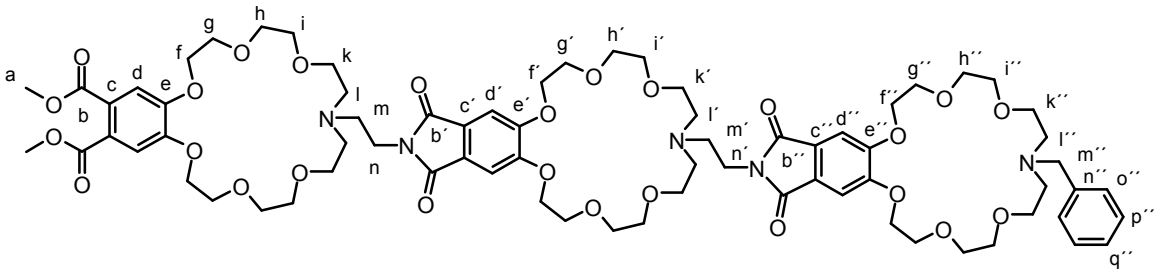
^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.41 (s, 9 H, r´´), 2.68 – 2.93 (m, 14 H, l, l´, l´´, m´´), 3.25 (m, 2 H, n´´), 3.48 – 3.80 (m, 40 H, k, m, n, o, m´, k´´), 3.82 – 3.96 (m, 14 H, g, g´, n´, g´´), 3.85 (s, 6 H, a), 4.12 – 4.28 (m, 12 H, f, f´, f´´), 5.64 (bs, 1 H), 7.15 (s, 2 H, d), 7.19 (m, 2 H, d´), 7.24 (bs, 2 H, d´´); - **^{13}C-NMR** (150 MHz, $CDCl_3$): δ [ppm] = 28.5 (+, 3 C, r´´), 35.9 (-, 2 C, n, n´), 52.5 (+, 2 C, a), 52.8 (-, 1 C, m), 53.0 (-, 1 C, m´´), 54.1 (-, 1 C, m´), 54.2 (-, 2 C, l´), 54.4 (-, 2 C, l), 54.6 (-, 2 C, l´´), 69.1 – 69.5 (-, 12 C, f, g, f´, g´, f´´, g´´), 70.0 (-, 2 C, k), 70.6 – 70.8 (-, 10 C, i, i´, k´, i´´, k´´), 71.0 – 71.2 (-, 6 C, h, h´, h´´), 80.0 (C_{quat}, 1 C, q´´), 106.7 (+, 4 C, d´, d´´), 113.7 (+, 2 C, d), 125.3 (C_{quat}, 2 C, c), 125.4 (C_{quat}, 2 C, c´), 125.5 (C_{quat}, 2 C, c´´), 150.5 (C_{quat}, 2 C, e), 153.2 (C_{quat}, 2 C, e´), 153.3 (C_{quat}, 2 C, e´´), 156.2 (C_{quat}, 1 C, p´´), 167.7 (C_{quat}, 4 C, b´, b´´), 163.1 (C_{quat}, 2 C, b); - **IR** (KBr): ν [cm^{-1}] = 3458 (bm),

3018 (m), 2952 (m), 2881 (w), 1677 (s), 1599 (m), 1501 (m), 1438 (s), 1391 (s), 1299 (s), 1178 (s), 1116 (s), 1052 (m), 946 (m), 799 (m), 719 (m), 659 (m); MS (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 487.3 (63, $(M - t\text{-}Bu + 3\ H^+)^{3+}$), 506.0 (80, $(M + 3H^+)^{3+}$), 758.6 (100, $(M + 2H^+)^{2+}$), 1516.1 (3, MH^+);$^+$); - **UV** (MeOH): λ (ε) = 247 (59100), 224 (42600); - **MF:** $C_{73}H_{106}N_6O_{28}$ – **FW:** 1515.68 g/mol;

Alternative procedure:

A solution of **6d** (106 mg, 0.10 mmol) in chloroform (5 mL) and DMF (2 mL) containing HOBt (34 mg, 0.25 mmol) and EDC (39 mg, 0.25 mmol) was stirred at 0 °C under nitrogen atmosphere for 15 min. A mixture of **4** (77 mg, 0.13 mmol) and DIPEA (78 mg, 0.60 mmol) in chloroform (5 mL) was added drop wise under cooling. The mixture was stirred 5 h under nitrogen at ambient temperature and was then refluxed over night. The solvent mixture was evaporated and the residue was dissolved in 30 mL of DCM. The solution was washed with brine (3 x 10 mL) and dried over $MgSO_4$. Further purification was achieved by repeated column chromatography (chloroform / methanol, 6:1; 8:1 → 5:1, 6:1 → 5:1). The product is a clear, yellow glass (44 mg, 0.029 mmol, 29 %) [R_f ($CHCl_3$ / MeOH 6:1) = 0.4].

*Compound **7b***



A solution of **5b** (165 mg, 0.30 mmol) in chloroform (10 mL) containing HOBt (55 mg, 0.40 mmol) and EDC (63 mg, 0.40 mmol) was stirred under nitrogen atmosphere for 15 min at 0 °C. A mixture of **6c** (215 mg, 0.2 mmol) and DIPEA (156 mg, 1.20 mmol) in chloroform (10 mL) was added drop wise under cooling. The mixture was stirred 5 h under nitrogen at ambient temperature and refluxed over night. After cooling to room temperature 20 mL of chloroform was added, the solution was washed with brine (3 x 10 mL) and dried over $MgSO_4$. After evaporation of the solvent, the sticky residue was purified by repeated column chromatography (chloroform / methanol, 10:1 → 6:1, 8:1 → 6:1, 6:1) to yield a yellow glass (108 mg, 0.074 mmol, 37 %) [R_f ($CHCl_3$ / MeOH 6:1) = 0.3].

^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 2.71 – 2.84 (m, 16 H, l, m, l′, m′, l′′), 3.54 – 3.60 (m, 12 H, k, k′, k′′), 3.62 – 3.73 (m, 26 H, h, i, n, h′, i′, n′, i′′, m′′), 3.74 (m, 4 H, h′′), 3.85 (s, 6 H, a), 3.89 (m, 4 H, g), 3.90 – 3.96 (m, 8 H, g′, g′′), 4.13 – 4.25 (m, 12 H, f, f′, f′′), 7.16 (s, 2 H, d), 7.18 (s, 2 H, q), 7.20 (s, 2 H, d′′), 7.23 – 7.28 (m, 3 H, p′′, q′′), 7.32 (m, 2 H, o′′); - **^{13}C-NMR** (150 MHz, $CDCl_3$): δ [ppm] = 36.0 (-, 1 C, n′), 36.2 (-, 1 C, m′′), 52.5 (+, 2 C, a), 52.9 (-, 1 C, m), 53.0 (-, 1 C, m′), 53.8 (-, 2 C, l), 54.2 (-, 2 C, l′), 54.6 (-, 2 C, l′′), 69.2 – 69.6 (-, 12 C, f, g, f′, g′, f′′, g′′), 70.1 (-, 1 C, n), 70.5 – 70.8 (-, 6 C, k, k′, k′′), 70.9 – 71.3 (-, 12 C, h, i, h′, i′, h′′, i′′), 106.8 (+, 4 C, d′, d′′), 113.7 (+, 2 C, d), 125.3 (C_{quat}, 2 C, c), 124.4 (C_{quat}, 2 C, c′), 124.5 (C_{quat}, 2 C, c′′), 128.2 (+, 2 C, o′′), 129.0 (+, 3 C, p′′, q′′), 150.5 (C_{quat}, 2 C, e), 153.3 (C_{quat}, 4 C, e′, e′′), 167.8 (C_{quat}, 4 C, b′, b′′), 168.4 (C_{quat}, 4 C, b); - **IR** (KBr): ν [cm^{-1}] = 3448 (bm), 3020 (m), 2970 (m), 2644 (w), 1688 (s), 1605 (s), 1599 (m), 1500 (m), 1438 (s), 1391 (s), 1301 (s), 1196 (s), 1113 (s), 1052 (m), 945 (m), 828 (m), 799 (m), 745 (m), 719 (m), 641 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 488.4 (96, $(M + 3H^+)^{3+}$), 732.2 (100, $(M + 2H^+)^{2+}$), 1463.0 (3, MH^+);$^{+}$); - **UV** (MeOH): λ (ε) = 247 (58300), 223 (42100); - **MF:** $C_{73}H_{99}N_5O_{26}$ – **FW:** 1462.62 g/mol;

II. 4.3. Solid Phase Synthesis of the Test Peptides

All peptides were synthesized on Rink Amide resin using Fmoc protecting group strategy. Coupling was done by HBTU / HOBt / DIPEA. HOBt and HBTU were used as a 0.45 M solution, DIPEA as a 1.2 M solution in DMF. The Fmoc protected amino acids were dissolved in NMP as 0.5 M solutions. Every peptide was synthesized on 150 mg of resin in standard 10 mL syringe. The lot of the resin used had a loading of 0.72 mmol/g (manufacturer's claims). The resin was allowed to preswell in DMF for 30 min. Each coupling was done twice using 1.0 ml of each solution per step and shaking for 2 h each. Fmoc deprotection was done by shaking the resin with 40 % piperidine in DMF for 5 minutes, subsequent washing and addition of 20 % piperidine in DMF followed by shaking for 15 minutes. After completion of the syntheses, the resin was washed with DMF and DCM (5 x 5 mL each). From the resin was cleaved by shaking for 3 h after addition of 4.0 mL of TFA / TIS / H_2O (95:3:2) (vol/vol/vol). After filtering off the resin, the TFA solution was reduced in volume to about 1.0 mL. It was then transferred to a Falcon tube and precipitated with cold Et_2O. The precipitate was centrifuged at –10 °C for 20 minutes. The solution was then carefully decanted off and the precipitate resuspended in cold Et_2O before being centrifuged again. This resuspending / centrifuging step was repeated five times. Finally, the Et_2O was

decanted off again and the peptide dried under vacuum. The peptides were analysed by ESI-MS.

Analytical control of Lys-Lys-Gly-Gly-NH_2: **MF:** $C_{22}H_{36}N_7O_{10}F_3$ – **FW:** 729.55 g/mol; **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 388.4 (100 %, MH^+);

Analytical control of Lys-Gly-Lys-Gly-NH_2: **MF:** $C_{22}H_{36}N_7O_{10}F_3$ – **FW:** 729.55 g/mol; **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 388.4 (100 %, MH^+);

Analytical control of Lys-Gly-Gly-Lys-NH_2: **MF:** $C_{22}H_{36}N_7O_{10}F_3$ – **FW:** 729.55 g/mol; **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 388.4 (100 %, MH^+);

II. 4.4. ^{1}H – and ^{13}C-NMR Spectra of Selected New Compounds

NMR spectra were recorded on Bruker Avance spectrometers 300, 400 and 600. Measuring temperature was 300 K. TMS was used as external standard. For solvents and conditions of measurement, see experimental procedures.

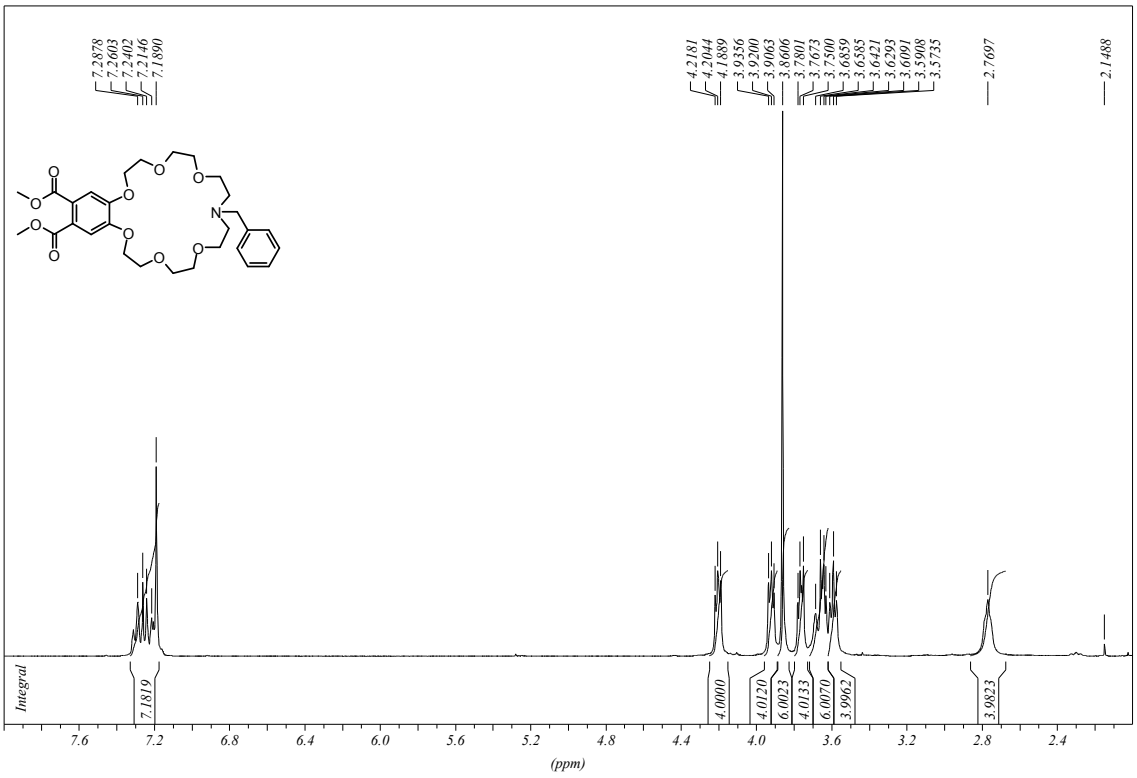


Figure 2: ^{1}H-NMR spectrum of compound **3e**

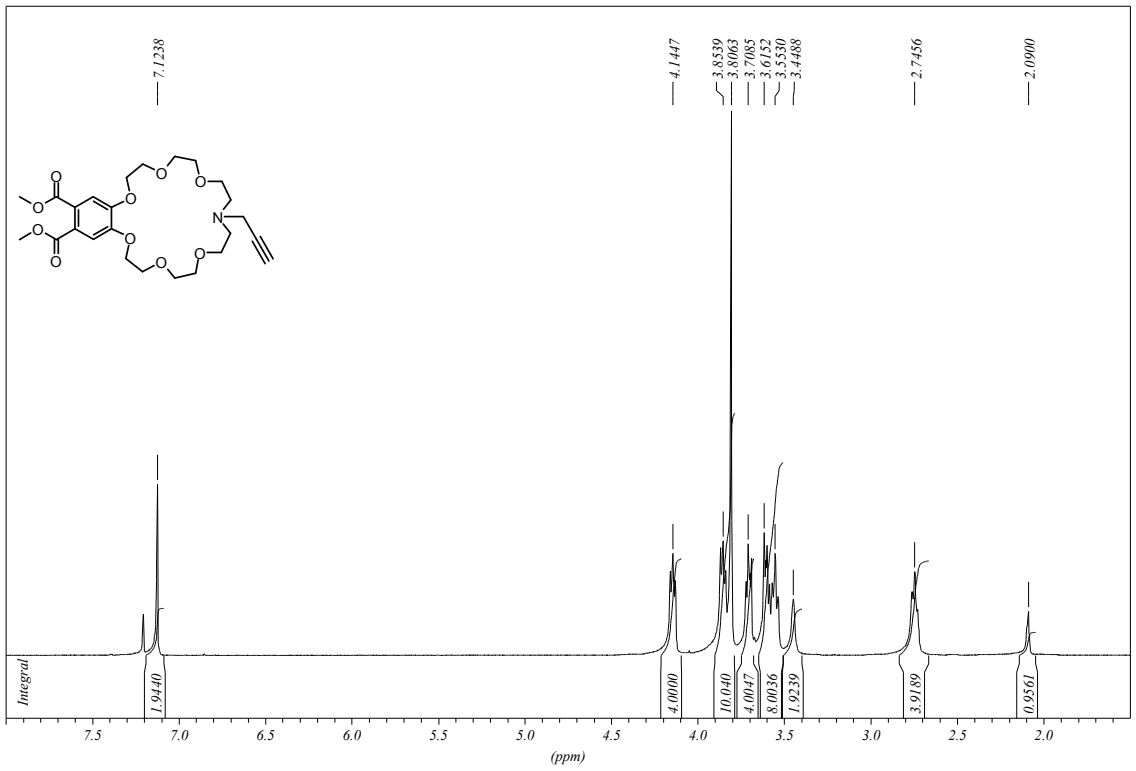


Figure 3: ^{1}H-NMR spectrum of compound **3g**

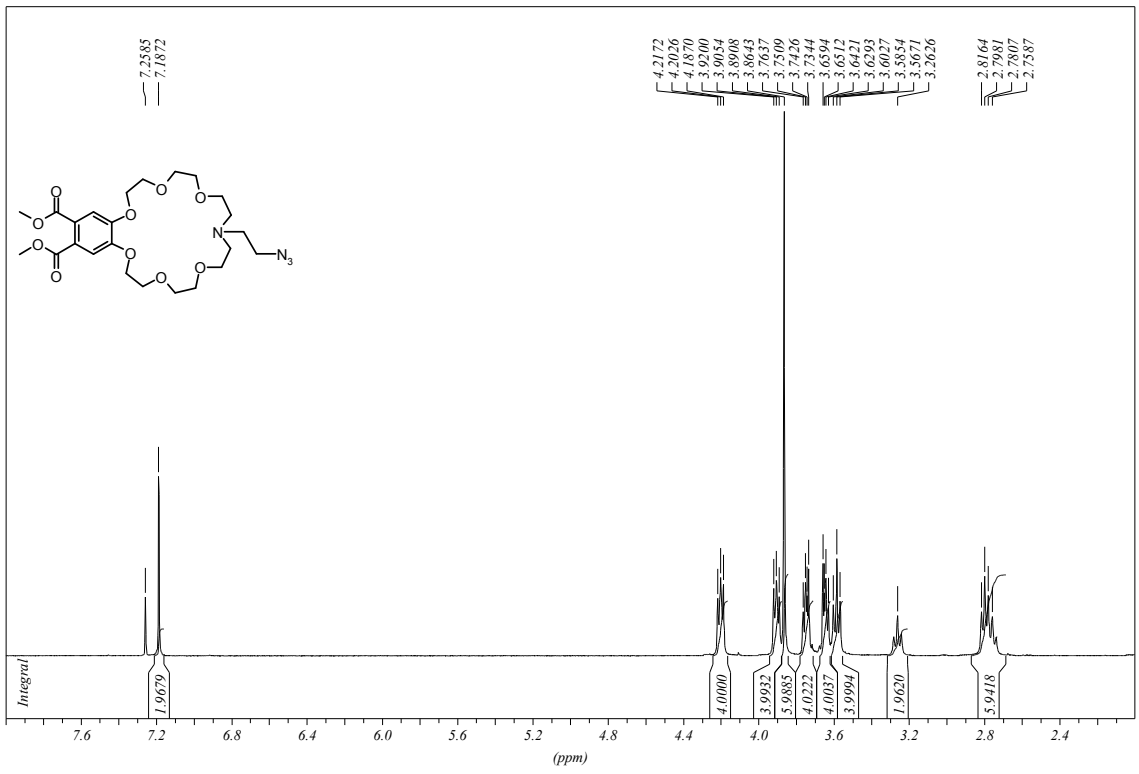


Figure 4: ^{1}H-NMR spectrum of compound **3h**

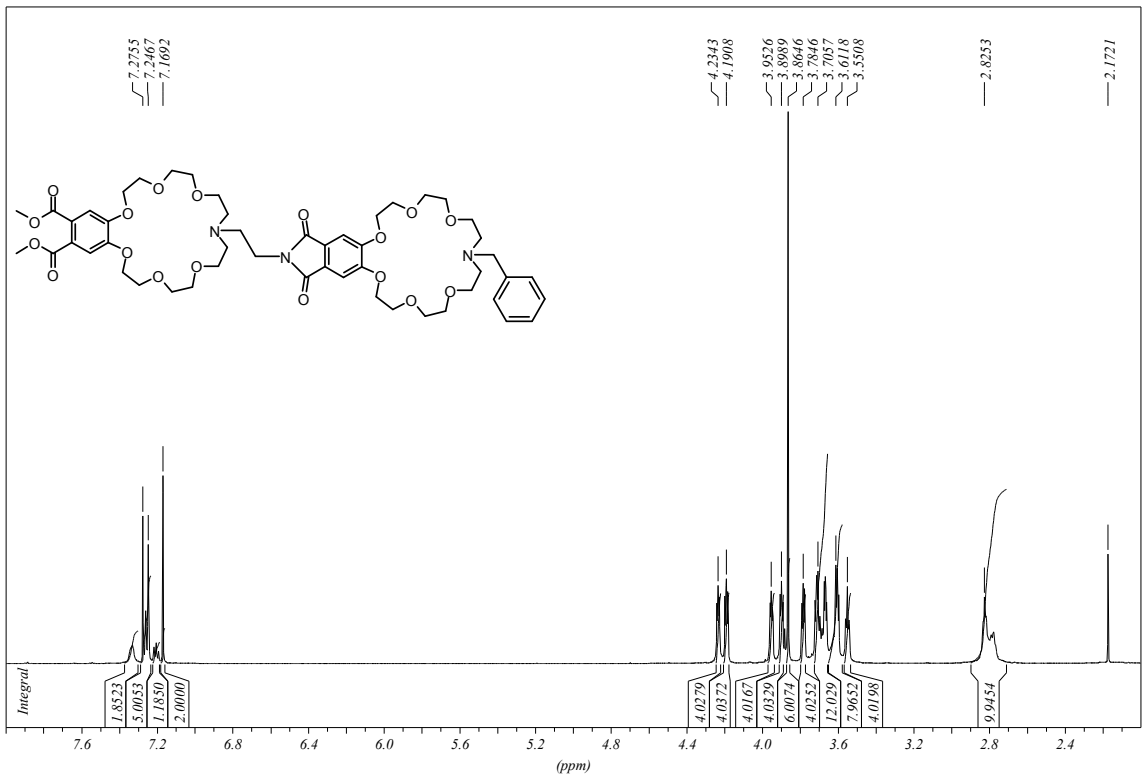


Figure 5: ^{1}H-NMR spectrum of compound **6b**

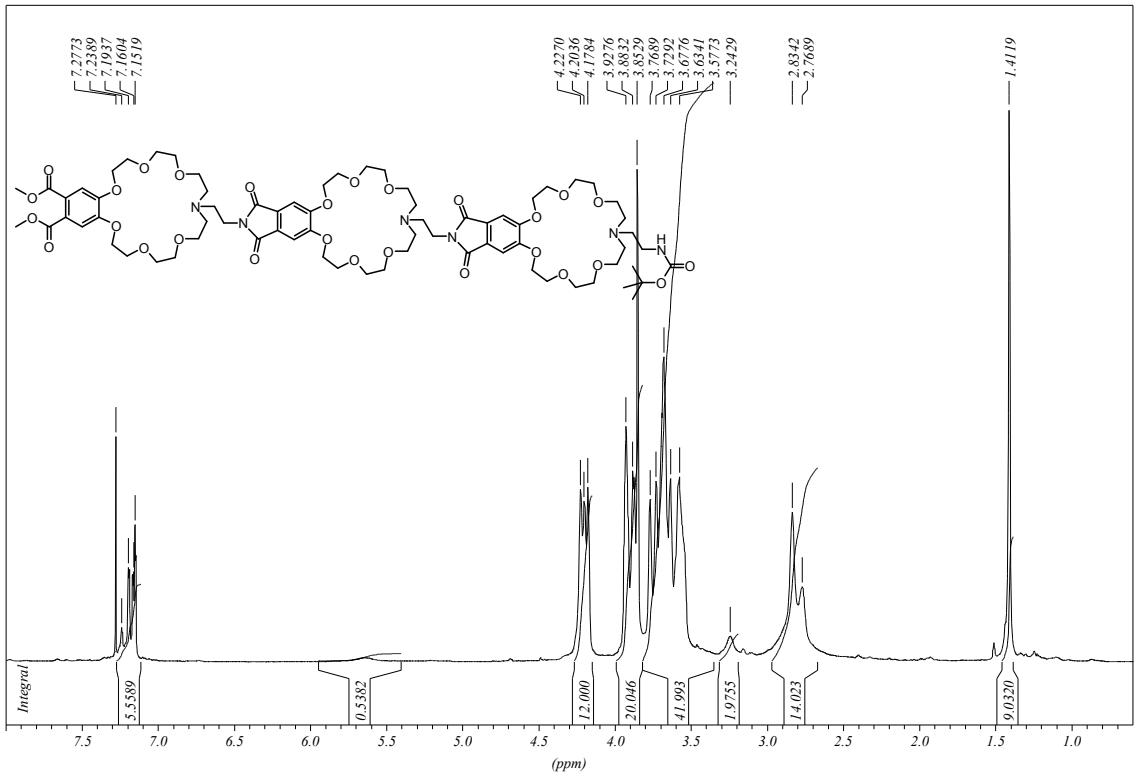


Figure 6: ^{1}H-NMR spectrum of compound **7a**

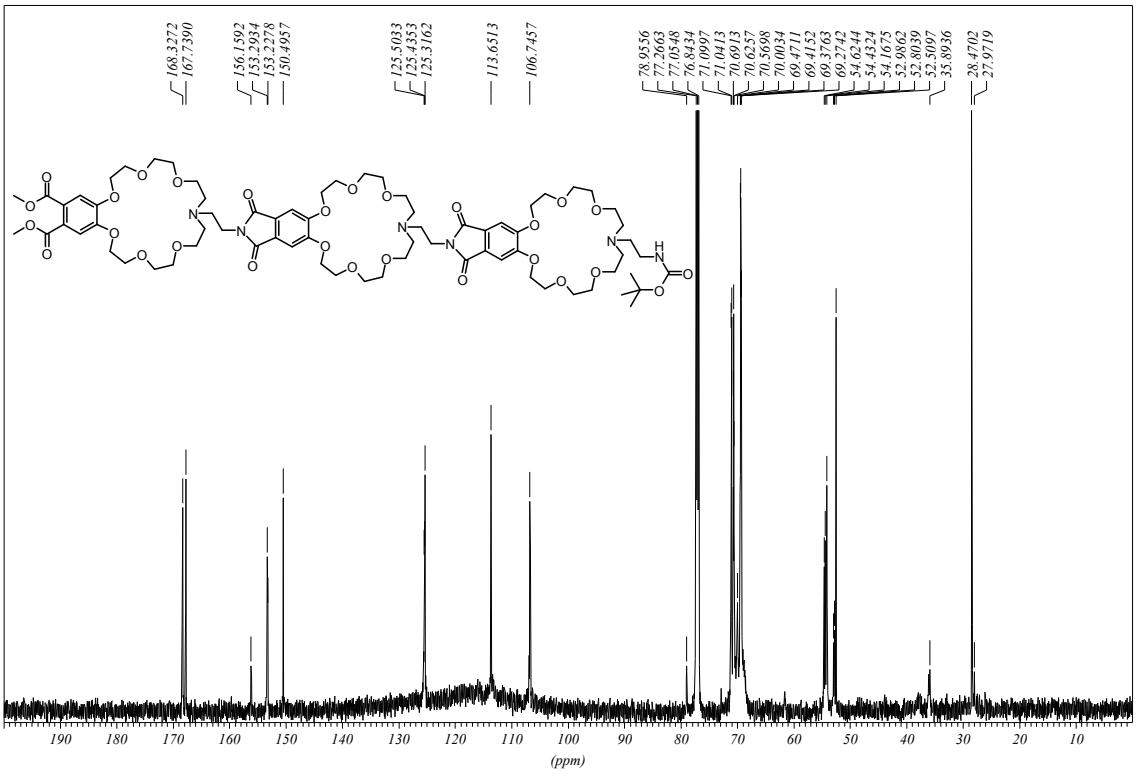


Figure 7: ^{13}C-NMR spectrum of compound **7a**

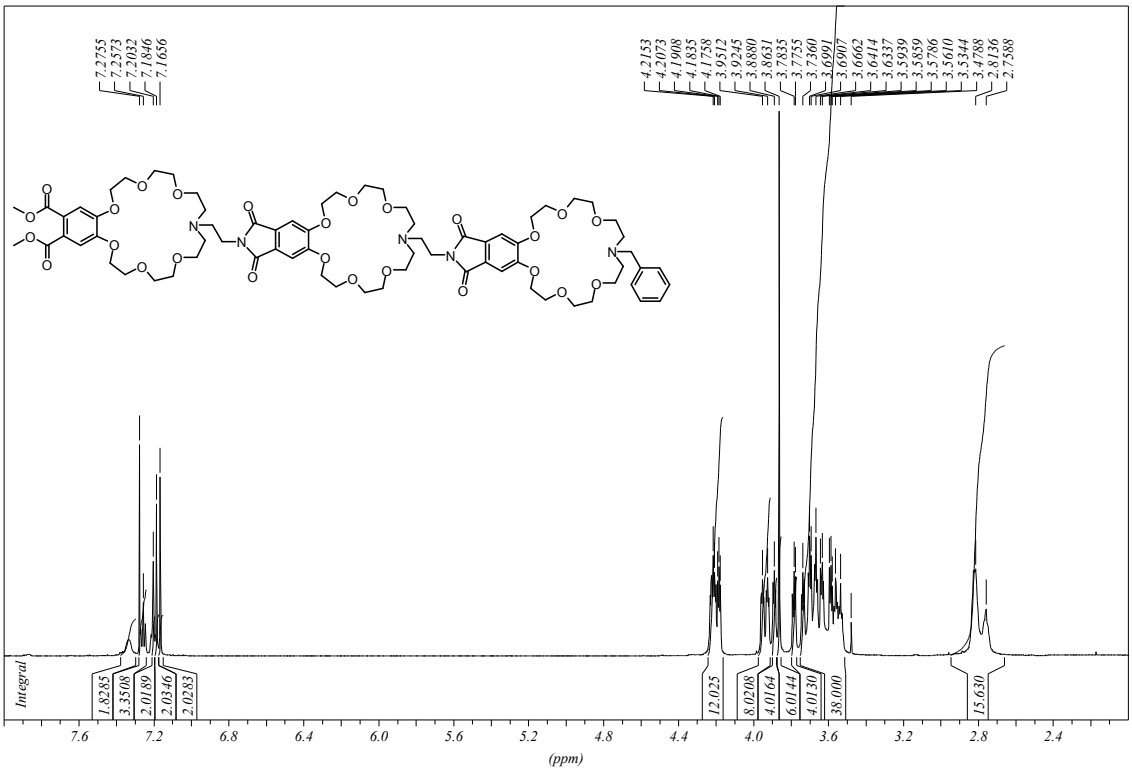


Figure 8: ^{1}H-NMR spectrum of compound **7b**

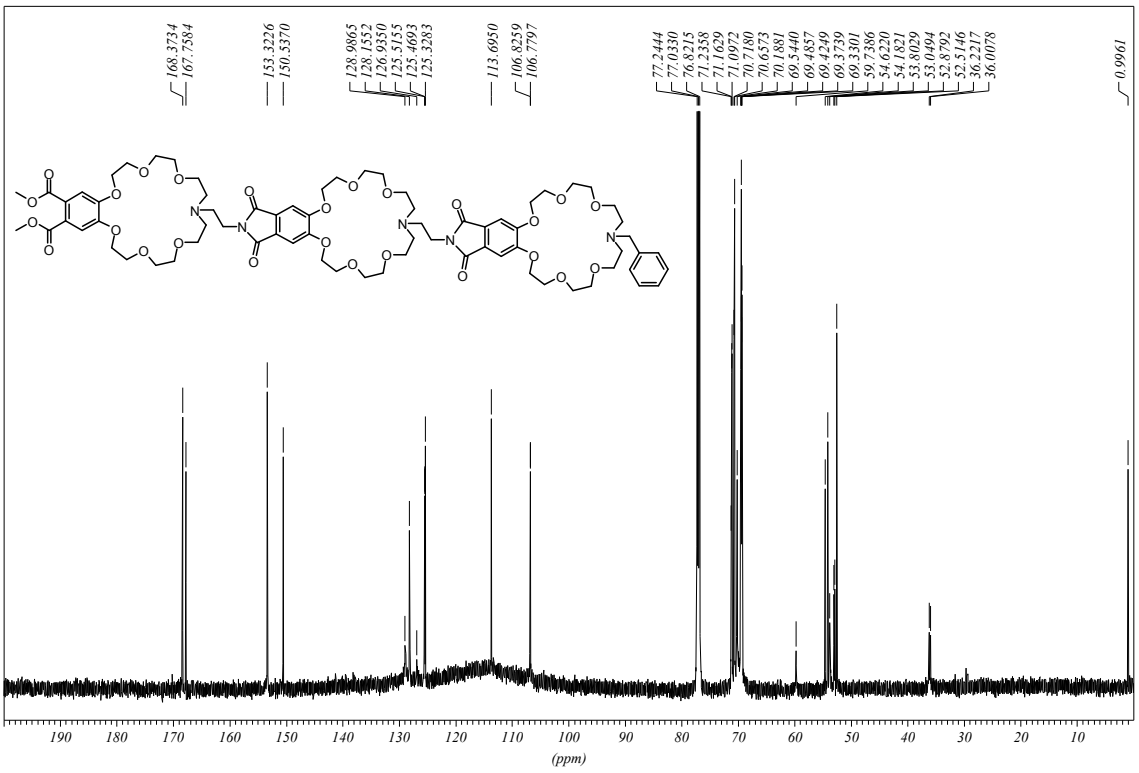


Figure 9: ^{13}C-NMR spectrum of compound **7b**

II. 4.5. Fluorescence Titration Data

Emission Spectroscopy. Fluorescence measurements were performed with UV-grade solvents (Baker or Merck) in 1 cm quartz cuvettes (Hellma) and recorded on a Varian 'Cary Eclipse' fluorescence spectrophotometer with temperature control. To determine the binding constants and Job´s plots fluorescence titration experiments were carried out. The emission intensity was volume corrected, plotted against guest concentration and analysed by nonlinear fitting.

Instrument Parameters	Excitation wavelength:	λ_{ex} = 309 nm
	Detection wavelength:	λ = 320 – 600 nm
	Concentration [receptor]:	c = $2*10^{-5}$ mol/L
	Concentration [guest]:	c = $1*10^{-4} – 5*10^{-3}$ mol/L
	Temperature:	T = 298 K
	PMT voltage:	U = 800 volts

II. 4.5.1. Emission Titrations and Job´s Plot Analyses with Lysine Esters

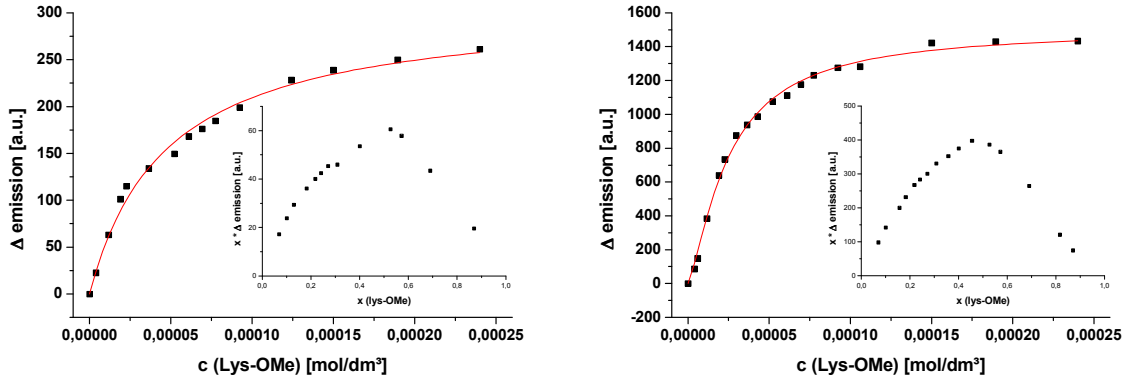


Figure 10: Emission titration of **6b** with H-Lys-OMe hydrochloride in methanol; Small inserts: Job´s plots

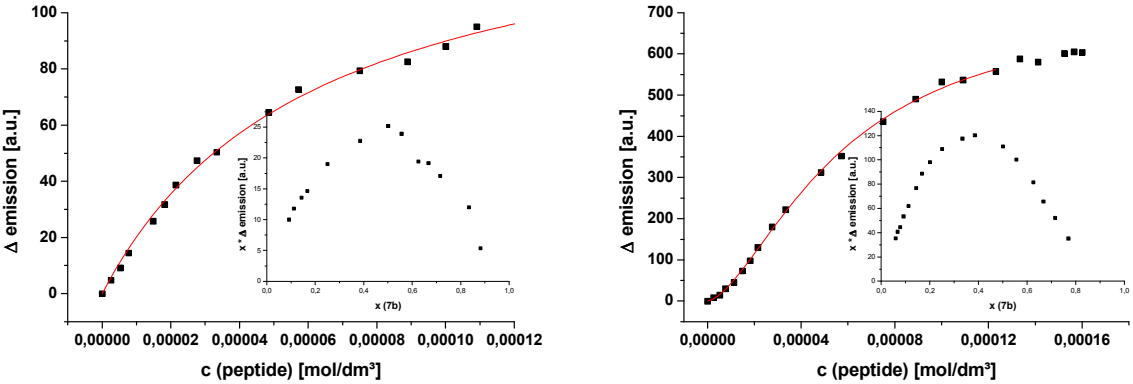


Figure 11: Emission titration of **7b** with H-Lys-Lys-OMe hydrochloride in methanol; Small inserts: Job´s plots

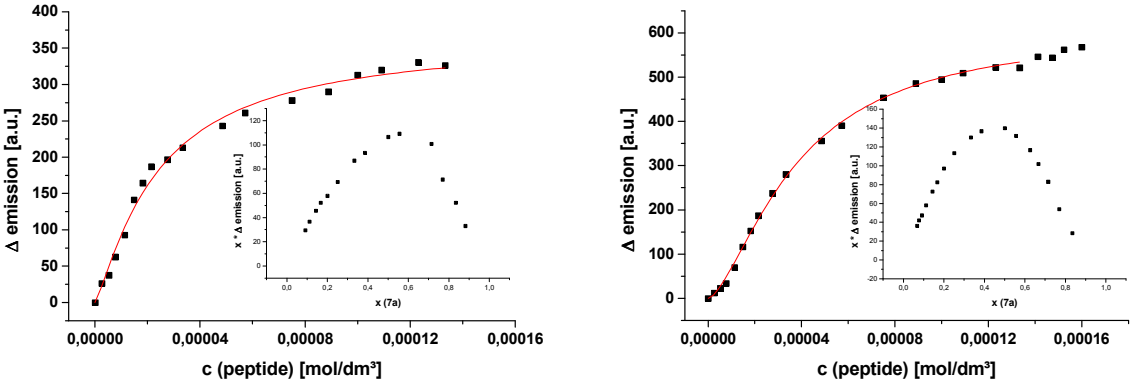


Figure 12: Emission titration of **7a** with H-Lys-Lys-OMe hydrochloride in methanol; Small inserts: Job´s plots

II. 4.5.2. Titration Curves for Isomeric Lysine Tetrapeptides

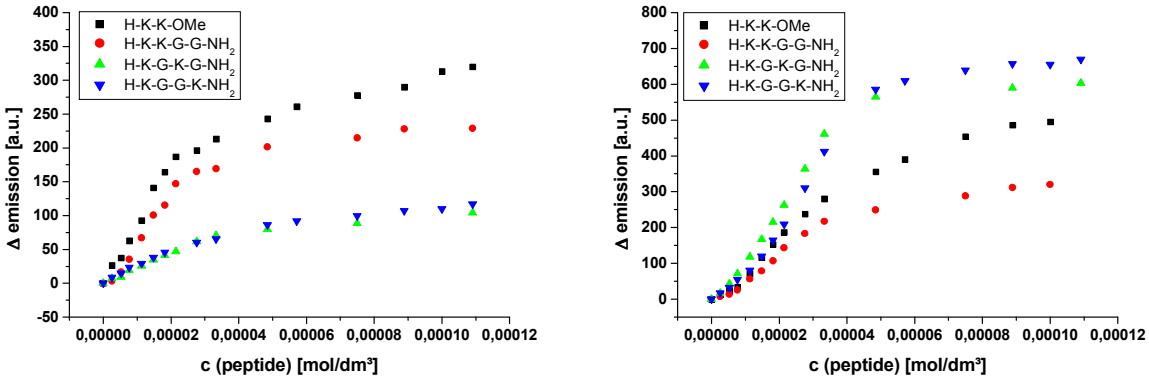


Figure 13: Titration curves for the trimeric receptor **7a** (c = $2*10^{-5}$ M^{-1}) with tetrapeptides trihydrotriflates and H-K-K-OMe hydrochloride (c = $1*10^{-4}$ M^{-1}) in methanol (phthalic ester moiety left and phthalimide moieties right)

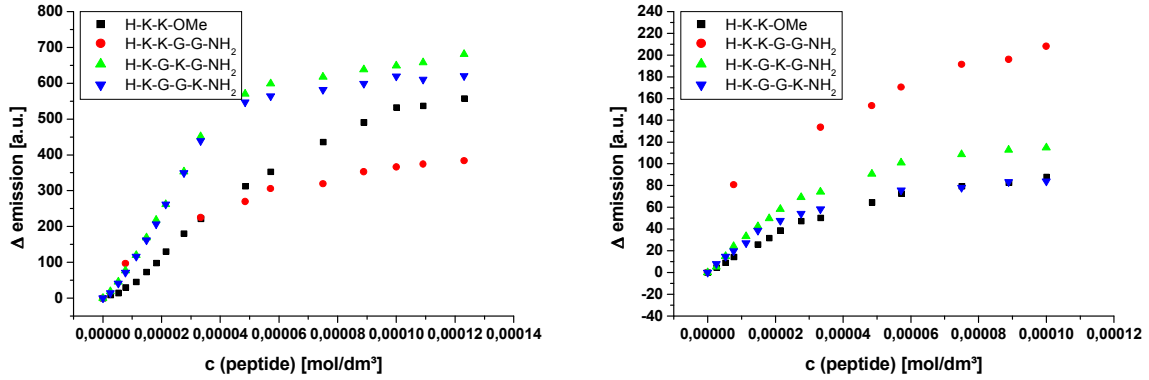


Figure 14: Titration curves for the trimeric receptor **7b** (c = $2*10^{-5}$ M^{-1}) with tetrapeptides trihydrotriflates and H-K-K-OMe hydrochloride (c = $1*10^{-4}$ M^{-1}) in methanol (phthalic ester moiety left and phthalimide moieties right)

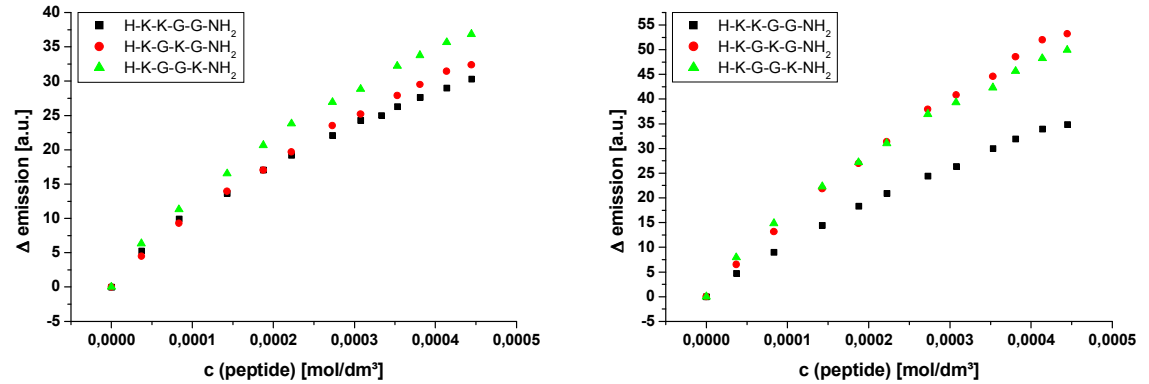


Figure 15: Comparison of the binding affinity of **7b** (left diagram) and **7a** (right diagram) to three isomeric Lys-tetrapeptides in HEPES buffered aqueous media

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III. Ditopic Crown Ether – Guanidinium Ion Receptors for the Molecular Recognition of Amino Acids and Small Peptides[i]

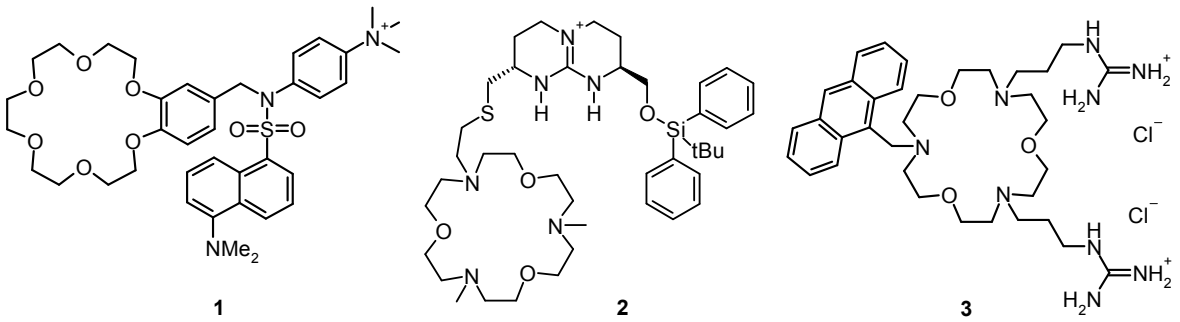
A series of ditopic synthetic receptors based on a crown ether - guanidinium ion recognition motif is reported. The compounds show binding affinity to selected amino acids, including important neurotransmitters. The effect of the distance of the ammonium and the carboxylate ion, the rigidity of the spacer and the use of pre-organized pyrrole- and pyrene-guanidinium groups on binding affinity and selectivity are discussed.

[i] A. Späth, B. König, *Tetrahedron* **2010**, *66*, 1859-1873.

III. 1. Introduction

The selective recognition of amino acids and small peptides by synthetic receptors is still a challenge, whereby the detection of analytes which are directly related to a biological function is of particular interest. Neurotransmitters are one group of such analytes. They play a critical role in living organisms:[1,2] A typical example is γ-aminobutyric acid (GABA), a non-proteinogenic γ-amino acid, which binds specifically to GABA receptors[3] located in nerve cells.[4]

The combination of crown ethers with ammonium or guanidinium ion binding sites has been successfully demonstrated in the design of synthetic amino acids receptors[5,6,7,8,9] by several examples. Crown ether **1** with a per-alkylated ammonium group was tested for peptide binding: Triglycine showed the highest binding affinity in water (K = 200 M^{-1}) and methanol (K = 13000 M^{-1}). Tripeptides bearing aromatic side chain functionalities showed a significantly increased affinity (K = 2150 M^{-1} for Gly-Trp-Gly), which was explained by additional π-stacking and hydrophobic interactions with the luminescent dansyl group.[5] Compound **2** combines a chiral bicyclic guanidinium salt for carboxylate ion binding, a triaza-crown ether as an ammonium binding moiety and a hydrophobic silyl ether. The compound was used to bind amino acid zwitterions and transfers them into an organic phase. Small hydrophilic (Ser, Gly) and aromatic (Phe, Trp), but no charged amino acids were extracted.[6] Receptor **3** binds preferably glycine, lysine and γ-aminobutyric acid. The binding is indicated by an emission intensity increase.[7] (see chapter I.3.5.)



1 2 3

Figure 1: Reported ditopic amino acid receptors containing crown ether and guanidinium or quaternary ammonium ion binding sites

We have recently developed a luminescent crown ether amino acid, which represents a particular suitable building block for the construction of amino acid receptors when combined

with guanidinium ion groups. The 21-azacrown-7 structure was optimized for ammonium ion binding.[10] Figure 2 shows the general structure of the envisaged amino acid receptors. We discuss in the following the synthesis of a series of such compounds with systematically altered structure and their binding ability towards selected amino acids.

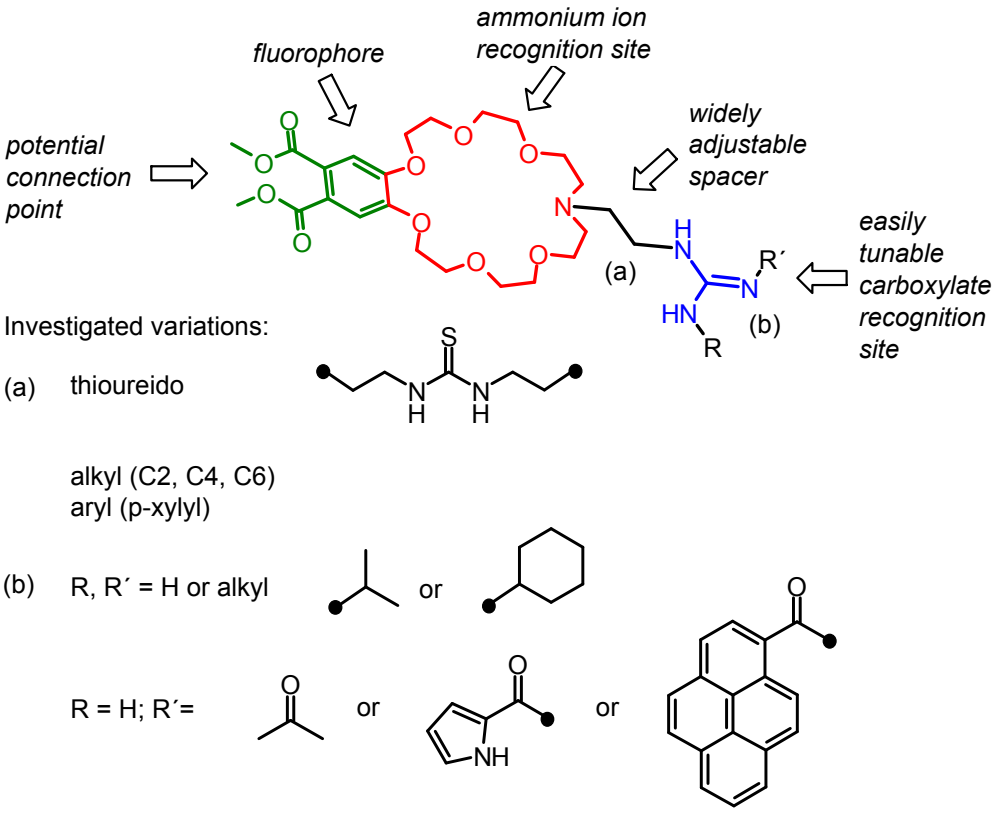
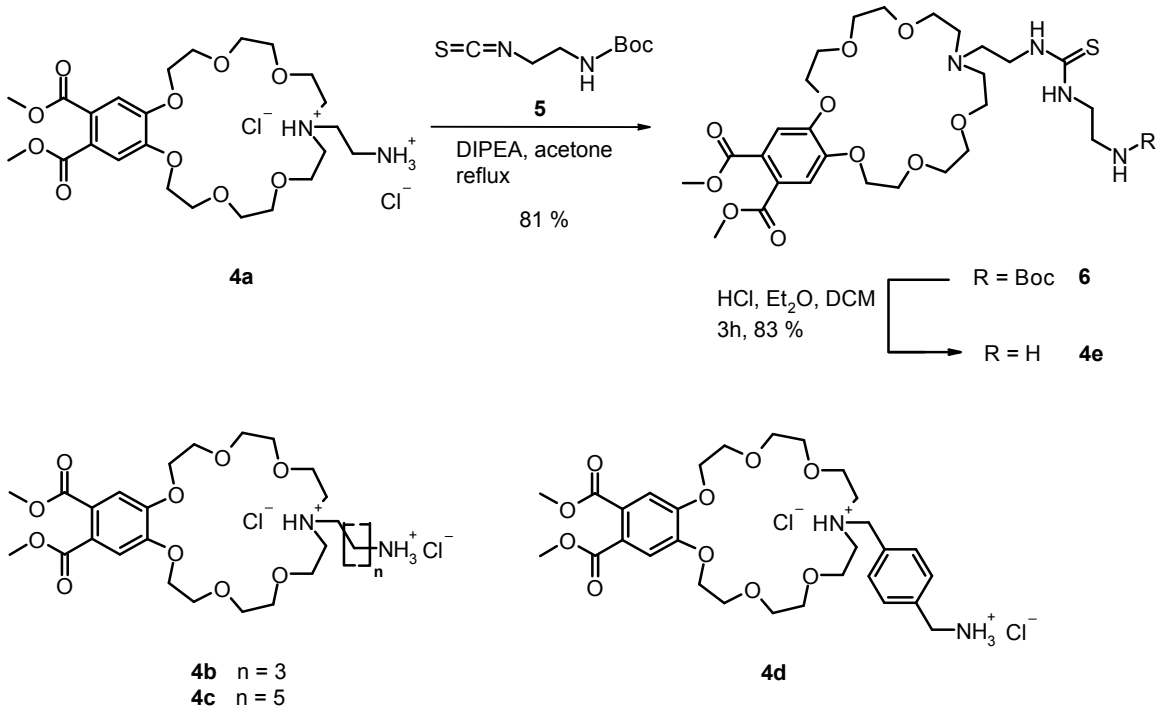


Figure 2: General structure and variations of the investigated crown ether guanidine conjugates

III. 2. Results and Discussion

III. 2.1. Syntheses

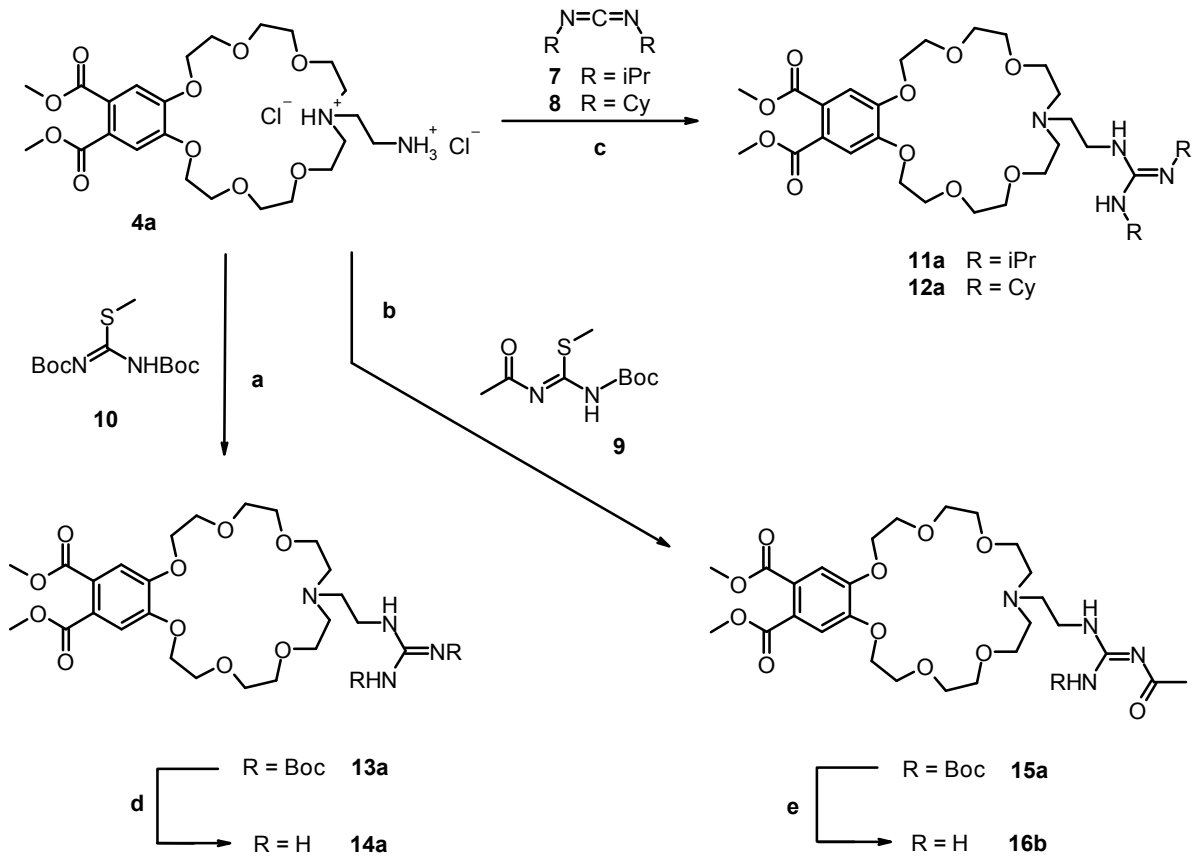
The synthesis of the amino acids receptors starts from crown ether amino esters **4a**, **4b**, **4c** and **4d** with ethyl, butyl, hexyl and *p*-xylyl spacer, respectively, connecting the crown ether dimethyl phthalate to the amino moiety. A general synthetic route for their preparation[11] and several examples[10,11] have been published previously (see also chapter II). A crown ether amino ester with thioureido **4e** was prepared from **4a** using the isothiocyanate of *N*-Boc-ethylenediamine.[12]



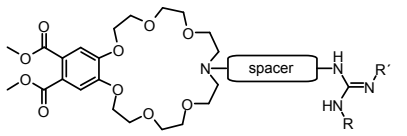
Scheme 1: Structures of crown ether amino esters **4a-e** and the synthesis of **4e** from **4a**

The synthesis of guanidines involves treatment of an amine with an electrophilic amidine species. Several reviews have been published on this topic.[13] The most commonly used reagents include derivatives of pyrazole-1-carboxyamidine,[14] triflylguanidines,[15] S-alkylisothioureas,[16] and protected thiourea[17] derivatives, the latter often activated by mercury salts[18] or EDC.[19] Alternatively phase transfer catalytic substitution[20] or the Mitsunobu reaction on carbamate-protected guanidines[21] can be employed. For the conversion of an amine to a symmetrically substituted guanidine carbodiimides[22] are often used.

Amines **4** were converted into the corresponding guanidines **11** and **12** using symmetrically substituted carbodiimides **7** or **8** under dry conditions. Guanidines **14** and **16** were obtained from reaction with methyl-isothiourea and subsequent deprotection.[23,24] The routes allow the introduction of a broad variety of substitutents in good overall yield. The reactions are shown exemplarily for the conversion of **4a** into **11a**, **12a, 14a**[25] and **16a** in scheme 2. Compound **4b** was converted analogously into **11b**, **12b, 14b** and **16b**, compound **4c** into **11c, 12c, 14c** and **16c**, compound **4d** into **11d, 12d, 14d** and **16d** and compound **4e** into **11e, 12e, 14e** and **16e**. Table 1 summarizes the yields of the transformations.



Scheme 2: Routes to the crown ether guanidine receptors; *conditions: a) NEt_3, $CHCl_3$ (dry), 2 d, RT to 40 °C; 78 %; b) NEt_3, $HgCl_2$, DMF (dry), $CHCl_3$ (dry), RT 2 - 4 h, over night 40 °C; 71 %; c) 5 eq. DIC or DCC, DIPEA, dry MeCN or THF, 4 d, reflux under nitrogen; 52 - 57 %; ^{i}Pr = isopropyl; Cy = cyclohexyl; d) CH_2Cl_2, HCl in Et_2O, 5 h; 95 %; e) CH_2Cl_2, HCl in Et_2O, 4 – 6 h, RT; quant.*

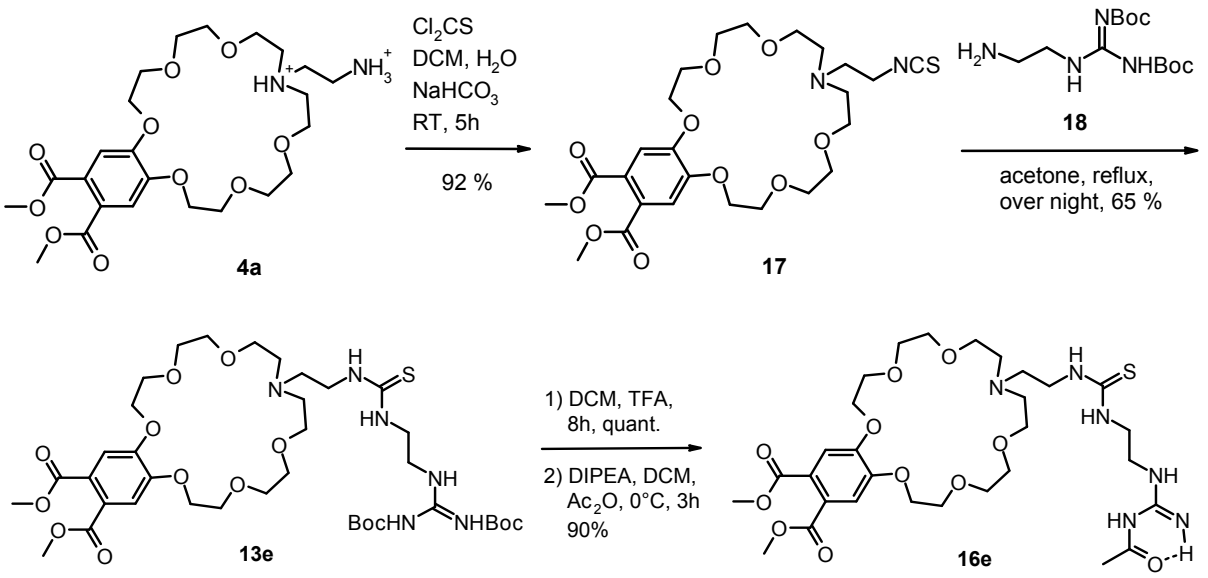
		**Yields of corresponding guanidines [%]**			
Amino ester	**Spacer**	**11** (R, R´= ^{i}Pr)	**12** (R, R´= Cy)	**14** (R, R´= H)	**16** (R = H, R´= Ac)
4a	Ethyl	**11a:** 69	**12a:** 76	**14a:** 78 (96)[a]	**16a:** 69 (91)[a]
4b	Butyl	**11b:** 58	**12b:** 62	**14b:** 75 (94)[a]	**16b:** 74 (90)[a]
4c	Hexyl	**11c:** 29	**12c:** 34	**14c:** 51 (78)[a]	**16c:** 43 (86)[a]
4d	*p*-Xylyl	**11d:** 61	**12d:** 68	**14d:** 82 (95)[a]	**16d:** 73 (93)[a]
4e	Bis-ethyl-thioureido	**11e:** 36	**12e:** 39	**14e:** 42 (76)[a]	-[b]

[a] The yield of the deprotection reaction is given in brackets. [b] Not prepared.

Table 1: Structures and yields of prepared crown ether guanidinium compounds with different spacer units

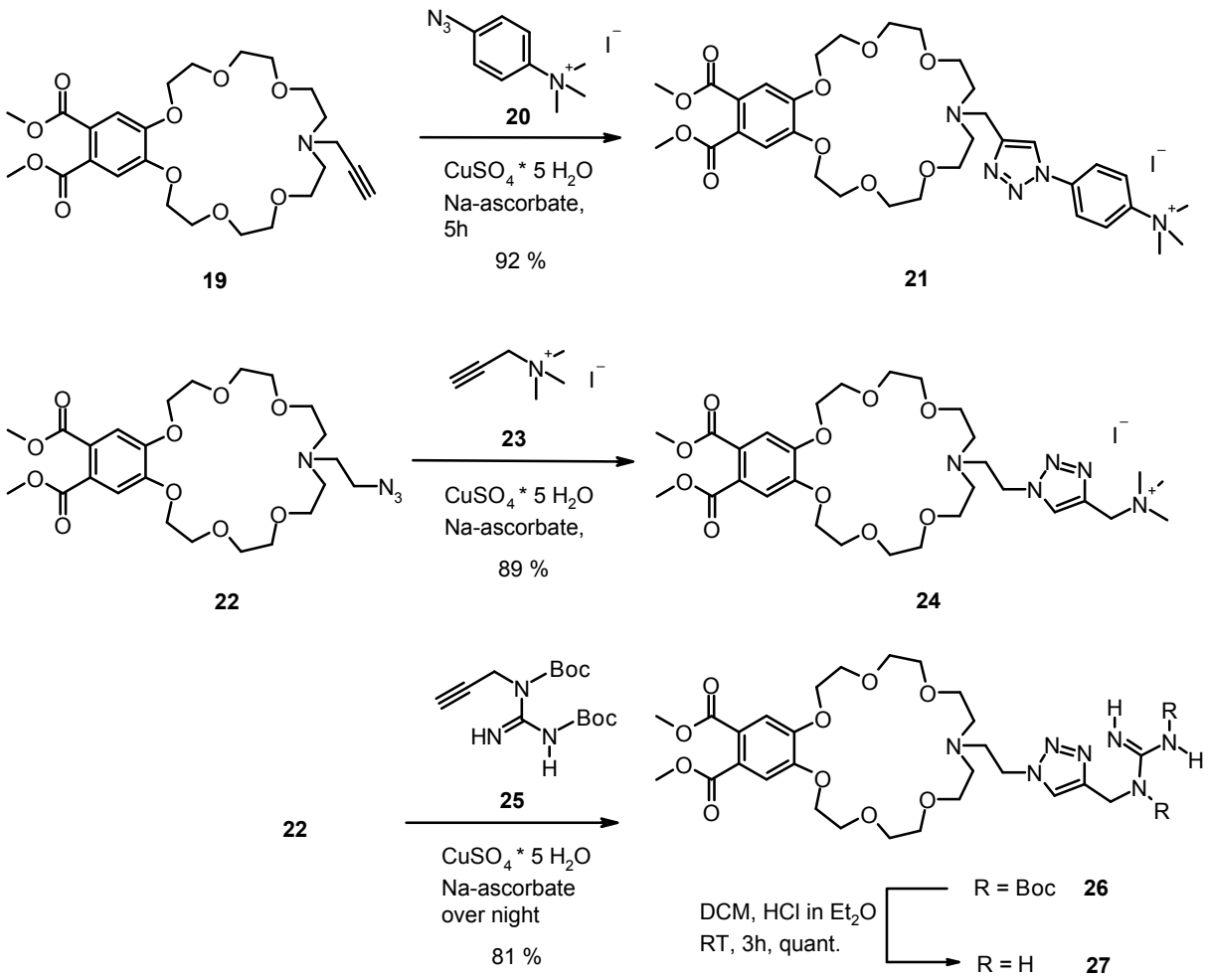
Acetylation as in compounds **16** increases the acidity of the guanidinium NHs and pre-organizes the host by intramolecular hydrogen bonds, resulting in more directional H-bonds aiding the guest binding.[32] In competitive solvents the binding strength depends mainly on the energy penalty necessary to remove the solvation shell around the host.[26] Large alkyl and aryl substitution patterns can serve as an unpolar shielding at the guanidinium site, excluding the more destabilizing influences of the surrounding polar solvent.[27]

For the synthesis of compound **16e** a different route (Scheme 3) was used: **4a** was converted into isothiocyanate **17**, reacted with the Boc-protected amine **18** to give **13e**, which was deprotected and acylated to yield **16e**.



Scheme 3: Alternative synthesis for crown ether guanidinium combinations (**13e** and **16e**) with thioureido spacer

For comparison with the effect of the guanidinium moieties, quaternary amines were introduced as carboxylate binding sites.[5,28] Two such receptors **21** and **24**, and one guanidinium substituted compound **27** were prepared by the Huisgen cycloaddition reaction. The starting alkyne **19** and azide **22** have been published recently.[11] 4-Azidoaniline[29] and propargylamine were converted into their corresponding iodide salts **20** and **23** in high yields. The protected guanidine **25** resulted from the reaction of propargylbromide with bis-Boc-protected guanidine under phase transfer conditions in excellent yield.[30]

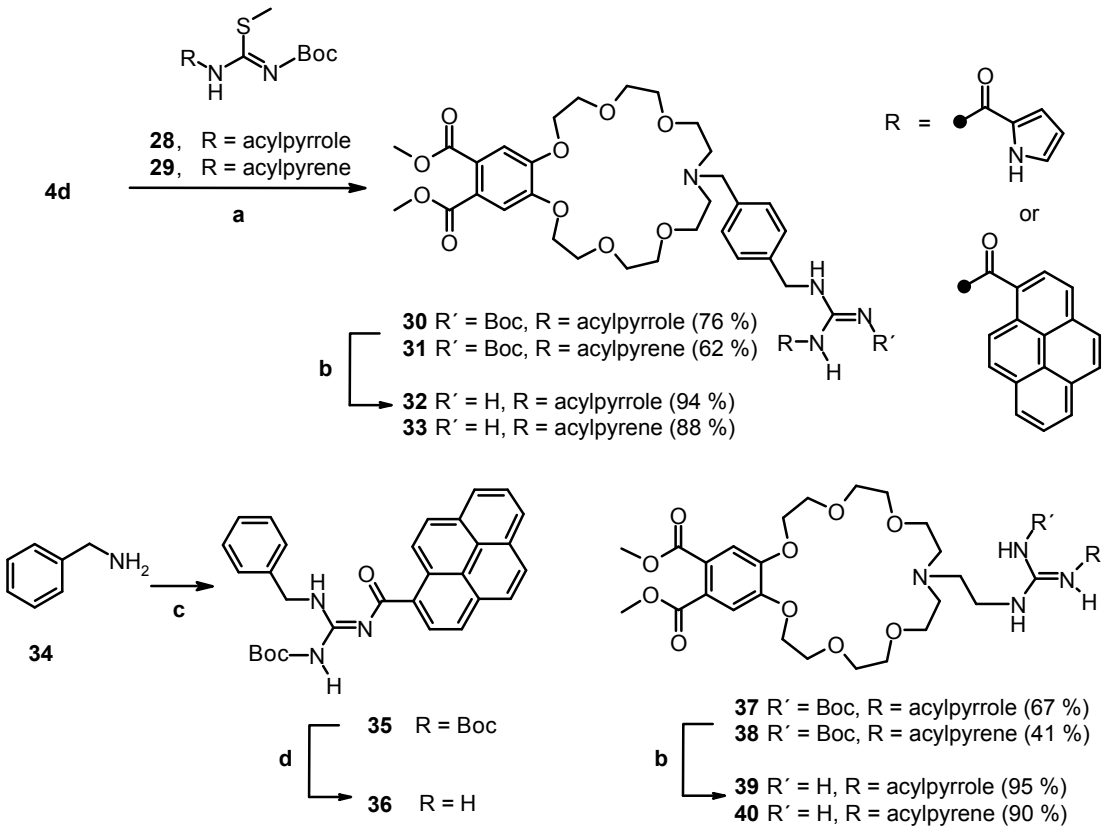


Scheme 4: Receptors **21**, **24** and **27** for ammonium and carboxylate ion binding obtained by "click reaction"

The Schmuck group developed a binding site for carboxylates by connecting a guanidinium-group with pyrrole building blocks.[31,32] It has been demonstrated, that additional H-bonds, hydrophobic effects and π-interactions enhance the binding strength, but mainly lead to a better selectivity of recognition. Pyrene features π-stacking abilities and can supply an unpolar shielding area. The fluorophore has been extensively used, e.g. developing fluorescent sensors for phosphates or DNA.[33,34] As binding site, the anticipated complexation with carboxylate anions will alter the redox potential of the guanidinium ion, which may change the emission properties of the fluorophore.

The guanidinium - pyrrole and guanidinium - pyrene compounds were prepared starting from amines **4a** and **4d**. In Scheme 5 the reactions are shown for **4d**; the synthesis using **4a** is analogous leading to compounds **37** - **40**. The methyl-iso-thiourea building blocks were prepared in good yields by peptide coupling of 1-*N*-Boc-2-methyl-iso-thiourea with pyrrole-2-

carboxylic acid and pyrene-1-carboxylic acid, respectively. Compound **36** was prepared for comparison to probe the effect of the crown ether moiety on the binding.



Scheme 5: Synthesis of crown ether receptors **32**, **33, 39** and **40** with pyrrolecarbonyl-guanidinium and pyrenecarbonyl-guanidinium groups; Synthesis of model compound **36**; *conditions: a) NEt_3, Hg^{2+}, DMF, $CHCl_3$, over night, RT; 69 %; b) CH_2Cl_2, HCl in Et_2O, 4 – 6 h, RT; quant.; c) DMF, Hg^{2+}, RT, then 40°C, 6 h, 91 %; d) MeOH, $HCl_{(aq)}$, 3 h, RT; quant.*

Peptides for the determination of binding selectivity were prepared in solution and by standard solid phase methods (see experimental part).

III. 2.2. Recognition Properties of Ditopic Amino Acid Receptors

III. 2.2.1. Photophysical Properties

Compounds **11** to **16** show absorption maxima in methanol at 220 nm and 270 nm (Figure 3), and emit upon excitation at 390 nm (Figure 4) with a quantum yield of about $\phi = 0.1$.[35] The absorption and emission properties are only marginally affected by the nature of the substituent R.[36] Compounds **21**, **24** and **27** behave similar, only **21** with its triazolyl-anilinium system in the side chain shows a stronger absorption at 270 nm. The emission maximum and quantum yield of these compounds match with the values observed for the receptors **11** to **16**.

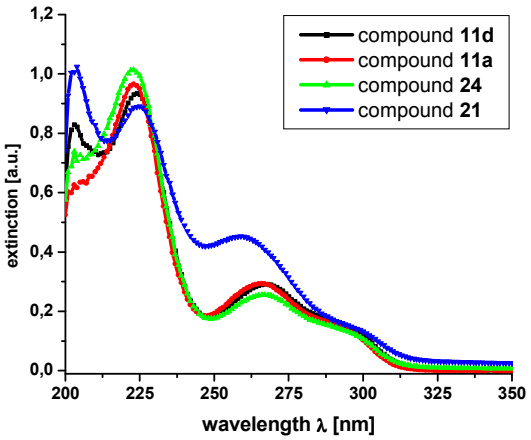


Figure 3: Absorption spectra of compounds **11a**, **11d** (c = $3.4*10^{-5}$ mol/L) and **21**, **24** (c = $3.5*10^{-5}$ mol/L) in methanol solution

The pyrrole compounds **32** and **39** absorb at 220 nm and 300 nm in methanol, and emit upon excitation at 390 nm with a quantum yield of about $\phi = 0.1$.[35] The emission spectrum of the parent molecule **14a** is given for comparison with **39**. The receptors **32** and **39** consist of two electronically not coupled parts, therefore the UV spectrum is the sum of the spectra of the pyrrole-guanidinium- (λ_{max} = 300 nm) and the crown ether unit (λ_{max} = 220 and 270 nm).

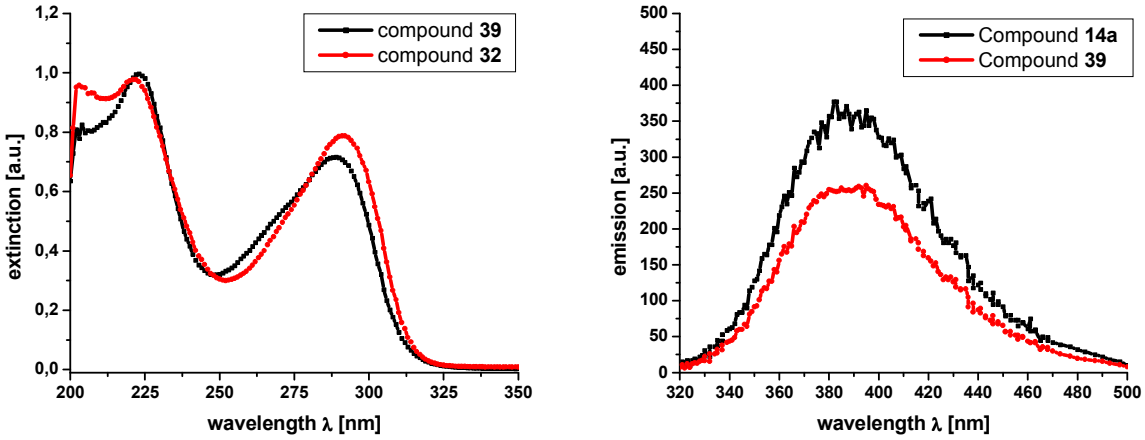


Figure 4: Absorption spectra of compounds **32** and **39** ($3.6*10^{-5}$ mol/L) and the emission of compounds **14a** and **39** in comparison (c = $2.0*10^{-5}$ mol/L) in methanol solution (λ_{ex} = 300 nm)

Compounds **33** and **40** show absorption maxima in methanol at 240 nm, 280 nm and 350 nm, and emit upon excitation at 390 nm with a quantum yield of about ϕ = 0.1.[35] Emission maxima and quantum yield are pH dependent. The emission intensity increases upon addition of acid and decreases upon base addition (Figure 5, right).

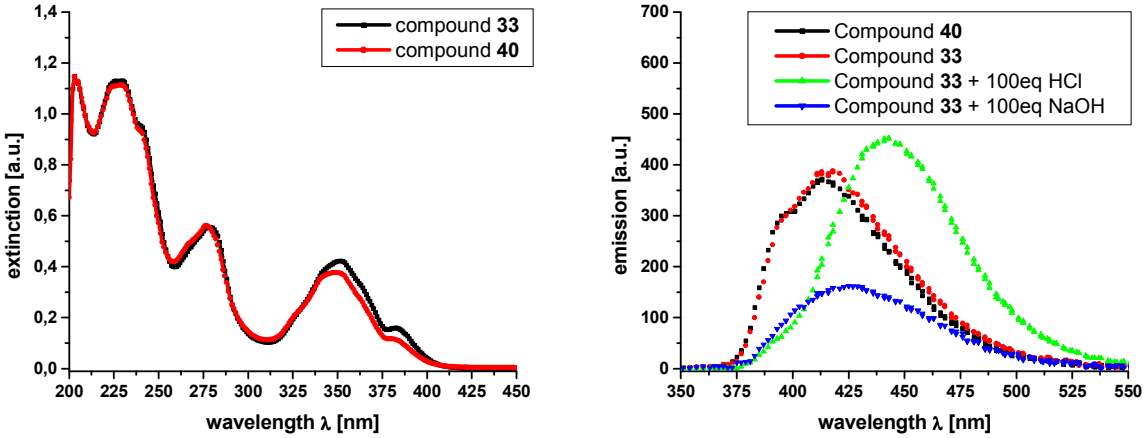


Figure 5: Absorption- (left) and emission (right) spectra of compounds **33** and **40** in methanol solution (c = $1.6*10^{-5}$ mol/L); excitation wavelength (λ_{ex}) 390 nm.

III. 2.2.2. Influence of the Protonation on the Emission – pH Range for the Measurements

Receptor protonation increases the luminescent output of the receptor compounds[37] and can therefore interfere with the amino acid binding measurements. To minimize this effect, the optimum pH range for the investigations was evaluated by recording the emission intensity of selected receptor compounds depending on the pH value. A receptor solution in methanol ($5*10^{-5}$ mol/L) was mixed with aqueous buffer solutions from pH 3 to pH 10 in the ratio 9:1.[38] After short equilibration time the fluorescence spectrum was recorded.

