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Supramolecular Complexation of Oxoanions by Novel Guanidinium Receptors: Insights from Calorimetric Trend Analysis.

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To

my parents

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Table of Contents

Abbreviations

ADP	Adenosine 5′-diphosphate
AMP	Adenosine 5′-monophosphate
ATP	Adenosine 5′-triphosphate
BTSA	bis(trimethylsilyl) acetamide
DBU	1,8-Diaza-7-bicyclo[5.4.0]undecene
DMA	Acetdimethylamide
DMAB	N,N-dimethylamino-4-benzoate
DMF	N,N-dimethylformamid
DMSO	dimethylsulfoxide
EDIPA	ethyldiisopropylamine
ESI	electrospray ionization
HPLC	high-performance liquid chromatography
HPNPP	2-hydroxypropyl-p-nitro phenyl phosphate
ITC	isothermal titration calorimetry
K_{ass}	association constant
mp	melting point
MPLC	medium pressure liquid chromatography
MS	mass spectrometry
NCS	N-chlorosuccinimide
NMR	nuclear magnetic resonance
RP	reversed phase
RT	room temperature
R_v	retention volume
SPE	solid phase extraction
TBA-PNB	Tetra-butylammonium para-nitrobenzoate
TBDPS	t-butyldiphenylsilyl
TEA	tetraethylammonium
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TMG	tetramethylguanidine

1. Introduction

1.1 Supramolecular interactions

Supramolecular chemistry is based on the weak non–covalent interactions between the molecules. In many biological processes such as substrate binding to an enzyme, assembling of protein complexes, intermolecular reading of the genetic code, signal induction by neurotransmitters, cellular recognition and so on, the non-covalent interactions between the molecules play an important role.[1-3]

The non-covalent interactions in the living system have inspired the scientific community to mimic these interactions in designing artificial receptors that are capable of binding a guest molecule or substrate strongly and selectively to form a host-guest complex of well defined structure and functions.[1] Based on these concepts a wide range of artificial host-guest systems has been developed and successfully used to perform functions like catalysis, molecular recognition, transportation etc.

Molecular recognition processes can be arbitrarily subdivided into two events as overviewed in figure 1. In the first process the host binds selectively to the guest forming a well defined host-guest complex; in the second process transformation of this binding event is translated into the desirable functions.[4]

Most of the time in designing the artificial receptors or hosts, the high affinity (association constants) achievement is given prominent importance. But most of the functions where these host or receptor complexes are to be used demand precise structuring in the binding to the guest species. From the thermodynamic point of view, these functions demand enthalpy-driven host-guest complexation in relatively competing solvents.

Many times the successful complexation is judged from the high Gibb's free energy (ΔG) of complexation and equilibrium constant for association (K_{ass}), not paying attention to the entropic components and solvent contributions. Since from the Gibbs-Helmholtz equation, $\Delta G = \Delta H - T\Delta S$, the Gibb's free energy is the combination of enthalpic and entropic components, one should not only focus on the free energy of association ΔG, which may not reflect a real picture of host-guest association and the effect of solvent contribution due to enthalpy-entropy compensation.[5,6] Moreover, entropy plays an important role in complexations in solution and therefore must not be overlooked.

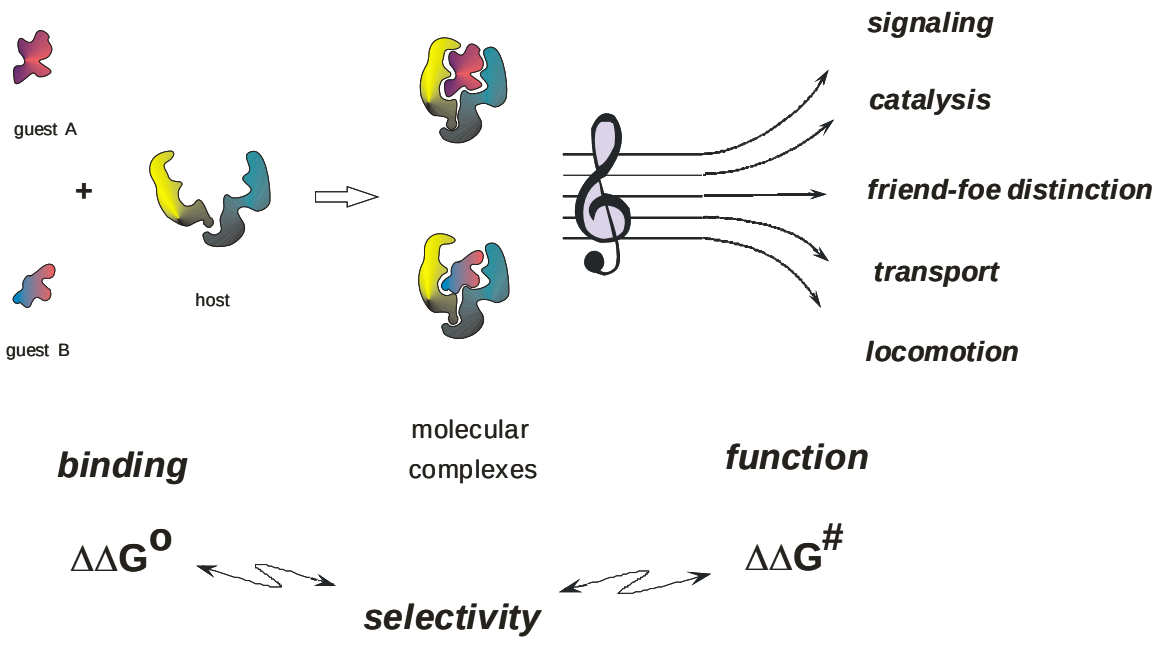


Figure 1. Overview of the host-guest binding and its applications.

Thus, it is necessary to dissect the free energy ΔG in to its enthalpy ΔH and entropy ΔS components. This would help to get detailed knowledge on the energetic parameters that allow a better determination of the nature of the complexation, *i.e.*, whether it is enthalpy- or entropy-driven. This aspect has only recently been taken into account[4,7-9] and it is one of the main factors involved in the design and complexation studies of the receptors synthesized in this dissertation.

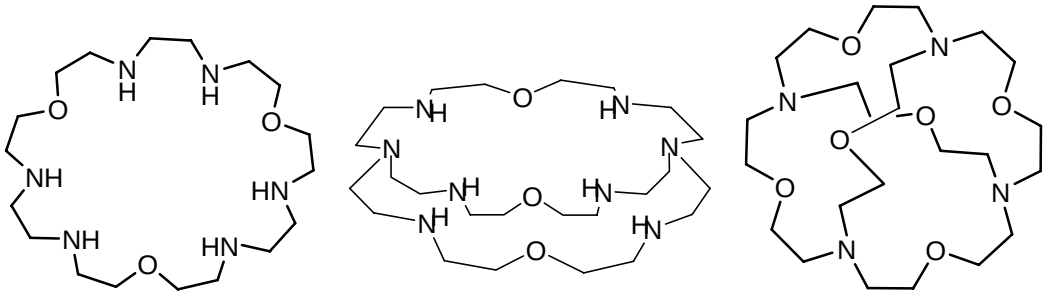
1.2 Hosts for oxoanions like phosphates and carboxylates.

The supramolecular complexation of anions has gained importance in recent years.[10] Nearly two thirds of enzyme substrates and cofactors are anions, e.g. the adenosine triphosphate (ATP) anion is the main energy source of life and DNA is a polyanion. The sulphate and carboxylate anions frequently occur in biochemical systems. Nitrates are the important component of fertilizers, pertechnetate anions (TcO_4^-) are a by-product of the nuclear power industry and it is important in biological imaging.[3] Many elements are more stable at higher oxidation states so they combine with water to form oxoanions in which the net charge is distributed over few atoms. Usually, anions possess larger ionic radii and higher solvation energy in protic solvents relative to cations of the same size. Anions are stabilized in polar protic solvents through hydrogen-bonding interactions.[11] Although anions are strongly hydrated, the binding of positively charged species is not suppressed. Lewis base character of anions is an essential feature for stabilization through hydrogen-bonding interactions.[12] The presence of lone electron pairs serve as H-acceptor sites apart from exceptions like AlH_4^-, BPh_4^-, etc.; their Lewis basicity, however, varies within broad limits. Nevertheless, it is a common feature of anions and may be used as a basic interaction type in the construction of anion hosts. Anions also possess diverse shapes like spherical, linear, angular etc.

Anion receptors can be of different type: Neutral Lewis acids, neutral proton donors, metal cations or positively charged organic groups, such as ammonium or guanidinium have been constructed. The basic idea of anions forming ion pairs with cations in solution was extensively utilized in developing cationic hosts. These hosts can be prepared by protonation of suitable basic compounds. Since many anions possess some basic properties as well, host-guest binding in these cases depends on relative proton affinities in interconnected multiple equilibria.

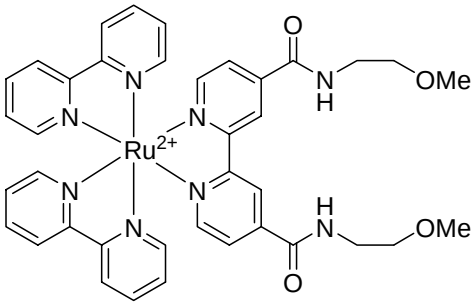
Initially open chain polyamines, like spermine or spermidine were studied, which are known[13-15] to bind phosphate anions or polyanions in water at neutral pH, but most likely adopt a flexible extended conformation rendering it difficult to incorporate all ammonium sites in the binding to anions. To overcome this drawback in open chain polyamines, polyprotonated azacrown ethers were designed, which possess a greater charge density and thus a greater predisposition to anion binding. A logical extension of this idea would incorporate as many ammonium sites as synthetically feasible in close proximity to each other in order to maximize the electrostatic

attraction for the negatively charged guest. A typical example of this class is the macrocycle **1**.[16] The hexa-protonated azacrown host **1** shows strong complexation with a variety of anions in water (log K_{ass} = 4.7 for AMP^{2-}, 7.7 for ADP^{3-}, 9.1 for ATP^{4-}). The general trend shows that the complex stability increases with the charge. This is an indication of the dominance of coulombic attractions. This contention is also supported by the dependence of stabilities on structure, which is only moderate.[17] Host **1** also shows good distinction between the anions of the same charge (for $fumarate^{2-}$, log K_{ass} = 2.2 and for $oxalate^{2-}$ log K_{ass} = 4.7). A much greater improvement in stability and selectivity of anion complexation was achieved by rigidification of the binding sites.[18] The Lehn group prepared several bicyclic cryptands like **2**, which, in their penta- or hexaprotonated forms, readily complex a variety of well-solvated anions in aqueous solutions.[19-21] Host **2** complexes anions like oxalate and malonate strongly (log K_{ass} = 4.95, log K_{ass} = 3.10 respectively) and trend analysis indicates that the strong complexation must be due to an encapsulation process, in which the guest anion invades the molecular cavity and is held there by an oriented set of H-bonds. This was confirmed by several crystal structures[19,21] showing that that the ellipsoidal cavity and topology of nitrogen sites provide optimal complementarity to azide anion resulting in high complex stability (log K_{ass} = 4.3). The interaction with halides was less dramatic and the trend from fluoride to iodide (log K_{ass} = 4.1 to 2.15) reflects the importance of H-bonding as the main attractive binding force. Extending this concept further, a macrotricyclic aza-crown ether **3** was prepared, when protonated, **3** forms a very stable anion complex[22] by virtue of an array of 4 H-bonds converging on to the centre of the molecular cavity expanded by the electrostatic repulsion of the positive charges. A chloride ion can be encapsulated as shown by the X-ray structure.[23] The discrimination of bromide versus chloride exceeds a factor of 10^3, while nitrate, acetate, perchlorate (the wrong shape) and iodide (too large) do not form complexes at all.



1 **2** **3**

A new class of anionic hosts has been developed based on metal cation ligation. The basic idea behind this class was to replace cationic charge that was obtained on protonation of the basic amine compounds. In this class, a suitable transition metal cation is used as an anion binding site, which most of the time has the ability to give readily detectable spectral or electrochemical signals upon binding. Therefore, these can act as anion sensors. The tris(2,2'-bipyridyl)ruthenium(II) system is the most extensively investigated system, which is popular due to its chemical stability, redox properties, excited-state reactivity and luminescent emission.[24,25] Beer and co-workers have incorporated this moiety into acyclic and macrocyclic structural frameworks to produce new classes of anion receptors **4-7** capable of optical and electrochemical sensing.[26-28]



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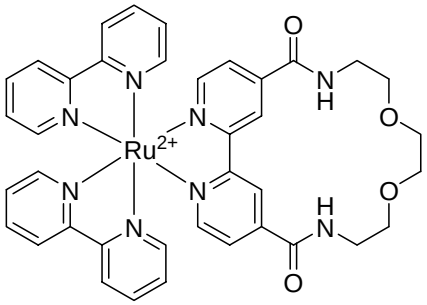

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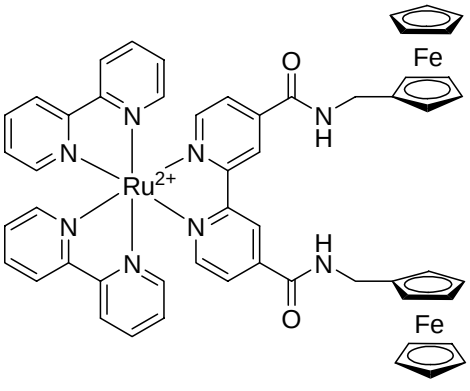

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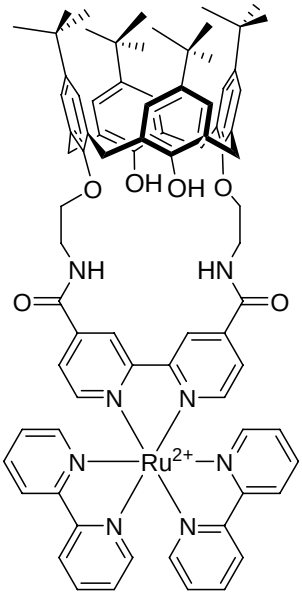
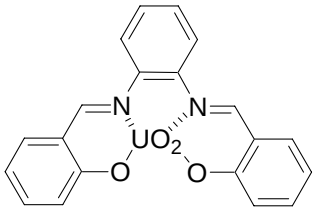


6

The single X-ray structure of the chloride complex of **4** demonstrates the importance of hydrogen bonding to the binding process. The host **4** forms stable complexes with chloride and $H_2PO_4^-$ ions in DMSO. On the other hand cyclic receptors **5** and **6** form highly selective and thermodynamically stable complexes with $H_2PO_4^-$ ion, even in presence of a tenfold excess of HSO_4^- and Cl^- ions. Anion binding of these receptors involves electrostatic and hydrogen bonding contributions. In the free acyclic ferrocene receptor **7,** the emission of the ruthenium center was quenched by the ferrocene units, but addition of $H_2PO_4^-$ ion enhanced it back by 20-fold in acetonitrile. This increase was not observed with Cl^- or HSO_4^- ions. Receptor **7,** thus can be considered a $H_2PO_4^-$ - luminescent anion receptor.



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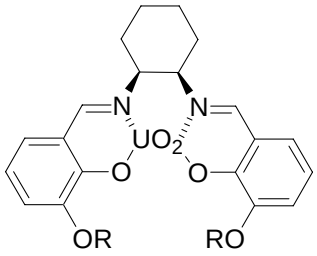

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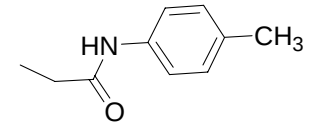

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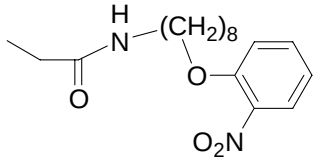
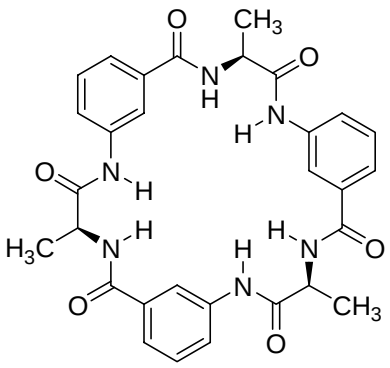
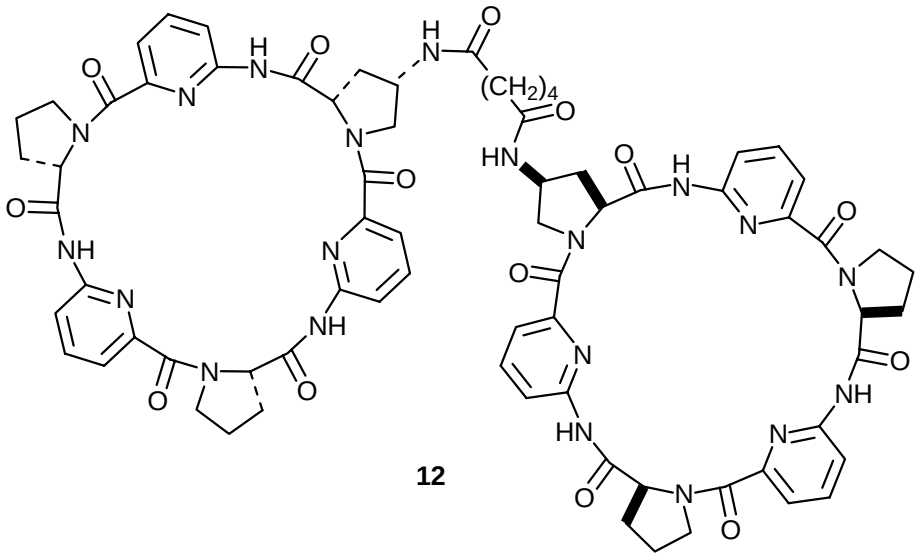


Another class of anion receptors uses electron deficient atoms; an example of this concept is the host **8.** The anion complexation of **8** in an organic solvent[29,30] (acetonitrile) shows general selectivity for $H_2PO_4^-$ over Cl^- ion. Additional hydrogen bonding was exploited as a means to enhance the selectivity toward $H_2PO_4^-$. The receptors **9** and **10,** supplemented with additional two secondary carboxamide functions show an increase in absolute association constants for $H_2PO_4^-$ ($K_{ass} > 10^5\ M^{-1}$) and set a discrimination factor between $H_2PO_4^-$ and Cl^- to 100 in acetonitrile.[29,30] The host **10** proved useful in transporting $H_2PO_4^-$ across a supported liquid membrane, demonstrating the advantage of an electroneutral anion receptor.[31]



11

Another class of anion receptors was designed by making use of an organised array of peptide bond dipoles for complexation. Ishida et al. synthesized the cyclic peptide **11**, composed of dipeptide building blocks containing *m*-aminobenzoic acid as a rigid, structure enforcing element.[32] The host **11**, by UV spectroscopic analysis, showed an extraordinary high association constant (K_{ass} = 1.2 x 10^6 M^{-1}) with p-nitrophenyl phosphate in DMSO. The structural variations of amino acid and the ring size show a strong preference for the cyclohexapeptide structure.

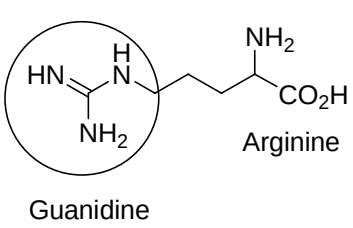


12

Recently an interesting neutral anion receptor **12**, in which two cyclohexapeptide subunits containing L-proline and 6-aminopicolinic acid subunits in an alternating sequences are connected via an adipinic acid spacer was made available.[33] This receptor shows remarkable sulfate affinity in aqueous solution (Kass = 3.5 x 10^5 M^{-1}) with strict 1:1 stoichiometry and

selectivity over halide anions. This has been attributed to the ability of sulfate to form stronger hydrogen bonds to the NH groups of receptor

One of the most widely explored classes of anion hosts contains guanidinium groups as the primary binding site. The idea of using guanidinium was inspired from nature. Enzymes often use the guanidinium containing side chain of arginine **13** as the active site to bind anionic substrates.[34-37] The advantage of using guanidinium group in anion binding arises from its high pKa value (≈ 13.5 in water). This makes guanidinium remain in the protonated state over a wide range of pH and offers, apart from the electrostatic attraction, a pair of hydrogen bonds for interaction with oxoanions such as phosphates, carboxylates, etc. This is also the recurring motif seen in the X-ray crystal structures of enzyme complexes with oxoanionic substrates and as well as in simple guanidinium salts.[34,38-40]



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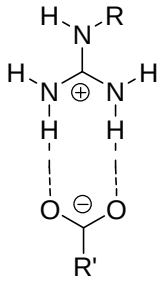
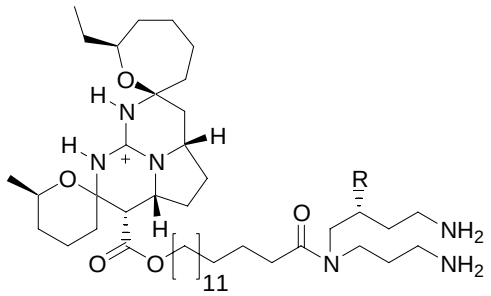


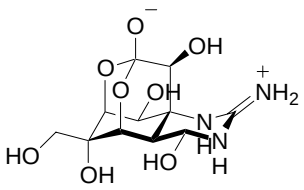
The guanidinium moiety is highly solvated by water due to its hydrophilic nature. Therefore the electrostatic and hydrogen bonding interactions with oxoanions are almost negligible[41] (K_{ass} < 5 M^{-1}). Even two cationic guanidinium moieties are known to dimerize through water molecule bridges.[42] In spite of these solvation properties which hamper the use of guanidinium in artificial receptors, the attractive features of the guanidinium group along with support from the natural host-guest binding has attracted the attention of researchers to design artificial guanidinium receptors.

The guanidinium group is a part of some natural products as well. Some alkaloids containing a guanidinium moiety were isolated from marine organisms. The alkaloid ptilomycalin A **14** was isolated from the Caribbean sponge, ptilocaulis spiculifer and the red sponge Hemimycale.[43] The related alkaloid crambescidines **15,** were isolated from the mediterranean sponge Crambe crambe. The pentacyclic guanidinium core, with a hydroxyspermidine residue attached by a long chain of a ω-hydroxycarboxylic acid spacer is a part of many pharmacologically important

drugs.[44] The guanidinium moiety is also responsible for the toxicity of some compounds, e.g., the puffer fish poison tetrodotoxin **16**[45]**,** the paralytic shellfish poison saxitoxin **17**[46] etc.



14 R= H ptilomycalin A
15 R= OH Crambescidin 800



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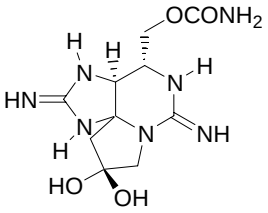
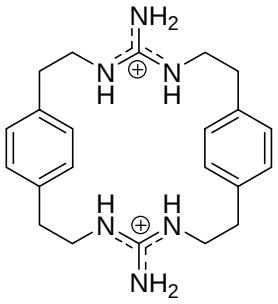


17

To compare the anion binding abilities of azacrown ethers, analogous macrocyclic guanidinium compounds **18-20** were made available by Lehn et al.[47] The complexation studies with tribasic anion PO_4^{3-} in methanol/water were disappointingly weak with log K_{ass} in the range of 1.7 to 2.5. The extensive study comparing more different guanidinium compounds set the conclusion that the electrostatic attractions dominates anion binding in these type of hosts.[48]



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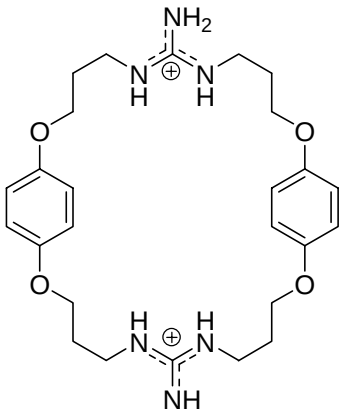

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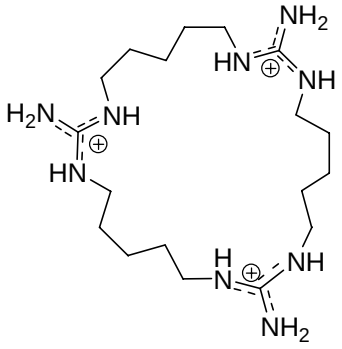
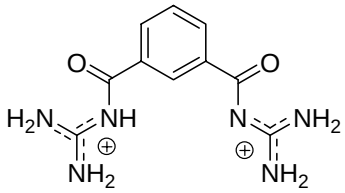


20

In order to mimic the enzymatic cleavage of phosphordiesters, the host **21** was made available.[49] This receptor was designed on the basis that the two guanidinium moieties separated by a spacer should allow them to orient in a way that resembles active sites of a staphylococcal nuclease. The host **21** bound to the phosphordiester guest with high affinity (K_{ass} = 5 x 10^4 M^{-1}) in acetonitrile

and enhanced the catalytic cleavage of phosphordiester by a factor of 700.[49] However, host **21** also showed higher order binding when concentration of guest applied exceeded one equivalent.



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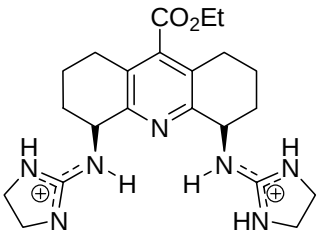

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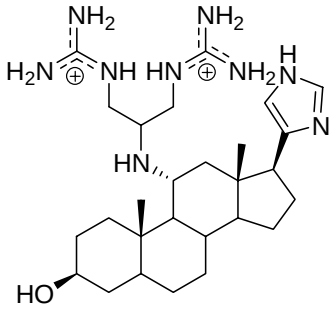
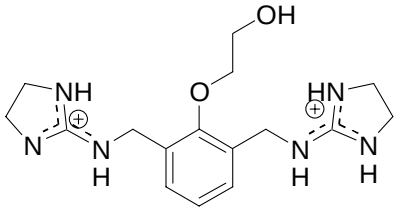


23

The structural improvement in enzyme mimetic receptors led to the design of the host **22.** This was prepared using an octahydroacridine spacer between two aminoimidazoline groups.[50] The host **22** showed binding in competitive media (DMSO/ water) and enhanced the catalysed mRNA hydrolysis by 20 fold in water.[51]

Another enzyme mimic receptor **23** that contains bis(guanidinium) groups appended on the steroid framework was made available.[52] In the class of structurally related compounds, the host **23** showed 10-fold greater rate in the cleavage of 2-hydroxypropyl-p-nitrophenylphosphate (HPNPP). Göbel and co-workers used the phenyl alcohols **24** and **25** to study the binding and catalysis of synthetic phosphordiester hydrolysis.[53] The host **24** bound to a cyclic phosphate guest with much higher affinity (K_{ass} = 2.9 x 10^3 M^{-1}) than **25** (K_{ass} = 190 M^{-1}) and catalysed phosphorylation of the alcohol 800 times faster than **25**, indicating the ability of guanidinium to enhance the binding and the reactivity of phosphate guests.