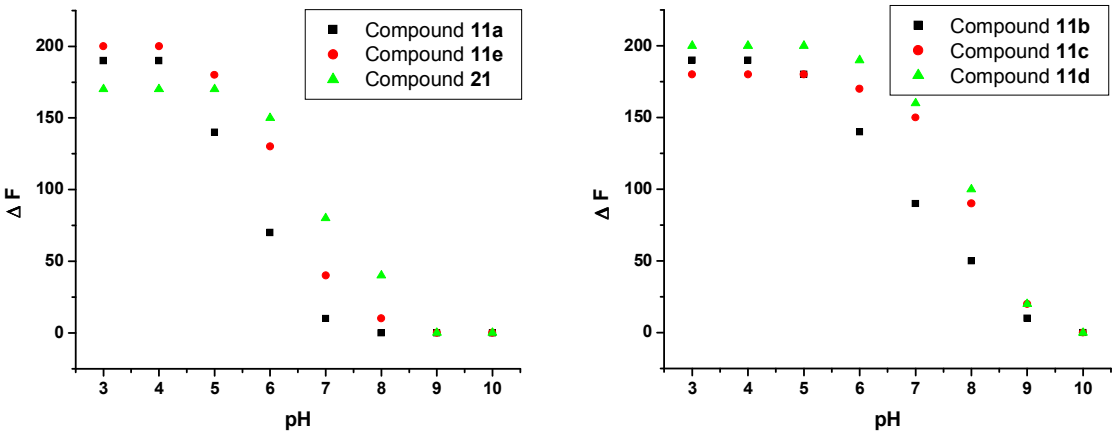


Figure 6: pH dependence of the emission intensity of selected receptors in methanol / water 9:1; λ_{max} = 300 nm

The emission of most receptors increases from neutral pH values with increasing acidity. Therefore a weakly basic pH (7.5 to 8.5) is suitable for binding measurements. Ethylene and thioureido bridged systems can be used for emission binding titrations down to a pH = 6.5 without interference. The pH range of the receptors for binding studies depends on the guanidine moiety, which has to be protonated for carboxylate ion binding.[39]

III. 2.2.3. Binding of Guanidinium and Ammonium Ions

The crown ether binding affinity for ammonium ions in aqueous solution at physiological pH is rather weak.[40] The ammonium binding properties were investigated in pH adjusted water-methanol systems using *n*-butylammonium chloride and acetyl-lysine methyl ester

hydrochloride with free side chain[41] by emission titration. Both ammonium species bind with comparable strength (K ~ 200 - 300 M^{-1} in methanol, < 100 M^{-1} in methanol / water 9:1) matching the expected value for the crown ether alone determined in previous studies.[11,10] Addition of guanidine and acetyl-guanidinium hydrochloride (up to 2000 eq.) gave a negligible response in the fluorescence study.

As guanidinium ions show affinity to crown ether moieties,[42] a potential inter- or intramolecular self-aggregation of the guanidinium-crown ether receptors **11a/c, 12a/c, 14a/c** and **16a/c** under the experimental conditions was investigated. Self-aggregation would lead to competing equilibria and altered stoichiometries, which may complicate the determination of affinity constants. NMR dilution experiments revealed that unsubstituted moieties (**11a** and **11c**) showed minimal chemically induced shifts and weak aggregation in chloroform, whereas in aqueous methanol no aggregation was observed.

III. 2.2.4. Screenings of the Receptor Library with Amino Acids and Small Peptides

Selectivities and relative response depending on the spacer length and the substitution pattern of the carboxylate binding sites were screened with compounds **11a-e, 12a-e, 14a-e, 16a-e, 21, 24** and **27**.

Different amino acids were selected as guests representing every mode of distance dependent binding, all kinds of polarity, charge, basicity and acidity.[43] To study the influence of rigidity of the guest on binding, differently long glycine sequences and β-, γ- and ε-amino acids, such as β-alanine, GABA and AHX, were chosen. Upon binding the fluorescence increased. Taking into account the error of the spectrometer and the well plate reading system, only events with more than 10 % increase were registered as binding and the fluorescence enhancement factors (z) were calculated as:

$$z = \frac{F}{F0}\left(\frac{v0 + v}{v0}\right)$$

F: Observed Fluorescence
F_0: Fluorescence of blanc sample
v_0: Volume before addition
v: Volume addition

Equation 1: Calculation of fluorescence enhancement factors with volume correction

The increase of emission increase is a relative indicator for the binding strength.

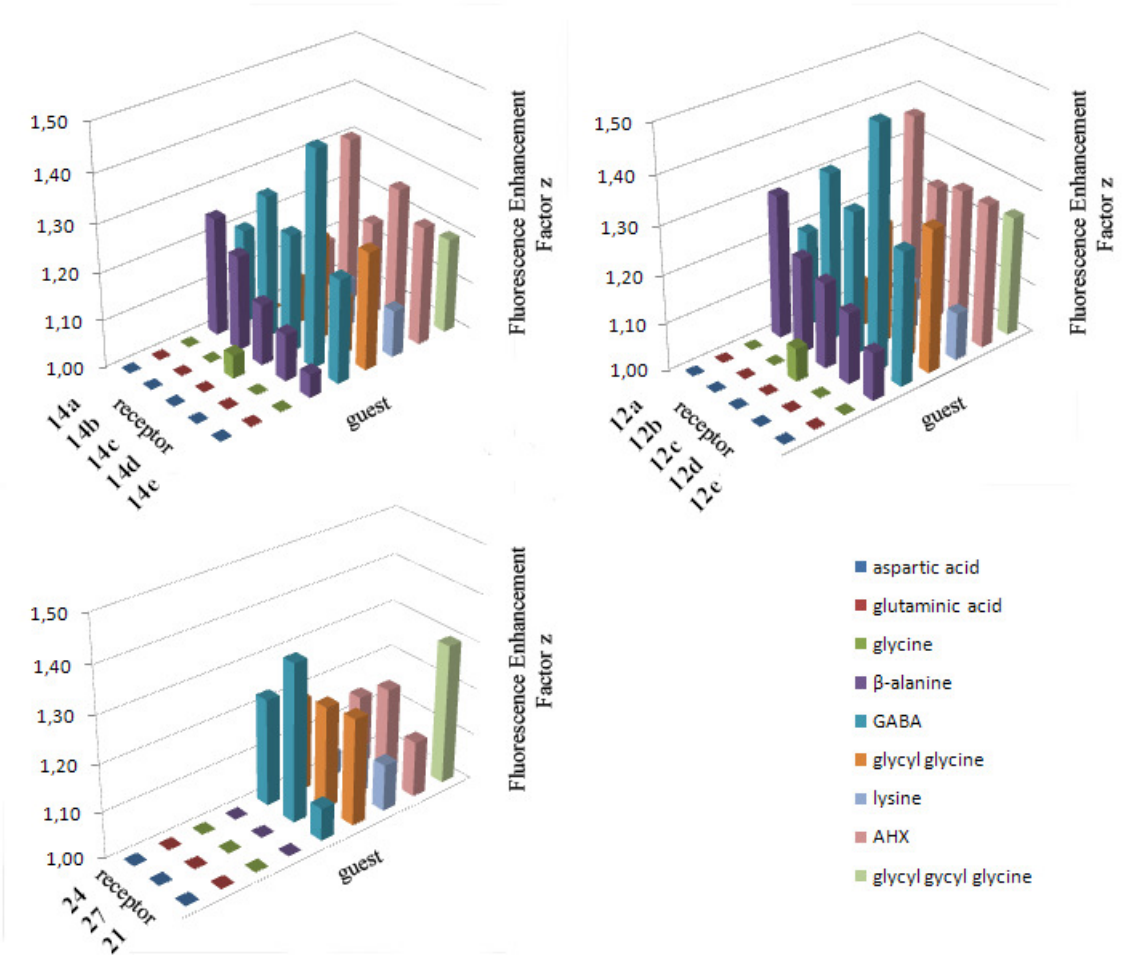


Figure 7: Selectivities and fluorescence enhancement factors of representative molecules; *conditions: 2*10^{-5} molar receptor in MeOH / water 9:1 at pH 7.5 to 8.5 adjusted with Et_4NOH or HCl; excitation wavelength 300 nm, emission wavelength 390 nm; the errors are estimated as ~ 10 %; [guest] = 0.02 M.*

Asparagine, glutamine, leucine, phenylalanine, serine, cysteine, methionine, guanidine hydrochloride and tetrabutylammonium acetate were also tested in the microtiter array and gave no response. In addition, the fluorescence response upon strong increase of the polarity[44] in the solution was investigated with 0.1 M sodium perchlorate in aqueous methanol and no change in emission intensity or shift of the fluorescence was observed.[45]

The receptors display distance dependent recognition ability. Only compounds with the short ethylene (**11a**, **12a**, **14a** and **16a**), the rigid xylylene (**11d**, **12d**, **14d** and **16d**) spacer or the triazole system with an appended aromatic quaternary amine (**21**) showed moderate to good binding strengths and selectivities. Especially the xylylene spacered molecules responded very well and a significant increase of the emission intensity was observed with GABA (n =

4). It is especially noteworthy, that glycine and glutamic acid, the physiological precursor of GABA, induced a negligible fluorescence response, even if added in large excess with respect to the sensor. With receptors **24** and **27** good selectivities, but weak binding was observed. Receptors bridged by longer alkyl chains were rather unselective and weak binders. The guanidines carrying cyclohexyl substituents showed no enhanced binding. All acetyl-guanidines were stable under the experimental conditions.[46]

III. 2.2.5. Selectivities of Crown-Guanidino-Pyrroles and -Pyrenes

Upon the addition of a carboxylate guest, the UV bands of **33**, **36** and **40** became broader and red shifted. In the fluorescence spectra a change in emission from 410 nm to 440 nm accompanied by quenching is observed. No excimer formation can be found in the presence of the charged guanidinium moiety. These receptors bind with the same selectivities as their isopropyl substituted analogues. Guest addition results in a gradual decrease of luminescence intensity, which allows monitoring of the binding event by optical spectroscopy.

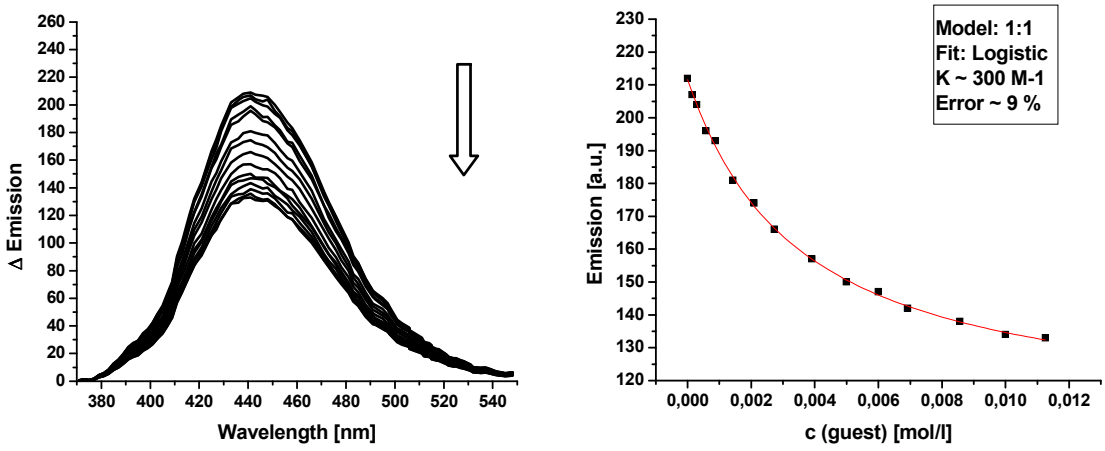


Figure 8: Emission spectrum of **33** ($8*10^{-6}$ molar, λ_{ex} = 350 nm, pH 6.5) at different ratios of γ-aminobutyric acid in aqueous methanol (see binding studies).

The binding of the receptors with pyrrole substituents **32** and **39** cannot be screened in the microtiter array due to the strong absorbance of the pyrrole system at 300 nm overlapping with the crown ether luminophore. No interpretable change in fluorescence intensity arises when a guest is added. The receptors were therefore investigated by UV spectroscopy in a cuvette. Following the results of the first screening only β-alanine, γ-aminobutyric acid and ε-

aminohexanoic acid were compared to glycine, glycyl glycine, lysine, aspartic and glutamic acid. In cases where a ditopic binding is possible due to the right distance, the strongest change is observed.

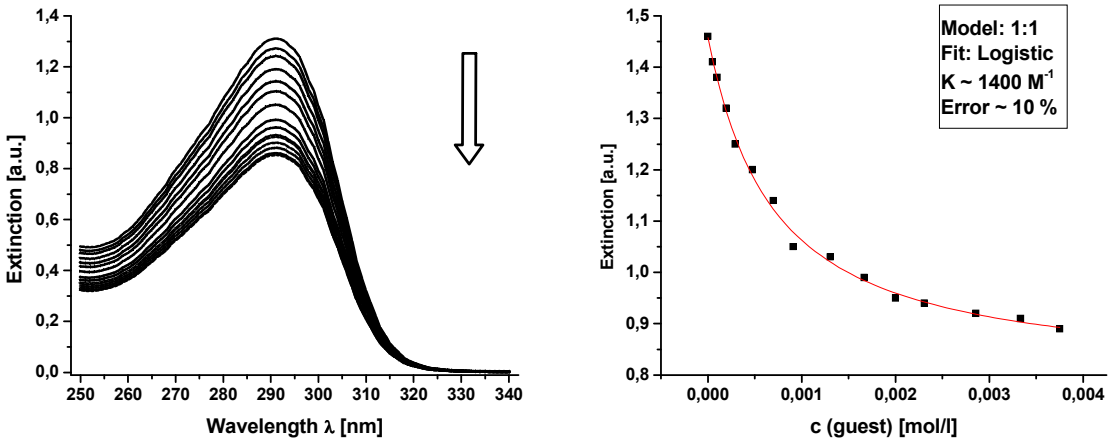


Figure 9: UV spectrum of the pyrrole part of **32** ($2*10^{-5}$ molar, pH 6.4) at different ratios of γ-aminobutyric acid in aqueous methanol (see binding studies).

III. 2.2.6. Binding of Short Peptide Sequences

The peptide binding selectivity of the crown ether with xylylene bis-isopropyl-guanidinium-binder (**11d**) was evaluated with small peptide sequences different in rigidity, distance of the ammonium and carboxylate group and substituents. A lysine-glutamic acid dipeptide was chosen due its biological relevance[47] and compared to the related tri- and tetrapeptide with one or respectively two inserted glycines in the sequence. The *N*-terminus of the peptides is acylated and the *C*-terminus functionalized as amide to allow only the interaction of the peptides side chains with the receptor. Additionally, four isomeric tetrapeptides containing glutamic acid and three glycines were used to investigate the influence of the ammonium-to-carboxylate ion distance on the binding. To quickly select the peptide sequences with highest affinity a screening was performed in aqueous methanol solution (pH adjusted to pH 8 - 8.5) using a microtiter array. Figure 10 summarizes the results.

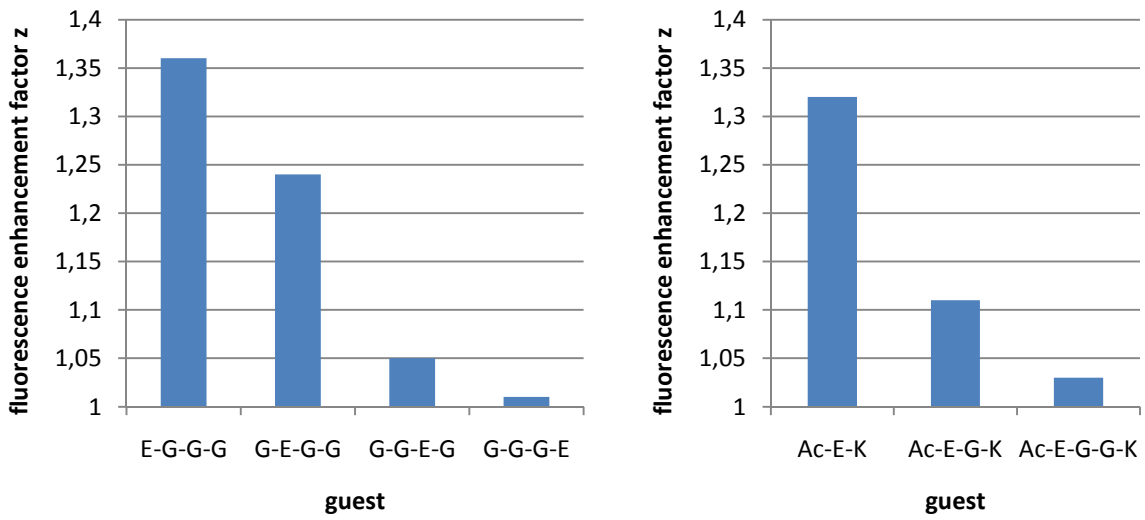


Figure 10: Fluorescence enhancement factors for **11d** with isomeric glutamic acid (E) – glycine (G) – tetrapeptides (left) and Ac-E-K-NH_2, Ac-E-G-K-NH_2, Ac-E-G-G-K-NH_2 sequences; the error is assumed as ~ 10 %

Glu-Lys-peptides induced a significant increase in emission, revealing a preference for the shortest example (Figure 10, right). The four isomeric glutamic acid tetrapeptides show a luminescence enhancement depending on their carboxyl to *N*-terminus distance (Figure 10, left). Corresponding sequences containing glutamine instead of glutamic acid did not show an increase in emission (data not shown), supporting the need of the additional coordination to the guanidine for recognition.

The peptide sequences with significant responses in the screening assay were investigated by emission titrations with **11d** (pH 8 - 8.5) or UV measurement with **32** (pH 6.5) in aqueous methanol solution. Their binding constants (log *K*) were derived from the titration data by non linear fitting methods. The stoichiometry of the binding events was determined by Job´s plot[48] analyses. Table 2 summarizes representative values for the isomeric Glu tetrapeptides.

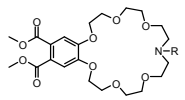
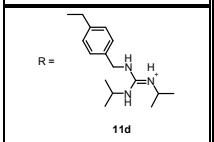
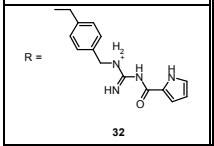
	**Binding constant *K* [M^{-1}] in methanol (10 % water)**		
	H-E-G-G-G-OH	**H-G-E-G-G-OH**	**H-G-G-E-G-H**
	400	200	< 100
	1300	1100	1000

Table 2: Binding strengths and selectivities of selected receptors versus short peptide sequences. The errors are assumed to be ~ 10 %, *conditions: T = 25 °C, counter ion is chloride, pH values for the measurements were adjusted with HCl or Et_4NOH*

Only peptide sequences which bind with their carboxyl donor site to the guanidinium group, and at the same time have an ammonium ion in the right distance available, show strong and specific response. E-G-G-G shows the highest affinity, as it provides an optimal geometry and distance to undergo bidentate coordination. Larger distances between both binding sites lead to a decrease in affinity.

Figure 11 shows the emission titration curve for Ac-Glu-Lys-amide and **11d**. Peptide Ac-Glu-Lys-amide and other Glu-Lys sequences are binding to **11d** with a stoichiometry differing from 1:1 (the red line indicates the theoretical 1:1 binding curve). Therefore, no binding affinity could be derived, but saturation in the range of a 10^{-2} molar guest concentration indicates low millimolar affinities (log *K* ~ 2 - 3).

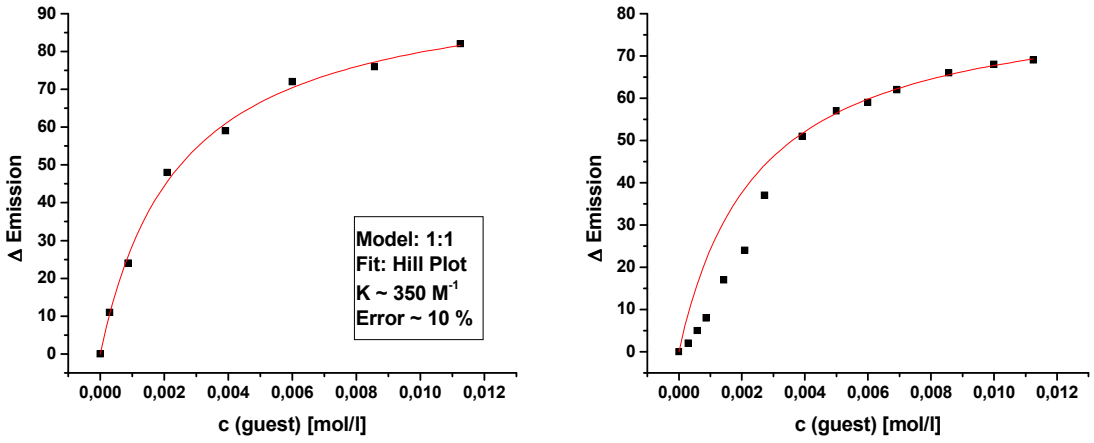


Figure 11: Emission titration curve of H-E-G-G-G-amide (left) and Ac-Glu-Lys-amide (right) with **11d** in aqueous methanol

III. 2.2.7. Fluorescence Titrations with Amino Acids

The binding affinities of the best performing receptors using the same conditions against selected amino acids were investigated. Depending on the receptor, β-alanine, γ-aminobutyric acid, ε-aminohexanoic acid, lysine and glycyl glycine were added. Lysine did not show any detectable binding event with one of the receptors. Table 3 summarizes the results. The best binding value for a particular receptor/guest-couple is indicated in bold numbers:

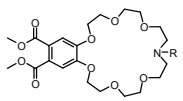
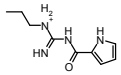
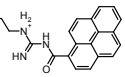
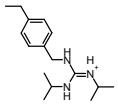
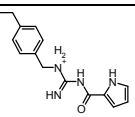
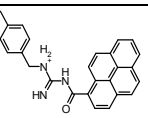
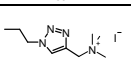
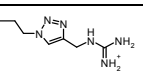
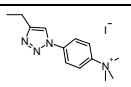
		**Binding constant *K* [M^{-1}] in methanol/water 9:1**				
		β-Ala	*GABA*	*Gly-Gly*	*AHX*	*Gly-Gly-Gly*
R =  **11a**	a)	300	200	---	< 100	---
R =  **39**	b)	**1300**	1000	---	1000	---
R =  **40**	c)	300	200	---	200	---
R =  **11d**	d)	100	**500**	---	400	---
R =  **32**	b)	n.d.	**1300**	---	1100	---
R =  **33**	c)	200	300	---	300	---
R =  **24**	a)	n.d.	100	200	< 100	---
R =  **27**	a)	n.d.	200	< 100	200	---
R =  **21**	a)	---	< 100	200	200	**600**

Table 3: Binding constants of the receptors against various guests; --- no binding detected, n.d. means not determined. The errors are assumed to be ~ 10 %, *conditions: T = 25 °C, counter ion is chloride, pH values for the measurements were adjusted with HCl or Et_4NOH to a) pH = 7.6; b) values determined by UV titration in methanol / water 9:1; c) pH = 6.4; d) pH = 8.5.*

Although the binding affinities and differences are small, a few general trends can be noted: The guanidines bind slightly better under the experimental conditions than the quaternized ammonium ions (receptors **21** and **24**). The flexibility of the binding site bridge is of importance. For the hexyl spaced receptors (**11c**, **12c**, **14c** and **16c**) all responses were too small to extract binding values (log K << 2). For the xylyl spaced materials (**11d**, **12d**, **14d** and **16d**) higher binding constants are always achieved with GABA as the preferred guest. This finds its reason in a better pre-organization of the two binding sites by the aromatic platform. The binding constants of the isopropyl-guanidines (**11a/d**) and the pyrene substituted moieties (**33** and **40**) are comparable. Figure 12 shows exemplarily the rise of luminescence in the fluorescence spectrum and the according titration curve for **11d** with GABA. Pyrrole-substituted receptors **32** and **39** (for a titration curve see Figure 9) show a twofold higher affinity for a specific guest compared to crown guanidinium receptors with isopropyl residues (**11a/d**). The pyrrole group stabilizes the aggregate by an additional H-bond and a less polar microenvironment.

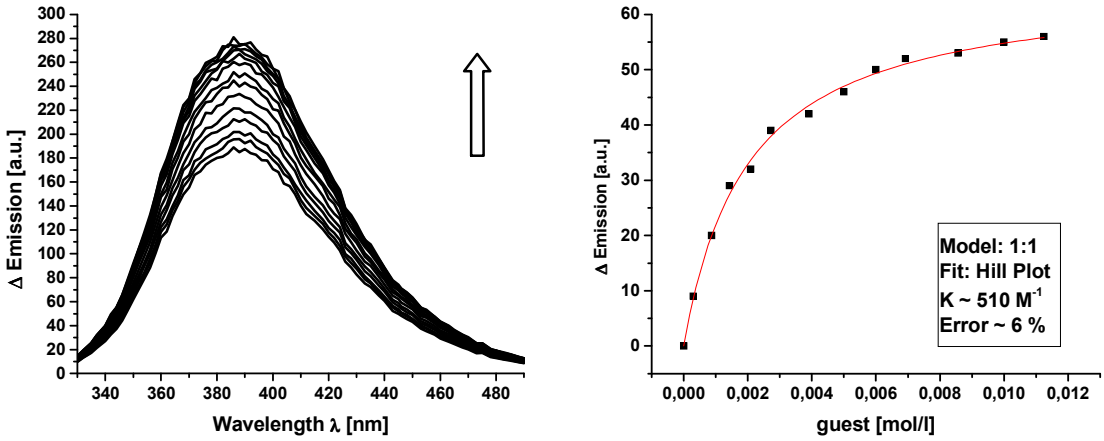


Figure 12: Fluorescent spectrum of **11d** ($2*10^{-5}$ molar, λ_{ex} = 300 nm) at different ratios of γ-aminobutyric acid in aqueous methanol

III. 3. Conclusions

We have reported receptors for zwitterionic amino acids and for the recognition of the neurotransmitter γ-aminobutyric acid (GABA) in polar protic solvents. Compound **11d** and **32** show selectivity towards GABA compared to similar amino acids and distinguish GABA from its biological precursor glutamic acid. In addition, molecule **21** was found to be suitable for recognition of tripeptides. The distance dependence of the binding affinity was demonstrated with different amino acids and small peptide sequences. Figure 13 summarizes typical titration curves and Job´s plot analyses[48] (small insertions) for the isopropyl guanidinium receptor **11d** with GABA and AHX, as well as **21** and $(Gly)_3$:

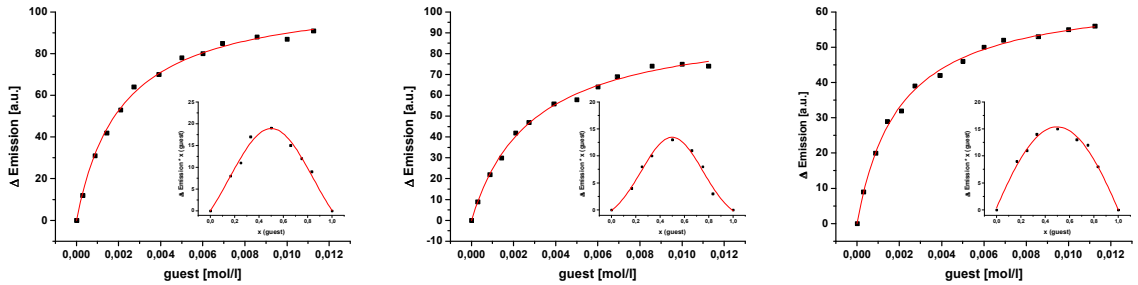


Figure 13: Comparison of the emission titrations of **11d** with GABA (left), **11d** with the similar guest AHX (middle) and **21** with Gly-Gly-Gly (right)

Based on these results, also taking into account comparable literature examples[6,8] and energy minimization studies with the aid of the program package SPARTAN, the following structure for the receptor – peptide aggregates are proposed:

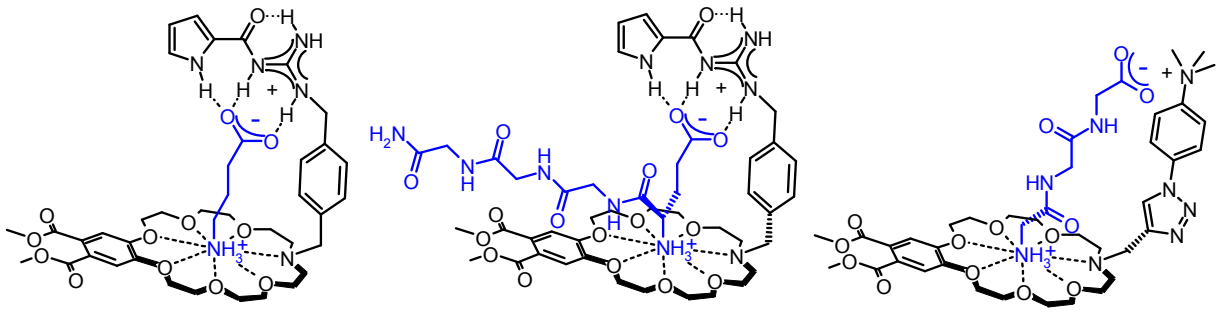


Figure 14: Illustrations of proposed structures[49] of stable peptide aggregates of GABA, Gly-Gly-Gly and Glu-Gly-Gly-Gly

In the course of the studies, we were able to lower the pH value for investigations with such systems by more than one order of magnitude in comparison to literature known examples.[5,6,7,8] Aza crown ether guanidinium systems, which are stable, applicable and insensitive to fluctuations over a broad pH range should carry alkyl chains with a maximum length of C2 appended to a carbamide, thioureido, ureido or triazole substituent[50] at the aza crown ether nitrogen atom. They allow the determination of amino acid binding at pH values close to 6.

The selectivity and affinity of the receptors is currently not sufficient for practical use in sensing of unprotected amino acids or peptides, but the investigations clearly show that the interaction of such ditopic compounds with amino acids and peptides can be rationalized on the basis of established binding motifs. This contributes to the development of a more rational design of sequence selective peptide chemosensors.

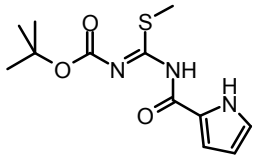
III. 4. Experimental Section

CAUTION: the noxious gas CH_3SH is produced during the synthesis of compounds **13a-e**, **15a-d**, **30**, **31**, **35**, **37** and **38.** These reactions should be only handled in the fume hood or the gas should be trapped with aqueous sodium hydroxide and destroyed with sodium hypochlorite.

Compounds **4a**,[10] **9**,[51] **18**,[52] **19**,[11] **22**,[11] **23**[53] and pyrene-1-carboxylic acid[54] were prepared in solution according to published procedures. The preparations of compounds **4b-e, 9, 11b, 11c, 11e, 12a-d, 13b, 13c, 13e, 14b, 14c, 14e, 15a-e, 16a-e, 17, 18, 20, 23, 29, 35, 36, 37, 38, 39** and **40** and the synthesis of the isomeric peptides on solid phase can be found in the supporting information of this chapter.

III. 4.1. Preparation of Building Blocks

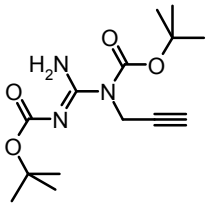
*1-(tertButyloxycarbonyl)-3-(pyrrole-2-carbonyl)-2-methyl-2-isothiourea (**28**)*



Pyrrole-2-carboxylic acid[55] (245 mg, 2.2 mmol) was dissolved in a 1:1 mixture of DMF and DCM (2.0 mL), triethylamine (0.66 mL, 0.50 g, 5.0 mmol), HOBt (405 mg, 3.0 mmol) and DCC (618 mg, 3.0 mmol) were added and it was stirred for 30 mins in an ice bath. 1-*N*-Boc-2-methyl-2-isothiourea (380 mg, 2.0 mmol) was added in one portion and the mixture was stirred for 2 h at room temperature, then heated to 40 °C and stirred for additional 4 h under nitrogen. It was diluted with 28.0 mL of ethyl acetate, the solution was filtered and the filtrate was washed with 5 % ammonium chloride solution and three times with water. The organic phase was dried over $MgSO_4$ and evaporated to dryness. The remaining solid was purified by column chromatography with ethyl acetate / petrol ether 1:2 to yield 440 mg of a pale brown solid (1.55 mmol, 78 %).

M.p. (uncorrected) = 89 – 91°C; - **^{1}H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.43 (s, 9 H), 2.48 (s, 3 H), 6.25 (m, 1 H), 6.93 (m, 1 H), 7.02 (m, 1 H), 9.28 (bs, 1 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 13.7 (+, 1 C), 27.0 (+, 3 C), 82.3 (C_{quat}, 1 C), 109.9 (+, 1 C), 113.7 (+, 1 C), 116.3 (+, 1 C), 129.3 (C_{quat}, 1 C), 149.9 (C_{quat}, 1 C), 168.0 (C_{quat}, 1 C), 169.5 (C_{quat}, 1 C); - **MS** (ESI(+), DCM/MeOH + 10 mmol NH_4OAc): m/z (%) = 284.1 (100, MH^+); - **HRMS** (EI-MS 70 eV): calc. for $C_{12}H_{17}N_3O_3S^{*+}$: 283.0991, found: 283.0988; $^+$); - **MF:** $C_{12}H_{17}N_3O_3S$ – **FW:** 283.35 g/mol;

*1,3-bis-(tertButyloxycarbonyl)-2-propargyl-guanidine (**25**)*

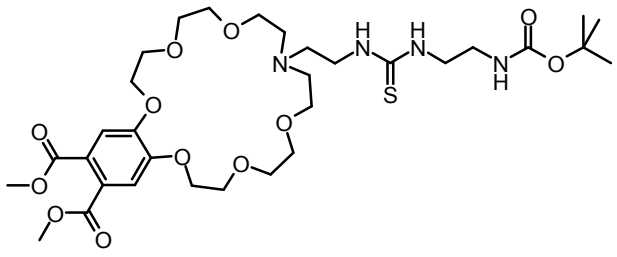


Bis-Boc-protected guanidine (519 mg, 2.0 mmol) was dissolved in DCM (5 mL). A cold solution of potassium hydroxide (336 mg, 6.0 mmol) and tetrabutylammonium bromide (228 mg, 1.0 mmol) in water (5 mL) was added. To the vigorously stirred mixture

propargylbromide (0.26 mL, 2.2 mmol, 80 % in toluene) in 1 mL of DCM was added drop wise. Stirring was continued for 4 h. The mixture was partitioned between DCM and saturated aqueous ammonium chloride solution (20 mL, 1:1), the organic layer was separated and the aqueous layer was extracted twice with DCM (5 mL). The combined organic phases were washed twice with brine, dried over $MgSO_4$ and evaporated at reduced pressure. The remaining solid was purified by flash chromatography with ethyl acetate / petrol ether 1:6 to give a yellow solid (491 mg, 0.827 mmol, 83 %).

M.p. (uncorrected) = 46 – 49 °C; - **^{1}H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.51 (s, 9 H), 1.59 (s, 9 H), 2.19 (s, 1 H), 4.73 (s, 2 H), 9.13 (s, 2 H), 9.38 (s, 2 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 28.0 (+, 3 C), 28.3 (+, 3 C), 34.3 (+, 1 C), 70.4 (-, 1 C), 79.1 (C_{quat}, 1 C), 80.0 (C_{quat}, 1 C), 84.7 (C_{quat}, 1 C), 154.2 (C_{quat}, 1 C), 159.7 (C_{quat}, 1 C), 163.5 (C_{quat}, 1 C); - **IR** (KBr): ν [cm^{-1}] = 3388 (bm), 3274 (bm), 2978 (m), 2934 (m), 1717 (m), 1646 (m), 1609 (m), 1507 (m), 1457 (m), 1367 (m), 1288 (s), 1239 (s), 1143 (s), 1122 (s), 1088 (m), 984 (m), 936 (m), 887 (m), 852 (m), 812 (m), 782 (m), 746 (m), 699 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 298.2 (100, MH^+), 242.1 (19, MH^+-C_4H_8), 198.1 (24, MH^+-CO_2 -C_4H_8); - **MF:** $C_{14}H_{23}N_3O_4$ – **FW:** 297.36 g/mol;

*14-[2-(3-(2-N-(^{tert}Butyloxycarbonyl)-aminoethyl)thioureido)ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester hydrochloride (**6**)*



Compound **4a** (0.59 g, 1.0 mmol) was stirred with DIPEA (0.25 g, 2.0 mmol) in dry ethanol (5 mL) for 15 minutes at room temperature. 1-(^{tert}Butyloxycarbonylamino)-2-isothiocyanato-ethane (1.02 g, 5.0 mmol) was added. The mixture was stirred for two days at 40 °C under dry conditions. The solvents were evaporated and the residue was re-dissolved in 50 mL of DCM. The organic phase was washed with 10 mL of saturated ammonium chloride solution, then two times with 20 mL of brine. After drying over $MgSO_4$, the solvent was evaporated and the

residue was purified via column chromatography (chloroform / ethanol 8:1) to yield the product **6** (570 mg, 0.83 mmol, 81 %) as a clear, thick, yellow oil (R_f = 0.35).

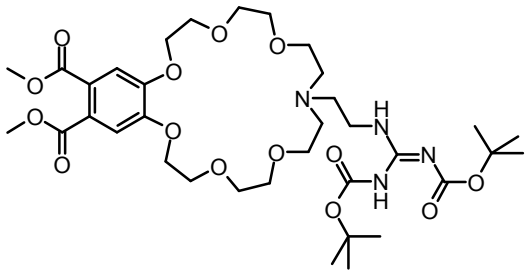
^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.43 (s, 9 H), 2.67 (m, 6 H), 3.21 (m, 2 H), 3.49 (m, 4 H), 3.56 – 3.63 (m, 8 H), 3.68 – 3.71 (m, 4 H), 3.80 – 3.86 (m, 4 H), 3.84 (s, 6 H), 4.13 – 4.19 (m, 4 H), 5.11 (s, 2 H), 7.14 (s, 2 H) - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 28.4 (+, 2 C), 29.7 (-, 1 C), 40.6 (-, 1 C), 43.0 (-, 1 C), 52.6 (+, 2 C), 54.8 (-, 1 C), 69.1 (-, 4 C), 69.4 (-, 4 C), 70.1 (-, 2 C), 71.1 (-, 2 C), 79.1 (C_{quat}, 1 C), 113.8 (+, 2 C), 125.6 (C_{quat}, 2 C), 150.3 (C_{quat}, 2 C), 167.7 (C_{quat}, 2 C); - **IR** (KBr): ν [cm^{-1}] = 3340 (bm), 2930 (m), 2880 (m), 2340 (m), 1716 (m), 1598 (m), 1516 (m), 1434 (m), 1349 (m), 1285 (s), 1195 (s), 1179 (s), 1125 (s), 1053 (m), 978 (m), 946 (m), 780 (m), 731 (m), 700 (m); - **MS** (ESI-MS, CH_2CL_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 717.5 (100, MH^+); - **UV** (MeOH): λ (ε) = 267 (7300), 224 (29700); - **HRMS** (EI-MS 70 eV): calc. for $C_{32}H_{53}N_4O_{12}S^{*+}$: 717.3381, found: 717.3394; - **MF:** $C_{32}H_{52}N_4O_{12}S$ – **FW:** 716.85 g/mol;

III. 4.2. Preparation and Deprotection of the Receptors

III. 4.2.1. General method for the preparation of aza-benzo-21-crown-7-ethers with appended bis-Boc-protected guanidines (GP III)

The according crown ether amino acid hydrochloride (0.3 mmol, approx. 200 mg) or TFA salt (0.3 mmol, approx. 300 mg) was dissolved in 5.0 mL of dried chloroform, freshly distilled triethylamine (1.0 mmol, 101 mg, 0.14 mL) was added slowly and the mixture was stirred for 20 minutes at room temperature. After addition of 290 mg of 1,3-bis(Boc)-2-methyl-2-isothiourea (**10**, 1.0 mmol), the well stirred solution was heated to reflux under nitrogen atmosphere and held at this temperature for two days. The solution was cooled to room temperature, diluted with 30 mL of ethyl acetate and filtered. The filtrate was washed with saturated ammonium chloride solution (10 mL) and water (10 mL), dried over magnesium sulfate and the solvent was evaporated. After vacuum drying the crude product was purified by column chromatography on silica gel with ethyl acetate/ethanol or chloroform / methanol as eluent. (R_f ca. 0.2 to 0.3 in ethyl acetate / ethanol 3:1).[56]

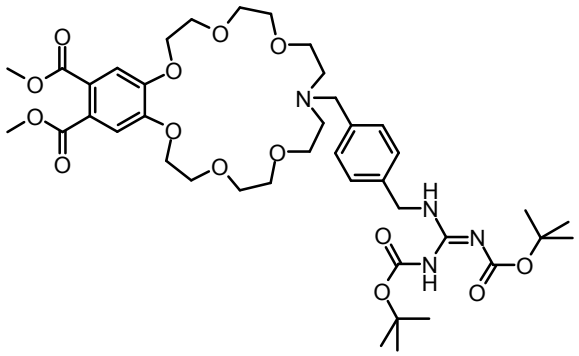
*14-[2-[2,3-di-(^{tert}Butyloxycarbonyl)-guanidino]-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**13a**)*



Compound **4a** (176 mg, 0.30 mmol) was converted to the product by *GP III*. The substance is a colourless glass (195 mg, 0.260 mmol, 78 %) (R_f [$CHCl_3$ / MeOH 12:1] = 0.27; R_f [EtOAc / EtOH 3:1] = 0.16).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.42 (s, 9 H), 1.43 (s, 9 H), 2.63 (t, 2 H, *J* = 5.4 Hz), 2.71 – 2.74 (t, 4 H, *J* = 5.4 Hz), 3.37 – 3.40 (q, 2 H), 3.51 – 3.54 (t, 4 H), 3.56 – 3.58 (m, 4 H), 3.67 – 3.70 (m, 4 H), 3.80 (s, 6 H), 3.83 – 3.86 (t, 4 H), 4.12 – 4.15 (t, 4 H), 7.12 (s, 2 H), 8.56 (bs, 1 H), 11.38 (bs, 1 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 28.0 (+, 3 C), 28.3 (+, 3 C), 38.8 (-, 1 C), 52.5 (+, 2 C), 53.8 (-, 1 C), 54.1 (-, 2 C), 69.3 (-, 2 C), 69.5 (-, 2 C), 70.1 (-, 2 C), 70.7 (-, 2 C), 71.1 (-, 2 C), 79.0 (C_{quat}, 1 C), 82.6 (C_{quat}, 1 C), 113.7 (+, 2 C), 125.3 (C_{quat}, 2 C), 150.5 (C_{quat}, 2 C), 152.8 (C_{quat}, 1 C), 156.0 (C_{quat}, 1 C), 163.5 (C_{quat}, 1 C), 167.7 (C_{quat}, 2 C); - **IR** (KBr): ν [cm^{-1}] = 3312 (bm), 2938 (m), 2866 (m), 1792 (m), 1720 (m), 1633 (m), 1521 (m), 1434 (m), 1347 (m), 1285 (s), 1251 (s), 1125 (s), 1058 (s), 982 (m), 949 (m), 875 (m), 773 (m), 657 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 757.4 (100, MH+); - **UV** (MeOH): λ (ε) = 268 (7400), 224 (30200); - **HRMS** (EI-MS 70 eV): calc. for $C_{35}H_{56}N_4O_{14}$*+: 757.3871, found: 757.3856; - **MF:** $C_{35}H_{56}N_4O_{14}$ – **FW:** 756.85 g/mol;

*14-[4-[2,3-di-(^{tert}Butyloxycarbonyl)-guanidino-methyl]-benzyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**13d**)*



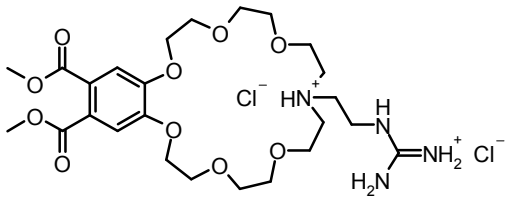
Compound **4d** (203 mg, 0.30 mmol) was reacted after *GP III*. The product appears as a yellow glass (205 mg, 0.247 mmol, 82 %) (R_f [$CHCl_3$ / MeOH 12:1] = 0.3; R_f [EtOAc / EtOH 3:1] = 0.18).

^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.46 (s, 9 H), 1.50 (s, 9 H), 2.76 (m, 4 H), 3.52 – 3.60 (m, 4 H), 3.62 – 3.66 (m, 6 H), 3.73 – 3.77 (m, 4 H), 3.86 (s, 6 H), 3.89 – 3.93 (m, 4 H), 4.13 – 4.21 (m, 4 H), 4.56 (d, 2 H, *J* = 5.3 Hz), 7.18 (s, 2 H), 7.20 (d, 2 H, *J* = 4.6 Hz), 7.27 (d, 2 H, *J* = 4.6 Hz), 8.53 (bs, 1 H), 11.51 (bs, 1 H); - **^{13}C-NMR** (150 MHz, $CDCl_3$): δ [ppm] = 28.1 (+, 3 C), 28.3 (+, 3 C), 44.8 (-, 1 C), 52.6 (+, 2 C), 53.7 (-, 2 C), 59.3 (-, 1 C), 69.3 (-, 2 C), 69.5 (-, 2 C), 69.8 (-, 2 C), 70.7 (-, 2 C), 71.2 (-, 2 C), 79.4 (C_{quat}, 1 C), 83.1 (C_{quat}, 1 C), 113.6 (+, 2 C), 125.3 (C_{quat}, 3 C), 127.7 (C_{quat}, 2 C), 129.3 (C_{quat}, 2 C), 135.8 (C_{quat}, 1 C), 150.5 (C_{quat}, 2 C), 153.2 (C_{quat}, 1 C), 156.1 (C_{quat}, 1 C), 163.6 (C_{quat}, 1 C), 167.8 (C_{quat}, 2 C); - **IR** (KBr): ν [cm^{-1}] = 3327 (bm), 2933 (m), 2877 (m), 2053 (w), 1970 (w), 1721 (m), 1612 (m), 1518 (m), 1433 (m), 1410 (m), 1353 (m), 1325 (m), 1286 (s), 1125 (s), 1058 (m), 981 (m), 914 (m), 803 (m), 778 (m), 731 (m), 649 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 833.4 (22 %, MH^+), 417.2 (100 %, $(M+2H^+)^{2+}$); - **UV** (MeOH): λ (ε) = 268 (7700), 224 (32100); - **HRMS** (PI-LSIMS FAB, glycerine): calc. for $C_{41}H_{61}N_4O_{14}^+$: 833.4184, found: 833.4191; - **MF:** $C_{41}H_{66}N_4O_{14}$ – **FW:** 832.95 g/mol;

III. 4.2.2. General procedure for Boc deprotection of the crown ether guanidines (GP V)

The according starting material (0.1 mmol) was dissolved in 0.5 mL of dry dichloromethane and a saturated solution of hydrochloric acid in diethyl ether (0.3 mL) was added. After four hours of stirring at room temperature the HCl gas was removed by bubbling N_2 through the solution. All volatiles were evaporated at reduced pressure. The residue was taken up in a minimum amount of dichloromethane and the product was precipitated carefully by slow addition of diethyl ether. The solid was allowed to settle completely, the solution was decanted off, the precipitate was washed once with diethyl ether, the solvent was decanted off again and the product was dried in the vacuum to furnish a hygroscopic powder.

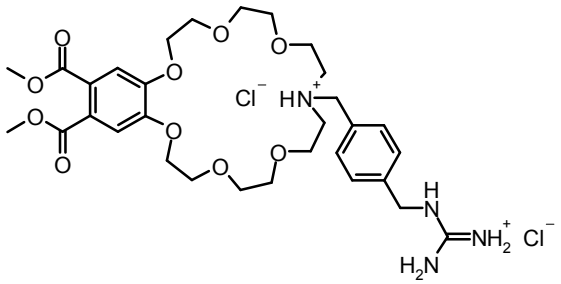
14-[2-Guanidino-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester hydrochloride ***(14a)***



A fine yellow powder (96 mg, 0.96 mmol, 96 %) was obtained.

M.p. (uncorrected) = 72 – 74°C; - **^{1}H-NMR** (300 MHz, MeOD): δ [ppm] = 2.68 – 2.73 (m, 6 H), 3.24 (m, 2 H), 3.52 – 3.59 (m, 4 H), 3.61 – 3.66 (m, 4 H), 3.74 – 3.79 (m, 4 H), 3.85 (s, 6 H), 3.89 – 3.93 (m, 4 H), 4.25 – 4.28 (m, 4 H), 7.32 (s, 2 H); - **^{13}C-NMR** (75 MHz, MeOD): δ [ppm] = 42.3 (-, 1 C), 53.2 (+, 2 C), 56.5 (-, 2 C), 58.4 (-, 1C), 70.2 (-, 2 C), 70.5 (-, 2 C), 70.6 (-, 2 C), 71.8 (-, 2 C), 72.3 (-, 2 C), 114.6 (+, 2 C), 126.9 (C_{quat}, 2 C), 151.7 (C_{quat}, 2 C), 159.3 (C_{quat}, 1 C), 166.2 (C_{quat}, 2 C); - **IR** (KBr): ν [cm^{-1}] = 3340 (bm), 2947 (m), 2882 (m), 1719 (m), 1659 (m), 1520 (m), 1439 (m), 1352 (m), 1292 (m), 1198 (s), 1130 (s), 1052 (m), 946 (m), 812 (m), 729 (m), 650 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 557.1 (100, MH^+), 298.4 (23, $(M+2Na^+)^{2+}$), 278.9 (23, $(M+2H^+)^{2+}$); - **UV** (MeOH): λ (ε) = 268 (7200), 223 (29100); - **HRMS** (PI-LSIMS FAB, glycerine): calc. for $C_{25}H_{41}N_4O_{10}^+$: 557.2823, found: 557.2825; - **MF:** $C_{25}H_{40}N_4O_{10}Cl_2$ – **FW:** 625.51 g/mol;

*14-[4-(Guanidino-methyl)-benzyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester hydrochloride (**14d**)*



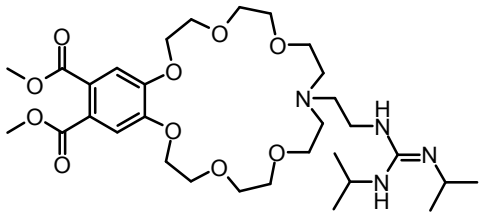
A fine, pale yellow powder (103 mg, 0.95 mmol, 95 %) was obtained.

M.p. (uncorrected) = 81 – 83°C; - **^{1}H-NMR** (300 MHz, MeOD): δ [ppm] = 3.21 – 3.28 (m, 4 H), 3.69 – 3.80 (m, 8 H), 3.86 (s, 6 H), 3.88 - 4.23 (m, 12 H), 4.29 (m, 2H), 4.69 (m, app. s, 2 H), 7.02 (s, 2 H), 7.11 (d, 2 H, *J* = 4.6 Hz), 7.34 (d, 2 H, *J* = 4.6 Hz); - **^{13}C-NMR** (75 MHz, MeOD): δ [ppm] = 45.3 (-, 1 C), 53.3 (+, 2 C), 53.6 (-, 2 C), 57.0 (-, 1 C), 65.5 (-, 2 C), 69.5 (-, 2 C), 70.6 (-, 2 C), 71.0 (-, 2 C), 71.2 (-, 2 C), 113.4 (+, 2 C), 126.1 (C_{quat}, 2 C), 128.3 (+, 2 C), 130.8 (C_{quat}, 1 C), 133.2 (+, 2 C), 139.2 (C_{quat}, 1 C), 151.5 (C_{quat}, 2 C), 158.9 (C_{quat}, 1 C), 169.8 (C_{quat}, 2 C); - **IR** (KBr): ν [cm^{-1}] = 3411 (bm), 2924 (m), 2071 (w), 1672 (m), 1600 (m), 1521 (m), 1436 (m), 1351 (m), 1288 (s), 1181 (s), 1126 (s), 1061 (m), 974 (m), 916 (m), 882 (m), 800 (m), 720 (m), 653 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 633.3 (19, MH^+), 317.0 (100, $(M+2H^+)^{2+}$); - **HRMS** (PI-LSIMS FAB, glycerine): calc. for $C_{31}H_{45}N_4O_{10}^+$: 633.3136, found: 633.3147; - **MF:** $C_{31}H_{50}N_4O_{10}$ – **FW:** 701.61 g/mol;

III. 4.2.3. Synthesis of aza-benzo-21-crown-7-ethers with N,N-dialkyl-substituted guanidine motifs (GP VI)

The according crown ether amino acid hydrochloride (0.3 mmol, approx. 200 mg) was dissolved in 10.0 mL of dry acetonitrile in nitrogen atmosphere, freshly distilled DIPEA (1.0 mmol, 129 mg, 0.17 mL) was added drop wise and the mixture was stirred for 30 min. While cooling to 2 - 5 °C DIC (190 mg, 1.5 mmol) or DCC (310 mg, 1.5 mmol) were added in one portion and the solution was, after 2 hours of stirring in the ice bath, carefully heated to reflux temperature and held at this temperature under dry conditions for four days. After cooling to room temperature, the reaction mixture was filtered over celite and the filter cake was washed several times with acetonitrile and dichloromethane. The solvent was evaporated, the crude product was thoroughly dried and purified by column chromatography with silica gel and chloroform/methanol as eluent. (R_f ca. 0.2 to 0.3 in chloroform / methanol 4:1).

*14-[2-[2,3-di-isopropyl-guanidino]-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**11a**)*

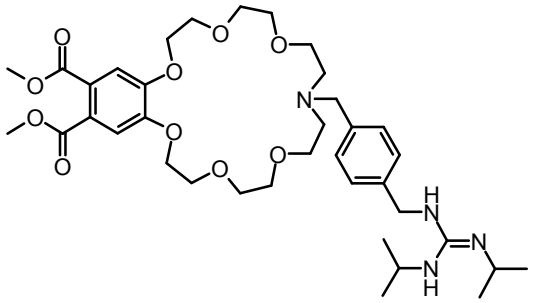


Converting compound **4a** (176 mg, 0.30 mmol) after *GP VI* to the product gives a colourless glass (133 mg, 0.207 mmol, 69 %) (R_f [EtOAc / MeOH 5:2] = 0.39; R_f [EtOAc / EtOH 3:1] = 0.1).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.18 – 1.22 (d, 12 H, J = 5.4 Hz), 2.79 (m, 6 H), 3.32 (m, 2 H), 3.49 – 3.59 (m, 8 H), 3.61 – 3.69 (m, 4 H), 3.78 – 3.87 (m, 4 H), 3.86 (s, 6 H), 4.01 (heptett, 2 H, J = 5.4 Hz), 4.12 – 4.22 (m, 4 H), 7.86 (bs, 2 H);- **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 21.9 (+, 2 C), 22.1 (+, 2 C), 43.8 (+, 2 C), 51.6 (+, 2 C), 53.2 (-, 1 C), 54.6 (-, 1 C), 68.1 (-, 4 C), 68.5 (-, 2 C), 68.6 (-, 2 C), 69.4 (-, 2 C), 70.1 (-, 2 C), 112.7 (+, 2 C), 124.4 (C_{quat}, 2 C), 149.4 (C_{quat}, 2 C), 153.9 (C_{quat}, 1 C), 167.7 (C_{quat}, 2 C); - **IR** (KBr): ν [cm^{-1}] = 3402 (bm), 2940 (m), 2874 (m), 1719 (m), 1615 (m), 1519 (m), 1435 (m), 1348 (m), 1285 (s), 1183 (s), 1124 (s), 1061 (m), 976 (m), 945 (m), 878 (m), 782 (m), 767 (m), 730 (m), 698

(m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 641.4 (100, MH^+); - **UV** (MeOH): λ (ε) = 268 (8500), 224 (28800); - **HRMS** (PI-LSIMS FAB, glycerine): calc. for $C_{31}H_{53}N_4O_{10}^+$: 641.3762, found: 641.3745; - **MF:** $C_{31}H_{52}N_4O_{10}$ – **FW:** 640.78 g/mol;

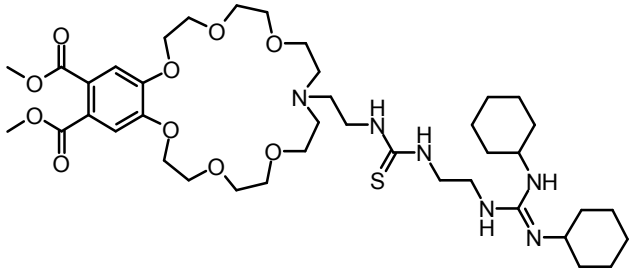
*14-[4-[2,3-Di-isopropyl-guanidino-methyl]-benzyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**11d**)*



Compound **4d** (203 mg, 0.30 mmol) was submitted to *GP VI* to give the according product as a yellow glass (131 mg, 0.183 mmol, 61 %) (R_f [$CHCl_3$ / MeOH 6:1] = 0.2).

^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.12 (d, 12 H, J = 6.4 Hz), 2.77 (m, 4 H), 3.56 – 3.61 (m, 8 H), 3.68 – 3.71 (m, 4 H), 3.66 – 3.73 (m, 2 H), 3.81 (s, 6 H), 3.83 – 3.87 (m, 4 H), 3.96 – 4.03 (heptett, 2 H, J = 6.4 Hz), 4.10 – 4.15 (m, 4 H), 4.46 (d, 2 H, J = 5.3 Hz), 6.51 (d, 2 H, J = 4.6 Hz), 7.09 (s, 2 H), 7.24 (dd, 4 H, J = 4.6 Hz, 2.1 Hz), 8.41 (bs, 1 H) - **^{13}C-NMR** (150 MHz, $CDCl_3$): δ [ppm] = 22.8 (+, 4 C), 45.3 (-, 1 C), 45.4 (+, 2 C), 52.7 (+, 2 C), 53.5 (-, 2 C), 59.0 (-, 1 C), 69.1 (-, 2 C), 69.2 (-, 2 C), 69.4 (-, 2 C), 70.5 (-, 2 C), 71.0 (-, 2 C), 113.3 (+, 2 C), 125.3 (C_{quat}, 2 C), 125.3 (C_{quat}, 1 C), 127.4 (+, 2 C), 129.9 (+, 2 C), 150.4 (C_{quat}, 2 C), 154.3 (C_{quat}, 1 C), 167.9 (C_{quat}, 2 C); - **IR** (KBr): ν [cm^{-1}] = 3180 (bm), 2931 (m), 2872 (m), 2194 (w), 1721 (m), 1612 (m), 1517 (m), 1437 (m), 1350 (m), 1288 (s), 1197 (s), 1125 (s), 1049 (m), 979 (m), 913 (m), 796 (m), 728 (m), 643 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 717.5 (13 %, MH^+), 359.2 (100 %, $(M+2H^+)^{2+}$); - **UV** (MeOH): λ (ε) = 268 (8600), 224 (29000); - **HRMS** (PI-LSIMS FAB, glycerine): calc. for $C_{37}H_{56}N_4O_{10}^+$: 716.3996, found: 716.3986; - **MF:** $C_{37}H_{56}N_4O_{10}$ – **FW:** 716.88 g/mol;

*14-[2-(3-(2-(2,3-Di-cyclohexyl-guanidino)ethyl)thioureido)ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**11e**)*



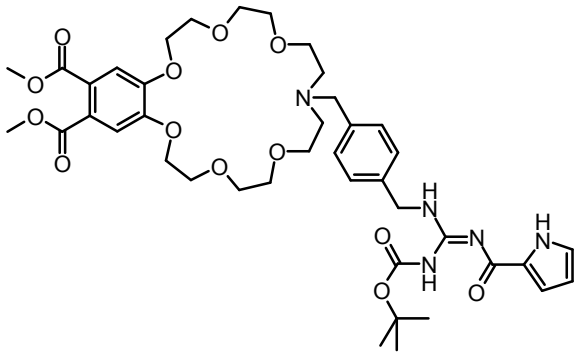
Reacting **4e** (210 mg, 0.30 mmol) after *GP VI* gives a yellow glass as the product (96 mg, 0.13 mmol, 39 %) (R_f [$CHCl_3$ / MeOH 4:1] = 0.31).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.13 – 1.23 (m, 2 H), 1.32 – 1.43 (m, 8 H), 1.54 – 1.66 (m, 2 H), 1.69 – 1.80 (m, 4 H), 1.89 – 2.03 (m, 4 H), 2.75 (m, 6 H), 3.48 – 3.63 (m, 10 H), 3.61 – 3.66 (m, 4 H), 3.73 – 3.79 (m, 4 H), 3.87 (s, 6 H), 3.88 – 3.93 (m, 4 H), 4.19 – 4.24 (m, 4 H), 6.41 (m, 1 H), 7.20 (s, 2 H), 7.63 (bs, 2 H), 8.70 (bs, 1 H) - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 24.8 (-, 4 C), 25.0 (-, 2 C), 33.0 (-, 4 C), 40.0 (-, 1 C), 41.8 (-, 1 C), 51.9 (+, 2 C), 52.6 (+, 2 C), 54.1 (-, 1 C), 54.4 (-, 2 C), 54.9 (-, 1 C), 68.8 (-, 2 C), 69.4 (-, 2 C), 69.5 (-, 2 C), 70.4 (-, 2 C), 70.9 (-, 2 C), 113.5 (+, 2 C), 125.4 (C_{quat}, 2 C), 150.3 (C_{quat}, 2 C), 153.3 (C_{quat}, 1 C), 155.1 (C_{quat}, 1 C), 166.7 (C_{quat}, 2 C); - **IR** (KBr): ν [cm^{-1}] = 3220 (bm), 3196 (bm), 3086 (m), 2931 (m), 2859 (m), 2202 (w), 2028 (w), 1722 (m), 1614 (m), 1556 (m), 1521 (m), 1438 (m), 1350 (m), 1287 (s), 1197 (s), 1123 (s), 1052 (m), 981 (m), 915 (m), 799 (m), 729 (m), 659 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 823.5 (11, MH^+), 412.1 (100, $(M+2H^+)^{2+}$); - **UV** (MeOH): λ (ε) = 268 (7900), 224 (28700); - **HRMS** (PI-LSIMS FAB, glycerine): calc. for $C_{40}H_{67}N_6O_{10}S^+$: 823.4639, found: 823.4649; - **MF:** $C_{40}H_{66}N_6O_{10}S$ – **FW:** 823.07 g/mol;

III. 4.2.4. Synthesis of protected aza-benzo-21-crown-7-ethers with pyrrole- and pyrene substituted guanidine motifs (GP VII)

The according crown ether amino acid hydrochloride or TFA salt (0.2 mmol, approx. 200 mg as the TFA salt) was dissolved in 3.0 mL of dry DMF, freshly distilled triethylamine (1.0 mmol, 101 mg, 0.14 mL) was dropped in and the mixture was stirred for 20 min. at room temperature. After addition of the appropriate 2-methyl-2-isothiourea (0.3 mmol), the well stirred solution was heated to 40 °C under nitrogen atmosphere and held at this temperature over night. The solution was cooled to room temperature, diluted with 30 mL of DCM and filtered. The filtrate was washed with saturated ammonium chloride solution (10 mL) and water (10 mL), dried over magnesium sulfate and the solvent was evaporated. After vacuum drying the crude product was purified by column chromatography on silica gel with ethyl acetate / ethanol as eluent. (R_f ca. 0.3 in EtOAc / EtOH 6:1).

14-[4-[2-(^{tert}*Butyloxycarbonyl)-3-(pyrrole-2-carbonyl)-guanidino-methyl]-benzyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**30**)*

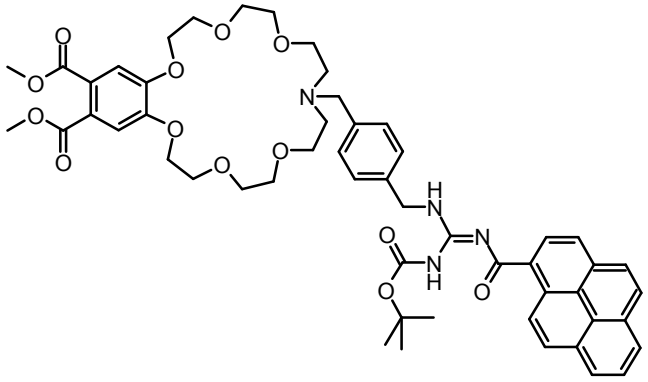


Reacting **4d** (136 mg, 0.20 mmol) after *GP VII* a yellow glass is obtained (125 mg, 0.151 mmol, 76 %). Ethanol / ethyl acetate 1:6 was used for purification.

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 149 - 1.51 (s, 9 H), 2.62 – 2.81 (m, 4 H), 3.49 – 3.70 (m, 10 H), 3.71 – 3.80 (m, 4 H), 3.83 – 3.96 (m, 4 H), 3.82 (s, 6 H), 4.11 – 4.21 (m, 4 H), 4.61 (m, 2 H), 6.21 (m, 1 H), 6.85 (m, 1 H), 6.95 (m, 1 H), 7.17 (s, 2 H), 7.21 – 7.33 (m, 4 H), 8.62 (bs, 1 H), 9.39 (bs, 1 H), 12.39 (s, 1 H); - **^{13}C-NMR** (100 MHz, $CDCl_3$): δ [ppm] = 28.0 (+, 2 C), 28.2 (+, 1 C), 44.7 (-, 1 C), 52.5 (+, 2 C), 53.8 (-, 2 C), 59.2 (-, 1 C), 69.3 (-, 2 C), 69.4 (-, 2 C), 69.8 (-, 1 C), 70.6 (-, 2 C), 71.2 (-, 2 C), 83.1 (C_{quat}, 1 C), 110.4 (+, 1 C), 113.6 (+, 1 C), 114.1 (+, 2 C), 121.8 (+, 1 C), 125.3 (C_{quat}, 2 C), 127.4 (+, 2 C), 129.3 (+, 2 C), 131.1 (C_{quat}, 1

C), 139.0 (C_{quat}, 1 C), 150.5 (C_{quat}, 2 C), 153.3 (C_{quat}, 1 C), 155.8 (C_{quat}, 1 C), 167.8 (C_{quat}, 2 C), 171.0 (C_{quat}, 1 C); - **IR** (KBr): ν [cm^{-1}] = 3319 (bm), 2941 (m), 2873 (m), 1718 (m), 1614 (m), 1577 (m), 1407 (m), 1352 (s), 1287 (s), 1195 (m), 1125 (s), 1061 (m), 1027 (s), 980 (m), 911 (m), 846 (m), 780 (m), 731 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 826.3 (81, MH^+), 413.6 (100, $(M+2H^+)^{2+}$); - **UV** (MeOH): λ (ε) = 303 (22900), 222 (27500); - **HRMS** (PI-LSIMS FAB, glycerine): calc. for $C_{41}H_{56}N_5O_{13}^+$: 826.6870, found: 826.3866; - **MF:** $C_{41}H_{55}N_5O_{13}$ – **FW:** 825.92 g/mol;

14-[4-[2-(^{tert}*Butyloxycarbonyl)-3-(pyrene-1-carbonyl)-guanidino-methyl]-benzyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**31**)*



Conversion of **4d** (136 mg, 0.20 mmol) by *GP VII* yields a yellow glass (119 mg, 0.124 mmol, 62 %). Ethanol / ethyl acetate 1:9 was used for purification.

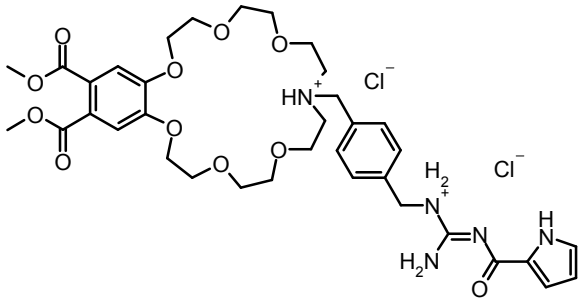
^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.48 (s, 3 H), 1.52 (s, 6 H), 1.86 (m, 2 H), 2.71 (m, 4 H), 3.59 – 3.61 (m, 8 H), 3.62 – 3.71 (m, 4 H), 3.72 – 3.93 (m, 4 H), 3.82 (s, 6 H), 4.09 – 4.18 (m, 4 H), 4.71 (d, 2 H, *J* = 5.3 Hz), 7.09 – 7.18 (m, 2 H), 7.25 – 7.32 (m, 4 H), 8.68 (d, 1 H, *J* = 4.6 Hz), 8.91 (bs, 1 H), 9.30 (d, 1 H, *J* = 4.6 Hz), 9.56 (bs, 1 H); - **^{13}C-NMR** (150 MHz, $CDCl_3$): δ [ppm] = 28.1 (+, 2 C), 28.3 (+, 1 C), 44.8 (-, 1 C), 52.5 (-, 1 C), 52.6 (+, 2 C), 53.6 (-, 2 C), 69.3 – 71.2 (-, 10 C), 83.4 (C_{quat}, 1 C), 114.0 (+, 2 C), 124.1 (+, 1 C), 124.6 (C_{quat}, 1 C), 124.8 (+, 1 C), 125.0 (C_{quat}, 1 C), 125.2 (+, 1 C), 125.2 (C_{quat}, 2 C), 125.6 (+, 1 C), 125.7 (C_{quat}, 1 C), 126.0 (+, 1 C), 126.4 (C_{quat}, 1 C), 126.7 (C_{quat}, 1 C), 127.1 (C_{quat}, 1 C), 127.4 (+, 1 C), 127.7 (+, 2 C), 127.9 (+, 2 C), 128.3 (+, 1 C), 128.4 (+, 1 C), 128.7 (+, 1 C), 130.3 (C_{quat}, 1 C), 150.2 (C_{quat}, 2 C), 153.3 (C_{quat}, 1 C), 156.0 (C_{quat}, 1 C), 163.9 (C_{quat}, 1 C), 167.7 (C_{quat}, 2 C), 180.9 (C_{quat}, 1 C); - **IR** (KBr): ν [cm^{-1}] = 3312 (bm), 2943 (m), 2873 (m), 1719 (m), 1598 (m), 1575 (m), 1514 (m), 1434 (m), 1398 (m), 1346 (m), 1287 (s), 1193 (m), 1125 (s), 1050

(s), 982 (m), 911 (m), 847 (m), 808 (m), 730 (a), 646 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 960.1 (100, MH+), 480.6 (100, $(M+2H^+)^{2+}$); - **UV** (MeOH): λ (ε) = 348 (35600), 281 (42100), 223 (74600); - **HRMS** (PI-LSIMS FAB, glycerine): calc. for $C_{53}H_{61}N_4O_{13}^+$: 961.4235, found: 961.4263; - **MF:** $C_{53}H_{60}N_4O_{13}$ – **FW:** 961.09 g/mol;

III. 4.2.5. Deprotection of aza-benzo-21-crown-7-ethers with pyrrole- and pyrene substituted guanidine motifs (GP VIII)

The according starting material (0.1 mmol) was dissolved in 2.0 mL of dry, cold dichloromethane and a cold solution of HCl in diethylether (1.0 mL) was added. After four to six hours of stirring at room temperature under moisture protection, all volatiles were removed at reduced pressure. The residue was dissolved in a minimum amount of dichloromethane and the product was precipitated carefully by slow addition of diethyl ether. The solution was centrifuged, the diethyl ether was decanted off and the process was repeated. The product was dried in the vacuum to give a fine hygroscopic, faintly yellow powder.

*14-[4-[2-(Pyrrole-2-carbonyl)-guanidino-methyl]-benzyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester hydrochloride (**32**)*

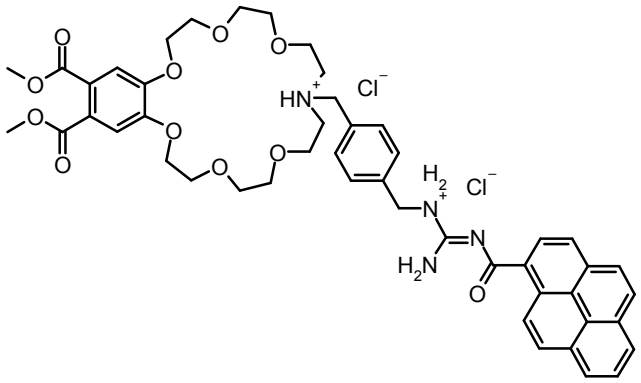


Deprotection of **30** (83 mg, 0.10 mmol) by *GP VII* gives a yellow, hygroscopic, glassy solid (68 mg, 0.094 mmol, 94 %).

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 3.12 – 3.30 (m, 4 H), 3.49 – 3.69 (m, 8 H), 3.71 – 3.96 (m, 8 H), 3.84 (s, 6 H), 4.06 – 4.25 (m, 4 H), 4.41 (m, 2 H), 4.52 (m, 2 H), 6.21 (m, 1 H), 6.90 – 7.11 (m, 5 H), 7.28 – 7.32 (m, 3 H), 9.71 (bs, 1 H), 10.30 (bs, 1 H), 11.12 (bs, 1 H); - **^{13}C-NMR** (100 MHz, $CDCl_3$): δ [ppm] = 44.1 (-, 1 C), 52.5 (-, 2 C), 52.8 (+, 2 C), 57.1 (-, 1

C), 65.0 (-, 2 C), 68.1 (-, 2 C), 69.1 (-, 2 C), 69.9 (-, 2 C), 70.1 (-, 2 C), 111.3 (+, 1 C), 112.3 (+, 2 C), 118.4 (+, 1 C), 123.4 (C_{quat}, 1 C), 125.1 (C_{quat}, 2 C), 126.2 (+, 2 C), 127.2 (+, 2 C), 128.9 (C_{quat}, 1 C), 132.0 (+, 1 C), 136.2 (C_{quat}, 1 C), 149.7 (C_{quat}, 2 C), 155.4 (C_{quat}, 1 C), 168.0 (C_{quat}, 2 C); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 726.3 (23, MH^+), 363.7 (100, $(M+2H^+)^{2+}$); - **UV** (MeOH): λ (ε) = 298 (22000), 220 (26700); - **HRMS** (PI-LSIMS FAB, glycerine): calc. for $C_{36}H_{48}N_5O_{11}^+$: 726.3350, found: 726.3346; - **MF:** $C_{36}H_{51}N_5O_{11}Cl_2$ – **FW:** 800.74 g/mol;

*14-[4-[2-(Pyrene-1-carbonyl)-guanidino-methyl]-benzyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester hydrochloride (**33**)*



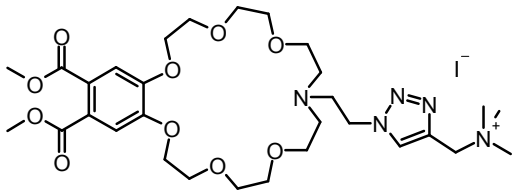
Compound **31** (96 mg, 0.10 mmol) was deprotected according to *GP VII* to give the product **33** as a yellow glass (76 mg, 0.088 mmol, 88 %).

^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 3.21 (m, 4 H), 3.61 – 3.68 (m, 8 H), 3.81 – 3.96 (m, 8 H), 3.86 (s, 6 H), 4.09 – 4.14 (m, 4 H), 4.51 (m, 2 H), 4.63 (m, 2 H), 6.93 (s, 2 H), 7.11 (d, 2 H, *J* = 4.6 Hz), 7.41 (d, 2 H, *J* = 4.6 Hz), 7.97 – 8.11 (m, 3 H), 8.13 – 8.21 (m, 2 H), 8.22 – 8.28 (m, 2 H), 8.36 (m, 1 H), 8.71 (m, 1 H), 10.23 (bs, 1 H), 10.96 (bs, 1 H), 13.51 (bs, 1 H); - **^{13}C-NMR** (150 MHz, $CDCl_3$): δ [ppm] = 44.3 (-, 1 C), 52.2 (-, 2 C), 52.9 (+, 2 C), 56.8 (-, 1 C), 65.6 (-, 2 C), 68.2 (-, 2 C), 69.2 (-, 2 C), 70.0 (-, 2 C), 70.2 (-, 2 C), 112.3 (+, 2 C), 123.9 (+, 1 C), 124.2 (C_{quat}, 1 C), 124.3 (+, 1 C), 124.9 (C_{quat}, 2 C), 125.2 (C_{quat}, 1 C), 126.4 (+, 1 C), 126.5 (+, 1 C), 126.6 (C_{quat}, 1 C), 126.9 (C_{quat}, 1 C), 127.0 (+, 2 C), 127.4 (+, 1 C), 129.9 (+, 1 C), 130.2 (+, 1 C), 130.6 (C_{quat}, 1 C), 130.7 (C_{quat}, 1 C), 132.2 (+, 5 C), 134.7 (+, 1 C), 135.8 (+, 1 C), 131.3 (C_{quat}, 1 C), 150.0 (C_{quat}, 2 C), 155.8 (C_{quat}, 1 C), 168.2 (C_{quat}, 2 C), 172.2 (C_{quat}, 1 C); - **IR** (KBr): ν [cm^{-1}] = 3302 (bm), 2916 (m), 2878 (m), 1675 (s), 1596 (m), 1515 (m), 1436 (m), 1351 (m), 1267 (m), 1197 (s), 1129 (s), 1066 (m), 977 (m), 913 (m), 832

(m), 725 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 861.4 (21, MH+), 431.8 (100, $(M+2H^+)^{2+}$); - **UV** (MeOH): λ (ε) = 346 (22500), 279 (35000), 227 (68700); - **HRMS** (PI-LSIMS FAB, glycerine): calc. for $C_{48}H_{53}N_4O_{11}^+$: 861.3711, found: 861.3686; - **MF:** $C_{48}H_{56}N_4O_{11}Cl_2$ – **FW:** 931.88 g/mol;

III. 4.2.6. Synthesis of receptors via Huisgen cycloaddition reaction

{1-[2-(2,3-Bis-methoxycarbonyl-6,7,9,10,12,13,15,16,18,19,21,22-dodecahydro-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosen-14-yl)-ethyl]-1H-[1,2,3]triazol-4-ylmethyl}-trimethyl-ammonium iodide (**24**)

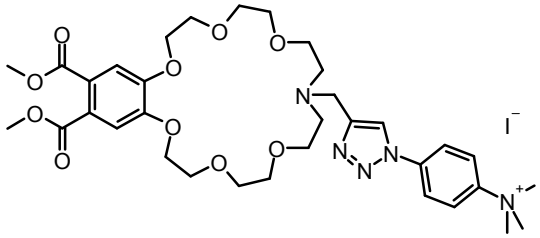


Compound **22** (130 mg, 0.24 mmol) and compound **23** (45 mg, 0.2 mmol) were dissolved in 1.0 mL of methanol. A solution of copper(II)sulfate pentahydrate (20 mg, 0.04 mmol) in 0.5 mL of water containing 32 mg sodium ascorbate (0.2 mmol) was added drop wise. After stirring for 2 h at room temperature, the same amount of $CuSO_4$ * 5 H_2O and sodium ascorbate in 0.5 mL water was added a second time and the vigorously stirred reaction mixture held for 4 h at 50 °C in nitrogen atmosphere. After cooling to room temperature 10 mL dichloromethane were added, the aqueous layer was separated and the organic phase was washed with brine (3 mL). After drying over $MgSO_4$ the solvent was evaporated and the solid residue was purified on a small pellet of silica gel. All impurities were washed from the column with chloroform / methanol 9:1 (TLC control); the product was eluted with chloroform / methanol 3:1. The solvent was evaporated, the residue was sonicated in 5 mL of chloroform and the silica gel was filtered off. The filtrate was evaporated to give the pure product as yellow oil (136 mg, 0.178 mmol, 89 %).

^{1}H-NMR (300 MHz, MeOD): δ [ppm] = 2.81 (m, 4 H), 3.10 (s, 9 H), 3.48 – 3.59 (m, 4 H), 3.60 – 3.68 (m, 4 H), 3.71 – 3.81 (m, 4 H), 3.72 (s, 2 H), 3.86 (s, 6 H), 3.84 – 3.91 (m, 4 H), 4.21 – 4.28 (m, 4 H), 4.50 – 4.69 (m, 4 H), 7.22 (s, 2 H), 8.43 (s, 1 H); - **^{13}C-NMR** (75 MHz, MeOD): δ [ppm] = 50.3 (-, 1 C), 53.4 (+, 3 C), 53.6 (+, 2 C), 56.1 (-, 2 C), 56.3 (-, 1 C), 61.7 (-, 1 C), 70.5 (-, 2 C), 70.6 (-, 2 C), 70.7 (-, 2 C), 71.4 (-, 2 C), 71.9 (-, 2 C), 114.8 (+, 2 C),

126.8 (C_{quat}, 2 C), 130.4 (+, 1 C), 136.3 (C_{quat}, 1 C), 151.7 (C_{quat}, 2 C), 169.4 (C_{quat}, 2 C); - **IR** (KBr): ν [cm^{-1}] = 3370 (bm), 2948 (m), 2872 (m), 1720 (m), 1639 (m), 1599 (m), 1519 (m), 1436 (m), 1350 (m), 1288 (s), 1200 (s), 1126 (s), 1058 (m), 1025 (s), 975 (m), 945 (m), 895 (m), 769 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 638.3 (23, M^+), 319.5 (100, $(M^+ + H^+)^{2+}$); - **UV** (MeOH): λ (ε) = 266 (7400), 226 (29400); - **HRMS** (PI-LSIMS FAB, glycerine): calc. for $C_{30}H_{48}N_5O_{10}^+$: 638.3401, found: 638.3411; - **MF:** $C_{30}H_{48}N_5O_{10}I$ – **FW:** 765.64 g/mol;

{4-[4-(2,3-Bis-methoxycarbonyl-6,7,9,10,12,13,15,16,18,19,21,22-dodecahydro-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosen-14-ylmethyl)-2,3-dihydro-[1,2,3]triazol-1-yl]-phenyl}-trimethyl-ammonium iodide (**21**)

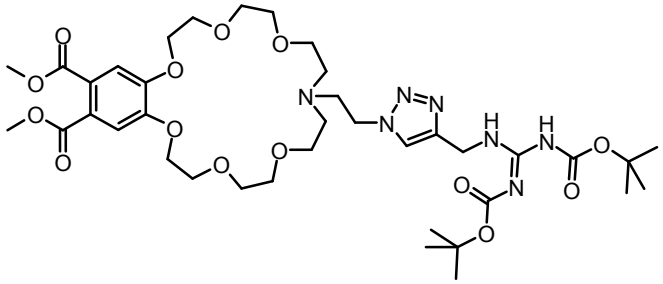


Compound **19** (122 mg, 0.24 mmol) was dissolved together with compound **20** (61 mg, 0.2 mmol) in 2.0 mL of methanol. A solution of copper(II)sulfate pentahydrate (25 mg, 0.05 mmol) and sodium ascorbate (32 mg, 0.2 mmol) in water (0.5 mL) was added drop wise. After stirring for 1 h at room temperature, $CuSO_4$ * 5 H_2O and sodium ascorbate were added in the same amount again and the vigorously stirred reaction mixture was warmed 4 h at 65 °C under nitrogen. After cooling to room temperature, 10 mL dichloromethane were added, the aqueous layer was separated off and the organic phase was washed with brine (3 mL). After drying the solution over $MgSO_4$ the solvent was distilled off and the solid residue was purified by column chromatography with a small amount of silica gel. All impurities were washed from the column with chloroform / methanol 9:1 (TLC control); the product was eluted with chloroform / methanol 3:1. The solvent was evaporated, the residue was sonicated in 5 mL of chloroform and the silica gel was filtered off. The filtrate was evaporated to give the pure product as orange, sticky oil (150 mg, 0.184 mmol, 92 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 3.08 – 3.15 (m, 4 H), 3.71 (s, 9 H), 3.66 – 3.87 (m, 8 H), 3.84 (s, 6 H), 3.91 – 3.98 (m, 4 H), 4.01 – 4.09 (m, 4 H), 4.12 – 4.19 (m, 4 H), 4.76 (m, 2 H), 7.02 (s, 2 H), 7.86 (d, 2 H, *J* = 4.6 Hz), 8.07 (d, 2 H, *J* = 4.6 Hz), 8.52 (bs, 1 H); - **^{13}C-**

NMR (75 MHz, $CDCl_3$): δ [ppm] = 47.0 (-, 1 C), 53.2 (+, 2 C), 54.7 (-, 2 C), 58.0 (+, 3 C), 67.8 (-, 2 C), 69.8 (-, 2 C), 70.7 (-, 2 C), 71.1 (-, 2 C), 71.7 (-, 2 C), 113.5 (+, 2 C), 121.9 (+, 1C), 122.3 (+, 2 C), 123.2 (+, 2 C), 125.8 (C_{quat}, 1 C), 126.2 (C_{quat}, 2 C), 138.7 (C_{quat}, 1 C), 147.8 (C_{quat}, 1 C), 151.4 (C_{quat}, 2 C), 169.4 (C_{quat}, 2 C); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 686.3 (12, M^+), 343.6 (100, $(M^+ + H^+)^{2+}$); - **UV** (MeOH): λ (ε) = 260 (13400), 227 (25100), 204 (31100); - **HRMS** (PI-LSIMS FAB, glycerine): calc. for $C_{34}H_{48}N_5O_{10}^+$: 686.3401, found: 686.3391; - **MF:** $C_{34}H_{48}N_5O_{10}$ – **FW:** 813.69 g/mol;

14-[2-(4-(2,3-di-(^{tert}Butyloxycarbonyl)-guanidino)-methyl-[1,2,3]triazol-1-yl)-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethyl ester (**26**)

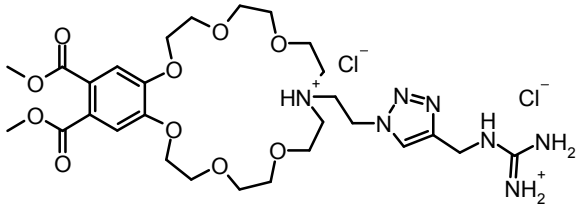


Compound **22** (112 mg, 0.20 mmol) and compound **25** (59 mg, 0.2 mmol) were dissolved in 0.5 mL of methanol. A solution of copper(II)sulfate pentahydrate (25 mg, 0.05 mmol) and sodium ascorbate (40 mg, 0.25 mmol) in 0.5 mL of water was added drop wise. After stirring for 2 h at room temperature, the vigorously stirred reaction mixture was warmed to 50 °C and kept at this temperature over night at under nitrogen atmosphere. The methanol was allowed to evaporate and the residue was partitioned between water and ethyl acetate (15 mL, 1:2). The aqueous layer was separated and the organic phase was washed with 3 mL of brine. After drying over $MgSO_4$ the solvent was evaporated and the solid residue was purified by flash chromatography on silica gel with ethyl acetate / ethanol 4:1 to give the pure product as yellow glass (136 mg, 0.162 mmol, 81 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.45 (s, 9 H), 1.48 (s, 9 H), 2.76 (m, 4 H), 3.02 (m, 2 H), 3.44 – 3.56 (m, 4 H), 3.58 – 3.63 (m, 4 H), 3.69 – 3.77 (m, 4 H), 3.86 (s, 6 H), 3.85 – 3.93 (m, 4 H), 4.16 – 4.23 (m, 4 H), 4.31 – 4.42 (m, 4 H), 5.16 (s, 2 H), 7.18 (s, 2 H), 7.68 (s, 1 H), 9.23 (bs, 2 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 26.9 (+, 3 C), 27.4 (+, 3 C), 39.2 (-, 1 C), 47.7 (-, 1 C), 51.6 (+, 2 C), 53.5 (-, 2 C), 54.2 (-, 1 C), 68.1 (-, 2 C), 68.5 (-, 2 C), 69.0 (-

, 2 C), 69.7 (-, 2 C), 70.1 (-, 2 C), 77.7 (C_{quat}, 1 C), 83.4 (C_{quat}, 1 C), 112.5 (+, 2 C), 122.5 (+, 1 C), 124.3 (C_{quat}, 2 C), 143.6 (C_{quat}, 1 C), 149.4 (C_{quat}, 2 C), 153.7 (C_{quat}, 1 C), 159.3 (C_{quat}, 1 C), 162.6 (C_{quat}, 1 C), 166.8 (C_{quat}, 2 C); - **IR** (KBr): ν [cm^{-1}] = 3370 (bm), 2948 (m), 2872 (m), 1720 (m), 1639 (m), 1599 (m), 1519 (m), 1436 (m), 1350 (m), 1288 (s), 1200 (s), 1126 (s), 1058 (m), 1025 (s), 975 (m), 945 (m), 895 (m), 769 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 838.5 (100, MH^+); - **UV** (MeOH): λ (ε) = 268 (7500), 226 (25700); - **MF:** $C_{38}H_{59}N_7O_{14}$ – **FW:** 837.93 g/mol;

14-[2-(4-Guanidinomethyl-[1,2,3]triazol-1-yl)-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethyl ester hydrochloride (**27**)

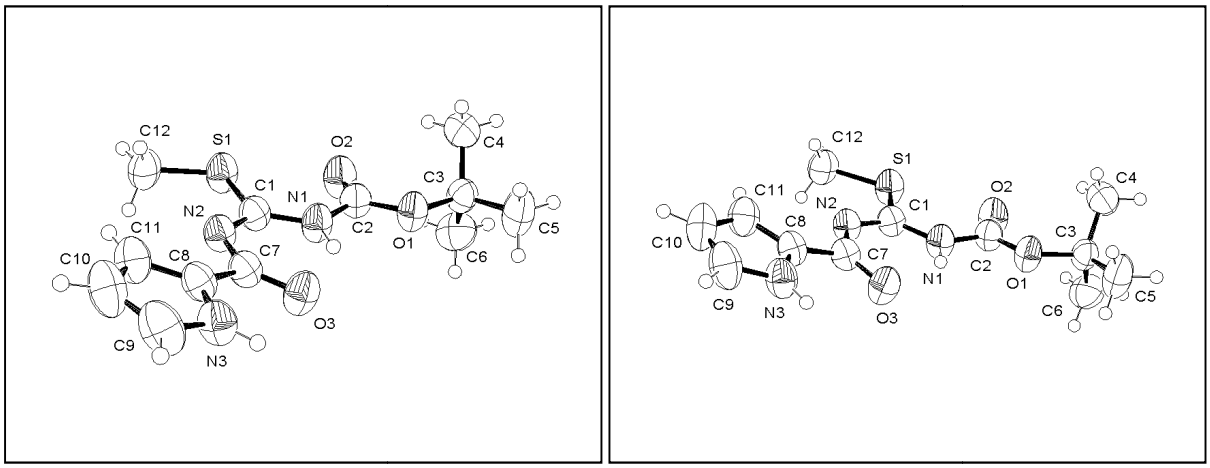


Compound **26** (84 mg, 0.10 mmol) was deprotected after *GP VIII*. The product was dried under vacuum at room temperature to yield **27** as yellow, sticky oil (49 mg, 0.077 mmol, 77 %).

^{1}H-NMR (300 MHz, MeOD): δ [ppm] = 3.48 (m, 4H), 3.56 (m, 2 H), 3.66 – 3.78 (m, 10 H), 3.80 – 3.92 (m, 8 H), 3.86 (s, 6 H), 4.19 – 4.31 (m, 6 H), 7.29 (s, 2 H), 8.24 (s, 1 H); - **^{13}C-NMR** (75 MHz, MeOD): δ [ppm] = 39.8 (-, 1 C), 46.9 (-, 1 C), 53.2 (-, 1 C), 53.3 (+, 2 C), 54.9 (-, 2 C), 65.7 (-, 2 C), 70.1 (-, 2 C), 70.8 (-, 2 C), 71.3 (-, 2 C), 71.9 (-, 2 C), 114.3 (+, 2 C), 120.1 (+, 1 C), 126.7 (C_{quat}, 2 C), 143.4 (C_{quat}, 1 C), 151.8 (C_{quat}, 2 C), 153.1 (C_{quat}, 1 C), 166.4 (C_{quat}, 2 C); - **IR** (KBr): ν [cm^{-1}] = 3349 (bm), 2923 (m), 2872 (m), 1786 (m), 1673 (s), 1520 (m), 1437 (m), 1350 (m), 1288 (s), 1182 (s), 1134 (s), 1059 (s), 911 (m), 835 (m), 799 (m), 724 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 638.3 (29, M^+), 340.1 (16, $(M+Na^+)^{2+}$), 319.5 (100, $(M+2H^+)^{2+}$); - **MF:** $C_{28}H_{47}N_7O_{10}Cl_2$ – **FW:** 712.63 g/mol;

III. 4.3. Selected Crystal Structures of New Compounds

*X-Ray structure and crystal data of **28**:*



Triclinic; Space group: P -1; unit cell dimensions: a = 5.8636(9) Å, α = 102.043(17)°, b = 9.7355(15) Å, β = 102.225(18)°, c = 13.7186(19) Å, γ = 93.521(19)°; V = 743.9(2) $Å^3$; Z = 2, Dx = 1.265 Mg/m^3; μ = 0.225 mm^{-1}; F(000) = 300.

Data collection: T = 296 K; graphite monochromator. A colourless plate with dimensions of 0.320 x 0.280 x 0.120 mm was used to measure 7804 reflections (2975 unique reflections, R_{int} = 0.0414) from 2.35° to 26.86° on a STOE-IPDS diffractometer with the rotation method.

Structure refinement: The F2 value was refined using the full-matrix least squares refinement method, with a goodness-of-fit of 0.88 for all reflections and 176 parameters.

III. 4.4. Investigation of the Binding Properties

Absorption Spectroscopy. Absorption spectra were recorded on a Varian Cary BIO 50 UV/VIS/NIR Spectrometer with temperature control by use of a 1 cm quartz cuvettes (Hellma) and Uvasol solvents (Merck, Baker or Acros).

Emission Spectroscopy. Fluorescence measurements were performed with UV-grade solvents (Baker or Merck) in 1 cm quartz cuvettes (Hellma) and recorded on a Varian 'Cary Eclipse' fluorescence spectrophotometer with temperature control.

Screening of peptide binding affinities: The estimation of binding affinities were performed in 96er half area UV star wellplates using a methanol / water mixture 9:1.[57] Depending on the expected binding strength hundred to five hundred equivalents of peptide or amino acid

($5*10^{-2}$ M to $1*10^{-3}$ M) were added to a $2*10^{-5}$ M solution of a particular receptor compound. Every solution - crown-guanidine receptors or amino acids - was, if necessary, adjusted[58] in it´s pH to weakly basic (pH 7.5 - 8.5) with Et_4NOH or HCl according to table 3.[59] The mixtures were quickly pipetted row by row, mixed with the aid of the Eppendorf pipette and allowed to equilibrate for five minutes. The fluorescence spectrum was recorded (λ_{ex} = 300 nm) and compared to a blanc sample of the receptor with the same concentration. All measurements were repeated twice.

Binding affinity titrations. In every titration 1.0 mL of a $2*10^{-5}$ M solution of receptor was used and titrated according to the following procedure in a cuvette: To the pH adjusted solutions[58] of the crown ether receptors in 9:1 methanol/water rising equivalents of the amino acids or peptides in the according solvent at the same pH were successively added in 10 µL (≙ 5 to 25 eq.) aliquots ($5*10^{-2}$ M to $1*10^{-2}$ M). After each addition the solution was allowed to equilibrate for 6 minutes and the emission intensity or a absorption spectrum was recorded at 25 °C (λ_{ex} = 300 nm). To determine the binding constant the obtained fluorescence intensities or absorption values were volume corrected, plotted against the concentration of peptide and evaluated by non linear fitting methods.

All titrations and the according curve fittings can be found in the supporting information of this chapter.

III. 5. References and Notes

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3 The $GABA_A$ receptor is very widely distributed in the brain and spinal cord and the major inhibitory receptor in the central nervous system (CNS). In the spinal cord the GABA receptors are involved in motoric control. In the thalamus, GABA acts at the initiation and maintenance of sleep. Pharmacologically, shortly summarized, these receptors can mediate the sedative effects of benzodiazepines (diazepam), are connected with an anxiolytic function or can have a muscle relaxant effect. For a brief review, see: M. Chebib, G.A.R. Johnston, *Clin. Exp. Pharm. Phys.*, **1999**, *26*, 937-940.

4 Most interestingly GABA is synthesized by means of glutamate decarboxylase (GAD) from glutamate. So in one step the main excitatory neurotransmitter is converted to most important inhibitory neurotransmitter, or in other words the γ-aminobutyric acid (GABA) can be seen as the biogenic amine of the glutamic acid.

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[35] All quantum yields were determined with quinine disulfate in 1 N H_2SO_4 as the reference compound (Φ = 0.546). The solution was degassed and measured in a closed, nitrogen flushed cuvette with septum.

[36] All compounds show due to their close structural relationship very similar photophysical properties: ε (nm) = 220 (27000 – 30000), 270 (7000 – 8500)

[37] a) The increase in emission by guest binding is most likely caused by the intercepted photoinduced electron transfer from the nitrogen atom of the crown ether to the fluorophore. b) The pK_a values of the tertiary amine in the crown ether in dependency on the spacer were evaluated by titration with perchloric acid (see supporting information). The aliphatic amines possess about the same basicity and are all between 5.6 and 6; only the aromatic substituent differs slightly with a value of 6.6.

[38] To prevent a potential cleavage of the methyl esters and decomposition of the acetyl-guanidine moiety, the measurements are restricted to the range pH > 5 to max. pH = 10.

[39] The pK_a value of the guanidinium moiety may vary depending on the adjacent group effects, in general it will be lowered by aromatic acyl > aliphatic acyl > phenyl > alkyl; K.A. Schug, W. Lindner, *Chem. Rev.* **2005**, *105*, 67-113; B.T. Storey, W.W. Sullivan, C.L. Moyer, *J. Org. Chem* **1964**, *29*, 3118-3120. Briefly summarized, the pK_a of aromatic acyl-guanidines is between 6 and 7 [see also 35], of aliphatic examples it is between 7 and 8. The alkylated or unsubstituted guanidines with about five orders of magnitude higher values can be assumed as always protonated under the given conditions.

[40] V. Rüdiger, H.-J. Schneider, V.P. Solov'ev, V.P. Kazachenko, O.A. Raevsky, *Eur. J. Org. Chem.* **1999**, *8*, 1847–1856.

[41] Commercially available, prepared by acylation with Ac_2O, NEt_3 DCM and esterifacion by TMS-Cl in MeOH of H-Lys(Cbz)-OH, followed by hydrogenolysis with H_2, Pd/C in MeOH, 20 bar, 1d. Flash chromatography with ethyl acetate gave the product in high purity. **^{1}H-NMR** (300 MHz, MeOD) [ppm] = 1.48 (m, 2 H), 1.70 (m, 2 H), 1.84 (m, 2 H), 2.02 (s, 3 H), 2.91 (m, 2 H), 3.91 (s, 3 H), 4.38 (m, 1 H); 1eq of 0.01 molar aqueous hydrochloric acid was added and the resulting salt was lyophylised before it was used for the measurement.

[42] Examples for guanidine binding by crown ethers smaller than 27-crown-9: R.E. Gawley, S. Pinet, C.M. Cardona, P.K. Datta, T. Ren, W.C. Guida, J. Nydick, R.M. Leblanc, *J. Am. Chem. Soc.* **2002**, *124*, 13448–13453.; H.-J. Buschmann, H. Dong, L. Mutihac, E. Schollmeyer, *J. Therm. Anal. Cal.* **1999**, *57*, 487–491.

[43] Tryptophane was exluded, as it absorbs in the same range as the crown ether fluorophore. The binding of tyrosine was not investigated; it is not sufficiently soluble under the experimental conditions.

[44] Solvatochromatic effects may lead to additional changes in the emission.

[45] The crown ether guanidinium combinations with thioureido chain or with triazole rings coordinate mercury ions weakly. Stable mercury compounds of triazoles are known: E. Müller, H. Meier, *Liebigs Ann. Chem.* **1968**, *716*, 11–18.

[46] Most acetyl-guanidines are comparable in their stability with esters. Acyl-guanidines are in general isolable compounds and can be stored as salts in non nucleophilic solvents over a long time without any decomposition. A possible intramolecular nucleophilic attack can accelerate the decomposition reaction enormously: A. Brennauer, M. Keller, M. Freund, G. Bernhard, A. Buschauer, *Tetrahedron Lett.* **2007**, *48*, 6996–6999. The acyl-guanidines proved to be stable for at least several days in solution. No different behaviour was observed if the screenings are repeated after two to three days. No strong nucleophile is present and the pH of the measurement never exceeded the range between 6 and 8.

[47] The combination Glu-Lys as in the dipeptide is found in functional regions of many important peptides as ubiquitin: C.M. Pickart, M.J. Eddins, *Biochim. Biophys. Acta.* **2004,** *1695(1–3)*, 55–72. It is highly conserved in nearly all human endorphins, for examples see: N. Ling, R. Burgus, R. Guillemin, *Proc. Natl. Acad. Sci. USA*, **1976**, *73(11)*, 3942-3946; K. Ramabadran, *Singapore Med. J.* **1983**, *24(4),* 235-240.

[48] P. Job, *Ann. Chim.* **1928**, *9*, 113–203.

[49] Energy minimization molecular modelling studies with the aid of the program package SPARTAN were used to obtain structural information for various receptor amino acid or peptide complexes and served as a basis to explain the observed differences in the binding constant. The depicted schemes are illustrative pictures, the modelling results can be found in the supporting information.

[50] Reactions like peptide coupling, thiourea- or urea synthesis or the versatile Huisgen cycloaddition are especially suitable for the assembly. A broad variety of synthetic prescriptions, which are compatible with almost any substituent, are existent. For example: R.B. Merrifield, *J. Am. Chem. Soc.* **1963**, *85*, 2149 – 2154; E. Atherton, R.C. Sheppard; *Solid Phase peptide synthesis: a practical approach.* Oxford, England: IRL Press. **1989**; D.A. Powell, P.D. Ramsden, R.A. Batey, *J. Org. Chem.* **2003**, *68*, 2300-2309; C.M. Starks, C. Liotta, *Phase-Transfer-Catalysis: Principles and Techniques*, Academic Press, New York, **1978**; H.C. Kolb, M.G. Finn, K.B. Sharpless, *Angew. Chem. Int. Ed.* **2001**, *40* , 2004–2021; A.J. Dirks, S.S. van Berkel, N.S. Hatzakis, J.A. Opsteen, F.L. van Delft, J.J.L.M. Cornelissen, A.E. Rowan, J.C.M. van Hest, F.P.J.T. Rutjes, R.J.M. Nolte, *Chem. Comm.* **2005**, *33*, 4172–4174.

[51] R.M. Williams, C. Yuan, V.J. Lee, S. Chamberland, *J. Antibiot.* **1998**, *51(2)*, 189-201.

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[53] K.E. Schulte, M. Goes, *Arch. Pharm. Ber. Dtsch. Pharm. Ges.* **1957**, *290*, 118-130; I. Marszak, J.P. Guermont, R. Epsztein, J. Jacob, *Compt. rend.* **1951**, *233*, 530-532.

[54] H. Vollmann, H. Becker, M. Corell, H. Streeck, *Liebigs Ann. Chem.* **1937**, *531*, 108.

[55] This material is commercially available. It can be easily prepared by refluxing the according aldehyde in acetone/water 1:1 with an excess of potassium permanganate for two hours. After filtration and evaporation of the acetone, it is carefully precipitated with diluted hydrochloric acid at 2 - 5 °C. Filtration by suction and drying in a dessicator furnishes the product. It can be recrystallised from ethanol if necessary. Beige needles with **M.p.** (not corr.) = 203 – 205°C; **^{1}H-NMR** (MeOD, 300 MHz) δ [ppm] = 6.18 (1 H, d), 6.86 (1 H, dd), 6.95 (1 H, d);

[56] The analogous receptors carrying one acetyl group were prepared after a similar procedure (*GP IV*). The 1-Boc-3-acetyl-2-methyl-2-isothiourea (145 mg, 0.5 mmol) was used instead. The well stirred solution was heated to 40°C under nitrogen atmosphere and held at this temperature over night. The workup is the same.

[57] Amino acids in their zwitterionic form are not sufficiently soluble in pure methanol, therefore the measurements have to be conducted in aqueous mixtures

[58] A pH electrode for methanolic solutions was used and calibrated once a day.

[59] Salt addition is avoided; it increases the polarity of the solvent systems and interferes with the guanidinium binding.

IV. Crown Ether Guanidinium Ion Synthetic Receptor for a Hemoregulatory Tetrapeptide[i]

The results gained from the extensive studies with ditopic crown ether guanidinium combinations (chapter III) were utilized to further improve the recognition motif for simultaneous ammonium and carboxylate recognition in its binding strength as well as in its pH dependence.

A synthetic receptor for the molecular recognition of a tetrapeptide in aqueous buffer was obtained by combining a luminescent crown ether with two pyrrole-guanidinium moieties. The compound interacts with ammonium carboxylates of complementary geometry and binds the hemoregulatory peptide Ac-Ser-Asp-Lys-Pro with $K = 7*10^3$ M^{-1} at physiological pH. Shorter fragments and other tetrapeptides show no or significant reduced affinity. The binding of the target peptide to the functionalized crown ether is signalled by an increase of its emission intensity.

[i] A. Späth, B. König, *Tetrahedron* **2010**, accepted.

IV. 1. Introduction

The naturally occurring hemoregulatory peptide Ac-Ser-Asp-Lys-Pro (**1**) shows anti-inflammatory and antifibrotic properties, thus decreasing hypertension-induced target organ damage.[1] It is known to inhibit the proliferation of hematopoietic stem cells[2] and has recently been reported to inhibit cardiac fibrosis.[3] It has been shown to reduce the damage to specific compartments in the bone marrow resulting from treatment with chemotherapeutic agents, ionizing radiations, hyperthermy, or phototherapy.[4] The peptide acts as a specific inhibitor for the *N*-terminal site of the angiotensin I-converting enzymes (ACE). The effect is characterized by the conversion activity of ACE.[5] Fluorescent substrates like coumaryl-acetyl-Ser-Asp-Lys-Pro were used for the determination of the kinetic constants and to develop a sensitive assay for ACE activity in human plasma.[6]

We report a synthetic receptor capable of indicating the presence of the peptide by change of its luminescence. The emission increase of the host depends on the concentration of the peptide which allows determining its concentration without further functionalisation with a fluorophore or radiolabel.

The structure of the peptide Ac-Ser-Asp-Lys-Pro (**1**) contains two carboxylate groups and the amino acid lysine bearing an ammonium ion side chain. Recently, we presented different receptors for zwitterionic amino acids, recognizing combinations of ammonium and carboxylate ions, based on luminescent crown ethers with guanidinium ion binding sites.[7] Here we extended this modular approach to a tridentate receptor **2** with complementary binding sites for the target peptide **1**.

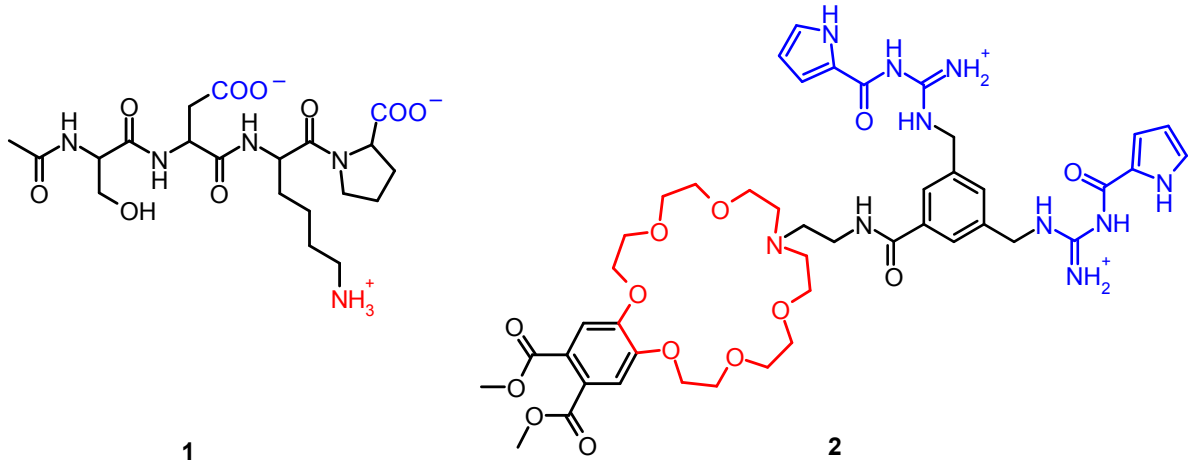


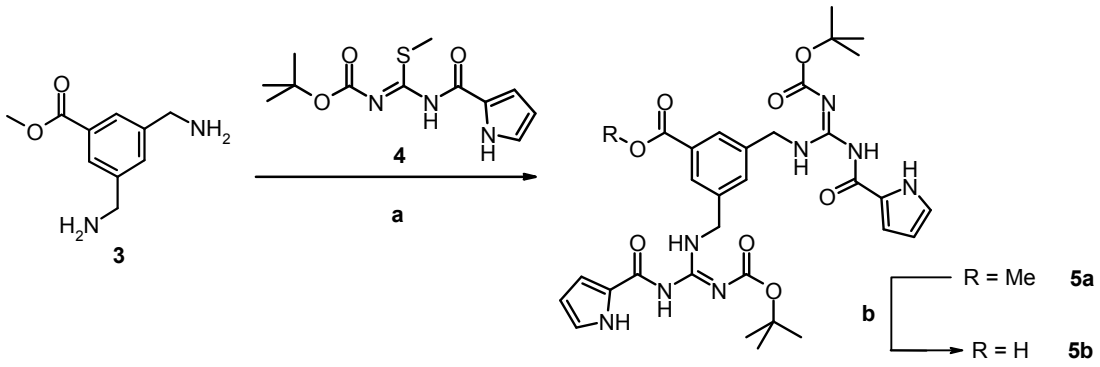
Figure 1: The hemoregulatory peptide **1** and the artificial receptor structure **2**; counterion is chloride.

The crown ether moiety[8,9] of compound **2** is a luminescent ammonium ion indicator based on photoinduced electron transfer,[10] while the guanidinium ion group strongly interacts with anions through charge pairing and hydrogen bonding.[11] It is used as recognition motif for carboxylates[12] and its interactions are well-studied.[13] A pyrrole substituent improves the binding strength of the recognition motif further.[14] Supramolecular studies in water[15] are especially difficult, because solvent hydrogen bonds may disturb the binding process and diminish or even prevent molecular recognition. Multiple binding sites may overcome the effect: The spacer units between the binding sites of compound **2** were designed to complement the hydrogen-bonding pattern of the target peptide zwitterion without significant re-organization of the receptor (fig. 1).

IV. 2. Results and Discussion

IV. 2.1. Synthesis

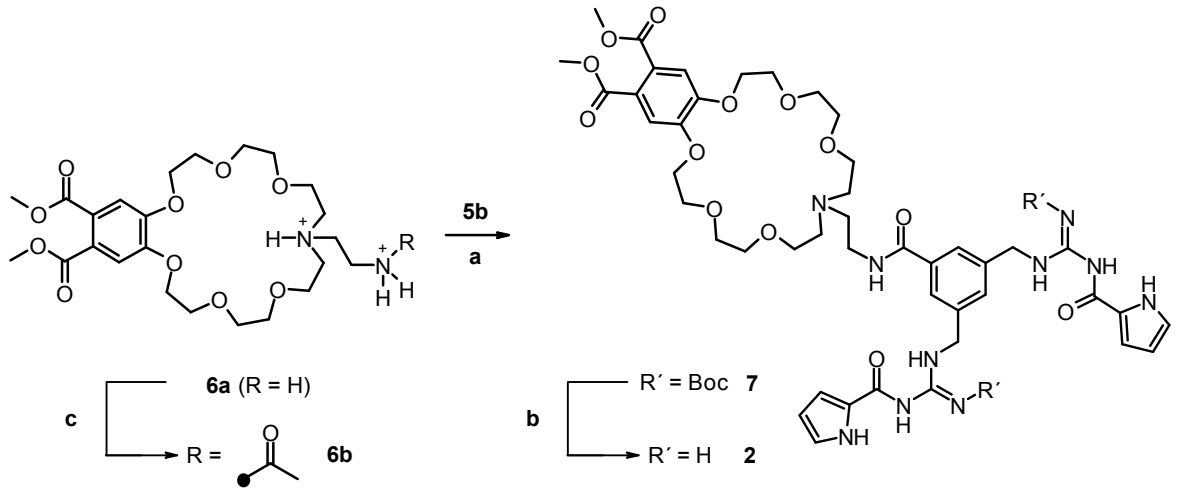
The synthesis of the substructures of receptor **2**, the crown ether amino acid ester **6**[9] and pyrrole substituted isothiourea **4**[7] were published before. 3,5-Bis-aminomethyl-benzoic acid methyl ester (**3**)[16] was reacted with methyl-isothiourea **4**, deprotected and coupled to the crown ether amino acid ester **6**.



Scheme 1: Synthesis of the protected pyrrole-guanidine **5b**; conditions: *a) DCM, MeOH, NEt_3, EDC*HCl, RT, then 40 °C over night, 77 %; b) NaOH, MeOH, H_2O, RT, 20 h, quant.*

The guanidilation reaction can also be achieved by mercury(II) catalysis in DMF in 6 h.[17] The EDC promoted reaction is slower, but superior: Toxic metal ions are avoided, the overall

yield is higher and less side products are formed.[18] A small amount of methanol has to be added in the reaction step due to solubility reasons.[19] The subsequent deprotection step gives quantitative yields of **5b**.



Scheme 2: Peptide coupling of compound **5b** with **6a**; conditions: *a) EDC*HCl, HOBt, $CHCl_3$, DMF, N_2, RT, then 40 °C, over night, 75 %; b) CH_2Cl_2, HCl in Et_2O, 5 h, RT, quant.; c) DCM, NEt_3, Ac_2O, 3 h, RT, quant.*

The peptide coupling step proceeds well in good yield by adding a second portion of coupling reagents after some time and rising the temperature.[20] The deprotection to the according hydrochloride proceeds in quantitative yield. Compound **6b** was prepared for comparison by acylation under standard conditions.

Peptides for the determination of binding selectivity were prepared in solution and by standard solid phase methods.

IV. 2.2. Physical Properties of Crown-Guanidino-Pyrrole Receptor 2

Compound **2** showed absorption maxima in methanol at 205 nm, with a shoulder at 220 nm, and at 290 nm, corresponding to the crown ether and pyrrole moieties, respectively. Upon excitation at 310 nm, the system emits at 390 nm.[21] The quantum yield is $\phi = 0.1$.[22]

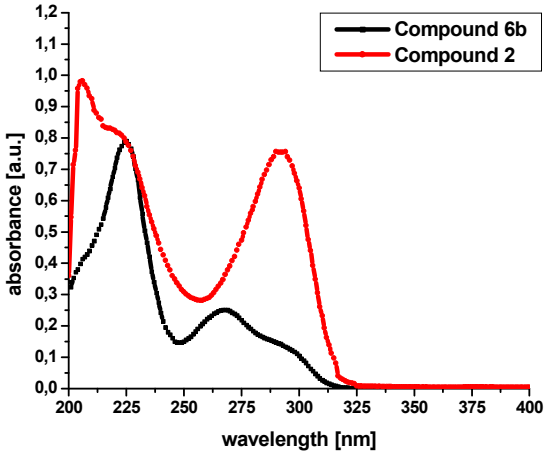


Figure 2: Absorption spectra of compound **2** and **6b** (c = $3.4*10^{-5}$ and $3.2*10^{-5}$ mol/L)

The compounds emission intensity is sensitive to pH: Protonation of the crown ether nitrogen atom interrupts PET fluorescence quenching. The pK_a value for the crown ether nitrogen atom was determined to be 5.6 by titration of the protected derivative **7** with perchloric acid.[23] In fluorescence experiments with **2** in methanol / water 4:1 the same value was found. Solutions of the compound in methanol were mixed with aqueous buffer at distinct pH values and the emission of the crown ether was recorded. Plotting of emission versus pH reveals a sigmaoidal curve shape (fig 3).[24]

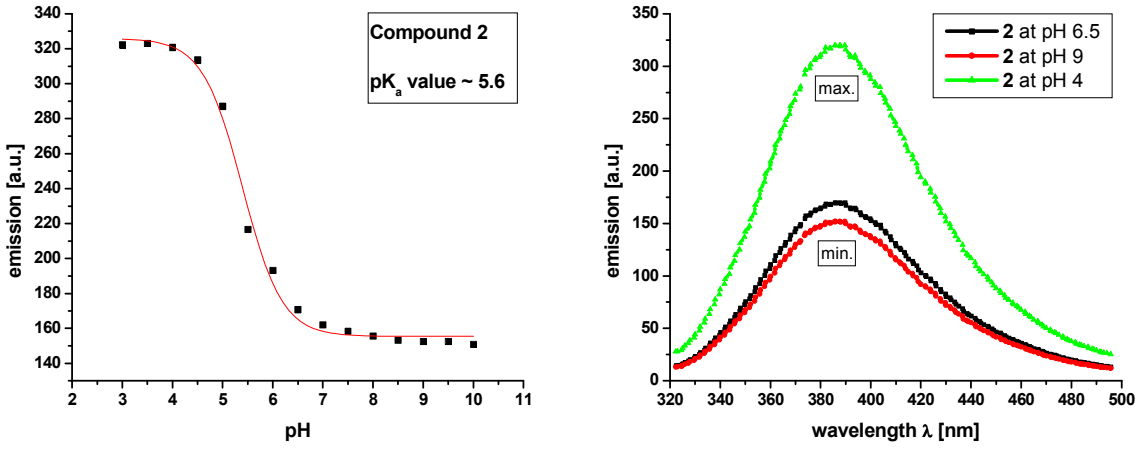


Figure 3: pH-Dependence of the emission intensity of receptor **2** in methanol / water 4:1; λ_{ex} = 300 nm, λ_{em} = 390 nm; (c = $3.0*10^{-5}$ mol/L)

The pK_a value of the guanidinium part was determined from pH titration experiments by the half-equivalence method: A 1.0 mM solution of a Boc-deprotected sample[25] of **5a** in water

was mixed with half the molar amount of sodium hydroxide solution (10.0 mM) and the pH value was measured. The experiment was repeated once. The averaged pK_a value observed for the pyrrole-guanidinium units was 6.5.

A pH value just in the middle between the pK_a values of both binding sites should meet the requirement of the guanidines being protonated and the crown ether moiety being nearly completely deprotonated. The binding is studied in buffered solution at pH 6.3 to minimize the interference by protons, while maintaining the guests largely in their zwitterionic form.[26] Lowering the pH value to 6.3 gives only small changes in emission (fig 3), indicating a protonation of the signalling and ammonium ion binding part to an inferior degree.[27]

IV. 2.3. Binding Studies

Peptide binding to compound **2** was investigated in methanol / aqueous buffer mixtures adjusted to pH 6.3. This ensures a large gain in emission intensity upon ammonium ion binding to the crown ether, the aza-crown nitrogen atom quenching the emission is mainly unprotonated at this pH. The acyl guanidines are protonated under these conditions and interact with carboxylate ions. First, the interaction of **2** with ammonium and carboxylate ions was investigated separately: The addition of up to 1000 eq. guanidine hydrochloride or tetrabutylammonium acetate in methanol / aqueous buffer 4:1 (10 mM HEPES adjusted to pH 6.3 with dilute hydrochloric acid) did not affect the emission at 390 nm. *n*-Butylammonium chloride addition increases the emission intensity only negligible ($F/F_0 < 1.05$).[28] The possible effect of ionic strength on the emission intensity was probes by addition of sodium perchlorate in large excess (1000 eq.), which induced no observable emission changes. Sodium acetate[29] was added to investigate the pyrrole-guanidinium carboxylate binding. Analysis of changes in the UV-vis spectrum revealed a binding constant of $< 10^3$ M^{-1} with 1:1 stoichiometry, but no changes of the emission intensity at 390 nm are observed.[30]

The binding of compound **2** to the hemoregulatory peptide **1** and related peptide structures was investigated by emission titration. Ac-Glu-Lys-amide (**8**)[31], Cbz-Lys-Gly and Lys-Gly (**9**) are partial structures of the target peptide. For comparison glycine (**11a**), γ-aminobutyric acid (**11b**), ε-aminohexanoic acid (**11c**), Gly-Gly-OH (**12a**), Gly-Gly-Gly-OH (**12b**), Gly-Gly-Gly-Gly-OH (**12c**) and the isomeric peptides Glu-Gly-Gly-Gly-NH_2 (**10a**), Gly-Glu-Gly-Gly-NH_2 (**10b**), Gly-Gly-Glu-Gly-NH_2 (**10c**) and Gly-Gly-Gly-Glu-NH_2 (**10d**) were investigated.

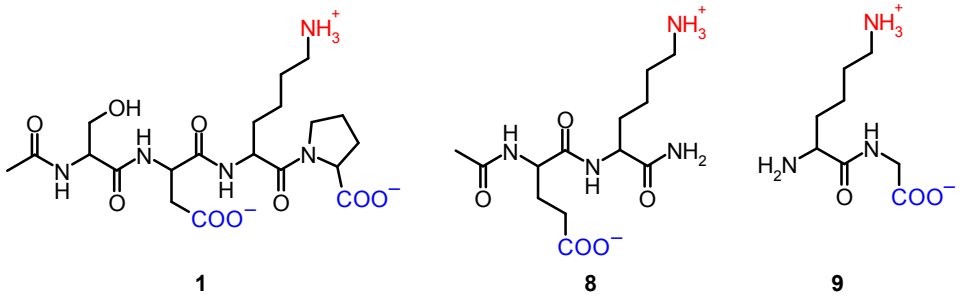


Figure 4: The hemoregulatory peptide **1** and fragment model peptides for binding to compound **2** in methanol / aqueous buffer 4:1 solution

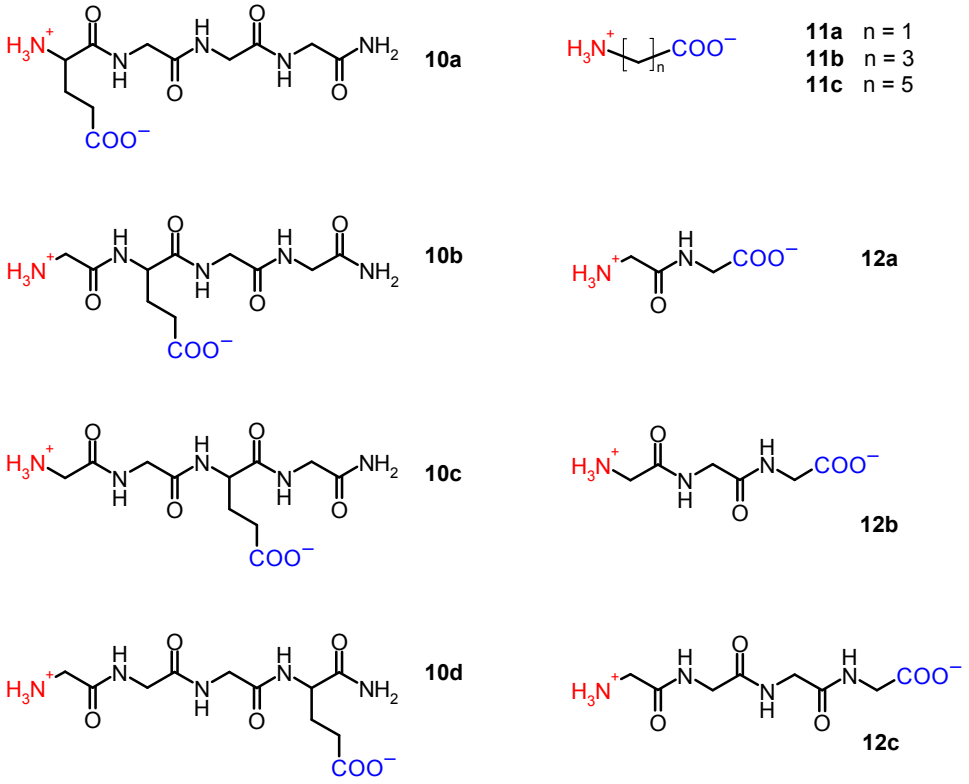


Figure 5: Different peptides investigated for binding to compound **2** in methanol / aqueous buffer 4:1 solution

To quickly identify the strongest interactions, 200 and 500 equivalents of the peptides were added to a solution of **2** (0.5 mL, $3*10^{-5}$ molar) in methanol / aqueous buffer 4:1 (10 mM HEPES; pH 6.3). All peptides were soluble in the used concentration range.[32] Compound **2** shows a significant 1.7-fold fluorescence enhancement with **1** in 20 % aqueous, buffered methanol (fig. 5, left diagram). Addition of $(Gly)_3$, $(Gly)_4$, (Cbz, H)-Lys-Gly, Gly-Glu-Gly-

Gly-amide and Gly-Gly-Glu-Gly-amide results in a weak response of the emission intensity (F/F_0 = 1.1 – 1.3). Increasing the amount of buffer to 50 % in the mixture with methanol (fig 5, right diagram), the emission change upon addition of **1** is still evident (F/F_0 = 1.5), all other substances induce negligible increase in emission (F/F_0 = < 1.1).[33]

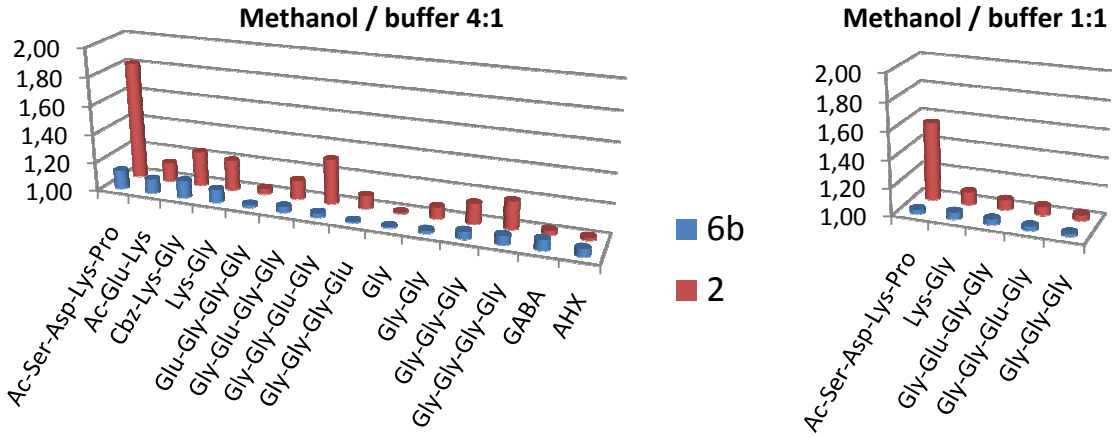


Figure 6: Response of compound **2** in comparison to the sole crown ether (**6b**) upon guest addition (200 eq.); [receptor] = *3*10^{-5} M in MeOH / buffer (10 mM HEPES) 4:1 (left) and 1:1 (right) at pH 6.3 adjusted with Et_4NOH and HCl; quantum yield when ion-free = 0.1; error is assumed to be around 5 %; counterion is chloride; [guest] = 0.006 M.*

The zwitterions glycine, γ-aminobutyric acid (GABA) and ε-aminohexanoic acid (AHX) give no response; Gly-Gly-OH, Glu-Gly-Gly-Gly-NH_2 and Glu-Gly-Gly-Gly-NH_2 elicit a weak response (F/F_0 ~ 1.1).

The binding affinities of **2** to all peptides showing an emission enhancement F/F_0 > 1.1 were determined under the conditions of the screening experiment (solvent, concentration, equivalents). Methanol / HEPES buffer was used as the solvent.[34]

To solutions of the crown ether receptor (3*10^{-5} M) in 4:1 methanol / aqueous buffer mixture (10 mM HEPES, pH 6.3) equivalents of the peptides were added. Fluorescence spectra were recorded at 25 °C after 5 min. The binding affinity was evaluated with the aid of the program package ORIGIN as a plot of the concentration corrected emission maxima (390 nm) versus the concentration of guest added.

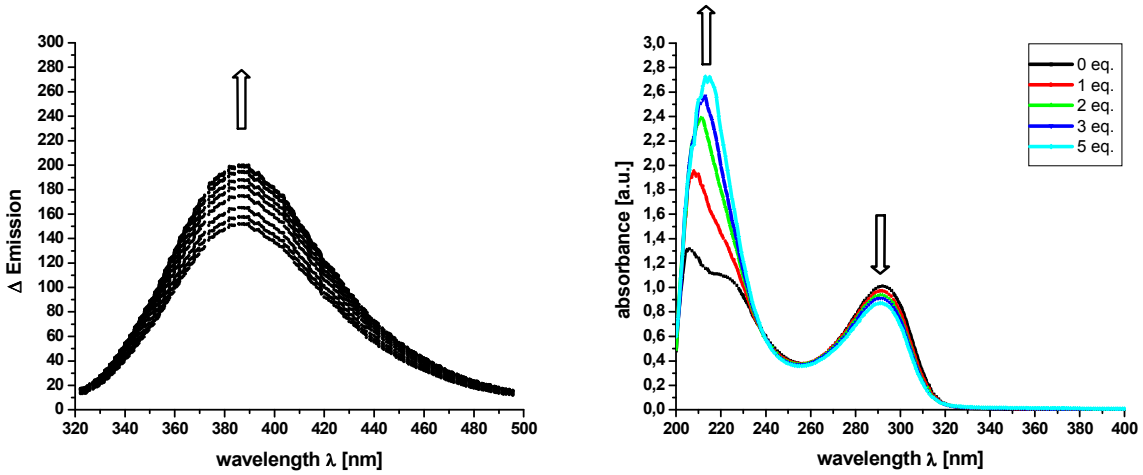


Figure 7: Changes in the emission- (left) and absorption spectra (right) of compound **2** upon titration with the tetrapeptide **1** in methanol / aqueous buffer 4:1 at pH 6.3

To study its interaction to the crown ether without additional coordination by the carboxylate binding sites, compound **6b** was titrated with **1** after the same schedule.

Additionally, due to giving a positive screening result, the binding constant of Ac-Ser-Asp-Lys-Pro (**1**) with **2** was evaluated in 1:1 methanol / aqueous buffer. Dependence of fluorescence intensity upon guest concentration was analysed according to the Hill-plot, which also allows determination of cooperativity (factor n).[35]

$$Y = (V_{max} * x^n) / (k^n + x^n);$$

Equation 1: Hill plot

Figure 7 shows the F/F_0 curves of compound **2** titrated with Ac-Ser-Asp-Lys-Pro (**1**) in methanol / aqueous buffer 4:1 and 1:1, where F_0 represents the fluorescence intensity when the guest is absent, F in the presence of the guest, K the binding constant. The red line is the fit by the Hill equation (equation 1).

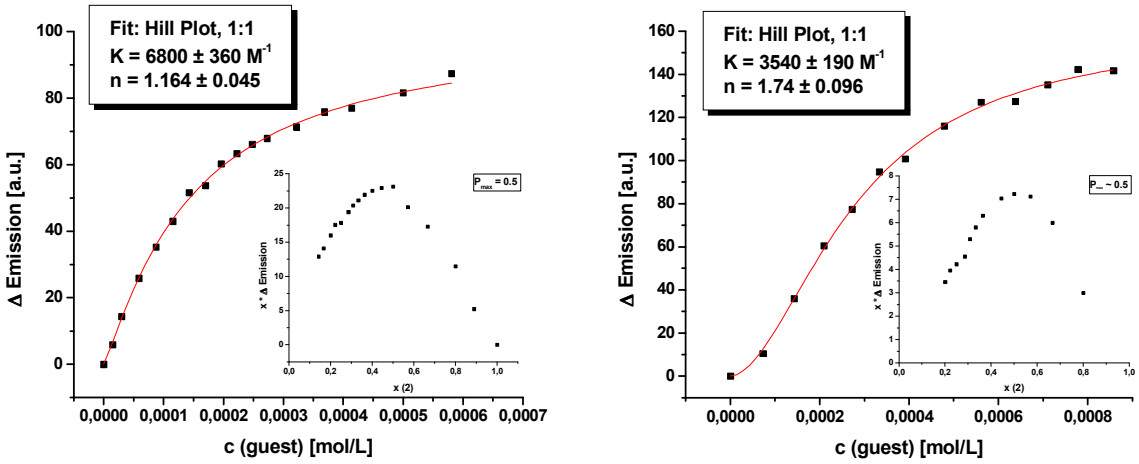


Figure 8: Titration of compound **2** with the tetrapeptide **1** in methanol / aqueous buffer 4:1 (left) and 1:1 (right) at pH 6.3; Small inserts: Job´s plot analyses

The Hill coefficient with a value of > 1.0 indicates weakly cooperative binding to the hemoregulatory peptide **1**. For all other guests no cooperativity was found (see supporting information for curves and fits). Table 1 summarizes the results of all titrations with receptor **2**. The values for GABA (**11b**) are given for comparison.

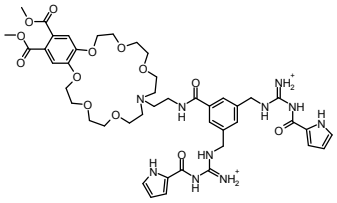
	**Binding constant** $K\ [M^{-1}]$	**Relative binding** $K / K(1)$	**Fluorescence enhancement** F / F_0
Ac-Ser-Asp-Lys-Pro (**1**)	6800 ± 360 (3540 ± 190)*	1 (1)*	1.62 (1.69)*
Lys-Gly (**8**)	930 ± 50	0.137	1.41
Gly-Glu-Gly-Gly-NH_2 (**10b**)	220 ± 30	0.032	1.32
Gly-Gly-Glu-Gly-NH_2 (**10c**)	800 ± 60	0.117	1.34
$(Gly)_3$ (**12b**)	510 ± 60	0.075	1.21
$(Gly)_4$ (**12c**)	390 ± 40	0.057	1.17
GABA (**11b**)	<< 100[36]	<< 0.01	1.04

Table 1: Binding constants, correlation to **1** and fluorescence enhancement for chemosensor **2** in methanol / aqueous buffer 4:1; pH 6.3; *) values for 1:1 methanol / aqueous buffer

It was not possible to derive a binding constant for **1** versus **6b**, the value is too small. The obtained titration curve does not reach saturation, even if a large excess of the guest (> 2000 eq.) is added.

All peptides that were compared, Lys-Gly (**8**), Gly-Glu-Gly-Gly-NH_2 (**10b**) and Gly-Gly-Glu-Gly-NH_2 (**10c**), $(Gly)_3$ (**12b**) and $(Gly)_4$ (**12c**), bind with affinities of 220 to 930 M^{-1} to **2**, which reflects a 6 – 30 fold weaker coordination with respect to **1** (table 1).

The enhanced binding affinity of **2** for peptide **1** of log K = 3.8 can be rationalized by the additional electrostatic interactions due to a second carboxylate group in **1**. The dipeptide guest Lys-Gly (**8**), which reflects a fragment of the structure of **1**, is missing this additional carboxylate guanidinium interaction and therefore shows an affinity to **2** of log K < 3. All other zwitterion guests, which can undergo ditopic binding, are even weaker coordinating to **2**. The importance of the guanidinium – carboxylate interaction is illustrated by the interaction of compound **6b** with **1**: The interaction of the crown ether with the ammonium ion in aqueous, buffered methanol (20 % water content) is too weak to derive a binding constant. The crown ether binding affinity for ammonium ions in aqueous solution is known to be small (log K < 2).[37]

IV. 3. Conclusions

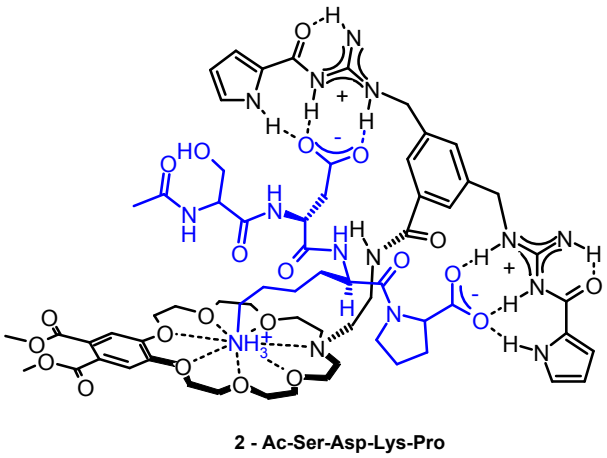


Figure 9: Proposed complex structure of compound **2** with the tetrapeptide **1**

Compound **2** is a synthetic receptor for the hemoregulatory peptide **1** binding the lysine ammonium side chain,[38] the terminal and the aspartic acid side chain carboxylate of the

peptide.[39] The binding of the peptide is signalled by an increase in emission intensity of the crown ether fluorophor, as the ammonium ion binding intercepts the photoinduced electron transfer (PET) quenching mechanism. The combination of the crown ether to ammonium ion interaction with the guanidinium to carboxylate ion interaction is essential for the binding process. The individual part does not show a sufficient interaction with the target peptide, as shown by investigations using the substructure **6b**. The same is true for simple ammonium of carboxylate ions, which interact with **2** only weakly. Small peptides, which were used for comparison, revealed that the additional carboxylate group in **1** and the ammonium – carboxylate distance complementary to **2** are of importance to achieve a high affinity. Figure 9 shows the proposed structure of the peptide – receptor aggregate.

The results provide another example that the combination of several weak reversible interactions may lead to synthetic receptors with affinity and selectivity of biological targets. Although the binding and emission properties of compound **2** are not sufficient for analytical applications in complex matrices, its use as an indicator with some selectivity in less complex mixtures is already possible.

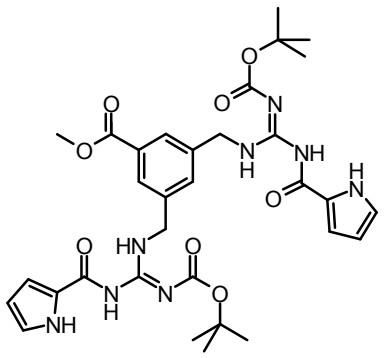
IV. 4. Experimental Part

IV. 4.1. Syntheses

Compounds **6**,[9] **4**[40] and **3**[16] were synthesized in solution according to published procedures. Ac-Ser-Asp-Lys-Pro (**1**) was purchased from GENSCRIPT in a purity > 95 % and used as is.

CAUTION: the noxious gas CH_3SH is produced during the synthesis of compound **5a.** This reaction should be only handled in the fume hood or the gas should be trapped with aqueous sodium hydroxide and destroyed with sodium hypochlorite.

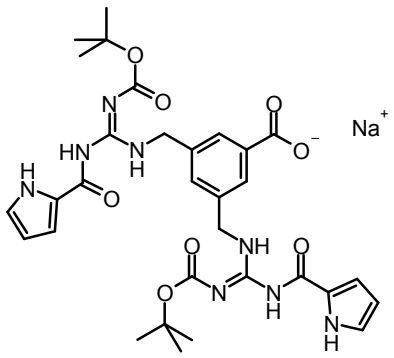
*3,5-bis-(1-(tertButyloxycarbonyl)-3-(pyrrol-2-carbonyl)-guanidino-methyl)benzoic acid methyl ester (**5a**)*



To a cooled solution (2 - 5°C) of compound **4** (312 mg, 1.1 mmol) in 3.0 ml DCM and methanol (19:1), containing triethylamine (0.66 mL, 500 mg, 5.0 mmol), 3,5-bis-aminomethyl-benzoic acid methylester (**3**)[41] (105 mg, 0.5 mmol) and EDC*HCl (272 mg, 1.5 mmol) were subsequently added. The reaction was stirred for 3 h whilst it was slowly reaching room temperature, then refluxed for another two hours and cooled to room temperature again. A second portion EDC*HCl (272 mg, 1.5 mmol) was added, it was stirred for 30 min at room temperature and then refluxed for 2 h. The reaction mixture was diluted with 30 mL of DCM and filtered. It was washed with 10 % citric acid (10 mL) and water (10 mL), dried over $MgSO_4$ and the solvent was evaporated. The residue was purified by column chromatography with ethyl acetate / ethanol 6:1 to give the product as an orange solid (256 mg, 0.385 mmol, 77 %), (R_f [EtOAc / EtOH 6:1] = 0.4).

M.p. (uncorrected) = 127 - 128°C; - **^{1}H-NMR** (300 MHz, $CDCl_3$):[42] δ [ppm] = 1.44 – 1.54 (m, 18 H), 3.90 (s, 3 H), 4.68 – 4.81 (m, 4 H), 6.22 (m, 2 H), 6.71 – 7.01 (m, 4 H), 7.59 – 7.72 (m, 1 H), 7.85 – 8.01 (m, 2 H), 8.80 (m, 2 H), 9.23 (m, 1 H), 9.53 (bs, 1 H), 9.76 (bs, 1 H), 9.98 (bs, 1 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$):[42] δ [ppm] = 28.1 & 128.3 (+, 6 C), 44.5 (-, 2 C), 52.3 (+, 1 C), 79.8 & 83.3 (C_{quat}, 2 C), 110.4 (+, 2 C), 114.5 (+, 2 C), 122.1 (+, 2 C), 128.2 (+, 2 C), 131.0 (C_{quat}, 2 C), 131.6 (+, 1 C), 139.1 (C_{quat}, 2 C), 153.3 (C_{quat}, 2 C), 156.0 & 156.3 (C_{quat}, 2 C), 160.6 (C_{quat}, 1 C), 164.0 (C_{quat}, 1 C), 166.5 (C_{quat}, 1 C), 171.1 (C_{quat}, 1 C); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 665.2 (90, MH^+), 333.0 (100, $(M+2H^+)^{2+}$); - **HRMS** (PI-LSIMS FAB, glycerine): calc. for $C_{32}H_{41}N_8O_8^+$: 665.3047, found: 665.3033; - **MF:** $C_{33}H_{43}N_8O_8$ – **FW:** 679.76 g/mol;

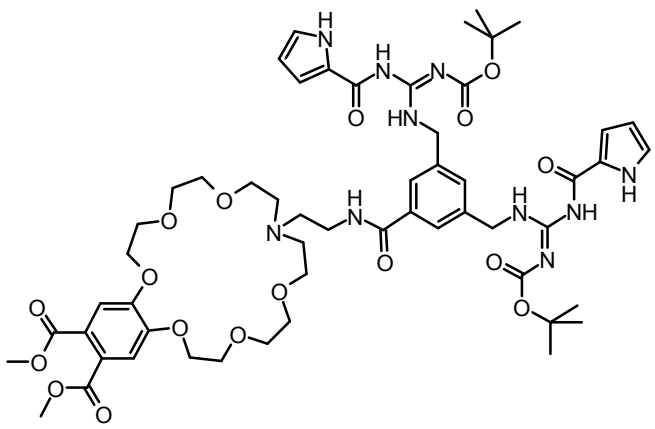
*3,5-bis-(1-(tertButyloxycarbonyl)-3-(pyrrol-2-carbonyl)-guanidino-methyl)benzoic acid sodium salt (**5b**)*



Compound **5a** (166 mg, 0.25 mmol) in methanol (1.0 mL) was treated with 1 M aqueous sodium hydroxide solution (0.25 mL) by vigorous stirring at room temperature over night. The methanol was evaporated and the residue was lyophilised to give the product as a yellowish solid (164 mg, 0.244 mmol, 98 %).

M.p. (uncorrected) = 145 - 146 °C (decomp.); - **^{1}H-NMR** (300 MHz, $CDCl_3$):[42] δ [ppm] = 1.47 – 1.53 (s, 18 H), 4.69 – 4.78 (m, 4 H), 6.20 – 6.31 (m, 2 H), 6.83 – 6.89 (m, 4 H), 7.64 (m, 1 H), 7.90 - 8.05 (m, 2 H), 8.84 (m, 2 H), 9.41 (m, 2 H), 9.86 (bs, 1 H), 12.43 (bs, 1 H); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 651.2 (94, MH^+), 326.0 (100, $(M+2H^+)^{2+}$); - **HRMS** (PI-LSIMS FAB, glycerine): calc. for $C_{31}H_{39}N_8O_8^+$: 651.2891, found: 651.2897; - **MF:** $C_{31}H_{37}N_8O_8$ – **FW:** 672.68 g/mol;

14-[2-[3,5-bis-(1-(tertButyloxycarbonyl)-3-(pyrrol-2-carbonyl)-guanidino-methyl)benzamido]-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (7)

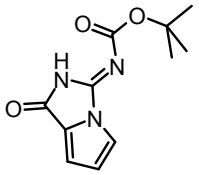


Compound **5b** (135 mg, 0.20 mmol) was dissolved in 1.0 ml of DMF and DCM (1:1) at 5° C, triethylamine (0.06 mL, 0.05 g, 0.5 mmol), HOBt (29 mg, 0.22 mmol) and EDC hydrochloride (36 mg, 0.21 mmol) were added subsequently and the mixture was stirred for 30 mins in the ice bath. The TFA salt of compound **6a** (163 mg, 0.22 mmol) dissolved in 1.0 mL of DCM containing 0.06 mL triethylamine (0.05 g, 0.5 mmol) was added slowly, then the mixture was stirred for 2 h at room temperature. Another portion of triethylamine (0.06 mL, 0.05 g, 0.5 mmol), HOBt (29 mg, 0.22 mmol) and EDC hydrochloride (36 mg, 0.21 mmol) was added, stirring was continued for 1 h, then the mixture was warmed to 40 °C and stirred for additional 8 h under nitrogen. The solvents were removed and the residue was re-dissolved in 10.0 mL of ethyl acetate. The solution was filtered and the filter cake was washed with a small portion of ice-cold ethyl acetate. The clear filtrate was washed with 5 % aqueous ammonium chloride solution and three times with water (3 mL). The organic phase was dried over $MgSO_4$ and evaporated to dryness. The remaining solid was purified by column chromatography with ethyl acetate / ethanol 10:1 to yield 173 mg of a pale yellow glass (0.151 mmol, 75 %), (R_f [EtOAc / EtOH 10:1] = 0.2).

^{1}H-NMR (600 MHz, $CDCl_3$):[42] δ [ppm] = 1.42 – 1.54 (m, 18 H), 2.56 (bm, 4 H), 3.49 – 3.68 (bm, 6 H), 3.59 (m, 4 H), 3.65 – (m, 4 H), 3.81 (m, 4 H), 3.82 – 3.88 (m, 8 H), 4.62 – 4.78 (m, 8 H), 6.15 – 6.33 (m, 2 H), 6.81 – 6.95 (m, 4 H), 7.04 (m, 1 H), 7.12 (m, 2 H), 7.46 (m, 1 H), 7.70 (m, 1 H), 7.82 (bs, 1 H); - **^{13}C-NMR** (150 MHz, $CDCl_3$):[42] δ [ppm] = 28.0 & 28.3 & 30.9 (+, 6 C), 40.9 (-, 1 C), 44.2 & 44.4 (-, 2 C), 46.3 (-, 1 C), 52.6 (+, 2 C), 54.5 (-, 2 C), 68.8 (-, 2 C), 69.4 (-, 2 C), 70.2 (-, 4 C), 70.8 (-, 2 C), 79.6 & 82.2 (C_{quat}, 2 C), 110.3 & 110.8 (+, 2 C), 113.1 & 113.6 (+, 2 C), 114.3 (+, 2 C), 121.9 (+, 2 C), 124.2 (+, 1 C), 125.3 (C_{quat}, 2 C), 126.1 (+, 2 C), 130.1 (C_{quat}, 2 C), 138.9 (C_{quat}, 1 C), 150.0 & 153.1 (C_{quat}, 2 C), 155.7 & 155.8 (C_{quat}, 2 C), 165.1 (C_{quat}, 1 C), 167.8 (C_{quat}, 2 C), 170.9 (C_{quat}, 2 C); - **IR** (KBr): ν [cm^{-1}] = 3300 (bm), 2948 (m), 2929 (m), 2876 (m), 2356 (w), 2247 (w), 1718 (m), 1607 (s), 1578 (s), 1546 (s), 1406 (s), 1349 (s), 1289 (s), 1256 (m), 1229 (m), 1187 (m), 1124 (s), 1027 (m), 980 (m), 910 (m), 843 (m), 780 (m), 729 (s), 646 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 1147.6 (21, MH^+), 574.2 (100, $(M+2H^+)^{2+}$), 383.2 (22, $(M+3H^+)^{3+}$); - **HRMS** (PI-LSIMS FAB, glycerine): calc. for $C_{56}H_{75}N_{10}O_{17}^+$: 1147.5312, found: 1147.5307; - **UV** (MeOH): λ (ε) = 298 (18900), 205 (30500); - **MF:** $C_{55}H_{74}N_{10}O_{17}$ – **FW:** 1147.26 g/mol;

If the reaction is carried out at 70°C or mercury(II) is employed the following side product is formed in the reaction in differing amounts:

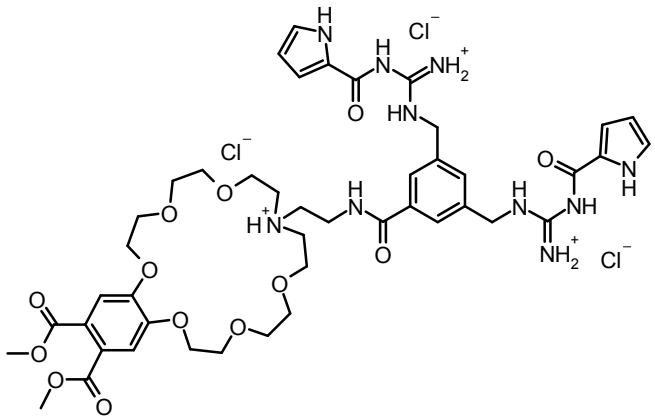
(1-Oxo-1,2-dihydro-pyrrolo[1,2-c]imidazol-3-ylidene)-carbamic acid ^{tert}butyl ester



R_f [EtOAc / EtOH 6:1] = 0.8

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.53 (s, 9 H), 6.43 (dd, 1 H, 5.8 Hz), 6.84 (d, 1 H, 5.8 Hz), 7.41 (d, 1 H, 5.8 Hz), 9.72 (bs, 1 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 26.9 (+, 3 C), 81.5 (C_{quat}, 1 C), 113.7 (+, 1 C), 116.9 (+, 1 C), 118.3 (+, 1 C), 123.0 (C_{quat}, 1 C), 149.4 (C_{quat}, 1 C), 156.3 (C_{quat}, 1 C), 159.8 (C_{quat}, 1 C); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 471.0 (6, $2MH^+$), 276.9 (100, MH^+ + MeCN), 235.9 (74, MH^+); - **MF:** $C_{11}H_{13}N_3O_3$ – **FW:** 235.24 g/mol;

*14-[2-[3,5-bis-(1-(Pyrrol-2-carbonyl)-guanidino-methyl)benzamido]-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester hydrochloride (**2**)*

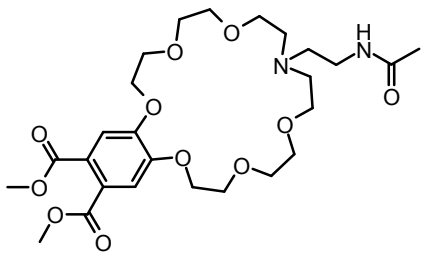


Compound **7** (115 mg, 0.10 mmol) was dissolved in 1.0 ml of dry DCM, 0.3 mL of HCl saturated diethylether was added and the mixture was stirred for 3 h at room temperature under moisture protection. The product was fully precipitated by slow addition of diethylether. The solvent was decanted off, the solid was suspended in a small volume of diethylether, was allowed to settle completely and the ether was decanted off again. This step

was repeated once. After drying in vacuo the product was isolated as a fine yellow powder (96 mg, 0.091 mmol, 91 %).

^{1}H-NMR (400 MHz, CD_3OD): δ [ppm] = 3.48 – 3.59 (m, 6 H), 3.65 – 3.71 (m, 8 H), 3.75 (t, 2 H, 6.4 Hz), 3.82 (s, 6 H), 3.84 (m, 4 H), 3.91 (m, 4 H), 4.21 (m, 4 H), 6.30 (m, 2 H), 7.16 (m, 2 H), 7.21 (s, 2 H), 7.26 (m, 2 H), 7.61 (s, 1 H), 7.82 (s, 2 H); - **^{13}C-NMR** (100 MHz, $CDCl_3$): δ [ppm] = 45.6 (-, 2 C), 51.7 (-, 1 C), 53.2 (+, 2 C), 55.3 (-, 2 C), 64.1 (-, 1 C), 70.2 (-, 4 C), 70.8 (-, 2 C), 71.4 (-, 2 C), 71.7 (-, 2 C), 111.8 (+, 2 C), 114.3 (+, 2 C), 115.9 (C_{quat},, 1 C), 116.6 (+, 2 C), 126.5 (C_{quat}, 2 C), 127.0 (+, 2 C), 130.9 (C_{quat}, 2 C), 151.7 (C_{quat}, 2 C), 161.7 (C_{quat}, 2 C), 169.4 (C_{quat}, 2 C), further signals were not detectable; - **IR** (KBr): ν [cm^{-1}] = 3200 (bm), 2946 (m), 2883 (m), 2361 (m), 2347 (m), 1678 (s), 1632 (m), 1546 (m), 1434 (m), 1293 (s), 1186 (m), 1120 (s), 1051 (m), 974 (m), 948 (m), 887 (m), 746 (m), 667 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 947.7 (6, MH^+), 531.2 (40, $(M+2H^+)^{2+}$ + TFA), 474.2 (100, $(M+2H^+)^{2+}$), 330.1 (98, $(M+3H^+)^{3+}$ + MeCN), 316.4 (14, $(M+3H^+)^{3+}$ + TFA); - **HRMS** (PI-LSIMS FAB, glycerine): calc. for $C_{45}H_{59}N_{10}O_{13}^+$: 947.4263, found: 947.4241; - **UV** (MeOH): λ (ε) = 291 (22300), 206 (29400); - **MF:** $C_{45}H_{61}N_{10}O_{13}Cl_3$ – **FW:** 1056.40 g/mol;

14-[2-N-(Acetyl)-aminoethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester ***(6b)***



Compound **6a** (135 mg, 0.2 mmol) was stirred with triethylamine (0.12 mL, 0.1 g, 1.0 mmol) and acetic anhydride (0.06 mL, 0.06 g, 0.5 mmol) in DCM (1.0 ml) for 3 h at room temperature, finally it was warmed to 40 °C and the dichloromethane was allowed to evaporate. All volatiles were removed under reduced pressure and the residue was re-dissolved in 10 mL of ethyl acetate. The organic phase was washed with saturated ammonium chloride solution, then four times with brine (3 mL). After drying over $MgSO_4$, the solvent

was evaporated to yield the product **6b** (108 mg, 0.194 mmol, 97 %) as a yellow glass (R_f [EtOAc / EtOH 3:1] = 0.2).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 2.07 (s, 3 H), 2.68 (m, 2 H), 3.22 (m, 2 H), 3.48 (m, 4 H), 3.55 – 3.64 (m, 8 H), 3.66 – 3.72 (m, 4 H), 3.81 – 3.89 (m, 4 H), 3.86 (s, 6 H), 4.14 – 4.21 (m, 4 H), 5.45 (bs, 1 H), 7.21 (s, 2 H) - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 25.1 (+, 1 C), 32.1 (-, 1 C), 40.5 (-, 1 C), 52.6 (+, 2 C), 54.8 (-, 2 C), 69.1 (-, 2 C), 69.4 (-, 2 C), 69.8 (-, 2 C), 70.1 (-, 2 C), 71.1 (-, 2 C), 79.2 (C_{quat}, 1 C), 114.1 (+, 2 C), 125.5 (C_{quat}, 2 C), 150.3 (C_{quat}, 2 C), 167.7 (C_{quat}, 2 C), 171.2 (C_{quat}, 1 C); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 557.6 (100, MH^+); - **UV** (MeOH): λ (ε) = 268 (7900), 224 (25600); - **MF:** $C_{26}H_{40}N_2O_{11}$ – **FW:** 556.62 g/mol;

IV. 4.2. Fluorescence Screening, Absorption- and Emission Titrations

Screening of amino acid and peptide binding affinities. The *s*creenings were performed in UV star wellplates with 96 cells (400 µL volume per cell) in a 4:1 mixture of methanol and aqueous HEPES buffer (10 mM)[43] at pH 6.3. To a $3*10^{-5}$ M solution of a particular receptor compound hundred equivalents of peptide or amino acid ($1.2*10^{-2}$ M) were added (1:1 vol/vol). If no change could be detected, the measurement was repeated with five hundred equivalents of guest ($6*10^{-2}$ M). The mixtures were quickly pipetted row by row, mixed with the aid of the pipette and allowed to equilibrate for five minutes. This time was sufficient to reach the equilibrium and guaranteed a constant volume in the wells. The fluorescence spectrum was recorded (λ_{ex} = 310 nm) and compared to the receptor blanc sample with the same concentration. All measurements were repeated twice.

The screenings in a 1:1 mixture of methanol and aqueous HEPES buffer (10 mM) were conducted after the same schedule.

Binding affinity titration with peptides in the cuvette. To a cuvette with 1.0 mL of the receptor ($3*10^{-5}$ M) in a 4:1 or a 1:1 mixture of methanol and aqueous HEPES buffer (10 mM, pH 6.3) were successively added small aliquots of the peptide solution, 5 – 40 µL for peptide **1** ($1*10^{-3}$ M) or 60 – 200 µL for G-E-G-G-NH_2, G-E-G-G-NH_2, K-G-OH, G-G-G-OH and G-G-G-G-OH ($1*10^{-2}$ mol/L) as guests. After each addition the solution was allowed to equilibrate for 5 min before the fluorescence intensity (λ_{ex} = 310 nm) and the UV spectrum

(see Figures 6 for a representative example) were recorded at 25 °C. The stoichiometry was determined by Job´s plot analysis separately titrated.[44] To determine the binding constant the obtained fluorescence intensities or extinction values were volume corrected, plotted against the concentration of peptide and evaluated by non linear fitting methods. The pH of the buffer mixtures was checked before and after measurement. Measurements with deviations of more than 0.5 pH units were not used for binding constant determination.

The titration curves and the according non-linear fittings are found in the supporting information of this chapter.

IV. 5. References and Notes

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2 M.-N. Lombard, D. Sotty, J. Wdzieczak-Bakala, M. Lenfant, **1989**, *Cell Proliferation*, 23(2), 99-103.

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platform molecule, complicating the purification of the desired product. For more information and characterization of the side product please see the supporting information.

[19] If the molecule is not properly dissolved, the side product *1-Oxo-1,2-dihydro-pyrrolo[1,2-c]imidazol-3-ylidene* is observed in increased amount.

[20] Small scale test reactions proved the reagent couple HBTU / TBTU as slightly more efficient, but the effort involved in purification and the reagents are more expensive. EDC is certainly one of the best and most versatile coupling reagents, giving an adequate yield for the receptor assembly. Using reagents especially suited for sterically hindered or inreactive amino acids, not much better yields are expected: N. Akaji, N. Kuriyama, Y. Kiso, *Tetrahedron Lett.* **1994**, *35*, 3315-3319.; F. Albericio, J.M. Bofill, A. El-Faham, S.A. Kates, *J. Org. Chem.* **1998**, 63, 9678-9683.; Y.M. Angell, C. García-Echeverría, D.H. Rich, *Tetrahedron Lett.* **1994**, *35*, 5981-5984.; L.A. Carpino, *J. Am. Chem. Soc.* **1993**, *115*, 4397-4398.; L.A. Carpino, A. El-Faham, C.A. Minor, F. Albericio, *J. Chem. Soc. Chem. Commun.* **1994**, 201-202.; J. Coste, E. Frérot, P. Jouin, *J. Org. Chem.* **1994**, *59*, 2437-2446.; P. Li, J.C. Xu, *Tetrahedron* **2000**, *56*, 8119-8131.; J.C.H.M. Wijkmans, F.A.A. Blok, G.A. van der Marel, J.H. van Boom, W. Bloemhoff, *Tetrahedron Lett.* **1995**, *36*, 4643-4646.