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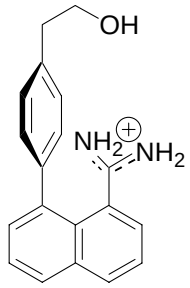

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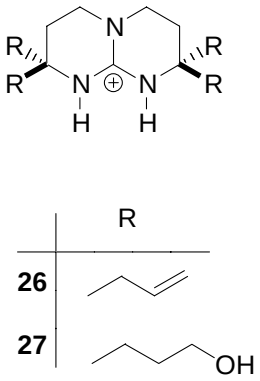
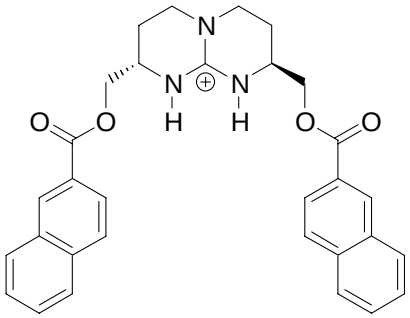
The above discussed simple bis-guanidinium salts showed catalytic effects in phosphate ester hydrolysis, they are much less efficient compared to metalloenzyme mimics and showed higher binding order complexes, too.

More than two decades ago, an interesting class of anionic hosts **26, 27** was made available by the Schmidtchen group[54] by incorporating the guanidinium group in to the bicyclic framework. The design was made to overcome the ambiguity in the binding mode that is possible with open chain analogues. The insertion of the guanidinium moiety into the strain-free bicyclic framework will make the host more chemically stable and basic than the parent guanidine. The addition of hydrocarbon residues should hinder the hydration of the charged moiety, adding a beneficial effect to oxoanion binding.

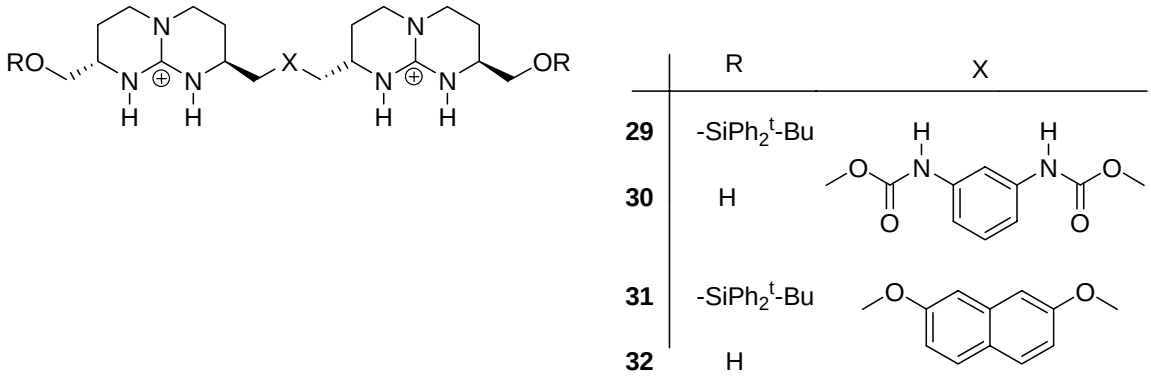




28

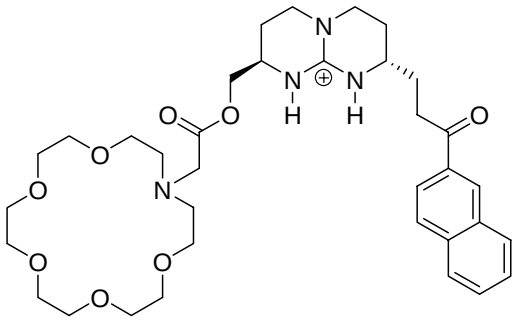
The host **26** was shown to form a very stable 1:1 complexes with *p*-nitrobenzoate in both acetonitrile and chloroform[54] (K_{ass} = 1.4 x 10^5 M^{-1}). The host **27** with four hydroxypropyl functions showed strong affinity towards a variety of oxoanions like phosphates, carboxylates and sulphates. The X-ray structure of host **27** complex with acetate showed strong evidence[55] supporting the fact that the binding motif is the combination of electrostatic interactions and a set of two parallel hydrogen bonds. Later, chiral analogues of bicyclic guanidinium hosts were made available.[56,57] The bisnaphthoyl host **28** showed strong binding towards aromatic carboxylates and was the first anion receptor showing chiral discrimination. The two point attractive interaction comprising the guanidinium carboxylate salt bridge and π-staking of the aromatic moiety of the guest to the naphthalene residue of the host was responsible for enantiorecognition.[58]

The successful attempts of bicyclic guanidinium as an anchor for oxoanionic species forced to search for more selective species. In the earlier study Schmidtchen employed the bicyclic guanidinium anchor to study the binding of tetrahedral oxoanions by a ditopic receptor.[59-63] In the hosts **29-32**, the two bicyclic guanidinium moieties were linked by a linear and flexible spacer so that the tetrahedral oxoanion binding would fold the bicyclic framework perpendicular to each other making NH-bond donor sites to converge towards the binding centre. The host **29** with nucleotides formed 1:1 complexes in methanol whereas host **30** formed complexes even in water.[60] The host **31** has been used to bind several different mono- and dinucleotides in water and methanol.[60] It was shown that the solvent has much more impact on the binding of host **31** with 5'-AMP, in almost similar solvents like methanol and water. The binding affinity of 5'-AMP with host **31** in water was nearly 40 times less relative to methanol. The host **32** forms a 1:1 complex in water, but shows higher complexes in methanol.



The design improvement of bicyclic guanidinium receptor for enantioselective recognition of zwitterionic aromatic amino acids led to the preparation of bifunctionalized chiral bicyclic guanidinium **33.** The azacrown ether was included to bind the ammonium group while the guanidinium moiety was expected to bind carboxylate. The naphthoyl arm would add a third binding interaction with π-stacking to the aromatic side chains. The host **33** displayed enantioselectivity extracting the L-enantiomer with 80% ee.[64] In a more systematic study, the host **34** bearing better triazacrown ether[65] for complexing the primary ammonium group of amino acid guest, attached with a more chemically stable thioether bridge to the guanidine. The host **34** was able to extract even the more hydrophilic species like glycine and serine into the organic phase. However, the low enantioselectivity (40% ee) compared to host **33** was attributed to the lack of planar aromatic surface that is present in host **33.** In the same class the calixarene-based

host **35** showed chiral recognition of various zwitterionic amino acids. The 90% ee was shown for L-phenylalanine over D-isomer.[66]



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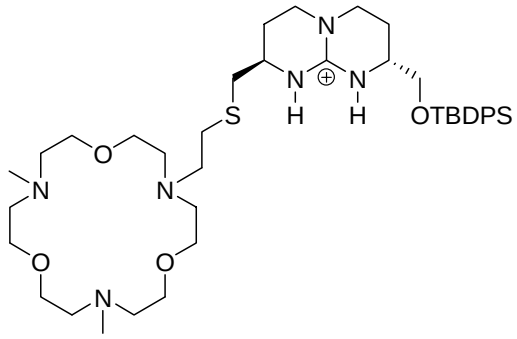

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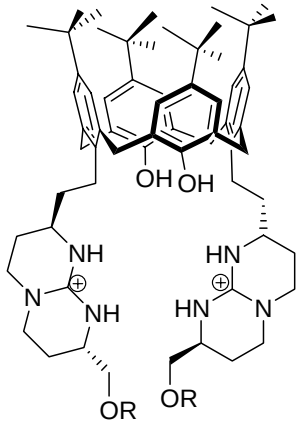
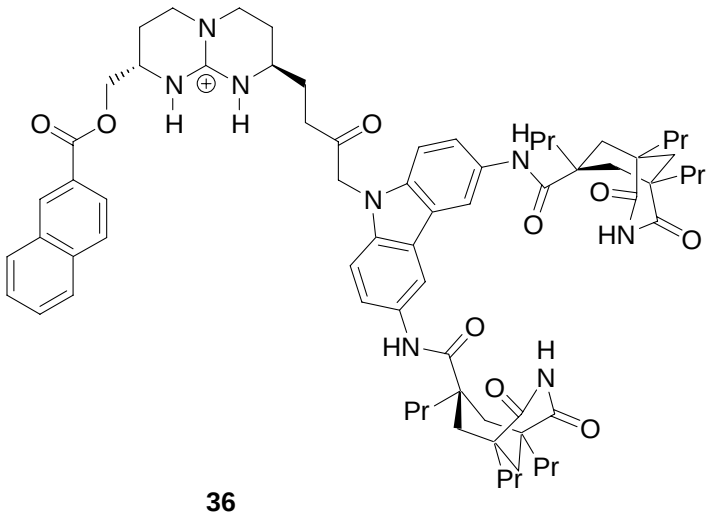


35 R= TBDPS



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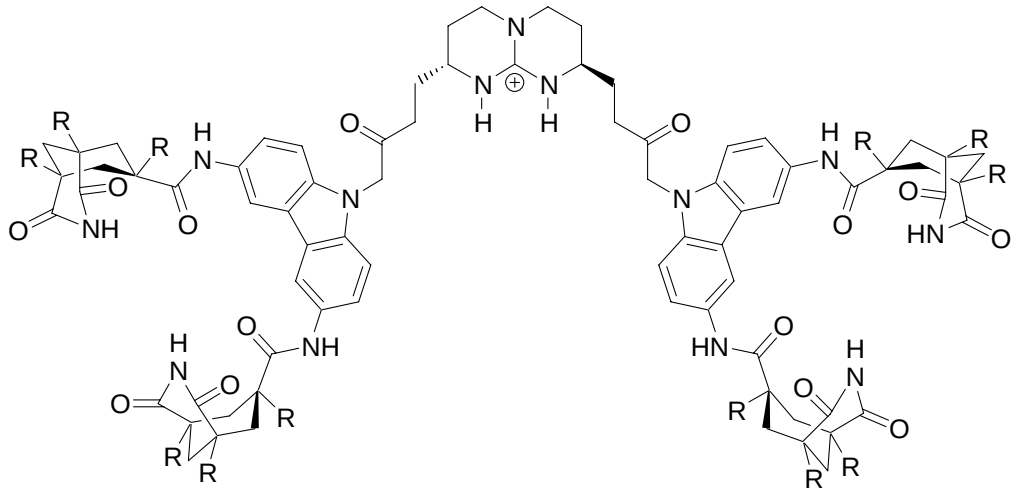
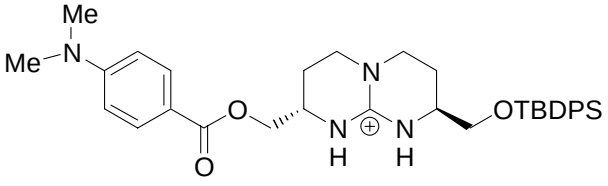


37 R= C_3H_7

38 R= CH_2OBn

In order to improve nucleotide binding selectivity, several guanidinium receptors were designed using the concept of supplementing groups that would interact by complementary base pairing with nucleotide guest.[67] The host **36** showed preference for cyclo-adenosine monophosphates over the guanosine analogues in two-phase extraction. The complexes formed were strictly 1:1 stoichiometric. Evidence from the NMR spectra suggests the binding in the complex to be a combination of electrostatic pairing, π-stacking and a network of hydrogen bonds. To increase the affinity for di- and oligo-nucleotides, the hosts **37** and **38** were made available.[68] The receptor **37** having exactly complementary sets of functions as in dinucleotide phosphate dApA showed high affinity for dApA. The similar host **38** was able to transfer in a two phase extraction, a nucleic acid up to molecular weight 25 kDa into a dichloromethane phase.

Recently many examples of receptors incorporating the bicyclic guanidinium moiety as the primary binding site has been made availble. A new chiral host **39,** possessing DMAB group tethered to a chiral bicyclic guanidinium subunit was designed for sulfate anion.[69]



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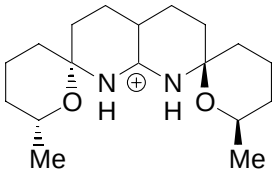

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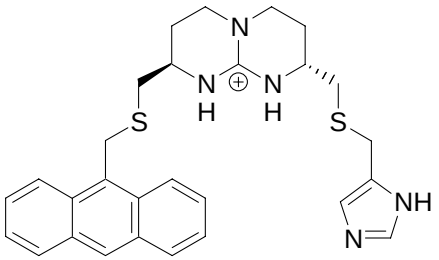

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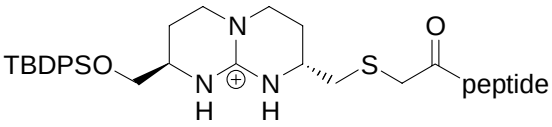

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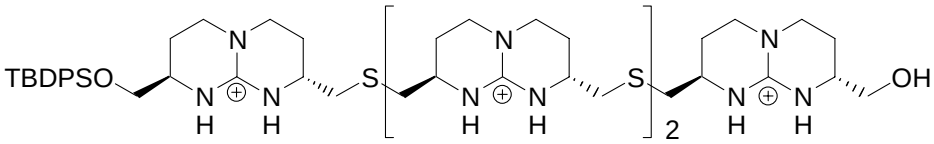


43

The host **39** showed typical successive 1:1 and 2:1 host-guest complexation with the divalent sulfate anion. The stepwise 1:1 and 2:1 association constants in acetonitrile as determined by NMR titration were 1.5 x 10^6 and 4.84 x 10^4 M^{-1} respectively. The C_2-symmetric guanidinium host **40** was used as catalyst in the conjugate addition of pyrrolidine to the unsaturated lactones.[70] The host **40** enhanced the rate of reaction by 16 fold relative to the uncatalysed process. The bicyclic guanidinium compound **41** was used as a catalyst in the addition of pyrrolidine to 2-(5*H*)-furanone.[71] The catalysis is likely due to the development of strong ion pairing and hydrogen-bonding in the transition state. The more interesting bicyclic guanidinium receptor **42** are known for their inhibitory role in the dimerization of HIV-1 protease.[72] In this receptor chiral guanidinium scaffold was linked to the short peptide mimic of the terminal protease sequence and to a lipophilic group. The more recent example is the tetraguanidinium **43** has been classified as the cell penetrating vector and efficiently internalized in human tumor cells.[73] The study by flow cytometry has shown high internalization as well as specific accomumulation of **43** in mitochondria. The vector **43** can likely to be vehicle for the targeted delivery of anticancer drugs to mitochondria.

2. Aim of this work

The guanidinium group plays a prominent role in biological[34] and artificial receptors[74] predominantly for its binding to oxoanions. Successful examples of artificial receptors containing a guanidinium group incorporated into the bicycle have been developed depicting the prime binding motif with an oxoanion as shown in figure 2.

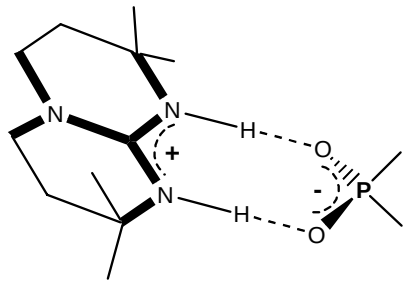


Figure 2: A sketch of the guanidinium oxoanion binding motif as it occurs in many biological and abiotic receptors.

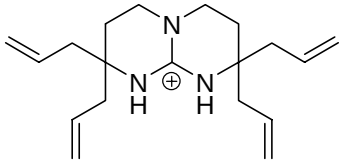
The use of the bicyclic guanidinium framework in anionic hosts helped in reducing the variety of binding modes possible with the oxoanionic guest (approach towards lock-and-key model) and the supplementation of additional substitutions on the peripheral system may lead to polytopic hosts. Based on this concept many artificial receptors have been made available.[75-82] In particular tetraallyl guanidinium host **79** is known to form 1:1 complexes with a variety of oxoanions exhibiting high affinity in chloroform and acetonitrile, moreover, the associations are highly enthalpy-driven.[8,55]

The main aim of this work is the elaboration of the concept discussed above to design and prepare artificial receptors that would meet the following points:

1) High selectivity and affinity towards oxoanions
2) The complexation in a highly competitive solvent like methanol, water and DMSO
3) The host-guest complexation must be enthalpy-driven (precise structuring in the host-guest complex) as demanded in many processes like self-assembly, catalysis etc.

The replacement of allyl substituents in **79** by hydrogen-bonding donor functions that would interact additionally by means of hydrogen bonding on to the bound guest. This should form a well-structured complex resulting in enhanced binding. Amides are the neutral hydrogen bond donors and have been extensively used solely or in combination with electrostatic interaction in designing artificial hosts for anions.[83,84] The amide binding units are most commonly

preorganised to act cooperatively within some convergent molecular architecture. Based on these facts the hosts **101-103** were designed by supplementing the bicyclic guanidinium core with four sec.-carboxyamido groups, so that all hydrogen bond donors will converge on to the bound guest anion resulting in a unique mode of interaction that is illustrated in figure 2. The formation of such a singular complex should surpass the exothermic effect observed in guanidinium host **79** which lacks these functions. Moreover, the congested disposition of the carboxamide anchor groups must hinder their proper solvation, thus, adding another favorable enthalpic contribution to the guest binding, because of the reduced costs of desolvation. Hence tetracarboxamido hosts **101-103** were expected to show enhanced enthalpic binding compared to the tetraallyl guanidinium host **79**.



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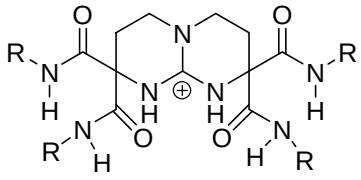


101 R= n-C_3H_7

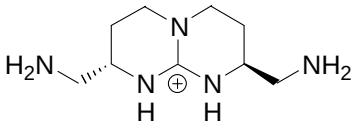
102 R= Ph

103 R= CH_2CH_2OMe

Thus, the development of a synthetic strategy that would give quick and easy access to this series of bicyclic guanidinium compounds was one of the prime tasks.

Further elaborating this concept, the combination of two bicyclic guanidinium units with suitable spacers in a ring would result in the macrocyclic hosts, e.g. **125** or **77**. The construction of a macrocycle using tetrasubstituted bicyclic guanidinium compounds would be too complicated. Therefore an alternative use of the known chiral amino bicyclic guanidinium core **120** can serve as a substitute.[85,86] The two macrocyclic hosts **125** and **77** where designed assuming that construction of an urea bridge in **125** would help by additional hydrogen bonding on to the bound guest and make the macrocycle rather flexible in contrast to the more rigid counterpart **77** that would result from using perfluoromethylene spacer groups. Moreover, these macrocyclic hosts are chiral and can be used in chiral recognition.

The bicyclic guanidinium compound **120** needed to be prepared by a shortened synthetic strategy relative to the established synthesis in order to be of use as a building block for the preparation of macrocycles **125** and **77**.



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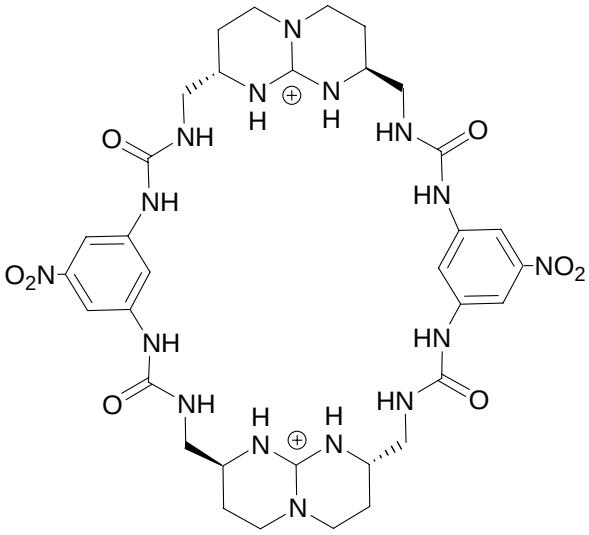

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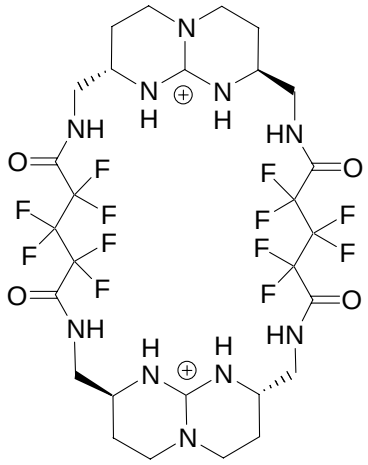

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3. Synthesis:

3.1 Synthesis of the tetra-substituted bicyclic guanidinium hosts 101, 102, and 103.

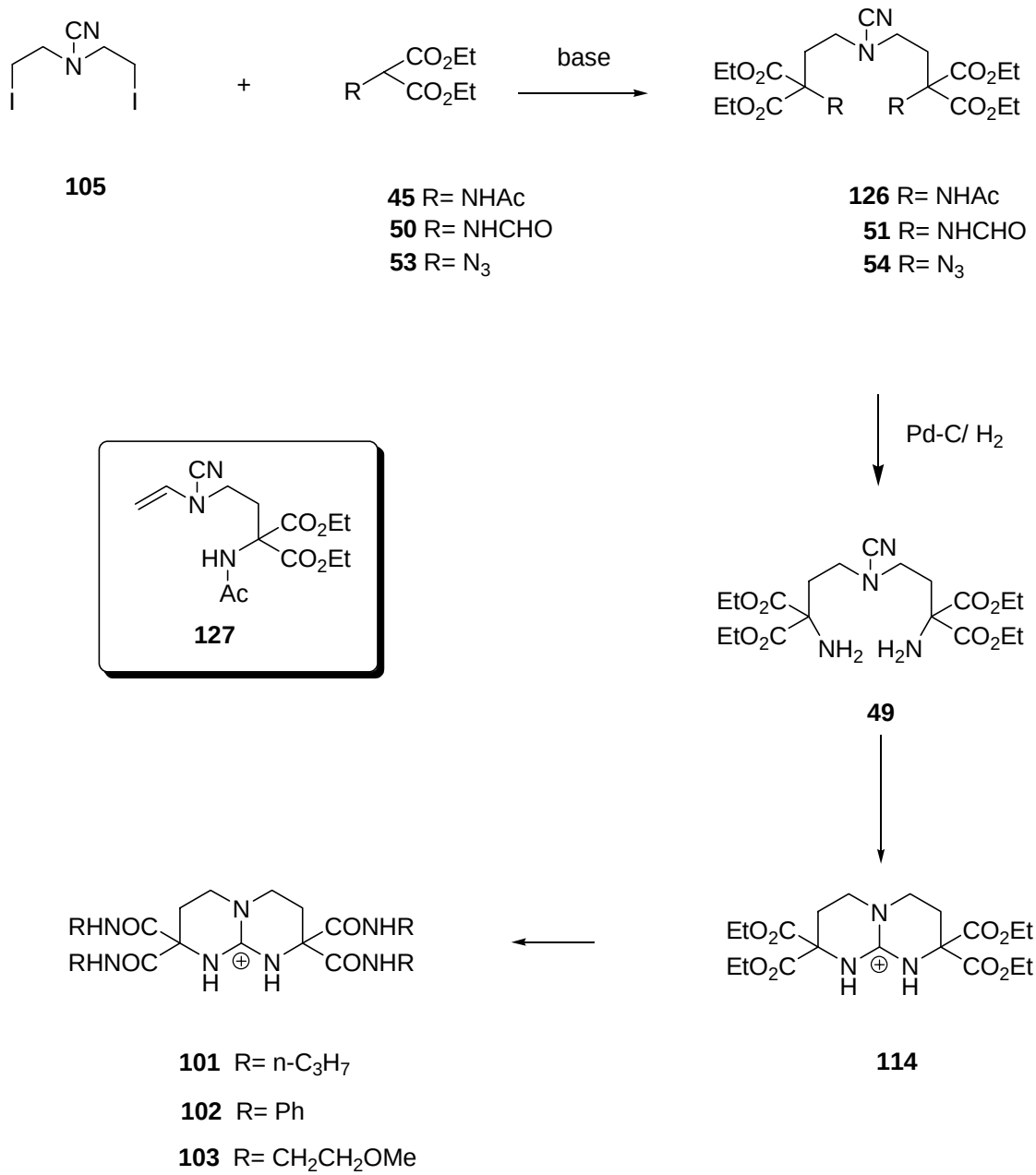
The synthetic strategies for the bicyclic guanidinium building block 104.

The synthesis of the bicyclic guanidinium hosts **101-103** was planned as a series of stepwise reactions outlined in scheme 1. The key intermediate in this route is the tetra-substituted bicyclic guanidinium ester **104**. In this section the synthetic strategies for preparing building block **104** are discussed**.** The reagent bis-iodoethylcyanamide **105** (scheme 1) was used expecting that the nitrile group can serve as the guanidinium carbon donor, thus avoiding the requirement of an extra guanidylating reagent. The strategy in scheme 1 is that the reaction between the known bis-iodoethylcyanamide **105**[87] and commercially available diethyl acetamidomalonate **45** should give compound **126**. The deprotection of the acetyl groups in compound **126** should result in the bisamino compound **49,** which on bicyclization using catalysts[88] should afford the guanidinium building block **104.**

The reagent bis-iodoethylcyanamide **105** was prepared in 74% yield from the reaction of bis(2-chloroethyl)amine hydrochloride with cyanogen bromide and the subsequent treatment of the product obtained with sodium iodide in acetone. The first step of scheme 1, however was less straightforward than expected. The reaction of bis-iodoethylcyanamide **105** with a large excess of diethyl acetamidomalonate **45** in the presence of potassium tert-butylate in THF afforded only 10 % of the desired product **126** and higher fraction of the elimination product **127.**

Many attempts to optimize the yield of the desired compound **126** were tried by varying the base and reaction conditions. The results are summarized in table 1.

Although the yield of the desired compound **126** (40% isolated) was satisfactory the next step in scheme 1 was probed. The attempted deprotection of **126** using HBr in acetic acid did not give the desired bis-amino product **49.** Another attempt in ethanol saturated with HCl afforded a mixture of several products that proved difficult to separate.



Scheme 1. A schematic synthetic strategy for the guanidinium hosts **101-103.**

Table 1. Various conditions used for optimizing the yield of compound **126.**

exp.	solvent	base	T (^{o}C)	relative % of*	
				126	**127**
1	THF	potassium tert-butylate	70	15	85
2	THF	potassium tert-butylate	RT	25	75
3	ethanol	NaOEt	85	-	-
4	acetonitrile	K_2CO_3	85	-	-
5	THF	K_2CO_3	70	15	85
6	DMF	NaH	100	33	67
7	DMF	NaH	50	65	35

*Relative percentage was determined by HPLC analysis.