[21] As expected from former studies [7], no pyrrole emission at 340 nm is observed exciting from 270 to 320 nm. Energy transfer from the pyrrole units to the crown fluorophore leads to an observed emission of 390 nm. Upon binding the emission signal increase at 390 nm corresponding to the guest concentration giving saturation type curves. Following the binding event by UV titration, a value in good accordance to fluorescence data was obtained.

[22] All quantum yields were determined with quinine disulfate in 1 N H_2SO_4 as the reference compound (Φ = 0.546).

[23] The titration was repeated with the sole crown ether (**6b**) giving a comparable pK_a value of 5.8. (see supporting information).

[24] The buffer is present in large excess over **2** and only the emission of the crown ether can be followed. Thus a disturbance by the guanidinium part of the compound is avoided. A crossing of the pK_a value of the crown ethers nitrogen with the one of the guanidinium pyrroles is not visible.

[25] Compound **5a** (68 mg, 0.1 mmol) was dissolved in dichloromethane (0.8 mL), TFA (0.2 mL) was added and the mixture was stirred until TLC showed complete deprotection (5 h). All volatiles were removed at reduced pressure to obtain the product in quantitative yield (71 mg, 98 %). **^{1}H-NMR** (300 MHz, CD_3OD): δ [ppm] = 3.82 (s, 3 H), 4.26 (m, 4 H), 6.28 (m, 2 H), 7.13 (m, 2 H), 7.29 (m, 2 H), 7.64 (s, 1 H), 7.89 (s, 2 H);

[26] Most stable host-guest complexes only arise from a primary ammonium ion bound in the deprotonated aza-crown moiety, and on the other site the deprotonated carboxyl-termini coordinated by the protonated guanidines, respectively.

[27] If the additional carboxyl function present in the substrate is bound by the anion receptor site, the pK_a of the ammonium group may shift and alter its binding features. The dependence of the complex stability on pH is supposed to be rather small between the relevant pK_a´s of host and guest, causing undetectable small changes in the observed binding upon small pH changes during the measurement. See: F.P. Schmidtchen, *J. Org. Chem.* **1986**, *51*, 5161-5168.

[28] In pure methanol the crown ether binds this ammonium guest weakly (log K (**2**) = 2.2 and log K (**6b**) = 2.3).

[29] Sodium is not bound by the crown ether (21-crown-7). Even if a 2000 fold excess is added in aqueous methanol no change in luminescence can be observed.

[30] C. Schmuck, *Chem. Eur. J.* **2006**, *6*, 709-718.

[31] Glu is used instead of Asp due to preparation reasons. The difference in methylene group should not influence the result of the selectivity and binding strength determination to a large extent.

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[33] A further increase in the water content, so measuring in pure buffer, only **1** shows a weak response ($I/I_0 \sim$ 1.3) with the receptor **2**, for all other guests no difference in luminescence was observed within the errors of the method. A fluorescence titration in this medium gave a flat saturation type curve reaching a plateau in

the range of 10^{-2} molar guest concentration. The binding strength was too small to derive a binding constant.

[34] For ditopic crown ether guanidinium systems binding constants can only be derived in methanol / water or more unpolar mixtures: A.P. de Silva, H.Q.N. Gunaratne, C. McVeigh, G.E.M. Maguire, P.R.S. Maxwell, E. O´Hanlon, *Chem. Commun*., **1996**, 2191–2192.; S.-i. Sasaki, A. Hashizume, D. Citterio, E. Fujii, K. Suzuki, *Tetrahedron Lett.* **2002**, *43*, 7243-7245.; Ch.R. Cooper, T.D. James, *Chem. Commun*., **1997,** 1419–1420.

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[36] GABA was the preferred guest for similar ditopic receptors [see 7]. Here, its binding strength can be only roughly estimated. No constant can be derived from fluorescence titration methods. The value is too small.

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[41] The free base was prepared from the hydrochloride by shaking its chloroform solution several times with an aqueous solution of potassium carbonate (2 M). The combined aqueous phases were re-extracted three times with chloroform.

[42] The splitting of some signals in the spectrum indicates the presence of unequal populations of two conformers at room temperature. This observation caused by hindered rotation in the molecule is literature known: D.R. Dalton, K.E. Ramey, H.J. Gisler, Jr., L.J. Lendvay, A. Abraham, J. Am. Chem. Soc. **1969**, *91(23)*, 6367-6370 and literature therein.

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[44] P. MacCarthy, *Anal. Chem.* **1978**, *50,* 2165.; C. Schmuck, P. Wich, *Angew. Chem. Int. Ed.* **2006**, *45*, 4277-4281.

V. Luminescent Metal Complex - Crown Ether Hybrid Receptors bind Phosphate and Ammonium Ions simultaneously[i]

Hybrid compounds combining a luminescent aza-crown ether and 1,4,7,10-tetraaza-cyclododecanes (cyclene) or bis-pyridin-2-yl-methyl-amines (bpa) metal complexes were prepared. These synthetic receptors signal the presence of peptidic ammonium phosphates in buffered aqueous solution by changes of their emission intensity. Reversible phosphate ion coordination to the metal complex binding sites decreases the emission intensity of the phthalic ester fluorophore.[ii] Ammonium phosphates with suitable structure, such as the *C*-terminal domain of RNA polymerase II **1** or protein kinase G target sequence **2**, bind to the synthetic receptors by an additional intramolecular ammonium ion and aza-crown ether interaction. The ammonium ion binding to the aza-crown ether increases the emission intensity of the fluorophor. As this weak interaction can in aqueous solution only occur within phosphate - metal complex receptor aggregates of matching geometry, it allows the distinction of structurally related phosphorylated peptides in aqueous buffer.

Acknowledgement

We thank Dr. A. Grauer for preparation of compound **13b** and Dr. S. Stadlbauer for supplying 2,2-*N,N*-dipicolylamine (**20**). Special thanks to F. Schmidt for preparation of the CTD heptapeptide (**1**) and helpful annotations related to the molecule, as well as to A. Riechers for discussions and annotations.

[i] A. Späth, C. Koch, B. König, *Eur. J. Inorg. Chem.* **2010**, accepted.

[ii] Complexation of the zinc-biscyclene motifs and pre-eliminary phosphate binding studies were performed by C. Koch in her bachelor thesis, University of Regensburg, **2009**.

V. 1. Introduction

Molecular recognition of molecules or ions of biological relevance in aqueous media by synthetic receptors is still a challenge in current research.[1] Ammonium ions are found in all amino acids[2] and in many compounds of biological interest such as the neurotransmitters dopamine[3] or γ-aminobutyric acid.[4] Of equal importance are phosphate esters,[5] found in nucleoside phosphates (nucleotides) as components of RNA and DNA[6], and in ATP[7] or phosphorylated amino acids in proteins involved in intracellular signalling and regulation.[8] Phosphorylation is one of the most biologically relevant and ubiquitous posttranslational modifications of proteins.[9] This reversible process is used by all organisms to regulate many cellular activities: Phosphorylation occurs at the hydroxyl residues of serine, tyrosine and threonine,[10] having a key role in cell growth regulation like apoptosis or cell cycle progression and various cellular expression processes,[11] proliferation and energy metabolism,[12] enzyme activity control[13] or in many signal transduction pathways as well as in gene expression and regulation.[14] Misregulated phosphorylation is directly connected to severe diseases like cancer and neuropathogenesis.

Two important enzymes in the context of phosphorylation are the cyclic GMP-dependent protein kinase G (PKG) and the RNA polymerase II (Pol II). Protein kinase G catalyzes the phosphate transfer from ATP to specific serine or threonine residues in target proteins. It phosphorylates a number of biologically important targets involved in the regulation of smooth muscle relaxation, cell division and nucleic acid synthesis.[15] Studies revealed highly specific substrate sequences like Arg-Lys-Arg-pSer-Arg-Ala-Glu (**2**), which shows a strong preference for PKG Iα (Km = 59 μM).[16]

RNA polymerase II (Pol II) unwinds the DNA double helix, synthesizes the messenger RNA and proofreads the nascent transcript in the eukaryotic transcription cycle. It is precisely regulated by phosphorylation in all transcription steps: initiation, elongation and termination.[17] Its *C*-terminal domain (CTD) seems to be the key to the complex reaction sequence of gene expression.[18] The CTD sequence consists of the heptapeptide repeats Tyr-Ser-Pro-Thr-Ser-Pro-Ser (**1**). The binding of regulatory factors in the transcription elongation process depends on a specific CTD phosphorylation pattern, which changes during the transcription cycle.[19] Mainly serine residues in position 2 and 5 of the heptapeptide repeat are phosphorylated and dephosphorylated during the transcription cycle. In average only one phosphate is present per repeat.[20] Both phosphorylations trigger different functions:[21] For

example, phosphorylation at serine residue in 5-position takes place during transcription initiation, while transcription elongation is connected with CTD phosphorylation at serine residue number 2.[22] During transcription termination, the CTD is dephosphorylated.

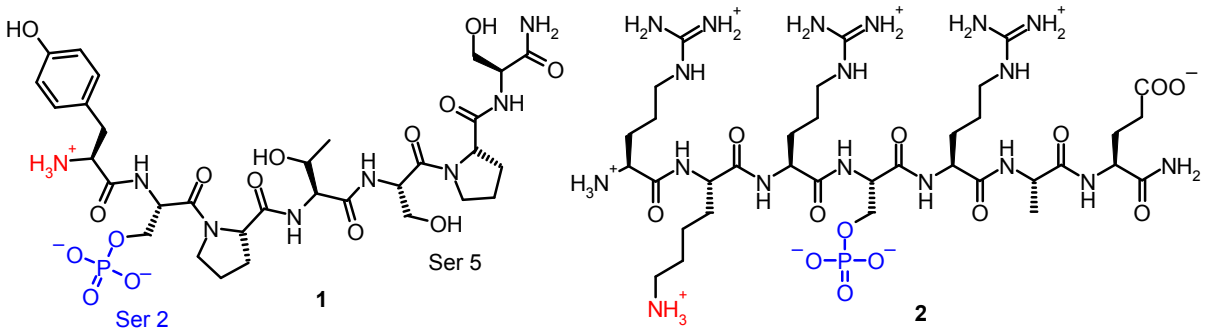


Figure 1: Phosphorylated target peptides

The phosphorylated target peptide sequences of PKG and Pol II are shown in Figure 1. A common element in both structures is the close proximity of the phosphate ester and an ammonium ion. A luminescent synthetic receptor binding simultaneously to ammonium and to phosphate ions, which are in close distance, should be useful to monitor the phosphorylation state of the proteins as a chemosensor. As a strong interaction we select the reversible coordination of a Lewis acidic metal complex to the phosphate anion. This places an aza-crown ether moiety in close proximity of the ammonium group. Although ammonium ion to crown ether interaction is weak in aqueous solution the intramolecular arrangement facilitates the binding. The ammonium ion binding is signalled by changes of the emission properties of the crown ether unit.

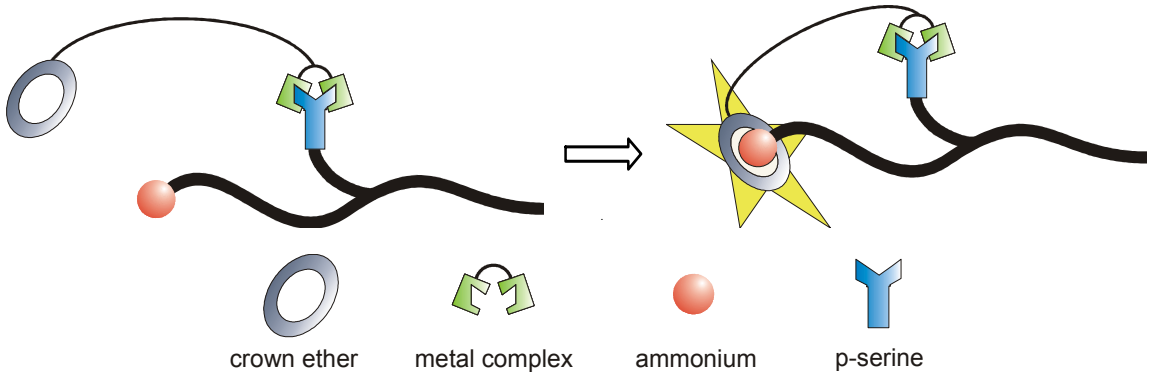


Figure 2: Schematic two step binding of a metal complex – aza-crown ether receptor to a peptide bearing ammonium and phosphate ions. 1. Reversible coordination of the metal complex to the phosphate ion. 2. Intramolecular interaction of ammonium ion and crown ether leads to an increase in emission intensity

Zinc di-picolylamine (Zn(II)-dpa) receptors,[23,24] binuclear Zn(II)-dpa,[25] and zinc 1,4,7,10-tetraazacyclododecane (Zn(II)-cyclene) complexes[26] have proven their ability as strong phosphate binding moieties in aqueous solution.[27,28,29,30] A comprehensive review on the molecular interactions of Zn(II)-cyclene and its derivatives was recently published.[31] Zn(II)-di-picolylamine-stilbazoles have been shown to bind to a natural phosphoprotein recognition domain,[32] or were used in conjugates of peptide nucleic acids (PNA) and metal binding ligands recognizing phosphate in DNA.[33] Bis-picolylamine metal complexes found application as artificial phosphoprotein sensors in protein separation and analysis by SDS-PAGE gel electrophoresis retaining phosphorylated proteins stronger than corresponding non-phosphorylated proteins,[34,35,36] or in fluorescent chemosensing arrays for phosphate derivatives,[23,37] for chemosensing of phosphatidylserine-containing membranes and membranes of bacterial cells.[38] Several dpa based fluorescent chemosensors for ATP were also published.[39]

We combine such metal complexes now with luminescent crown ether amino acids (CEAAs), which respond to the presence of ammonium ions by changes of their emission properties.[40,41] Both units are connected by peptidic linkers to allow variation in distance. Figure 3 shows the general structure of the receptors for simultaneous ammonium – phosphate binding.

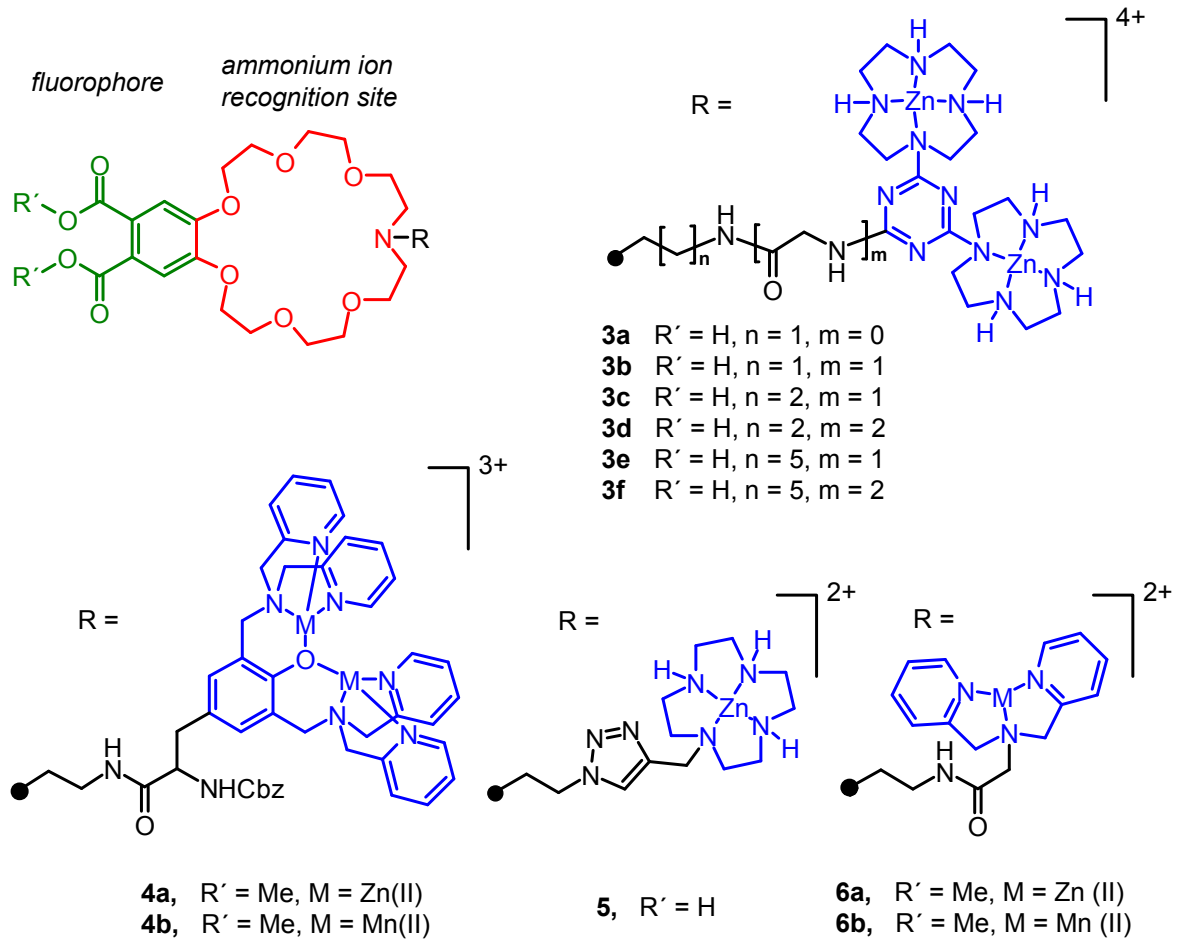


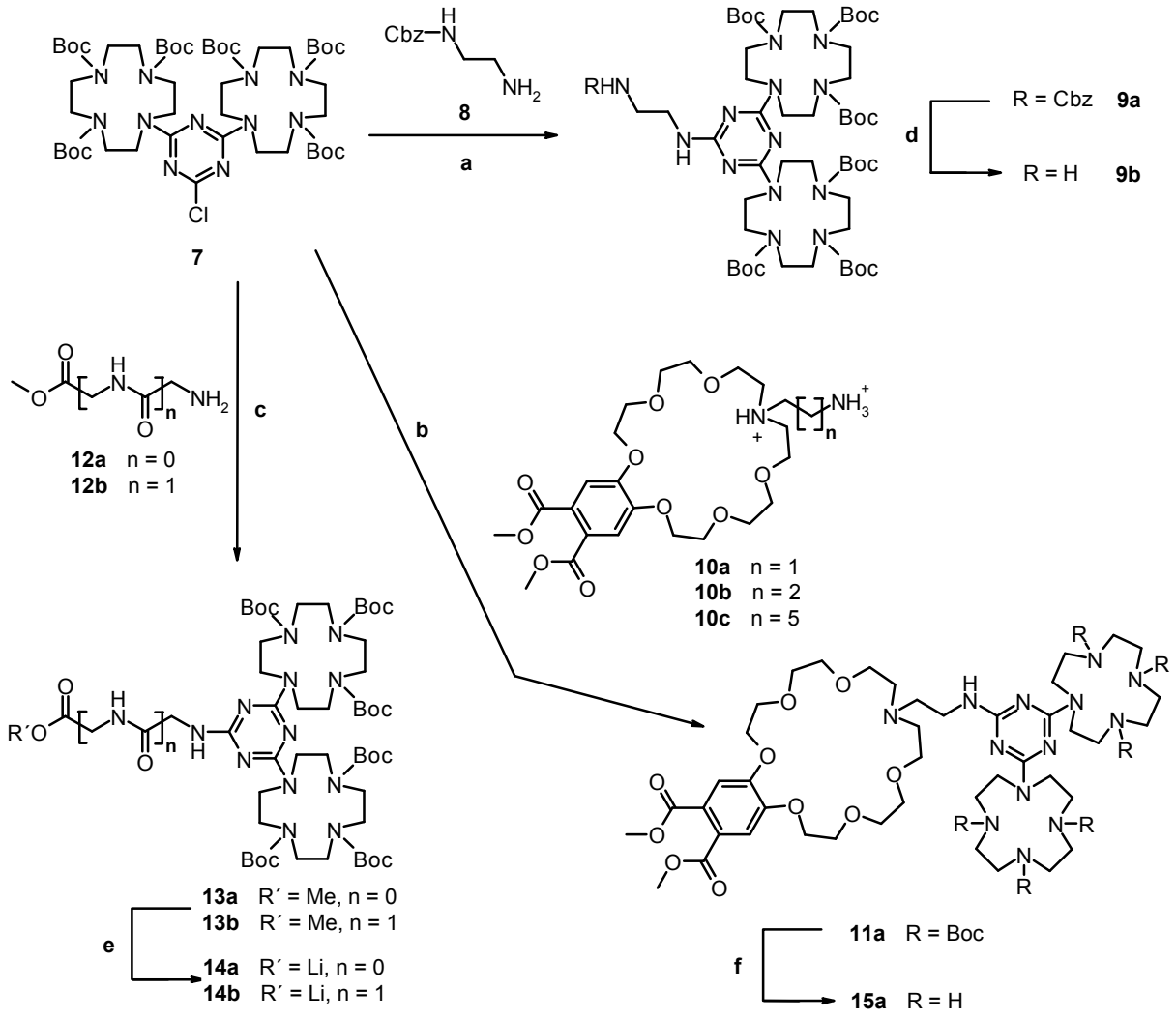
Figure 3: General structure of synthetic receptors with ammonium and phosphate ion affinity: The luminescent crown ether is connected to zinc-bis-cyclene **3a-f**, zinc-cyclene **5**, bis-pyridin-2-yl-methyl-amine-complex (bpa[M]) **6** or Tyr-bis-bpa-complex (BPA[M]) **4**; perchlorate or chloride counter ions are not shown.

V. 2. Results and Discussion

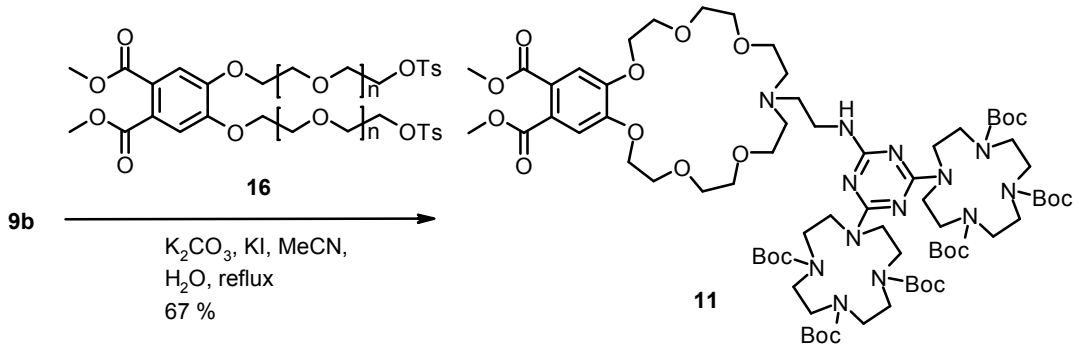
V. 2.1. Syntheses

The synthesis starts with protected bis-cyclene **7**, which was substituted by orthogonally mono-protected 1-*N*-benzyloxycarbonyl-ethylenediamine (**8**), glycine methyl ester (**12a**) or glycyl glycine methyl ester (**12b**). The following deprotection steps proceeded in excellent yields (Scheme 1). Starting materials and crown ether amino acid esters (**10**) were prepared and deprotected as previously described.[42,43] Crown ether – bis-cyclene **11a** can be also

prepared by this route as depicted in scheme 1. It is better prepared by reacting **9b** with the tosylate **16** (Scheme 2).



Scheme 1: Synthesis routes to compound **9**, **14** and **15**; *conditions: a) K_2CO_3, dioxane, 2 d, 120°C; 86 %*; b*) K_2CO_3, dioxane, 2 d, 140 °C; 41 %; c) K_2CO_3, dioxane, 2 d, 140 °C; 76 - 79 %; d) EtOH Pd/C 10 %, 20 bar H_2, 4 d; 97 %; e) LiOH, acetone, 2 d, RT; quant.; f) TFA, DCM, 4 h, RT, then ion exchange (strongly basic resin) in H_2O, 91 %.*

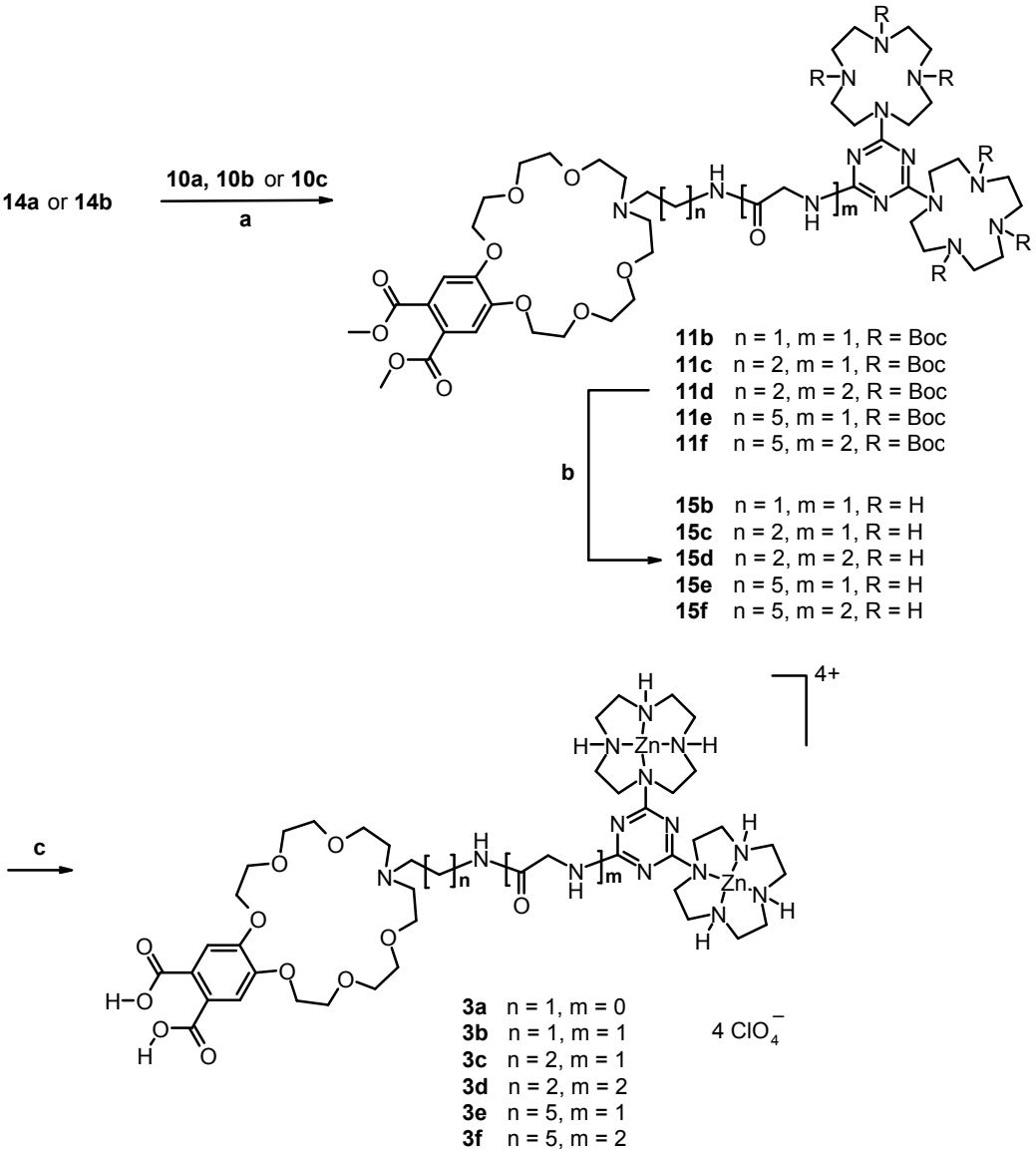


Scheme 2: Synthesis of ligand **11a** by ring closing S_N reaction

Peptide coupling of the building blocks **10** and **14** gave the protected ditopic crown ether bis-cyclene receptor ligands **11b - 11f**.[44] All compounds were deprotected with TFA[45] and, after liberating the free bases with the aid of a strongly basic ion exchange resin, complexed with zinc ions under reflux conditions in water – methanol solution (Scheme 3).

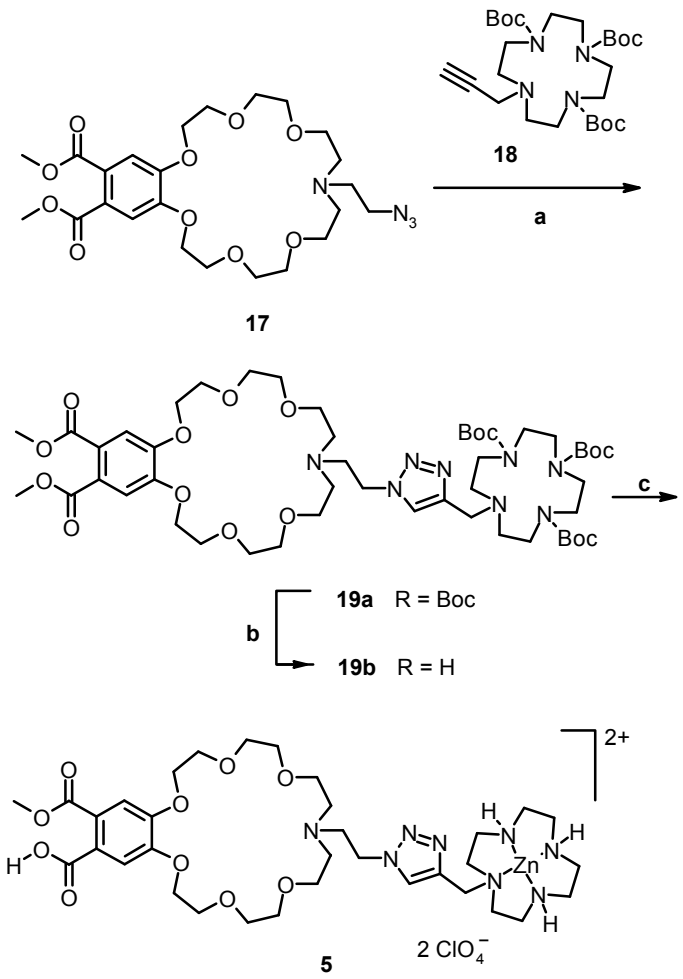
The dinuclear Zn(II) complexes (**3a** – **3f**) were isolated in analytical purity with good yield (79 – 95 %) from the reaction of the ligand with zinc perchlorate salt in aqueous solution and subsequent precipitation from water with ethanol. Characterization by different methods (^{1}H-NMR, UV/Vis, IR and ESI-MS) confirmed a metal cation to ligand ratio of 2:1.

The methyl esters are partially cleaved to the free acids under the conditions of the complexation or storage of the complexes in buffer medium. Therefore reaction conditions for the complexation were chosen, which result in complete ester cleavage avoiding compound mixtures.[46]



Scheme 3: Synthesis of complexes **3** by peptide coupling, deprotection and complexation; *conditions: a) EDC, HOBt, DIPEA, $CHCl_3$, 0 °C, RT→ 40 °C, over night, 51 – 65 %;* b*) TFA, DCM, 4 h, RT, then ion exchange (strongly basic resin) in H_2O, 86 - 93 %; c) $Zn(ClO_4)_2$ * 6 H_2O, $NaHCO_3$, MeOH, H_2O, 60 °C → 80 °C, 6 h, 79 – 95 %.*

A mono-cyclene-crown **5** was prepared for comparison (Scheme 4). The compound was obtained by Huisgen azide **17** – alkyne **18** cycloaddition. Acidic cleavage of Boc protecting groups and subsequent treatment of the TFA salt with a basic anion exchanger resin yielded the free amine cyclene ligand. Complex formation with one equivalent of $Zn(ClO_4)_2$ gave the mononuclear Zn(II)-cyclene derivative **5**.[47]

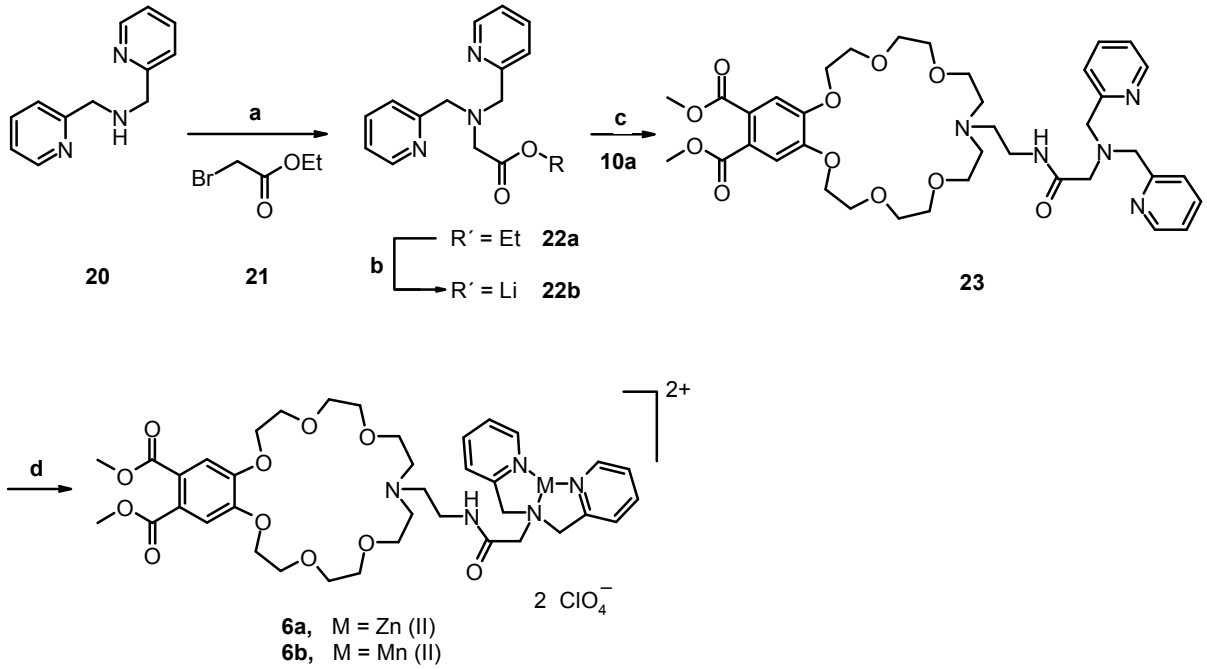


Scheme 4: Synthesis of complex **5**; *conditions: a) $CuSO_4$, Na-ascorbate, MeOH, H_2O, RT then reflux, 5 h, 93 %;* b*) TFA, DCM, 4 h, RT, then ion exchange (strongly basic resin) in H_2O, 97 %; c) $Zn(ClO_4)_2$, $NaHCO_3$, MeOH, H_2O, 60 °C → 80 °C, 6 h, quant.*

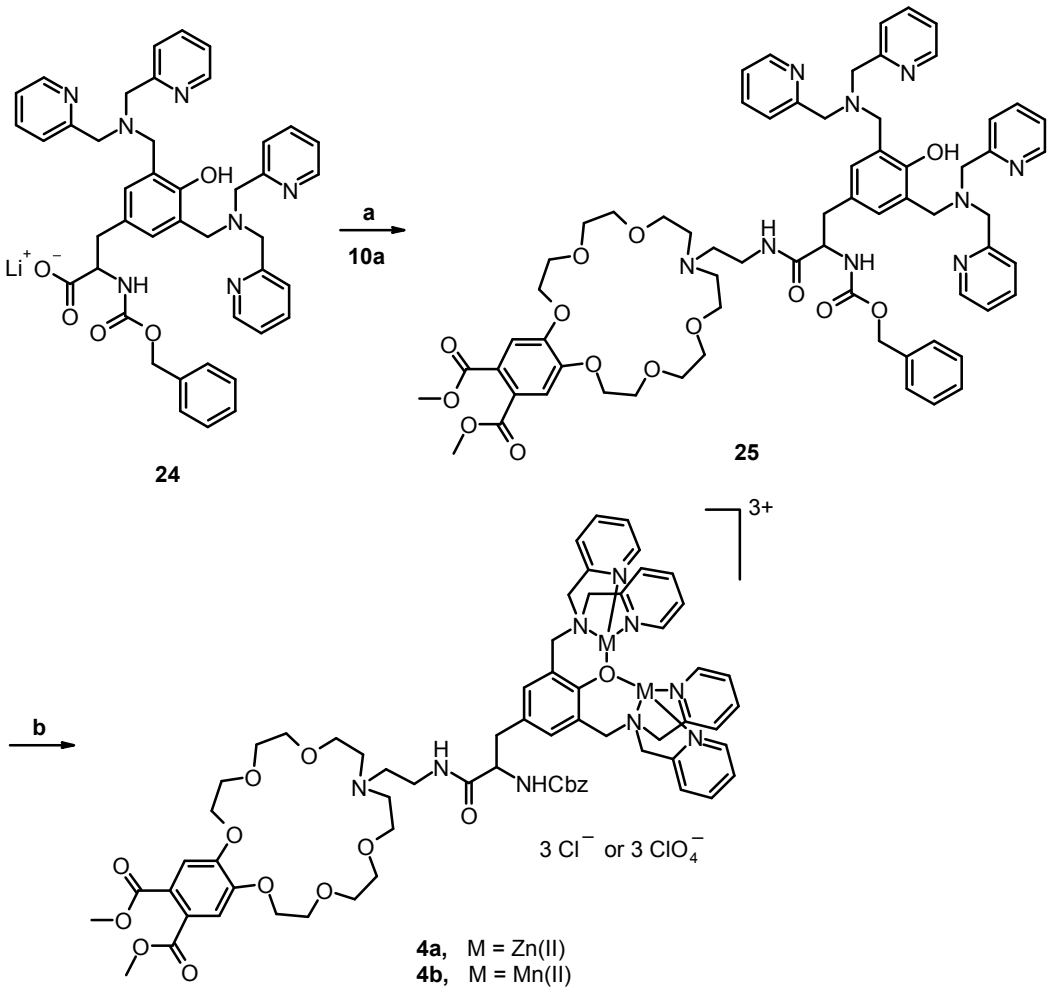
Zinc and manganese complexes **6** of bis-pyridylmethylamine (bpa) - crown ether ligand **23** were prepared starting from 2,2-bis-picolylamine (**20**). Carboxylic acid **22b** is coupled with the crown ether amino acid TFA salt (**10a**) by peptide bond formation. The manganese or zinc complexes **6** were obtained in methanol at ambient temperature (Scheme 5). The complexes are fluorescent, as zinc and manganese ions do not quench the emission.

Analogously, the lithium salt of a Cbz-protected tyrosine-bis-bpa (**24**) was reacted with crown ether **10a** yielding the desired crown appended bis-bpa ligand **25**. The ligand is converted in aqueous methanol solution into the bis-manganese(II) (**4b**) or bis-zinc(II) (**4a**) complex by treatment with zinc perchlorate or manganese chloride, respectively (Scheme 6). Overall, the complexes were prepared in good yield.

Peptides for the determination of binding selectivity were prepared in solution and by standard solid phase methods or were commercially available.



Scheme 5: Synthesis of complexes **6**; *conditions: a) MeCN, K_2CO_3, 0 °C then RT, 81 %; b) NaOH, MeOH, H_2O, 6 h, RT; quant.; c) EDC, HOBt, DIPEA, $CHCl_3$, DMF, 0 °C, RT → 40 °C, over night, 61 %; d) $Zn(ClO_4)_2$ * 6 H_2O, MeOH, H_2O, 40 °C, 2 h, quant. or $MnCl_2$ * 4 H_2O, MeOH, H_2O, 40 °C, 2 h, quant.*



Scheme 6: Preparation of complexes **4**; *conditions: a) EDC, HOBt, DIPEA, DMF (dry), $CHCl_3$ (dry), 0 °C, RT→ 60 °C, over night, 47 %; d) $Zn(ClO_4)_2$ * 6 H_2O, MeOH, H_2O, 40 °C, 2 h, quant. or $MnCl_2$ * 4 H_2O, MeOH, H_2O, 40°C, 2 h, quant.*

V. 2.2. Recognition Properties of the Crown Ether Metal Complex Receptors

V. 2.2.1. Photophysical Properties

Compounds **3a - 3f** and **5** show absorption maxima in water at 220 nm and 270 nm, and emit upon excitation at 380 nm with a quantum yield of about $\phi = 0.05$.[48] The absorption properties are only marginally affected by the nature of the ether.[49] Compounds **4a/b** and **6a/b** show a similar behaviour, having absorption bands at 220 nm and 270 nm. The emission maximum is observed at 390 nm; the quantum yield was determined to be $\phi = 0.1$ (Figure 4).

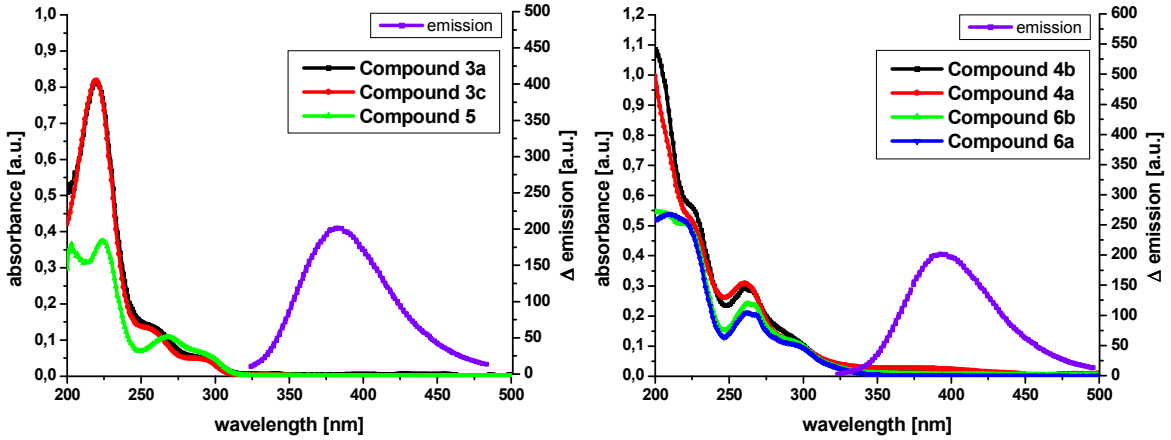


Figure 4: Absorption- and emission spectra of selected metal complexes in water; left: compound **3a**, **3c** and **5** (c = $2.1*10^{-5}$ mol/L), right: compound **4a/b** and **6a/b** (c = $2.2*10^{-5}$ mol/L)

V. 2.2.2. Binding Properties of the Individual Binding Sites

To evaluate the ammonium ion affinity of the crown ether binding site the protected ligands **11a - f** were titrated with *n*-butylammonium chloride and potassium isothiocyanate in methanol, giving log *K* values of 2.5 for *n*-butylammonium chloride and log *K* = 3.5 for potassium isothiocyanate, respectively, which matches literature data.[42] In HEPES buffered aqueous solution no interaction of the cations with the crown ether was detectable, even if 2000 equivalents of the salts were added (see the supporting information of this chapter).

Compound **3e** was titrated in aqueous HEPES buffer (50 mM, pH 7.4) with adenosine monophosphate (AMP, **26**) yielding a binding constant of log *K* = 4.1. The phosphate ion binding to the metal complex leads to a luminescence decrease (Figure 5).

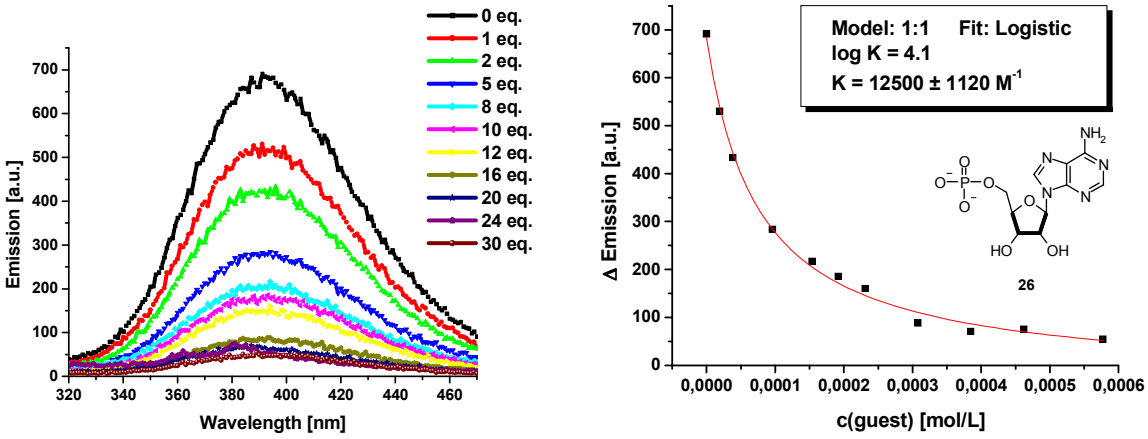


Figure 5: Titration of compound **3e** with AMP (**26**); left: observed emission changes; right: non-linear fit results to a 1:1 binding model.

V. 2.2.3. Binding of Ammonium Phosphates

The simultaneous binding ability to phosphate and ammonium ions of receptors **3** to **6** was investigated with phosphoserine (**27**), the PKG substrate **2** and the mono-phosphorylated CTD heptapeptide **1** (Figure 6). The distance between the phosphate and the ammonium ion increases from **27** to **1**, but the structure of the peptides is very flexible in all cases. To quickly identify the best matches between synthetic receptors and target peptides a well plate screening of the library of complexes **3** to **6** against **1**, **2** and **27** was performed in buffered aqueous solution (HEPES 50 mM, pH 7.4, 25 °C).

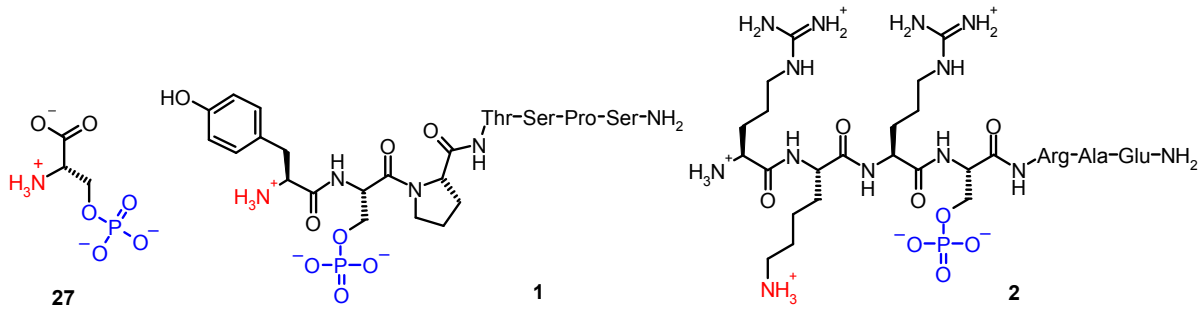


Figure 6: Ammonium phosphates used to evaluate the binding properties of compounds **3** to **6**: Phosphoserine (**27**), CTD sequence repeat (pSer2) **1** and PKG-substrate (pSer4) **2**.

Twenty equivalents of peptide **1** or **2**, or hundred equivalents of **27** were added to a solution of the synthetic receptor ($1*10^{-5}$ M), and the fluorescence emission intensity of **3a – 3f**, **5** at 380 nm or of **4a/b** and **6a/b** at 390 nm (λ_{ex} = 300 nm) was recorded. The fluorescence enhancement factors were calculated as:

$$z = \frac{F}{F0}\left(\frac{v0 + v}{v0}\right)$$

F: Observed Fluorescence
F_0: Fluorescence of blanc sample
v_0: Volume before addition
v: Volume addition

Equation 1: Calculation of fluorescence enhancement factors with volume correction

A value of 1.00 indicates no significant interaction, a value smaller than 1.00 coordination of the metal complex to a phosphoserine residue and a value larger than 1.00 additional intramolecular interaction of ammonium ion and crown ether. Figure 7 summarises the results.

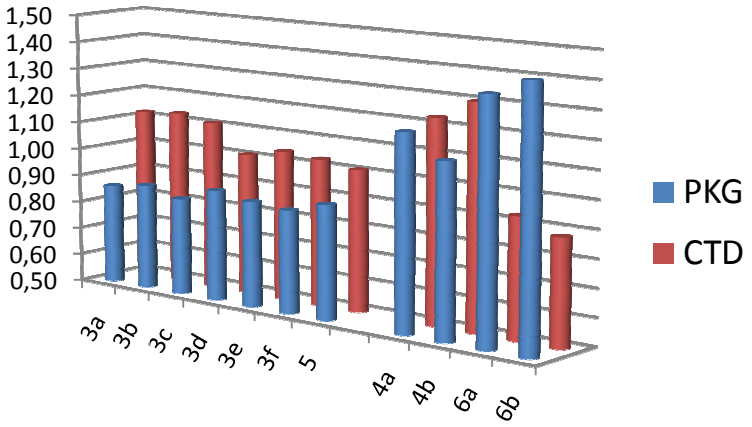


Figure 7: Ammonium phosphate induced fluorescence enhancement factors of receptors **3** to **6**. *[receptor] = $1*10^{-5}$ M in 50 mM HEPES buffer at pH = 7.4, excitation wavelength 300 nm, emission wavelength 390 nm, the errors are estimated as ~ 10 %; [guest] = $2*10^{-4}$ M.*

The presence of phosphoserine (**27**) decreases the luminescence intensity of all investigated complexes. Phosphate coordination to the metal complex occurs, but the distance or geometry of the ammonium ion group is not favourable for intramolecular inclusion into the crown ether. A significant fluorescence enhancement was observed for **6a/b** with both peptides **1** and **2**, and for **4a/b** binding to **2**. Synthetic receptors **3a**, **3b** and **3c** show a weak increase of the

fluorescence intensity ($F/F_0 < 1.2$) in the presence of CTD motif **1**. To confirm that the phosphorylated site is essential in the target peptides to trigger emission changes, complexes **3**, **4** and **6** were treated under identical conditions with the amino acids cysteine, serine, arginine, histidine, glutamic acid, glutamine and tetrabutylammonium acetate. The presence of the amino acids or the ammonium salt resulted in only negligible changes in the emission intensities of the complexes.

The peptide - receptor combinations with largest fluorescence enhancement response in the screening assay were investigated by emission titrations in HEPES-buffered (50 mM, pH 7.4) aqueous solution. The stoichiometry was determined by Job´s plot analyses extracted from the titration data.[50] Fluorescence intensities were volume corrected, plotted against the concentration of the peptide and evaluated by non linear fitting methods to determine the binding constants. Figure 8 shows as a representative example of the emission titration curve and Job´s plot analysis for the titration of **4b** with peptide **2**.

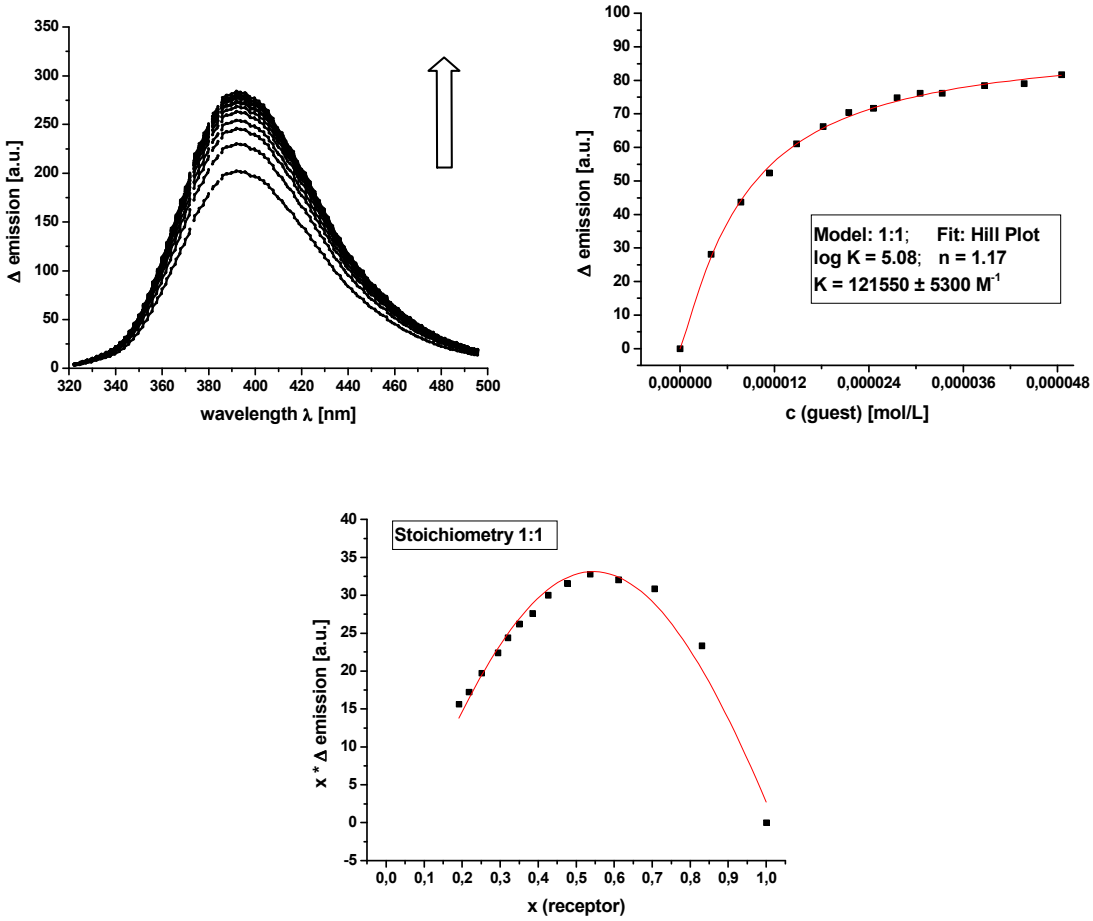


Figure 8: Emission intensity changes of **4b** ($2*10^{-5}$ molar, λ_{ex} = 300 nm) upon addition of **2**. Bottom: Job´s plot analysis

Table 1 summarises all binding data derived from the titrations. The affinities of **4a** and **4b** for peptide **2** and of **6a**, **6b**, **3a**, **3b** and **3c** for peptide **1** are in the micromolar range and identical within the error of the experiment. Job´s plot analyses[50] confirmed stoichiometric ratios of the receptor–peptide aggregates of 1:1 in all cases. All receptors show a similar affinity to phosphoserine (**27**) which is 1 - 2 orders of magnitude smaller than to **1** or **2**. Emission intensities decrease during titration (Figure 9). The difference in emission response indicates the mode of binding: Phosphate to metal coordination for **27** and ditopic ammonium phosphate binding in the case of **1** and **2**. Hill plot analysis[51] shows a weak cooperativity of the supramolecular aggregate formation for the binding of the metal complexes with CTD heptapeptide (**1**), Hill coefficient n = 1.4 – 1.8, and PKG substrate (**2**), Hill coefficient n = 1.1 – 1.4.

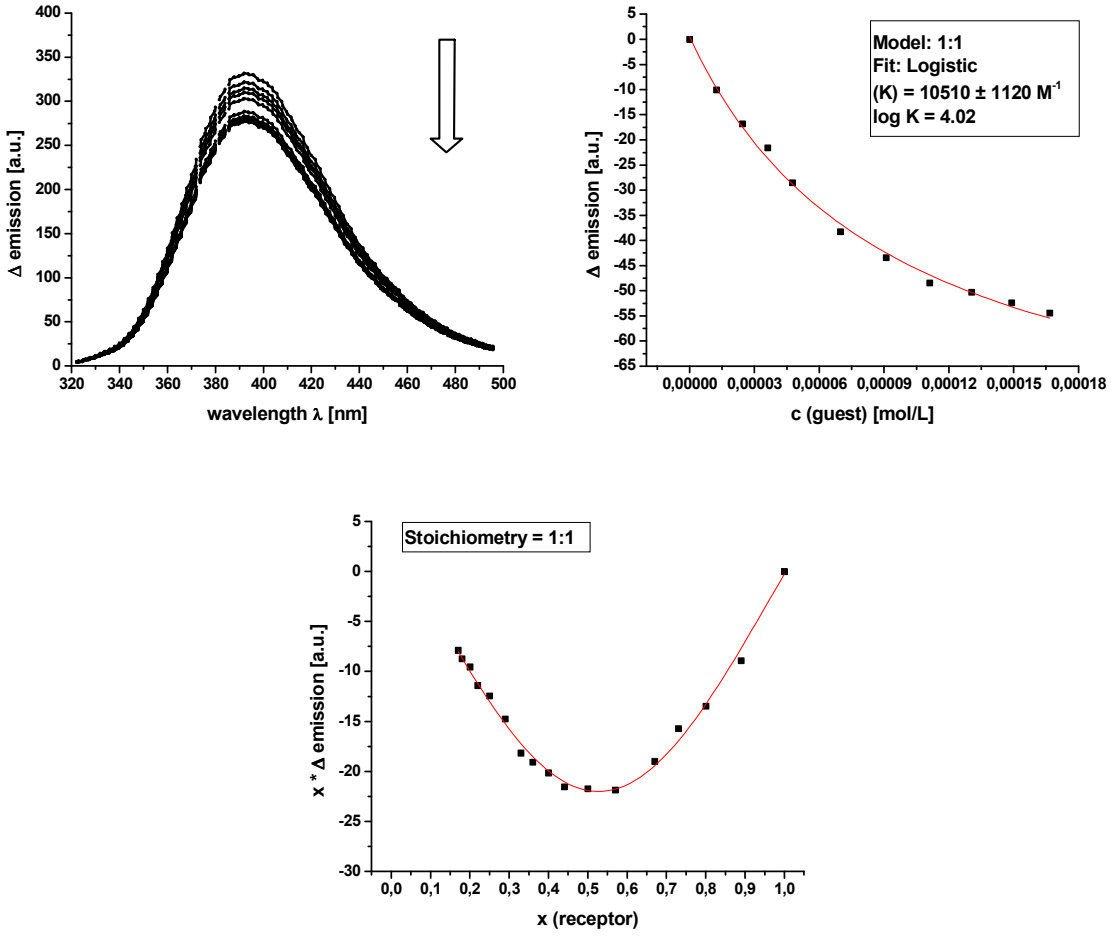


Figure 9: Emission titration of **4b** with phosphoserine (**27**). Bottom: Job´s plot analysis

Receptor[a]	PKG substrate (2)[b]		CTD heptapeptide (1)[b]		Phosphoserine (27)[b]	
	K [$*10^3$ M^{-1}]	F/F_0	K [$*10^3$ M^{-1}]	F/F_0	K [$*10^3$ M^{-1}]	F/F_0
4a	81 ± 6 *log K = 4.91*	1.39	-[c]	-[c]	13 ± 2 *log K = 4.12*	0.92
4b	121 ± 5 *log K = 5.08*	1.40	-[c]	-[c]	10 ± 1 *Log K = 4.02*	0.84
6a	33 ± 3 *log K = 4.52*	1.17	64 ± 2 *log K = 4.80*	1.23	7 ± 1 *log K = 3.86*	0.82
6b	78 ± 6 *log K = 4.89*	1.11	70 ± 3 *log K = 4.84*	1.31	8 ± 1 *log K = 3.92*	0.79
3a	-[c]	-[c]	72 ± 4 *log K = 4.86*	1.16	5 ± 1 *log K = 3.7*	0.69
3b	-[c]	-[c]	82 ± 5 *log K = 4.91*	1.14	5 ± 1 *log K = 3.7*	0.72
3c	-[c]	-[c]	92 ± 4 *log K = 4.96*	1.12	5 ± 1 *log K = 3.7*	0.71

Table 1: Binding constants K and fluorescence enhancement (F/F_0) values for selected combinations of complexes **3**, **4** and **6** with peptides **1** and **2** and phosphoserine: a) *$2*10^{-5}$ M receptor in HEPES buffer (50 mM, pH 7.4, no salt added, adjusted with Et_4NOH and HCl); counterion is chloride; b) [guest] = 0.0002 M or 0.001 M; c) not determined.*

V. 3. Conclusions

We have prepared luminescent synthetic receptors **3**, **4** and **6** for the binding of peptidic ammonium phosphates **1**, **2** and **27** in aqueous buffer. The compounds consist of a metal complex, which reversibly coordinates phosphate anions, and a luminescent aza-crown ether with weak ammonium ion affinity. The binding of ammonium ions to the crown ether in aqueous solution is only possible in an intramolecular fashion subsequent to phosphate ion coordination. The coordination of phosphate ions to the metal complex decreases the emission intensity of the fluorophor, while ammonium ion binding to the aza-crown ether increases the

emission intensity. This allows a distinction between phosphate coordination and ditopic ammonium phosphate binding. Phosphoserine coordinates to all synthetic receptors with millimolar affinity, but its geometry does not favour the simultaneous interaction of phosphate and ammonium ion with the receptor, as indicated by decreased emission intensity. Phosphorylated peptides **1** and **2** bind to the synthetic receptors in ditopic fashion with micromolar affinity. The increase in emission intensity clearly indicates the aza-crown ether ammonium ion interaction. The conformationally flexible structure of the short peptides **1** and **2** and the receptors does not originate large differences in selectivity upon structural variation. However, from the screening experiment best matches were identified for the combinations of receptor **4** and peptide **2**, and receptors **3a - c** or **6** and peptide **1**, respectively.

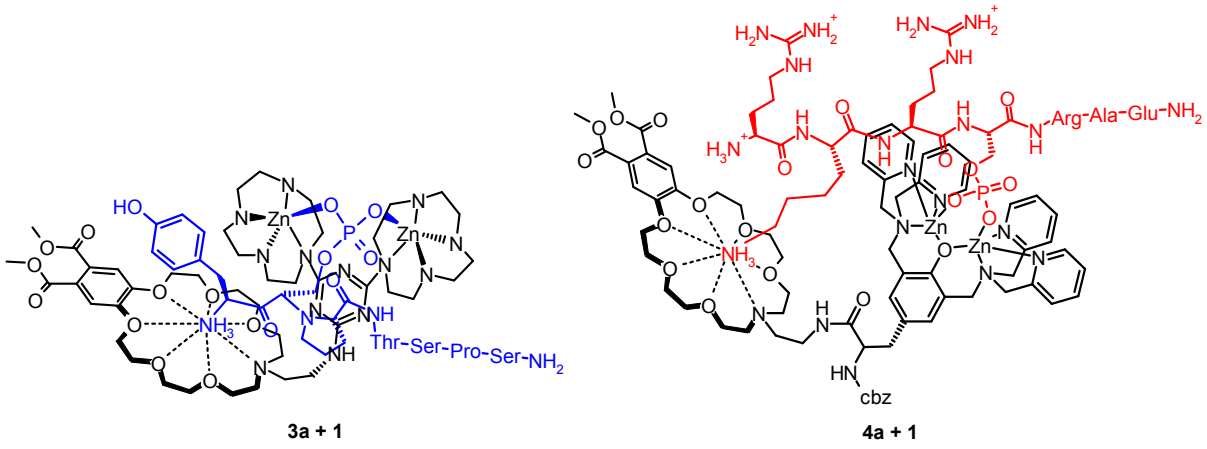


Figure 10: Illustrations of proposed structure of stable aggregates of the PGK substrate (**2**) with **4a** (right) and the CTD heptapeptide (**1**) with **3a** (left)

Our examples show that by combining weak and strong reversible interactions the selective detection of phosphorylated peptide sequences in aqueous buffered solution becomes possible. Non-phosphorylated amino acids do not interfere and phosphate coordination alone is indicated by emission intensity decrease. The here presented synthetic receptors may not fit the requirements for practical chemosensor applications in bioanalytics, such as excitation and emission wavelength, membrane permeability or tolerance to other species present, but they prove that combinations of strong and weak binding sites allow a distinction between phosphorylated peptides in aqueous buffer.

V. 4. Experimental Part

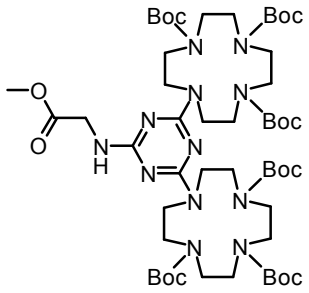
V. 4.1. Syntheses

Compound **7**[29,52], **8**[53], **10a**[54], **10c**[43], **16**[54], **17**[42], **18**[55], **20**[56] and **24**[57] were prepared following literature known procedures. The PKG substrate (pSer4) was purchased from Genscript (> 95 %) and used as is.

Caution: Although no problems were encountered in this work, metal perchlorate complexes are potentially explosive. They should be handled with care and the complexes should be prepared in small quantities.

V. 4.1.1. Syntheses of the Building Blocks

*[4,6-Bis-(4,7,10-tri-(^{tert}butyloxycarbonyl)-1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-acetic acid methyl ester (**13a**)*

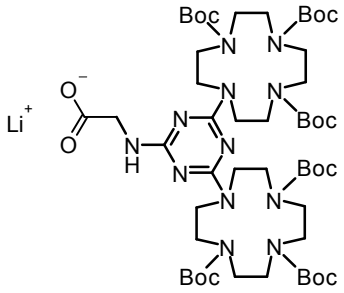


Glycine methyl ester hydrochloride (2.50 g, 20.0 mmol) and potassium carbonate (4.16 g, 30.0 mmol) were suspended in 50 mL of dioxane. The solution was stirred for one hour, then compound **7** (2.11 g, 2.0 mmol) was added and the solution was stirred for two days at 130 °C. The precipitated solids were filtered off and the solution was evaporated to dryness. The crude product was purified by column chromatography on silica gel with a mixture of petrol ether and ethyl acetate (1:1, R_f = 0.3) as eluent to give **13a** as colourless solid (1.76 g. 1.59 mmol, 79 %).

M.p. (uncorrected) = 129 – 133 °C; - **^{1}H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.36 - 1.42 (m, 54 H), 3.06 - 3.69 (m, 32 H), 3.66 (s, 3 H), 4.07 (m, 2 H), 5.07 (bs, 1 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 28.5 (+, 18 C), 42.7 (-, 1 C), 50.2 (-, 16 C), 52.0 (+, 1 C), 79.7

(C_{quat}, 6 C), 156.4 (C_{quat}, 6 C), 165.5 (C_{quat}, 6 C), 171.9 (C_{quat}, 1 C); - **IR** (KBr): ν [cm^{-1}] = 3374 (w), 2975 (m), 2946 (m), 2886 (m), 1752 (m), 1687 (s), 1538 (m), 1468 (m), 1407 (m), 1363 (m), 1247 (m), 1156 (s), 1105 (m), 1028 (m), 971 (m), 936 (m), 859 (m), 814 (m), 775 (m), 624 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 1109.8 (100, MH^+), 1009.1 (11, MH^+ - Boc), 564.0 (14, $(MH^+ + MNH_4^+)^{2+}$), 555.0 (9, $(M + 2\ H^+)^{2+}$); - **HRMS** (EI-MS 70 eV): calc. for $C_{52}H_{92}N_{12}O_{15}^{*+}$: 1108.6856, found: 1108.6857; - **$C_{52}H_{92}N_{12}O_{14}$:** calc. C 56.3, H 8.4, N 15.2, found. C 56.1, H 8.9, N 14.8.; - **MF:** $C_{52}H_{92}N_{12}O_{14}$ – **FW:** 1109.39 g/mol;

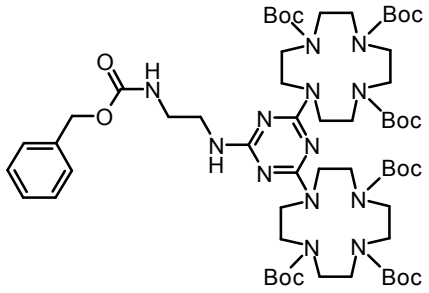
*[4,6 Bis-(4,7,10-tri-(^{tert}butyloxycarbonyl)-1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-acetic acid lithium salt (**14a**)*



Compound **13a** (0.66 g, 0.6 mmol) was dissolved in 20 mL of acetone. A freshly prepared aqueous solution of lithium hydroxide (2 M, 0.3 mL) was added and the mixture was stirred for two days at ambient temperature. The solvent was evaporated and the residue was lyophilised to yield **14a** as colourless solid (0.65 g. 0.59 mmol, 98 %).

M.p. (uncorrected) = 198 - 203 °C; - **^{1}H-NMR** (300 MHz, DMSO-d6): δ = 1.29 - 1.48 (m, 54 H), 3.12 - 3.63 (m, 36 H), 5.85 (bs, 1 H); - **^{13}C-NMR** (75 MHz, DMSO-d6): δ = 27.9 (+, 18 C), 40.2 (-, 1 C), 49.2 (-, 16 C), 78.8 (C_{quat}, 6 C), 155.3 (C_{quat}, 6 C), 164.2 (C_{quat}, 6 C), 171.0 (C_{quat}, 1 C), further signals were not detectable; - **IR** (KBr): ν [cm^{-1}] = 3425 (bm), 2975 (m), 2931 (m), 2868 (m), 1687 (s), 1539 (m), 1472 (m), 1408 (m), 1364 (m), 1298 (m), 1247 (m), 1158 (s), 1108 (m), 1030 (m), 971 (m), 938 (m), 860 (m), 813 (m), 775 (m), 629 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 1095.8 (100, MH^+), 557.0 (93, $(MH^+ + MNH_4^+)^{2+}$), 551.5 (41, $(MH^+ + Li^+)^{2+}$); - **HRMS** (PI-LSIMS FAB, glycerine): calc. for $C_{51}H_{91}N_{12}O_{14}^+$: 1095.6778, found: 1095.6785; - **MF:** $C_{51}H_{89}N_{12}O_{14}Li$ – **FW:** 1101.29 g/mol;

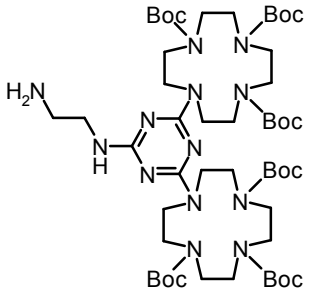
*1-[4,6 Bis-(4,7,10-tri-(^{tert}butyloxycarbonyl)-1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-2-(benzyloxycarbonyl-amino)-ethane (**9a**)*



Compound **8** (0.97 g, 5.0 mmol) and potassium carbonate (6.91 g, 50.0 mmol) were suspended in 80 mL of dioxane and stirred for 5 min. Compound **7** (3.81 g, 3.60 mmol) was added and the solution was vigorously stirred for three days at 140 °C. After filtering the solvent was distilled off and the crude product was purified by column chromatography on silica gel (PE / EtOAc 1:1, R_f = 0.2) to obtain **9a** as colourless solid (3.76 g. 3.1 mmol, 86 %).

M.p. (uncorrected) = 130 - 132 °C; - **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 1.23 - 1.45 (m, 54 H), 3.12 - 3.72 (m, 34 H), 4.87 (bs, 1 H), 5.05 (s, 2 H), 7.30 (m, 5 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 28.5 (+, 18 C), 40.9 (-, 1 C), 50.3 (-, 17 C), 60.4 (-, 1 C), 79.8 (C_{quat}, 6 C), 127.8 (+, 1 C), 128.2 (+, 2 C), 128.3 (+, 2 C), 136.9 (C_{quat}, 1 C), 156.3 (C_{quat}, 6 C), 166.0 (C_{quat}, 1 C), further signals were not detectable; - **IR** (KBr): ν [cm^{-1}] = 3306 (m), 3238 (m), 2972 (m), 2924 (m), 2854 (m), 2323 (m), 1683 (s), 1539 (m), 1503 (m), 1465 (m), 1407 (m), 1364 (m), 1287 (m), 1248 (m), 1146 (s), 1107 (m), 1048 (m), 947 (m), 858 (m), 813 (m), 775 (m), 753 (m), 693 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 1215.0 (100, MH^+), 616.6 (12, $(MH^+ + MNH_4^+)^{2+}$); - **HRMS** (EI-MS 70 eV): calc. for $C_{59}H_{99}N_{13}O_{14}^{*+}$: 1213.7434, found: 1213.7414; **- $C_{59}H_{99}N_{13}O_{14}$:** calc. C 58.3, H 8.2, N 15.0, found. C 58.0, H 8.8, N 14.6. - **MF:** $C_{59}H_{99}N_{13}O_{14}$ – **FW:** 1214.53 g/mol;

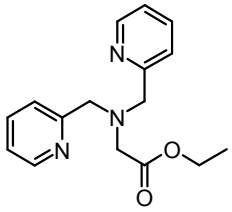
*(1-[4,6 Bis-(4,7,10-tri-(^{tert}butyloxycarbonyl)-1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-2-amino-ethane) (**9b**)*



Compound **9a** (1.21 g, 1.0 mmol) was dissolved in 20 mL of methanol and three spatula tips of palladium catalyst on charcoal were added. The solution was stirred for four days at 20 bar pressure of hydrogen at room temperature. After filtering over celite the solution was evaporated to dryness to yield **9b** as colourless solid (1.05 g. 0.97 mmol, 97 %).

M.p. (uncorrected) = 120 – 123 °C; - **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 1.27 - 1.49 (m, 54 H), 2.60 (m, 2 H), 2.89 (m, 2 H), 3.20 - 3.70 (m, 34 H), 5.01 (bs, 1 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 28.5 (+, 18 C), 41.9 (-, 1 C), 50.3 (-, 17 C), 79.8 (C_{quat}, 6 C), 156.4 (C_{quat}, 6 C), 166.1 (C_{quat}, 6 C); - **IR** (KBr): ν [cm^{-1}] = 3371 (bm), 2974 (m), 2931 (m), 2871 (m), 1686 (s), 1538 (m), 1469 (m), 1407 (m), 1363 (m), 1247 (m), 1158 (s), 1106 (m), 1030 (m), 971 (m), 934 (m), 858 (m), 813 (m), 775 (m), 624 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 1080.8 (100, MH^+), 549.6 (21, $(MH^+ + MNH_4^+)^{2+}$), 541.1 (54, $(M + 2 H^+)^{2+}$); - **HRMS** (EI-MS 70 eV): calc. for $C_{51}H_{93}N_{13}O_{12}^{*+}$: 1079.7079, found: 1079.7074; - **$C_{59}H_{99}N_{13}O_{14}$ + 3 H_2O:** calc. C 54.0, H 8.8, N 16.0, found. C 53.8, H 8.4, N 15.6. - **MF:** $C_{51}H_{93}N_{13}O_{12}$ – **FW:** 1080.39 g/mol;

*2,2-N,N-Dipicolyl-glycine ethyl ester (**22a**)*

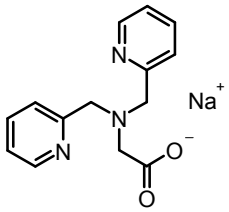


2,2-*N,N*-Dipicolylamine (1.0 g, 5.0 mmol) was dissolved in 50 mL of acetonitrile, potassium carbonate (1.38 g, 10.0 mmol) was added and the suspension was cooled in an ice bath. Under vigorous stirring a solution of bromoacetic acid ethyl ester (1.00 g, 0.66 mL, 6.0 mmol) in

20 mL of acetonitrile was dropped in over a course of 2 h at 2 – 5 °C. The mixture was stirred 4 h at room temperature, then refluxed for 2 h. After cooling to room temperature, the solution was filtered over celite and the filter cake was washed with acetonitrile and DCM. The solvents were evaporated and the residue was dried in vacuo. The crude product was purified by column chromatography with chloroform / methanol (1 % $NH_{3(aq)}$ content) 16:1 to give 1.15 g of an orange oil (4.04 mmol, 81 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.18 (t, 3 H, 7.14 Hz), 3.39 (s, 2 H), 3.92 (s, 4 H), 4.09 (q, 2 H, 7.14 Hz), 7.05 (dt, 2 H, 1.92 Hz & 4.38 Hz), 7.49 (d, 2 H, 7.68 Hz), 7.57 (dt, 2 H, 1.92 Hz & 7.68 Hz), 8.44 (d, 2 H, 4.6 Hz); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 13.2 (+, 1 C), 53.9 (-, 1 C), 58.9 (-, 2 C), 59.4 (-, 1 C), 121.1 (+, 2 C), 122.1 (+, 2 C), 135.5 (+, 2 C), 148.0 (+, 2 C), 158.1 (C_{quat}, 2 C), 170.2 (C_{quat}, 1 C); - **IR** (neat): ν [cm^{-1}] = 2936 (m), 2886 (m), 2846 (m), 2370 (w), 1588 (s), 1477 (m), 1432 (m), 1398 (s), 1332 (m), 1268 (m), 1152 (m), 1094 (m), 1053 (m), 1023 (m), 982 (m), 906 (m), 758 (m), 731 (s), 626 (m); - **MS** (CI-MS, NH_3): m/z (%) = 286.2 (100, MH^+); - **HRMS** (EI-MS 70 eV): calc. for $C_{16}H_{19}N_3O_3^{*+}$: 285.1477, found: 285.1474; - **MF:** $C_{16}H_{19}N_3O_2$ – **FW:** 285.35 g/mol;

*2,2-N,N-Dipicolyl-glycine sodium salt (**22b**)*



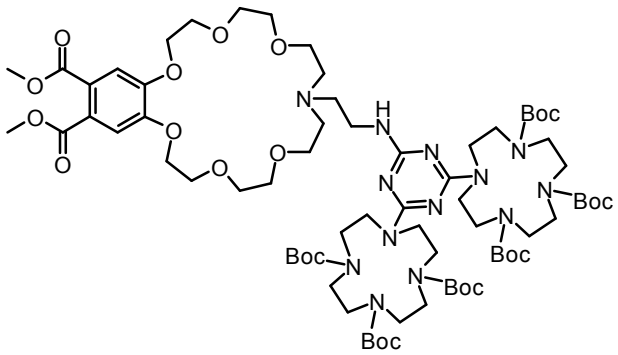
2,2-*N,N*-Dipicolyl-glycine ethyl ester (570 mg, 2.0 mmol) was dissolved in THF (6 mL) and aqueous NaOH (1 M, 2 mL, 2.0 mmol) was added. After stirring for 3 h at room temperature, the THF was evaporated and the remaining aqueous solution was lyophilised to give the product as fine yellow powder (558 mg, 1.973 mmol, 99 %).