The disappointing results from the deprotection of compound **126** prompted us to look for a different N-protecting group that was more easily deprotected to get the bis-amino compound **49** as starting reagent. Diethyl acetamidomalonate **45** (scheme 1) was replaced by diethyl formamidomalonate **50** and the same synthetic sequence depicted in scheme 1 was followed. Unfortunately, the reaction of bis-iodoethylcyanamide **105** with formamidomalonate **50** did not afford the desired product **51**, despite all variations in base and reaction conditions summarized in table 2.

Table 2 Conditions used in the attempted synthesis of compound **51**

exp.	solvent	base	T (^{o}C)
1	THF	potassium tert-butylate	70
2	THF	sodium bis(trimethylsilyl)amide	RT
3	acetonitrile	K_2CO_3	85
4	THF	K_2CO_3	70
5	DMF	NaH	100
6	DMF	NaH	50

The disappointing results from the very beginning steps of scheme 1 led to look for the use of an alternative reagent that would give a convenient access to the bis-amino compound **49.** It is well known that amines can be obtained under mild conditions by reduction of azides.[89] The use of azido reagent **53** (scheme 1) to furnish compound **54**, thus seemed worth a try.

This route was abandoned, too, since the preparation of starting azido reagent **53** was unsuccessful. In an attempt to convert compound **52** to the azido reagent **53** (scheme 2) we observed the formation of diethylmalonate **55** as a major product and only minor conversion to the desired product **53**.[90]

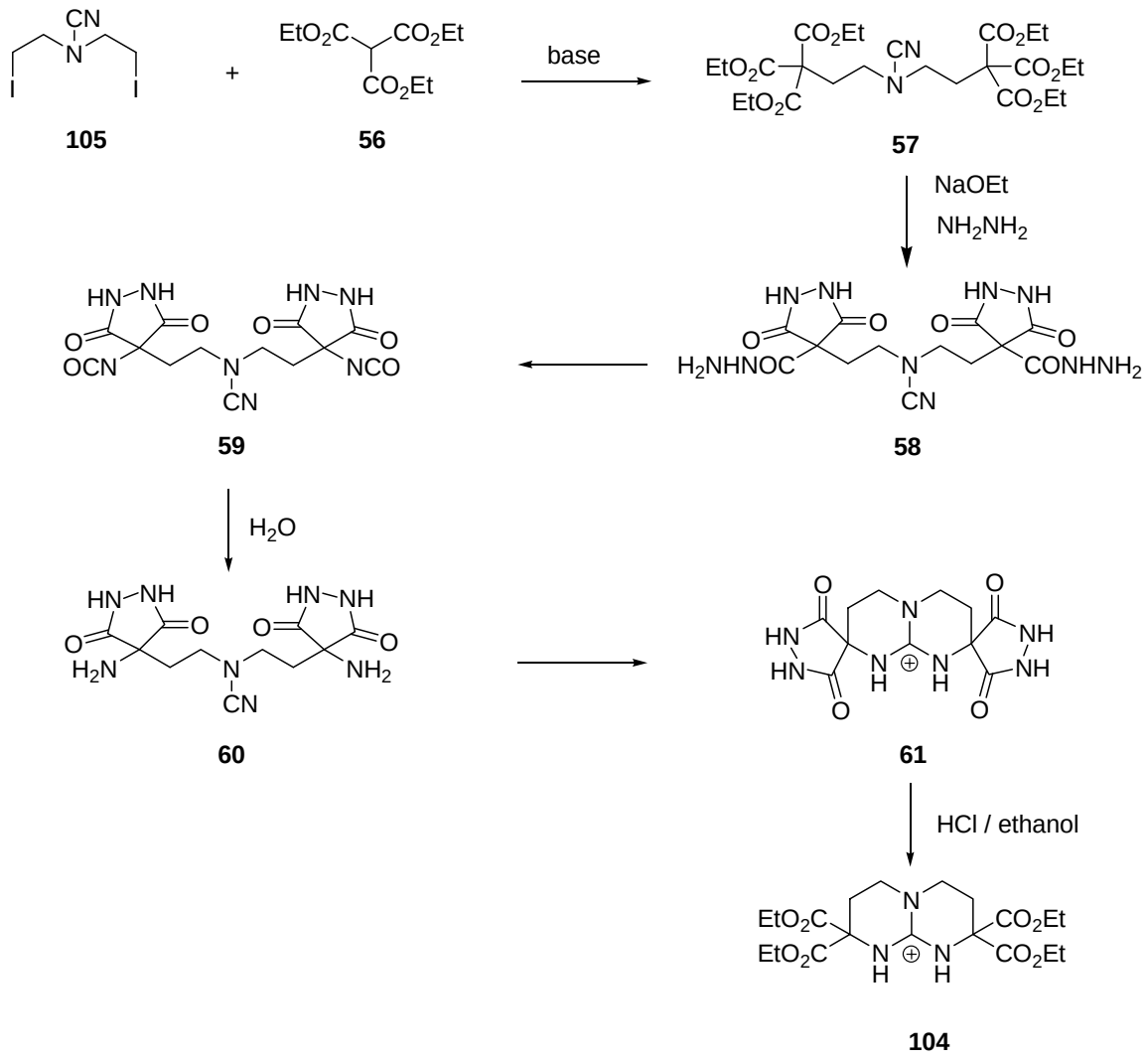
52 **53** **55**

Scheme 2

Since none of the synthetic attempts above described lead to the desired tetra-substituted bicyclic guanidinium compound **104**. A new synthetic strategy was designed still making use of the reagent bis-iodoethylcyanamide **105.** The systematic outline of this pathway is shown in scheme 3. The preparation of compound **57** was carried out successfully (above 90% yield) by the addition of bis-iodoethylcyanamide **105** to the mixture of tri-ester **56** and NaH in DMF at 80 ^{o}C. All the attempts to obtain compound **58** from the reactions of compound **57** with hydrazine afforded a mixture of several products that proved difficult to separate (table 3).

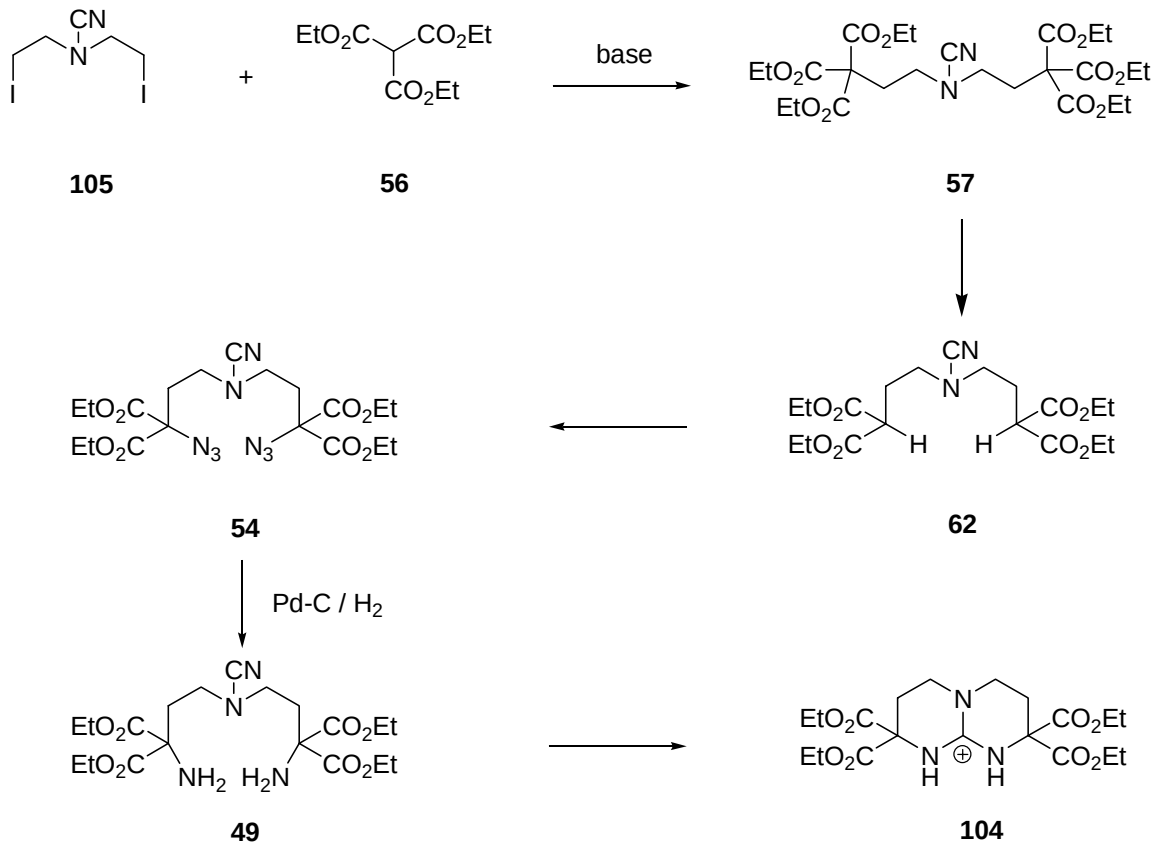
Table 3 Conditions used in the reaction of **57** with hydrazine.

exp.	solvent	base	T(^{o}C)
1	ethanol	-	80
2	ethanol	NaOEt	75
3	DMF	NaOEt	RT



Scheme 3. A new synthetic strategy for the guanidinium building block **104.**

Since compound **57** was obtained in high yield (above 90%), this was to be converted in stepwise fashion to the final building block **104** as shown in scheme 4. However, this route was found not to be fertile and thus was abandoned due to failure in converting compound **57** to compound **62.**
The NMR spectra of the crude product using sodium in ethanol showed that the nitrile group of the molecule **57** is being affected. Fragility of the nitrile group in the starting reagent **105** could be the reason for the failure of previous synthetic strategies.
A new synthetic strategy was design avoiding the use of the cyanamide reagent **105.** The designed synthetic route is shown in scheme 5. The Boc-protected bis-hydroxy compound **64** was obtained in quantitative yield from the reaction of bis-hydroxy amine **64** with Boc-anhydride.[91]



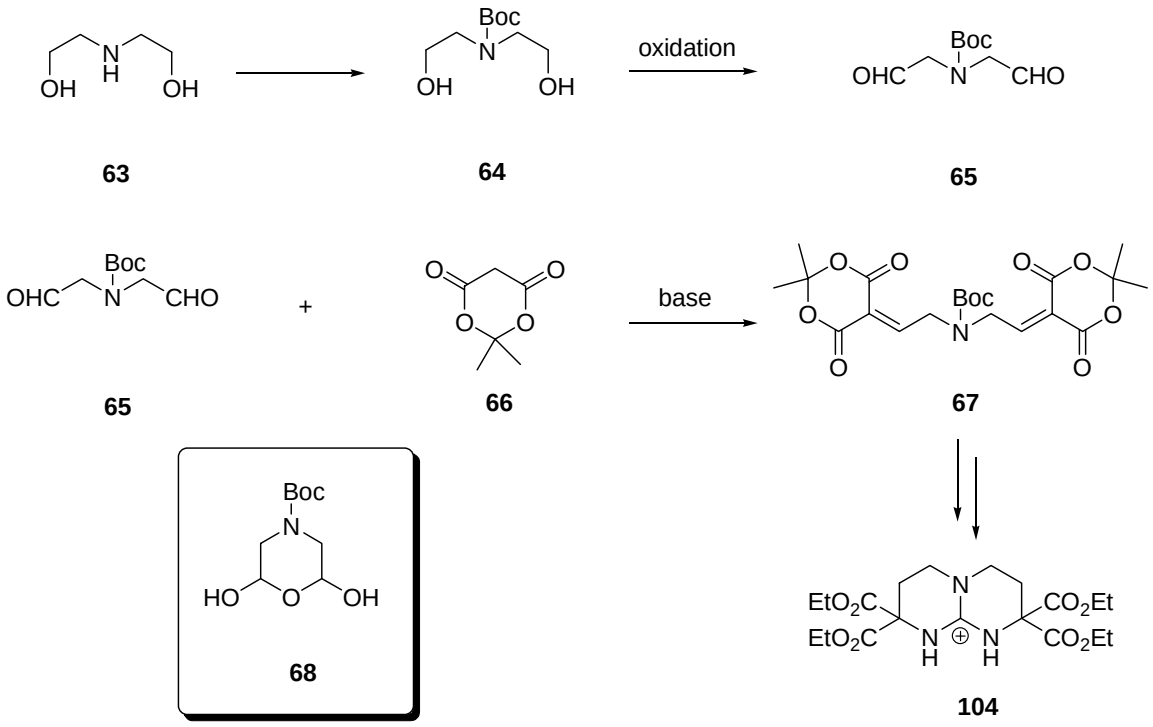
Scheme 4. A new approach for the guanidinium building block **104.**

The oxidation of bis-hydroxy compound **64** (scheme 5) to get compound **65**[92] was not as expected but resulted in the formation of mixture of compounds. The NMR spectra of the crude material indicated the cyclic product **68** as the one of the component in a low proporsion. The unexpected results in the synthesis of reagent **65** forced us to give-up this approach too.

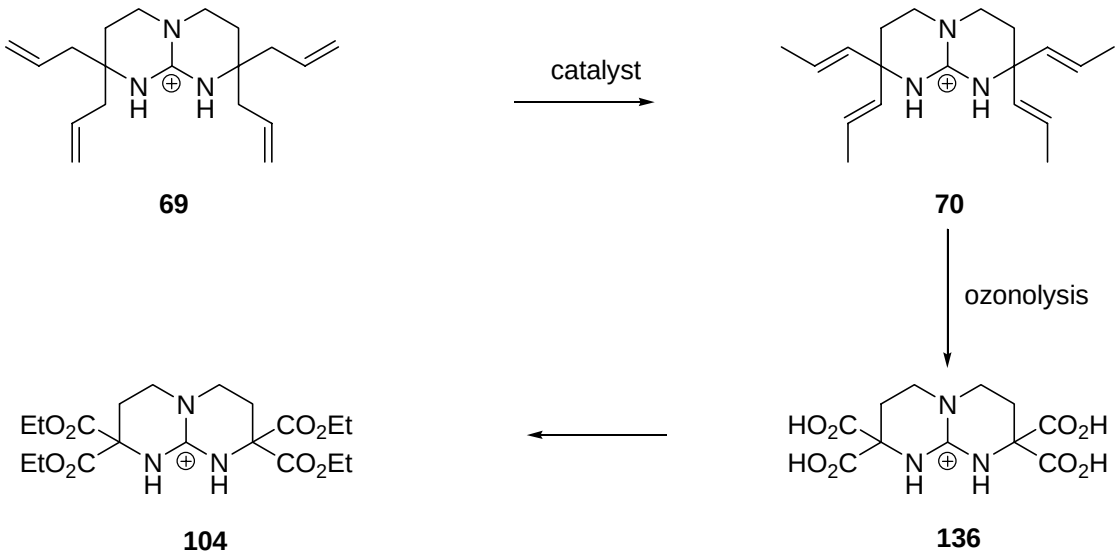
In the search of a good starting compound that would give quick access to the bicyclic guanidinium compound **104**, the well-known tetra-allyl guanidinium compound **69** (scheme 6) was considered.[54] The novel synthetic strategy using allyl guanidinium compound **69** to obtain compound **104** is depicted in scheme 6.

The idea of the new pathway was to isomerize double bonds in the allyl guanidinium compound **69** to get the more stable isomer **70** which on ozonolysis should furnish tetra-acid **136.** The acid **136** on esterification should result in the building block **104.** However, the attempts to bring about isomerisation of the double bonds in **69** were unsuccessful. The various catalysts were tried

that are known to isomerize double bonds in the olefinic systems are collected in table 4, but none of the experiments gave the desired product **70**.



Scheme 5. A new approach for the guanidinium building block **104**



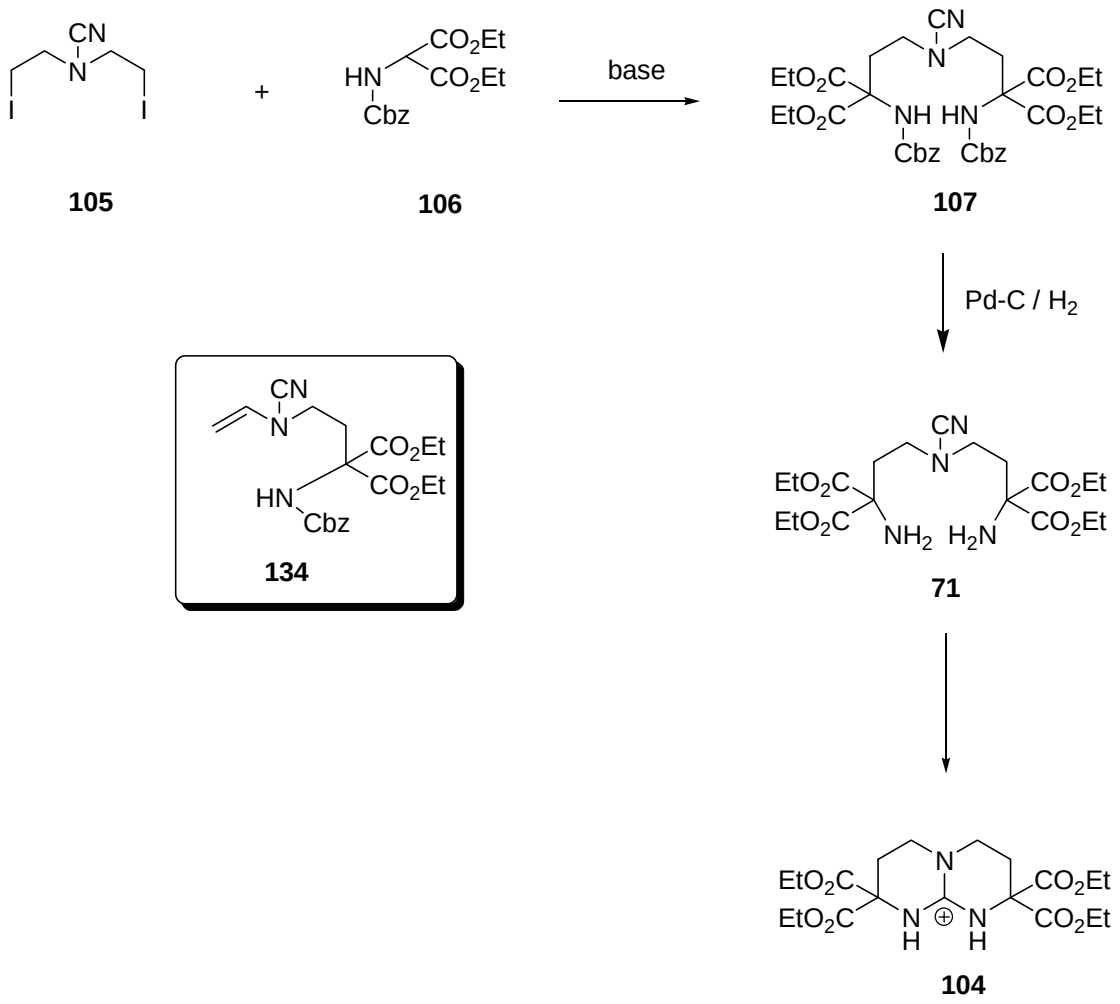
Scheme 6. Another synthetic strategy for the preparation of the guanidinium compound **104.**

Table 4 Different catalysts used in the attempted isomerization of double bonds in compound **69**.

exp.	solvent	catalyst	T (^{o}C)	result
1	ethanol	Dowex®/$RhCl_3$[93]	80	no reaction
2	water	Dowex®/$RhCl_3$	100	no reaction
3	DMA	bis(triphenylphosphine) palladium(II) chloride	150	no reaction
4	DMA	tris(triphenylphosphine) palladium(I) chloride	150	no reaction
5	DMF	tris(triphenylphosphine) palladium(I) chloride	150	no reaction

Since none of the various strategies examined so far furnished the target compound **104** it was decided to have a closer look at the route of scheme 1 and find out about the concrete problems preventing the successful elaboration of this path. As already mentioned, the nitrile group in reagent **105** is fragile and creates problems in the clean cleavage of the acetyl groups in compound **126** (scheme 1). To overcome this problem a N-protecting group was sought that could be removed under milder conditions. On screening the literature[94] the benzyloxycarbonylamino malonic acid diethylester **106** (scheme 7) was deemed suitable to replace diethyl acetamidomalonate **45** (scheme 1)**.** A new synthetic strategy using reagent **106** is shown in scheme 7. The advantage of using benzyloxycarbonylamino malonic acid diethylester **106** over diethyl acetamidomalonate **45** is that the deprotection step can be performed under mild conditions hoping to overcome the problem that we were facing in scheme 1.

The reagent benzyloxycarbonylamino malonic acid diethylester **106** was obtained in 85 % yield from the reaction of diethyl 2-aminomalonate hydrochloride with benzylchloroformate in the presence of BTSA in diethylether.[94] The preparation of compound **107** from the reaction of bis iodoethylcyanamide **105** and the benzyloxycarbonylamino malonic acid diethylester **106** using NaH as a base in DMF (scheme 7) was not straightforward since we also observed the formation of the undesired elimination product **134.** Optimization of the conditions (table 5) finally afforded compound **107** in 72 % isolated yield.



Scheme 7. A new approach for the guanidinium building block **104**

The deprotection of the benzyloxy carbonyl groups was performed at first by hydrogenation using 15 % Pd-C in ethanol. The product obtained, however, was not the desired compound **71,** but the monocyclic compound **128** formed in almost quantitative yield (scheme 8). The series of reactions tried varying the catalysts and reaction conditions to effect the cleavage of benzyloxy protecting groups in the compound **107** are presented in table 6.

When deprotection was performed in acidic media (table 6, exp. 6), instead, the monocyclic guanidinium compound **130** was obtained in high yield. The subsequent cyclization of the monocyclic compound **130** to the desired bicyclic product **104** was probed using different catalysts (table 7) in acetonitrile while heating the mixture in pressure tubes. Monitoring the

reactions by HPLC analysis for several days, we did not observe any conversion to the desired product **104.**

Table 5. Different conditions tested for optimizing the yield of the compound **107.**

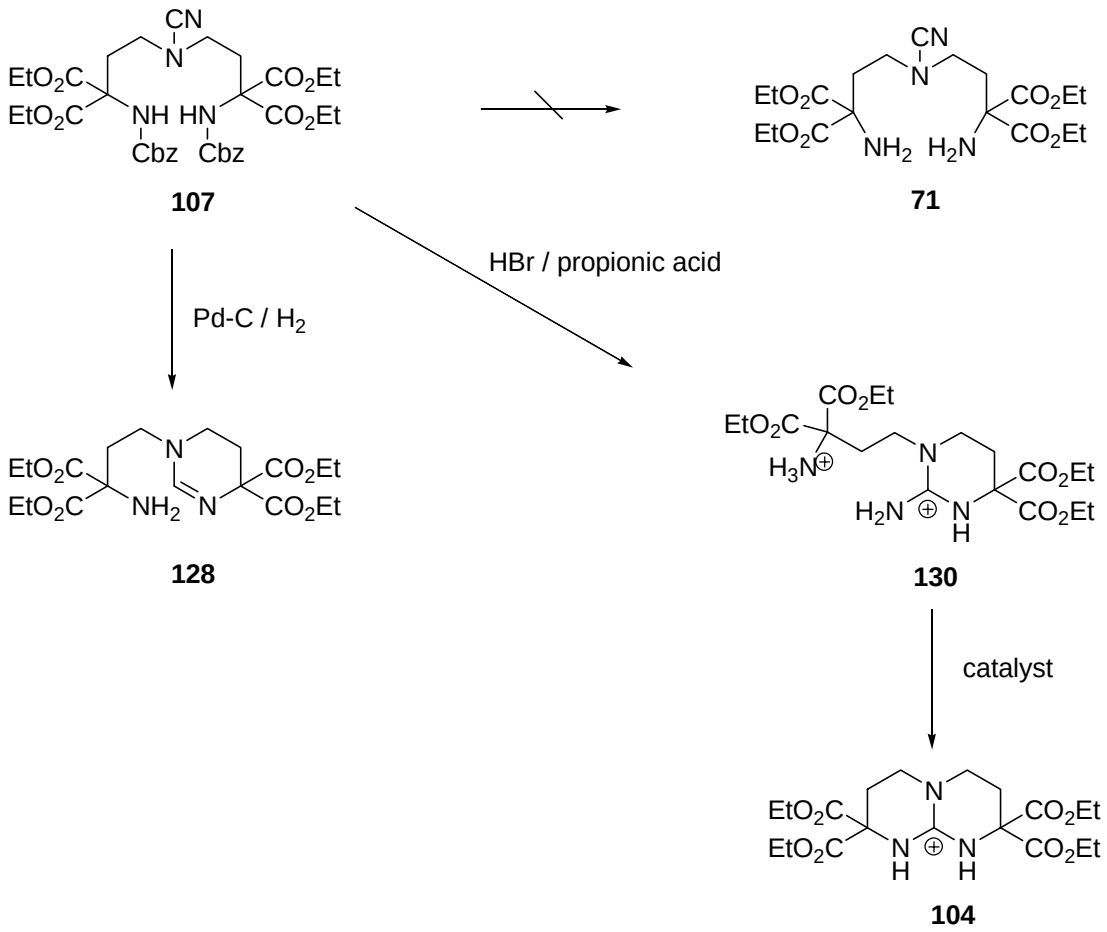
exp.	addition temperature* (^oC)	reaction temperature (^oC)	reaction time	relative yields of** **107**	**134**
1	50	50	4 hours	50	50
2	RT	RT-40	12 hours	60	40
3	0	0-50	12 hours	68	32
4	0	0-RT	2 days	85	15
5	0	0-12	4 days	95	05

* Temperature at which the reagent **105** was added to the reaction mixture containing reagent **106** and NaH in DMF. **Relative percentage was determined by HPLC analysis.

Table 6. Conditions tested for the deprotection of Cbz groups in compound **107.**

exp.	conditions	solvent	T (^oC)	product	yield % of **128** or **130**
1	Pd-C/ H_2	ethanol, 5% CH_3CO_2H	RT	**128**	99
2	Pd-C/ H_2	ethanol	RT	**128**	99
3	Pd-C/ cyclohexene	ethylacetate	80	**128**	90
4	Pd-C/ cyclohexadiene	ethylacetate	80	**128**	85
5	$(CH_3)_3SiI$[95]	chloroform	65	**128**	-
6	HBr in propionic acid	dichloromethane	RT	**130**	95

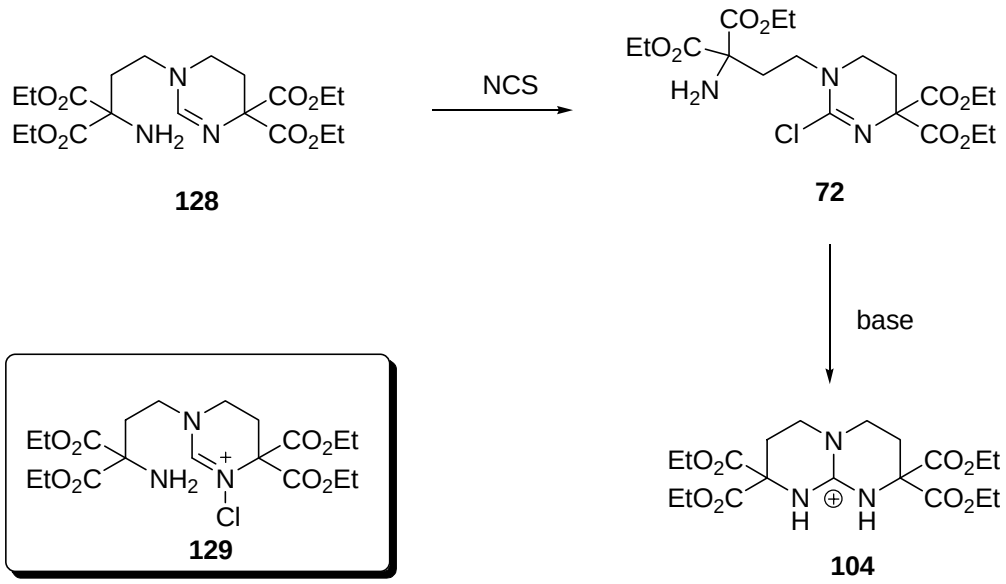
Prompted by this failure the monocyclic formamidine **128** which can be prepared in almost quantitative yield by the hydrogenation of compound **107**, was examined in guanidine formation. The cyclization to the desired product **104** was attempted by oxidizing the formamidine double bond in the compound **128** (scheme 9). Unfortunately, the oxidation using NCS ended up with formation of the undesired product **129** instead of the expected imidoyl chloride **72.**



Scheme 8. A new approach for the guanidinium building block **104**

Table 7. Different catalysts tested for the reaction shown in scheme 7.

exp.	catalyst	result
1	-	no reaction
2	bis(triphenylphosphine) Palladium(II) Chloride	no reaction
3	tetrakis(acetonitrile)palladium(II) chloride	no reaction



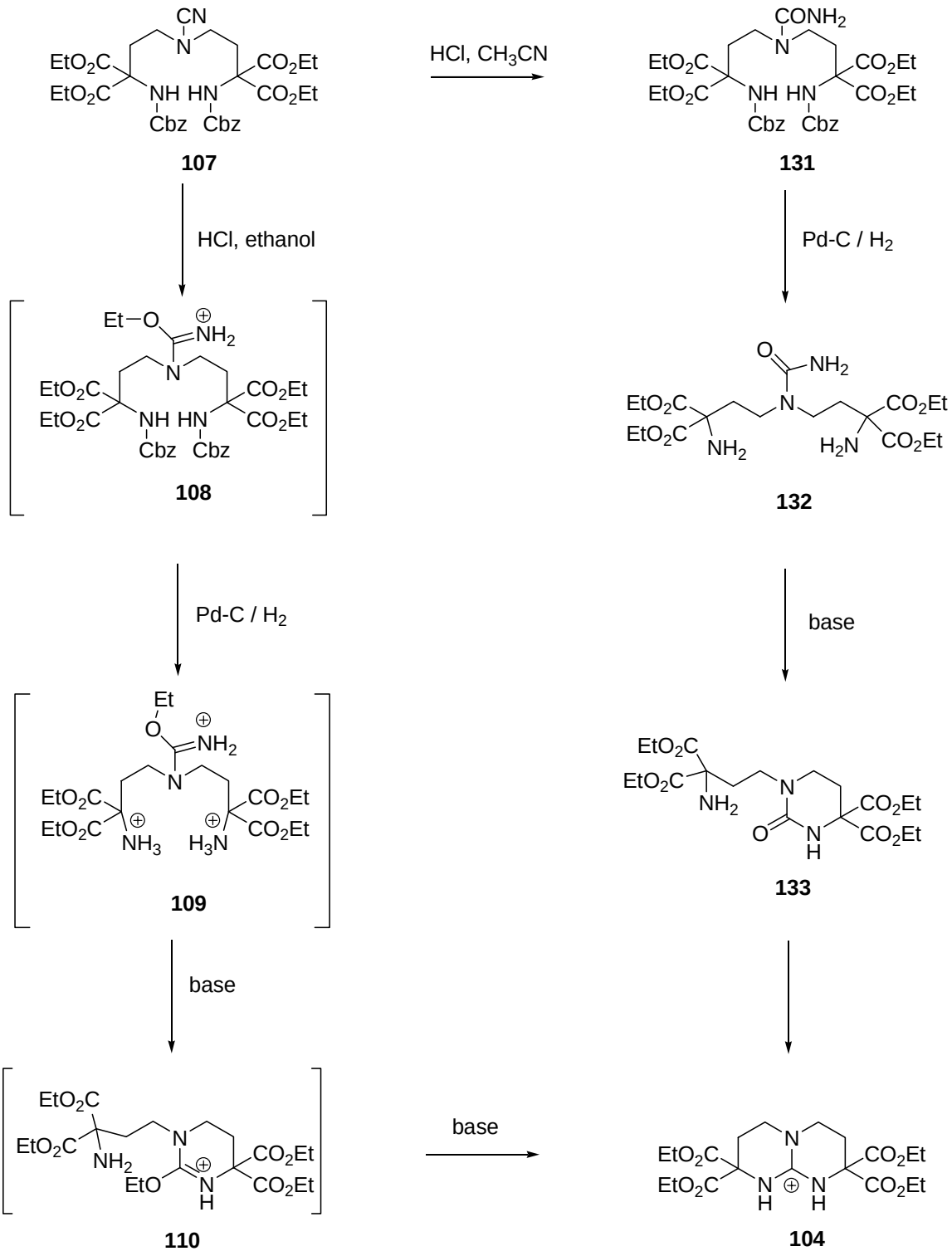
Scheme 9. Alternative approach for cyclization of **128** into desired product **104**

Since the nitrile group in compound **107** appeared to be quite fragile some more stable derivative was desirable before deprotection of the benzyloxy groups. The immediate choice was to transform it into an urea as shown in scheme 10.

The reaction of compound **107** in ethanol in presence of HCl at reflux condition resulted in the formation of 1:1 mixture of urea **131** and the ethylimidate **108.** Switching the solvent to acetonitrile removed the side product **108** and the urea **131** was formed in quantitative yield. It was later found that the use of H_2SO_4 instead of HCl results in much cleaner reaction. Using standard hydrogenation with H_2/ Pd-C in ethanol the bisamino compound **132** was obtained in quantitative yield.

The results from the reactions (table 8) to obtain monocyclic urea **133** from the bisamino compound **132** were not encouraging. At best the monocyclic **133** was obtained in 27% yield (table 8, exp. 6). Also the subsequent cyclizations of the monocyclic urea **133** to the bicyclic guanidinium building block **104** were unsuccessful.

The low yield of monocyclic urea **133** and the failure to afford the bicyclic guanidinium compound **104** disqualified the approach via a urea function. A more promising transformation consisted of the Pinner reaction to the ethylimidate **108** and its subsequent conversion according to scheme 10. The four-step sequence (scheme 10) does not require the isolation of intermediates, but can conveniently be monitored by HPLC analysis.



Scheme 10. A new approach for cyclization to furnish the guanidinium building block **104**

Table 8. Conditions tested for the synthesis of monocycle **133**

exp.	solvent	base/acid	T (^{o}C)	product **133**
1	ethanol	-	80	no
2	DMSO	-	155	no
3	nitropropane	triethylamine	RT-135	no
4	nitropropane	acetic acid	RT-135	no
5	nitropropane	pyridinium tosylate	90-135	no
6	nitropropane	-	135	yes

The reaction of compound **107** with 5% HCl in ethanol resulted in the formation of the imidate **108** as confirmed by MS-ESI. Deprotection of the amino groups by hydrogenation using Pd-C afforded the ammonium salt **109**. Addition of triethylamine base (**caution**: use of excess base led pathway in a different reaction to the monocyclic guanidinium compound **130**) to the reaction mixture resulted in the stepwise conversion to the bicyclic guanidinium compound **104** via the intermediate isourea **110** (confirmed by MS-ESI). The bicyclic guanidinium compound **104** thus was obtained in an overall 74% yield after four successive steps. The X-ray crystal structure of guanidinium compound **104** is represented in figure 3.

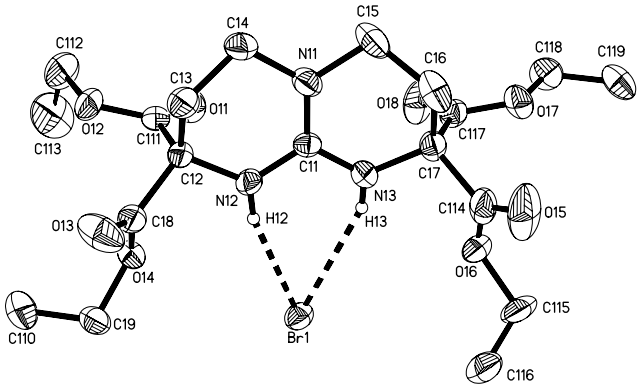
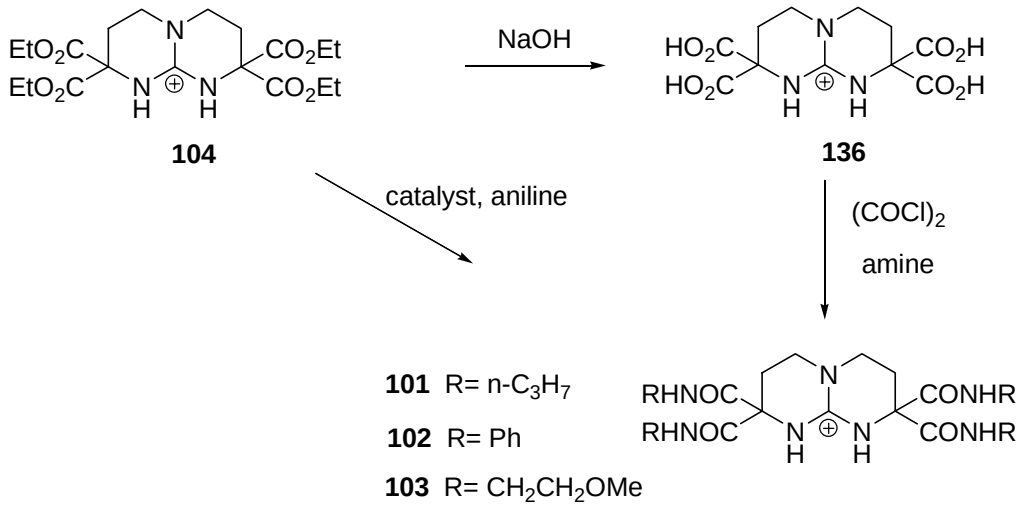


Figure 3. The X-ray crystal structure of the guanidinium compound **104**.

3.2 Synthesis of carboxyamido bicyclic guanidinium host 101-103

The next task was to convert the bicyclic guanidinium building block **104** to the guanidinium hosts **101-103** as outlined in scheme 11.



Scheme 11. Synthetic strategy for the guanidinium hosts **101-103**.

The strategy of the above scheme was to hydrolyze the guanidinium compound **104** to get the tetra-acid **136** followed by amidation after activation of the acid **136** into a more reactive derivative. Hydrolysis of the guanidinium compound **104** using 4 N NaOH in ethanol at room temperature afforded tetra-acid **136** in 88 % yield. The X-ray crystal structure of the tetra-acid **136** is representated in figure 4.

The preparation of amides, however, was unsuccessful. Several attempts were made to transform acid **136** into an activated derivative (table 9) for amidation. The futile attempts were most likely attributable to the lack of solubility of **136** in all of the solvents tried.

An alternative to the stepwise amidation (scheme 11) opened by the catalyzed action of amine onto the ester producing the required amide directly. Various catalysts in the amidation of the tetra-ester **104** with aniline (scheme 11, table 10) were tested. In the end trimethylaluminium[96] in dichloromethane at reflux temperature proved to be the catalyst of choice and afforded the

desired bicyclic guanidinium compound **102** in 77% yield. The X-ray crystal structure of guanidinium compound **102** is shown in figure 5.

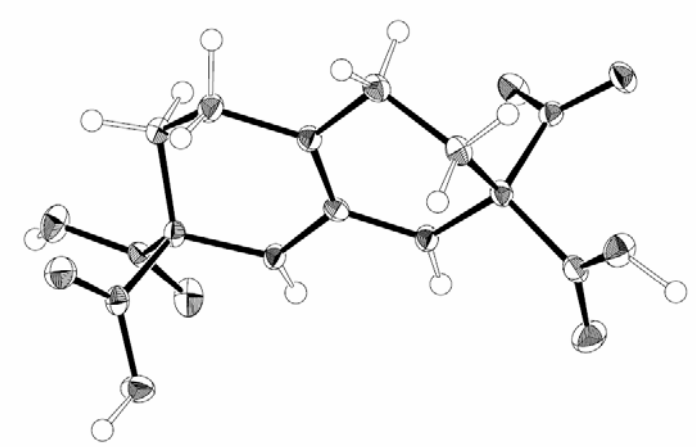


Figure 4 The X-ray crystal structure of **136** (anion is not shown).

Table 9. Different reagents tried in the activation reaction of tetra-acid **136**.

exp.	reagent/solvent	T (^{o}C)	result
1	$SOCl_2$	75	no reaction
2	$(COCl)_2$, cat. DMF	65	no reaction
3	iosobutyl chloroformate, pyridine	50	no reaction

Table 10. Different catalysts tested for the reaction shown in scheme 11.

exp.	solvent	catalyst	T (^{o}C)	product **102**
1	ethanol	acetic acid	RT-80	no
2	THF	$SnCl_2$	70	no
3	THF	$MgCl_2$	70	no
4	dichloromethane	$AlMe_3$	40	yes

The same strategy was applied to get the bicyclic guanidinium hosts **101** and **103** in 85 and 75 % yields, respectively. The X-ray crystal structure of guanidinium compound **101** is represented in figure 6.

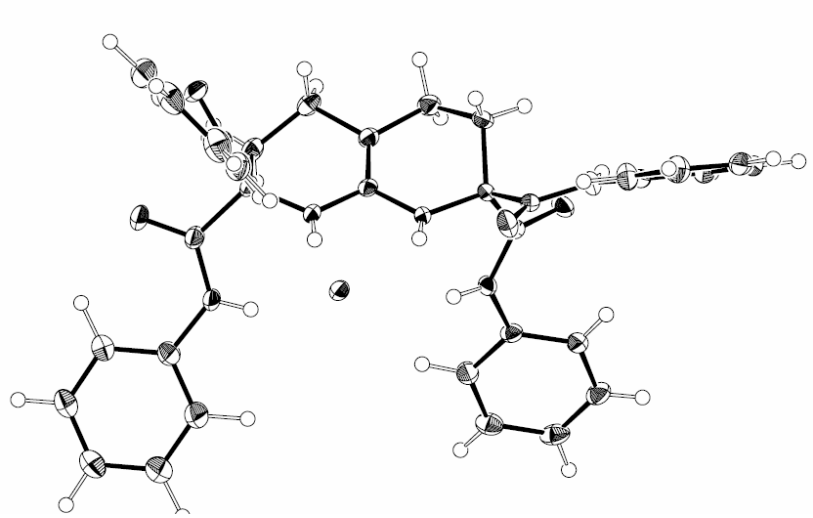


Figure 5. The X-ray crystal structure of guanidinium compound **102.**

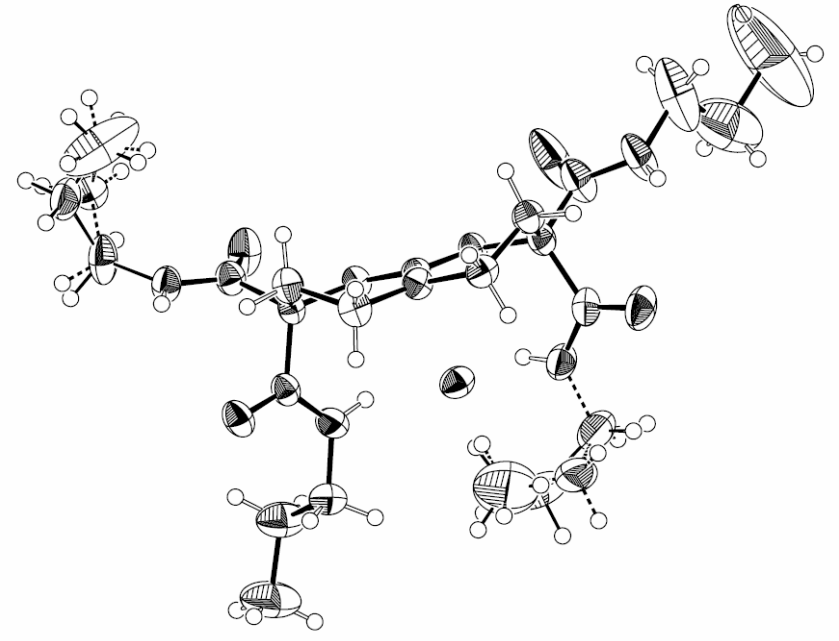


Figure 6. The X-ray crystal structure of guanidinium compound **101**.

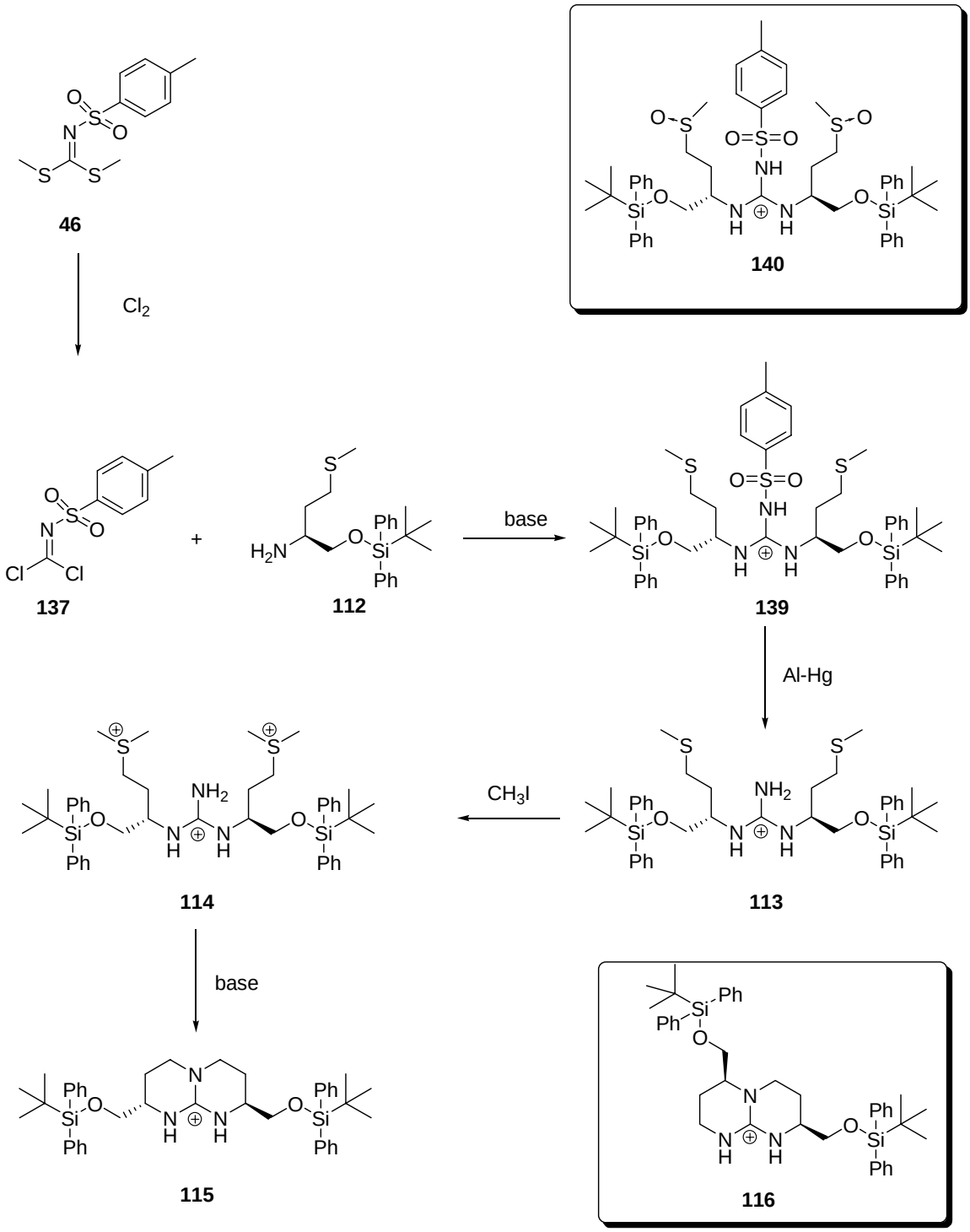
3.3 The synthetic strategies for the bicyclic guanidinium compound 115:

To prepare the bicyclic guanidinium compound **115** the synthetic strategy shown in scheme 11 was designed. The main goal behind this strategy was to achieve a quick and shorter access to the guanidinium compound **115** than already known from literature.[97] The concept bearing the scheme 12 was the reaction of tosyl reagent **137** with amine **112,** which should result in the tosyl protected guanidinium compound **139.** The detosylation of compound **139** should give compound **113**, that lends itself to double cyclization after alkylation to furnish the target guanidinium **115**. The guanidinium compound **115** can be prepared in one pot from the compound **113**.

The tosyl reagent **137** was prepared in 85% yield from the reaction of the commercial compound **46** with chlorine gas.[98] The amino reagent **112** was prepared by known literature method[99] in 78% yield. The tosyl protected guanidinium compound **139** was obtained successfully in high yield (76%) from the reaction of tosyl reagent **137** with amino compound **112**. The detosylation of compound **139** needed to take place under neutral conditions, because an acidic medium would affect the silyl protection whereas basic conditions would harm the guanidinium skeleton. First choice was the use of aluminium amalgam which is known to deprotect guanidinium tosyl groups.[100,101] But unfortunately, this method did not bring about detosylation of **139**. The electrochemical reductive detosylation[97] of compound **139** conducted at a mercury cathode in buffered methanol did not give the desired product **113,** but instead resulted in the quantitative formation of compound **140**.

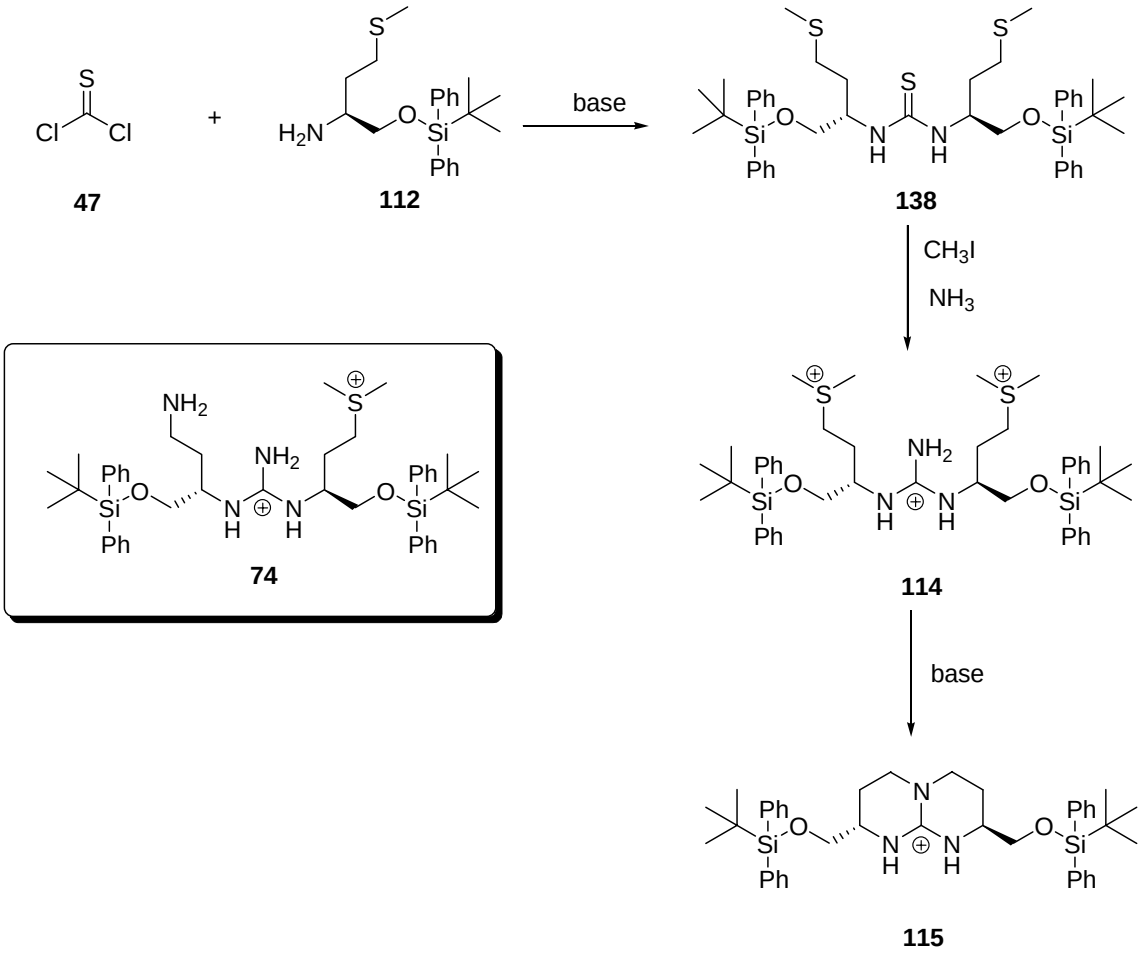
Since the desired product **113** could not be obtained by the two detosylation methods described, metal-ammonia reduction was examined next. The reduction of tosyl guanidinium compound **139** using sodium in liq. ammonia did not bring about detosylation. This was most likely caused by insufficient solubility of the starting guanidinium compound **139** in liquid ammonia. When THF as a co-solvent was used reaction occurred. The NMR spectra of the crude material showed the desired product **113** but gave also evidence of unknown impurities. In order to confirm the validity of the reaction sequence the next steps were investigated without purification of the crude product **113.** Treatment of the crude product **113** with methyl iodide in dichloromethane showed stepwise conversion to the more hydrophilic compound **114** by HPLC analysis. The reaction was much faster when methyl iodide was used as a solvent. After removing excess of methyl iodide, the crude product **114** was redissolved in acetonitrile. On addition of base (DBU)

to the acetonitrile solution, the formation of the desired product **115** (compared with authentic sample) and its structural isomer **116** was detected (scheme 12) in the ratio of 60:40.



Scheme 12. A synthetic strategy for the guanidinium compound **115**

Although the route to get compound **115** was feasible on a small scale problems occurred on scaling up. The detosylation of compound **139** by using sodium in liq ammonia was capricious and very often failed to reproduce the initial results. Frustrated by the unreliable character of this sequence an alternative strategy depicted in scheme 13 was designed. This approach holds the advantage over the previous one (scheme 12) that it does not contain any additional deprotection steps.