M.p. (uncorrected) = 128 – 130 °C; - **^{1}H-NMR** (300 MHz, MeOD): δ [ppm] = 3.14 (s, 2 H), 3.84 (s, 4 H), 7.31 (dt, 2 H, 1.92 Hz & 4.38 Hz), 7.39 (d, 2 H, 7.68 Hz), 7.73 (dt, 2 H, 1.92 Hz & 7.68 Hz), 8.56 (d, 2 H, 4.38 Hz); - **^{13}C-NMR** (75 MHz, MeOD): δ [ppm] = 59.6 (-, 1 C), 60.1 (-, 2 C), 124.1 (+, 2 C), 124.4 (+, 2 C), 139.0 (+, 2 C), 150.5 (+, 2 C), 160.2 (C_{quat}, 2 C), 179.7 (C_{quat}, 1 C); - **IR** (KBr): ν [cm^{-1}] = 3370 (bm), 3218 (bm), 2934 (m), 2887 (m), 2844 (m), 2360 (m), 1588 (s), 1477 (m), 1432 (m), 1398 (s), 1332 (m), 1267 (m), 1151 (m), 1093

(m), 1050 (m), 984 (m), 905 (m), 756 (s), 730 (s), 627 (m), 584 (m), 527 (m), 450 (m), 405 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 258.1 (100, MH^+); - **HRMS** (PI-LSIMS FAB, glycerine): calc. for $C_{14}H_{16}N_3O_2^+$: 258.1243, found: 258.1242; - **$C_{14}H_{14}N_3O_2Na$ * 2.5 H_2O:** calc. C 51.8, H 5.9, N 13.0, found. C 51.7, H 6.0, N 12.6.; - **UV** (MeOH): λ (ε) = 262 (8840), 205 (15100); - **MF:** $C_{14}H_{14}N_3O_2Na$ – **FW:** 279.28 g/mol;

V. 4.1.2. Syntheses of the Crown Appended Ligands

*14-[4,6-bis-(4,7,10-tri-(^{tert}butyloxycarbonyl)-1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazinyl-amino-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethyl ester **(11a)***



Method 1

Compound **10a** (0.31 g, 0.6 mmol) and potassium carbonate (1.38 g, 10.0 mmol) were suspended in 30 mL of dioxane and the solution was stirred for 2 h. Compound **7** (0.53 g, 0.5 mmol) was added and the solution was stirred for three days at 130 °C. After filtering the solvent was evaporated and the crude product was purified by column chromatography with silica gel (EtOH / EtOAc 1:3, R_f = 0.3) to give **11a** as pale yellow solid (0.32 g. 0.2 mmol, 41 %).

Method 2

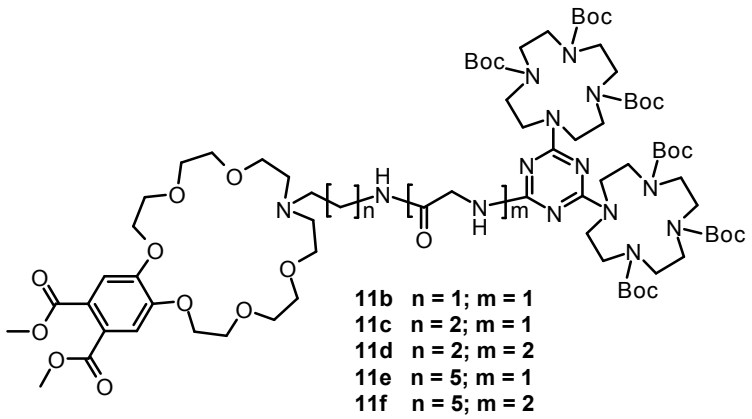
To a solution of compound **16** (0.80 g, 1.0 mmol) in acetonitrile (30 mL) containing 0.2 mL of water were successively added compound **9b** (1.08 g, 1.0 mmol), potassium iodide

(250 mg, 1.5 mmol) and potassium carbonate (1.38 g, 10.0 mmol). The mixture was refluxed over night and filtered over celite after cooling.

The filter cake was washed with acetonitrile and dichloromethane. After removal of the solvent at reduced pressure, the residue was purified by column chromatography (chloroform / ethanol 8:1) to yield the product **11a** (970 mg, 0.63 mmol, 63 %) as pale yellow solid (R_f = 0.48).

M.p. (uncorrected) = 94 – 97 °C; - **^{1}H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.23 - 1.47 (m, 54 H), 2.59 (m, 2 H), 2.73 (m, 2 H), 3.08 - 3.75 (m, 34 H), 3.51 (m, 4 H), 3.59 (m, 4 H), 3.69 (m, 4 H), 3.81 (s, 6 H), 3.85 (m, 4 H), 4.14 (m, 4 H), 5.31 (bs, 1 H), 7.12 (s, 2 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 28.5 (+, 18 C), 38.7 (-, 1 C), 50.2 (-, 16 C), 52.6 (+, 2 C), 54.0 (-, 2 C), 54.5 (-, 1 C), 69.3 (-, 2 C), 69.5 (-, 2 C), 70.0 (-, 2 C), 70.6 (-, 2 C), 71.2 (-, 2 C), 79.7 (C_{quat}, 6 C), 113.6 (+, 2 C), 125.4 (C_{quat}, 2 C), 150.5 (C_{quat}, 2 C), 156.7 (C_{quat}, 6 C), 165.7 (C_{quat}, 2 C), 167.8 (C_{quat}, 2 C); - **IR** (KBr): ν [cm^{-1}] = 2974 (m), 2932 (m), 2878 (m), 1691 (s), 1543 (m), 1499 (m), 1471 (m), 1410 (m), 1366 (m), 1288 (m), 1255 (m), 1166 (s), 1143 (m), 1035 (m), 971 (m), 947 (m), 919 (m), 857 (m), 777 (m), 730 (m), 663 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 768.2 (100, $(M+2H^+)^{2+}$), 1535.2 (68, MH^+); - **$C_{73}H_{123}N_{13}O_{22}$ * H_2O:** calc. C 56.5, H 8.1, N 11.7, found. C 56.5, H 8.3, N 11.2. - **UV** (MeOH): λ (ε) = 266 (7100), 224 (48700); - **MF:** $C_{73}H_{123}N_{13}O_{22}$ – **FW:** 1534.87 g/mol;

General procedure for peptide coupling of crown ether amino acids to bis-cyclene carboxylates (GP I)



The crown ether amino acid hydrochloride (0.5 mmol) was dissolved in 10 mL of dichloromethane, freshly distilled DIPEA (1.6 mmol, 207 mg, 0.27 mL) was added in one

portion and the mixture was stirred for 20 mins at room temperature. To an ice cold solution of the glycyl-bis-cyclene (0.5 mmol) in dichloromethane (10.0 mL) containing 0.5 mL of NMP, HOBt (0.6 mmol, 81 mg) and EDC (0.6 mmol, 94 mg) were subsequently added. After stirring for 30 mins at 2 to 5°C in a nitrogen atmosphere, the first solution was dropped in and stirring was continued over night at room temperature in the protective gas atmosphere. Another portion of EDC (0.3 mmol, 47 mg) was added and stirring was continued for two hours at room temperature, then one hour at 40°C. The organic solution was filtered, washed three times with brine (10 mL) and once with water (10 mL), dried over magnesium sulfate and the solvent was removed at reduced pressure. The crude product was purified by column chromatography with silica gel and chloroform / methanol or chloroform / ethanol as eluent.

*14-[4,6-bis-(4,7,10-tri-(^{tert}butyloxycarbonyl)-1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl-amino]-N-(2-methylamino-ethyl)-acetamido-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethyl ester (**11b**)*

Compound **10a** (290 mg, 0.5 mmol) was reacted with compound **14a** (550 mg, 0.5 mmol) after *GP I.* Chloroform / ethanol 8:1 was used for the purification to obtain **11b** as faintly yellow solid (516 mg, 0.317 mmol, 65 %) (R_f = 0.38).

M.p. (uncorrected) = 103 – 108 °C; - **^{1}H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 1.27 - 1.53 (m, 54 H), 2.62 (m, 2 H), 2.75 (m, 2 H), 3.17 - 3.71 (m, 34 H), 3.54 (m, 4 H), 3.64 (m, 4 H), 3.76 (m, 4 H), 3.80 – 3.96 (m, 6 H), 3.81 (s, 6 H), 4.20 (m, 4 H), 5.45 (bs, 1 H), 7.18 (s, 2 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 28.5 (+, 18 C), 38.6 (-, 1 C), 50.1 (-, 16 C), 52.6 (+, 2 C), 54.1 (-, 1 C), 54.2 (-, 2 C), 69.3 (-, 2 C), 69.5 (-, 2 C), 69.9 (-, 2 C), 70.5 (-, 2 C), 71.2 (-, 2 C), 79.8 (C_{quat}, 6 C), 113.5 (+, 2 C), 125.4 (C_{quat}, 2 C), 150.4 (C_{quat}, 2 C), 156.4 (C_{quat}, 6 C), 165.7 (C_{quat}, 2 C), 167.8 (C_{quat}, 2 C), further signals were not detectable; - **IR** (KBr): ν [cm^{-1}] = 2976 (m), 2931 (m), 2876 (m), 1685 (s), 1539 (m), 1498 (m), 1469 (m), 1408 (m), 1363 (m), 1289 (m), 1247 (m), 1168 (s), 1106 (m), 1055 (m), 972 (m), 948 (m), 858 (m), 812 (m), 776 (m), 625 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 796.7 (100, $(M+2H^+)^{2+}$), 1592.5 (17, MH^+); **- $C_{75}H_{126}N_{14}O_{23}$ * 3 H_2O:** calc. C 54.7, H 8.1, N 11.9, found. C 54.7, H 8.0, N 12.3. **UV** (MeOH): λ (ε) = 266 (7000), 225 (49200); - **MF:** $C_{75}H_{126}N_{14}O_{23}$ – **FW:** 1591.92 g/mol;

*14-[4,6-bis-(4,7,10-tri-(^{tert}butyloxycarbonyl)-1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl-amino-N-(2-methylamino)-acetamido-3-propyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethyl ester (**11c**)*

Compound **10b** (298 mg, 0.5 mmol) was reacted with compound **14a** (550 mg, 0.5 mmol) according to *GP I*. Chloroform / methanol 10:1 was used for the purification to give the product as colourless glass (506 mg, 0.312 mmol, 63 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.42 (s, 36 H), 1.43 (s, 18 H), 1.72 (m, 2 H), 3.08 – 3.78 (m, 46 H), 3.63 (m, 4 H), 3.72 (m, 4 H), 3.79 – 3.98 (m, 6 H), 3.86 (s, 6 H), 4.15 – 4.22 (m, 4 H), 5.46 (bs, 1 H), 7.18 (s, 2 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 28.5 (+, 12 C), 28.5 (+, 6 C), 37.4 (-, 1 C), 44.8 (-, 1 C), 49.8 – 51.1 (-, 16 C), 52.6 (+, 2 C), 52.8 (-, 1 C), 53.5 (-, 2 C), 69.1 (-, 2 C), 69.5 (-, 2 C), 69.5 (-, 2 C), 70.4 (-, 2 C), 71.0 (-, 2 C), 79.9 (C_{quat}, 6 C), 113.5 (+, 2 C), 125.4 (C_{quat}, 2 C), 150.4 (C_{quat}, 2 C), 156.4 (C_{quat}, 6 C), 165.7 (C_{quat}, 2 C), 167.8 (C_{quat}, 2 C), further signals were not detectable; - **IR** (KBr): ν [cm^{-1}] = 3300 (bm), 2975 (m), 2933 (m), 2891 (m), 1720 (m), 1681 (s), 1538 (m), 1494 (m), 1466 (m), 1408 (m), 1364 (m), 1323 (m), 1287 (m), 1248 (m), 1161 (s), 1134 (m), 1105 (m), 1054 (m), 973 (m), 945 (m), 918 (m), 859 (m), 815 (m), 777 (m), 730 (s), 646 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 803.6 (78, $(M+2H^+)^{2+}$), 1606.3 (100, MH^+); **UV** (MeOH): λ (ε) = 265 (7100), 224 (49300); - **MF:** $C_{76}H_{128}N_{14}O_{23}$ – **FW:** 1605.95 g/mol;

*14-[4,6-bis-(4,7,10-tri-(^{tert}butyloxycarbonyl)-1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl-amino-N-(2-methylamino)-acetamido-N-(2-methylamino)-acetamido-3-propyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethyl ester (**11d**)*

Compound **10b** (298 mg, 0.5 mmol) was reacted with compound **14b** (580 mg, 0.5 mmol) according to *GP I*. Chloroform / methanol 10:1 was used for the purification to give the product as colourless glass (456 mg, 0.274 mmol, 55 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.36 (s, 36 H), 1.38 (s, 18 H), 1.75 (m, 2 H), 2.71 – 3.06 (m, 6 H), 3.08 – 3.71 (m, 38 H), 3.63 (m, 4 H), 3.66 (m, 4 H), 3.74 – 3.88 (m, 6 H), 3.84 (s, 6 H), 4.05 (m, 2 H), 4.13 – 4.19 (m, 4 H), 5.68 (bs, 1 H), 7.13 (s, 2 H), 7.56 (bs, 1 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 24.4 (-, 1 C), 28.4 (+, 12 C), 28.5 (+, 6 C), 36.6 (-, 1

C), 43.1 (-, 1 C), 45.0 (-, 1 C), 49.8 – 51.2 (-, 16 C), 52.0 (-, 1 C), 52.6 (+, 2 C), 53.4 (-, 2 C), 68.7 (-, 2 C), 69.4 (-, 2 C), 70.2 (-, 2 C), 70.8 (-, 4 C), 79.9 (C_{quat}, 6 C), 113.1 (+, 2 C), 125.4 (C_{quat}, 2 C), 150.1 (C_{quat}, 2 C), 156.4 (C_{quat}, 6 C), 167.7 (C_{quat}, 2 C), 169.4 (C_{quat}, 2 C), 171.7 (C_{quat}, 2 C), 178.6 (C_{quat}, 1 C); - **IR** (KBr): ν [cm^{-1}] = 3330 (bm), 2975 (m), 2933 (m), 2887 (m), 1722 (m), 1678 (s), 1538 (m), 1496 (m), 1468 (m), 1409 (m), 1365 (m), 1324 (m), 1287 (m), 1248 (m), 1161 (s), 1136 (m), 1108 (m), 1054 (s), 1034 (m), 972 (m), 946 (m), 911 (m), 858 (m), 815 (m), 777 (m), 726 (s), 646 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 832.2 (100 %, $(M+2H^+)^{2+}$), 1663.4 (21 %, MH^+); **UV** (MeOH): λ (ε) = 264 (7000), 225 (49100); - **MF:** $C_{78}H_{131}N_{15}O_{24}$ – **FW:** 1663.00 g/mol;

*14-[4,6-bis-(4,7,10-tri-(^{tert}butyloxycarbonyl)-1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl-amino-N-(2-methylamino)-acetamido-6-hexyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethyl ester (**11e**)*

Compound **10c** (319 mg, 0.5 mmol) was coupled with compound **14a** (550 mg, 0.5 mmol) according to *GP I*. Chloroform / methanol 12:1 was used for the purification to yield the product as faintly yellow glass (470 mg, 0.285 mmol, 57 %).

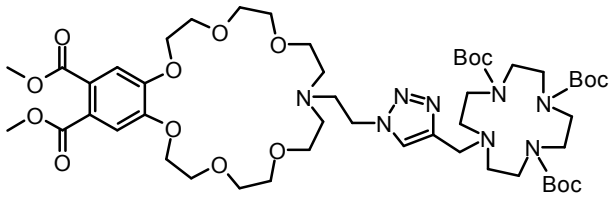
^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.19 – 1.26 (m, 4 H), 1.42 (s, 18 H), 1.44 (s, 36 H), 1.32 – 1.44 (m, 4 H), 3.17 (m, 2 H), 3.06 – 3.68 (m, 44 H), 3.66 (m, 4 H), 3.71 (m, 4 H), 3.87 (s, 6 H), 3.85 – 3.94 (m, 6 H), 4.20 (m, 4 H), 5.34 (bs, 1 H), 7.19 (s, 2 H); - **^{13}C-NMR** (150 MHz, $CDCl_3$): δ [ppm] = 26.6 (-, 1 C), 26.8 (-, 1 C), 28.5 (+, 12 C), 28.5 (+, 6 C), 29.6 (-, 1 C), 30.6 (-, 1 C), 39.2 (-, 1 C), 41.5 (-, 1 C), 45.0 (-, 1 C), 49.8 – 51.4 (-, 16 C), 52.6 (+, 2 C), 53.3 (-, 2 C), 59.3 (-, 1 C), 69.1 (-, 2 C), 69.5 (-, 4 C), 70.5 (-, 2 C), 70.9 (-, 2 C), 79.9 (C_{quat}, 6 C), 113.5 (+, 2 C), 125.4 (C_{quat}, 2 C), 150.4 (C_{quat}, 2 C), 156.5 (C_{quat}, 6 C), 165.7 (C_{quat}, 2 C), 167.8 (C_{quat}, 2 C), 170.4 (C_{quat}, 1 C); - **IR** (KBr): ν [cm^{-1}] = 3300 (bm), 2974 (m), 2933 (m), 2873 (m), 1730 (m), 1684 (s), 1537 (m), 1502 (m), 1466 (m), 1409 (m), 1365 (m), 1323 (m), 1287 (m), 1248 (m), 1161 (s), 1136 (m), 1108 (m), 1053 (m), 1035 (m), 973 (m), 944 (m), 919 (m), 858 (m), 815 (m), 777 (m), 730 (s), 645 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 824.6 (100, $(M+2H^+)^{2+}$), 1684.4 (27, MH^+); - **UV** (MeOH): λ (ε) = 265 (7300), 226 (50200); - **MF:** $C_{79}H_{134}N_{14}O_{23}$ – **FW:** 1648.03 g/mol;

*14-[4,6-bis-(4,7,10-tri-(^{tert}butyloxycarbonyl)-1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl-amino-N-(2-methylamino)-acetamido-N-(2-methylamino)-acetamido-6-hexyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethyl ester (**11f**)*

Compound **10c** (319 mg, 0.5 mmol) was coupled with compound **14b** (550 mg, 0.5 mmol) according to *GP I*. Chloroform / methanol 12:1 was used for the purification to obtain the product as faintly yellow glass (437 mg, 0.256 mmol, 51 %).

^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.01 – 1.12 (m, 4 H), 1.26 (s, 18 H), 1.28 (s, 36 H), 1.32 – 1.39 (m, 2 H), 1.30 – 1.38 (m, 2 H), 2.82 – 3.06 (m, 6 H), 3.07 – 3.62 (m, 36 H), 3.53 (m, 8 H), 3.64 – 3.78 (m, 10 H), 3.72 (s, 6 H), 3.89 (m, 2 H), 4.03 – 4.12 (m, 4 H), 5.79 (bs, 1 H), 7.05 (s, 2 H), 7.91 (bs, 1 H); - **^{13}C-NMR** (150 MHz, $CDCl_3$): δ [ppm] = 23.5 (-, 1 C), 25.5 (-, 1 C), 25.9 (-, 1 C), 28.4 (+, 12 C), 28.4 (+, 6 C), 38.8 (-, 1 C), 43.2 (-, 1 C), 44.9 (-, 1 C), 49.4 – 52.1 (-, 16 C), 52.6 (+, 2 C), 53.1 (-, 1 C), 53.7 (-, 2 C), 54.5 (-, 1 C), 65.8 (-, 2 C), 68.6 (-, 2 C), 69.4 (-, 2 C), 70.3 (-, 2 C), 70.6 (-, 2 C), 79.9 (C_{quat}, 6 C), 113.0 (+, 2 C), 125.3 (C_{quat}, 2 C), 150.0 (C_{quat}, 2 C), 156.2 (C_{quat}, 6 C), 165.6 (C_{quat}, 2 C), 167.7 (C_{quat}, 2 C), 169.1 (C_{quat}, 2 C), further signals were not detectable; - **IR** (KBr): ν [cm^{-1}] = 3300 (bm), 2975 (m), 2936 (m), 2878 (m), 1723 (m), 1686 (s), 1535 (m), 1501 (m), 1466 (m), 1407 (m), 1364 (s), 1326 (m), 1248 (s), 1161 (s), 1134 (s), 1102 (s), 1053 (m), 1024 (m), 971 (m), 946 (m), 853 (m), 812 (m), 778 (m), 737 (m), 699 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 853.2 (100 %, $(M+2H^+)^{2+}$), 1705.5 (19 %, MH^+); - **UV** (MeOH): λ (ε) = 267 (7200), 225 (49600); - **MF:** $C_{81}H_{137}N_{15}O_{24}$ – **FW:** 1705.08 g/mol;

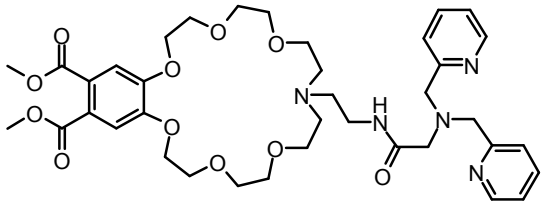
*14-[4,6-bis-(4,7,10-tri-(^{tert}butyloxycarbonyl)-1,4,7,10-tetraaza-cyclododec-1-yl)-N-(2-methylamino)-triazolyl-2-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethyl ester (**19a**)*



Compound **17** (162 mg, 0.3 mmol) and compound **18** (152 mg, 0.3 mmol) were dissolved in 1.0 mL of methanol. A solution of copper(II)sulfate pentahydrate (10 mg, 0.02 mmol) in 0.5 mL of water containing 16 mg sodium ascorbate (0.1 mmol) was added drop wise. After stirring for 2 h at room temperature, the reaction mixture was heated to 60 °C for 5 h in nitrogen atmosphere (TLC control). After cooling to room temperature, 20 mL of ethyl acetate was added, the aqueous layer was separated and the organic phase was washed with 5 mL of brine. After drying over $MgSO_4$ the solvent was evaporated and the solid residue was purified by column chromatography with ethyl acetate / ethanol 3:1. The product appears as a colourless glass (292 mg, 0.279 mmol, 93 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.42 (s, 9 H), 1.43 (s, 9 H), 1.46 (s, 9 H), 2.59 (m, 2 H), 2.66 (m, 2 H), 2.78 (m, 4 H), 3.02 (m, 2 H), 3.21 – 3.39 (m, 8 H), 3.40 – 3.51 (m, 8 H), 3.52 – 3.64 (m, 4 H), 3.67 – 3.76 (m, 4 H), 3.78 – 3.88 (m, 6 H), 3.87 (s, 6 H), 4.14 – 4.22 (m, 4 H), 4.40 (m, 2 H), 7.18 (s, 2 H), 7.61 (s, 1 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 27.5 (+, 6 C), 27.7 (+, 3 C), 46.6 (-, 8 C), 47.8 (-, 2 C), 48.9 (-, 1 C), 51.6 (+, 2 C), 52.1 (-, 1 C), 53.6 (-, 2 C), 54.3 (-, 1 C), 68.1 (-, 2 C), 68.5 (-, 2 C), 69.0 (-, 2 C), 70.1 (-, 2 C), 78.1 (C_{quat}, 1 C), 78.3 (C_{quat}, 1 C), 78.5 (C_{quat}, 1 C), 112.4 (+, 2 C), 123.1 (+, 1 C), 125.3 (C_{quat}, 2 C), 140.2 (C_{quat}, 1 C), 150.4 (C_{quat}, 2 C), 155.4 (C_{quat}, 1 C), 155.7 (C_{quat}, 1 C), 156.1 (C_{quat}, 1 C), 167.7 (C_{quat}, 2 C); - **IR** (KBr): ν [cm^{-1}] = 2994 (m), 2952 (m), 2926 (m), 2874 (m), 1724 (m), 1685 (s), 1598 (m), 1521 (m), 1458 (m), 1436 (m), 1414 (m), 1405 (m), 1364 (m), 1285 (m), 1252 (m), 1176 (s), 1129 (m), 1049 (m), 979 (m), 943 (m), 883 (m), 857 (m), 773 (m), 638 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 1051.8 (41, MH^+), 526.4 (100, $(M+2H^+)^{2+}$); - **HRMS** (PI-LSIMS FAB, glycerine): calc. for $C_{50}H_{83}N_8O_{16}^+$: 1051.5927, found: 1051.5921; - **UV** (MeOH): λ (ε) = 264 (6900), 223 (39300); - **$C_{50}H_{82}N_8O_{16}$ * H_2O:** calc. C 56.2, H 7.9, N 10.5, found. C 56.6, H 7.8, N 8.9. - **MF:** $C_{50}H_{82}N_8O_{16}$ – **FW:** 1051.26 g/mol;

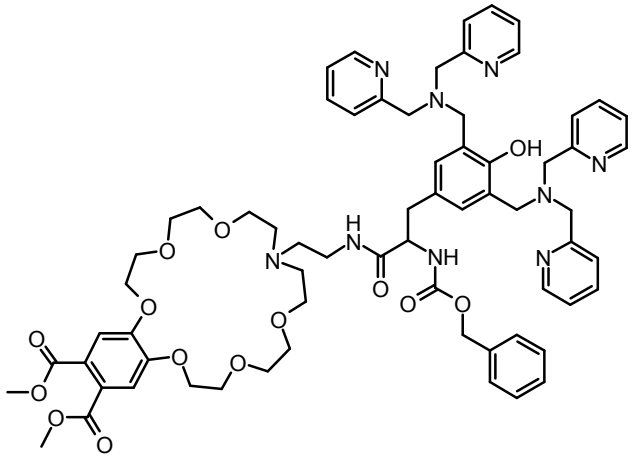
*14-[2,2-Bis-picolyl-2-methylamino)-acetamido-3-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethyl ester (**23**)*



2,2-*N,N*-Dipicoly-glycine sodium salt (**22b**) (307 mg, 1.1 mmol) was dissolved together with HOBt (162 mg, 1.2 mmol) in DCM (6 mL). The solution was cooled in an ice bath and DIPEA (169 mg, 0.21 mL, 1.3 mmol) followed by EDC (218 mg, 1.2 mmol) were added. The mixture was allowed to stir for 30 mins in the ice bath under nitrogen atmosphere, then a solution containing compound **10a** (587 mg, 1.0 mmol) and DIPEA (169 mg, 0.21 mL, 1.3 mmol) in DCM (4 mL) was added in small portions at 2 – 5 °C. The ice bath was removed and the mixture was stirred for 2 h at room temperature, then refluxed over night. The solvent was evaporated and the residue was purified by column chromatography on the reversed phase (RP 18, 35 µm) with acetonitrile / water 1:19 → 4:6, then 4:1. The product is yellow sticky oil, which solidifies as a glass after prolonged standing (460 mg, 0.610 mmol, 68 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 2.95 (m, 2 H), 3.04 (m, 4 H), 3.22 (s, 2 H), 3.53 (m, 4 H), 3.43 (m, 2 H), 3.48 – 3.57 m, 8 H), 3.74 (s, 4 H), 3.68 – 3.84 (m, 4 H), 3.79 (s, 6 H), 4.13 (m, 4 H), 7.07 (m, 2 H), 7.12 (s, 2 H), 7.27 (m, 2 H), 7.53 (m, 2 H), 8.45 (m, 2 H), 8.74 (bs, 1 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 35.6 (-, 1 C), 52.3 (+, 2 C), 53.4 (-, 2 C), 53.8 (-, 1 C), 57.8 (-, 2 C), 60.1 (-, 2 C), 67.7 (-, 1 C), 68.8 (-, 2 C), 69.2 (-, 2 C), 70.2 (-, 2 C), 70.6 (-, 2 C), 113.3 (+, 2 C), 122.2 (+, 2 C), 123.1 (+, 2 C), 123.8 (C_{quat}, 2 C), 125.1 (+, 2 C), 136.5 (+, 2 C), 149.0 (C_{quat}, 2 C), 150.1 (C_{quat}, 2 C), 157.8 (C_{quat}, 2 C), 167.5 (C_{quat}, 2 C), 171.5 (C_{quat}, 1 C); - **IR** (neat): ν [cm^{-1}] = 3300 (bm), 2936 (m), 2878 (m), 1720 (m), 1664 (m), 1594 (m), 1520 (m), 1435 (m), 1350 (m), 1287 (m), 1196 (m), 1124 (m), 1054 (m), 1027 (m), 978 (m), 909 (m), 885 (m), 823 (m), 764 (s), 616 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 754.3 (24, MH^+), 377.6 (100, $(M+2H^+)^{2+}$); - **HRMS** (EI-MS 70 eV): calc. for $C_{38}H_{51}N_5O_{11}^+$: 753.3585, found: 753.3574; - **UV** (MeOH): λ (ε) = 263 (12400), 222 (30400); - **MF:** $C_{38}H_{51}N_5O_{11}$ – **FW:** 753.86 g/mol;

*14-[2-(2-(Benzyloxycarbonyl-amino)-3-{3,5-bis-[(bis-pyridin-2-ylmethyl-amino)-methyl]-4-hydroxy-phenyl}-propionylamino)-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethyl ester (**25**)*



Compound **24** (295 mg, 0.4 mmol) was dissolved in chloroform (3 mL) and DMF (3 mL). The solution was cooled in an ice bath and DIPEA (130 mg, 0.16 mL, 1.0 mmol) followed by HOBt (68 mg, 0.5 mmol) and EDC*HCl (90 mg, 0.5 mmol) were added. After stirring for 30 mins in the ice bath under nitrogen atmosphere, a solution containing compound **10a** (220 mg, 0.4 mmol) and DIPEA (100 mg, 0.14 mL, 0.8 mmol) in chloroform (2 mL) was quickly dropped in. The ice bath was removed and the mixture was stirred for 2 h at room temperature, then heated to 60 °C for 2 h. The mixture was cooled to 2 – 5 °C, a second portion EDC*HCl (90 mg, 0.5 mmol) was added and the mixture was stirred for 2 h, while it was allowed to reach room temperature. It was then heated to 60 °C over night under nitrogen atmosphere. The solvent was evaporated and the residue was purified by column chromatography on the reversed phase (RP 18, 35 μm) with acetonitrile / water 1:19 → 4:6, then 9:1. The product is a yellow glassy solid (281 mg, 0.228 mmol, 57 %).

^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 2.97 (m, 1 H), 3.08 (m, 1 H), 3.38 – 3.57 (m, 6 H), 3.59 – 3.71 (m, 8 H), 3.75 – 3.90 (m, 6 H), 3.86 (s, 6 H), 3.97 (m, 4 H), 4.08 (app. q, 4 H, 13.2 Hz), 4.15 – 4.29 (m, 12 H), 4.48 (m, 2 H), 4.86 (s, 1 H), 4.92 (m, 1 H), 6.31 (m, 1 H), 7.12 (s, 1 H), 7.14 – 7.25 (m, 11 H), 7.47 (d, 4 H, 7.68 Hz), 7.64 (dt, 2 H, 1.92 Hz & 7.68 Hz), 8.55 (d, 2 H, 4.38 Hz); - **^{13}C-NMR** (150 MHz, $CDCl_3$): δ [ppm] = 34.3 (-, 1 C), 37.4 (-, 1 C), 52.6 (+, 2 C), 53.8 (-, 2 C), 55.1 (-, 1 C), 56.5 (-, 1 C), 58.1 (-, 2 C), 65.5 (-, 4 C), 66.3 (+, 1 C), 68.7 (-, 2 C), 69.4 (-, 2 C), 70.2 (-, 2 C), 70.5 (-, 2 C), 113.3 (+, 2 C), 122.3 (+, 4 C), 124.2 (+, 4 C), 125.3 (C_{quat}, 2 C), 127.5 (+, 1 C), 127.8 (+, 6 C), 128.3 (+, 2 C), 133.0 (C_{quat}, 1 C),

136.5 (C_{quat}, 1 C), 137.4 (+, 4 C), 148.6 (C_{quat}, 2 C), 150.2 (C_{quat}, 2 C), 154.7 (C_{quat}, 1 C), 155.4 (C_{quat}, 1 C), 156.0 (C_{quat}, 1 C), 167.7 (C_{quat}, 2 C), 172.1 (C_{quat}, 1 C); - **IR** (neat): ν [cm^{-1}] = 3325 (bm), 3050 (m), 3036 (w), 2991 (w), 2944 (m), 2925 (m), 2884 (m), 2362 (m), 2348 (m), 1719 (s), 1672 (m), 1594 (m), 1520 (m), 1476 (m), 1436 (m), 1381 (m), 1350 (m), 1288 (m), 1258 (s), 1199 (m), 1125 (s), 1049 (m), 1027 (m), 913 (m), 898 (m), 823 (w), 763 (s), 616 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 412.2 (74, $(M+3H^+)^{3+}$), 618.2 (100, $(M+2H^+)^{2+}$), 1234.9 (6, MH^+) - **UV** (MeOH): λ (ε) = 268 (15300), 224 (30100), 206 (54800); - **MF:** $C_{67}H_{79}N_9O_{14}$ – **FW:** 1234.43 g/mol;

Purification protocol for reversed phase:

For the purification of compound **23** and **25** a Varian 971-FP Flash System equipped with a reversed phase column (C18 gel, Superflash 1409-1, SF10-10g, 35 μm) was used. The column was conditioned with acetonitrile, then with acetonitrile / water 1:19.

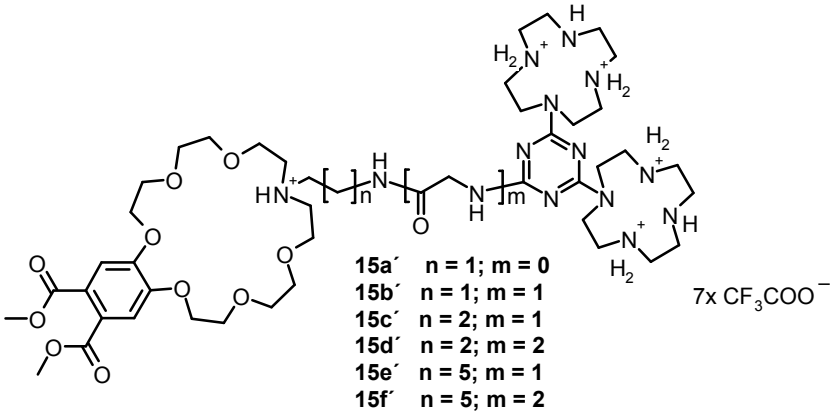
The crude compound was dissolved in acetonitrile / water 1:19 (0.5 mL) and was transferred to the column with the aid of a syringe. Acetonitrile / water 1:19 (0.5 mL) was used to rinse the syringe once and transfer the material completely to the column.

Flow rate:	20 mL/min	
Gradient:	0 min:	5 % acetonitrile
	30 min:	40 % acetonitrile
	40 min:	90 % acetonitrile

Retention time:	Compound **23**	19.5 – 24.5 min
	Compound **25**	14.0 – 18.5 min

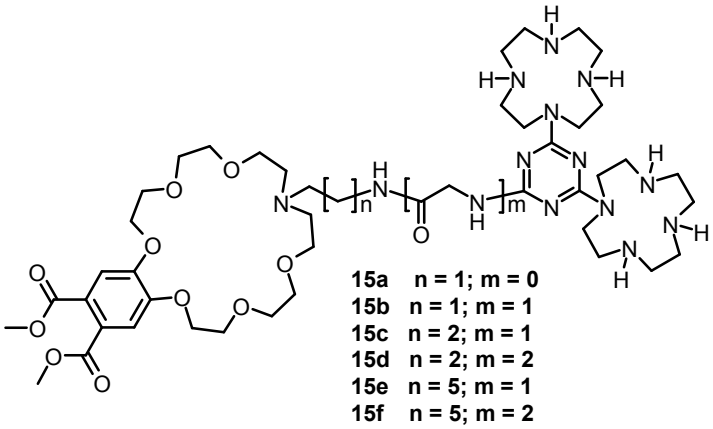
V. 4.1.3. Preparation of the Cyclene Freebases

General procedure for the deprotection of crown ether cyclenes to the free ligand salts (GP II)[58]



To a well stirred solution of the Boc-protected ligand (0.1 mmol) in dichloromethane (2 mL) a solution of TFA in dichloromethane (2 mL, 20 % vol/vol) was added. Stirring was continued for 4 h (TLC control), then the solvent was evaporated. The residue was re-dissolved in a minimum amount of dichloromethane and the product was precipitated by drop wise addition of diethyl ether. The solvent mixture was decanted off carefully, the precipitate was suspended in diethyl ether, allowed to settle completely and the solvent was decanted off again. After drying a yellow under reduced pressure, a yellow, deliquescent solid is obtained in excellent yield. (yield and characterisation of the salts can be found in the supporting information of this chapter).

General procedure for liberation of the cyclene free base amines (GP III):



A small column was packed with 1.1 mL of a strongly basic anion exchanger (OH^--form, loading: 0.9 mmol/mL, 0.99 mmol, 10 eq.) and the resin was rinsed with water till it gave a neutral reaction against universal pH-indicator paper. The ligand TFA-salt (0.10 mmol) was dissolved in 0.5 mL of water and eluted over the column. It was rinsed with distilled water until no more fractions with the typical blue-violet fluorescence could be collected. The fractions were combined and lyophilized to give the free base as colourless hygroscopic fine powder in quantitative yield.

14-[4,6-Bis-(1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazinyl-amino-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethyl ester **(*15a*)**

^{1}H-NMR (300 MHz, MeOD): δ [ppm] = 2.84 – 2.90 (m, 10 H), 2.86 – 3.28 (m, 16 H), 3.32 – 3.48 (m, 6 H), 3.54 – 3.78 (m, 20 H), 3.84 (s, 6 H), 3.89 (m, 4 H), 4.22 (m, 4 H), 7.24 (m, 2 H); – **^{13}C-NMR** (75 MHz, D_2O): δ [ppm] = 44.8 (-, 1 C), 48.6 (-, 8 C), 48.7 (-, 4 C), 49.3 (-, 4 C), 53.2 (+, 2 C), 53.4 (-, 1 C), 57.9 (-, 2 C), 68.8 (-, 2 C), 68.9 (-, 2 C), 69.7 (-, 2 C), 69.9 (-, 2 C), 70.0 (-, 2 C), 124.3 (C_{quat}, 2 C), 149.8 (C_{quat}, 2 C), 163.5 (C_{quat}, 1 C), 164.6 (C_{quat}, 1 C), 165.0 (C_{quat}, 1 C), 169.3 (C_{quat}, 2 C) - **IR** (neat): ν [cm^{-1}] = 3370 (bm), 2930 (m), 2880 (m), 1717 (m), 1541 (s), 1484 (s), 1411 (m), 1350 (s), 1298 (s), 1194 (m), 1118 (s), 1058 (m), 1030 (m), 949 (m), 899 (m), 809 (s), 599 (m), 517 (m); - **MS** (ESI-MS, H_2O/MeOH + 0.1 % TFA): m/z (%) = 312.3 (32, $(M + 3H^+)^{3+}$), 467.9 (100, $(M + 2H^+)^{2+}$), 934.4 (45, (MH^+)); - **MF:** $C_{43}H_{75}N_{13}O_{10}$ – **FW:** 934.16 g/mol;

14-[4,6-Bis-(1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl-amino-N-(2-methyl-amino)-acetamido-3-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17, 20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethyl ester **(*15b*)**

^{1}H-NMR (300 MHz, MeOD): δ [ppm] = 2.62 (m, 2 H), 2.68 – 2.86 (m, 8 H), 2.87 – 3.18 (m, 16 H), 3.41 (m, 6 H), 3.48 – 3.79 (m, 20 H), 3.84 (s, 6 H), 3.81 - 3.93 (m, 6 H), 4.23 (m, 4 H), 7.26 (m, 2 H) – **^{13}C-NMR** (75 MHz, MeOD): δ [ppm] = 44.9 (-, 1 C), 48.4 (-, 8 C), 48.6 (-, 4 C), 49.5 (-, 4 C), 50.4 (-, 4 C), 53.2 (+, 2 C), 55.3 (-, 2 C), 55.4 (-, 1 C), 70.3 (-, 2 C), 70.6 (-, 2 C), 70.7 (-, 2 C), 71.5 (-, 2 C), 71.6 (-, 2 C), 72.0 (-, 1 C), 114.5 (+, 2 C), 126.0 ($C_{quat,}$ 2 C), 151.8 (C_{quat}, 2 C), 161.5 (C_{quat}, 1 C), 169.4 (C_{quat}, 2 C), further signals were not detectable; -

IR (neat): ν [cm^{-1}] = 3357 (bm), 2929 (m), 2866 (m), 1721 (m), 1654 (m), 1550 (s), 1487 (s), 1349 (s), 1279 (s), 1199 (m), 1123 (s), 1066 (m), 1022 (m), 919 (m), 812 (m), 729 (s), 641 (m); - **MS** (ESI-MS, H_2O/MeOH + 0.1 % TFA): m/z (%) = 331.3 (100, $(M + 3H^+)^{3+}$), 496.4 (87, $(M + 2H^+)^{2+}$), 991.7 (4, (MH^+)); - **MF:** $C_{75}H_{78}N_{14}O_{11}$ – **FW:** 991.21 g/mol;

14-[4,6-Bis-(1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl-amino-N-(2-methyl-amino)-acetamido-3-propyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17, 20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethyl ester **(*15c*)**

^{1}H-NMR (300 MHz, MeOD): δ [ppm] = 1.61 (m, 2 H), 2.40 – 2.55 (m, 2 H), 2.53 – 2.72 (m, 16 H), 2.73 – 3.06 (m, 8 H), 3.22 (m, 2 H), 3.44 – 3.78 (m, 20 H), 3.79 – 3.96 (m, 6 H), 3.85 (s, 6 H), 4.20 (m, 4 H), 7.28 (m, 2 H) – **IR** (neat): ν [cm^{-1}] = 3287 (bm), 2929 (m), 2875 (m), 1721 (m), 1655 (m), 1542 (s), 1488 (s), 1415 (m), 1357 (s), 1275 (s), 1198 (m), 1122 (s), 1050 (m), 1008 (m), 913 (m), 811 (m), 704 (s), 643 (m); - **MS** (ESI-MS, H_2O/MeOH + 0.1 % TFA): m/z (%) = 335.9 (100, $(M + 3H^+)^{3+}$), 503.4 (41, $(M + 2H^+)^{2+}$), 1005.6 (7, (MH^+)); - **MF:** $C_{46}H_{80}N_{14}O_{11}$ – **FW:** 1005.24 g/mol;

14-[4,6-Bis-(1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl-amino-N-(2-methylamino)-acetamido-N-(2-methylamino)-acetamido-3-propyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethyl ester **(*15d*)**

^{1}H-NMR (300 MHz, MeOD): δ [ppm] = 1.52 – 1.68 (m, 2 H), 2.50 (m, 2 H), 2.60 – 2.80 (m, 16 H), 2.80 – 3.00 (m, 12 H), 3.22 (m, 2 H), 3.33 – 3.39 (m, 2 H), 3.56 – 3.76 (m, 20 H), 3.80 – 3.92 (m, 10 H), 4.00 (s, 2 H), 4.18 – 4.28 (m, 4 H), 7.27 (s, 2 H) – **IR** (neat): ν [cm^{-1}] = 3265 (bm), 2927 (m), 2868 (m), 2362 (m), 1719 (m), 1655 (m), 1546 (s), 1493 (s), 1350 (s), 1147 (m), 1128 (s), 1106 (m), 1046 (m), 920 (m), 811 (m), 731 (m), 622 (m); - **MS** (ESI-MS, H_2O/MeOH + 0.1 % TFA): m/z (%) = 355.0 (100, $(M + 3H^+)^{3+}$), 532.0 (52, $(M + 2H^+)^{2+}$), 1062.5 (3, (MH^+)); - **MF:** $C_{48}H_{83}N_{15}O_{12}$ – **FW:** 1062.29 g/mol;

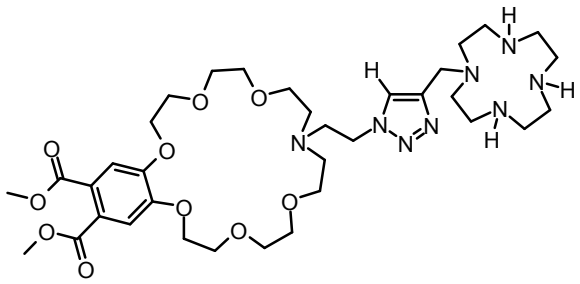
14-[4,6-Bis-(1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl-amino-N-(2-methyl-amino)-acetamido-6-hexyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17, 20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethyl ester **(*15e*)**

^{1}H-NMR (300 MHz, MeOD): δ [ppm] = 1.10 – 1.31 (m, 4 H), 1.33 – 1.51 (m, 4 H), 2.38 – 2.52 (m, 2 H), 2.63 – 2.81 (m, 12 H), 2.93 – 3.22 (m, 14 H), 3.37 – 3.51 (m, 4 H), 3.53 – 3.80 (m, 20 H), 3.85 (s, 6 H), 3.83 - 3.89 (m, 6 H), 4.23 (m, 4 H), 7.30 (s, 2 H) – **IR** (neat): ν [cm^{-1}] = 3377 (bm), 2931 (m), 2859 (m), 1721 (m), 1654 (m), 1547 (s), 1485 (s), 1415 (s), 1349 (s), 1283 (s), 1197 (m), 1123 (s), 1056 (m), 1029 (m), 915 (m), 870 (m), 809 (m), 703 (s), 640 (m); - **MS** (ESI-MS, H_2O/MeOH + 0.1 % TFA): m/z (%) = 350.0 (100, $(M + 3H^+)^{3+}$), 524.4 (48, $(M + 2H^+)^{2+}$), 1047.7 (1, (MH^+)); - **MF:** $C_{49}H_{86}N_{14}O_{11}$ – **FW:** 1047.32 g/mol;

14-[4,6-Bis-(1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl-amino-N-(2-methylamino)-acetamido-N-(2-methylamino)-acetamido-6-hexyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethyl ester **(*15f*)**

^{1}H-NMR (300 MHz, MeOD): δ [ppm] = 1.20 – 1.32 (m, 4 H), 1.34 – 1.49 (m, 4 H), 2.47 (t, 2 H, 6.4 Hz), 2.58 – 2.74 (m, 20 H), 2.75 – 2.97 (m, 10 H), 3.14 (t, 2 H, 6.4 Hz), 3.48 – 3.71 (m, 20 H), 3.82 – 3.94 (m, 6 H), 3.85 (s, 6 H), 3.99 (m, 2 H), 4.23 (m, 4 H), 7.29 (s, 2 H); - **IR** (neat): ν [cm^{-1}] = 3370 (bm), 2934 (m), 2858 (m), 1719 (m), 1656 (m), 1548 (s), 1486 (s), 1416 (s), 1350 (s), 1284 (s), 1198 (m), 1122 (s), 1053 (m), 1027 (m), 916 (m), 871 (m), 806 (m), 704 (s), 642 (m); - **MS** (ESI-MS, H_2O/MeOH + 0.1 % TFA): m/z (%) = 369.0 (100, $(M + 3H^+)^{3+}$), 553.0 (39, $(M + 2H^+)^{2+}$), 1104.6 (1, (MH^+)); - **MF:** $C_{51}H_{89}N_{15}O_{12}$ – **FW:** 1104.37 g/mol;

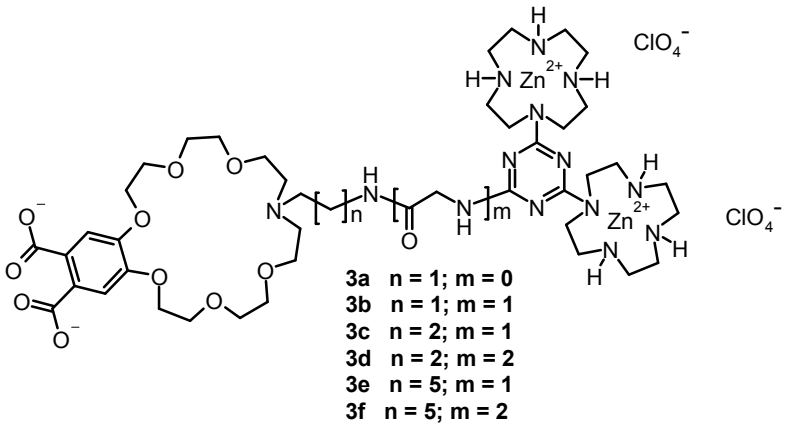
14-[3-(1,4,7,10-Tetraaza-cyclododec-1-yl)-[1,4,5]triazol-amino-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethyl ester **(*19b*)**



^{1}H-NMR (300 MHz, MeOD): δ [ppm] = 2.51 (m, 6 H), 2.61 (m, 4 H), 2.73 (m, 12 H), 3.02 (t, 2 H, 6.6 Hz), 3.52 (m, 4 H, 4.6 Hz), 3.61 (m, 4 H), 3.64 – 3.75 (m, 6 H), 3.78 – 3.91 (m, 4 H), 3.87 (s, 6 H), 4.19 (m, 4 H), 4.48 (t, 2 H, 6.6 Hz), 7.23 (s, 2 H), 7.91 (s, 1 H); – **^{13}C-NMR** (75 MHz, MeOD): δ [ppm] = 45.0 (-, 2 C), 46.4 (-, 2 C), 47.2 (-, 2 C), 49.5 (-, 1 C), 49.9 (-, 1 C), 50.1 (-, 1 C), 51.4 (-, 2 C), 53.1 (+, 2 C), 56.2 (-, 1 C), 56.4 (-, 1 C), 70.5 (-, 2 C), 70.7 (-, 2 C), 71.1 (-, 2 C), 71.7 (-, 2 C), 72.1 (-, 2 C), 114.4 (+, 2 C), 125.9 (+, 1 C), 126.6 (C_{quat}, 2 C), 144.4 (C_{quat}, 1 C), 151.9 (C_{quat}, 2 C), 169.4 (C_{quat}, 2 C); - **IR** (neat): ν [cm^{-1}] = 3292 (bm), 2942 (m), 2863 (m), 1719 (m), 1595 (m), 1517 (m), 1438 (m), 1286 (s), 1100 (s), 802 (m), 730 (s), 645 (m); - **MF:** $C_{35}H_{58}N_8O_{10}$ – **FW:** 750.90 g/mol;

V. 4.1.4. Complexation of the Ligands

General procedure for bis-zinc-cyclene complexes (GP IV)



The free base (approx. 100 mg, 0.1 mmol) was dissolved in water (0.5 mL). An aqueous solution of $Zn(ClO_4)_2$ hexahydrate (2.2 eq., 1 M, 0.22 mL, 0.22 mmol) was added. The mixture was adjusted to a weakly basic pH value (~ 7.5 - 8) with 0.01 M aqueous $NaHCO_3$ and stirred over night at 80 °C. The solution was cooled to room temperature and lyophilized. The residue was suspended in 0.5 ml dry EtOH, treated with ultra sound for 10 min, filtered and washed with dry EtOH. The product was dried at reduced pressure to give the zinc-complex as colourless hygroscopic solid.

14-[4,6-Bis-(1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazinyl-amino-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid bis-zinc complex ***(3a)***[59]

By complexation of compound **15a** (93 mg, 0.1 mmol) following *GP IV*, a fine, colourless powder is obtained (117 mg, 0.095 mmol, 95 %).

M.p. (uncorrected) = 225 °C (decomp.); - **^{1}H-NMR** (300 MHz, CD_3CN): δ [ppm] = 2.50 – 4.00 (m, 56 H), 4.05 – 4.70 (m, 8 H), 7.24 (s, 2 H); - **IR** (neat): ν [cm^{-1}] = 3490 (bm), 3295 (bm), 2942 (m), 2874 (m), 1685 (m), 1560 (m), 1424 (m), 1347 (m), 1280 (m), 1199 (m), 1048 (s), 813 (m), 806 (m), 742 (m), 619 (m); - **MS** (ESI-MS, H_2O/MeCN): m/z (%) = 344.9 (68, $(M^{2+} + H^+)^{3+}$), 517.8 (100, (M^{2+}), 567.8 (12, $(M^{2+} + ClO_4^- - H^+)^{2+}$), 1134.1 (5, $(M^{2+} - H^+)^+$); - **UV** (H_2O): λ (ε) = 261 (7400), 225 (49200); - **MF:** $C_{41}H_{61}N_{13}O_{18}Zn_2Cl$ – **FW:** 1233.71 g/mol;

14-[4,6-Bis-(1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl-amino-N-(2-methyl-amino)-acetamido-3-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17, 20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid bis-zinc complex ***(3b)***

Complexation of compound **15b** (94 mg, 0.1 mmol) according to *GP IV* yields 116 mg of a colourless solid (0.090 mmol, 90 %).

M.p. (uncorrected) = 169 °C (decomp.); - **^{1}H-NMR** (300 MHz, CD_3CN): δ [ppm] = 2.50 – 4.00 (m, 58 H), 4.05 – 4.70 (m, 8 H), 7.23 (s, 2 H); - **IR** (neat): ν [cm^{-1}] = 3480 (bm), 3293 (bm), 2943 (m), 2872 (m), 1686 (m), 1562 (m), 1425 (m), 1346 (m), 1281 (m), 1198 (m), 1046 (s), 813 (m), 804 (m), 743 (m), 619 (m); - **MS** (ESI-MS, H_2O/MeCN): m/z (%) = 364.4 (100, $(M^{2+} + H^+)^{3+}$), 546.3 (21, (M^{2+})), 596.3 (14, $(M^{2+} - H^+ + ClO_4^-)^{2+}$); - **UV** ($H_2O$): λ (ε) = 260 (7300), 224 (48700); - **MF:** $C_{43}H_{72}N_{14}O_{19}Zn_2Cl_2$ – **FW:** 1290.76 g/mol;

14-[4,6-Bis-(1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl-amino-N-(2-methy-lamino)-acetamido-3-propyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17, 20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid bis-zinc complex ***(3c)***

Compound **15c** (95 mg, 0.1 mmol) complexed by *GP IV* gives a fine, colourless powder (121 mg, 0.093 mmol, 93 %).

M.p. (uncorrected) = 217 °C (decomp.); - **^{1}H-NMR** (300 MHz, CD_3CN): δ [ppm] = 1.43 (m, 2 H), 2.50 – 3.10 (m, 34 H), 3.15 – 3.45 (m, 6 H), 3.50 – 3.72 (m, 14 H), 3.75 – 3.96 (m, 6 H), 4.00 – 4.40 (m, 8 H), 7.04 (s, 2 H); - **IR** (neat): ν [cm^{-1}] = 3480 (bm), 3293 (bm), 2946 (m), 2880 (m), 1688 (m), 1564 (m), 1426 (m), 1348 (m), 1282 (m), 1196 (m), 1048 (s), 812 (m), 806 (m), 743 (m), 618 (m); - **MS** (ESI-MS, H_2O/MeCN): m/z (%) = 369.1 (100, $(M^{2+} + H^+)^{3+}$), 553.3 (54, (M^{2+})), 603.3 (21, $(M^{2+} - H^+ + ClO_4^-)^{2+}$), 1205.2 (3, $(M^{2+} + ClO_4^-)^+$), 1305.3 (2, $(M^{2+} + H^+ + 2\ ClO_4^-)^+$); - **UV** ($H_2O$): λ (ε) = 260 (7400), 223 (49600); - **MF:** $C_{44}H_{74}N_{14}O_{19}Zn_2Cl_2$ – **FW:** 1304.79 g/mol;

14-[4,6-Bis-(1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl-amino-N-(2-methylamino)-acetamido-N-(2-methylamino)-acetamido-3-propyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid bis-zinc complex ***(3d)***

By complexation of compound **15d** (101 mg, 0.1 mmol) following *GP IV*, a fine, colourless powder is yielded (121 mg, 0.089 mmol, 89 %).

M.p. (uncorrected) = 182 °C (decomp.); - **^{1}H-NMR** (300 MHz, CD_3CN): δ [ppm] = 1.40 (m, 2 H), 2.50 – 4.00 (m, 62 H), 4.05 – 4.65 (m, 6 H), 7.21 (s, 2 H); - **IR** (neat): ν [cm^{-1}] = 3375 (bm), 2918 (m), 1630 (m), 1553 (m), 1420 (m), 1274 (m), 1095 (s), 816 (m), 621 (s) - **MS** (ESI-MS, H_2O/MeCN): m/z (%) = 388.1 (100, $(M^{2+} - H^+)^{3+}$), 581.8 (40, M^{2+}), 631.8 (24, $(M^{2+} + H^+ + ClO_4^-)^{2+}$), 1262.2 (1, $(M^{2+} + ClO_4^-)^+$), 1384.5 (1, $(M^{2+} + 2\ H^+ - ClO_4^- + 2\ OAc^-)^+$); - **UV** ($H_2O$): λ (ε) = 262 (7100), 224 (49900); - **MF:** $C_{46}H_{77}N_{15}O_{20}Zn_2Cl_2$ – **FW:** 1361.84 g/mol;

14-[4,6-Bis-(1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl-amino-N-(2-methyl-amino)-acetamido-6-hexyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17, 20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid bis-zinc complex ***(3e)***

Converting compound **15e** (99 mg, 0.1 mmol) to its zinc-complex by *GP IV*, gives a colourless powder (117 mg, 0.087 mmol, 87 %).

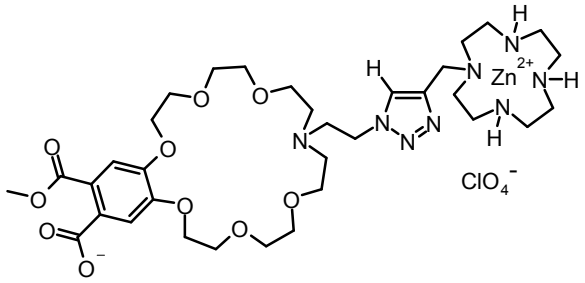
M.p. (uncorrected) = 152 °C (decomp.); - **^{1}H-NMR** (300 MHz, CD_3CN): δ [ppm] = 0.82 – 1.12 (m, 6 H), 1.50 (m, 2 H), 2.60 – 3.10 (m, 30 H), 3.12 – 3.50 (m, 8 H), 3.52 – 3.98 (m, 20 H), 4.00 – 4.40 (m, 8 H), 7.01 (s, 2 H); - **IR** (neat): ν [cm^{-1}] = 3496 (bm), 3391 (bm), 3276 (bm), 2930 (m), 2890 (m), 1631 (m), 1551 (m), 1420 (m), 1349 (m), 1273 (m), 1206 (m), 1082 (s), 960 (m), 810 (m), 620 (m); - **MS** (ESI-MS, H_2O/MeCN): m/z (%) = 382.6 (100, $(M^{2+} + H^+)^{3+}$), 574.4 (30, (M^{2+}), 624.4 (31, $(M^{2+} + H^+ + ClO_4^-)^{2+}$); - **UV** ($H_2O$): λ (ε) = 260 (7500), 224 (48800); - **MF:** $C_{47}H_{80}N_{14}O_{19}$ – **FW:** 1346.87 g/mol;

14-[4,6-Bis-(1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-yl-amino-N-(2-methylamino)-acetamido-N-(2-methylamino)-acetamido-6-hexyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethyl ester bis-zinc complex ***(3f)***

Complexation of compound **15f** (104 mg, 0.1 mmol) by *GP IV* yields a fine, faintly yellow powder (110 mg, 0.079 mmol, 79 %).

M.p. (uncorrected) = 155 °C (decomp.); - **^{1}H-NMR** (300 MHz, CD_3CN): δ [ppm] = 0.90 – 1.70 (m, 8 H), 2.50 – 4.00 (m, 58 H), 4.10 – 4.60 (m, 6 H), 7.19 (s, 2 H); - **IR** (neat): ν [cm^{-1}] = 3588 (bm), 2930 (m), 2890 (m), 1655 (m), 1560 (m), 1437 (m), 1350 (m), 1274 (m), 1208 (m), 1042 (s), 958 (m), 821 (m), 618 (m); - **MS** (ESI-MS, H_2O/MeCN): m/z (%) = 402.3 (100, $(M^{2+} + H^+)^{3+}$), 602.9 (32, (M^{2+})), 652.9 (20, $(M^{2+} + H^+ + ClO_4^-)^{2+}$), 1304.2 (1, $(M^{2+} + ClO_4^-)^+$); - **UV** (H_2O): λ (ε) = 261 (7300), 225 (49600); - **MF:** $C_{49}H_{83}N_{15}O_{20}Zn_2Cl_2$ – **FW:** 1403.92 g/mol;

14-[3-(1,4,7,10-Tetraaza-cyclododec-1-yl)-[1,4,5]triazol-amino-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid mono-methylester zinc complex ***(5)***

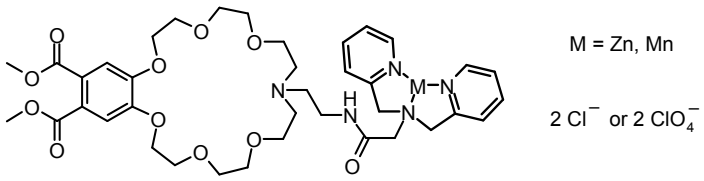


A procedure analogous to *GP IV* was followed starting from the free base **19b** (75 mg, 0.1 mmol) and admitting a solution of 1.1 eq. of 1 M $Zn(ClO_4)_2$ * 6 H_2O (0.11 mL, 0.11 mmol). The product was obtained in quantitative yield as a colourless hygroscopic solid (89 mg, 0.1 mmol).

M.p. (uncorrected) = 164 °C (decomp.); - **^{1}H-NMR** (300 MHz, D_2O): δ [ppm] = 2.48 – 2.96 (m, 20 H), 3.07 (m, 2 H), 3.55 (m, 8 H), 3.61 (m, 4 H), 3.70 (m, 2 H), 3.74 (s, 3 H), 3.79 (m, 4 H), 4.11 (m, 4 H), 4.44 (m, 2 H), 7.17 (s, 2 H), 7.77 (s, 1 H); - **^{13}C-NMR** (75 MHz, CD_3CN): δ [ppm] = 43.1 (-, 1 C), 44.4 (-, 1 C), 51.2 (-, 1 C), 52.0 (+, 1 C), 67.4 (-, 2 C), 68.0 (-, 2 C),

68.6 (-, 2 C), 68.7 (-, 2 C), 69.2 (-, 2 C), 69.5 (-, 2 C), 112.6 (+, 2 C), 125.1 (+, 1 C), 125.7 (C_{quat}, 1 C), 126.1 (C_{quat}, 1 C), 141.8 (C_{quat}, 1 C), 149.4 (C_{quat}, 1 C), 166.9 (C_{quat}, 1 C), 167.2 (C_{quat}, 1 C), further signals were not detectable - **IR** (neat): ν [cm^{-1}] = 3526 (bm), 2937 (m), 1711 (m), 1627 (m), 1599 (m), 1440 (m), 1281 (m), 1078 (s), 780 (m), 620 (s) - **MS** (ESI-MS, H_2O/MeCN): m/z (%) = 400.3 (20, M^{2+}), 498.3 (100, $(M^{2+} + H^+ + ClO_4^-)^{2+}$), 799.3 (19, $(M^{2+} - H^+)^+$), 898.2 (6, $(M^{2+} + ClO_4^-)^+$); - **UV** (H_2O): λ (ε) = 267 (7700), 228 (26100); - **MF:** $C_{34}H_{59}N_8O_{14}ZnCl$ – **FW:** 900.64 g/mol;

14-[2,2-Bis-picolyl-2-methylamino)-acetamido-3-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethyl ester metal complexes



Compound **23** (151 mg, 0.2 mmol) was dissolved in methanol (2 mL) and the according metal salt (40 mg manganese(II)chloride tetrahydrate or 74 mg zinc(II)perchlorate hexahydrate, 0.2 mmol) in water (3 mL) was added drop wise. The solution was warmed to 40 °C for 2 h, whilst the methanol was allowed to evaporate slowly. After stirring at room temperature for 30 min, the remaining solution was lyophilised to give the complex salt in quantitative yield.

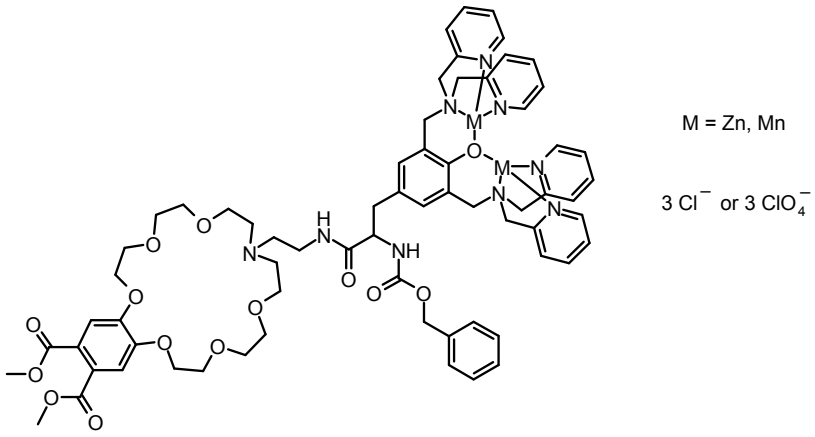
Zinc complex diperchlorate (**6a**):

M.p. (uncorrected) = 126 - 130 °C (decomp.); - **^{1}H-NMR** (300 MHz, MeOD): δ [ppm] = 3.40 – 3.95 (m, 24 H), 3.83 (s, 6 H), 4.07 (m, 2 H), 4.30 (m, 4 H), 4.38 – 4.60 (m, 4 H), 7.31 (s, 2 H), 7.53- 7.75 (m, 2 H), 8.10 – 8.27 (m, 2 H), 8.82 (m, 2 H); - **IR** (neat): ν [cm^{-1}] = 3470 (bm), 2960 (m), 2941 (m), 2874 (m), 1699 (m), 1615 (m), 1577 (m), 1440 (m), 1350 (m), 1297 (s), 1203 (m), 1052 (bs), 981 (m), 911 (m), 767 (m), 654 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 408.7 (100, M^{2+}), 816.4 (3, $(M^{2+} - H^+)^+$), 876.5 (8, $(M^{2+} + OAc^-)^+$), 916.4 (6, $(M^{2+} + ClO_4^-)^+$); - **UV** (H_2O): λ (ε) = 266 (12200), 220 (31200); - **MF:** $C_{38}H_{51}N_5O_{19}ZnCl_2$ – **FW:** 1018.51 g/mol;

Manganese complex dichloride (**6b**):

M.p. (uncorrected) = 165 - 169 °C (decomp.); - **IR** (neat): ν [cm^{-1}] = 3360 (bm), 2955 (m), 2889 (m), 1712 (m), 1633 (m), 1601 (m), 1520 (m), 1438 (m), 1351 (m), 1291 (m), 1198 (m), 1128 (m), 1102 (m), 1052 (m), 1019 (m), 973 (m), 943 (m), 889 (m), 766 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 408.7 (100, M^{2+}), 816.4 (3, $(M^{2+} - H^+)^+$), 876.5 (8, $(M^{2+} + OAc^-)^+$), 916.4 (6, $(M^{2+} + ClO_4^-)^+$); - **UV** (H_2O): λ (ε) = 266 (12900), 221 (32100); - **MF:** $C_{38}H_{51}N_5O_{11}MnCl_2$ – **FW:** 879.70 g/mol;

14-[2-(2-(Benzyloxycarbonyl-amino)-3-{3,5-bis-[(bis-pyridin-2-ylmethyl-amino)-methyl]-4-hydroxy-phenyl}-propionylamino)-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylic acid dimethyl ester



Compound **25** (123 mg, 0.1 mmol) was dissolved in methanol (2 mL) and the according metal salt (40 mg manganese(II)chloride tetrahydrate or 74 mg zinc(II)perchlorate hexahydrate, 0.2 mmol) in water (3 mL) was added drop wise. The solution was warmed to 40 °C for five hours, whilst the methanol was allowed to evaporate. After slow cooling to room temperature, the remaining solution was lyophilised to give the complex salt as a sticky solid in quantitative yield.

Bis-zinc complex triperchlorate (**4a**):

^{1}H-NMR (300 MHz, MeOD): δ [ppm] = 2.61 - 3.20 (m, 6 H), 3.50 – 4.16 (m, 17 H), 3.81 (s, 6 H), 4.82 (m, 1 H), 5.00 (m, 1 H), 6.52 (m, 1 H), 6.98 - 7.13 (m, 4 H), 7.16 - 7.42 (m, 5 H), 7.59 – 7.92 (m, 8 H), 8.08 – 8.30 (m, 4 H), 9.04 – 9.32 (m, 4 H); - **IR** (neat): ν [cm^{-1}] = 2962

(m), 2943 (m), 2878 (m), 1715 (m), 1608 (m), 1520 (m), 1475 (m), 1437 (m), 1349 (m), 1289 (m), 1259 (m), 1198 (m), 1082 (s), 1051 (s), 1022 (s), 879 (m), 798 (s), 764 (s), 703 (m), 621 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 1482.9 (3, M^{4+} $-H^+$ $+2CH_3COO^-$), 711.9 (100, $(M^{4+}$ $-H^+$ $+CH_3COO^-)^{2+}$); - **UV** (H_2O): λ (ε) = 263 (16900), 229 (33200); - **MF:** $C_{67}H_{79}N_9O_{26}Zn_2Cl_3$ – **FW:** 1664.09 g/mol;

Bis-manganese complex trichloride (**4b**):

IR (neat): ν [cm^{-1}] = 2961 (m), 2946 (m), 2876 (m), 1716 (m), 1610 (m), 1521 (m), 1473 (m), 1436 (m), 1352 (m), 1287 (m), 1261 (m), 1199 (m), 1076 (s), 1049 (s), 1020 (s), 878 (m), 799 (s), 762 (s), 701 (m), 622 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 1460.9 (7, M^{4+} $-H^+$ $+2CH_3COO^-$), 1378.7 (3, M^{4+} $-2H^+$ $+HCl$), 731.0 (100, $(M^{4+}$ $+2CH_3COO^-)^{2+}$), 690.9 (50, $(M^{4+}$ $-H^+$ $+CH_3COO^-)^{2+}$), 689.9 (58, $(M^{4+}$ $-H^+$ $+Cl^-)^{2+}$); - **UV** (H_2O): λ (ε) = 262 (16200), 227 (32000); - **MF:** $C_{67}H_{79}N_9O_{14}Mn_2Cl_3$ – **FW:** 1450.66 g/mol;

Synthetic details for preparation of molecule **1**, **8**, **10b**, **13b** and **14b** are available in the supporting information of this chapter.