Scheme 13. Another pathway to the guanidinium compound **115**

The thiourea compound **138** was successfully prepared in 78 % yield from the reaction of thiophosgene **47** with amino compound **112** in the presence of triethylamine as a base. The treatment of the thiourea **138** with methyl iodide and the subsequent reaction with aqueous

ammonia did not furnish the desired product **114,** but led to a mixture of products containing mainly compound **74** as confirmed by HPLC-MS.

Since this overreaction could not be controlled or elaborated in the desired sense this approach was discontinued. Instead, a guanidylating reagent that would react with the amino compound **112** to directly give guanidinium compound **113** was required bypassing the need for the problematic detosylation step.

Recently Wu, Yong-Qian et al. had demonstrated that di(1H-imidazol-1-yl)methanimine **111** can be used as a guanidylating reagent primary and secondary amines.[102,103] Under similar conditions the reactions of guanidylating reagent **111** with amino compound **112** should give guanidinium compound **113**. Based on this premise a new synthetic strategy for guanidinium compound **115** was designed (scheme 14).

The guanidylating reagent **111** was prepared in 70 % yield by the reaction of two moles of imidazole **75** with one mole of cyanogen bromide.[102] In the pilot run to prepare guanidinium compound **113,** the same conditions used by Wu, Yong-Qian et al. were tried. The reaction of an excess of amino compound **112** with reagent **111** in DMF at 100 ^{o}C did not give the desired guanidinium product **113** but exclusively formed cyanamide **76** (scheme 14).

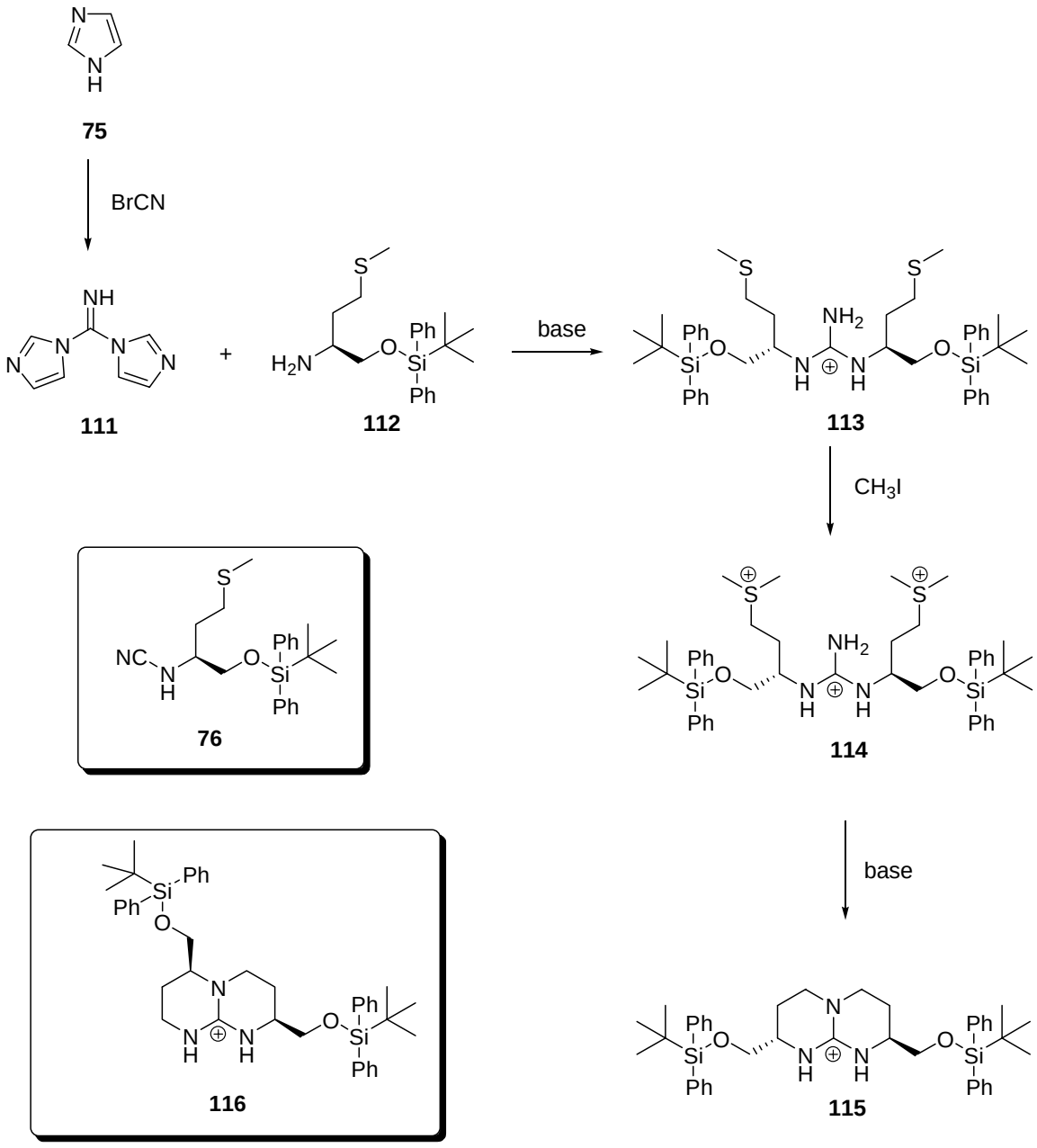
Various conditions in the reaction between guanidylating reagent **111** and amine **112** were explored and results are shown in table 11.

Table 11. Conditions examined for the synthesis of compound **113**.

exp.	solvent / catalyst	T (^{o}C)	reaction time	product **113**
1	THF	RT-70	12 hours	no
2	DMSO	100	12 hours	no
3	THF/ cat. acetic acid	RT	4 days	yes
4	DMSO / cat.acetic acid	100	12 hours	no
5	THF / cat. TFA	70	48 hours	yes
6	Dioxane / cat. TFA	100	24 hours	yes
7	neat / cat. TFA	100	4 hours	yes

Thus, the optimal condition for the preparation of guanidinium compound **113** was to heat the reagents **111**, **112** and a catalytic amount of TFA at 100 ^{o}C for 4 hours (exp. 7, table 11) furnishing the guanidinium compound **113** in 77% yield. The bissulfonium compound **114**

(scheme 14) was obtained in quantitative yield by stirring guanidinium compound **113** in methyl iodide at room temperature over night.



Scheme 14. A new approach to guanidinium compound **115**.

When the bissulfonium salt **114** was treated with base tetramethylguanidine (TMG) in acetonitrile at 40 ^{o}C the formation of the desired product **115** along with its structural isomer **116** in the ratio of 40:60 respectively was observed.

Several attempts to minimize the formation of the undesired guanidinium compound **116**, were under taken as listed in table 12.

Table 12. Reaction conditions explored for the cyclization of **114**.

exp.	solvent	base	T (^{o}C)	relative % of*	
				115	**116**
1	dichloromethane	TMG	RT	60	40
2	dichloromethane	TMG	0	60	40
3	THF	TMG	RT	45	55
4	THF	Sodium bis(trimethylsilyl)amide	-78 to RT	33	67
5	toluene	DBU	RT	30	70
6	dichloromethane	DBU	RT	60	40
7	dichloromethane	DBU	40	60	40
8	dichloromethane	DBU	- 18	65	35
9	dichloromethane	DBU	5 to RT	65	35
10	toluene	K_3PO_4	RT	30	70
11	chlorobenzene	DBU	RT	50	50
12	dichloromethane	DBU/ TBA-PNB**	RT	60	40

* Relative percentage was determined by HPLC analysis.

** TBA-PNB. Tetra-butylammonium para-nitrobenzoate was used as a templating reagent.

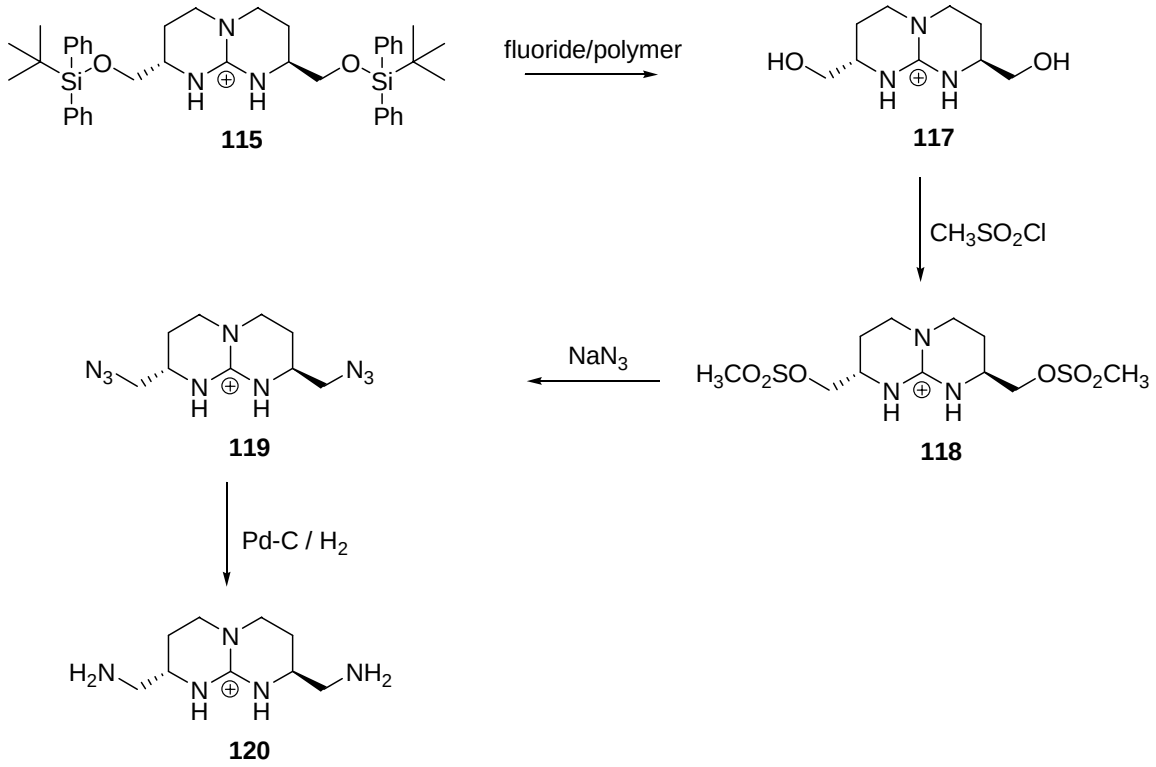
The optimal condition (table 12, exp. 9) favouring the desired product **115** over its isomer **116** consisted of using DBU as base in dichloromethane solvent at a temperature range from 5 ^{o}C to RT. The purification of the mixture of isomers was achieved by crystallization from acetonitrile

at -18 ^{o}C where the desired bicyclic guanidinium compound **115** crystallized preferentially, to give a 45% yield.

As a corollary, the versatile[59,62,97,104] and much desired guanidinium building block **115** is now accessible in an overall 30 % yield in a straightforward 4-step sequence from commercial chiral compounds.

3.4 Synthesis of the bisaminoguanidinium compound 120

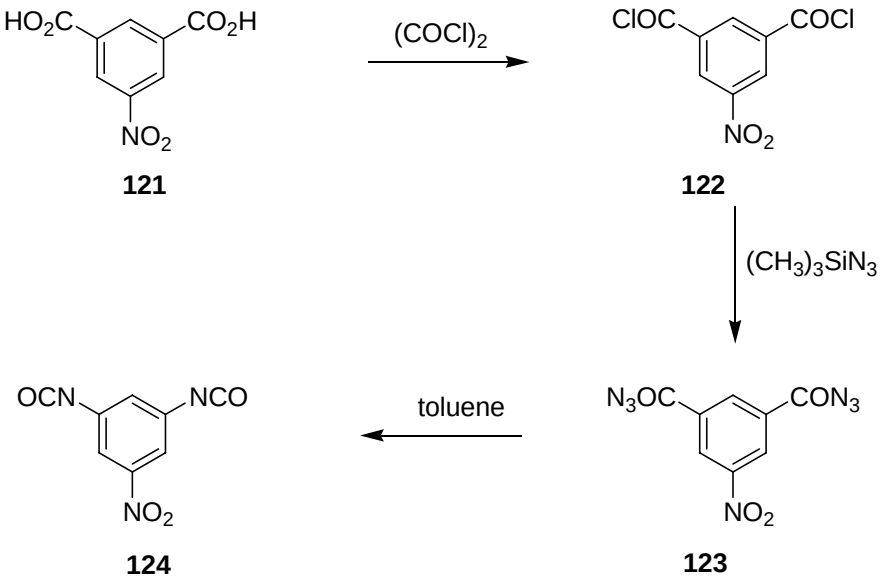
The bisaminoguanidinium compound **120** was synthesized by the series of stepwise reactions shown in scheme 15. The bishydroxy compound **117** was prepared by the cleavage of silyl ethers of **115** using fluoride supported on polymer. The reaction of the dihydroxymethylguanidinium compound **117** with excess of methanesulfonylchloride afforded mesylester **118**. The bis-(diazidomethyl)guanidinium compound **119** was obtained in 60 % (yield over the last three steps) from the reaction of **118** with sodium azide in DMF at 90 ^{o}C. Finally, bisaminomethyl derivative **120** was obtained in quantitative yield by the reduction of bisazide **119** using Pd-C under hydrogen atmosphere.



Scheme 15. A synthetic approach to the bisaminoguanidinium compound **120.**

3.5 Synthesis of the dicyanato reagent 124.

The bis isocyanato reagent **124** was prepared according to the synthetic approach shown in scheme 16. The 5-nitroisophthalic acid **121** was converted to its dichloride derivative **122** in 51 % yield by using oxalyl chloride and a catalytic amount DMF. The 5-nitroisophthalic acid azide **123** was prepared there from in 92 % yield by the reaction with an excess of trimethylsilyl azide. The acid azide **124** rearranges at 110 ^{o}C in toluene to afford bis-isocyanato reagent **124** in 70 % yield.[105]

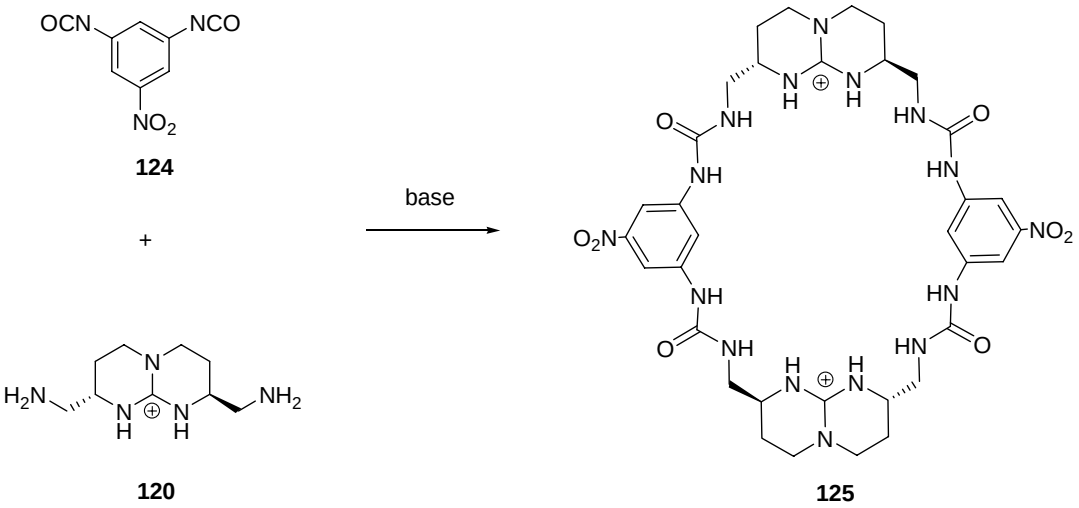


Scheme 16. Synthetic strategy for the compound **124.**

3.6 Synthesis of the macrocycle 125.

Usually the reaction between isocyanates and amines to form urea is catalysed by tertiary amine bases.[106-108] Triethylamine was thus used as a catalyst in the reaction of isocyanato compound **124** and bisamino compound **120** to prepare macrocycle **125** (scheme 17). In a pilot reaction the isocyanato compound **124** in acetonitrile was added to the solution of bisamino compound **120** and triethylamine in acetonitrile at 60 °C monitoring the reaction by HPLC-MS analysis. The product obtained was mainly a polymer. Several attempts were carried to optimize the reaction

for macrocyclization as collected in table 13. In general, in all reactions a freshly sublimed isocyanato reagent **124** was dissolved in a reaction solvent and added by using a syringe pump to the mixture of bisamino compound **120** and base.



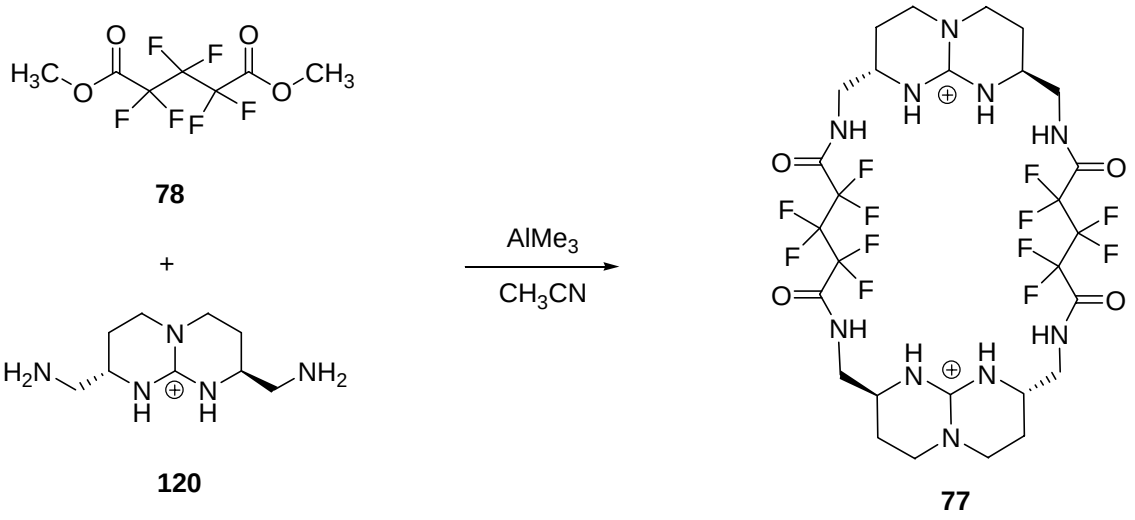
Scheme 17. Synthetic strategy for the compound **125.**

Table 13. Reaction conditions explored for macrocyclization of **120** and **124**.

exp.	solvent	base	T (^{o}C)	macrocycle **125** (%)
1	acetonitrile	triethylamine	60	no
2	acetonitrile	triethylamine	RT	no
3	acetonitrile / DMF	triethylamine	RT	no
4	acetonitrile/ TEA squarate*	triethylamine	RT	no
5	acetonitrile/ TEA squarate*	triethylamine	70	no
6	dichloromethane	triethylamine	RT	no
7	acetonitrile	triethylamine	-10	no
8	DMF/dichloromethane	EDIPA	-10	yes (10)
9	DMF/dichloromethane	EDIPA	-40	yes (10)

3.7 Attempted synthesis of the macrocycle 77.

Contrary to the rather flexible macrocycle **125** a more rigid counterpart was expected from the combination of two guanidinium units **120** with perfluoromethylene spacer groups. As was explored before, a direct amidation of an ester can be achieved by catalyzing the reaction of amine and ester by trimethylaluminium. Thus, macrocycle **77** should be prepared by the synthetic plan shown in scheme 18. The equimolar reaction of bisamino compound **120** and dimethylhexafluoroglutarate **78** in the presence of trimethylaluminium in acetonitrile at reflux however, did not give the desired macrocycle **77**. In spite of a broad variation of reaction conditions (table 14) macrocycle **77** could not be identified as a product in these reactions. Instead, it led to the degradation into the unidentified products.

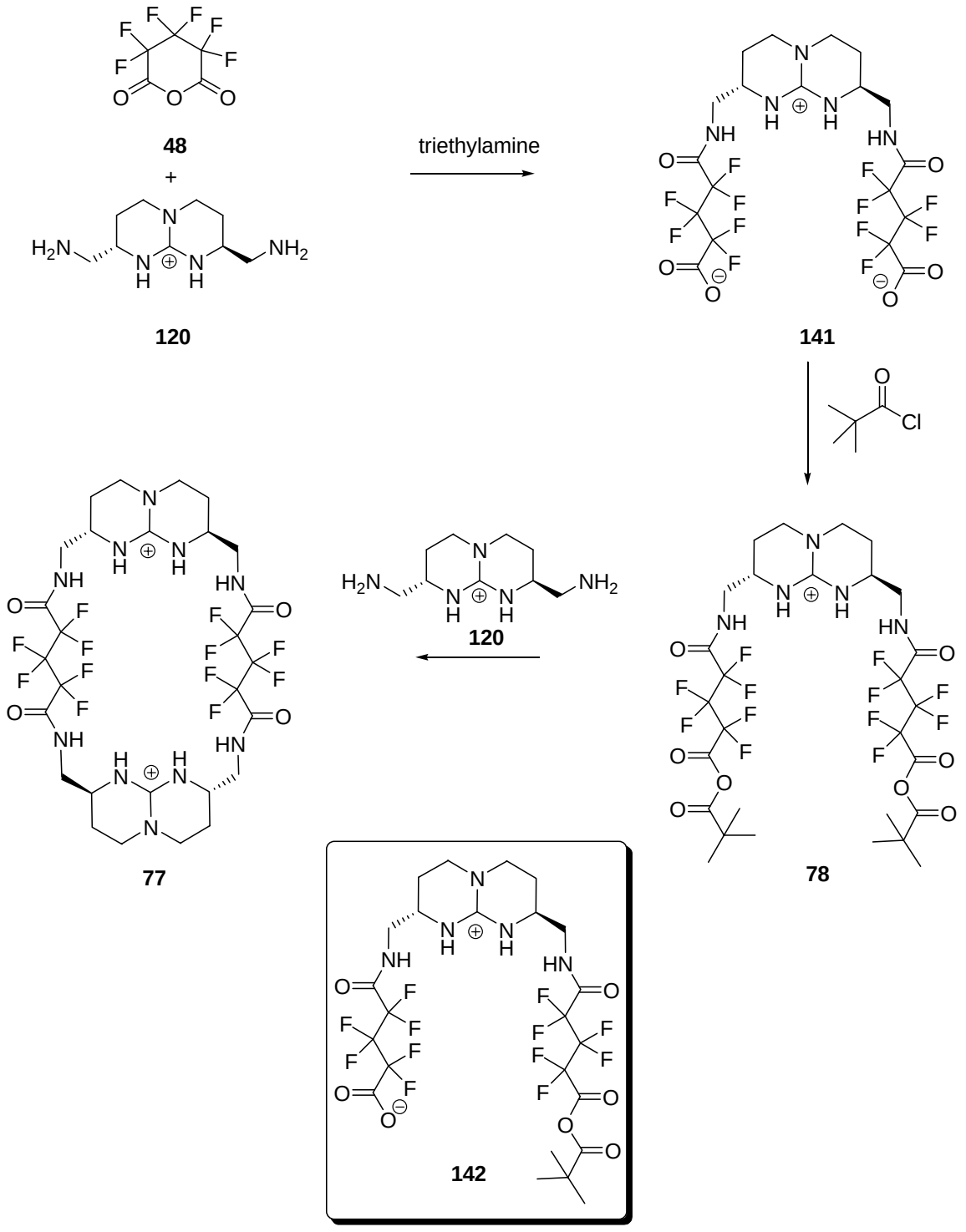


Scheme 18. Synthetic strategy for the macrocycle **77.**

Table 14. Different reaction conditions used for the reaction shown in scheme 32.

exp.	solvent	base/ catalyst	T (^{o}C)	macrocycle **77**
1	acetonitrile	triethylamine	80	no
2	acetonitrile	methanesulfonic acid	80	no
3	DMF	triethylamine	100	no
4	toluene	EDIPA	100	no

Since single step cyclizations to obtain macrocycle **77** were unsuccessful, a stepwise process was envisaged. The strategy as depicted in scheme 19 is based on the reaction of bisamino guanidinium compound **120** with hexafluoroglutaric anhydride **48** to give the bisacid **141**.



Scheme 19 The synthetic approach for the macrocycle **77**.

Converting bisacid **141** to its more reactive mixed anhydride derivative **78** should pave the way to the subsequent reaction with bisamino compound **120** and afford the macrocycle **77**.

The first step of step in this sequence was successfully carried out to deliver bisacid **141** (as a mono triethylammonium salt) in 62 % yield. An attempt to prepare the mixed anhydride **78** from the reaction of salt **141** with pivaloylchloride was unsuccessful and resulted in the formation of the monoanhydride **142** (scheme 19).

An attempt to react salt **141** with diazomethane to transform this compound into the corresponding dimethylester failed. In this case also only one of the two carboxy functions was converted to the corresponding methyl ester. These results strongly suggest that an intermolecular complexation (dimerization) between the carboxylate and guanidinium moiety in the compound **141** takes place**.** Such a complexation diminishes or eventually abolishes the reactivity of one of the carboxy functions. As a way out the reaction of **120** and **48** was conducted in presence of a strong fluoric oxoanion [$CF_3(CF_2)_3SO_3^-K^+$] hoping for competing complexation, but in this case also the formation of compound **142** was observed as the only product and thus suppression of dimerization did not work.

4. Results and discussion of supramolecular complexations

4.1 Molecular modeling of the guanidinium host 101-dihydrogenphosphate 80 complex.

The molecular modeling studies were carried out using HyperChem 7 software on Pentium 4 PCs. The formation of the complex between guanidinium host **101** and dihydrogenphosphate is expected to very much depend on the coulombic electrostatic force of attraction between the binding partners and the additional hydrogen bonding provided by the carboxyamido functions attached to the guanidinium core. The Amber force field was chosen for the energy minimizations of the complex due to its ability to take into account both charge and hydrogen bonding interactions. The global minimized structure obtained by static energy minimization function also was the most populated structure in the molecular dynamics stimulated annealing. On energy minimization and molecular dynamics annealing, the carboxyamido side arms of the guanidinium core rapidly arranged to form an array of hydrogen bonds on to the dihydrogenphosphate guest. The two different views of the global minimum structure of the complex obtained using the Amber force field in vacuo is shown in figure 7. Thus it is very clear from the minimized structure that the dihydrogenphosphate guest **88** is well located with respect to the molecular host with in the carboxyamido side arms forming a finely structured complex by electrostatic attraction and an hydrogen bonding network as originally anticipated.