V. 4.2. Selected NMR and Mass Spectra of New Compounds

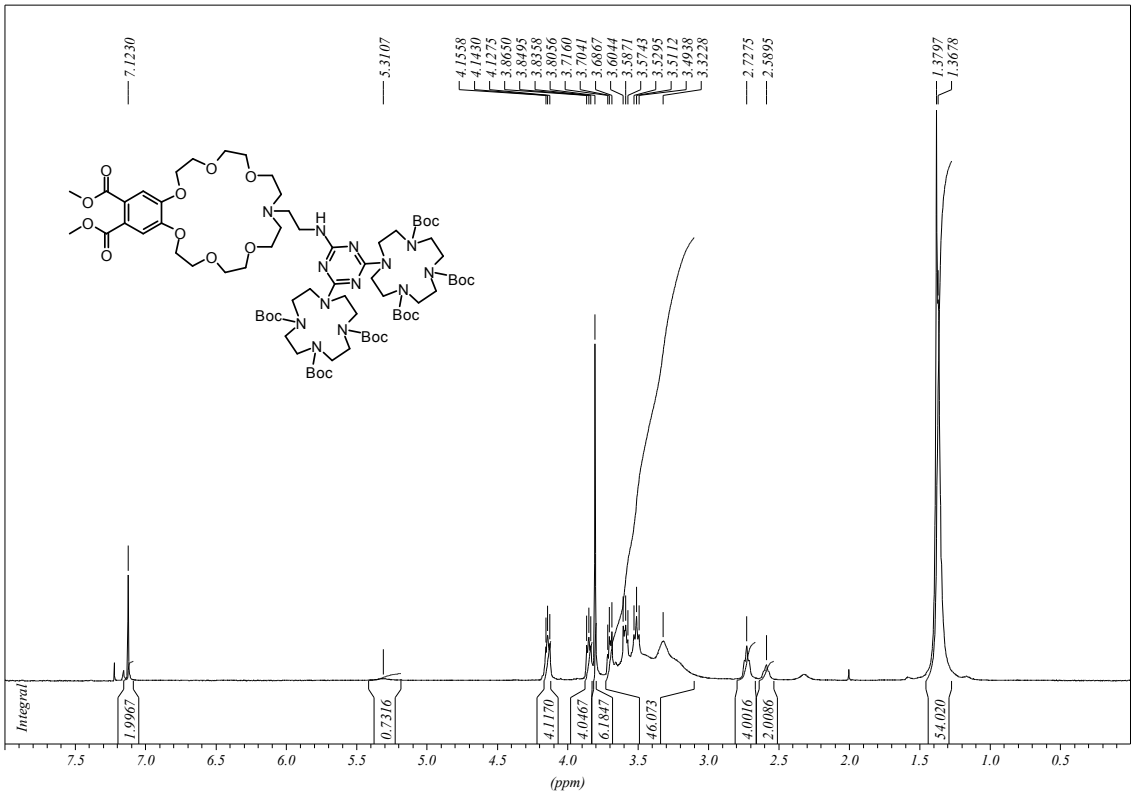


Figure 11: ^{1}H-NMR spectrum of compound **11a**

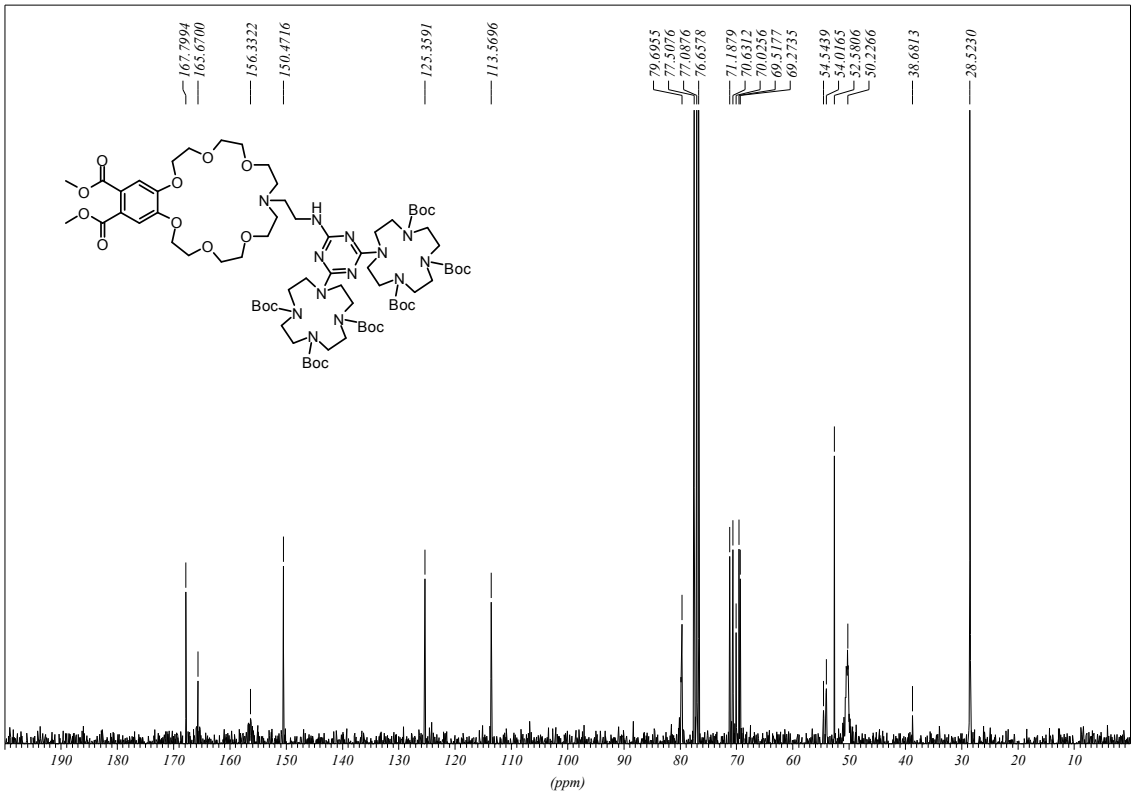


Figure 12: ^{13}C-NMR spectrum of compound **11a**

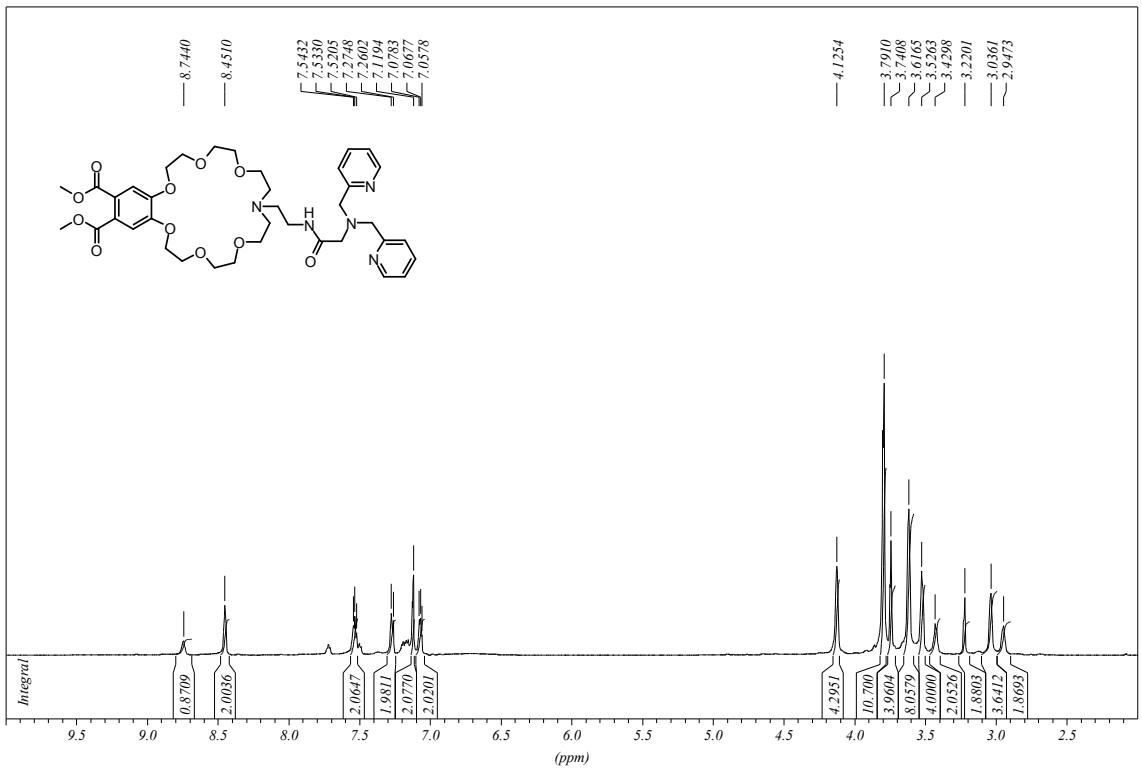


Figure 13: ^{1}H-NMR spectrum of compound **23**

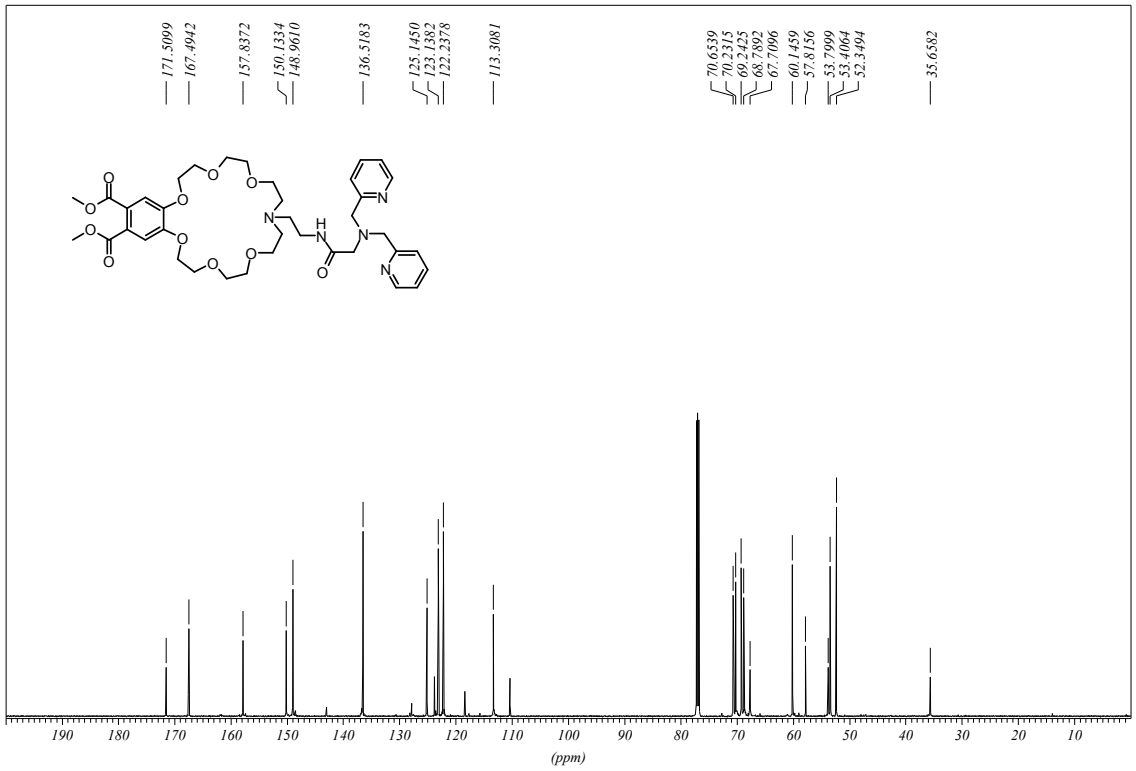


Figure 14: ^{13}C-NMR spectrum of compound **23**

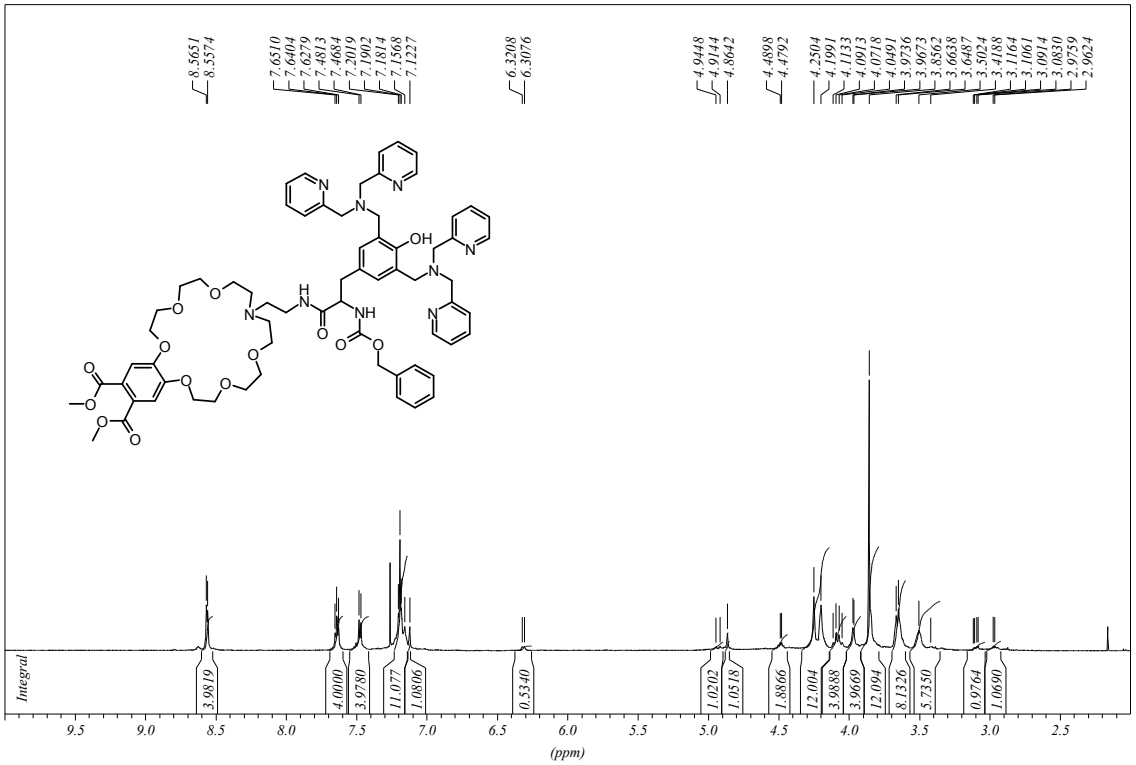


Figure 15: ^{1}H-NMR spectrum of compound **25**

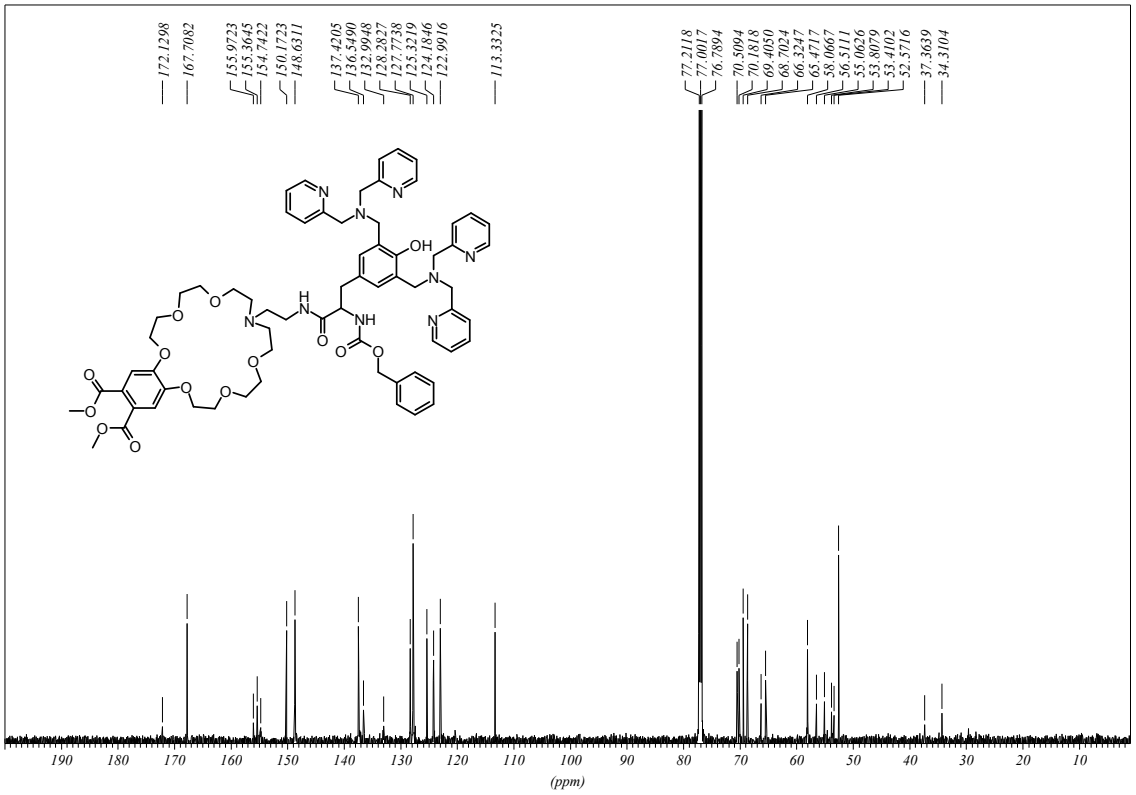


Figure 16: ^{13}C-NMR spectrum of compound **25**

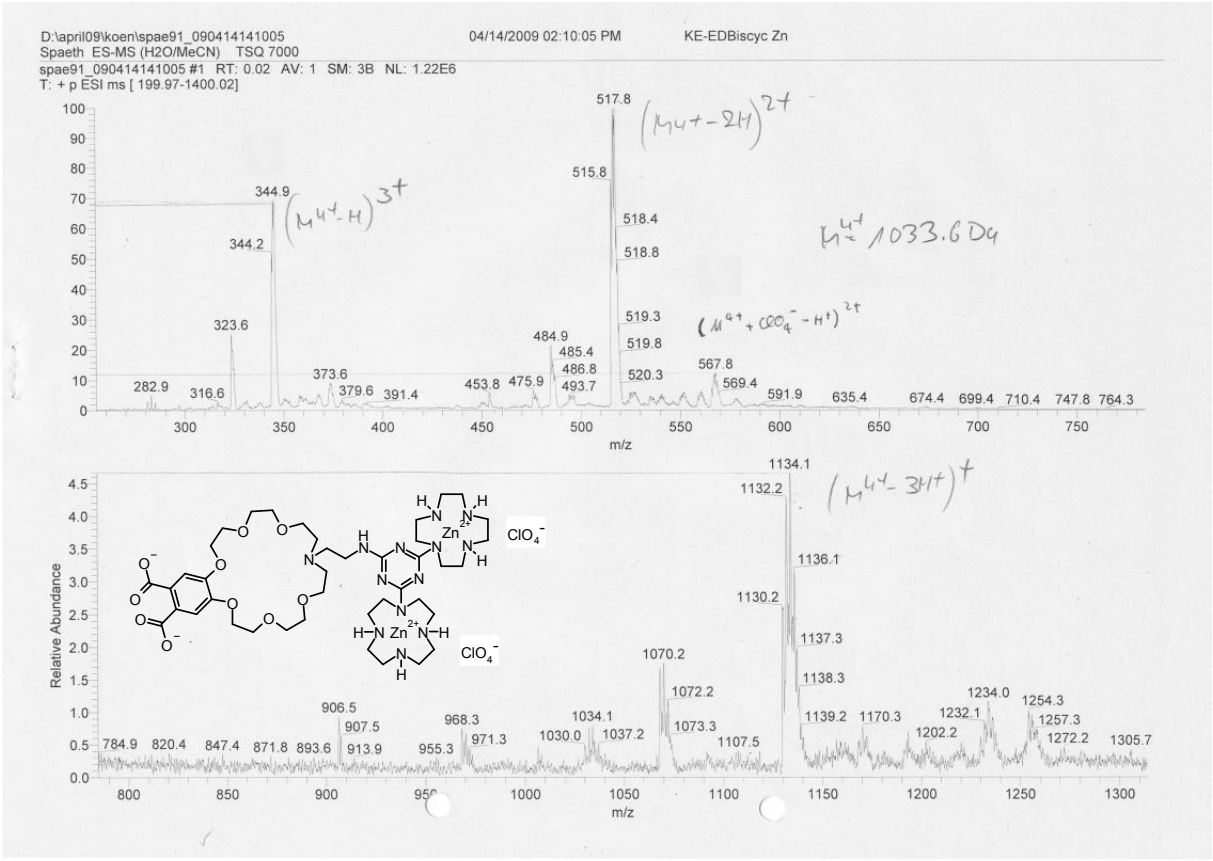


Figure 17: Mass spectrum of compound **3a**

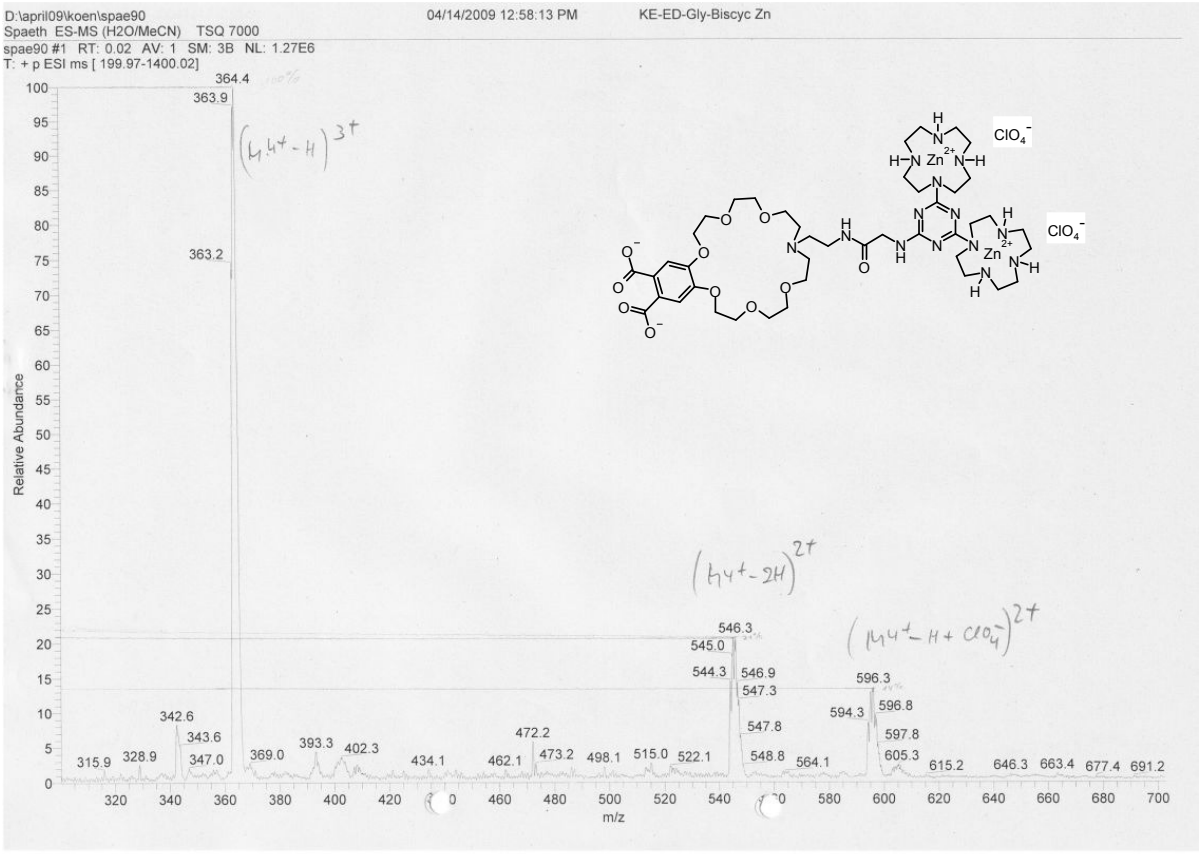


Figure 18: Mass spectrum of compound **3b**

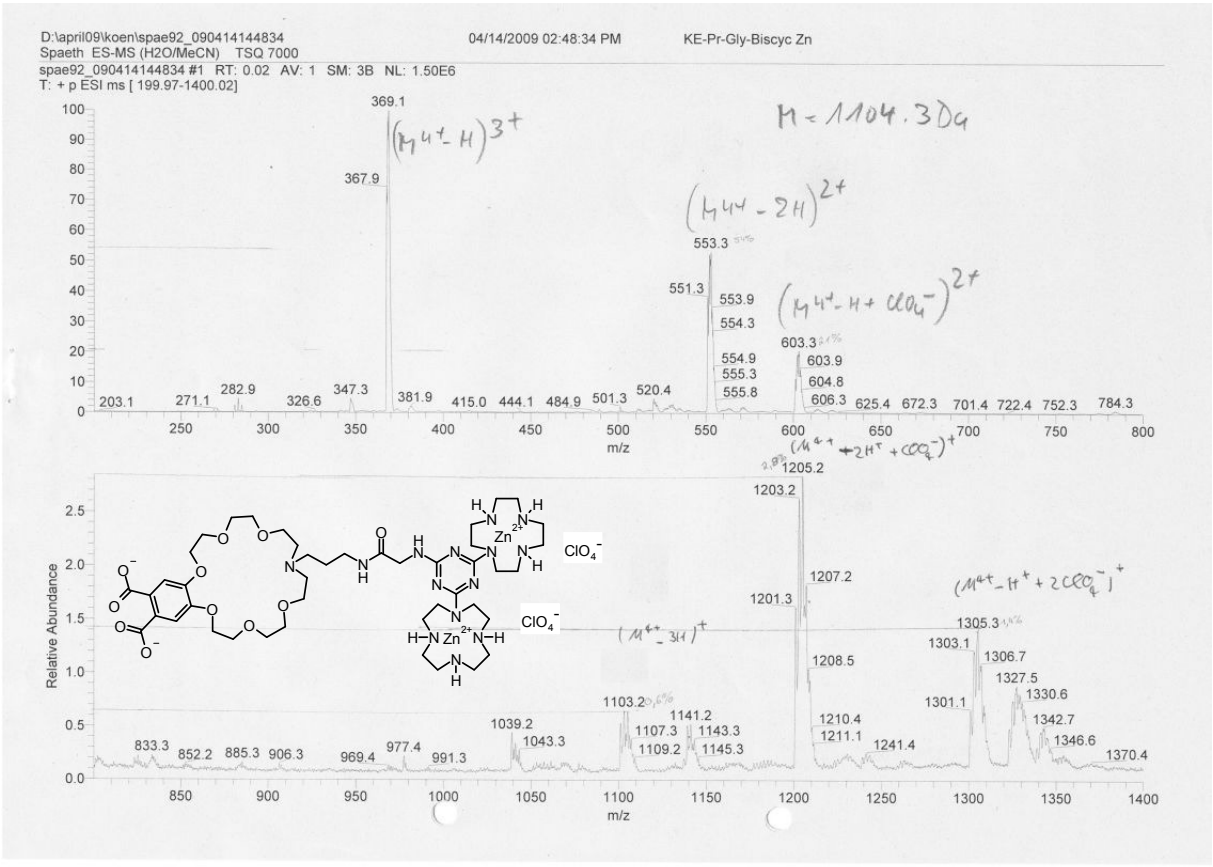


Figure 19: Mass spectrum of compound **3c**

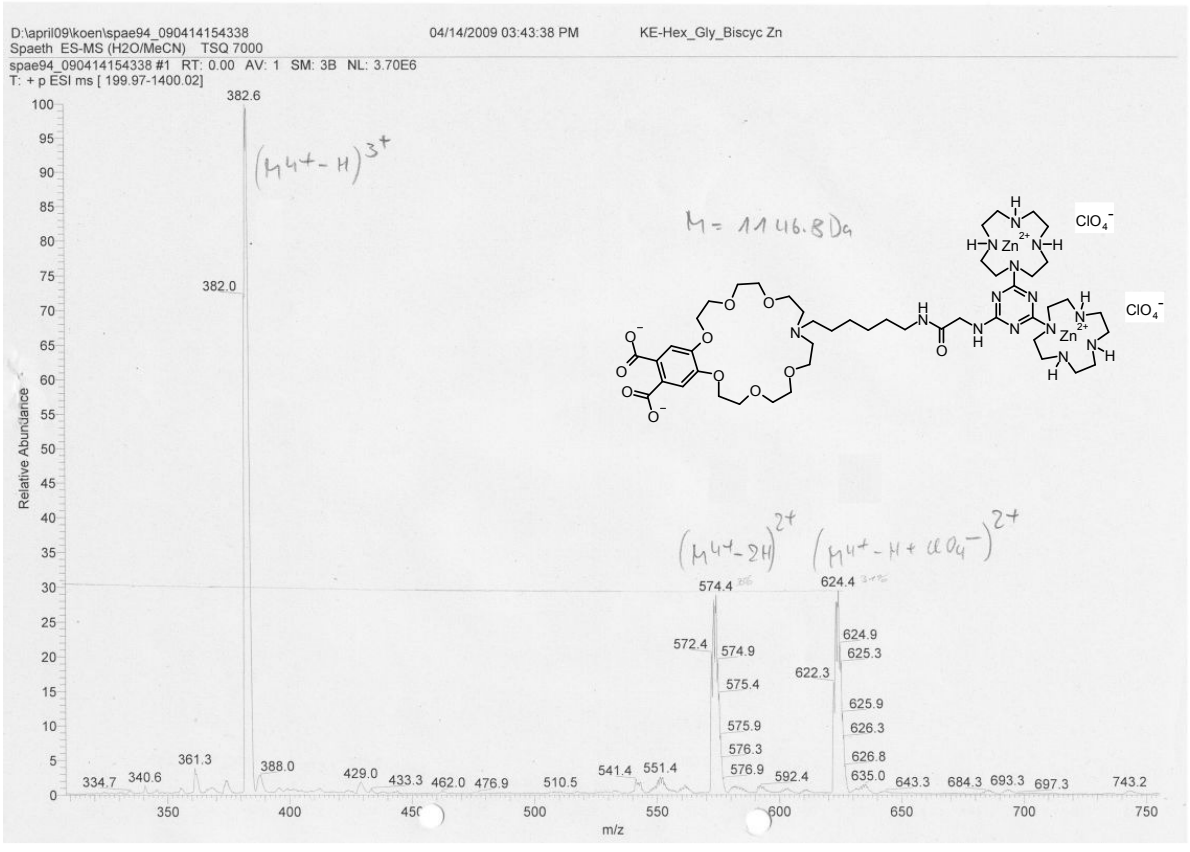


Figure 20: Mass spectrum of compound **3e**

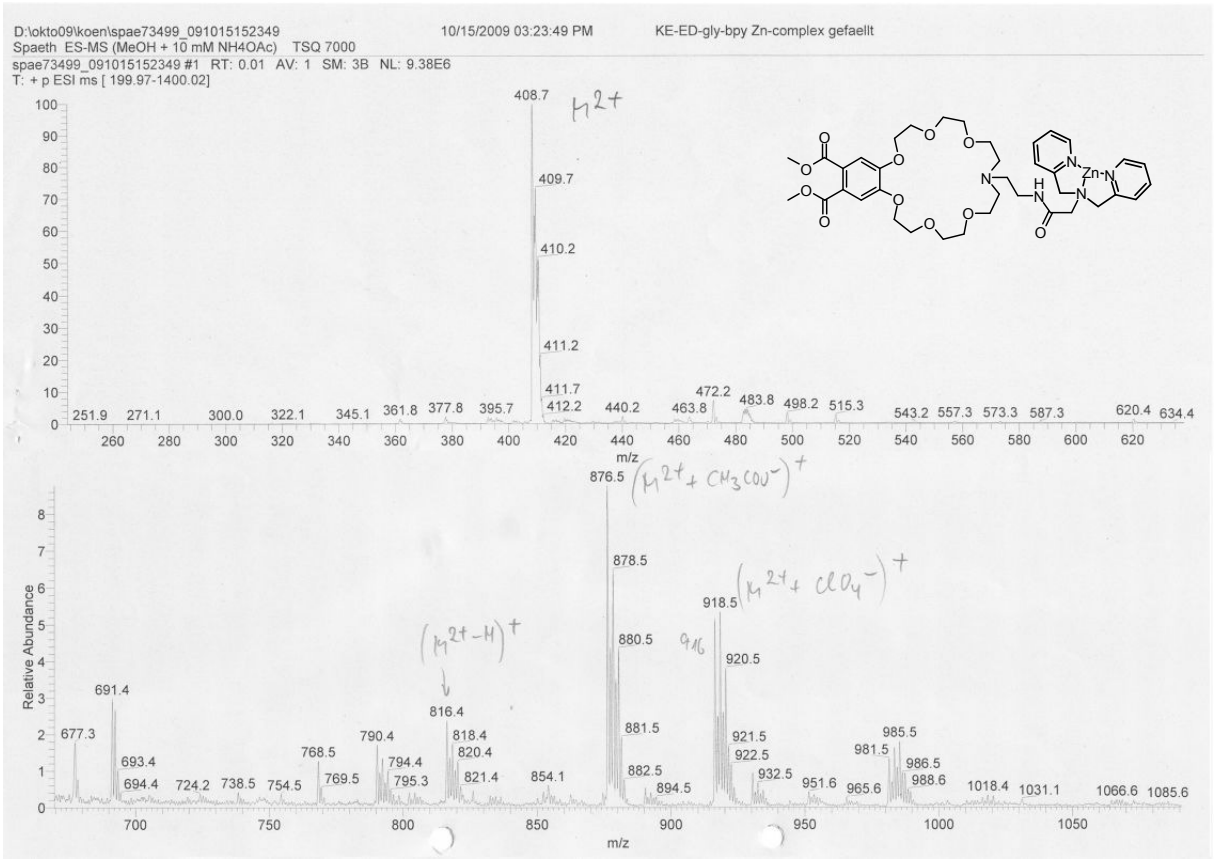


Figure 21: Mass spectrum of compound **6a**

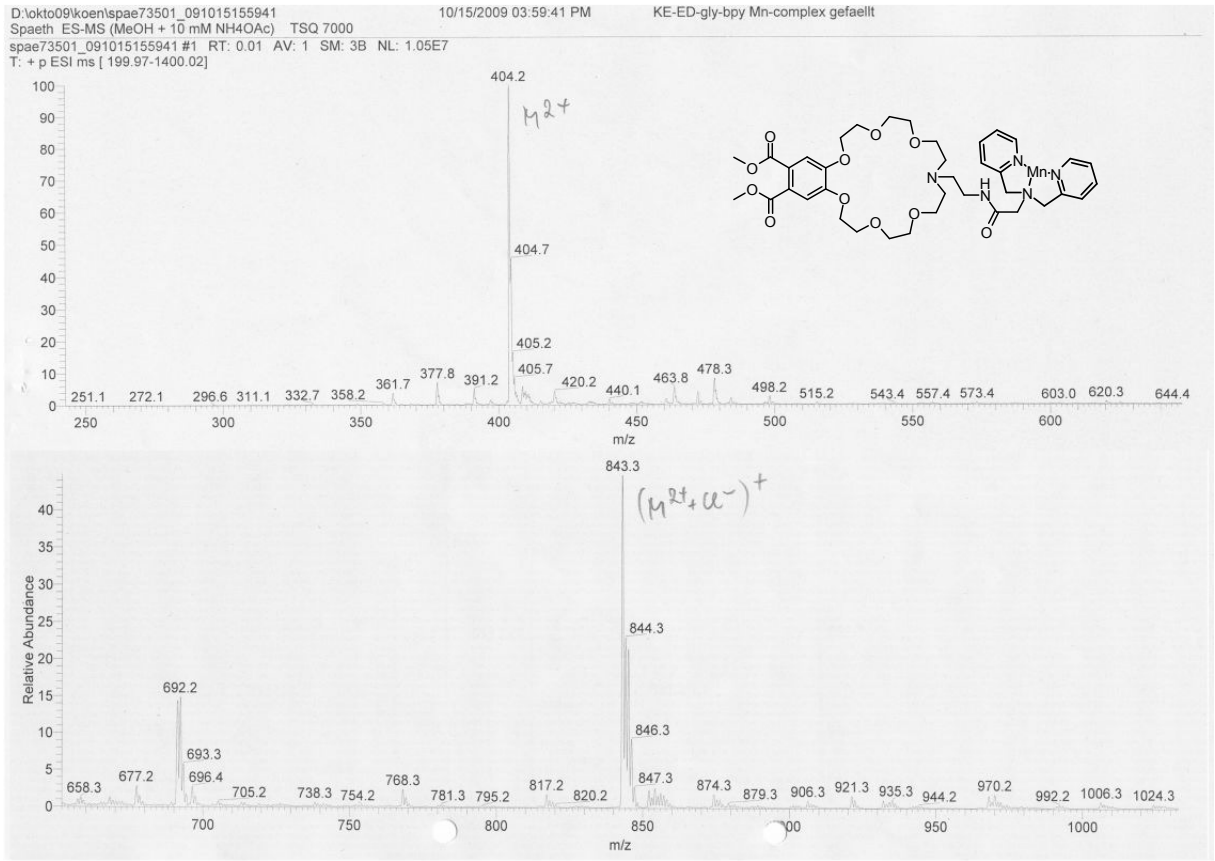


Figure 22: Mass spectrum of compound **6b**

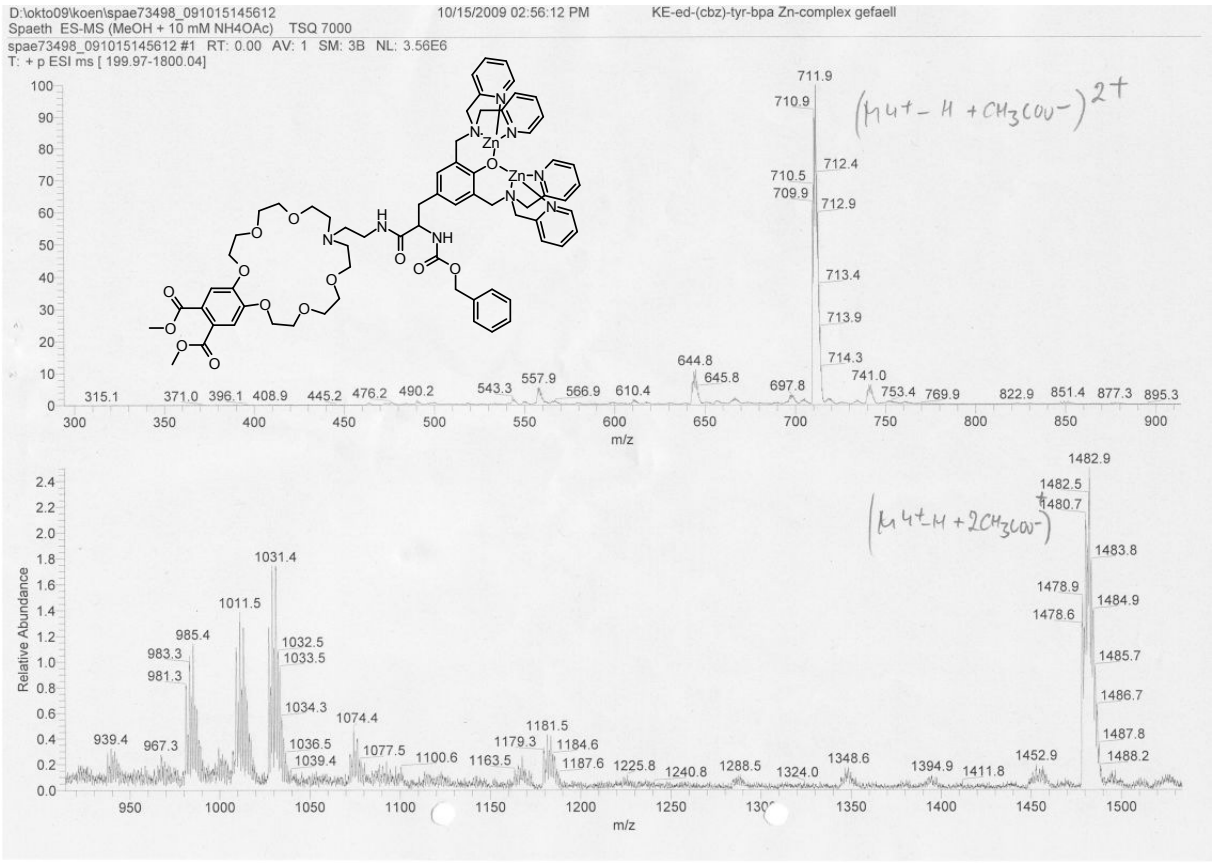


Figure 23: Mass spectrum of compound **4a**

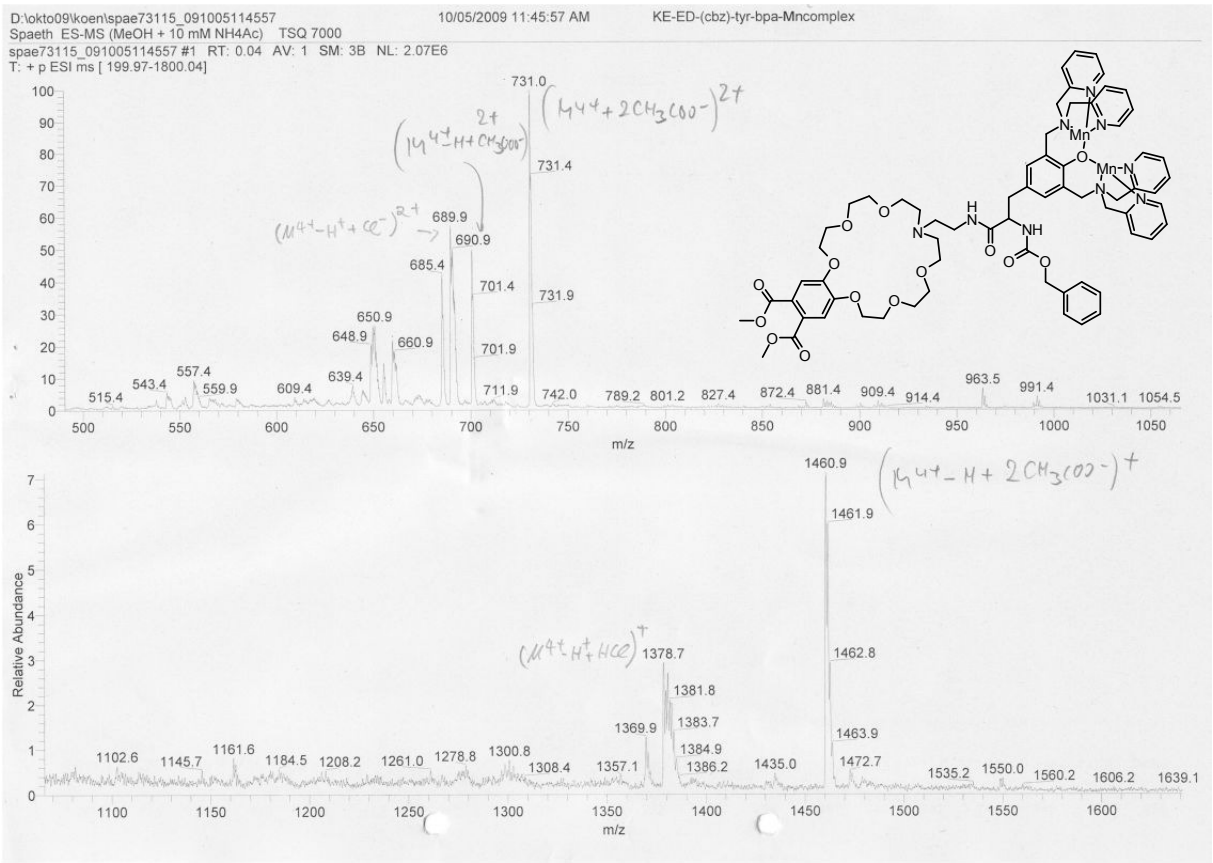


Figure 24: Mass spectrum of compound **4b**

V. 4.3. Fluorescence Screening, Absorption- and Emission Titrations

The fluorescence output was recorded in the wavelength range of 330 – 500 nm, below and above of this range no signal is detected. The excitation of the fluorophor is possible at 280 to 310 nm, below the nucleotide adenine or the amino acid tyrosine absorb light.

Screening of amino acid and peptide binding affinities. The *s*creenings were performed in black UV star well plates with 384 cells (130 µL volume per cell) in HEPES buffer (50 mM) at pH 7.4. To a $1*10^{-5}$ molar solution of a particular receptor compound twenty equivalents of peptide ($2*10^{-4}$ mol/L) or hundred equivalents of amino acid ($1*10^{-3}$ mol/L) were added (1:1 vol/vol). The mixtures were pipetted row by row, mixed with the aid of the pipette and allowed to equilibrate for 10 minutes. The fluorescence spectrum was recorded (λ_{ex} = 300 nm) and compared to diluted blank receptor solutions. All measurements were repeated twice.

Titration with nucleotides and phosphoserine in the well plate. The solution of the hosts ($5*10^{-5}$ mol/L) and guests ($5*10^{-5}$ mol/L) / ($1*10^{-3}$ mol/L) were prepared in aqueous HEPES-buffer (50 mM; pH = 7.4). The titrations were performed in black UV star well plates with 384 cells. Different small aliquots (0, 1, 2, 5, 8, 10, 12, 16, 20, 24 and 30 eq.) of the guest were added to the receptor. The final concentration of the receptor was $1.92*10^{-5}$ mol/L in every well. The luminescence output was plotted against the concentration of the guest and the binding constant was evaluated by non linear fitting methods.

Binding affinity titration with peptides in the cuvette. All binding studies were conducted in buffered aqueous solution (50 mM HEPES, pH 7.4, adjusted with Et_4NOH, no salt added) at a constant temperature of 298 K. The cuvette with 1.0 mL of the receptor ($2*10^{-5}$ mol/L) was titrated stepwise with small amounts (10 - 50 µL ≙ 0.1 – 1.0 eq.) of the peptide solution ($2*10^{-4}$ mol/L). After each addition the solution was allowed to equilibrate for 10 min before the fluorescence intensity (λ_{ex} = 300 nm) was recorded. The stoichiometry was determined by Job´s plot analysis extracted from titration data.[50] To determine the binding constant obtained fluorescence intensities were volume corrected, plotted against the concentration of peptide and evaluated by non linear fitting methods.

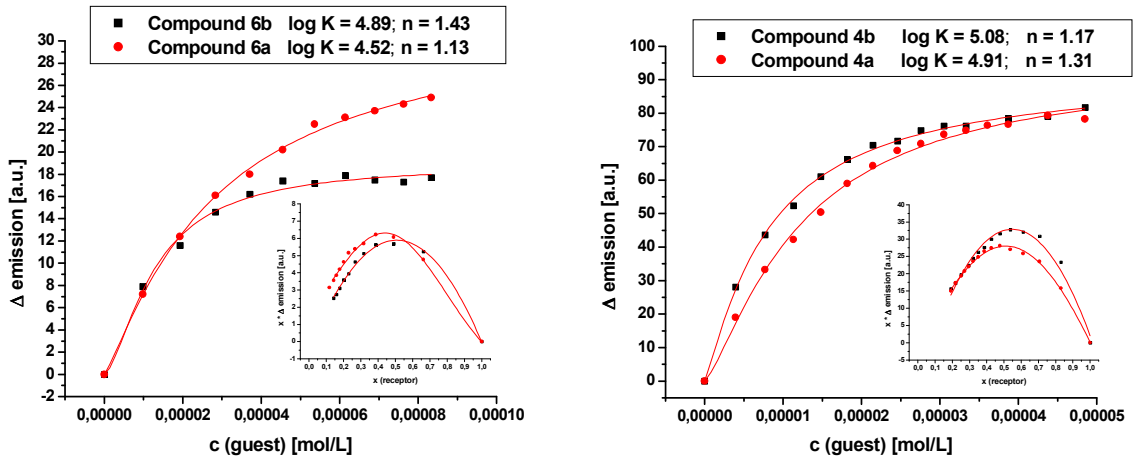


Figure 25: Fluorescence titrations of **2** versus **6a** or **6b** (left, $2*10^{-5}$ mol/L, λ_{ex} = 300 nm) and **4a** or **4b** (right, $2*10^{-5}$ mol/L, λ_{ex} = 300 nm); Small inserts: Job´s plots

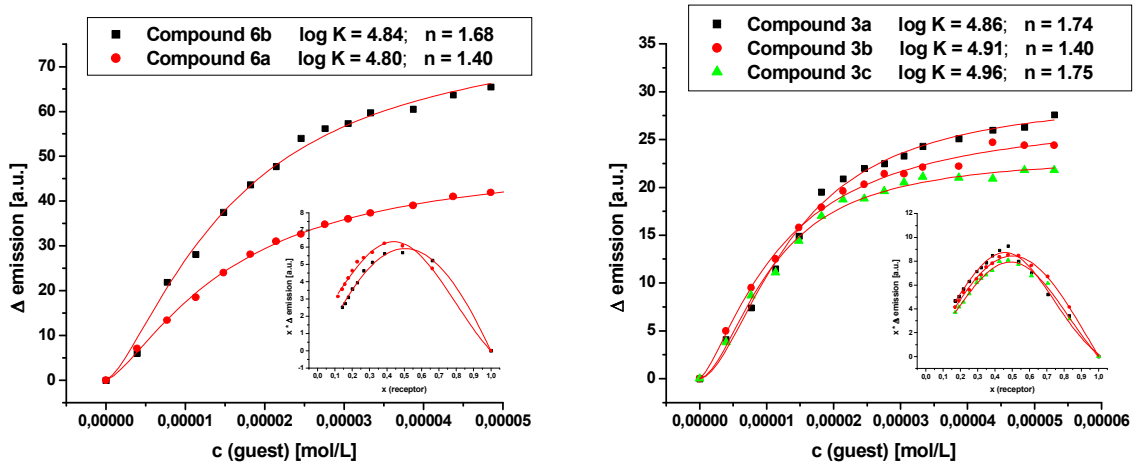


Figure 26: Fluorescence titrations of **1** versus **6a** or **6b** (left, $2*10^{-5}$ mol/L, λ_{ex} = 300 nm) and **3a** to **3c** (right, $2*10^{-5}$ mol/L, λ_{ex} = 300 nm); Small inserts: Job´s plots

All graphs and detailed non-linear fitting results can be found in the supporting information of this chapter.

V. 5. References and Notes

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[44] The amide can also be formed using TBTU and HOBt yielding the Boc-protected compounds in comparable yields. In synthesis with the glycyl glycine substituted building block, addition of NMP to the solution is crucial for achieving good yields. It breaks aggregates between the glycyl chains, which are known to cause the problems in oligo-glycine peptide coupling.

[45] If HCl in diethylether is used for Boc-cleavage, the product may precipitate so quickly that a second deprotection step is necessary to cleave all protecting groups. If traces of water are present, the methyl esters of the crown ether are quantitatively hydrolysed.

[46] It is known that zinc-cyclene complexes promote the hydrolysis of activated phosphate and carboxy esters, and even of certain peptide bonds. Typically the hydrolysis rate of carboxy esters at neutral pH is low. We suggest that the structure of the here described compounds promotes the intramolecular ester hydrolysis. Peptide bond hydrolysis: C. Bazzicalupi, A. Bencini, E. Berni, A. Bianchi, P. Fornasari, C. Giorgi, B. Valtancoli, *Eur. J. Inorg. Chem.* **2003**, 1974-1983. Carboxyester hydrolysis: M. Subat, K. Woinaroschy, S. Anthofer, B. Malterer, B. König, *Inorg. Chem.* **2007**, *46*, 4336-4356.

[47] The metal complex carrying one ester functionality was isolated with good yields from the reaction of the ligand with metal perchlorate salt in aqueous solutions. It was characterized by different analytical methods (^{1}H-NMR, ^{13}C-NMR, UV/Vis, IR, ESI). The diester is a minor byproduct (ESI-MS), which cannot be isolated, as it slowly converts in solution into the mono ester.

[48] All quantum yields were determined with quinine disulfate in 1 N H_2SO_4 as the reference compound (Φ = 0.546).

[49] All compounds show very similar photophysical properties: ε_{max} (λ) = 220 nm (27000 – 30000), 270 nm (7000 – 8500).

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[58] *Alternative procedure for the Boc deprotection with HCl in diethylether:* The respective starting material (0.1 mmol) was dissolved in 2 mL of dry dichloromethane and a saturated solution of hydrochloric acid in diethyl ether (0.4 mL) was added. After three hours of stirring at room temperature the HCl gas and the solvent was removed in a N_2 stream. The residue was taken up in a minimum amount of dichloromethane and the product was precipitated carefully by slow addition of diethyl ether. The solution was decanted off the precipitate, it was washed once with diethyl ether, decanted off again and the product was dried in the vacuum to furnish a hygroscopic powder. *Caution! Anhydrous conditions have to be maintained, else the ligands will decompose!*

[59] The high nitrogen content prohibited obtaining accurate elemental analysis. The high molecular mass and the highly charged structure did not allow for HRMS.

VI. Synthesis and Binding Properties of Guanidinium Bis-carboxylates[i]

The ammonium ion binding site of the enzyme glutaminase HisF inspired us to design guanidinium bis-carboxylates as potential self-organized ionophores in molecular recognition. The synthesis of the title compounds based on aliphatic and aromatic building blocks, along with a general method for preparation of the δ-aminoethoxy acetic acid building blocks are presented in this chapter.[ii] The fundamental properties were investigated by fluorescence spectroscopy and NMR methods.

[i] A. Späth, N. Gonschor, B. König, *Supramol. Chem.* **2010**, accepted.

[ii] These Syntheses were performed in collaboration with N. Gonschor; Bachelor Thesis, University of Regensburg, **2009**.

VI. 1. Introduction

Ionophores find applications in many fields of chemistry[1,2,3,4] including medical diagnostics.[5,6,7] Their pre-organization is of key importance achieving high binding affinity and selectivity as supramolecular binding enthalpies are typically small and disfavourable entropic effects of binding should be minimized.[8] The classical approach is the use of macrocycles, such as crown ethers, but clefts, [9,10] tripods[11] or tweezers[12] can be valuable alternatives. Tweezer ligands are pincer-like molecules carrying two side arms arranged in the form of a forceps, which can complement a particular guest by specific interactions. Many different examples like **1**[13], **2**[14], **3**[15], **4**[16] or **5**[17] with a rigid core and flexible side arms are known (Figure 1). Such clefts or tweezer-type molecules have been used for the recognition of carboxylate,[18] guanidinium[19] and metal ions,[20] amino acids[21,22] and ion pairs.[23]

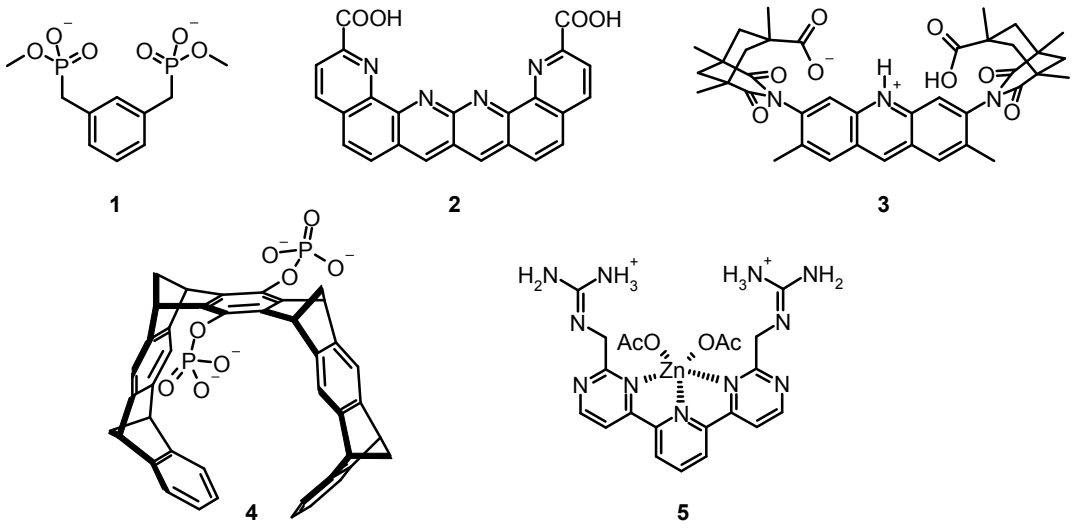
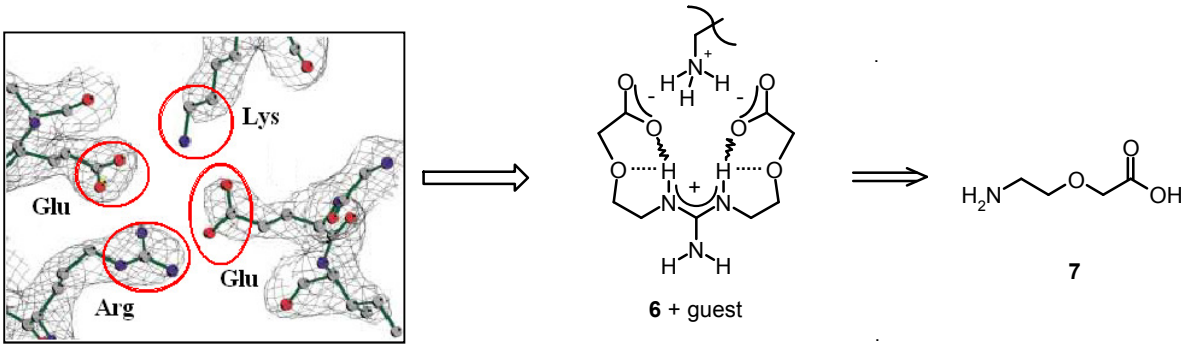


Figure 1: Examples of bifunctional synthetic receptors with tweezers or cleft structures

In the bottom region of the glutaminase subunit HisF two glutamic acid functionalities and one arginine side chain form a hydrogen bond network with the ammonium ion of a lysine side chain[24] in the centre of a stabilizing αβ-eight barrel.[25] Inspired by this, we derived a guanidinium bis-carboxylate **6** as a minimal tweezer-type structure for potential ammonium ion binding. Intramolecular hydrogen bonds should pre-organize the structure and expose the carboxylate groups in close proximity as potential cation binding site. Molecular modelling (DFT, BLYP 6-G-31*) confirmed that the structure is a stable conformer (Figure 2).



Scheme 1: Bottom region of HisF (modified illustration from ref. 25) and the derived simplified tweezer-type binding motif **6** available from glycol-δ-amino acid **7**

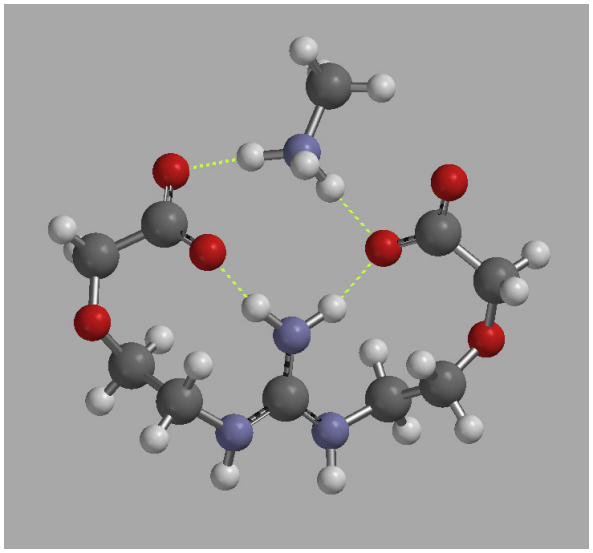


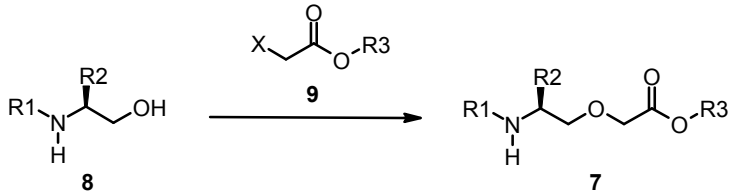
Figure 2: Optimized geometry of compound **6** in the presence of a methylammonium ion (DFT, BYPL 6-G-31*)

Unnatural amino acids like **7** are a suitable starting material for the synthesis of **6**. The parent aminoethoxy acetic acid[26] is commercially available, but substituted derivatives have not been reported. We describe the preparation of derivatives of **7** and their conversion into **6**. The ammonium ion binding of **6**, dynamics and fundamental properties were investigated by NMR and emission titration.

VI. 2. Results and Discussion

VI. 2.1. Syntheses

N-Protected 1,2-amino alcohols **8** were prepared by standard conditions and used as starting materials. Substituted aminoalcohols (**8**) were prepared by reduction of the corresponding amino acid methyl esters[27] with sodium boranate in dry THF.[28] The indole of tryptophanol was protected[29] by the orthogonal Cbz group to avoid formation of side products. Ether synthesis with bromo acetic esters **9** proceeds in moderate to good yields and the copper-mediated decomposition of azoesters[30] is a feasible alternative route to prepare derivatives of **7**.[31,32] Table 1 summarises the results.



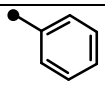
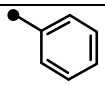
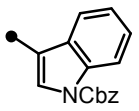
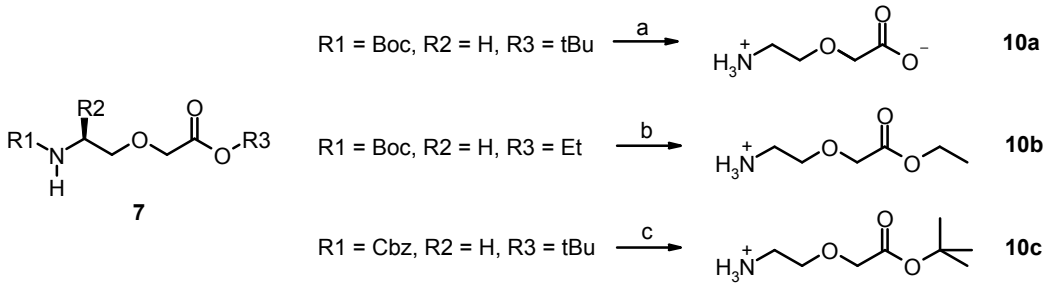
Entry	Amino alcohol **8**		Acetic ester **9**		Conditions	Yield of **7**
	R^1	R^2	R^3	X		[%]
1	Boc	H	tBu	Br	*a*	**7a)** 83
2	Boc	H	Et	Br	*b*	**7b)** 59
3	Boc	H	Et	N_2	*c*	**7b)** 78
4	Cbz	H	tBu	Br	*a*	**7c)** 87
5	Cbz	H	Et	N_2	*c*	**7d)** 61
6	Boc		tBu	Br	*a*	**7e)** 77
7	Boc		Et	Br	*b*	**7f)** 52
8	Boc		tBu	Br	*a*	**7g)** 89
9	Boc		Et	Br	*b*	**7h)** 50
10	Boc		tBu	Br	*a*	**7i)** 63

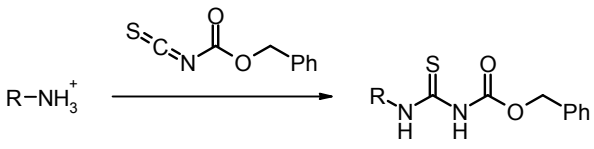
Table 1: Reaction partners and yields for preparation of δ-aminoethoxyacetic acid esters; *conditions: a) $Bu_4N^+HSO_4^-$, DCM, H_2O, NaOH, RT, over night; b) NaH, THF (dry), RT, 3 h; c) $Cu(OTf)_2$, PhHN-NH_2, TMEDA, DCM (dry, degassed), N_2, 0°C → RT, 8 h.*

Deprotection by standard conditions furnishes the according products in excellent yields, for example:



Scheme 2: Deprotection of differently protected glycol-δ-amino acids; *conditions: a) MeOH or THF or acetone, HCl 1N, RT, 5 h, quant.; b) DCM, TFA or HCl in Et_2O, RT, 2 - 4 h, 93 – 98 %; c) MeOH, HOAc, 10 bar H_2, Pd/C, RT, over night, 94 %.*

The materials are the side arms for the 1,3-bis-substituted-guanidines prepared in a two step procedure via the Cbz-protected thioureas. The versatility of benzyloxycarbonyl-isothiocyanate (Cbz-NCS)[33] or ethoxycarbonyl-isothiocyanate[34] has been demonstrated. Having tested several reactions for thiourea synthesis,[35] Cbz-NCS[36] **11** was used in our case, as the reaction to yield a thiourea proceeds rapid and in high yield.[37] Representative examples of amino esters **10** which were converted by **11** into the corresponding Cbz-thioureas in good to excellent yields are summarized in Table 2.[38]



10 **11** **12 - 16**

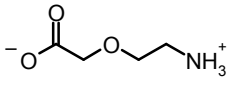
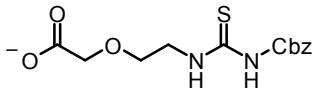
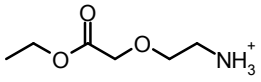
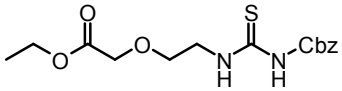
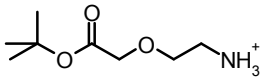
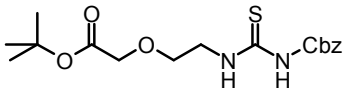
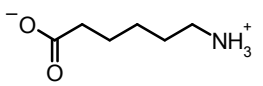
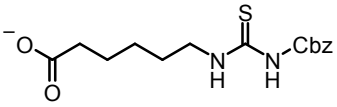
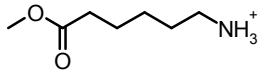
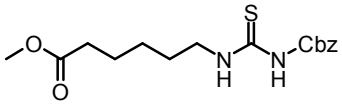
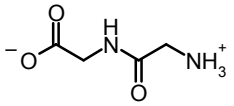
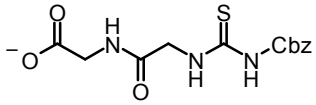
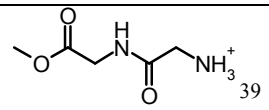
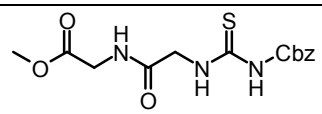
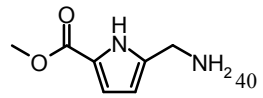
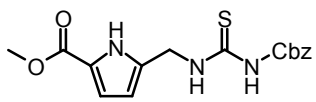
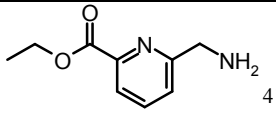
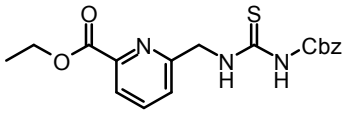
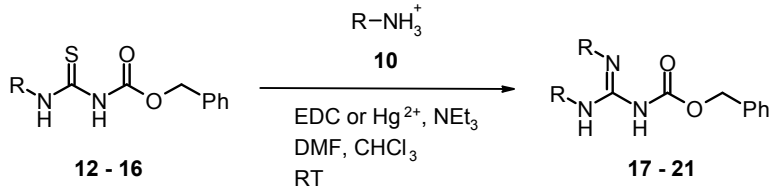
Entry	Amine compound	Conditions	Cbz-thiourea	Yield [%]
1		*a*	 **(12a)**	84
2		*b, 2 h*	 **(12b)**	91
3		*b, 2 h*	 **(12c)**	89
4		*a*	 **(13a)**	81
5		*b, 2 h*	 **(13b)**	83
6		*a*	 **(14a)**	78
7	 [39]	*b, 3 h*	 **(14b)**	84
8	 [40]	*b, 2 h*	 **(15)**	92
9	 [41]	*b, 2 h*	 **(16)**	81

Table 2: Conversion of amines with benzoyloxycarbonyl-isothiocyanate to their Cbz-isothioureas, *conditions: a) Dioxane, H_2O, NaOH, Cbz-NCS, 12 h, RT; b) DCM, NEt_3, Cbz-NCS, 2 – 3 h, RT.*

Pyrrole (entry 8) rigidifies the structure of the later ligand and can donate an additional hydrogen bond by each side arm.[42] Pyridine derivatives (entry 9) can be used with benefit as

substituent in tweezer structures:[43] The rigid aromatic ring, in combination with a meta-substituent may induce to a good pre-organisation of binding sites in the final ligand.

The next conversion step to the symmetric Cbz-guanidine moiety uses the esters to facilitate the product purification.[44] This step can be performed in DMF, with NEt_3 as base, using either mercury(II)-chloride[45] or EDC[46] as an activating agent for the thiourea, again observing good yields. All conversions and their corresponding yields are given in table 3:[36] Thiourea **16** cannot be converted into the corresponding twofold substituted guanidine, instead it yields by intramolecular reaction the cyclic guanidine[47] **21** as the only isolable product. This imidazo[1,5-a]pyridine derivative is a new unnatural amino acid with syn pre-organized residues and interesting spectroscopic properties (see the supporting information of this chapter).



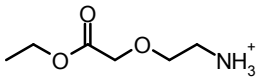
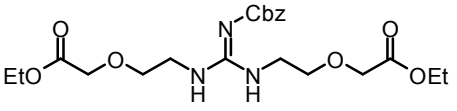
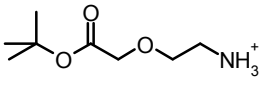
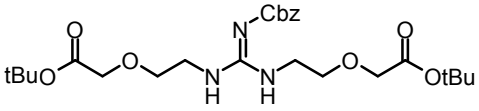
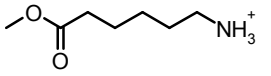
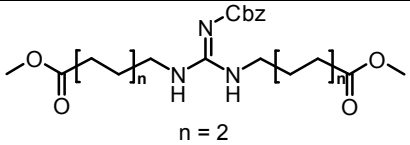
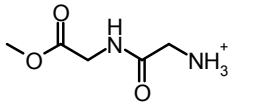
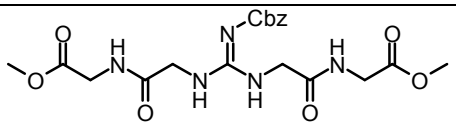
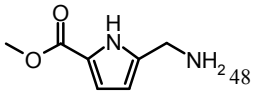
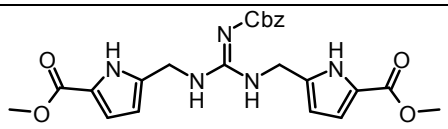
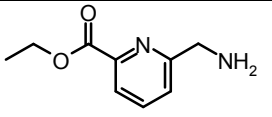
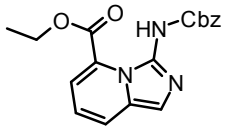
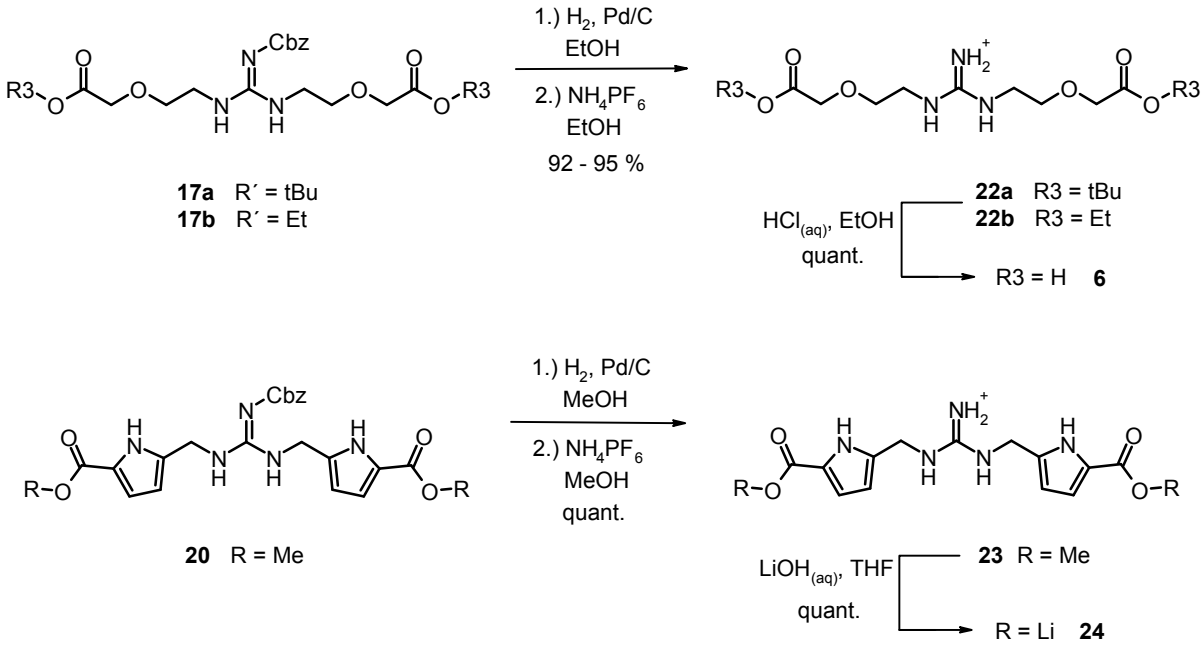
Entry	Amine compound	Cbz-thiourea	Cbz-guanidine		Yield [%]
1		**(12b)**		**(17b)**	73
2		**(12c)**		**(17a)**	78
3		**(13b)**		**(18)**	78
4		**(14b)**		**(19)**	76
5		**(15)**		**(20)**	87
6		**(16)**		**(21)**	66

Table 3: Preparation of 1,3-symmetrically substituted guanidines, *conditions: DMF, $CHCl_3$, NEt_3, EDC or $HgCl_2$, RT, over night*.

Alternatively, symmetrically substituted guanidines can be prepared via their according thiourea, reacting the amine compound and thiophosgene,[50] followed by guanidilation with trifluoroacetamide.[51] (see the supporting information for details)

The Cbz protection group was removed by hydrogenation using 10 % Pd on charcoal at 30 bar of hydrogen pressure. The guanidinium esters (**22 a/b** and **23**) are isolated as their hexafluorophosphate salts. Basic or acidic ester cleavage gives the deprotected tweezers **6** and **24** in excellent overall yields.[52]



Scheme 3: Deprotection of the Cbz-guanidines followed by ester cleavage

VI. 2.2. Fundamental Properties of the Tweezers and Pre-studies

Carboxy guanidinium tweezers exist as zwitterionic structures over a wide range of pH. The pK_a values of their guanidinium functionality are 12 and 11.5 for **6** and **24**, respectively. This is in good accordance to the value for the arginine side chain (pK_a = 12.5).[53] The acid functionalities in **6** show a pK_a value of 3.5, the carboxy groups in **24** of 4 (for pK_a determination experiments and simulations of fundamental properties see the supporting information). By the large differences to the values of amino acids (pK_a´s being typically 2 - 3

(-COOH) and 9 - 11 (-NH_3^+)[54]) transprotonation processes with these guest molecules are not expected: Similar values are observed for the according functional groups in *n*-butylammonium chloride[55] and tetrabutylammonium acetate[56]. Comparable relationships are valid for DMSO as solvent.[57]

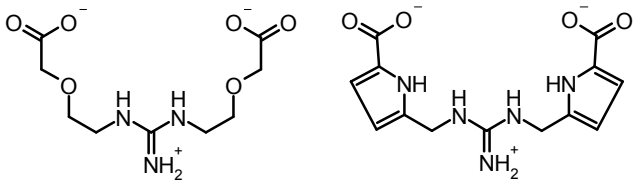


Figure 3: Zwitterionic tweezers

VI. 2.1.1. Photophysical Properties

Compounds **15, 20, 23** and **24** show absorption maxima in methanol at 270 nm and emit upon excitation at 340 nm with a quantum yield of about $\phi = 0.1$.[58] The absorption and emission properties are only marginally affected by the protection groups.

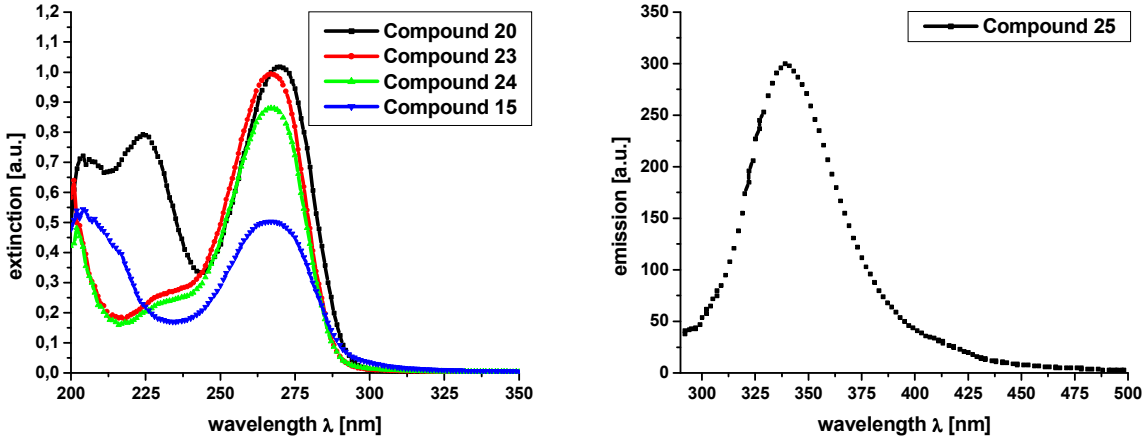


Figure 4: Absorption spectra of compounds **15**, **20**, **23** and **24** (c = 8*10^{-5} mol/L) and the emission spectrum of compound **24** (c = 4*10^{-5} mol/L) in methanol

VI. 2.1.2. Binding Properties of 22b and 23 versus Carboxylate Anions

The carboxylate binding properties of the guanidinium part were investigated separately with **23** by fluorescence titrations with tetrabutylammonium acetate in methanol and methanol / water (4:1 vol/vol) and glycine in methanol / water (4:1 vol/vol). Compounds **22b** and **23** were investigated in DMSO via NMR titration methods with tetrabutylammonium acetate as guest. The guanidines **22b** and **23** were employed as their hexafluorophosphate salts for all measurements.

A binding constant of $1.1*10^4$ M^{-1} in methanol and $4.2*10^3$ M^{-1} in methanol / water 4:1 is observed for **23**. The stoichiometry of all binding processes is 1:1 as determined by Job`s plot analysis. The value found by NMR titration in DMSO amounts $1.3*10^3$ M^{-1}, which is in good accordance with literature values for tetrabutylammonium acetate binding by alkylguanidines.[59]

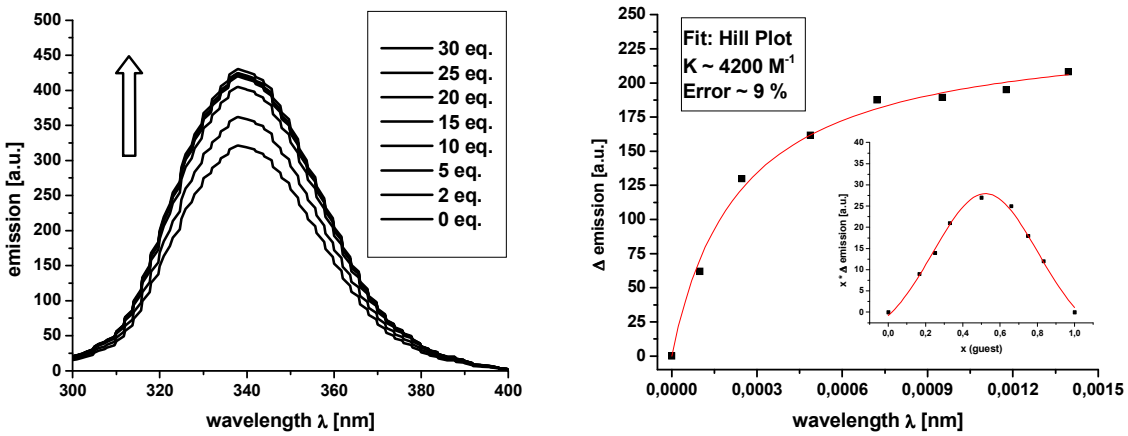


Figure 5: Fluorescence spectrum of **23** ($3*10^{-5}$ molar, λ_{ex} = 270 nm) at different ratios of tetrabutylammonium acetate in methanol / water 4:1; insert: Job´s plot

For **22b** an even higher binding constant of $1*10^4$ M^{-1} in DMSO was found by NMR titration.[60]

VI. 2.1.3. Aggregation and Dynamic Behaviour

The carboxylate ion of the zwitterionic compounds **6** and **24** can bind to the guanidinium centre of the tweezer. As expected from our initial modelling studies this should pre-organize the structure for ammonium ion recognition. We investigated the dynamic behaviour by

temperature dependent NMR of **24** in DMSO-d6 and by comparison of the NMR spectra in different solvents, which stabilize aggregation ($CDCl_3$-DMSO-d6 4:1/ DMSO-d6) differently strong or interfere with the aggregates (D_2O).

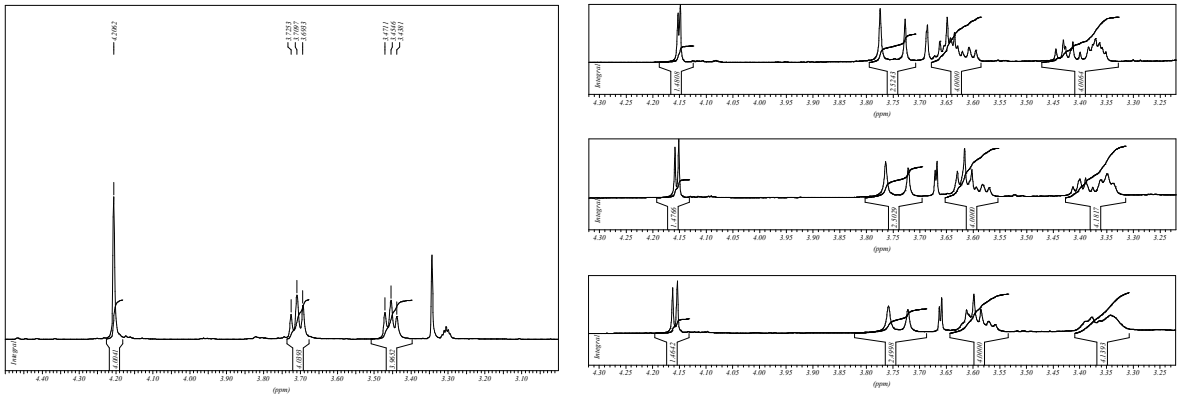


Figure 6: NMR spectrum of **6** in fully protonated form in CD_3OD (left), NMR spectra of the zwitterion form in DMSO-d6 at 333 K, 353 K and 393 K

Upon deprotonation of the acids – in the zwitterionic form – compound **6** can participate in guanidinium-carboxylate interaction forming aggregates with itself or other molecules, resulting in a more complicated NMR spectrum (compare fig. 6 left/right). Temperature dependent NMR measurements indicate an asymmetric, highly dynamic structure: In the spectrum at 333 K most signals overlap or are broadened, indicating exchange processes, which are fast on the NMR time scale ("Flipping").[61] By rising the temperature to 393 K a more defined spectrum results, but it is also not corresponding to C2-symmetric structure. The fact that coalescence can not be reached at the 100 K higher temperature indicates a strong interaction between carboxylate and guanidinium group. In the 2D-NOESY spectrum a weak coupling between one methylene-group directly at the guanidinium centre and the methylene-group located in α-position to the carboxyl function was found (see the supporting information). DFT calculation also indicated a strong intramolecular H-bond and confirmed the interpretation of the signal splitting in the NMR spectrum (fig. 7).

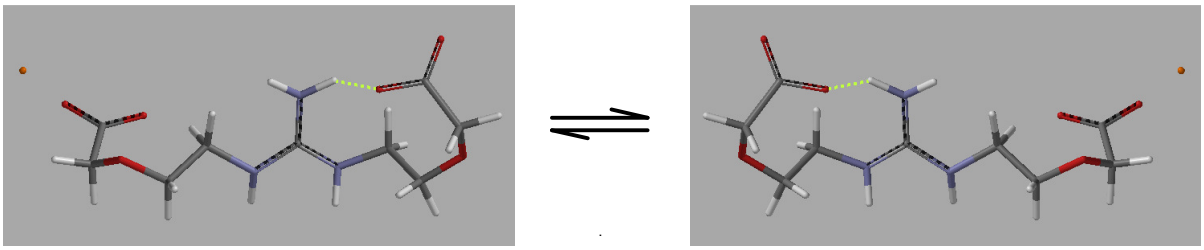


Figure 7: Dynamic flipping of compound **6** simulated in the gas phase (DFT, BYPL 6-G-31*); *nitrogen in violet, oxygen in red, lithium in orange, hydrogen in white colour*

A NMR spectrum recorded in water is similar in signal splitting with the spectrum recorded in DMSO at 393 K, the spectrum in chloroform / DMSO shows a similar signal splitting and broadened peaks as observed in DMSO below 333 K:

The structure of **6** is highly flexible and forms multiple aggregates in unpolar solution, which break at elevated temperature or in the highly competitive water. Only one strong intramolecular guanidinium-carboxylate interaction can remain. The intramolecular H-bond interaction is assisted by the charge attraction and is therefore much more stabilised than interaction of the functional groups with the uncharged water.[62] The second arm of the molecule is unbound and can coordinate a guest molecule. Nevertheless, it can be hardly predicted what happens if a guest like the ammonium ion is added. This has to be investigated separately. Structure **24** gives an interesting impression: The Li ion (orange) is pinched between both carboxylates in the modelling studies: At least one H-bond and intramolecular aggregation was also found by DFT methods, but no signal splitting can be observed in the NMR spectra of the molecule in zwitterionic form. Only the resonance for the bridging methylene groups located between guanidinium group and pyrrole ring is broadened in comparison to the ester **23**. This may be due to the flipping being fast on the NMR time scale.

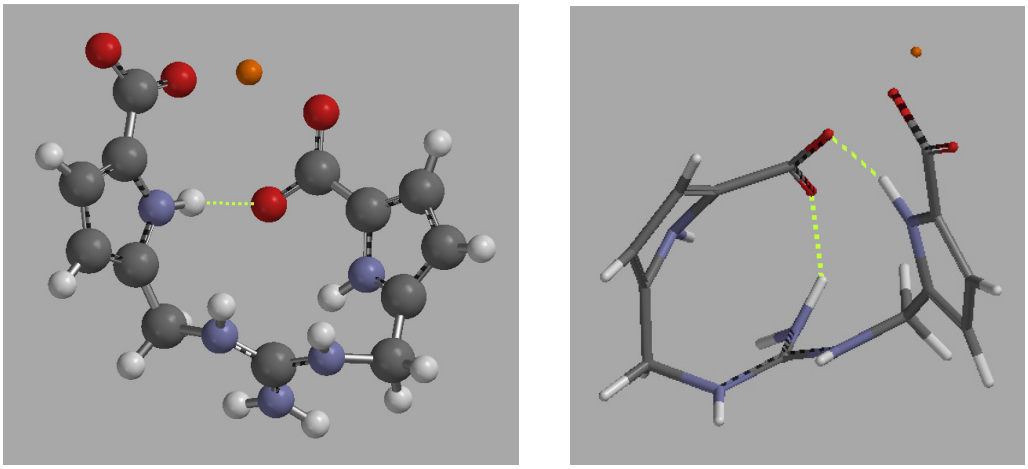


Figure 8: Possible intramolecular association of compound **25** simulated in the gas phase (DFT, BYPL 6-G-31*)

Amino acids or acetate will participate in the equilibrium and may form defined aggregates with the ligands.

VI. 2.3. Binding Properties of the Tweezers 6 and 24

VI. 2.3.1. Screening Studies: Amino Acid Guests and Concurrent Ions

The interaction of selected guests with the tweezer compounds equilibrium was screened with **24** and amino acids of different length, namely glycine, β-alanine, λ-aminobutyric acid (GABA) and ε-aminohexanoic acid (AHX), and the small peptides glycyl glycine and $(Gly)_3$. Sodium perchlorate and potassium isothiocyanate were employed to investigate the response to alkali metals and the effect of strong polarity increase. Methanol / water (4:1 vol/vol, at pH 6.5 - 7) was used; the guest was added in large excess (1000 eq.). Tetrabutylammonium acetate served as standard in the screening.

No binding event is observed with sodium or potassium ions, maybe due to the charge repulsion of the guanidinium group. Potassium needs an octahedral coordination for good binding,[63] which can not be supplied by these tweezers. Only with glycine, β-alanine and λ-aminobutyric acid **24** showed a weak response (F/F_0 = 1.1 – 1.2), the other guests were not bound under these conditions ($F = F_0$). Tetrabutylammonium acetate clearly induces the strongest luminescence enhancement (F/F_0 = 1.4).