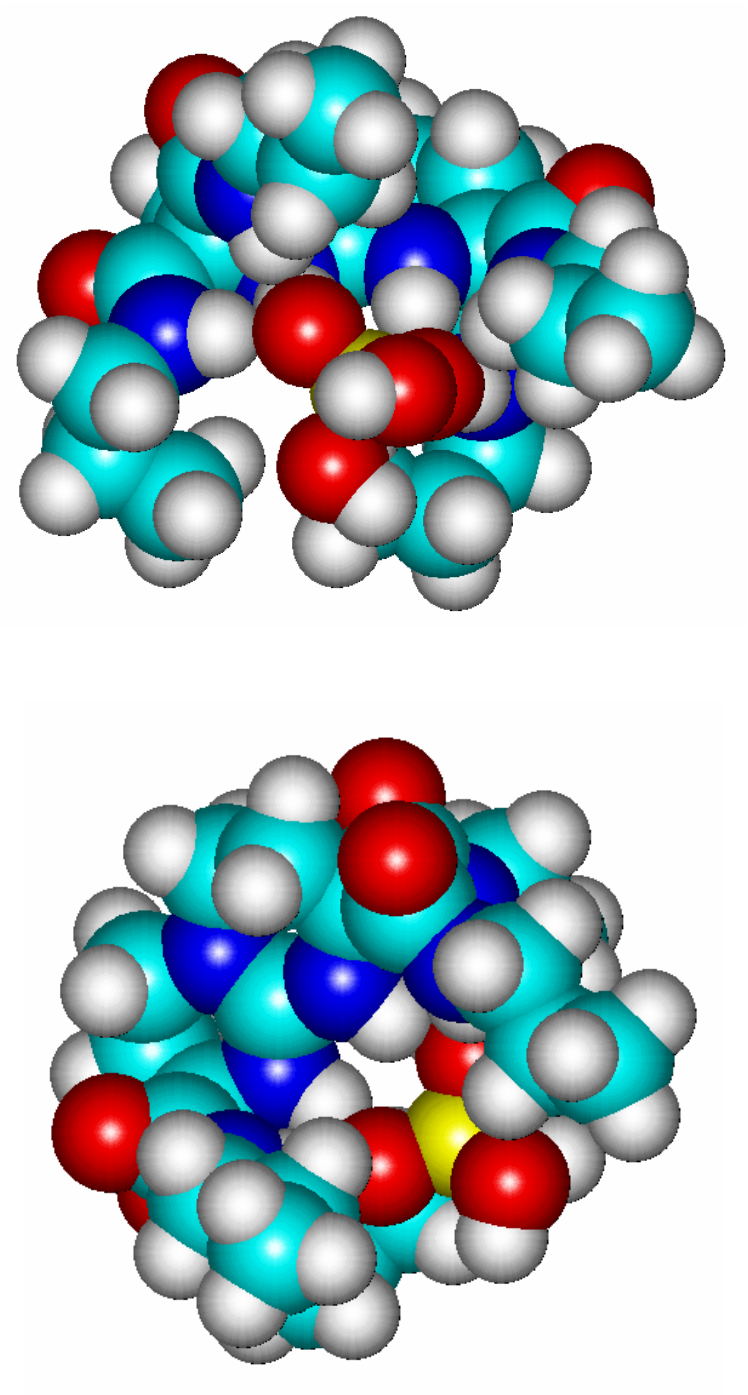
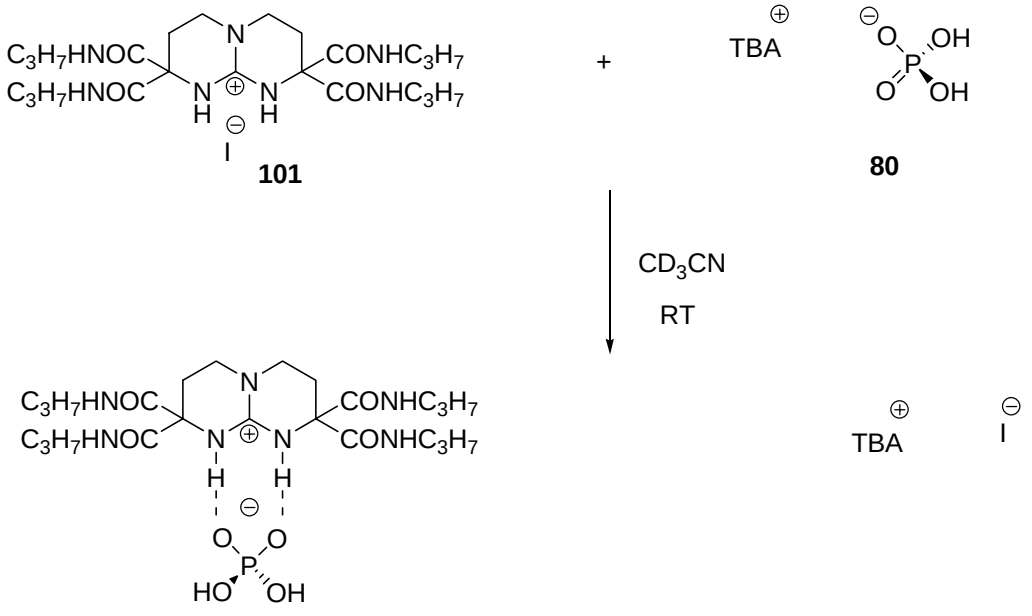


Figure 7. Two different view of the guanidinium **101**-dihydrogenphosphate **80** complex

4.2 ^{1}H-NMR titration of the bicyclic guanidinium host 101 with dihydrogenphosphate 80.

After successful preparations of the guanidinium hosts **101-103** by the novel and shortened synthetic strategy, the next attempt was to study the anion binding. The first attempt was to study the anion binding of the hosts by standard NMR titration experiments. Based on the known mode of interaction in bicyclic guanidinium host-guest systems, the guanidinium host **101** with dihydrogenphosphate **80** in acetonitrile should form the complex **81** as outlined in scheme 20. Since the counteranion plays an important role in complexation of guanidinium with oxoanions,[9] the iodide, a rather weak ion pairing ion was chosen as the counteranion in guanidinium host **101**. The tetrabutylammonium salt of the oxoanion was prepared for the titration in acetonitrile due to the insolubility of the corresponding sodium salt and, moreover, a tetrabutylammonium iodide formed will be a strong electrolyte ensuring the total effect observed in complexation is from the host-guest complex.



Scheme 20. Overview of the association between the guanidinium host **101** and guest **80.**

Usually in the ^{1}H-NMR titration experiment, the guest is added in a stepwise manner to the host and changes in chemical shifts are noted. The titration curve can be obtained by non linear regression methods which, for a typical 1:1 binding gives a hyperbolic curve.

When the anion dihydrogenphosphate **80** (as a TBA salt) was added to the host **101** in acetonitrile-d_3 (for the detailed procedure see experimental section of this dissertation), the signal of the guanidinium NH protons broadened and eventually vanished in the baseline (figure 8).

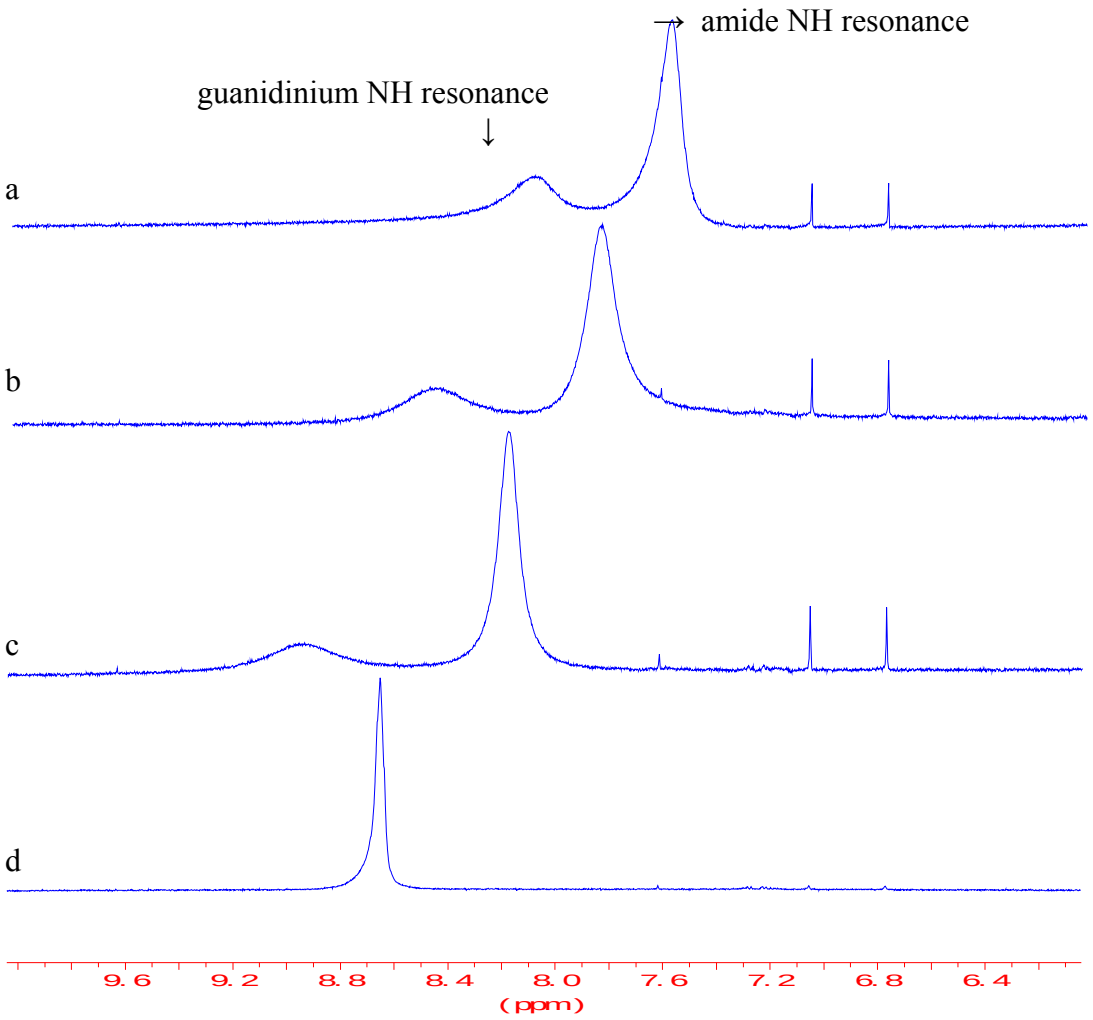
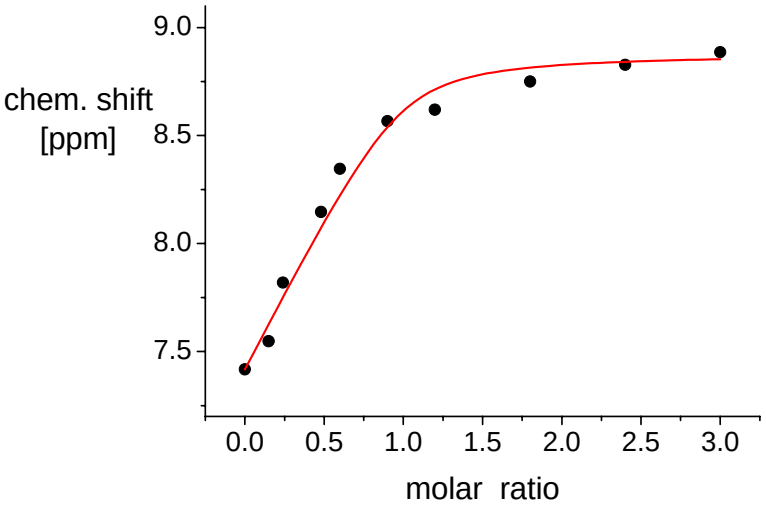
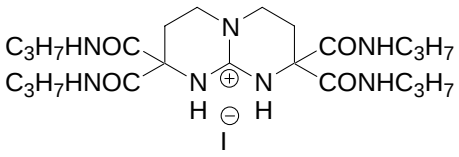


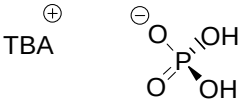
Figure 8. The lowfield region (amide-NH and guanidinium-NH) of the ^{1}H-NMR spectra (CD_3CN, 360 MHz) recorded for the complex formed by the guanidinium host **101** (1 mM) with (a) 0 mM (b) 0.24 mM, (c) 0.48 mM and (d) 1.2 mM of dihydrogenphosphate **80.**

The amide-NH resonances also experienced the expected downfield shift indicating their direct participation in guest complexation. The NMR titration curve represented in figure 9 was obtained by nonlinear regression methods using Origin 5 software.





101



80

Figure 9. NMR titration of guanidinium host **101** (amide NH signal) with dihydrogenphosphate **80** (as a TBA salt) in acetonitrile at 1.0 mM, ambient temperature. The solid line represents the best fit to a 1:1 binding model.

The change in chemical shifts with molar ratio (figure 9) does fit with the ordinary 1:1 binding model. But the careful observation of the experimental data shows the systematic deviations from the fit indicative of the additional binding events. The deconvolution of higher order complexation using this limited data was unsuccessful. But there was an indication of the fact that the host **101** does not strictly follow 1:1 binding model.

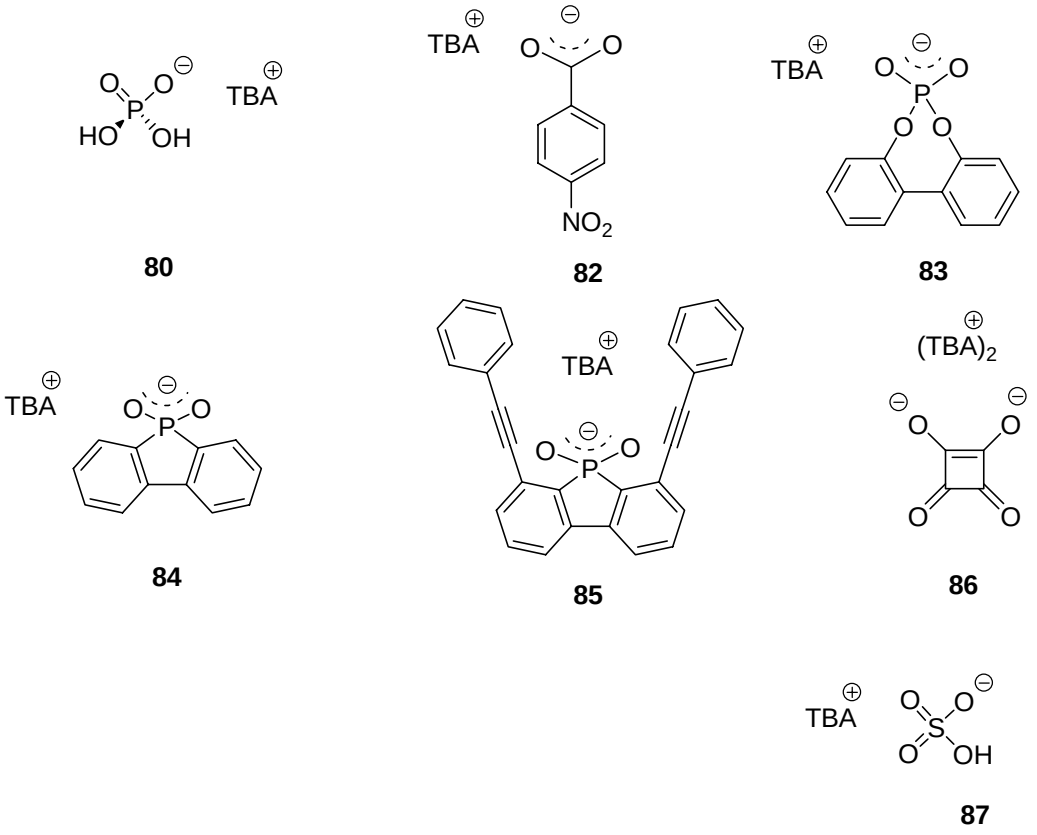
4.3 ITC-Titrations.

Complexation studies were performed using standard isothermal titration microcalorimetry (ITC). This technique provides a measure of association strength, stoichiometry of binding as well as thermodynamic parameters of association (enthalpy ΔH, entropy ΔS, free energy) from a single experiment.

4.3.1 ITC-measurements in acetonitrile.

Since the hydrogen bonds play an important role in the formation of the complex between the guanidinium host **101** and dihydrogenphosphate **80**, the formation of this complex is expected to depend on the competition with solvent. The non-hydrogen bonding solvent acetonitrile was, among other grounds, chosen due to its high dielectric permittivity ε (=36) which minimizes the unspecific ion pairing.

The different oxoanions that were used for complexation in acetonitrile are represented in scheme 21.



Scheme 21. Different oxoanion salts used for complexation to host **101** in acetonitrile.

The addition of the guanidinium host **101** into the solution of the dihydrogenphosphate **80** in acetonitrile produced a heat response characterized by various successive phases (panel A, figure 10).

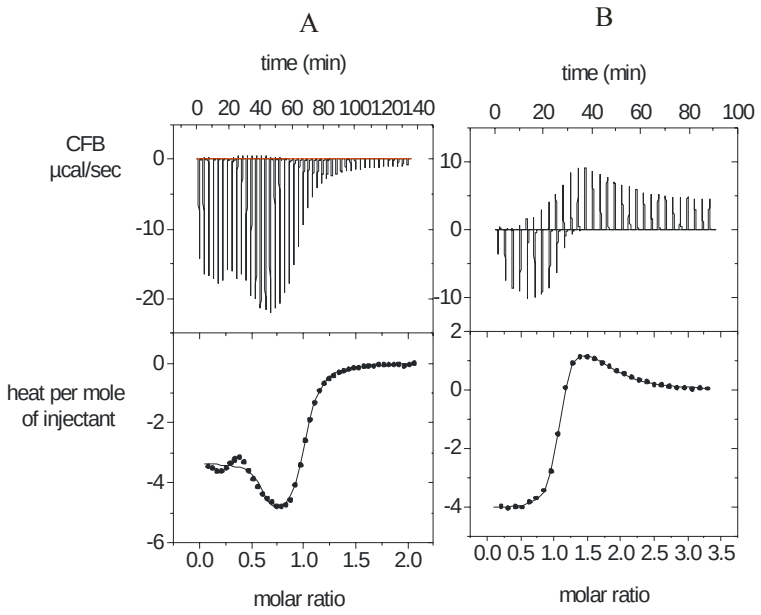


Figure 10. ITC-traces of the titration of **101** into dihydrogenphosphate **80** (1.53 mM, as a TBA salt) in acetonitrile at 298 K (panel A) or adding $H_2PO_4^-$ into host **101** solution at 0.69 mM (panel B, figure 10). The lines represent the best fit to a two-independent-site-model. The derived energetic constants for the 1:1 binding step are included in table 15.

The different processes occurred depending on the actual stoichiometric ratio of the host and guest partners. In the initial stages of panel A (host:guest ratio $n < 0.3$) there is a high excess of phosphate anion over the guanidinium host. The wiggles in the calorimetric response undoubtedly reflects higher order complexation. The stoichiometric phase with $n > 0.5$ was clearly described by a binding model in which a low affinity and endothermic 1:2 host-guest binding step is taken over by an exothermic 1:1 complexation. The validity of this sequence of events was probed by inverting the titration sequence (panel B). Under these conditions the exothermic formation of the 1:1 complex preceeds the endothermic 1:2 process after which the heat evolution ceases because higher complexes are not formed due to their weaker affinity and the moderate excess of guest anion applied. The energetic parameters determined for the 1:1 process in both titration modes are almost identical. The 1:2 step association constant was not determined due to the small fraction of the saturation isotherm detectable in the 1:2 stoichiometric step. The formation of higher order complexes suggests that the host-guest binding deviates from the originally predicted lock-and-key features as it shows variable binding modes

instead of one mode of interaction.. It was observed that the supplementation of hydrogen donor groups led to the predicted enhancement in the binding affinity as compaired to the tetraallyl analogue **79**. But at the same time the energetic results showed fuzziness in the structure of the complex due to formation of higher order complexes which were not observed in the tetraallyl host **79**.[9,82]

The thermodynamic state functions obtained in the calorimetric study of the complexation between the guanidinium host **101** with different oxoanions are presented in table 15.

Table 15. Energetics of host-guest binding of the guanidinium host **101** with different oxoanions in acetonitrile at 298 K.

Tit.	guest	n	K_{ass} [M^{-1}]	-ΔH [kcal mol^{-1}]	TΔS [kcal mol^{-1}]	-ΔG [kcal mol^{-1}]
1	**80**	1.02	1.52 E6	4.19	4.31	8.50
2	**82**	0.91	2.02 E5	4.21	3.15	7.36
3	**83**	1.0	4.82 E4	2.46	4.02	6.49
4	**84**	0.95	1.90 E5	4.92	2.40	7.32
5	**85**	0.80	1.92 E6	5.40	3.02	8.42
6	**86**	0.91	1.54 E7	2.80	7.22	10.00

For all anions probed, there was an indication of the formation of higher complexes. The energetic signature shows a positive entropy of association, indicating weak structuring in the complexes. The smaller guests **80** and **86** show a more positive entropic outcome. There is no apparent size relation with the energetic result among the binding partners.

To study the effect of the supplementation of carboxyamido functions in host **101**, the comparison of binding energetics with host **79**, which lacks these functions, where undertaken (figure 11, table 16). In the entire series of oxoanions, the 1:1 complex formation constants are higher for the host **101** compared to host **79** indicating that the increase in the number of attracting interaction leads to the observed enhanced affinity as originally expected. But when detailed calorimetric data are inspected, it is found that the enthalpic component in all the cases is smaller (less negative) for the carboxyamido host **101** than the tetraallyl host **79** (figure 11). The observed enhanced affinity is exclusively due to the increase in the entropic component of association.

Table16. Energetics of host-guest binding of the guanidinium host **79** with different oxoanions in acetonitrile at 298 K.

Tit.	guest	K_{ass} [M^{-1}]	-ΔH [kcal mol^{-1}]	TΔS [kcal mol^{-1}]	-ΔG [kcal mol^{-1}]
1	**80**	2.28 E4	5.59	0.26	5.84
2	**82**	7.10 E4	5.32	1.40	6.73
3	**83**	1.82 E4	3.50	2.39	5.90
4	**84**	4.90 E4	5.13	1.36	6.50
5	**85**	4.63 E5	7.93	-0.10	7.84
6	**86**	5.01 E4	2.84	3.46	6.32

The straightforward explanation for this surprising result is the release of the solvent shell, solvating the polar amido functions to the bulk solvent.[109,110] However, the desolvation cannot be the only reason for the observed positive entropy since there is no trend in the entropy gain among the guests measured. The release of solvent to the bulk is generally related to the interface area of the host and guest.[111] In this respect, the phosphinate **85** bearing the most extended interface area among the oxoanions probed should show the highest entropy gain. In contrast, the greatest entropic differences between host **101** and **79** were found in the association with the smallest anions, **80** and **86**. The significant entropy differences are thus believed to originate from variations in the number and stiffness of the binding modes encompassing the partners rather than from desolvation.

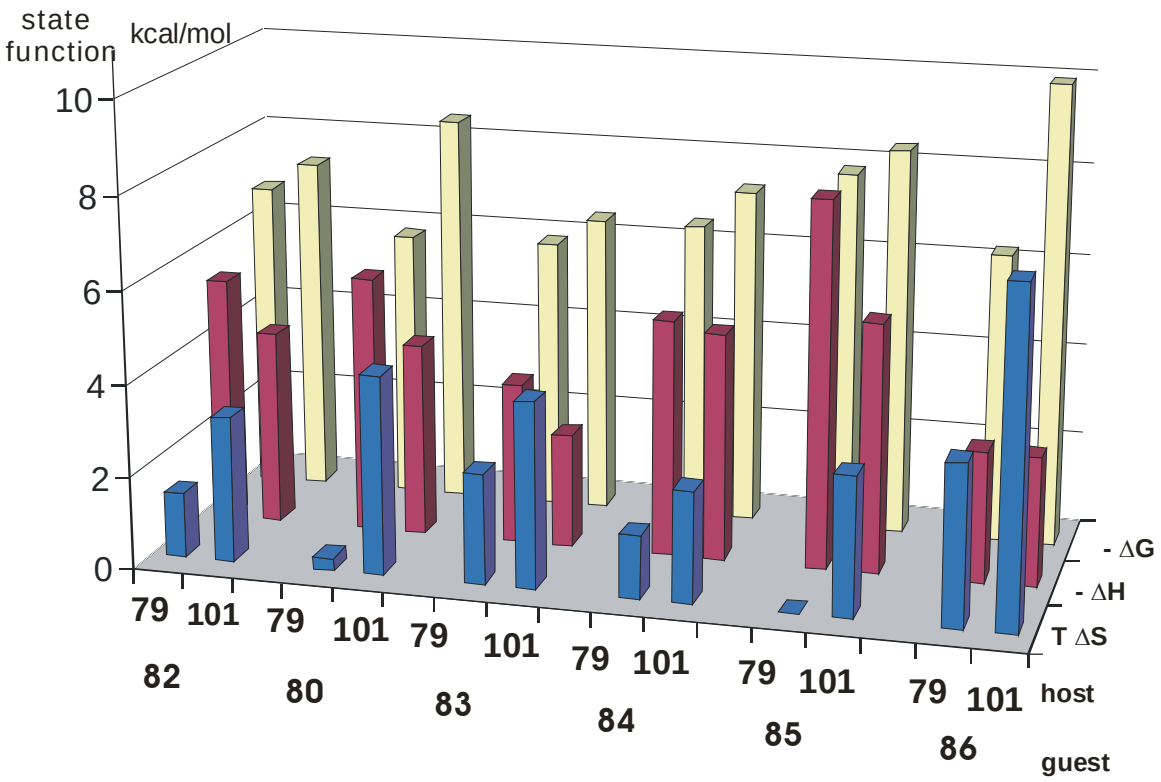


Figure 11. Comparison of binding energetics of 1:1 stoichiometric complexes of oxoanion guest species to the guanidinium host **101** and **79** in acetonitrile at 298 K.

The symmetrical anions phosphate **80** and squarate **86** experience the greatest entropy gain due to their capability of creating and populating a larger variety of energetically low-lying host-guest configurations. The binding energetics and the formation of higher stoichiometric complexes suggest that the enhanced affinity in guest binding by host **101** is a result of weakened structuring. The supplementation of several hydrogen bonding functions did not bring about well structured host-guest complexes as required in processes like self-assembly or catalysis. These results bear on the molecular design of the artificial receptors since those applications relying on the precise structuring of the host-guest complex, e.g., in enthalpy dominated processes such as self-assembly or catalysis, may be differentiated from others that primarily honor more negative Gibb free energies as e.g., in extraction.

4.3.2 ITC titrations of host 102 with different oxoanions.

In the titration of host **102** with guest **82** there was also indication of successive processes. Figure 12 represents the titration of guest **82** into the solution of host **102** in acetonitrile.

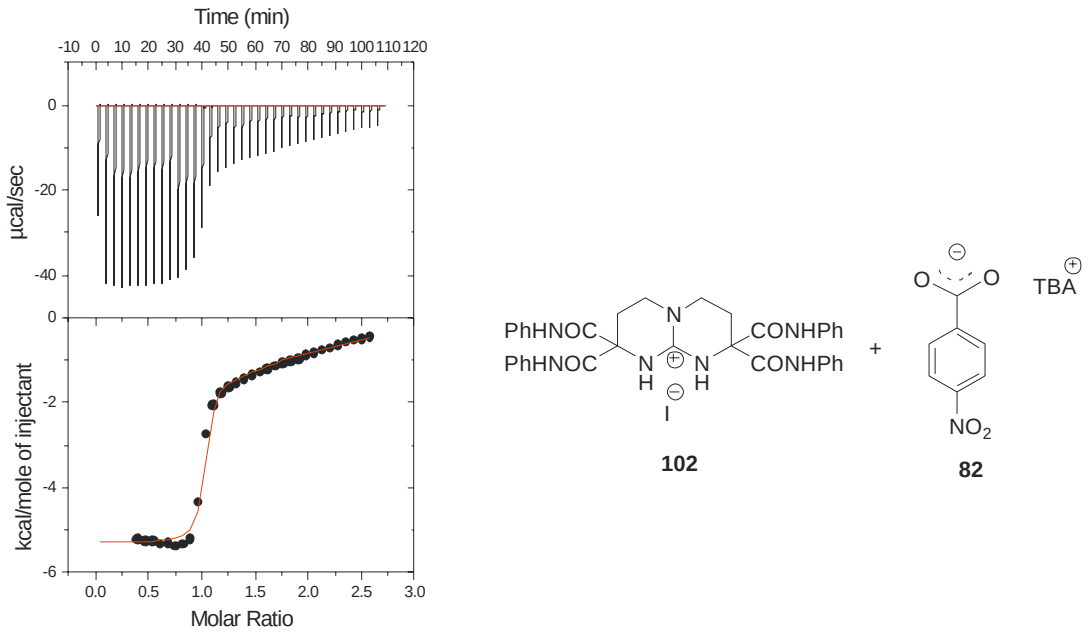


Figure 12. ITC-traces of the titration of guest **82** (40 mM) into the host **102** (1.3 mM) in acetonitrile at 298 K

The ITC traces clearly showed a sequential process and the fit-curve was obtained by applying a sequential binding model. Apart from the 1:1 binding stoichiometry, in the initial phases of titration where there was excess of host over the guest indicated the formation of 2:1 host-guest complex and in the latter phase of titration, where the concentration of guest becomes substantial it also showed 1:2 host-guest complexation. The 1:1 complexation was exothermic (ΔH = -6.0 kcal mol^{-1}) with a positive entropy component giving an association constant of K_{ass}= 1.2 x 10^6 M^{-1}. These results clearly indicate week structuring in the host-guest complexes formed.
The ITC traces of the titration of host **102** into the guest **80** are shown in figure 13.

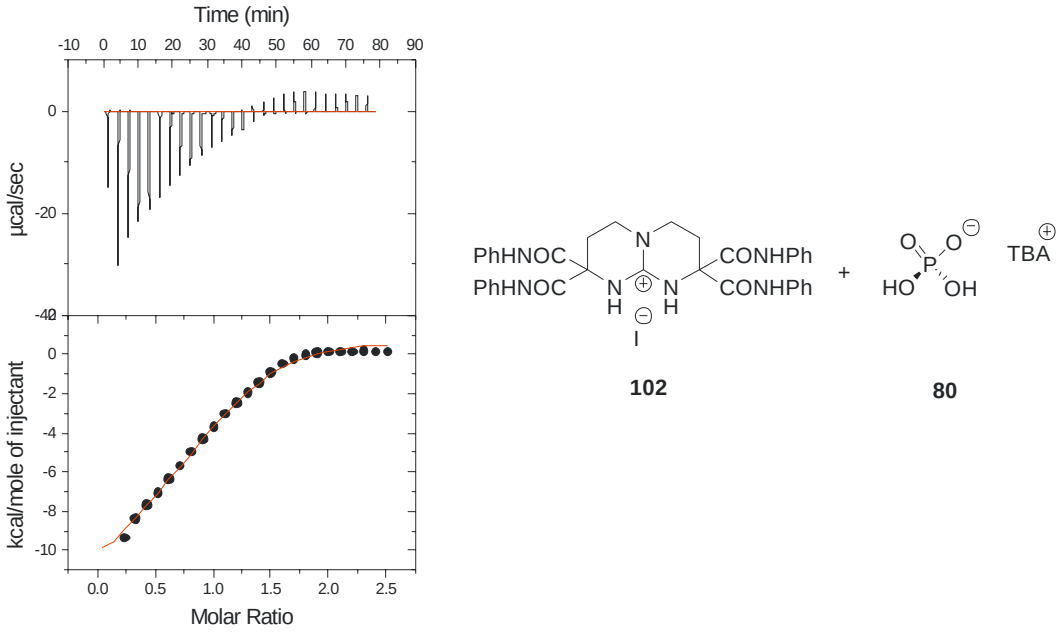
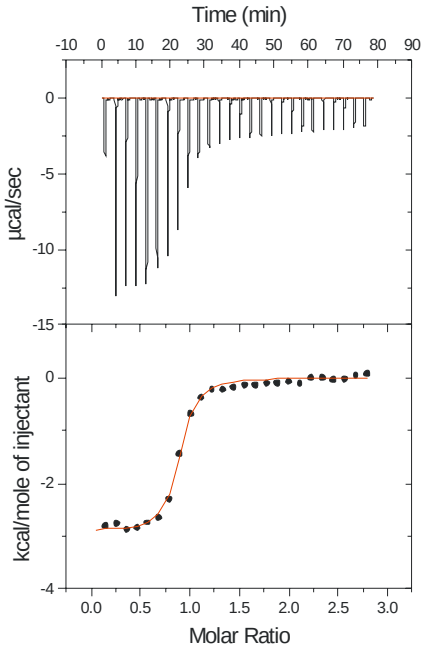
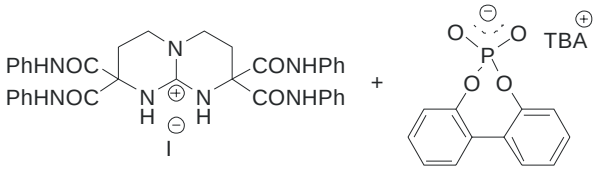


Figure 13. ITC-traces of the titration of guest **80** (12 mM) into the host **102** (0.43 mM) in acetonitrile at 298 K

The original titration did not show a sigmoidal curve. The initial phase of titration showed an exothermic interaction which, with the progress of titration, reverted into an endothermic effect (the blind titration was endothermic). The form of the curve in this case also indicates, that there occur at least two processes the first one being strongly exothermic and the second one endothermic. The curve fitting was possible by application of a sequential 2-site model only after deleting some initial data points. From the curve fitting, the first 1:1 complex step was exothermic with high affinity (K_1= 3.8 x 10^4 M^{-1}, ΔH_1 = -10.0 kcal mol^{-1}, ΔS_1 = -12.1 e.u.) and second step was exclusively entropy driven (K_2= 4600 M^{-1}, ΔH_2 = 3.1 kcal/mol, ΔS_2 = +27.2 e.u.).

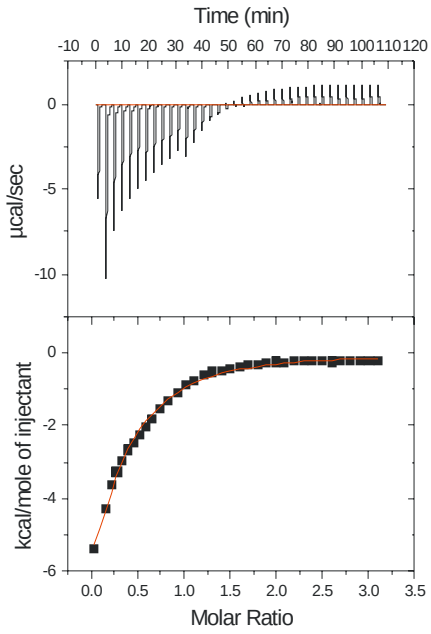
The titration of guest **83** in to the host **102** resulted in the ITC-traces shown in figure 14. Although the one-site-model fit gave a sigmoidal curve, there was an indication of other processes in the initial and latter phases of titrations. The 1:1 phase was well defined and indicative of the fact that the very high association constant (K_{ass}= 2.7 x 10^5 M^{-1}) arises from the more positive entropy of association (ΔS = +15.3 e.u.) and a relatively small enthalpy (ΔH = -2.9 kcal mol^{-1}).

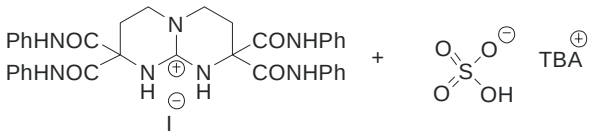




102 **83**

Figure 14. ITC-traces of the titration of guest **83** (12 mM) into the host **102** (0.43 mM) in acetonitrile at 298 K





102 **87**

Figure 15. ITC-traces of the titration of guest **87** (12 mM) into the host **102** (0.60 mM) in acetonitrile at 298 K

The titration of hydrogensulphate **87** into the solution of host **102** in acetonitrile is shown in figure 15. The form of the curve obtained did not comply with 1:1 stoichiometry, but indicated higher complexes. The one site model fitting model curve shows off stoichiometry indicating formation of host-guest 2:1 complex with very low association constant(K_{ass}= 6100 M^{-1}) but very high enthalpy of association (ΔH = -7.7 kcal mol^{-1}).

The results form the titrations of host **102** with oxoanions indicate that the host **102** also does not limit the possibilities of host-guest arrangements as compared to the tetraallyl guanidinium host **79**.

4.3.3 ITC titrations of host 103 with different oxoanions.

The ITC traces from the titration of guest **80** into the host **103** are represented in the figure 16.

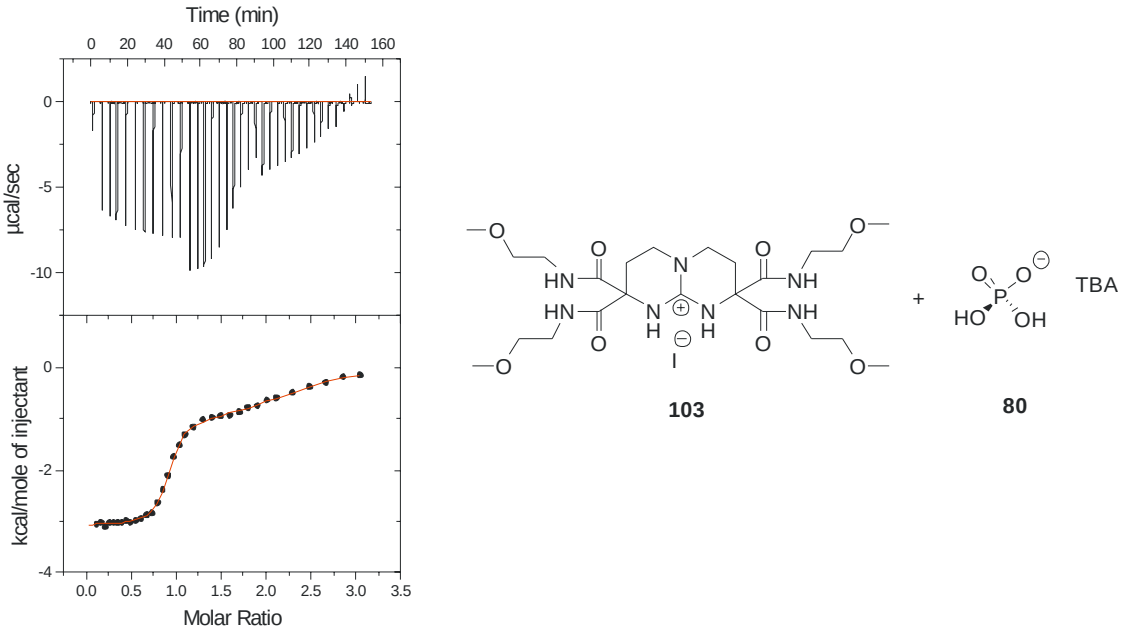


Figure 16. ITC-traces of the titration of guest **80** (15.3 mM) into the host **103** (1 mM) solution in acetonitrile at 298 K.

The picture was very clear from the system suggesting not only the 1:1 host-guest complex, but there was also the indication of higher complexes. But nevertheless the 1:1 step was well defined. The fit with a sequential model was not possible, but with the two-independent-site model it was

successful with the ligand-in-syringe settings. The curve fitting indicates the presence of two independent binding sites in the guanidinium host **103** with differences in affinities by the factor of 160 (K_1= 1.6 x 10^6 M^{-1}, ΔH_1 = -3.0 kcal mol^{-1}, ΔS_1 = +18.3 e.u.; K_2= 1.0 x 10^4 M^{-1}, ΔH_2 -10.0 kcal mol^{-1}, ΔS_2 = +14.86 e.u.).

The ITC traces from the titration of the guanidinium host **103** into the solution of guest **82** in acetonitrile are shown in figure 17. The curve fitting was obtained by applying the sequential-binding-site model. The picture from the curve is very clear, in the initial phases of titration where there was excess of carboxylate compared to guanidinium host **103**, indicated the formation of guest:host (2:1) complex with low affinity but high exothermicity (K_2= 9.1 M^{-1}, ΔH_2 = -16 kcal mol^{-1}, ΔS_2 = -48.7 e.u.). Increasing the addition of guanidinium host **103**, the 2:1 complex dissociates to form 1:1 complex (K_1= 9.3 x 10^4 M^{-1}, ΔH_1 = -3.1 kcal mol^{-1}, ΔS_1 = +12.32 e.u.).

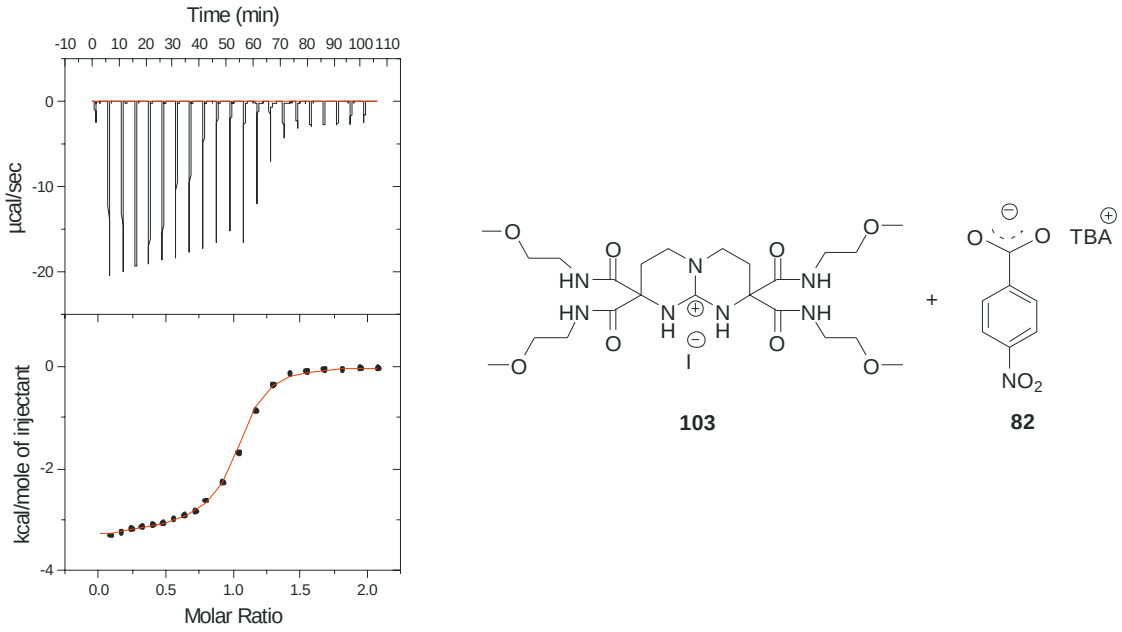
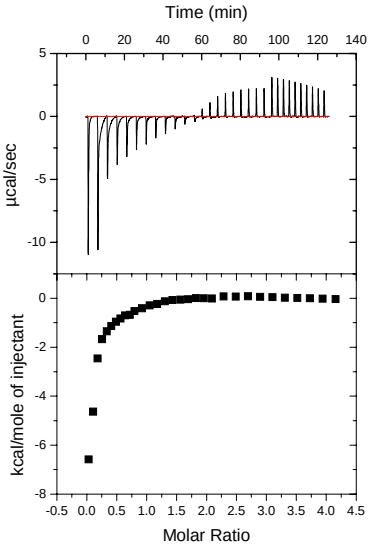
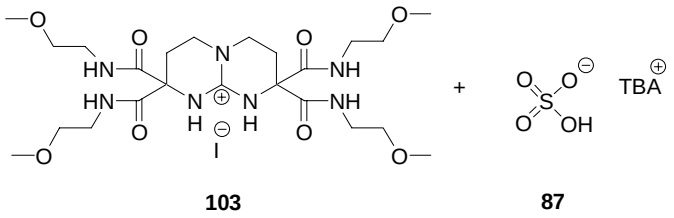


Figure 17. ITC-traces of the titration of the host **103** (10 mM) into the guest **82** (1 mM) solution in acetonitrile at 298 K.

The titration of TBA monohydrogensulphate **87** into the guanidinium host **103** was not that clear (figure 18). The beginning phase was exothermic and gradually over the titration showed

endothermic interactions. The appearance of the curve indicates higher complexes being formed, especially a host to guest 2:1 complex.





103 **87**

Figure 18. ITC-traces of the titration of guest **87** (20 mM) into the host **103** (1 mM) solution in acetonitrile at 298 K.

4.3.4 ITC titrations of macrocycle 125 with different oxoanions.

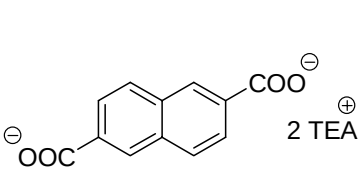
The different oxoanions used in the complexation of macrocycle **125** in acetonitrile are listed in scheme 22.

The titration of macrocycle **125** into the solution of tetrarethylammonium 2,6-naphthalenedicarboxylate **88** in acetonitrile at 25 ^{o}C produced an ITC-trace shown in figure 19. The sigmoidal curve was obtained by applying the one-site-fitting model. The large association constant (table 17, tit.9) was observed with high enthalpy of association and low entropic contribution indicating the formation of 1:1 stoichiometry well structured complex.

The ITC titration experiments of the macrocycle **125** with different oxoanions (scheme 22) were performed by titrating the macrocycle **125** into the guest solutions. The energetics of titration is represented in table 17.

The stoichiometries in all of the above titrations vary by a factor of 2 and the main average of n was 0.6-0.7. Among the guests that were probed, terephthalate **89** was the best bound guest (table

17, tit. 1). The range of affinities covers a factor of 18 and there was no size relation between the binding partners.



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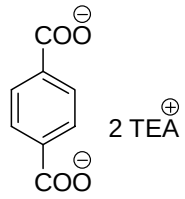

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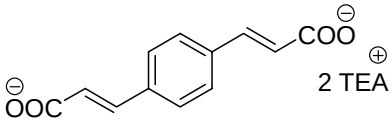

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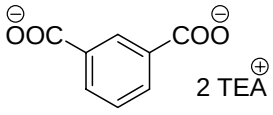

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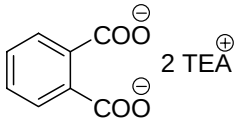

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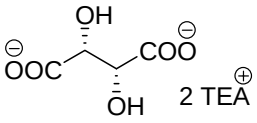

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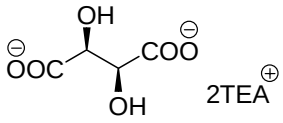

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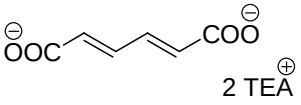

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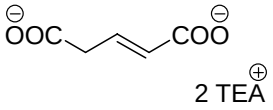

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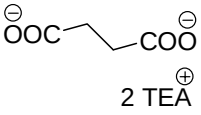

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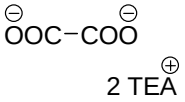

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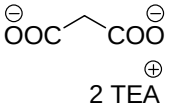

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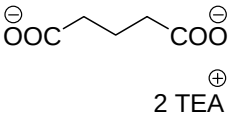

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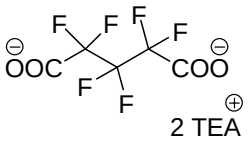

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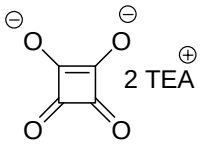

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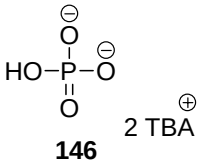

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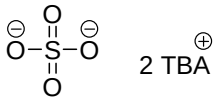

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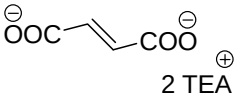


148

Scheme 22. Different oxoanions used for complexation studies.

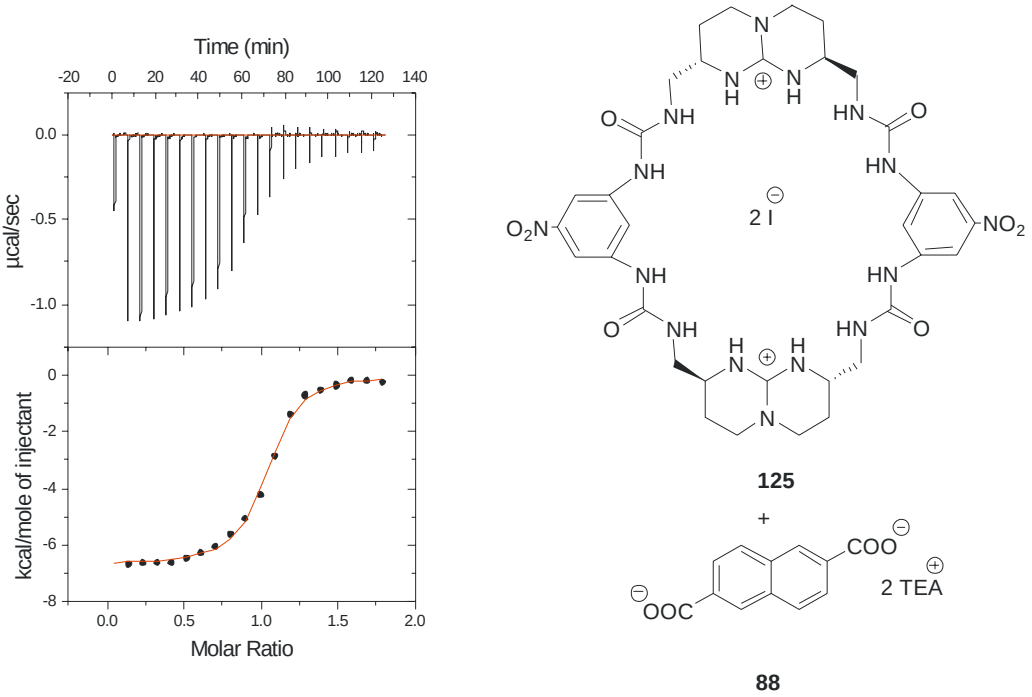


Figure 19. ITC-traces of the titration of host **125** (0.48 mM) into the guest **88** (0.032 mM) solution in acetonitrile at 298 K.

Table 17. Energetics of host-guest binding of the macrocycle **125** (0.48 mM) with different oxoanions in acetonitrile at 298 K.

Tit	**Guest**	**n**	**K_{ass}** [M^{-1}]	**-ΔG** [kcal mol^{-1}]	**-ΔH** [kcal mol^{-1}]	**TΔS** [kcal mol^{-1}]
1	**89**	0.65	7.17×10^6	9.34	8.55	0.81
2	**90**	0.59	4.33×10^6	9.04	10.00	-0.98
3	**91**	0.70	7.18×10^5	7.98	9.20	-1.20
4	**92**	-	1.65×10^5	7.12	0.74	6.38
5	**93**	0.75	3.22×10^6	8.87	8.42	0.46
6	**94**	0.81	7.56×10^5	8.01	10.05	-2.44
7	**95**	0.60	1.08×10^6	8.22	9.13	-0.89
8	**96**	0.43	3.95×10^5	7.62	8.75	-1.11
9	**88**	1.01	2.76×10^6	8.77	6.70	2.09

Comparing the three phthalates, the isophthalate **91** was most stringently bound, followed by the terephthalate **89**, leaving the phthalate dianion **92** as the most loosely bound. This sequence was clearly reflected by the energetic signature (table 17, tit. 1, 3-4). Naphthalene dicarboxylate **88** was more loosely bound compared to the smaller congener terephthalate **89**, but shows much improved positive entropy. This scenario resembles ion pairing indicating weak structuring. Among the unsaturated dianionic guests, all show negative entropies of association forming well structured complexes.

D(-) / L(+) tartrate display enantiomeric recognition in all three energetic parameters; here a surprising notion was observed that better structure leads to impaired affinity. Saturated dicarboxylic guests **97-100, 144** showed very small enthalpic interactions rendering the output difficult to analyse. The ITC-traces from the complexation of tetraethylammonium succinate **97** with macrocycle **125** are shown in figure 20. The picture from the titration curve suggests that there are higher complexes. The one-site-model fitting curve gave high association constant (K_{ass}=1.84 x 10^6 M^{-1}) with low enthalpic contribution (ΔH = -1.65 kcal mol^{-1}) and huge entropic outcome (ΔS = +23.11 e.u.). These results resemble unspecific ion pairing.