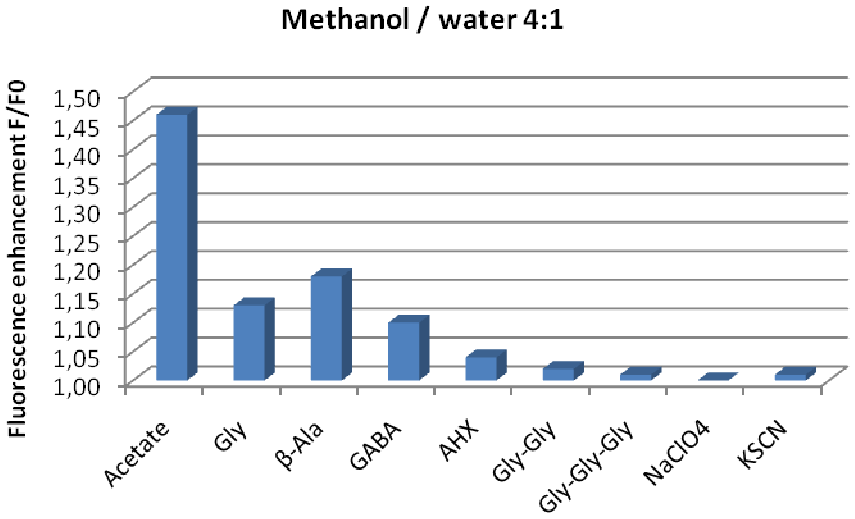


Figure 9: Selectivities and fluorescence enhancement factors (F/F_0) of compound **24** with selected guests; *conditions: [receptor] = 3*10^{-5} M, λ_{ex} = 270 nm, λ_{em} = 340 nm, the errors are estimated as ~ 10 %; [guest] = 3*10^{-2} M.*

VI. 2.3.2. Binding Constants of the Tweezers 6 and 24

The binding abilities of the tweezers versus an ammonium guest - *n*-butylammonium chloride - and the amino acids glycine, β-alanine and λ-aminobutyric acid, were investigated in succession and compared to their carboxylate binding properties. Receptor **6** and **24** were titrated with tetrabutylammonium acetate and *n*-butylammonium chloride in DMSO, monitored by ^{1}H-NMR spectroscopy. The binding constants for both guests and the amino acids were determined with **24** by fluorescence spectroscopy in methanol / water 4:1 at pH 6.5 - 7. Additionally and for better comparison the values for binding tetrabutylammonium acetate and *n*-butylammonium chloride with this receptor were investigated by the same method in methanol. Table 4 summarizes the results; in figure 10 typical titration curves are shown.

	^{1}H-NMR titration		*Fluorescence- / UV-vis-titration*	
	Compound 6	**Compound 24**	**Compound 24**	
Solvent	*DMSO*	*DMSO*	*MeOH*	*MeOH / H_2O 4:1*
$Bu_4N^+OAc^-$	1540	1780	2320	1220
$BuNH_3^+Cl^-$	*	*	---	---
Gly	n.d.	n.d.	n.d.	210
β-Ala	n.d.	n.d.	n.d.	250
GABA	n.d.	n.d.	n.d.	200

Table 4: Binding constants of **6** and **24** for different guests; *) *the shift in the ^{1}H-NMR spectrum results in a curve, but upon dilution linearity in the signal shift is observed;* ---) *the binding is too weak to be detected; n.d.) not determined due to solubility reasons.*

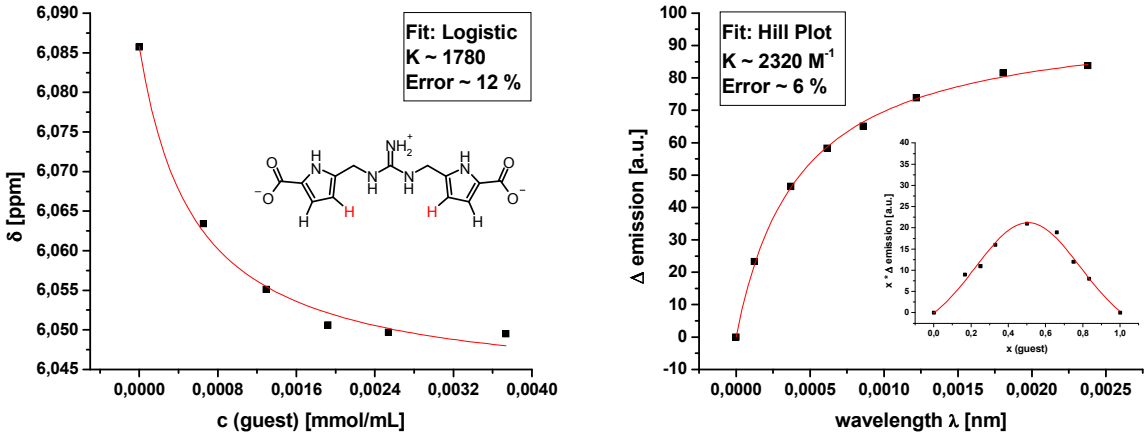


Figure 10: Binding studies of **24** with tetrabutylammonium acetate by ^{1}H-NMR (c_{host} = 3.33*10^{-3} molar) in DMSO (left) and fluorescence spectroscopy (c_{host} = 3*10^{-5} molar) in methanol (right); insert: Job´s plot

The acetate bound stronger to the guanidinium part than every amino acid, attributed to the strong self-association by ionic interactions of the amino acids to each other. In the competitive solvents even more interactions disturb the ditopic binding equilibrium to the host. Within the accuracy of the method, all amino acids bound to **24** with similar strengths (K ~ 200 - 300 M^{-1} in methanol / water 4:1, see the supporting information). The curves were too flat to extract rational Job´s plot. The binding value is surprisingly high in contrast to the expected value from the simple carboxylate-guanidinium interaction in comparable aqueous solvents,[59] especially if the self-aggregation of the amino acids is taken into account.

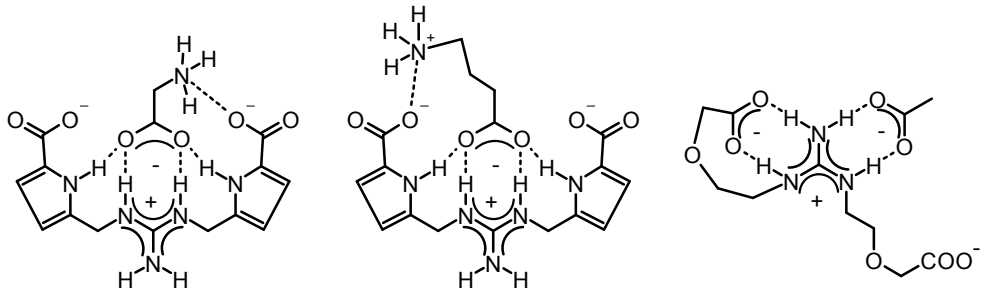
This observation may find its reason in additional H-bond donation by the pyrrole moieties[42] or, as suggested by simulations (MM2 or PM3), in an additional coordination of the amino acids´ ammonium group with the receptor´s carboxylates.

VI. 2.3.3. Suggestion of the Binding Mode

To elucidate the actual structure of **24**-aminoacid complexes, semiempirical (PM3 and MM2) calculations were performed with use of SPARTAN´06 V.112 and the CHEMOFFICE 3D ultra 7.0 packages. Different minimum geometries were found for the glycine and β-alanine complexes in the gas phase: in all of them the amino acid is bound via a guanidinium-

carboxylate interaction and hydrogen bonds between one or two of the hydrogen atoms of the ammonium group of the guest and the carboxylate oxygen atoms of the host (Figure 11).

Together with the experimental data, as well as taking into account recent literature about related systems[42,43] we propose the mode of interaction:



24 - Glycine **24** - GABA **6** - acetate

Figure 11: Illustrations of proposed structures[64] of stable complexes of glycine and GABA with compound **24**

The structural output of the modelling program is found in the supporting information of this chapter.

VI. 3. Conclusion

A straight forward synthesis strategy for symmetrically 1,3-substituted guanidines via the according Cbz-thioureas was presented. Starting from different substrates like esters, amides and carboxylic acids good overall yields are achieved. Based on glycol-δ-amino acids, many different substituents can be introduced.

Temperature dependent NMR studies revealed the structure of these compounds being highly dynamic, pointing towards a strong intramolecular guanidinium carboxylate interaction and self-assembly properties of **23**. Studying the fundamental properties of such tweezer molecules it became obvious, that they not only bound carboxylates, but also amino acids to a weak extent, which is remarkably in view of the polarity of the solvent and the self aggregation of the guest.

Such structures may not only find application as building blocks for carboxylate binding sites, also additional functional groups may be easily introduced to enhance their amino acid binding ability. Recognition motifs for β-strands or complementary peptide structures are imaginable building up on the tweezer molecules.

We also demonstrated a cost-efficient synthesis of a suitably protected glycol like spacers, for use under both solid-phase and solution-phase synthesis. The phase transfer catalysis is especially valuable for their preparation, the copper-mediated decomposition of azoesters proved to be a feasible alternative. The preparation is not more laborious than the commercial route for amino-PEG-acids, starting for example from 2-(2-chloroethoxy)-ethanol.[65] Our synthesis is more flexible and involves no toxic halogenated ether compound. It allows the introduction of a broad variety of substituents and different protecting groups.

Ketone- and PEG-containing amino acids belong to the most 'useful' unnatural amino acids. A major interest in clinical development is established with PEGylated proteins and peptides.[66] A variety of applications for unnatural glycol-δ-amino acids, for example in medical studies to enhance the therapeutic value and duration of peptide drugs or as spacer molecules with customized properties or building blocks for new macrocycles is imaginable.

VI. 4. Experimental Part

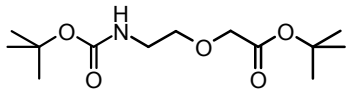
VI. 4.1. Syntheses

Compounds 1-*N*-Boc-ethanolamin[67], 1-*N*-Cbz-ethanolamin[68], *N*-Boc-L-serine-methylester[69], *N*-Boc-L-leucine-methylester[69], *N*-Boc-L-tryptophane-methylester[69], *N*-Boc-L-phenylalanine-methylester[69], 3-(2-(*N*-Boc-aminoethoxy)propionic acid ethylester[70], glycyl glycine methylester hydrochloride,[71] 2,6-pyridine-di-carboxylic acid ethyl ester[72], 5-Boc-amino-(toluoyl-4-sulfonyl)-methyl]-pyrrol-2-carboxlic acid methyl ester[73], 5-[Boc-amino-methyl]-pyrrol-2-carboxlic acid methyl ester[73] and 5-amino-methyl-pyrrol-2-carboxlic acid methyl ester[73] were prepared after literature known procedures.

VI. 4.1.1. General Procedure 1: Phase Transfer Catalytic Etherification of Substituted Amino Alcohols (GP I)

The *N*-Boc-amino alcohol was dissolved with tetrabutylammonium hydrogensulfate (0.4 mmol, 98.8 mg) in CH_2Cl_2 (6 mL). Aqueous NaOH (5.0 M, 6.0 mL) was added, bromoacetic acid *tert*butyl ester (1.2 mmol, 233 mg) in CH_2Cl_2 (1 mL) was dropped in and the reaction mixture was vigorously stirred for 3 h at room temperature. The same amount bromoacetic acid *tert*butyl ester was added again and the heterogeneous solution was stirred over night. The mixture was poured on ice (10 g) in a separation funnel, the organic phase was separated and the aqueous layer was extracted with dichloromethane (3 x 10 mL). The organic phases were combined, dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography with ethyl acetate / petrol ether 1:4 to obtain the pure compound.

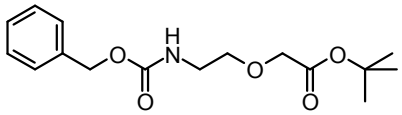
2-(2-tertButoxycarbonyl-aminoethoxy)acetic acid tertbutyl ester **(7a)**



N-Boc-ethanolamine (192 mg, 1.2 mmol) was submitted to *GP I* to give **7** as colourless oil (273 mg, 0.996 mmol, 83 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.37 (s, 9 H), 1.42 (s, 9 H), 3.26 (m, 2 H), 3.53 (t, *J* = 5.2 Hz, 2 H), 3.90 (s, 2 H), 5.12 (br s, 1 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 28.1 (+, 3 C), 28.4 (+, 3 C), 40.4 (-, 1 C), 68.7 (-, 1 C), 70.7 (-, 1 C), 79.1 (C_{quat}, 1 C), 81.8 (C_{quat}, 1 C), 156.0 (C_{quat}, 1 C), 169.6 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3371 (bm), 2978 (m), 2932 (m), 1761 (m), 1714 (s), 1509 (m), 1486 (m), 1416 (m), 1367 (m), 1250 (s), 1202 (s), 1137 (s), 762 (m); - **MS** (CI-MS, NH_3): m/z (%) = 181.1 (43, MNH_4^+ - 2 C_4H_8), 219.2 (24, MH^+ - C_4H_8), 237.2 (100, MNH_4^+ - C_4H_8), 276.2 (46, MH^+), 293.3 (60, MNH_4^+); - **MW** = 275.35; - **MF** = $C_{13}H_{25}NO_5$.

2-(2-(tertBenzyloxycarbonyl)-aminoethyloxy)acetic acid ethyl ester (7c)



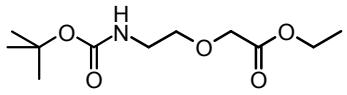
To a solution of 2-(*N*-^{tert}Benzyloxycarbonyl)-aminoethanol (2.0 g, 12.5 mmol) in toluene (60 mL) ^{tert}butyl bromoacetate (3.9 mL, 5.73 g, 25.0 mmol) and tetrabutylammonium hydrogensulfate (2.12 g, 6.25 mmol) were added. The reaction mixture was vigorously stirred and 30 mL of 30 % NaOH were slowly added. After 12 h of stirring at room temperature, another portion of ^{tert}butyl bromoacetate (0.78 mL, 1.15 g, 5.0 mmol) was added. Stirring was continued for 6 h then the organic phase was separated. It was washed with 20 mL of 5 % aqueous acetic acid and trice with 20 mL portions of water. After drying over $MgSO_4$ the solvent was removed at reduced pressure. The excess of ^{tert}butyl bromoacetate was evaporated in the vacuum and the oily raw material was purified by column chromatography with ethyl acetate / petrol ether 1:4 to yield the benzyloxycarbonyl amino acid ester as clear, colourless oil (3.36 g, 10.86 mmol, 87 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.41 (s, 9 H), 3.36 (t, 2 H, *J* = 5.2 Hz), 3.54 (t, 2 H, *J* = 5.2 Hz), 3.91 (s, 2 H), 5.07 (s, 2 H), 5.48 (bs, 1 H), 7.26 – 7.32 (m, 5H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 27.1 (+, 3 C), 40.0 (-, 1 C), 64,1 (-, 1 C), 65.6 (-, 1 C), 67.7 (-, 1 C), 69.6 (-, 1 C), 80.7 (C_{quat}, 1 C), 127.0 (+, 1 C), 127.7 (+, 2 C), 128.5 (+, 2 C), 135.6 (C_{quat}, 1 C), 155.5 (C_{quat}, 1 C), 168.7 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3351 (bm), 2978 (m), 2937 (m), 2886 (m), 1713 (s), 1518 (m), 1455 (m), 1368 (m), 1228 (s), 1131 (s), 1026 (m), 915 (m), 845 (m), 798 (m), 735 (m), 698 (m), 584 (m); - **MS** (CI-MS, NH_3): m/z (%) = 219.2 (90, MNH_4^+ - C_7H_8O), 254.2 (9, MH^+ - C_4H_8), 271.2 (63, MNH_4^+ - C_4H_8), 310.2 (9, MH^+), 327.2 (100, MNH_4^+); - **MW** = 309.37; - **MF** = $C_{16}H_{23}NO_5$.

VI. 4.1.2. General Procedure 2: NaH Catalysed Ether Synthesis (GP II)

In an nitrogen flushed Schlenck-flask the *N*-Boc-aminoalcohol (5.0 mmol) was added to a suspension of NaH (60 % susp., 380 mg, 8.0 mmol) and KI (150 mg, 0.8 mmol) in 30 mL of dry THF at 0 °C. After drop wise addition of ethyl bromoacetate (1.67 g, 1.11 mL, 10.0 mmol) in 10 mL of dry THF, the reaction mixture was stirred for 4 h at room temperature. The NaH suspension and precipitated solids were settled by a centrifuge. The THF solution was decanted off and evaporated to give the crude product. The residue was purified by column chromatography (petrol ether / ethyl acetate, 9:1 → 4:1) to give the according glycol-δ-amino acid ester.

2-(2-^{tert}Butyloxycarbonylaminoethoxy)acetic acid ethyl ester **(7b)**



N-Boc-ethanolamine (800 mg, 5.0 mmol) was reacted according to *GP II* to give **7b** as faintly yellow oil (727 mg, 2.955 mmol, 59 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.23 (t, 3 H, *J* = 7.1 Hz), 1.38 (s, 9 H), 3.28 (m, 2 H), 3.54 (t, 2 H, *J* = 5.2 Hz), 4.02 (s, 2 H), 4.15 (q, 2 H, *J* = 7.1 Hz), 5.11 (bs, 1 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 14.2 (+, 1 C), 28.4 (+, 3 C), 40.4 (-, 1 C), 61.0 (-, 1 C), 68.3 (-, 1 C), 70.8 (-, 1 C), 79.2 (C_{quat}, 1 C), 156.0 (C_{quat}, 1 C), 170.4 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3374 (bm), 2978 (m), 2935 (m), 1761 (m), 1706 (s), 1511 (m), 1455 (m), 1367 (m), 1248 (m), 1206 (s), 1136 (s), 1025 (s), 864 (m), 782 (m), 717 (m), 581 (m); - **MS** (CI-MS, NH_3): m/z (%) = 192.1 (98, MH^+ - C_4H_8), 248.2 (16, MH^+), 265.2 (40, MNH_4^+); - **HRMS** (PI-LSIMS FAB, glycerine): calc. for $C_{11}H_{22}NO_5^+$: 248.1498, found: 248.1493; - **MW** = 247.29; - **MF** = $C_{11}H_{21}NO_5$.

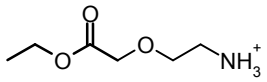
VI. 4.1.3. Ethersynthesis by Azo Acetic Acid Ethyl Ester/Cu(I)

To a solution of *N*-Boc-ethanolamine (1.60 g, 10.0 mmol) in DCM (30 mL) in nitrogen atmosphere at 5 °C was added copper(II)triflate (362 mg, 1.0 mmol, 10 mol%) and three drops of phenyl hydrazine, immediately followed by TMEDA (120 mg, 1.0 mmol, 10 mol%). After stirring for 30 minutes, 8.8 mL of 15 % (weight/weight) azo acetic acid ethyl ester in DCM (1.71 g, 15.0 mmol) was dropped in over a period of 6 h at 5 °C. The mixture was allowed to warm up slowly to room temperature and was stirred over night. The solution was filtered over alumina N, the filter cake was washed with DCM and the solvent was evaporated. The remaining oil was purified by column chromatography (ethyl acetate / petrol ether 2:1 → 1:1) to yield the product **12a** (1.93 g, 7.81 mmol, 78 %) as a clear yellow oil (EtOAc / PE 1:2 R_f = 0.3).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.23 (t, 3 H, *J* = 7.1 Hz), 1.38 (s, 9 H), 3.27 (t, 2 H, *J* = 5.2 Hz), 3.54 (t, 2 H, *J* = 5.2 Hz), 4.02 (s, 2 H), 4.15 (q, 2 H, *J* = 7.2 Hz), 5.11 (bs, 1 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 14.2 (+, 1 C), 28.4 (+, 3 C), 40.5 (-, 1 C), 61.0 (-, 1

C), 68.3 (-, 1 C), 70.8 (-, 1 C), 79.3 (C_{quat}, 1 C), 156.0 (C_{quat}, 1 C), 170.4 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3374 (bm), 2978 (m), 2935 (m), 1761 (m), 1706 (s), 1511 (m), 1455 (m), 1367 (m), 1248 (m), 1206 (s), 1136 (s), 1025 (s), 864 (m), 782 (m), 717 (m), 581 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 192.1 (98, MH^+ - C_4H_8), 247.1 (100, MNH_4^+ - C_4H_8), 248.2 (16, MH^+), 265.2 (40, MNH_4^+); - **MW** = 247.29; - **MF** = $C_{11}H_{21}NO_5$.

*(2-amino-ethoxy)-acetic acid ethyl ester hydrochloride (**10b**)*



To a solution of **7b** (0.52 g, 2.0 mmol) in dried DCM (10 mL) was slowly added 4 mL of HCl saturated diethylether. After 3 h of stirring at room temperature the solvent was evaporated. The residue was taken up in a little of DCM, and the solvent was removed under reduced pressure again. This process was repeated once. The remaining sticky solid was dried in the vacuum over night. The yield was 0.38 g of a yellow, deliquescent solid (1.96 mmol, 98 %).

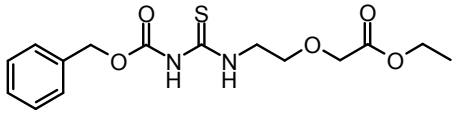
^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.26 (t, 3 H, *J* = 7.1 Hz), 3.32 (m, 2 H), 3.91 (m, 2 H), 4.09 – 4.21 (q, 2 H, *J* = 7.1 Hz), 4.18 (s, 2 H), 6.50 (bs, 3 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 14.1 (+, 1 C), 39.4 (-, 1 C), 61.3 (-, 1 C), 67.4 (-, 1 C), 68.2 (-, 1 C), 171.0 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3470 (bm), 2937 (m), 2916 (m), 1726 (m), 1669 (s), 1516 (m), 1430 (m), 1183 (s), 1127 (s), 1020 (m), 965 (m), 836 (m), 799 (s), 721 (m), 673 (m), 517 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 147.8 (100, MH^+), 294.9 ($2M+H^+$, 5%); - **MW** = 183.63; - **MF** = $C_6H_{14}ClNO_3$.

VI. 4.1.4. General Procedure 3: Preparation of Benzyloxycarbonylthioureas from Amino Acid Esters (GP III)

Benzyloxycarbonyl-isothiocyanat (500 mg, 2.6 mmol) in dichloromethane (10 mL) was added slowly to a solution of the according amine compound (2.0 mmol) and triethylamine (300 mg, 0.4 mL, 3.0 mmol or 500 mg, 0.68 mL, 5.0 mmol if the amine salt is employed) in dichloromethane (10 mL) at 2 – 5 °C. The solution was stirred at room temperature until TLC

indicated no further reaction (2 – 6 h). All volatiles were removed under reduced pressure. Ethyl acetate (30 mL) was added, the organic phase was washed with saturated ammonium chloride solution (5 mL) and twice with water (10 mL). After drying over $MgSO_4$, the solvent was evaporated and the residue was suspended in diethyl ether / petrol ether 1:4 (5 mL). The product was allowed to settle completely, the ether mixture was decanted off, and after drying the precipitate, it was purified by column chromatography if necessary (ethyl acetate / petrol ether 1:1 if not stated otherwise).

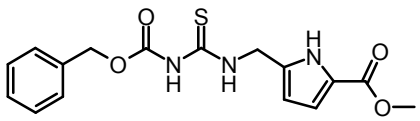
*2-(2-[3-N-(benzyloxycarbonyl)thioureido]ethyloxy)acetic acid ethylester (**12b**)*



The TFA salt of 2-(2-Amino-ethoxy)-acetic acid ethyl ester (520 mg, 2.0 mmol) was reacted according to *GP III* to give the thiourea (**12b**) as pale yellow oil (574 mg, 1.77 mmol, 89 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.20 (t, 3 H, *J* = 7.2 Hz), 3.70 (t, 2 H, *J* = 5.2 Hz), 3.83 (t, 2 H, *J* = 5.2 Hz), 4.05 (s, 2 H), 4.15 (q, 2 H, *J* = 7.2 Hz), 5.11 (s, 2 H), 7.22 – 7.35 (m, 5 H), 8.50 (bs, 1 H), 9.90 (bs, 1H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 14.2 (+, 1 C), 45.4 (-, 1 C), 61.0 (-, 1 C), 68.1 (-, 1 C), 68.4 (-, 1 C), 69.0 (-, 1 C), 128.3 (+, 1 C), 128.7 (+, 2 C), 128.8 (+, 2 C), 134.6 (C_{quat}, 1 C), 152.4 (C_{quat}, 1 C), 170.3 (C_{quat}, 1 C), 179.4 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3291 (bm), 2981 (m), 2942 (m), 2905 (m), 1721 (s), 1515 (s), 1453 (m), 1375 (m), 1208 (s), 1126 (s), 1022 (s), 911 (m), 839 (m), 796 (m), 740 (m), 696 (m), 580 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 340.9 (100, MH^+), 357.9 (8, MNH_4^+), 681.1 (22, 2M + H^+), 698.1 (14, 2M + NH_4^+); - **MW** = 340.40; - **MF** = $C_{15}H_{20}N_2O_5S$.

*5-([3-N-(benzyloxycarbonyl)thioureido]methyl)pyrrol-2-carboxylic acid methylester (**15**)*



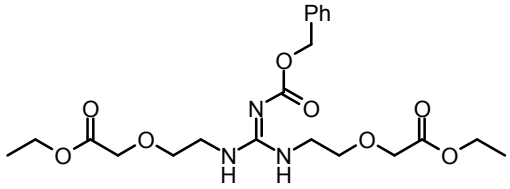
The TFA salt of 5-Aminomethyl-pyrrol-2-carboxylic acid methyl ester (534 mg, 2.0 mmol) was submitted to *GP III* to give its according thiourea as a beige solid (610 mg, 1.84 mmol, 92 %).

M.p. (uncorrected) = 155 – 156 °C; - **^{1}H-NMR** (300 MHz, $CDCl_3$): δ [ppm] = 3.82 (s, 3 H), 4.85 (d, 2 H, *J* = 5.8 Hz), 5.16 (s, 2 H), 6.15 (d, 1 H, *J* = 6.0 Hz), 6.81 (d, 1 H, *J* = 6.0 Hz), 7.28 – 7.41 (m, 5 H), 8.30 (s, 1 H), 10.01 (bs, 2 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 41.7 (-, 1 C), 51.6 (+, 1 C), 68.5 (-, 1 C), 110.1 (+, 1 C), 115.4 (+, 1 C), 122.8 (C_{quat}, 1 C), 128.4 (+, 2 C), 128.8 (+, 2 C), 129.0 (+, 1 C), 132.7 (C_{quat}, 1 C), 134.3 (C_{quat}, 1 C), 152.4 (C_{quat}, 1 C), 161.3 (C_{quat}, 1 C), 180.1 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3279 (bm), 2955 (m), 2926 (m), 2855 (m), 1701 (s), 1614 (m), 1519 (m), 1492 (s), 1452 (m), 1385 (m), 1315 (m), 1214 (s), 1089 (m), 1037 (m), 1003 (m), 910 (m), 796 (m), 765 (m), 733 (m), 697 (m), 605 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 348.0 (44, MH^+), 389.0 (12, MH^+ + MeCN), 695.0 (100, 2M + H^+), 712.1 (19, 2M + NH_4^+); - **HRMS** (EI-MS 70 eV): calc. for $C_{16}H_{17}N_3O_4S^{*+}$: 347.0940, found: 347.0946; **- $C_{17}H_{24}N_2O_5S$ + 0.5 EtOH:** calc. C 55.1, H 5.4, N 11.3, S 8.7, found. C 55.1, H 4.9, N 10.9, S 8.5; - **UV** (MeOH): λ (ε) = 264 (6900), 209 (6100); - **MW** = 347.40; - **MF** = $C_{16}H_{17}N_3O_4S$.

VI. 4.1.5. General Procedure 4: Preparation of Symmetric 1,3-substituted Benzyloxycarbonyl-guanidines (GP IV)

The benzyloxycarbonylthiourea (0.5 mmol) was dissolved together with its according amine compound (0.6 mmol) and triethylamine (130 mg, 0.17 mL, 1.3 mmol) in DCM (5.0 mL). EDC hydrochloride (110 mg, 0.6 mmol) was added at 2 – 5 °C in one portion[74]. The mixture was allowed to reach room temperature in 1 hour and was then stirred over night. In the case were TLC indicated thiourea still being present at this point, another portion of triethylamine (30 mg, 0.04 mL, 0.3 mmol) and EDC hydrochloride (54 mg, 0.3 mmol) was added and stirring was continued for 4 h. It was diluted with DCM (25.0 mL), the organic solution was washed with saturated ammonium chloride solution (5.0 mL) and twice with water (10.0 mL). After drying over $MgSO_4$, the solvent was evaporated and the oily residue was purified by column chromatography with ethyl acetate / petrol ether 4:1 to yield the guanidine.

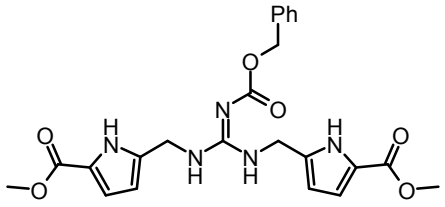
*(2-[N-(2-Ethyloxycarbonylmethyloxyethyl)-N-(benzyloxycarbonyl)guanidine]ethyloxy)acetic acid ethylester (**17b**)*



Thiourea **12b** (161 mg, 0.5 mmol) was reacted with the TFA salt of 2-(2-Amino-ethoxy)-acetic acid ethyl ester (**10b**) (157 mg, 0.6 mmol) after *GP IV* to give a clear, faintly yellow oil (166 mg, 0.366 mmol, 73 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.25 (t, 6 H, *J* = 7.1 Hz), 3.56 (m, 4 H), 3.70 (m, 4 H), 4.18 (q, 4 H, *J* = 7.4 Hz), 4.10 (s, 4 H), 5.12 (s, 2 H), 7.21 – 7.43 (m, 5 H), 9.00 (bs, 2 H); - **^{13}C-NMR** (75 MHz, $CDCl_3$): δ [ppm] = 14.2 (+, 2 C), 42.8 (-, 2 C), 47.5 (-, 2 C), 61.2 (-, 2 C), 67.2 (-, 2 C), 68.3 (-, 1 C), 70.8 (-, 2 C), 128.0 (+, 1 C), 128.1 (+, 2 C), 128.4 (+, 2 C), 136.8 (C_{quat}, 1 C), 160.0 (C_{quat}, 2 C), 161.5 (C_{quat}, 1 C), 170.1 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3284 (bm), 2981 (m), 2936 (m), 1740 (s), 1668 (m), 1598 (s), 1518 (m), 1451 (m), 1382 (m), 1275 (m), 1209 (s), 1132 (s), 1051 (m), 1025 (m), 912 (m), 852 (m), 799 (m), 729 (m), 691 (m), 582 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 454.0 (100, MH^+); - **MW** = 453.50; - **MF** = $C_{21}H_{31}N_3O_8$.

*5-[(N'-[(2-Methoxycarbonyl-1H-pyrrole)-5-methyl- N-(benzyloxycarbonyl)guanidino)-methyl]-1H-pyrrole-2-carboxylic acid methyl ester (**20**)*



Thiourea **15** (165 mg, 0.5 mmol) was reacted with the TFA salt of 5-Aminomethyl-pyrrol-2-carboxylic acid methyl ester (160 mg, 0.6 mmol) according to GP *IV* to yield a yellow solid (203 mg, 0.434 mmol, 87 %).

^{1}H-NMR (300 MHz, DMSO-d6): δ [ppm] = 3.54 (s, 6 H), 4.41 (s, 4 H), 5.14 (s, 2 H), 6.12 (d, 2 H, *J* = 6.0 Hz), 6.77 (d, 2 H, *J* = 6.0 Hz), 7.38 – 7.42 (m, 5 H), 8.16 (s, 2 H), 10.81 (bs, 1 H), 11.05 (bs, 1 H); - **^{13}C-NMR** (75 MHz, MeOD): δ [ppm] = 38.9 (-, 2 C), 51.8 (+, 2 C), 67.9 (-,

1 C), 109.9 (+, 2 C), 116.9 (+, 2 C), 122.1 (C_{quat}, 2 C), 128.8 (+, 1 C), 128.9 (+, 2 C), 129.5 (+, 2 C), 138.9 (C_{quat}, 1 C), 158.2 (C_{quat}, 1 C), 161.3 (C_{quat}, 2 C), 163.1 (C_{quat}, 2 C), 165.0 (C_{quat}, 1 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3415 (bm), 3281 (s), 2949 (m), 1679 (m), 1642 (m), 1584 (m), 1549 (m), 1490 (m), 1443 (m), 1390 (m), 1333 (m), 1277 (m), 1220 (s), 1127 (m), 1065 (m), 1002 (m), 927 (m), 799 (m), 764 (s), 701 (m), 627 (m), 597 (m), 508 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 468.0 (100, MH^+); - **HRMS** (PI-LSIMS FAB, glycerine): calc. for $C_{23}H_{26}N_5O_6^+$: 468.1883, found: 468.1879; - **UV** (MeOH): λ (ε) = 266 (12800), 226 (9800); - **MW** = 467.49; - **MF** = $C_{23}H_{25}N_5O_6$.

VI. 4.1.6. Deprotection of the Compounds

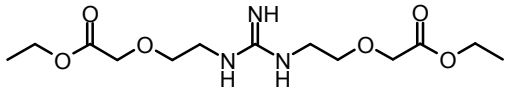
Deprotection of the benzyloxycarbonylguanidine esters (GP Va)

The symmetric 1,3-substituted benzyloxycarbonylguanidine ester (0.4 mmol) was dissolved, according to the molecules ester, in ethanol or methanol (3.0 mL). Three spatula tips of palladium on charcoal (10 % Pd) were added. It was stirred in a hydrogen atmosphere (30 bar) for two days at room temperature. The reaction mixture was diluted with the according alcohol (10 mL) and filtered over celite. The filter cake was washed with small portions of this alcohol. The clear filtrate was evaporated to dryness to give the guanidine free base.

Synthesis of the guanidinium hexafluorophosphate salts (GP Vb):

The guanidine free base (0.2 mmol) was dissolved in 6 mL of methanol or ethanol, according to its esters. Ammonium hexafluorophosphate (38 mg, 0.24 mmol) was added and the mixture was warmed to 40 °C for three hours. The solvent was evaporated and the residue was extracted with DCM (1 mL) trice. The solvent was distilled off and the remaining guanidinium salt was properly dried in vacuo. The products appear as colourless solids in nearly quantitative yield.

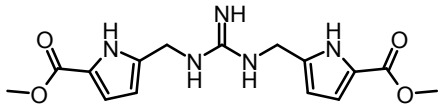
*(2-[N-(2-Ethyloxycarbonylmethyloxyethyl)-N-guanidine]ethyloxy)acetic acid ethyl ester hexafluorophosphate (**22b**)*



Compound **17b** (181 mg, 0.4 mmol) was deprotected after *GP Va* and reacted according to *GP Vb* to give a colourless, sticky oil (97 mg, 0.369 mmol, 92 %).

^{1}H-NMR (300 MHz, acetone-d6): δ [ppm] = 1.24 (t, 6 H, *J* = 7.1 Hz), 3.59 (m, 4 H), 3.79 (m, 4 H), 4.19 (q, 4 H, *J* = 7.1 Hz), 4.23 (s, 4 H), 5.16 (s, 2 H), 7.30 (m, 5 H); **^{19}F-NMR** (300 MHz, acetone-d6): δ [ppm] = -70.2 (+), -72.7 (+); - **^{13}C-NMR** (75 MHz, MeOD): δ [ppm] = 14.6 (+, 2 C), 43.2 (-, 2 C), 62.2 (-, 2 C), 69.1 (-, 2 C), 71.3 (-, 2 C), 158.8 (C_{quat}, 1 C), 172.3 (C_{quat}, 2 C), 171.0 (C_{quat}, 1 C); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 320.0 (100, MH^+); - **HRMS** (PI-LSIMS FAB, glycerine): calc. for $C_{13}H_{26}N_3O_6^+$: 320.1822, found: 320.1827; - **MW** = 465.33; - **MF** = $C_{13}H_{26}PF_6N_3O_6$.

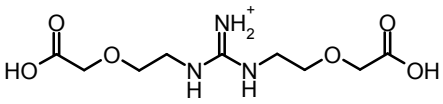
*5-[(N'-[(2-Methoxycarbonyl-1H-pyrrole)-5-methyl-guanidino)-methyl]-1H-pyrrole-2-carboxylic acid methyl ester hexafluorophosphate (**23**)*



Compound **20** (187 mg, 0.4 mmol) was reacted according to *GP Va* and *Vb* to yield a yellow solid (117 mg, 0.384 mmol, 96 %).

^{1}H-NMR (300 MHz, acetone-d6): δ [ppm] = 3.78 (s, 6 H), 4.67 (s, 4 H), 6.23 (d, 2 H, *J* = 3.6 Hz), 6.75 (d, 2 H, *J* = 3.6 Hz), 7.76 (bs, 1 H), 11.09 (bs, 1 H); - **^{13}C-NMR** (75 MHz, MeOD): δ [ppm] = 51.9 (+, 2 C), 110.3 (+, 2 C), 117.0 (+, 2 C), 124.1 (C_{quat}, 2 C), 133.4 (C_{quat}, 2 C), 157.2 (C_{quat}, 1 C), 163.0 (C_{quat}, 2 C); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 334.0 (100, MH^+); - **UV** (MeOH): λ (ε) = 263 (11600); - **MW** = 479.32; - **MF** = $C_{15}H_{20}PF_6N_5O_4$.

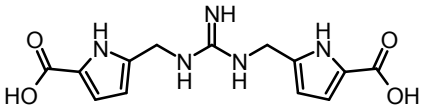
*{2-[N'-(2-Carboxymethoxy-ethyl)-guanidino]-ethoxy}-acetic acid hydrochloride (**6**)*



The guanidine free base **22b** (181 mg, 0.4 mmol) was dissolved in 5 mL of methanol or THF and aqueous hydrochloric acid (2 M, 2.0 mL, 4.0 mmol) was added. The mixture was stirred at room temperature until the TLC showed complete consumption of the starting material (4 to 6 h). The solvent was evaporated and the product was lyophilised to give the guanidine bis-acid as inner salt, a colourless, sticky oil (97 mg, 0.369 mmol, 92 %).

^{1}H-NMR (300 MHz, acetone-d6): δ [ppm] = 3.51 (m, 4 H), 3.71 (s, 4 H), 3.78 (m, 4 H), 7.60 (bs, 2 H), 8.71 (bs, 1 H); - **^{13}C-NMR** (75 MHz, MeOD): δ [ppm] = 43.2 (-, 2 C), 68.8 (-, 2 C), 71.3 (-, 2 C), 158.8 (C_{quat}, 1 C), 155.9 (C_{quat}, 1 C), 177.2 (C_{quat}, 2 C); - **IR** (FT-IR, film): ν [cm^{-1}] = 3178 (bm), 2935 (m), 2250 (m), 1736 (m), 1638 (m), 1435 (m), 1349 (m), 1218 (m), 1134 (s), 1070 (m), 1010 (s), 875 (m), 824 (m), 762 (m), 682 (m), 669 (m), 623 (m); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 264.1 (100, MH^+); - **MW** = 298.60; - **MF** = $C_9H_{18}N_3O_6Cl$.

*5-[(N'-[(2-Carboxy-1H-pyrrole)-5-methyl-guanidino)-methyl]-1H-pyrrole-2-carboxylic acid (**24**)*



Compound **23** (187 mg, 0.4 mmol) was dissolved in 5 mL methanol and aqueous LiOH (1 M, 0.8 mL, 0.8 mmol) was added. The mixture was stirred at room temperature until the TLC showed complete conversion (6 - 8 h). The solvent was evaporated and the product was lyophilised to obtain the guanidine bis-acid lithium salt as a fine pale beige powder (117 mg, 0.384 mmol, 96 %).

^{1}H-NMR (300 MHz, MeOD): δ [ppm] = 4.31 (s, 4 H), 6.02 (m, 2 H), 6.41 (m, 2 H); - **^{13}C-NMR** (75 MHz, MeOD): δ [ppm] = 39.9 (-, 2 C), 108.6 (+, 2 C), 113.5 (+, 2 C), 130.6 (C_{quat}, 2 C), 132.0 (C_{quat}, 2 C), 137.3 (C_{quat}, 1 C), 170.1 (C_{quat}, 2 C); - **MS** (ESI-MS, CH_2Cl_2/MeOH + 10 mmol NH_4OAc): m/z (%) = 306.0 (100, MH^+); - **UV** (MeOH): λ (ε) = 264 (11200); - **MW** = 305.30; - **MF** = $C_{13}H_{15}N_5O_4$.

VI. 4.2. ^{1}H – and ^{13}C-NMR Spectra of Selected New Compounds

NMR spectra were recorded on Bruker Avance spectrometers 300, 400 and 600. Measuring temperature was 300 K. TMS was used as external standard. For solvents and conditions of measurement, see experimental procedures.

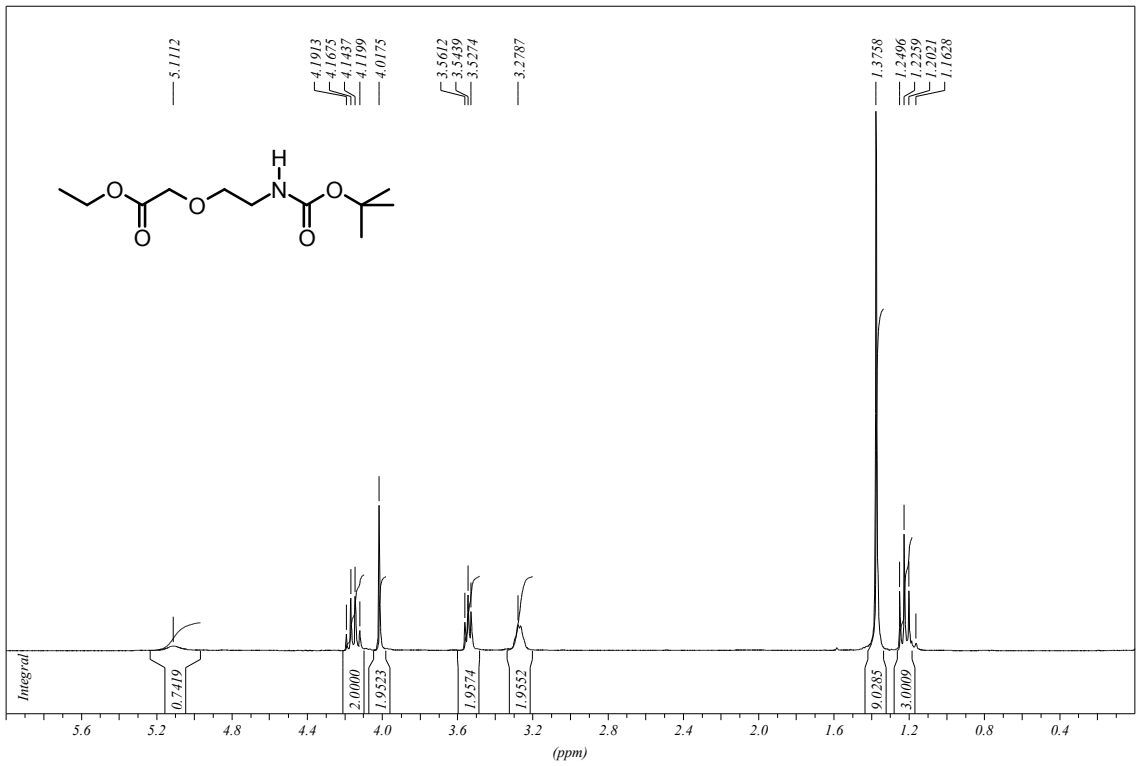


Figure 12: ^{1}H-NMR spectrum of compound **7b**

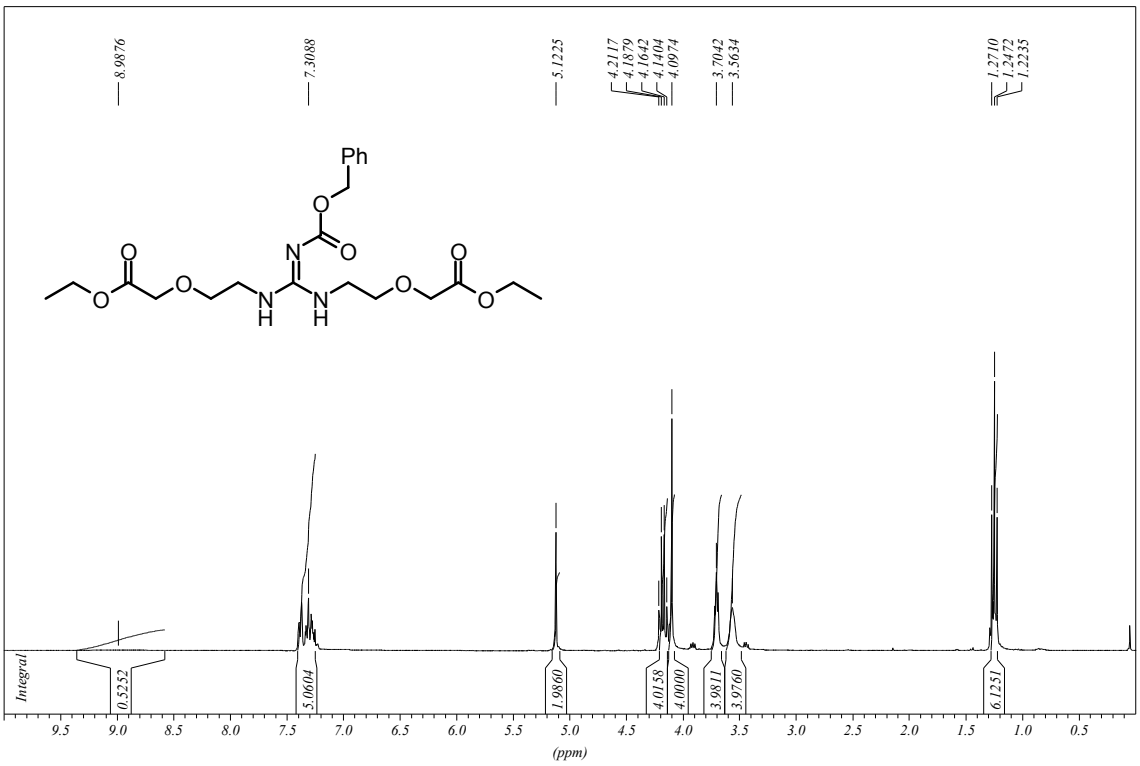


Figure 13: ^{1}H-NMR spectrum of compound **17b**

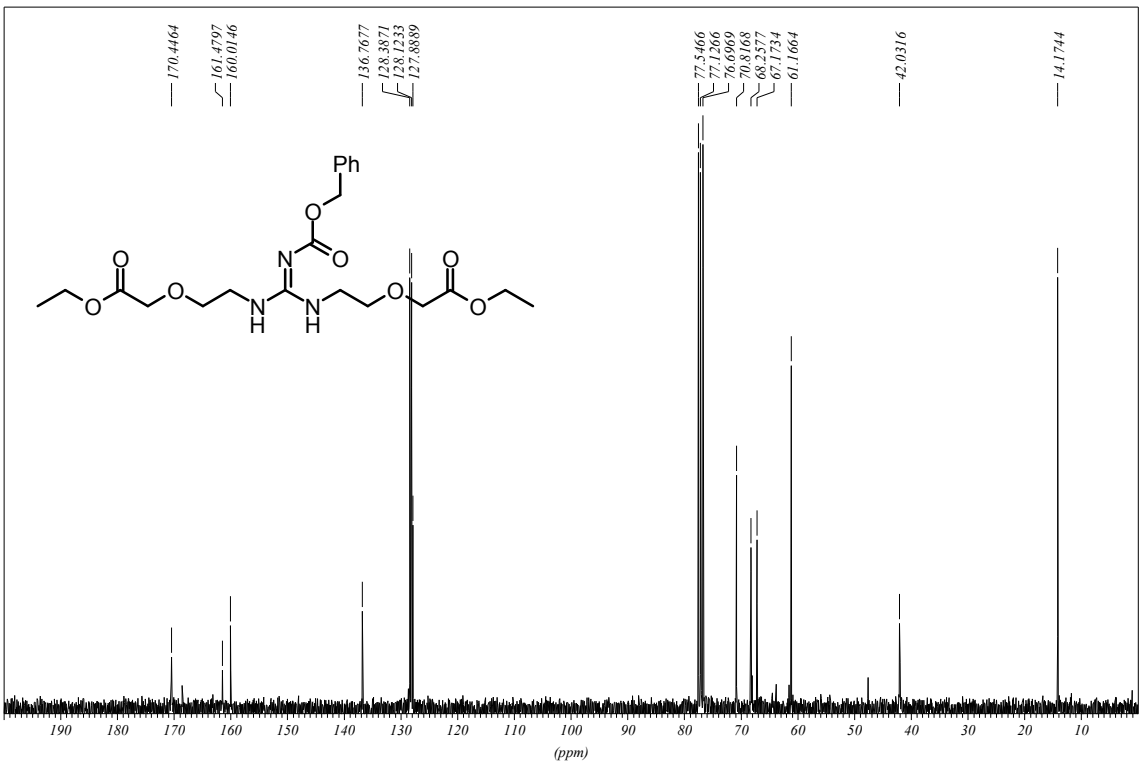


Figure 14: ^{13}C-NMR spectrum of compound **17b**

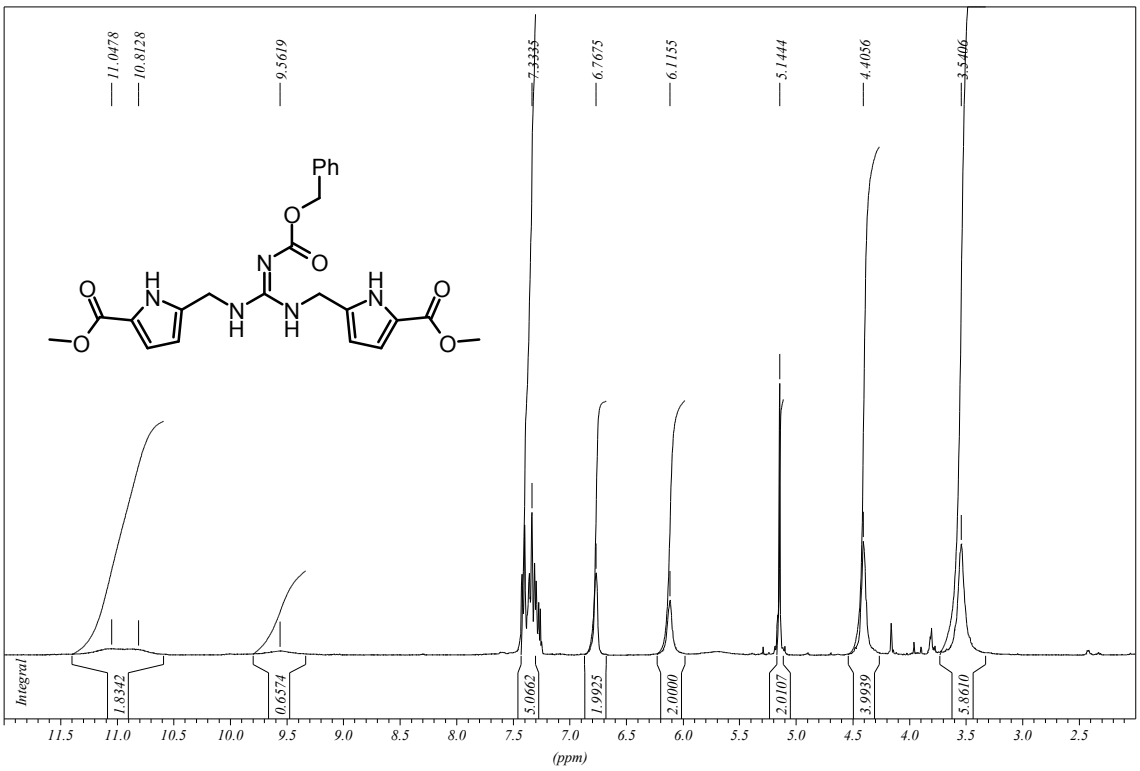


Figure 15: ^{1}H-NMR spectrum of compound **20**

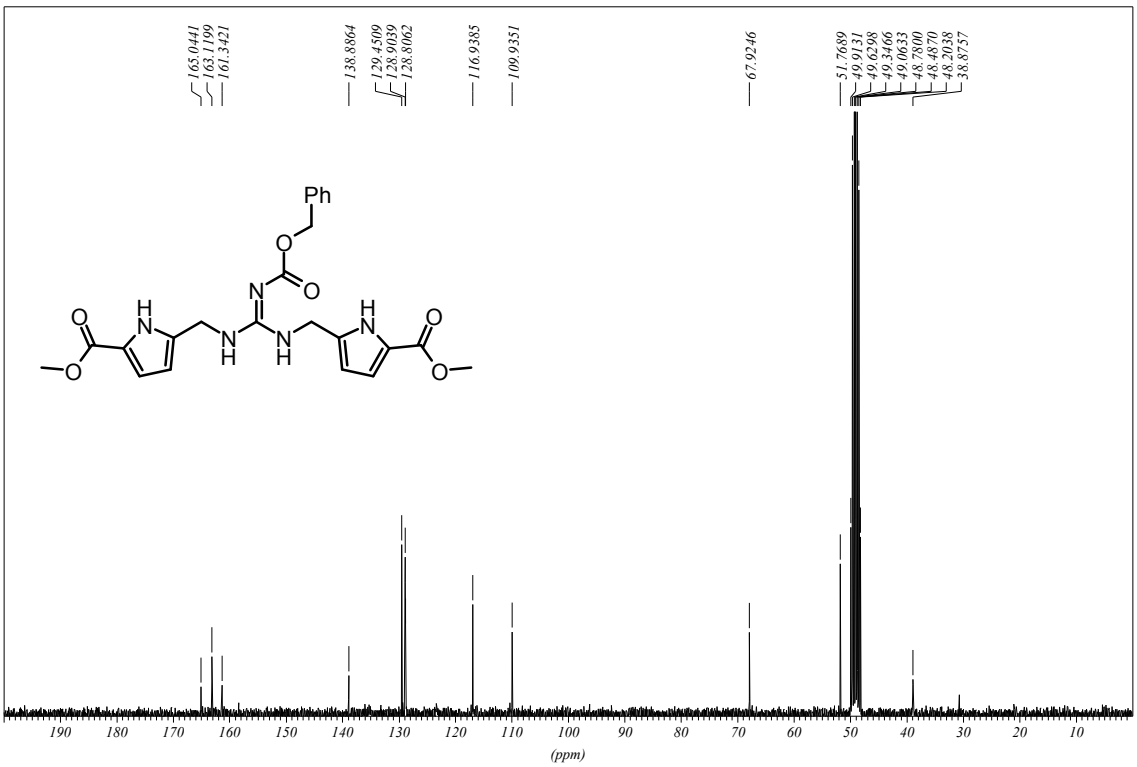


Figure 16: ^{13}C-NMR spectrum of compound **20**

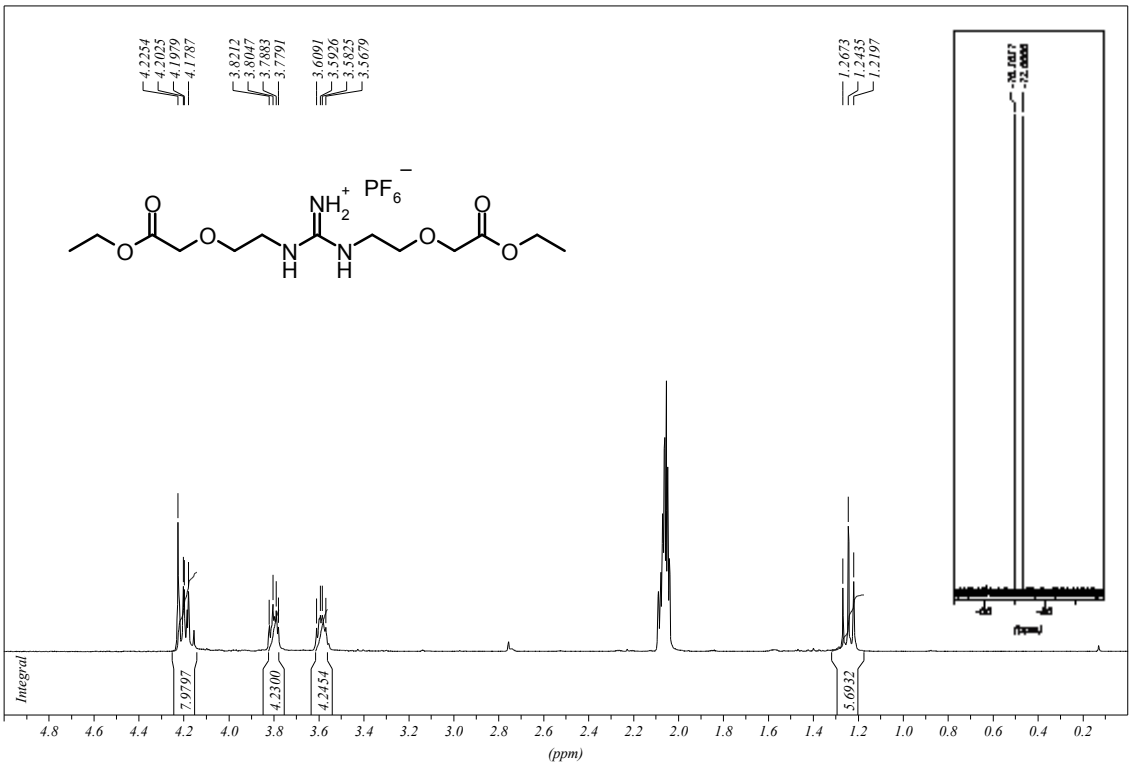


Figure 17: ^{1}H-NMR and ^{19}F-NMR spectrum (insert) of compound **22b**

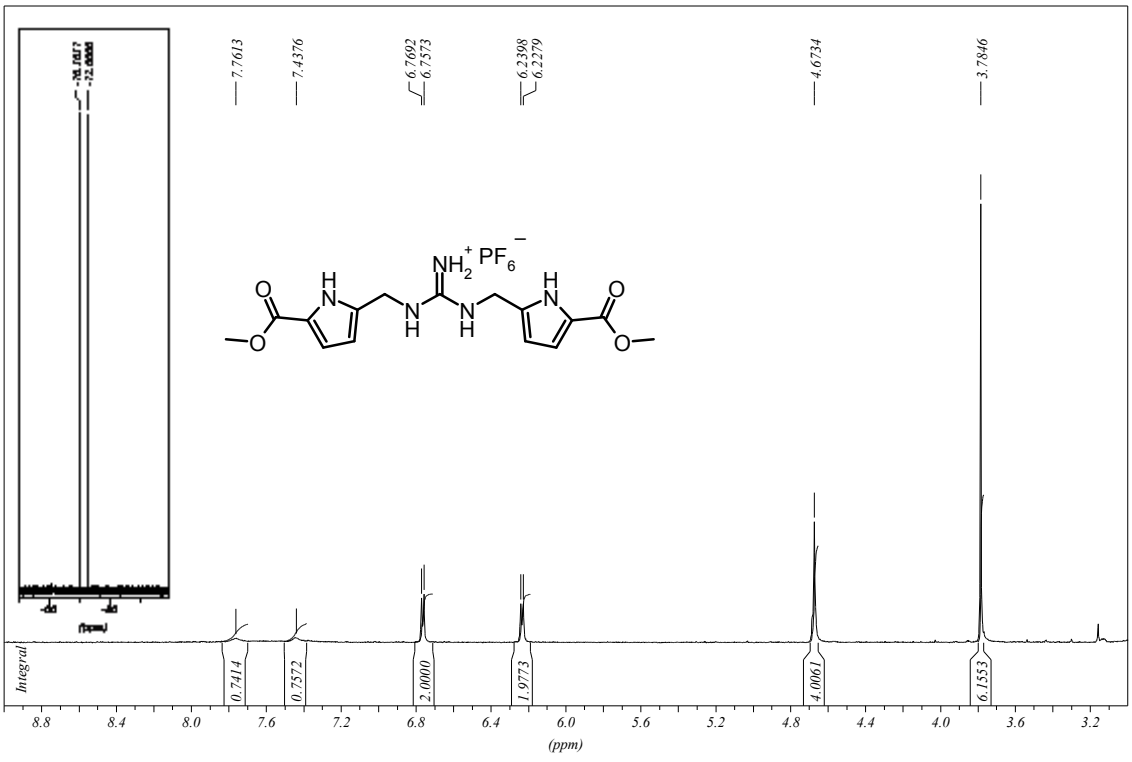


Figure 18: ^{1}H-NMR and ^{19}F-NMR spectrum (insert) of compound **23**

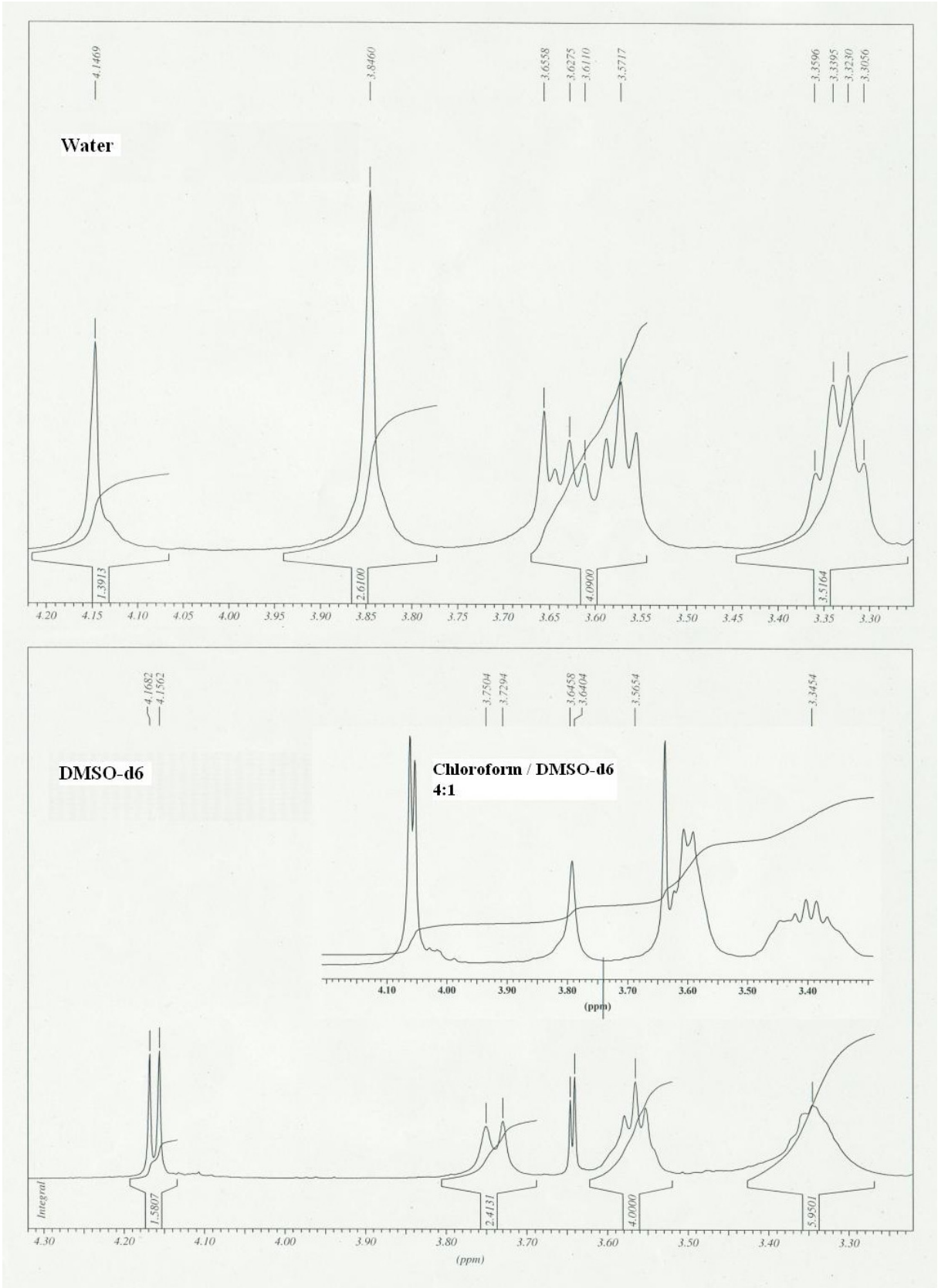


Figure 19: ^{1}H-NMR spectra of compound **6** in different solvents

VI. 4.3. General Methods and Receptor Titrations

Zwitterionic tweezers (General Procedure): The deprotected tweezer ligands were dissolved in water (0.01 molar). Compound **23** (acidic reaction, apparent pH of a dilute solution ~ 4 - 5) was carefully neutralized to pH 7 using not more than 1.1 eq. of $LiOH_{(aq)}$ (0.1 M). Compound **25** (basic reaction, apparent pH of a dilute solution ~ 8 - 9) was treated with dilute hydrochloric acid (0.1 M) in an analogue manner. The solutions were lyophilised to give the desired product as a white salt.

^{1}H-NMR Titrations: All salts and ligands were pre-dried under high vacuum and then kept under nitrogen in a glove box. DMSO-d6 of 99.8 % isotopic purity (Deutero), dried over molecular sieves 3 Å was used. All manipulations were carried out in the glove box under dry conditions and the NMR tubes were properly closed by a septum and parafilm. For the titration experiments, a Bruker Avance 300 (^{1}H: 300.1 MHz, ^{13}C: 75.5 MHz, T = 300 K) was used.

Stock solutions of the host molecule being studied were prepared in DMSO-d6 at a final concentration of $5.00*10^{-3}$ mmol/mL. Stock solutions (0.1 M) of *n*-butylammonium chloride or tetrabutylammonium acetate were prepared by dissolving approximately 10 eq. of the perchlorate salts in 0.5 mL of the host stock solution. From these solutions, 5 - 10 µL (0.2 – 0.4 equivalents) portions were titrated with the aid of a Hamilton syringe to the host stock solution of 0.5 mL and ^{1}H-NMR spectrum was recorded after shaking and a short equibrillation time. The shifts of the proton signals were monitored. The association constants were calculated from the changes in the chemical shifts of the protons of the guanidine (compound **23**) or the pyrrole (compound **25**). Nonlinear curve fitting for a 1:1 binding model was carried out with the ORIGIN program package.

Emission Spectroscopy. Fluorescence measurements were performed with UV-grade solvents (Baker or Merck) in 1 cm quartz cuvettes (Hellma) and recorded on a Varian 'Cary Eclipse' fluorescence spectrophotometer with temperature control.

Absorption Spectroscopy. Absorption were recorded on a Varian Cary BIO 50 UV/VIS/NIR Spectrometer with temperature control by use of a 1 cm quartz cuvettes (Hellma) and Uvasol solvents (Merck, Baker or Acros).

Screening. The fluorescence enhancement (F/F_0) was determined in a cuvette in three point measurements. The fluorescence intensity of the receptor ($3*10^{-5}$ M, 1.0 mL) in methanol / water (4:1 vol/vol, at pH 6.5 - 7) was recorded, the guest solution was added in excess (200 eq., 500 eq. and 1000 eq., 0.06 M), the solution was allowed to equilibrate and the emission intensity was recorded again. The obtained fluorescence intensity values were volume corrected and compared to the blanc sample. A binding event is registered at $F/F_0 > 1.1$.

Spectroscopic titrations: The binding constants were determined in a cuvette. To 1.0 mL of the receptor ($3*10^{-5}$ M) in methanol / water (4:1 vol/vol, at pH 6.5 - 7) or methanol were added small (≙ 1 to 20 eq.) aliquots of the guest solution (0.01 M). After each addition the solution was allowed to equilibrate and the emission intensity was recorded. To determine the binding constant the obtained fluorescence intensity values were volume corrected, plotted against the concentration of the guest and evaluated by non linear fitting methods.

All titration curves and the curve fitting for tetrabutylammonium acetate and amino acid guests can be found in the supporting information of this chapter.

VI. 5. References and Notes

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[32] The azoester (**9c**) **-** Cu(I) **-** TMEDA *(N,N,N′,N′*-tetramethylethylendiamine) couple proved it´s applicability in the synthesis of linear ether chains by the conversion of *N*-Boc-2-(2-aminoethoxy)-ethanol to the according analog to **7b** in good yield of 68 %.

[33] M.P. Groziak, L.B. Townsend, Org. Chem. 1986, 51, 1277-1282.; N.I. Martin, J.J. Woodward, M.A. Marletta *Org. Lett.* **2006**, *8(18)*, 4035-4038.; N.I. Martin, W.T. Beeson, J.J. Woodward, M. A. Marletta, *J. Med. Chem.* **2008**, *51*, 924–931.

[34] M.E. Lewellyn, S.S. Wang, P.J. Strydom, *J. Org. Chem.* **1990**, *55*, 5230-5231.; T. Suhs, B. König, *Chem. Eur. J.* **2006**, *12*, 8150-8157.

[35] A.R. Katritzky, N. Kirichenko, B.V. Rogovoy, J. Kister, H. Tao, *Synthesis* **2004**, *11*, 1799-1805 and literature therein.

[36] B.R. Linton, A.J. Carr, B.P. Orner, A.D. Hamilton, *J. Org. Chem.* **2000**, *65(5)*, 1566-1568.

[37] R. Esmail, F. Kurzer, *Synthesis* **1975**, 301-314.

[38] Two further, chiral building blocks – the diastereomers of a tetrahydrofurane amino acid alanine dipeptide – were converted by this method to the according thiourea and the symmetrically substituted guanidine with comparable good yields. The synthesis of these interesting molecules can be found in the supporting information. These tetrahydrofurane amino acid peptides building blocks (P. Maity, M. Zabel, B. König, *J. Org. Chem.* **2007**, *72*, 8046–8053) may open the way towards enantioselective recognition or may lead to interesting turn-inducing peptide like structures (P. Maity, B. König, *Pept. Sci.* **2007**, *90*, 8-27).

[39] Sterically demanding tetrahydrofuran amino acid alanine dipeptides were reacted under the same conditions in 6 h giving 64 – 71 % yield (see the supporting information).

[40] C. Bonauer, B. König, *Synthesis* **2005**, 2367–2372.; C. Bonauer, M. Zabel, B. König, *Org. Lett.* **2004**, *6*, 1349–1352.

[41] The route to this material starting from pyridine-2,6-dicarboxylic acid is literature known: T.H. Fife, T.J. Przystas, *J. Am. Chem. Soc*. **1982**, *104,* 2251-2257; R. Fornasier, D. Milani, P. Scrimin, U. Tonellato, *J. Chem. Soc. Perkins Trans II*, **1986,** 233-237. A complete, improved procedure is given in the supporting information.

[42] C. Schmuck, J. Lex, *Org. Lett.* **1999**, *1*, 1779-1781.; C. Schmuck, *Eur. J. Org. Chem.* **1999**, 2397-2403.; C. Schmuck, J. Dudaczek, *Tetrahedron Lett.* **2005**, *46*, 7101-7105.; C. Schmuck, *Coord. Chem. Rev.* **2006**, *250*, 3053–3067.; C. Schmuck, V. Bickert, *Org. Lett.* **2003**, *5*, 4579.; C. Schmuck, *Chem.–Eur. J.* **2000**, *6*, 709–718.; C. Schmuck, *Chem. Commun.*, **1999**, 843–844.

[43] G.M. Kyne, M.E. Light, M.B. Hursthouse, J. de Mendoza, J.D. Kilburn, *J. Chem. Soc., Perkin Trans. 1* **2001**, 1258–1263.; C.A. Hunter, D.H. Purvis, *Angew. Chem., Int. Ed.* **1992**, *31*, 792–795.; A. Ragusa, S. Rossi, J.M. Hayes, M. Stein, J.D. Kilburn, *Chem.–Eur. J.* **2005**, *5*, 5674–5688.; S.-Y. Chang, H.S. Kim, K.-J. Chang, K.-S. Jeong, *Org. Lett.* **2004**, *6*, 181–184.; C. Schmuck, U. Machon, *Chem.–Eur. J.* **2005**, *11*, 1109–1118.

[44] A mixed reaction, leading to a product with one ester and one free acid is easily possible, but it is already more difficult to purify the product. Employing two times the free acid leads to the same substitution on the guanidine without side reactions, bur purification is not possible without reversed phase column material.

[45] The mercury(II)-ion does not coordinate too strongly to the tweezer with the protected ester side chains. It can be removed by simple solvent extraction. Remaining traces of mercury can be separated from the now clean and nontoxic product by column chromatography. Caution! For the free bis acid a different behaviour is observed: The mercury(II)-ion binds to the built tweezer strongly and cannot be removed from it. These complexes are in part transported even over silica gel columns, leading to toxic products! EDC is always the better choice for catalyzing these reactions.

[46] M.A. Poss, E. Iwanowicz, J.A. Reid, J. Lin, Z. Gu, *Tetrahedron Lett.* **1992**, *33*, 5933-5934.; N.I. Martin, R.M.J. Liskamp, *J. Org. Chem.* **2008**, *73*, 7849–7851.

[47] Kilburn observed this with a similar amide compound. The imidazo[1,5-a]pyridine derivatives were the only product upon reaction of the corresponding thiourea precursors with amines: R.J. Fitzmaurice, F. Gaggini, N. Srinivasan, J.D. Kilburn, *Org. Biomol. Chem*. **2007**, 5, 1706–1714.; see also: J. Bourdais, A. Omar, M.E. Mohsen, *J. Heterocycl. Chem.* **1980**, *17*, 555–558.

[48] C. Bonauer, B. König, *Synthesis* **2005**, 2367–2372.; C. Bonauer, M. Zabel, B. König, *Org. Lett.* **2004**, *6*, 1349–1352.

[49] The route to this material starting from pyridine-2,6-dicarboxylic acid is literature known: T.H. Fife, T.J. Przystas, *J. Am. Chem. Soc*. **1982**, *104,* 2251-2257; R. Fornasier, D. Milani, P. Scrimin, U. Tonellato, *J. Chem. Soc. Perkins Trans II*, **1986,** 233-237. A complete, improved procedure is given in the supporting information.

[50] J.A. Martin, L. Ozores, B. List, J. Am. Chem. Soc. **2007**, *129*, 8976-8977.

[51] S. Bartoli, K.B. Jensen, J.D. Kilburn, *J. Org. Chem.* **2003**, *68*, 9416-9422.

[52] Before measurement both materials were neutralized with lithium hydroxide or hydrochloric acid to guarantee the zwitterionic forms at defined pH (see pK_a titrations in the supporting information). The according esters (**22b** and **24**) are employed for comparison of the carboxylate binding ability, as it is expected for guanidinium ions.

[53] R. Schwesinger, *Chimica* **1985**, *39*, 269.

[54] R.M.C. Dawson, Data for Biochemical Research, Oxford, Clarendon Press, **1959**.

[55] H.K. Hall, Jr., *J. Am. Chem. Soc.* **1957**, *79*, 5441-5444.

[56] J.F.J. Dippy, S.R.C. Hughes, A Rozanski, *J. Chem. Soc.* **1959**, 2492-2498.

[57] According to Bordwel, primary amines have pK_a values of ~ 40 (*J. Org. Chem.* **1981**, *46*, 632.), carboxy acids of ~ 11 - 12 (*J. Org. Chem.* **1976**, *41*, 2507., *J. Org. Chem.* **1980**, *45*,3299.) and guanidines of ~ 28 - 30 (*J. Am. Chem. Soc.* **1991**, *113*, 8398.)

[58] All quantum yields were determined with quinine disulfate in 1 N H_2SO_4 as the reference compound (Φ = 0.546).

[59] B. Linton, A.D. Hamilton, *Tetrahedron* **1999**, *55(19)*, 6027-6038 and literature therein.

[60] This value has to be taken with care, as the guanidinium proton signals significantly broaden with rising guest amounts added, leading to relatively high errors of ~ 20 %. A just sufficient number of points to reach saturation could be extracted to fit the data, before the signals were lost.

[61] This is entropically favoured: In average, the fixation of the carboxylate groups are less strongly rigidified leading to a gain in entropy by the dynamic exchange.

[62] T. Steiner, *Angew.Chem. Int. Ed.* **2002**, 41, 48-76.

[63] A. Pullman, *Chem. Rev.* **1991**, *91*, 793-812.

[64] The depicted schemes are illustrative pictures, the modeling results can be found in the supporting information.

[65] See introduction in: J.V. Aldrich, K. Vivek, United States Patent 7038078

[66] F.M. Veronese, G. Pasut; *Drug Discov Today*. **2005**, *10,* 1451-1458.; K.C. Lee, K.K. Tak, M.O. Park, *Pharm Dev Technol*. **1999**, *4*, 269-275.

[67] See *J. Med. Chem.* **1989**, *32*, 391-396.; This material is also commercially available. It is easily prepared by Boc protection at room temperature with 0.3 equivalents Boc anhydride in DCM. After the reaction (4 h), the organic solution is washed several times with brine, dried and evaporated to give the product high purity and nearly quantitative yield.

[68] This material is commercially available. It is easily prepared by dropping slowly 0.3 equivalents benzylorthochloroformate in DCM in an icecold solution of ethanolamine under vigorous stirring. After the reaction (3 h), the organic solution is washed several times with brine, dried and evaporated to give the product high purity and excellent yield.

[69] P. Garner, J.M. Park in *Organic Syntheses* **1998,** *Coll. Vol. 9*, 300-305.

[70] D. Srinivasa Reddy, D. Vander Velde, J. Aube, *J. Org. Chem.* **2004**, *69*, 1716–1719.

[71] Commercially available; For easy preparation, glycyl glycine (10.0 mmol) were suspended in methanol (200 mL) and trimethylchlorosilane (12.0 mmol) was dropped in the well stirred mixture at 0 °C over 1 h. Stirring was continued for 4 h slowly coming to room temperature. The solvent was evaporated, the residue thoroughly dried and the crude material was recrystallised from a small amount of methanol. The yield is nearly quantitative.

[72] Literature known [see 48]; Can be easily prepared by suspending 2,6-pyridine dicarboxylic acid an 3 equivalents of potassium carbonate in an excess of dried ethanol and slowly dropping in 3 equivalents of thionyl chloride under vigourous stirring. After stirring at room temperature for 3 h and short heating to reflux, the solvent is evaporated to give the ester in nearly quantitative yield.

[73] C. Bonauer, *PhD thesis* **2004***,* University of Regensburg.

[74] Instead of EDC, mercury(II)chloride can be used in this reaction. DCM has to be replaced by DMF then. For example: The benzyloxycarbonylthiourea (0.5 mmol) was added to a solution of its according amine compound (0.6 mmol) and triethylamine (130 mg, 0.17 mL, 1.3 mmol) in DMF (5 mL). Mercury(II)-chloride (170 mg, 0.62 mmol) was added and the reaction mixture was stirred for 15 h at room temperature. The mixture was filtered over Celite and the solvent was removed under reduced pressure. The crude product was dissolved in H_2O and acidified with 5 % $KHSO_4$ to pH = 3. The aqueous layer was extracted three times with EtOAc. The combined organic phases were dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product was purified by column chromatography.

VII. Metal-catalyzed Derivatization of C^{α}-tetrasubstituted Amino Acids and Their Use in the Synthesis of Cyclic Peptides[i]

C^{α}-Tetrasubstituted amino acids are important building blocks in the design and preparation of novel peptidomimetics.[ii] We report the functionalisation of the C^{α}-tetrasubstituted THF amino acid *rac*-**5** by copper(I) catalyzed *N*-arylation reactions. The aryl bromide substituent of *rac*-**5** is replaced by a variety of aliphatic and aromatic amines.[iii] Intramolecular *N*-arylation yielded only small amounts of a cyclic tripeptide **2**, while cyclic tripeptide ethers **4** and **50** were obtained enantiomerically pure from a palladium(0)-catalyzed intramolecular *O*-arylation.[iv]

Abstract in Chinese

摘要: 在设计和制备新的肽模拟物过程中, α-碳四取代氨基酸是重要的合成砌块。我们在这里报道通过铜催化的 N-芳基化反应使α-碳四取代THF类氨基酸*rac*-**5**官能团化。*rac*-**5**中的芳基溴单元可以被大量的脂肪胺或芳香胺所取代。分子内的 N-芳基化反应只给出少量的环三肽 **2**，而带有醚键的环三肽 **4** 和 **50** 可以通过钯催化的分子内 O-芳基化反应以光学纯的形式得到。

Acknowledgments

We would like to thank Prof. Diederich and her group from the Philipps-University Marburg for testing the cyclic compounds in their HIV 1 protease inhibitory assay and Dr. Manfred Zabel from the University of Regensburg for providing the X-ray crystal structures.

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[i] A. Grauer, A. Späth, D. Ma, B. König, *Chem. Asia. J.* **2009**, *4*, 1134-1140.

[ii] The tetrahydrofurane amino acids as starting materials were prepared by A. Grauer after literature known prescriptions.

[iii] Reactions were carried out in collaboration with A. Grauer at the Shanghai Institute for Organic Chemistry (SIOC) under the supervision of Prof. D. Ma.

[iv] All cyclisation reactions were performed by A. Grauer.

VII. 1. Introduction

The design and synthesis of small molecules which mimic natural peptide sequences have attracted much attention from organic, bioorganic and peptide chemists.[1] This is due to numerous advantages of peptidomimetics over short natural sequences, such as greater steric constrains leading to a more pronounced secondary structure and a higher biological and chemical stability. [2]

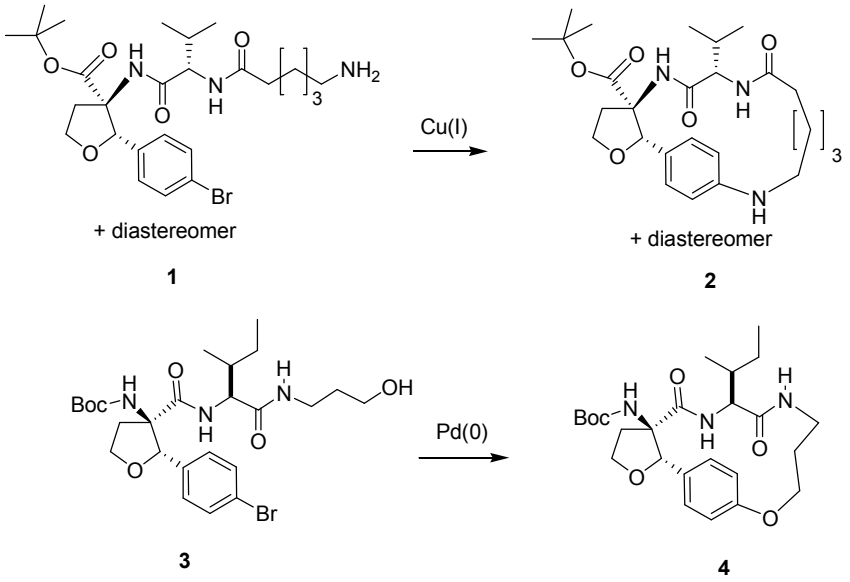
Many different ways are known for the synthesis of peptidomimetics[3] among which the preparation of cyclic peptides has received considerable attention. Cyclic peptides occur in a variety of natural bioactive compounds and they are of great interest for synthetic chemists and biologist, because they often show higher in vivo stability and reduced conformational mobility than their linear counterparts thus making them potential drug candidates.[4] A large number of cyclic peptides was designed and synthesized in the last years with some being biologically active natural products,[5] while others are mimics of natural peptide sequences like for example the large group of bio-active analogues of the natural RGD-sequence.[6] Cyclic peptidomimetics are also used as inhibitors for enzymes like HIV 1 proteases.[7]

Another strategy to peptidomimetics with enhanced proteolytic stability compared to their natural analogues uses unnatural constrained amino acids like C^{α}-tetrasubstituted α-amino acids which are incorporated into the peptide sequence.[8] A large number of such unnatural cyclic or acyclic amino acids with and without a stereocentre at the C^{α}-carbon has been reported[9] and used in the synthesis of peptidomimetics resulting in stable helices[10] and turn-like structures[11] of which some show biological activity.[12] One example of such a cyclic C^{α}-tetrasubstituted amino acid is the tetrahydrofuran derivative *rac*-**5** developed in our group, which introduces β-turn structures in tripeptides.[13] Despite the many known examples of cyclic α-amino acids and their applications as structure inducing elements in peptide strands,[14] to the best of our knowledge cyclic C^{α}-tetrasubstituted α-amino acids have not been used in the synthesis of cyclic peptidomimetics so far.

Cu^{I}-catalyzed *N*-arylations have proven to be a very versatile method for the conversion of aryl bromides providing the *N*-aryl products in good yields.[15] In recent years this methodology was also used for the synthesis of a variety of natural products and their analogues or substructures.[16]

Hence, we developed a methodology for the Cu^{I}-catalyzed *N*-arylation reaction of the C^{α}-tetrasubstituted tetrahydrofuran amino acid *rac*-**5** with a variety of different amines and used it to link the *N*-terminus of a tripeptide with the side chain aryl halide of *rac*-**5**

incorporated in the sequence (Scheme 1, **1** → **2**). A cyclic tripeptide mimetic containing an aromatic ether was obtained by an O-arylation reaction using palladium catalysis (Scheme 1, **3** → **4**).[17]

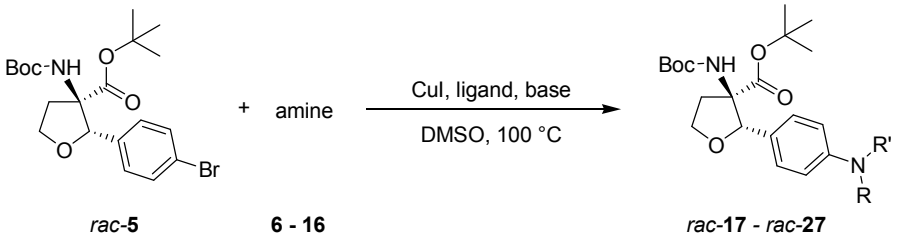


1 **2** **3** **4**

Scheme 1: Synthesis of cyclic peptide mimics by an *N*-arylation reaction using copper(I) (top) or by an *O*-arylation reaction using palladium(0) (bottom)

VII. 2. Results and Discussion

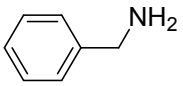
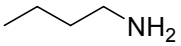
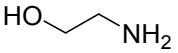
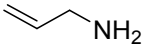
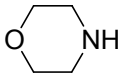
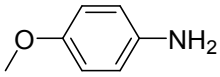
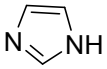
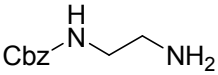
Initially, we explored the scope for Cu^{I}-catalyzed reactions of the unnatural amino acid *rac*-**5** with aliphatic amines. Previous studies served as a starting point for the optimization experiments.[18] The ligands *L*-proline **A** and *N,N*-dimethylglycine **B** were found to be good promoters for the Cu^{I}-catalyzed reaction. K_3PO_4 and K_2CO_3 were found to be suitable bases.



rac-**5** **6 - 16** *rac*-**17** - *rac*-**27**

Scheme 2: C-N bond formation reactions catalyzed by a combination of CuI and *L*-amino acids as ligands.

In Table 1, entries 1 to 5 summarize the reaction conditions for the model reaction of benzylamine with the unnatural amino acid *rac*-**5**. These results illustrate that when using K_3PO_4 as base with 10 mol% of catalyst and 20 mol% ligand **A** at 100 °C, a yield of about 50 % was obtained. The use of larger amounts of CuI in combination with ligand **A** gave no better results. Decreasing the temperature to about 50 °C resulted in no product formation (entry 3). A significant increase in yield by about 20 % was obtained when the base was changed from K_3PO_4 to the more basic K_2CO_3 (entry 4). Substitution of ligand **A** by ligand **B** while retaining the other conditions, gave the same amount of product (entries 4 and 5).
In entries 6 to 9 three different primary amines with and without functional groups were used. Butylamine was coupled in good yields to *rac*-**5** (entry 6). The reaction was conducted at a tenfold higher dilution (entry 7) to test the conditions of the cyclisation reaction for which a higher dilution is necessary to favour the intramolecular cyclisation reaction and to avoid polymerization. The product was obtained, although in poor yield. Entry 8 shows that 2-aminoethanol selectively undergoes the *N*-arylation reaction without an adverse effect of the unprotected hydroxyl moiety of the amino alcohol. This reaction and entry 9 show that amines containing different functional groups are compatible with the applied reaction conditions. Another example is shown in entry 21 where a mono-Cbz-protected alkyl diamine was successfully coupled introducing a second amine functionality with an orthogonal protecting group. Secondary amines were also tried as reaction partners for the amino acid *rac*-**5** but their increased steric hindrance decreased their reactivity: diethylamine (entries 10 to 12) showed no conversion. Neither the change from ligand **A** to **B** nor from the base K_2CO_3 to K_3PO_4 gave any product. Morpholine (entries 13 and 14) gave about 30 % yield. The yield did not increase with larger amounts of catalyst and ligand. Pyrrolidine (entry 15) produced over 70 % yield; the reactivity of diethyl amine and pyrrolidine differ significantly, explainable by the different geometry of the two amines.[18a] The formation of diarylamines was examined using *p*-anisidine together with 10 mol% CuI leading to the diphenylamine in about 50 % yield. The heterocyclic imidazole was coupled to *rac*-**5** under CuI catalysis giving the product in 45 % yield when using 10 mol% CuI and in a slightly higher yield when using 30 mol% CuI. Furthermore, amino acid esters (entry 20) can be coupled in the Ullmann-type reaction in moderate yields. The amino acid ester hydrochloride must be neutralized by extraction under basic conditions using sat. aqueous $NaHCO_3$ solution and ethyl acetate prior to use, because the salt itself was found to be unreactive under the applied reaction conditions, even with large excess of an inorganic base (entries 19 and 20).

entry	amine	CuI (eq.)	ligand	base	product	yield (%)
1	 **6**	0.1	A	K_3PO_4	*rac*-**17**, R = benzyl, R´= H	49
2		0.2	A	K_3PO_4		47
3		0.1	A	K_3PO_4		-[(a)]
4		0.1	A	K_2CO_3		70
5		0.1	B	K_2CO_3		70
6	 **7**	0.1	A	K_3PO_4	*rac*-**18**, R = Bu, R´= H	65
7		0.1	A	K_3PO_4		12[(b)]
8	 **8**	0.1	A	K_2CO_3	*rac*-**19**, R = C_2H_4OH, R´= H	42
9	 **9**	0.1	A	K_2CO_3	*rac*-**20**, R = allyl, R´= H	54
10	 **10**	0.1	A	K_2CO_3	*rac*-**21**, R, R` = Et	-
11		0.1	A	K_3PO_4		-
12		0.1	B	K_2CO_3		-
13	 **11**	0.1	A	K_2CO_3	*rac*-**22**, R-R´ = $O(C_2H_4)_2$	31
14		0.3	A	K_2CO_3		33
15	 **12**	0.1	A	K_2CO_3	*rac*-**23**, R-R´= C_4H_8	73
16	 **13**	0.1	A	K_2CO_3	*rac*-**24**, R = p-MeO-C_6H_4, R´= H	49
17	 **14**	0.1	A	K_2CO_3	*rac*-**25**, NRR´ = imidazole	45
18		0.3	A	K_2CO_3		49
19	H-Gly-OMe*HCl **15-HCl**	0.1	A	K_2CO_3		-[(c)]
20	H-Gly-OMe **15**	0.1	A	K_2CO_3	*rac*-**26**, R = Gly-OMe, R´= H	13
21	 **16**	0.1	A	K_2CO_3	*rac*-**27**, R = C_2H_4NHCbz, R´= H	23

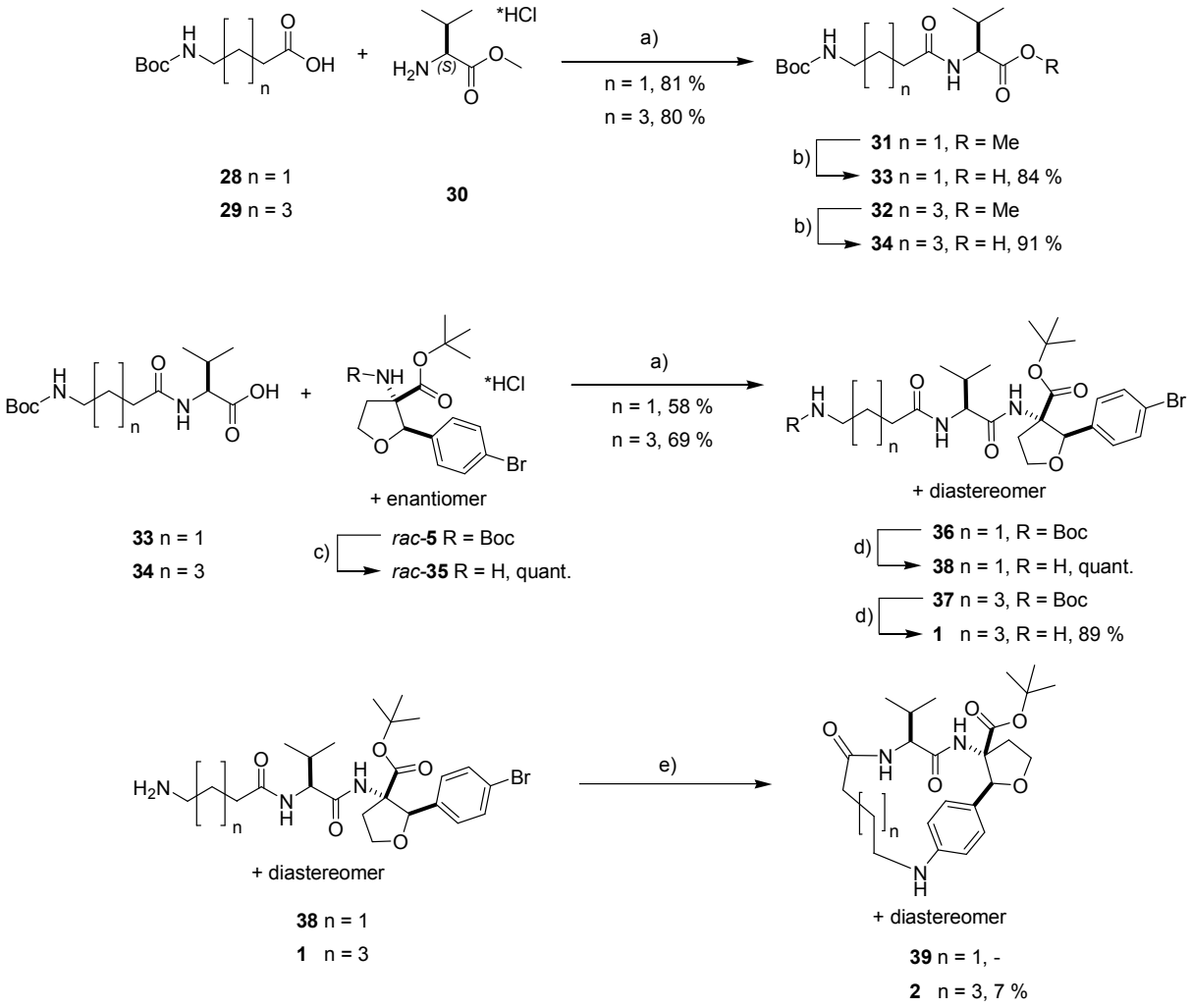
All reactions, unless otherwise indicated, were performed using 1 eq. *rac*-**5**, 1.5 eq. amine, 2 eq. base, 10 mol% CuI (based on the *rac*-**5**), 20 mol% ligand (based on the *rac*-**5**), c = 1 mol/L *rac*-**5** in dry DMSO, 100 °C, 48 hours; (a) reaction at 50 °C; (b) the reaction was performed at a concentration c = 0.1 mol/L of *rac*-**5** in dry DMSO; (c) the reaction was performed using 4 eq. of base

Table 1: CuI catalyzed C-N bond formation reaction conditions and yields.

All reactions shown in Table 1 where performed under conventional heating in an oil bath and required about 2 days to come to completion. Microwave irradiation has been used as an alternative energy input reducing in some cases the required reaction time.[19] For CuI promoted C-N bond formations one example of microwave heating has been reported.[20] Test reactions were performed using imidazole or benzylamine and *rac*-**5** and reaction times of 20-60 min, but the highest yield obtained under these conditions was 15 %. Hence, microwave heating does not improve the reaction in our case.[21] We conclude that it is possible to couple a variety of different mono- or dialkylated amines to *rac*-**5** in moderate to good yields up to 70 % using 10 mol% CuI as catalyst and *L*-amino acid ligands.

The cyclic tripeptide **2** and its diastereomer were synthesized applying the developed C-N bond formation reactions. A 15-membered ring system should result from the reaction of unnatural amino acid *rac*-**5**, *L*-valine **30** and 4-aminobutanoic acid **28**, while a 17-membered ring is expected if 6-aminohexanoic acid **29** was used instead of 4-aminobutanoic acid **28**.

Both tripeptides were synthesized using standard peptide coupling reactions starting from the Boc-protected amino acids **28** or **29** which were coupled with *L*-valine methyl ester **32** to afford the dipeptides **31** and **32**. After ester saponification using LiOH as base, the resulting dipeptide acids **33** and **34** were coupled to the Boc-deprotected TAA *rac*-**35**. Both tripeptides were obtained in good yields, but as a 1:1 mixture of diastereomers, because the racemic amine *rac*-**35** was used. A chromatographic separation of the diastereomers was not possible after this step of the synthesis. The following Boc-removal of compounds **36** and **37** was accomplished by the use of 8 % TFA solution in dry DCM. Higher concentrations of TFA or the use of HCl saturated diethyl ether lead to a partial cleavage of the *tert*-butyl ester moiety. The obtained diastereomeric mixtures of the amines **1** and **38** were used in the CuI catalyzed cyclisation reaction (10 mol% CuI, 20 mol% *L*-proline, K_2CO_3, DMSO) after deprotonation by a strongly basic ion exchange resin. The concentration of the starting materials in the reaction mixture was lowered for the cyclisation reactions form c = 1 mol/L to 0.1 mol/L to prevent the deprotected precursors **1** and **38** from intermolecular polymerisation instead of the desired intramolecular cyclisation.



Scheme 3: Reaction sequence leading to the cyclic tripeptides **2** and **39**: *a) DIPEA, EDC, HOBt, dry DMF, 24 h, RT; b) LiOH, water / THF, 24 h, RT; c) HCl sat. diethyl ether, DCM, 24 h, RT; d) 8 % TFA, dry DCM, 3 - 4 h, RT; e) CuI, L-proline, K_2CO_3, DMSO, 48 h, 100 °C.*

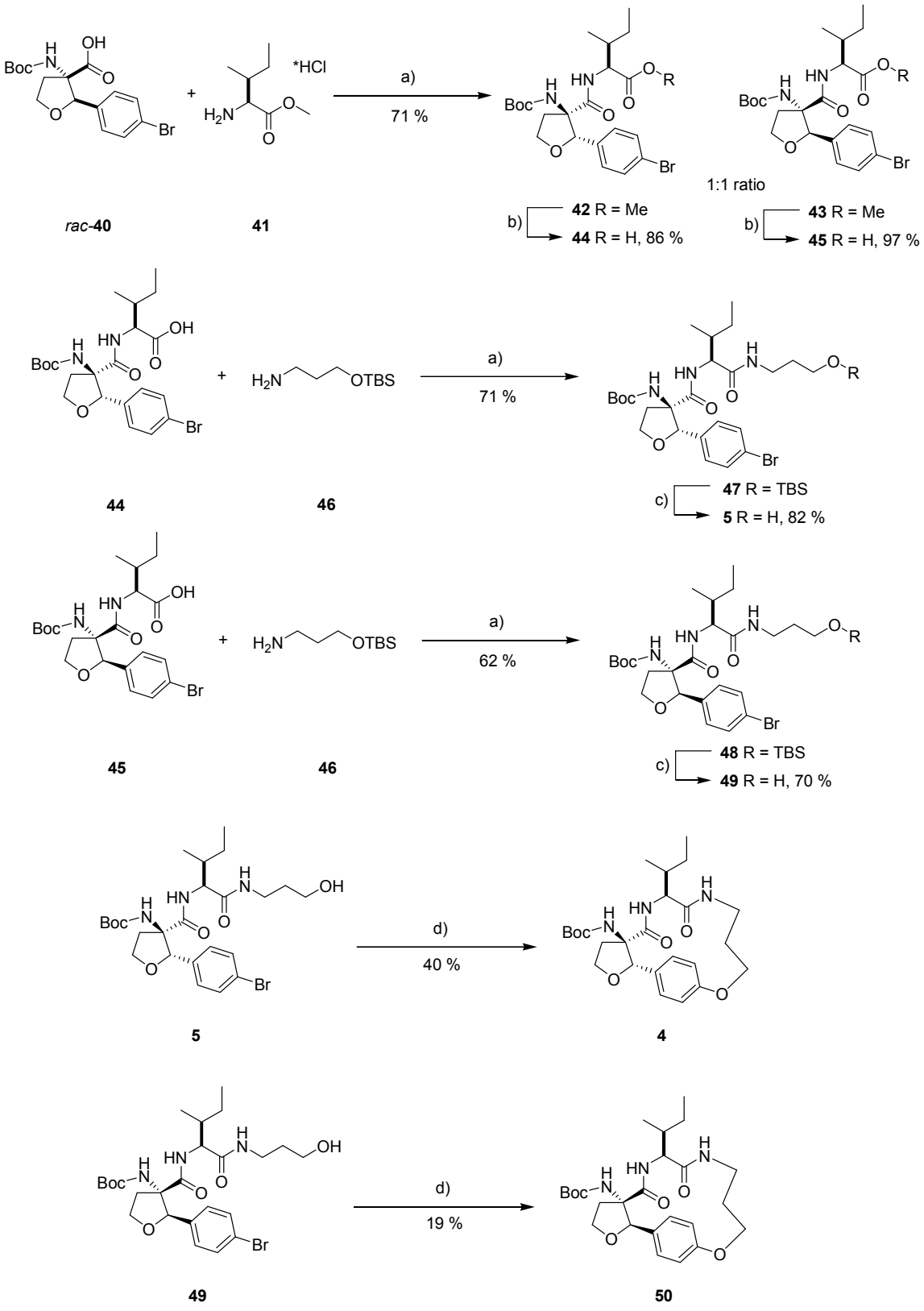
The shorter amine **38** was found to be unreactive under the applied conditions. Compound **1** gave product **2** although only in 7 % yield.[22]

The synthesis of cyclic peptides through a C-O arylation reaction was tested by the formation of a 15-membered ring system shown in Scheme 4. The group of Fairlie used a cyclic tripeptide mimic consisting of Phe, Ile and 3-bromopropan-1-amine to substitute the Phe-Ile-Val motif in the peptide Ac-Leu-Val-Phe-$CHOHCH_2$-{Phe-Ile-Val}-NH_2, which is a known inhibitor of HIV-1 protease derived from a substrate sequence. The substitution of the natural tripeptide by the unnatural cyclic group resulted in an enhanced inhibitory activity.[23]

Our goal was to synthesize a similar tripeptide macrocycle by the use of the unnatural amino acid *rac*-**40** instead of Phe leading to an even higher conformational constraint and to a more rigid secondary structure.

To form the acyclic precursors for the C-O arylation reaction, Boc-TAA-OH *rac*-**40** was coupled to *L*-isoleucine methyl ester hydrochloride **41** using EDC and HOBt as coupling reagents. Due to the racemic amino acid *rac*-**40,** two diastereomers **42** and **43** were formed, which could be easily separated by column chromatography. Crystals of compound **42** were obtained and an X-ray analysis was performed which revealed the absolute configuration (see Figure 1).[24] The subsequent reaction steps were carried out separately with both diastereomers leading to two stereochemically different macrocycles with different secondary structures. After ester saponification the resulting free acids **44** and **45** were coupled with 3-(*tert*-butyldimethylsilyloxy)propan-1-amine **46** using TBTU and HOAt as coupling reagents. The protected precursors **47** and **48** were formed in good yields. After deprotection of the TBS-group they were used in the metal-catalyzed cyclisation reaction. As catalytic system, $Pd(OAc)_2$ with dicyclohexyl(2',4',6'-triisopropylbiphenyl-2-yl)phosphine as ligand and Cs_2CO_3 as base were chosen. The reaction was conducted under nitrogen atmosphere at 80 °C in dry toluene. These reaction conditions were adopted from literature by changing the ligand to the dicyclohexyl(2',4',6'-triisopropylbiphenyl-2-yl)phosphine.[25] The use of a bulky ligand is important to prevent a β-hydride elimination in the temporarily formed oxidative addition product. Another crucial reaction parameter is the concentration of the starting materials: When the reaction mixture was diluted the yield dropped drastically, while high concentrations resulted in the formation of cyclic dimers instead of the intramolecular ring closing reaction.

The best results were obtained at a concentration of c = 25 mmol/L of the starting material (**3** or **49**). These reaction conditions yielded 40 % for compound **4** and about 19 % of its diastereomer **50**. The stereochemistry of both compounds (**4** and **50**) was confirmed by 2D-NMR spectroscopy.[26]



Scheme 4: Reaction sequence scheme leading to the macrocycles **4** and **50**: *a) DIPEA, TBTU, HOAt, dry DMF, 24 h, RT; b) LiOH, H_2O, THF, 24 h, RT; c) CH_3COOH, 1M TBAF in THF, MeCN, 12 h, 0 °C - RT; d) $Pd(OAc)_2$, dicyclohexyl(2',4',6'-triisopropylbiphenyl-2-yl)phosphine, Cs_2CO_3, toluene, 24 h, 80 °C.*

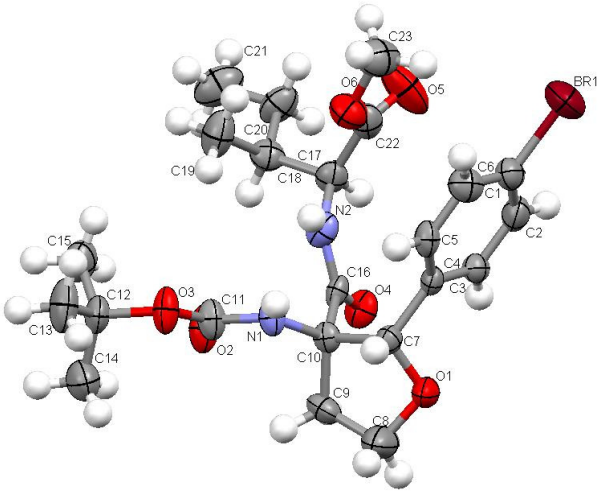


Figure 1: Structure of compound **20** in the solid state as determined by X-ray analysis.

VII. 3. Conclusion

The Cu^{I}-catalyzed *N*-arylation of *rac*-**5** proceeds in moderate to good yields with a variety of amines, allowing to introduce aliphatic aldehydes and different functional groups containing amines into the side chain of the protected TAA *rac*-**5**. An intramolecular Cu^{I}-catalyzed *N*-arylation reaction was used for the synthesis of the 17-membered macrocycle **2**, but the obtained yield was low. Pd^{0}-catalyzed *O*-arylations gave higher yields for the macrocylization of non-natural tripeptides containing TAA *rac*-**5**: The 15-membered macrocycle **4** was obtained in 40 % isolated yield using $Pd(OAc)_2$ and a sterically demanding ligand. The examples illustrate that copper(I)-catalyzed *N*-arylations and palladium(0)-catalyzed *O*-arylations permit access to side chain modified derivatives of the unnatural amino acid *rac*-***5*** and macrocyclic peptidomimetics.

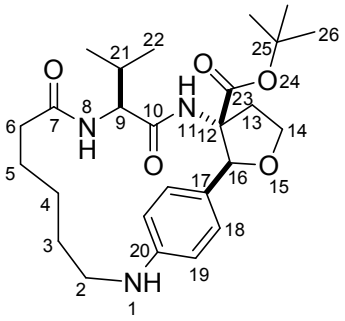
VII. 4. Experimental

VII. 4.1. General Procedure for the Cu^{I} catalyzed *N*-arylation (GP I):

An oven-dried Schlenk tube equipped with a Teflon septum was charged with a magnetic stir bar. The tube was evacuated and backfilled with argon three times. Compound *rac*-**5** (1 eq.),

the inorganic base K_2CO_3 or K_3PO_4 (2 eq.), CuI (10 - 30 mol%), the appropriate amino acid A or B (20 – 60 mol%) and the amine (if solid, 1.5 eq.) were added. The tube was again evacuated and backfilled with argon (this procedure was repeated three times). Under a counter flow of argon the amine (if liquid, 1.5 eq.) and the solvent DMSO (1 - 10 mL/mmol of compound *rac*-**5**, dry) were added via syringe. The tube was sealed and heated under stirring at the indicated temperature (50 – 100 °C) for 48 hours. After completion of the reaction the mixture was allowed to cool to room temperature and was then quenched with water (2 mL/mmol *rac*-**5**). The mixture was extracted three times with ethyl acetate (5 mL/mmol *rac*-**5**, each), the combined organic layers were dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure to give the crude product. Purification was accomplished by the use of column chromatography with the solvent mixture indicated for each reaction.

*Compound **2**:*



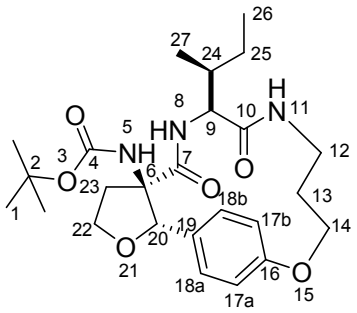
The synthesis followed *GP1* using compound **1** (40 mg, 0.07 mmol), K_2CO_3 (20 mg, 0.14 mmol), CuI (3 mg, 0.01 mmol), *L*-proline (3 mg, 0.03 mmol) and 0.7 mL DMSO. The crude product was purified using a 6:4 mixture of PE and EtOAc (R_f = 0.09) giving the product as a colourless solid in 7 % yield (2.4 mg, 0.004 mmol). R_f = 0.09 (PE / EtOAc, 6:4).

^{1}H-NMR (600 MHz, COSY, $CDCl_3$): δ [ppm] = 0.42 - 0.47 (m, 1 H; 4), 0.74 - 0.84 (m, 1 H; 4), 0.91 (d, $^3J_{H,H}$ = 6.6, 3 H; 22), 0.97 (d, $^3J_{H,H}$ = 7.0, 3 H; 22), 1.20 - 1.25 (m, 1 H; 5), 1.40 (s, 9 H; 26), 1.44 – 1.51 (m, 1 H; 5), 1.58 - 1.65 (m, 1 H; 3), 1.74 - 1.84 (m, 1 H; 3), 2.00 (ddd, $^3J_{H,H}$ = 3.1, $^3J_{H,H}$ = 9.7, $^2J_{H,H}$ = 16.8, 1 H; 6), 2.18 (ddd, $^3J_{H,H}$ = 3.3, $^3J_{H,H}$ = 6.8, $^2J_{H,H}$ = 16.6, 1 H; 6), 2.46 (q, $^3J_{H,H}$ = 10.2, 1 H; 13), 2.57 - 2.64 (m, 1 H; 21), 2.94 (ddd, $^3J_{H,H}$ = 3.5, $^3J_{H,H}$ = 8.5, $^2J_{H,H}$ = 12.4, 1 H; 13), 3.01 (dd, $^3J_{H,H}$ = 8.4, $^2J_{H,H}$ = 13.4, 1 H; 2), 3.35 (d, $^3J_{H,H}$ = 8.9, 1 H; 9), 3.56 (dd, $^3J_{H,H}$ = 8.1, $^2J_{H,H}$ = 13.4, 1 H; 2), 4.22 (ddd, $^3J_{H,H}$ = 3.3, $^3J_{H,H}$ = 7.6, $^2J_{H,H}$ = 10.9, 1 H; 14), 4.36 (q, $^3J_{H,H}$ = 8.1, 1 H; 14), 5.00 (s, 1 H; 16), 5.70 (d, $^3J_{H,H}$ = 7.7, 1 H; 8),

7.35 - 7.52 (m, 4 H; 18 + 19), 7.93 (s, 1 H; 11); - **^{13}C-NMR** (150 MHz, HSQC, HMBC, $CDCl_3$): δ [ppm] = 19.1 (+, 1 C; 22), 19.7 (+, 1 C; 22), 23.2 (-, 1 C; 5), 26.1 (-, 1 C; 3), 27.3 (-, 1 C; 4), 27.3 (+, 1 C, 21), 28.0 (+, 3 C; 26), 34.1 (-, 1 C; 6), 36.2 (-, 1 C; 13), 50.3 (-, 1 C; 2), 65.9 (-, 1 C; 9), 67.7 (-, 1 C; 14), 69.1 (C_{quat}, 1 C; 12), 82.8 (C_{quat}, 1 C; 25), 84.0 (+, 1 C; 16), 122.3 (+, 2 C; 19), 129.8 (+, 2 C; 18), 135.5 (C_{quat}, 1 C; 17), 136.9 (C_{quat}, 1 C; 20), 169.4 (C_{quat}, 1 C; 23), 172.8 (C_{quat}, 1 C; 10), 173.2 ppm (C_{quat}, 1 C; 7); - **MS** (LCMS, MeCN/H_2O+0.1% TFA): m/z (%) = 418.0 (37, $(M+H-C_4H_8)^+$), 474.1 (100, $M+H^+$), 515.1 (44, $(M+H+MeCN)^+$).

VII. 4.2. Cyclisation by Pd catalyzed *O*-arylation:

*Compound **4**:*

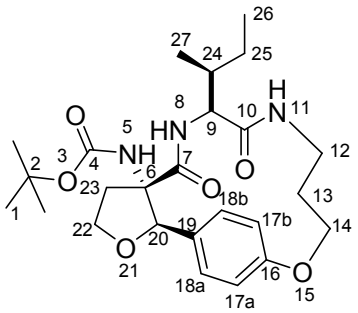


Compound **3** (100 mg, 0.18 mmol, 1 eq.), Cs_2CO_3 (52 mg, 0.27 mmol, 1.5 eq.), $Pd(OAc)_2$ (4 mg, 0.02 mmol, 0.1 eq.) and dicyclohexyl(2',4',6'-triisopropylbiphenyl-2-yl)phosphine (11 mg, 0.02 mmol, 1.25 eq.) were put into an oven dried Schlenk tube. The reaction vessel was evacuated and flushed with argon twice. To the mixture 7.2 mL of dry toluene (40 mL/mmol) were added and the tube was closed with a glass stopper and sealed. The mixture was heated under stirring to 80 °C for 24 h. The mixture was filtered and washed with 10 mL of ethyl acetate twice. The organic layers were concentrated under reduced pressure. The crude product was then purified by column chromatography on flash silica gel using a solvent gradient starting from diethyl ether / ethanol 98:2 up to 95:5 (EtOAc, R_f = 0.20). In a second purification step the compound was submitted to HPLC using a phenomenex Luna C18 and an acetonitrile / H_2O (0.0059 % TFA) gradient (5 % - 98 % acetonitrile) to give the product as colorless solid (34 mg, 0.07 mmol, 40 %). R_f = 0.20 (diethyl ether / EtOH, 98:2).

M.p. (uncorrected): 146 - 147°C; - **^{1}H-NMR** (600 MHz, COSY, NOESY, $CDCl_3$): δ [ppm] = 0.72 (d, $^3J_{H,H}$ = 6.6, 3 H; 27), 0.81 (t, $^3J_{H,H}$ = 6.9, 3 H, 26), 0.87 - 0.97 (m, 1 H; 25a), 1.35 -

1.42 (m, 1 H; 25b), 1.45 (s, 9 H; 1), 1.53 - 1.60 (m, 1 H; 24), 1.68 (bs, 1 H; 13a), 2.09 (ddd, $^{3}J_{H,H}$ = 4.8, $^{3}J_{H,H}$ = 8.0, $^{2}J_{H,H}$ = 13.1, 1 H; 13b), 2.41 - 2.55 (m, 1 H; 23b), 2.93 (ddd, $^{3}J_{H,H}$ = 3.3, $^{3}J_{H,H}$ = 7.2, $^{2}J_{H,H}$ = 10.4, 1 H; 12a), 3.34 (s, 1 H; 23a), 3.41 (s, 1 H; 9), 3.70 - 3.77 (m, 1 H; 12b), 4.01 (q, $^{3}J_{H,H}$ = 7.8, 1 H; 22a/b), 4.22 (dt, $^{3}J_{H,H}$ = 3.3, $^{2}J_{H,H}$ = 11.6, 1 H; 14a), 4.41 (dt, $^{3}J_{H,H}$ = 4.6, $^{3}J_{H,H}$ = 8.3, 1 H; 22a/b), 4.47 (dt, $^{3}J_{H,H}$ = 12.2, $^{2}J_{H,H}$ = 3.8, 1 H; 14b), 4.55 (s, 1 H; 20), 5.03 - 5.19 (m, 2 H; 5 + 11), 6.22 (d, $^{3}J_{H,H}$ = 7.5, 1 H; 8), 6.82 (dd, $^{3}J_{H,H}$ = 8.3, $^{4}J_{H,H}$ = 2.5, 1 H; 17a), 6.86 (dd, $^{3}J_{H,H}$ = 8.6, $^{4}J_{H,H}$ = 2.4, 1 H; 17b), 7.04 (s, 1 H; 18b), 7.37 (dd, $^{3}J_{H,H}$ = 8.6, $^{4}J_{H,H}$ = 2.0, 1H; 17a); - **^{13}C-NMR** (150 MHz, HSQC, HMBC, $CDCl_3$): δ [ppm] = 11.5 (+, 1 C; 26), 14.4 (+, 1 C; 27), 25.1 (-, 1 C; 25), 26.7 (-, 1 C; 13), 28.2 (+, 3 C; 1), 36.8 (-, 1 C; 23), 37.6 (-, 1 C; 12), 38.7 (+, 1 C; 24), 58.7 (+, 1 C; 9), 67.2 (-, 1 C; 14), 68.6 (-, 1 C; 22), 71.2 (C_{quat}, 1 C; 6), 81.2 (C_{quat}, 1 C; 2), 89.5 (+, 1 C; 20), 114.3 (+, 1 C; 17a), 117.5 (+, 1 C; 17b), 127.0 (+, 1 C; 18b), 127.7 (+, 1 C; 18a), 129.2 (C_{quat}, 1 C; 19), 154.6 (C_{quat}, 1 C; 4), 159.2 (C_{quat}, 1 C; 16), 169.8 (C_{quat}, 1 C; 10), 170.2 ppm (C_{quat}, 1 C; 7); - **MS** (ES, DCM/MeOH+10mmol/l NH_4OAc): m/z (%) = 458.3 (7, $M+H-Boc)^+$), 500.3 (55, $M+H-C_4H_8)^+$), 558.3 (100, $M+H^+$); - **HRMS** (PI-LSIMS FAB, MeOH/glycerine): *m/z* (%) calcd. for $C_{25}H_{37}N_3O_6$: 475.2684 [*M*]; found: 475.2682 (100).

*Compound **50**:*



Compound **49** (80 mg, 0.14 mmol, 1 eq.), Cs_2CO_3 (42 mg, 0.22 mmol, 1.5 eq.), $Pd(OAc)_2$ (3 mg, 0.01 mmol, 0.1 eq.) and dicyclohexyl(2',4',6'-triisopropylbiphenyl-2-yl)phosphine (9 mg, 0.02 mmol, 1.25 eq.) were put into an oven dried Schlenk tube. The reaction vessel was evacuated and back filled with argon twice. To the mixture 5.6 mL of dry toluene (40 mL/mmol) were added and the tube was closed with a glass stopper and sealed. The mixture was heated under stirring to 80 °C for 24 h. The mixture was filtered and washed with 10 mL of ethyl acetate twice. The organic layers were concentrated under reduced pressure. The crude product was then purified by column chromatography on flash silica gel using a

solvent gradient starting from diethyl ether / ethanol 98:2 and going to 95:5 (EtOAc, R_f = 0.20). In a second purification step the compound was submitted to HPLC using a phenomenex Luna C18 and an acetonitrile / H_2O (0.0059 % TFA) gradient (5 % - 98 % acetonitrile) to give the product as colorless solid (12.4 mg, 0.03 mmol, 19 %). R_f = 0.20 (diethyl ether/EtOH, 98:2).

M.p. (uncorrected) = 159 - 160°C; - **^{1}H-NMR** (400 MHz, COSY, NOESY, $CDCl_3$): δ [ppm] = 0.77 (d, $^3J_{H,H}$ = 6.7, 3 H; 27), 0.83 (t, $^3J_{H,H}$ = 7.3, 3 H; 26), 0.90 - 1.03 (m, 1 H; 25a), 1.30 - 1.39 (m, 1 H; 25b), 1.46 (s, 9 H; 1), 1.68 - 1.82 (m, 2 H; 13a + 24), 2.10 - 2.24 (m, 1 H; 13b), 2.31 (dt, $^2J_{H,H}$ = 12.8, $^3J_{H,H}$ = 9.4, 1 H; 23b), 2.67 - 2.80 (m, 1 H; 23a), 2.90 (ddd, $^2J_{H,H}$ = 10.7, $^3J_{H,H}$ = 7.5, $^3J_{H,H}$ = 3.5, 1 H; 12a), 3.58 (t, $^3J_{H,H}$ = 7.5, 1H; 9), 3.64 - 3.75 (m, 1 H; 12b), 4.22 (ddd, $^2J_{H,H}$ = 12.7, $^3J_{H,H}$ = 9.1, $^3J_{H,H}$ = 3.6, 1 H; 14a), 4.29 - 4.37 (m, 2 H; 22a/b), 4.42 (dt, $^2J_{H,H}$ = 12.8, $^3J_{H,H}$ = 4.9, 1 H; 14b), 4.73 (s, 1 H; 11), 5.29 (s, 1 H; 20), 5.77 (d, $^3J_{H,H}$ = 7.8, 1 H; 8), 6.02 (s, 1 H; 5), 6.86 (dd, $^3J_{H,H}$ = 8.4, $^4J_{H,H}$ = 2.6, 1 H; 17a), 6.97 (dd, $^3J_{H,H}$ = 8.6, $^4J_{H,H}$ = 2.6, 1 H; 17a), 7.12 (dd, $^3J_{H,H}$ = 8.6, $^4J_{H,H}$ = 2.1, 1 H; 18a), 7.39 (dd, $^3J_{H,H}$ = 8.5, $^4J_{H,H}$ = 1.8, 1 H; 18b); - **^{13}C-NMR** (100 MHz, HSQC, HMBC, $CDCl_3$): δ [ppm] = 11.3 (+, 1 C, 26), 15.5 (+, 1 C; 27), 24.6 (-, 1 C; 25), 28.2 (-, 1 C; 13), 28.3 (+, 3 C; 1), 35.1 (-, 1 C; 23), 37.3 (-, 1 C; 12), 37.7 (+, 1 C; 24), 59.2 (+, 1 C; 9), 67.6 (-, 1 C; 22), 68.6 (-, 1 C; 14), 70.2 (C_{quat}, 1 C; 6), 80.1 (C_{quat}, 1 C; 2), 83.2 (+, 1 C; 20), 117.1 (+, 1 C; 17b), 117.6 (+, 1 C; 17a), 126.0 (+, 1 C; 18a), 126.4 (+, 1 C; 18b), 131.7 (C_{quat}, 1 C; 19), 154.6 (C_{quat}, 1 C; 4), 158.6 (C_{quat}, 1 C; 16), 170.2 (C_{quat}, 1 C; 10), 171.5 (C_{quat}, 1 C; 7); - **MS** (ES, DCM/MeOH+10 mmol/l NH_4OAc): m/z (%) = 420.2 (42, $(M+H-C_4H_8)^+$), 476.2 (25, $M+H^+$), 493.4 (100, $M+NH_4^+$), 951.6 (21, $(2M+H)^+$); - **HRMS** (PI-LSIMS FAB, MeOH/glycerine): m/z (%) calcd. for $C_{25}H_{38}N_3O_6$: 476.2761 $[M+H]^+$; found: 476.2758.

Further data and complete analytics for all prepared compounds can be found in the supporting information of: A. Grauer, A. Späth, D. Ma, B. König, *Chem. Asia. J.* **2009**, *4*, 1134-1140.

VII. 5. References and Notes

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VIII. Summary

The detection of ammonium ions plays a critical role in nature. Amino acids, small peptides and biogenic amines are the most important signal transmitters in organisms. Their amino groups are protonated under physiological conditions.

To achieve the necessary selectivity and binding strengths for neurotransmitters, nature uses diverse, mutually supportive binding events. This concept of multiple binding sites can be transferred to artificial receptors. The linkage of different modules for different functional groups should lead to an increase in binding strength and selectivity of such systems. These di- and tritopic receptors could in return contribute to a better understanding of biologically important recognition processes.

The present work therefore focuses on linking recognition units for ammonium, carboxylate and phosphate groups for binding biologically important peptides. Especially ammonium ions are targeted, enabling also the detection of amino acids and peptides. Furthermore, a new biomimetic approach towards ammonium ion or amino acid recognition is investigated. A small part is synthesis and functionalisation of unnatural amino acids.

In the first part of the work a survey of current literature is given highlighting synthetic receptor systems for the detection of organic ammonium ions and relevant ammonium-containing guests, such as amino acids or biogenic amines in solution. Crown ethers, calixarenes and resorcarenes, cucurbiturils, clamps, tweezer molecules, cyclophanes, metal complexes, natural ionophores, cyclic peptides, and some other classes of compounds are comparatively discussed on the basis of their interactions to ammonium guests. Where available, enantioselective recognition is briefly discussed. Combinations with other binding sites are presented ordered by their ammonium ion detection unit.

In the practical part of this work di- and tritopic receptors with the central aspect of ammonium ion binding were investigated. They either contain more than one ammonium ion binding site or adjacent to this, recognition units for carboxylate or phosphate groups.

In a previous work (Ch.P. Mandl, *PhD thesis*, University of Regensburg **2004**), crown ether amino acids capable of ammonium ion binding in methanol with $K = 1.8*10^2$ M^{-1} were developed. A fluorescent phthalic ester or imide unit served as a fluorescent sensor indicating a recognition event. Binding an ammonium ion resulted in a four-fold luminescence

enhancement. By dimerization of these moieties a hundred-fold increase in the ammonium binding strength was achieved upon coordination of a guest such as lysine methyl ester.

In the present study, the effect by adding a third binding site was investigated (Chapter 2). The binding strength and selectivity towards ammonium guests or lysine peptides was expected to increase. A general synthesis for several crown ether amino acids (CEAAs) is presented. These building blocks allow the synthesis of the various receptors in subsequent chapters. The coupling to linear chains consisting of up to three of these units has been successfully demonstrated. The binding properties were investigated with isomeric tetrapeptides containing two lysine amino acids in different positions. It turned out that the third binding site at best behaves additive. A further increase in binding strength can not be achieved by increasing the number of ammonium ion receptor units. The combination with stronger binding sites for other functional groups is more promising.

In the third chapter amino acid receptors based on the luminescent crown ether amino acid (CEAA) and a guanidinium binding site for carboxylate groups are investigated. Selectivity for the neurotransmitter γ-aminobutyric acid (GABA) versus its biological precursor glutamic acid and similar amino acids in polar protic environment was shown with two examples. The maximum binding strength was $1.3*10^3$ M^{-1}, which is still too small for practical application. Another receptor proved to be a recognition site for tripeptides with comparable binding strength. A distance dependence of the binding strength and selectivity between ammonium and carboxylate groups of the respective spacers could be detected with different amino acids and small peptides. The aromatic *p*-xylylene spacer proved to be particularly suitable.
Furthermore, we succeeded in lowering the pH range for the use of such systems by more than one unit in comparison to similar systems in literature. A measurement down to a slightly acidic pH of 6 is possible. The results allowed deriving more rational design of the receptors:

A tritopic receptor based on the combination of crown ether with guanidinium binding sites was presented (chapter four). The ligand for the hemoregulatory effective peptide Ac-Ser-Asp-Lys-Pro was rationally synthesized taking into account the experience of the third chapter. It was demonstrated that the host binds cooperatively to his guest even in a buffered methanolic solution (50 % water content) with millimolar strength ($4*10^3$ M^{-1}). The receptor is selective over small peptides that represent the tetrapeptide´s fragments and other zwitterions and peptides, which can also bridge the gap between the binding sites.

The fifth chapter deals with crown ether receptors carrying metal complexes for the binding of phosphate groups. The strong interaction of the complexed zinc(II) or manganese(II) ions

to the side chain of phosphoserine was combined with the luminescent properties of CEAA. The PKG substrate, a highly selective guest for the enzyme protein kinase G could be detected with a combination of a thyrosine based bispicolyamine-Mn/Zn complex and the crown ether with a binding strength of log K = 5 in buffered water. The sequence of the RNA polymerase II CTD was *N*-terminally coordinated with two examples of this receptor class and also recognized with three combinations of a bis-zinc-cyclene and the luminescent crown ether with log K = 4 in aqueous buffer. Ditopic binding events are indicated by a luminescence increase, whereas monotopic phosphate binding is indicated by lowered intensity.

These examples and a further study (S. Stadlbauer, A. Riechers, A. Späth, B. König, "Utilizing Reversible Copper(II) Coordination in a Peptide Sequence Selective Luminescent Receptor", *Chem. Eur. J.* **2008**, *14*, 2536 -2541.) clearly show the need for a second, strong binding site at the crown ether system to make use of these luminescent receptors in aqueous environment.

Based on the structure of the enzyme glutaminase HisF a new biomimetic approach for an improved binding site for ammonium ions should be developed: A guanidinium-carboxylate (chapter six). These structures, however, proved to be too flexible to serve as a pre-organized binding site for ammonium ions. However, they showed weak amino acid binding in aqueous methanol (K = $1*10^2$ M^{-1}) and good carboxylate recognition abilities in pure methanol, aqueous methanol and DMSO. The dynamics of the systems were investigated and referred to self-assembly processes in solution. A versatile method for the preparation of bis-guanidinium-carboxylates, which allowed easy purification, multiple functionalisation and very good yields was developed. These molecules are versatile building blocks for peptide receptors or spacers with additional carboxylate binding properties.

In the last chapter (chapter seven), the copper(I) catalyzed *N*-arylation of unnatural tetrahydrofuran amino acids (TAA) and the cyclisation of tripeptides by the same reaction or palladium-mediated *O*-arylation was examined in collaboration with A. Grauer.

The CuI-catalyzed *N*-arylation of TAA with a variety of amines proceeded in moderate to good yields. Thus the introduction of different functional groups or of amines in the side chain of protected TAA was possible. An intramolecular Cu(I)-catalyzed *N*-arylation of a tripeptide achieved only low yields of the 17-membered ring. The Pd(0)-catalyzed *O*-arylation with racemic TAA was found to give isolated yields up to 40 % for the 15-membered cycle of the non-natural tripeptides. The macrocyclic peptidomimetic prepared were tested for a HIV-1 protease inhibitory effect. Unfortunately, they proved to be inactive.

IX. Zusammenfassung

Der Erkennung von Ammoniumionen kommt in der Natur eine herausragende Rolle zu. Aminosäuren, kleine Peptide und biogene Amine sind die wichtigsten Signalüberträger in jedem Organismus. Ihre Aminogruppen liegen unter physiologischen Bedingungen nahezu immer protoniert vor.

Um die nötigen Selektivitäten und Bindungsstärken z.B. für so einen Neurotransmitter sicherzustellen, nutzt die Natur vielfältige, sich gegenseitig unterstützende Bindungsereignisse. Dieses Konzept multipler Bindungsstellen lässt sich auch auf künstliche Rezeptoren übertragen. Die Verknüpfung verschiedener Bausteine für die unterschiedlichen funktionellen Gruppen sollte zur Steigerung von Bindungsstärke und Selektivität solcher Systeme führen. Solche ditopische oder tritopische Rezeptoren könnten umgekehrt zum besseren Verständnis natürlicher Bindungsprozesse und der Signalübertragungswege beitragen.

Die vorliegende Arbeit versucht hierzu einen Beitrag zu leisten und beschäftigt sich mit der Verknüpfung von Erkennungseinheiten für Ammonium-, Carboxylat- und Phosphatgruppen. Die zentrale Rolle spielt hierbei das Ammoniumion, sowie die Erkennung von Aminosäuren und bestimmten Peptiden mit biologischer Bedeutung. Daneben soll ein neues biomimetisches Konzept zur Ammoniumionen- oder Aminosäurebindung untersucht werden. Ein kleiner Teil kommt der Synthese und Funktionalisierung unnatürlicher Aminosäuren zu.

Im ersten Teil der Arbeit wird anhand aktueller Literatur eine Übersicht über synthetische Rezeptorsysteme zur Erkennung von Ammoniumionen und relevanter Gäste die Ammonium Ionen enthalten, wie Aminosäuren oder biogene Amine, in Lösung gegeben. Kronenether, Calixarene und Resorcarene, Cucurbiturile, Klammern und Pinzettenmoleküle, Cyclophane, Metallkomplexe, natürliche Ionophore, Cyclopeptide und einige weitere Substanzklassen werden anhand ihrer Wechselwirkungen zum Ammoniumgast vergleichend diskutiert. Wo verfügbar wird enantioselektive Erkennung jeweils kurz betrachtet. Kombinationen mit anderen Bindungsstellen werden sortiert nach ihrer Ammoniumionenerkennungseinheit vorgestellt.

Im praktischen Teil der Arbeit wurden di- und tritopische Rezeptoren mit dem zentralen Aspekt der Ammoniumionenbindung behandelt. Sie enthalten entweder mehrere

Ammoniumionenbindungsstellen oder neben dieser Erkennungseinheiten für Carboxylat- oder Phosphatgruppen.

In einer früheren Arbeit (Ch.P. Mandl, *Dissertation* **2004**, Universität Regensburg), wurden Kronenetheraminosäuren entwickelt die Ammoniumionen in Methanol mit $K = 1.8*10^2$ M^{-1} binden können. Eine fluoreszierende Phthalester oder Phthalimideinheit dient als Fluoreszenzsensor zur Anzeige von Bindungsprozessen. Die Erkennung des Gastes resultiert in einer bis zu vierfachen Lumineszenzsteigerung. Durch Dimerisierung dieser Einheiten konnte eine hundertfache Steigerung der Ammoniumbindung gegenüber einem Diammoniumgast wie Lysinmethylester erreicht werden.

In der vorliegenden Arbeit sollte zunächst der Effekt bei Hinzufügen einer weiteren solchen Bindungsstelle untersucht werden (Kapitel Zwei). Die Bindungsstärke und Selektivität gegenüber Ammoniumgästen bzw. Lysin-Peptiden sollten erhöht werden. Eine allgemeine Synthese zur Herstellung von Kronenetheraminosäuren (CEAAs) wird vorgestellt, mehrere Beispiele wurden synthetisiert. Diese Bausteine dienen den Synthesen verschiedener Rezeptoren in den Folgekapiteln. Die Kupplung zu linearen Ketten bestehend aus bis zu drei dieser Bausteine wurde erfolgreich gezeigt. Die Bindungeigenschaften wurden mit isomeren Tetrapeptiden, die zwei Lysinaminosäuren in verschieden Abständen enthielten, untersucht. Es zeigte sich, dass sich die dritte Bindungsstelle bestenfalls additiv verhält und nicht wie erwartet eine starke Steigerung der Koordinationsstärke für den entsprechenden Ammoniumgast eintritt. Eine weitere Steigerung der Bindungsstärke lässt sich also nicht durch Erhöhung der Zahl der Ammoniumrezeptoreinheiten erzielen. Die Kombination mit stärkeren Bindungstellen für andere funktionelle Gruppen wird deswegen eingehender untersucht.

Im dritten Kapitel werden Aminosäurerezeptoren basierend auf der lumineszenten Kronenetheraminosäure (CEAA) und einer Guanidiniumbindungstelle für Carboxylaterkennung untersucht. Selektivität für den wichtigen Neurotransmitter γ-Aminobuttersäure (GABA) gegenüber dem biologischen Vorläufer Glutaminsäure und ähnlicher Aminosäuren in polar protischer Umgebung konnte mit zwei Beispielen gezeigt werden. Die maximale Bindungsstärke betrug hierbei $1.3*10^3$ M^{-1}. Das ist für eine praktische Anwendung noch zu niedrig. Ein weiterer Rezeptor basierend auf der Kronenethereinheit und einer quaternären Ammoniumfunktion als Carboxylatbindungsstelle erwies sich als Erkennungseinheit für Tripeptide mit vergleichbarer Bindungsstärke. Eine Distanzabhängigkeit der Bindungsstärke und Selektivität zwischen Ammonium und

Carboxylatgruppen für den jeweiligen Spacer konnte mit verschieden Aminosäuren und kleinen Peptiden nachgewiesen werden. Der aromatische *p*-Xylylspacer erwies sich dabei als besonders geeignet.

Weiterhin gelang es den pH-Bereich in dem solche Systeme verwendet werden können um mehr als eine Einheit gegenüber ähnlichen, literaturbekannten Systemen zu senken. Eine Messung bis zu leicht saurem pH von 6 wird dadurch möglich. Die Ergebnisse ermöglichten auch ein mehr rationelles Design solcher Rezeptoren abzuleiten.

So wurde ein weiterer Rezeptor basierend auf der Verknüpfung von Kronenether- mit Guanidinbindungstellen vorgestellt (Kapitel Vier). Der tritopische Ligand für das hemoregulatorisch wirksame Peptid Ac-Ser-Asp-Lys-Pro wurde rational nach den gesammelten Erfahrungen des dritten Kapitels und basierend auf dem Bausteinprinzip synthetisiert. Es konnte gezeigt werden, dass der Rezeptor diesen Gast auch noch in gepufferter methanolischer Lösung (50 % Wasseranteil) kooperativ und mit millimolarer Stärke zu binden vermag ($4*10^3$ M^{-1}). Der Rezeptor ist selektiv gegenüber kleinen Peptiden, die Bruchstücke des Gastes darstellen, sowie gegenüber anderen Zwitterionen und Peptiden, die ebenfalls den Abstand zwischen beiden Bindungstellen überbrücken können.

Das fünfte Kapitel beschäftigt sich mit Kronenetherrezeptoren die Metallkomplexe zur Bindung von Phosphatgruppen tragen. Die starke Wechselwirkung von komplexierten Zink(II) und Mangan(II)ionen zur Seitenkette des Phosphoserins sollte mit den lumineszenten Eigenschaften der Kronenetheraminosäure kombiniert werden. Das PKG Substrat, ein hochselektiver Gast für das Enzym Proteinkinase G, konnte in reinem Puffer mit einer Kombination aus einem Thyrosinbasierten bispicolyamin-Mn/Zn Komplex und der Kronenetheraminosäure mit einer Bindungsstärke von log K = 5 erkannt werden. Die CTD Sequenz der RNA Polymerase II wurde mit mehreren Beispielen dieser Rezeptorklasse und mit drei Kombinationen eines bis-Zink-Cyclens und dem lumineszenten Kronenether in wässrigem Puffer *N*-terminal mit log K = 4 erkannt. Diese ditopischen Bindungsereignisse werden durch eine Lumineszensteigerung angezeigt, wohingegen monotopische Phosphatbindung durch eine Senkung der Intensität signalisiert wird.

Diese Beispiele und eine weitere Publikation (S. Stadlbauer, A. Riechers, A. Späth, B. König "Utilizing Reversible Copper(II) Peptide Coordination in a Sequence Selective Luminescent Receptor", *Chem. Eur. J.* **2008**, *14*, 2536-2541.) zeigen deutlich die

Notwendigkeit einer zweiten, starken Bindungsstelle am Kronenethersystem um es für eine Bindung in wässriger Umgebung nutzen zu können. Metallkomplexe sind dazu gut geeignet.

In Anlehnung an die Struktur eines Glutaminase Enzyms (HisF) sollte ein neues biomimetisches Konzept für eine verbesserte Bindungsstelle für Ammoniumionen, ein Guanidinium-bis-carboxylat, entwickelt werden (Kapitel Sechs). Die Strukturen erwiesen sich jedoch als zu flexibel und zu wenig vororganisiert um als Bindungsstelle für Ammoniumionen zu dienen. Sie zeigten jedoch schwache Aminosäurebindung in wässrigem Methanol ($K = 1*10^2\ M^{-1}$) und gute Carboxylatbindungseigenschaften in reinem Methanol, wässrigem Methanol und DMSO. Die Dynamik der Systeme wurde untersucht und wies auf „self-assembly“ Prozesse in Lösung hin. Eine vielseitige Methode zur Herstellung dieser Guanidinium-bis-carboxylate, die eine einfache Reinigung, vielfältige Funktionalisierung und sehr gute Ausbeuten erlaubt wurde erarbeitet. Diese Moleküle können als vielseitige Bausteine für Peptidrezeptoren oder Spacermoleküle mit Carboxylatbindungseigenschaften dienen.

Im letzten Kapitel (Kapitel Sieben) wurde die Kupfer(I) katalysierte *N*-Arylierung an unnatürlichen Tetrahydrofuranaminosäuren (TAA) sowie die Cyclisierung ihrer Tripeptide durch diese Reaktion oder Pd(0) vermittelte *O*-Arylierung zusammen mit A. Grauer untersucht.

Die CuI-katalysierte *N*-Arylierung der TAA verläuft mit einer Vielzahl von Aminen in mittleren bis guten Ausbeuten, wodurch die Einführung verschiedener funktioneller Gruppen bzw. von Amine in der Seitenkette der geschützten TAA möglich wird. Eine intramolekulare Cu(I)-katalysierte *N*-Arylierung des Tripeptides erzielte nur geringe Ausbeuten des 17-gliedrigen Rings. Die Pd(0)-katalysierte *O*-Arylierungen ergab Ausbeuten des isolierten Produkts von bis zu 40 % für die Makrocyclisierung nicht natürlicher Tripeptide mit racemischer TAA. Die so hergestellten makrocyclischen Peptidomimetika wurden auf HIV-1 Protease Inhibitor Wirkung getestet, erwiesen sich jedoch leider als inaktiv.

X. 1. General Methods and Material

Analytical control of the synthesized compounds was done by common methods. Melting points were determined on Büchi SMP or a Lambda Photometrics OptiMelt MPA 100 and are uncorrected. IR Spectra were recorded with a Bio-Rad FT-IR Excalibur FTS 3000. UV spectra were recorded on a Cary 50 BIO spectrometer with temperature control at 25°C. Electro spray mass spectra were performed on a Finnigan MAT TSQ 7000 ESI-spectrometer. Other Mass Spectra were recorded on Varian CH-5 (EI), Finnigan MAT 95 (CI; FAB and FD); Xenon serves as the ionization gas for FAB.

NMR spectra were recorded on Bruker Avance 600 (^{1}H: 600.1 MHz, ^{13}C: 150.1 MHz, T = 300 K), Bruker Avance 400 (^{1}H: 400.1 MHz, ^{13}C: 100.6 MHz, T = 300 K) or Bruker Avance 300 (^{1}H: 300.1 MHz, ^{13}C: 75.5 MHz, T = 300 K) relative to external standards.

Characterization of the signals: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet, dd = double doublet, dt = double triplet, ddd = double double doublet. Integration is determined as the relative number of atoms, the coupling constants are given in Hertz [Hz]. The multiplicity of the carbon atoms is given as (+] = CH_3 or CH, (-] = CH_2 and (C_{quat}) for quaternary carbon atoms. Structural assignments are based on DEPT and COSY experiments where applicable. Error of reported values: chemical shift: 0.01 ppm for ^{1}H-NMR, 0.1 ppm for ^{13}C-NMR and 0.1 Hz for coupling constants. The solvent used is reported for each spectrum.

Analytical TLC plates (silica gel 60 F_{254}) and silica gel 60 (70 - 230 or 230 - 400 mesh) were used for chromatographic separations. Visualization of the spots was by UV light and/or staining with phosphomolybdate or ninhydrin, both in ethanol. DMF was purchased anhydrous (Fluka) and used as received. Other solvents used were reagent or pro analysi grade. THF, EtOH, DCM, $CHCl_3$, MeCN and Et_2O were dried after standard procedures, distilled and stored over molecular sieves. PE means petrol ether with a boiling range of 70 - 90 ^{o}C. All other solvents and chemicals were of reagent grade (Merck, Aldrich, Fluka) and used with out further purification. Test substances were of pro analysi grade, checked by NMR or HPLC and used as purchased with out further purification.

Solvents for fluorescence measurements were from special spectroscopic purity purchased from Acros or Baker or Uvasol from Merck. Millipore water (18 MΩ, Milli Q_{Plus}) was used; the HEPES buffer was from according purity, suitable for biochemical optical screenings. As far as not stated otherwise analytical grade tetraethylammoniumhydroxide or hydrochloric acid were used to adjust the pH in titrations and screenings.

X. 2. Explanation of the PET Sensing Principle

The chromophore-containing aza-crown ether amino acids are able to participate in photoinduced electron transfer (PET), where, upon excitation an electron of the lone pair on the nitrogen is able to travel to the chromophore, quenching the fluorescence.[1]

Such a photo-induced electron transfer (PET) can occur over a distance of 10 Å.[2] Yoshida et al. showed, chromophore containing aza-crown ether compounds maintain their absorption and emission wavelengths and absorption intensity, while their fluorescence intensities are significantly altered upon cation complexation.[3]

Cation complexation disrupts the electrostatic field of the amine and thereby the PET process, causing an increase in fluorescence intensity proportional to the amount of complexation and therefore the concentration of cation present. However, the crown ethers suffer pH dependence problems, where protonation of the amine mimics cation complexation at low pH, so the pH has to be maintained by a base like tetraethylammonium hydroxide or all studies have to be conducted in a buffered medium (the system can be used at pH values $\geq$6).

The effect of the pH value on the luminescence[4] and cation selectivity[5] of aza-crown-ethers based on this signalling principle were studied (see literature citations).

X. 3. Abbreviations

A	anion
abs.	absolute
Ac	acetyl
aq	aqueous
ADP	adenosine-diphosphate
AMP	adenosine-monophosphate
ATP	adenosine-triphosphate
BEA	1-Phenyl-propyl-ammonium
Biscyclen	2-Chloro-4,6-bis(4,7,10-tris-*tert*-butoxycarbonyl-1,4,7-triaza-cyclododec-1-yl)-[1,3,5]triazin
Bn	benzyl
Boc	*tert*-butoxycarbonyl
Bpa	di(pyridin-2-ylmethyl)amine
Bpy	[2,2']Bipyridinyl
Bu	butyl
Bz	benzyl
calc	calculated
Cbz	benzoyloxycarbonyl
CC	column chromatography
CD	circular dichroism
CEAA	crown ether amino acid
CMP	cytosine-monophosphate
COSY	correlated spin spectroscopy
Cy	cyclohexyl
Cyclen	1,4,7,10-tetraazacyclo-dodecane
d	day
DBU	*N,N*-diazabicycloundecen
DCC	dicyclohexyl carbodiimide
DCM	dichloromethane
DFT	density functional theory
DIC	diisopropylcarbodiimide
DIPEA	ethyl diisopropyl amine
DMAP	4-(dimethylamino) pyridine
DMF	*N,N*-dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
EA	elemental analysis
ED	ethylene diamine
EDC	1-ethyl-3-(3-dimethyl-aminopropyl)carbodiimide
EI	electron impact
eq	equivalents
ESI	electron spray ionization
Et	ethyl
Et_2O	diethyl ether
EtOAc	ethyl acetate
EtOH	ethanol
F	fluorescence intensity
FAB	fast atom bombardment
fig	figure
Fmoc	fluorenylmethoxycarbonyl
FRET	fluorescence resonance energy transfer
GMP	guanosine-monophosphate
GP	general procedure
Gua	guanidine
h	hour
HATU	2-(1H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl

	uronium hexafluorophosphate
HBTU	O-benzotriazole-*N,N,N',N'*-tetramethyl-uronium-hexafluoro-phosphate
HEPES	4-(2-hydroxyethyl)-piperazine-1-ethane sulfonic acid
HMBC	heteronuclear multiple-bond correlation
HOAt	1-hydroxy-7-azabenzo-triazole
HOBt	1-hydroxybenzotriazole
HPLC	high performance liquid chromatography
HR-MS	high resolution mass spectroscopy
HSQC	heteronuclear single quantum coherence
IC50	half maximal inhibitory concentration
IDA	iminodiacetic acid
IR	infrared spectroscopy
J	coupling constant
K	cation
LB	Langmuir-Blodgett
LC	liquid chromatography
M	molecule
M	mol/L
Me	methyl
MeCN	acetonitrile
MeOH	methanol
MF	molecular formula
min	minutes
MP	melting point
MS	mass spectroscopy
MW	molecular weight
NEA	(1-napthyl)ethylamine perchlorate salt
NEt_3	triethyl amine
NIR	near infrared
NMA	*N*-methyl amino acid
NMP	*N*-methyl-pyrrolidone
NMR	nuclear magnetic resonance
NOE	nuclear overhauser effect
NOESY	nuclear overhauser effect spectroscopy
NTA	nitrilotriacetic acid
OAc	acetate
PAM	1-Methoxycarbonyl-2-phenylethylammonium
PE	petrol ether with a boiling point 50 - 60°C
PEA	(1-phenyl)ethylamine perchlorate salt
PET	photoinduced electron transfer
Ph	phenyl
Py	pyridinyl
rac	racemic
R_f	retention factor
RNA	ribonucleic acid
ROESY	rotating-frame NMR spectroscopy
RT	room temperature
SAM	self-assembled monolayers
sat	saturated

SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SN	nucleophilic substitution
SPR	surface plasmon resonance
TAA	tetrahydrofuran amino acid
TBA	tetrabutylammonium
TBABr	tetrabutylammonium bromide
TBACl	tetrabutylammonium chloride
TBAOAc	tetrabutylammonium acetate
TBDMS	*tert*-butyldimethylsily
TBDMSCl	*tert*-butyldimethylsily chloride
TBTU	2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyl-uronium tetrafluoro borate
tBu	*tert*-butyl
tBuOH	*tert*-butanol
TFA	trifluoroacetic acid
TIS	trisiopropylsilane
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
Tos	toluenylsulfonyl
UV	ultraviolet
vol	volume
Vis	visible
w	weight
x	mole fraction
Z	benzoyloxycarbonyl

Amino acids

Three letter code	*Amino acid*	*One letter code*
Aib	aminoisobutyric acid	
AHX	ε-aminohexanoic acid	
Ala	alanine	A
β-Ala	β-alanine	
Arg	arginine	R
Asn	asparagine	N
Asp	aspartic acid	D
Cys	cysteine	C
GABA	γ-aminobutyric acid	
Gln	glutamine	Q
Glu	glutamic acid	E
Gly	glycine	G
His	histidine	H
Ile	isoleucine	I
Leu	leucine	L
Lys	lysine	K
Met	methionine	M
Orn	ornithine	O
Phe	phenylalanine	F
Pro	proline	P
pSer	serine-o-phosphate	pS
Ser	serine	S
Thr	threonine	T
Trp	tryptophan	W
Tyr	tyrosine	Y
Val	valine	V

X. 4. Publications

S. Stadlbauer, A. Riechers, A. Späth, B. König "Utilizing Reversible Copper(II) Peptide Coordination in a Sequence Selective Luminescent Receptor", *Chem. Eur. J.* **2008**, *14*, 2536-2541.

A. Späth, B. König „Modular Synthesis of Di- and Tripeptides of Luminescent Crown Ether Aminocarboxylic Acids", *Tetrahedron* **2009**, *65*, 690-695.

A. Grauer, A. Späth, D. Ma, B. König „Metal-catalyzed Derivatization of C^{α}-tetrasubstituted Amino Acids and their Use in the Synthesis of Cyclic Peptides", *Chem. Asia. J.* **2009**, 4, 1134-1140.

A. Späth, B. König "Ditopic Crown Ether–Guanidinium Ion Receptors for the Molecular Recognition of Amino Acids and Small Peptides", *Tetrahedron* **2010**, *66*, 1859-1873.

A. Späth, B. König "Binding Organic Ammonium-Ions in Solution Using Synthetic Receptors" (Review), *Beilstein J. Org. Chem.* **2010**, *6*, No. 32.

A. Späth, B. König „Luminescent Metal Complex - Crown Ether Hybrid Receptors bind Phosphate and Ammonium Ions simultaneously", *Eur. J. Inorg. Chem.* **2010**, *accepted.*

A. Späth, B. König „Crown Ether Guanidinium Ion Synthetic Receptor for a Hemoregulatory Tetrapeptide", *Tetrahedron* **2010**, *accepted.*

A. Späth, B. König „Synthesis and Binding Properties of Guanidinium Bis-carboxylates", *Supramol. Chem.* **2010**, *accepted.*

B. Gruber, S. Stadlbauer, A. Späth, S. Weiss, B. König „Luminescent Vesicular Chemosensors by simple Mixing of Amphiphiles, Binding Sites and Reporter Dyes", *Angew. Chem.* **2010**, *submitted*.

A. Späth, B. König „Synthesis of a Luminescent Lariat Aza-Crown Ether Carboxylic Acid", *Molbank* **2010**, *1,* M663.

A. Späth, B. König „Synthesis of a Luminescent Lariat Aza-Crown Ether", *Molbank* **2010**, *1*, M662.

A. Späth, B. König „Synthesis of a Luminescent Aza-27-crown-9 Amino Acid", *Molbank* **2010**, *1*, M660.

X. 5. Conferences and Presentations:

"International Symposium of Macrocyclic Chemistry (ISMC 2005)" in Dresden, Germany, July 2005 (Poster Presentation)

"Summerschool of the Graduate College Sensory Photoreceptors" in Kostenz, Germany, July 2006 (Oral Presentation)

"1st EuCheMS Chemistry Congress", Budapest, Hungary, September 2006, Funded by the Karl-Ziegler-Institute (Poster Presentation)

"Summer School Medicinal Chemistry", Ho Chi Min City, Vietnam, November 2006, Funded by the EU-AsiaLink Medicinal Chemistry (Oral & Poster Presentation)

"Summerschool of the Graduate College Sensory Photoreceptors" in Regen, Germany, June 2007 (Oral Presentation)

"15th Symposium on Organic Chemistry (ESOC 2007)" in Dublin, Ireland, July 2007, Funded by the Graduate College "Sensory Photoreceptors" (Poster Presentation)

"Summerschool of the Graduate College Sensory Photoreceptors" in Kostenz, Germany, July 2008 (Oral Presentation)

"5th Symposium on Organic Chemistry (SupraChem 2009)" in Kiev, Ukraine, May 2009, Funded by the Graduate College "Sensory Photoreceptors" (Poster Presentation)

X. 6. Curriculum vitae

Personal details:

Name:	Andreas Späth
Date of Birth:	28.08.1980
Place of Birth:	Straubing
Status:	Single

Primary and Secondary Education:

09.1987 – 07.1991	Volkschule St. Josef, Straubing
09.1991 – 07.2000	Ludwigsgymnasium, Straubing
07.2000	Abitur, Majors in Chemistry and Economics

Tertiary Education:

10.2000 – 09.2005	Chemistry Studies at the University of Regensburg
09.2002	Intermediate Diploma
09.2002 – 01.2005	Advanced Study Period, Elective Course: "Polymer Physics"
11.2004 – 12.2004	Diploma examination
01.2005 – 09.2005	Diploma thesis in Organic Chemsitry under Prof. Dr. B. König, University of Regensburg, Germany, Thesis entitled: "Towards Extended Linear Chains of Luminescent Crown Ethers"
10.2005 – 03.2010	PhD in Organic Chemistry under Prof. Dr. B. König, Title: "Ammonium Ion Recognition: Luminescent Amino Acid and Peptide Receptors & Modification of Amino Acids: Artificial Amino Acids, Cyclopeptides and Guanidinium-bis-carboxylates"

Internships / Professional experience:

07.2000 – 08.2000	Student trainee, Quality Assurance, Thyssen Polymer GmbH, Hunderdorf, Germany
11.2002 – 05.2003	Student Assistant in the Research Group of Prof. Dr. A. Pfitzner, Inorganic Chemistry, University of Regensburg, Germany
02.2004 – 04.2004	Student trainee, Explorative Research on functional dyes, near IR chromophors, photo active pigments and colours, BASF AG, Ludwigshafen, Germany
04.2004 – 06.2004	Student Assistant in the Research Group of Prof. Dr. M. Scheer, Inorganic Chemistry, University of Regensburg, Germany
01.2005 – 07.2009	Teaching assistant in laboratory courses for chemistry, biology and biochemistry students, supervisor for bachelor and diploma students at the University of Regensburg
04.2006	Workshop "Environmental Management" at the BASF AG in Ludwigshafen, Germany
02.2008 – 04.2008	Research Internship in the Research Group of Prof. Dr. D. Ma, Shanghai Institute of Organic Chemistry, Shanghai, P. R. China

Academic continuing education

01.2007 – 05.2008	"Certified project manager in chemical economics" of the German Chemistry Society (GDCh), Prof. J. Leker, Westfälische Wilhelms-University of Münster, Germany
10.2005 – 09.2009	Associated Member of the graduate college "Sensory Photoreceptors" (GRK 640)
04.2006 – 02.2007	Language Course A1 and A2 "Finnish"

[1] The thermodynamics of Photoinduced Electron Transfer are described by a simplified Rehm-Weller equation ($\Delta G_{PET} = E_{ox} - E_{red} - e^2/\varepsilon_r - E_{oo}$). ΔG_{PET} is calculated to determine the spontaneity of the PET process for chemosensors with a fluorophore-spacer-receptor system. Here, ΔG_{PET} is the free energy for the PET process, E_{ox} is the oxidation potential of the amine, E_{red} is the reduction potential of the chromophore, and ε the dielectric constant of the solvent. To optically detect cation concentrations, the molecule must be able to participate in photo-induced electron transfer (PET) (H.-F. Ji, R. Dabestani, G.M. Brown, R.L. Hettich, *Photochem. Photobiol.* **1999**, *69*, 5, 513.). PET requires that an electron donor is able to travel to the fluorophore and thus quench fluorescence upon excitation (R.A. Bissell, A.P. de Silva, H.Q.N. Gunaratne, P.L.M. Lynch, G.E.M. Maguire, *Chem. Soc. Rev.* **1992**, 187.)

[2] K. Kubo, R. Ishige, N. Kato, E. Yamamoto, T. Sakurai, *Heterocycles* **1997**, *45*, 12, 2365.; In our case modellings of the crown ethers in the gas phase show a bowl like shape, orienting the crown ether´s luminophore even more close to the aza-nitrogen, which acts as the donating quencher.

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[4] R.A. Bissell, E. Calle, A.P. de Silva, *J. Chem. Soc. Perk. Trans. 2* **1992**, 1559.

[5] K. Kubo, N. Kato, T. Sakurai, *Bull. Chem. Soc. Jap.* **1997**, 70, 3041.