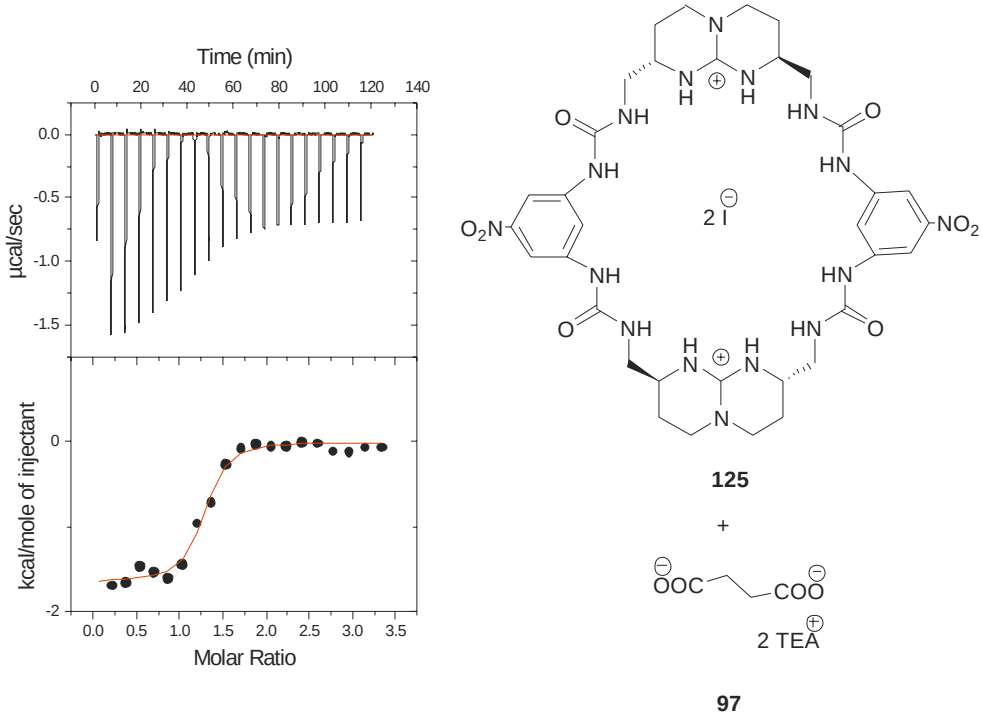
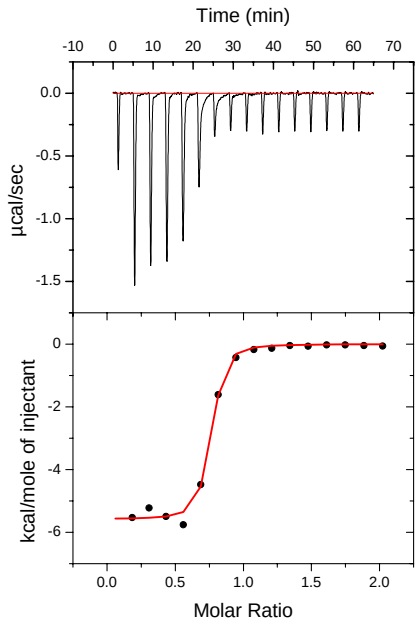
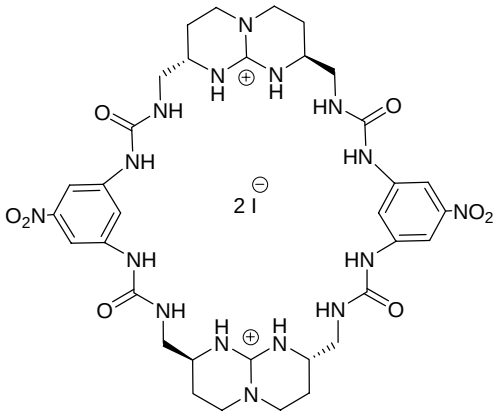


Figure 20. ITC-traces of the titration of guest **97** (0.50 mM) into the host **125** (0.037 mM) solution in acetonitrile at 298 K.

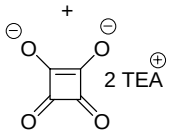
The complexation of squarate dianion **145** with macrocycle **125** was not a straight 1:1 stoichiometric complex, but indicated higher complexes, too (figure 21). The curve fitting by the one-site-model was only possible after making a y-translation of the data points and subtraction of a linear regression line of blind titration from the original titration data. If 1:1 complex was assumed, then the stoichiometry was off, indicating higher complexes; nevertheless macrocycle has a high affinity for the squarate anion **145** (K_{ass}=1.07 x 10^7 M^{-1}) with huge positive entropy of association (ΔS = +13.4 e.u.).

The titration of dihydrogenphosphate **80** into the solution of macrocycle **125** in acetonitrile produced ITC-traces shown in figure 22. The blind titration was endothermic and the original titration shows progressive interactions with a slope at relatively high stoichiometry. The form of the curve suggests higher order host-guest complexes. The host-guest system was too complex for deconvolution of data.





125



145

Figure 21. ITC-traces of the titration of guest **145** (0.50 mM) into the host **125** (0.037 mM) solution in acetonitrile at 298 K.

But the titration between monohydrogenphosphate dianion **146** and macrocycle **125** was informative (figure 23). The curve fitting by one site model was possible only after deleting initial two titration steps. The stoichiometry was well off indicating a host-guest 2:1 higher

complex. This complex has a high association constant (K_{ass}=9.01 x 10^5 M^{-1}) with a huge enthalpy of association (ΔH = -7.7 kcal mol^{-1}) and reduced counteracting entropy (ΔS = +1.3 e.u.).

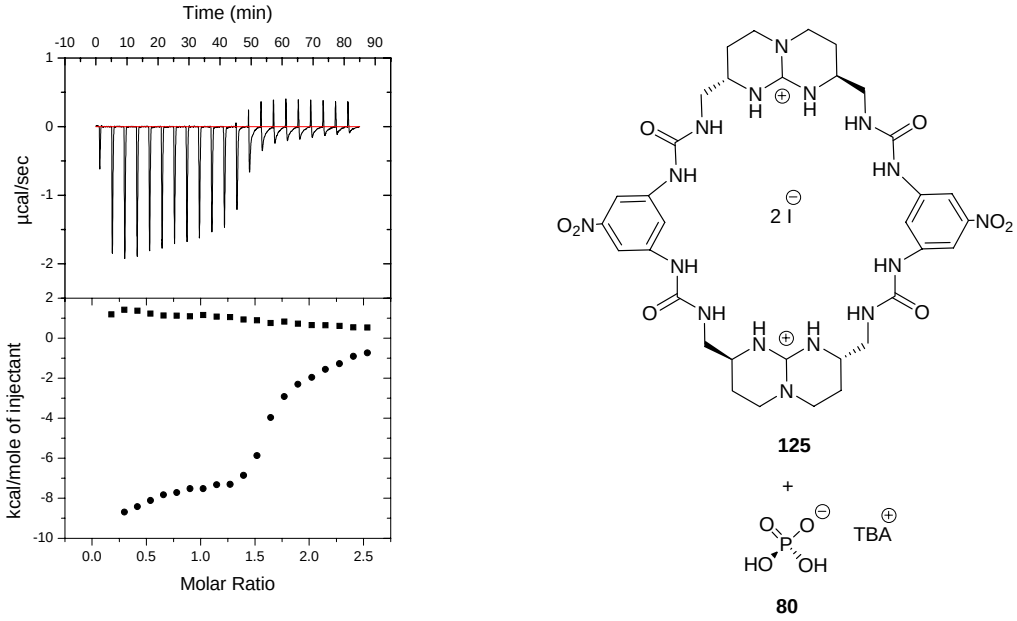


Figure 22. ITC-traces of the titration of guest **80** (0.90 mM) into the host **125** (0.037 mM) solution in acetonitrile at 298 K

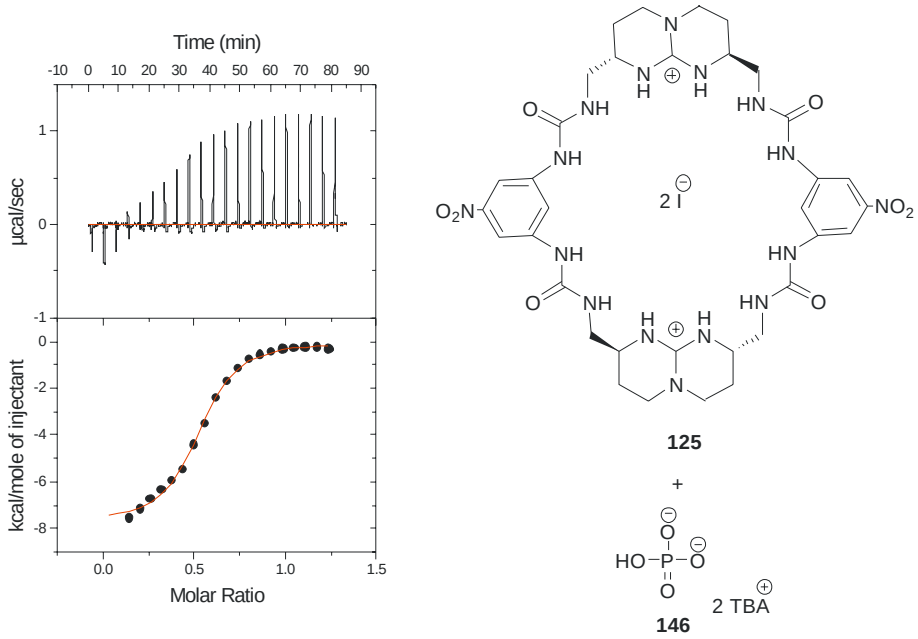


Figure 23. ITC-traces of the titration of macrocycle **125** (0.48 mM) into the guest **146** (0.05 mM) solution in acetonitrile at 298 K

The titration data of the macrocycle **125** with tetrabutylammonium sulphate **147** is represented in figure 24. The blind titration was endothermic and original titration shows dependence with an additional maximum at lower stoichiometry indicating higher complexes. The host-guest system was too complicated for deconvolution.

The ITC-traces from the titration of the macrocycle **125** into the solution of tetraethylammonium fumarate **148** are represented in figure 25. The data were indicative of higher guest-host complexes and the curve fitting was obtained by applying the sequential-2-site-binding model. The fit was better when calculated by ligand-in-cell conditions were applied. The fit shows two processes, the first with large affinity, huge enthalpic contributions and negative entropy of association (K_1= 3.7 x 10^6 M^{-1}, ΔH_1 = -12.9 kcal mol^{-1}, ΔS_1 = -13.2 e.u.). The second process has a relatively moderate affinity, low enthalpy of association and negative entropic contributions (K_2= 4753 M^{-1}, ΔH_2 = -0.9 kcal/mol, ΔS_2 = -13.77 e.u.).

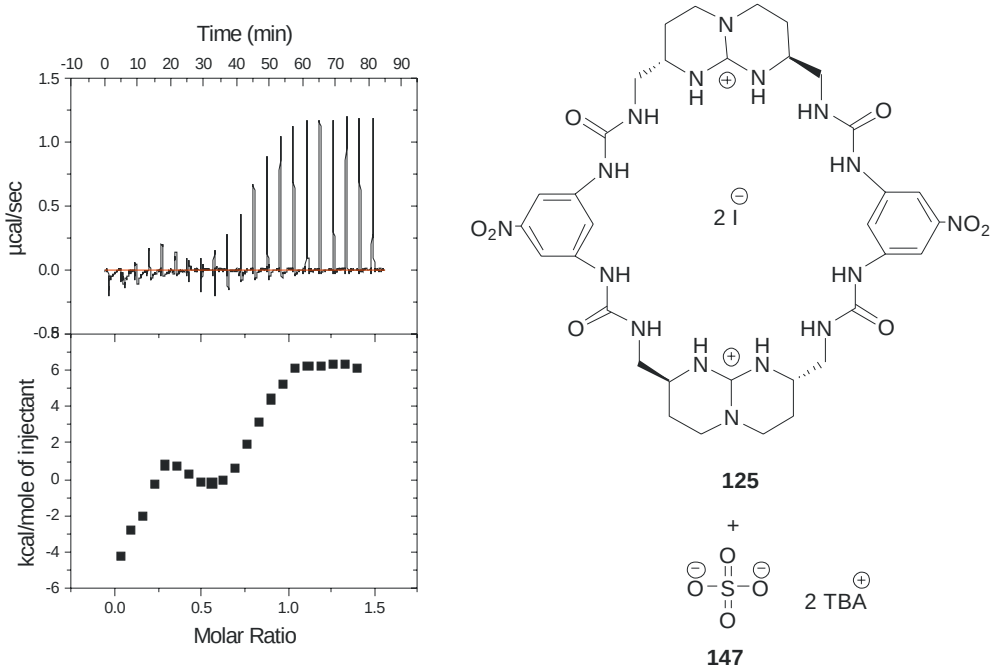


Figure 24. ITC-traces of the titration of macrocycle **125** (0.48 mM) into the guest **147** (0.045 mM) solution in acetonitrile at 298 K.

The ITC-traces for the D-aspartate **149** and L-aspartate **150** complexation with macrocycle **125** are shown in figure 26. The initial phases of titration in both of the anions were exothermic and by applying the one-site-binding model the curves obtained were almost identical. Although the

enantiomeric recognition of aspartate by macrocycle **125** was not observed from the association affinities, significant differences are reflected in the counteracting entropies of association (table 18).

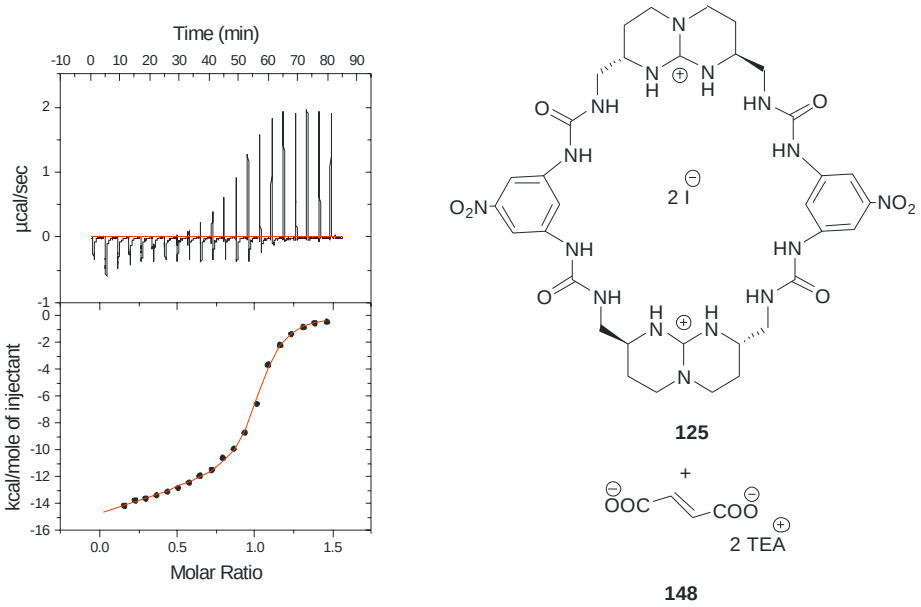


Figure 25. ITC-traces of the titration of macrocycle **125** (0.48 mM) into the guest **148** (0.045 mM) solution in acetonitrile at 298 K.

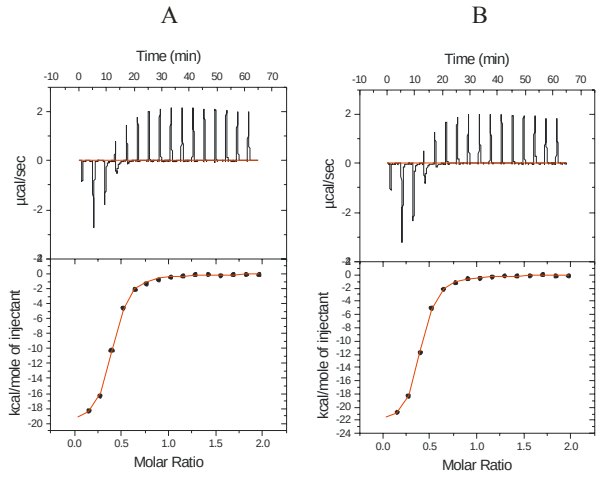


Figure 26. ITC-traces of the titration of macrocycle 25 (0.48 mM) into the guest, panel A for D-aspartate **149** (0.045 mM) and panel B for L-aspartate **150** (0.045 mM) solution in acetonitrile at 298 K.

The shape of the curve and the stoichiometry n reflect the more basic carboxylate in aspartate binding to the guanidinium groups in the macrocycle whilst the zwitterionic ends form dimer. In other words the dimer of aspartate binds to the macrocycle resulting in 1:2 (host–guest) stoichiometry.

Table 18. Energetics of host-guest binding of macrocycle **125** with aspartates.

Tit	**Guest**	**n**	**K_{ass}** [M^{-1}]	**-ΔG** [kcal mol^{-1}]	**-ΔH** [kcal mol^{-1}]	**TΔS** [kcal mol^{-1}]
1	TEA D-aspartate **149**	0.36	1.02 x 10^6	8.20	20.44	-12.22
2	TEA L-aspartate **150**	0.36	1.13 x 10^6	8.26	22.86	-14.70

4.4 ITC-measurements in DMSO.

Since most of the biological processes occur in relatively aqueous environment, the more competing solvent DMSO was chosen due to its more polarity and can be considered similar to water. The complexation of macrocycle **125** with terephthalate dianion **89** in DMSO at 25 ^oC is shown in figure 27. The nice sigmoidal curve was obtained by applying one-site-binding model. The picture from the curve indicates that the complexation of macrocycle **125** with **89** resembles strong ion-pairing with a very high association constant (Kass = 1.1 x 10^6 M^{-1}). The high affinity arises mainly from the large amount of positive entropy (ΔS = +33.20 e.u.) and a very small opposing enthalpic contribution (ΔH_1 = 1.7 kcal mol^{-1}). From the stoichiometry one must anticipate the formation of a host-guest 1:2 complex.

The complexation of macrocycle **125** with muconate **95** and the biologically important folic acid dianion showed similar effects as with terephthalate **89.**

The macrocycle **125** bound to a tetrahedral dihydrogenphosphate **80** with strong affinity. The picture from the ITC-titrations is represented in figure 28. In the initial phases of the titration higher order binding is observed. From the curve one can deduce a high affinity of macrocycle **125** with the phosphate **80** (Kass = 1.8 x 10^6 M^{-1}), but this too rather resembles ion pairing process since the high association constant arises from the respectable positive entropy (ΔS = +19.96 e.u.).

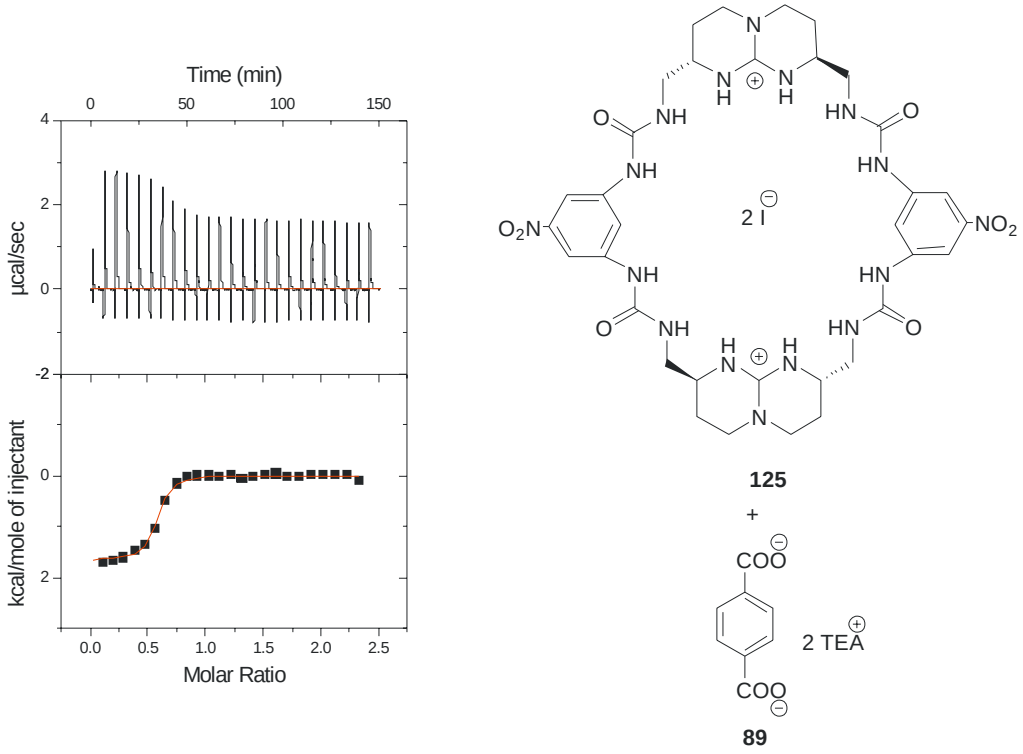


Figure 27. ITC-traces of the titration of the macrocycle **125** (1.4 mM) into the guest **89** (0.15 mM) solution in DMSO at 298 K.

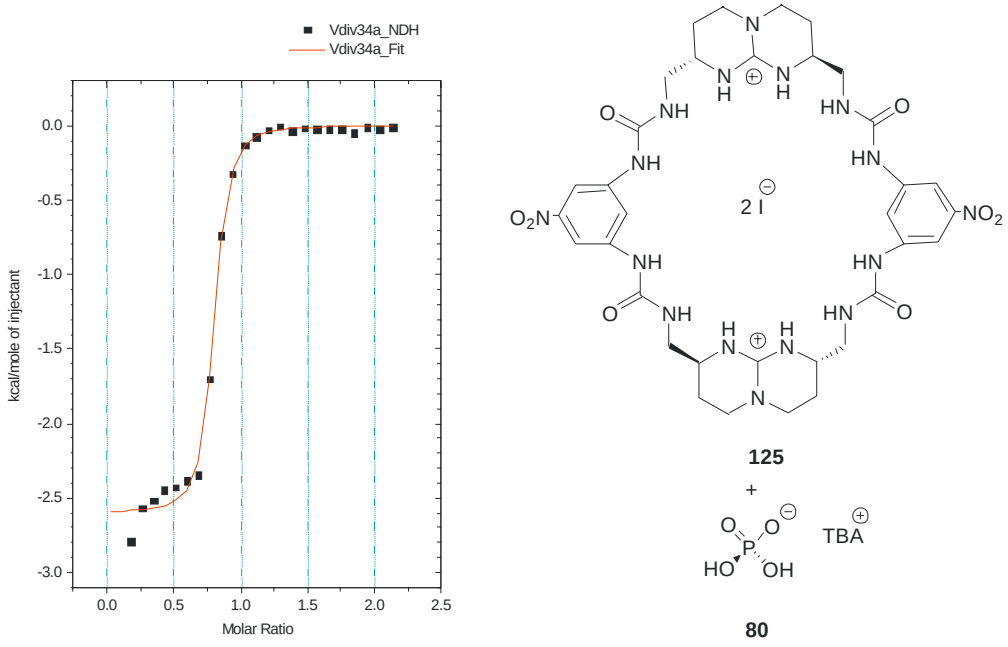
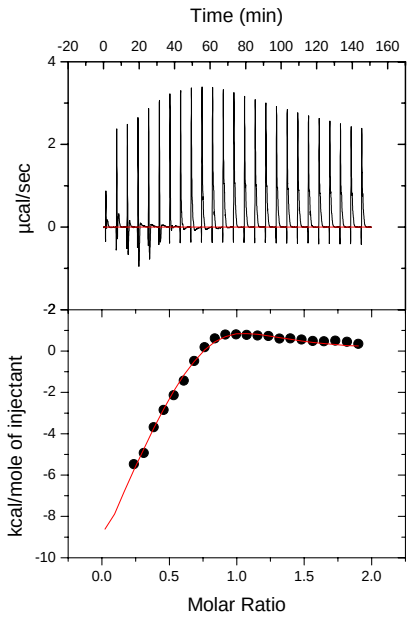


Figure 28. ITC-traces of the titration of the macrocycle **125** (1.4 mM) into the guest **80** (0.16 mM) solution in DMSO at 298 K.

The titration of macrocycle **125** with ADP^{2-} **151** also indicated strong ion-pairing. Although a clear sigmoidal dependence was not seen form the ITC data the rough fit obtained allowed to estimate a high association constant (K_{ass} = 4.3 x 10^5 M^{-1}) and a huge entropy component of association (ΔS = +24.10 e.u.) with a small favorable enthalpy contribution (ΔH = -0.5 kcal mol^{-1}).

The macrocycle did not show any heat effect in the complexation with naphthalenedicarboxylate **88** under the concentrations that the measurements were carried out.

The ITC-traces obtained from the complexation of macrocycle **125** with succinate **97** is shown is figure 29. The one site-binding-model fitting curve was not so clear but indicated from the stoichiometry (n= 0.5), the operation of at least two processes, the first one being exothermic followed by an endothermic second process. A better fit was obtained by applying the sequential-two-site-binding model. The first 1:1 association was strongly entropy-driven with a small negative enthalpy assisting the association (K_1= 1.7 x 10^5 M^{-1}, ΔH_1 = -1.9 kcal mol^{-1}, ΔS_1 = +16.75 e.u.). In contrast, the second step binding of succinate **97** as a dimer was 100 times less affinitive displaying a high enthalpy of association and more negative entropy. (K_2= 1200 M^{-1}). The absolute values of this second step do not seem to be reliable for the interpretation.



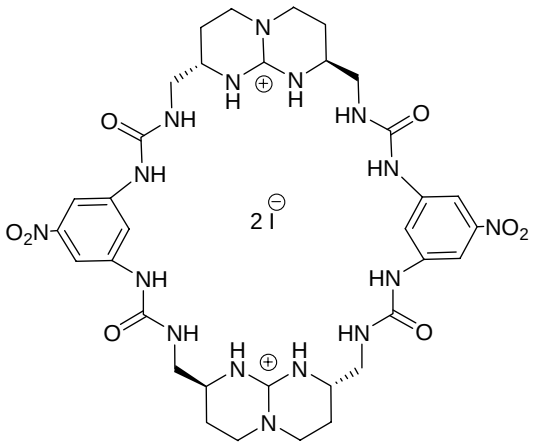
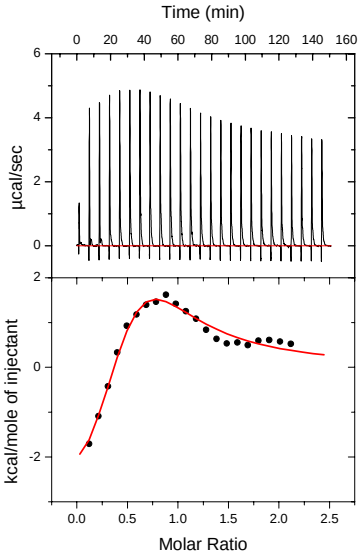
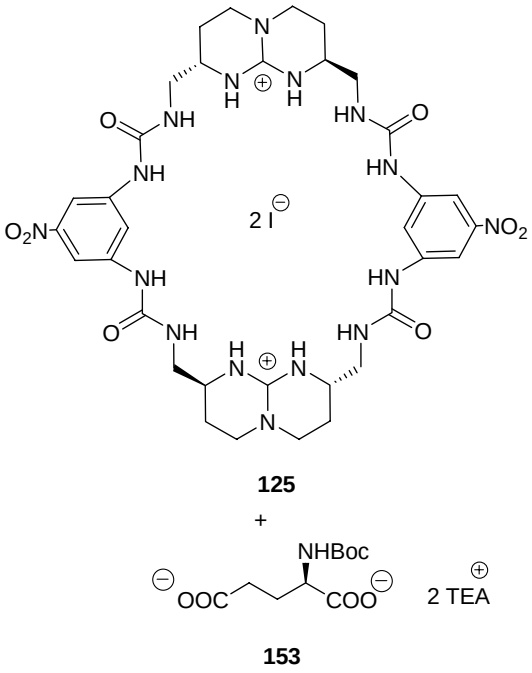


Figure 29. ITC-traces of the titration of the macrocycle **125** (1.4 mM) into the guest **97** (0.18 mM) solution in DMSO at 298 K.

The complexation of macrocycle **125** with L-Boc-glutamate **152** hardly yielded any heat effect rendering it impossible to analyse the data; D-Boc-glutamate **153** in turn showed an interpretable response (figure 30). The one-site-model did not fit the data points so the two-site-sequential model was applied using ligand-in-cell conditions. The form of the curve gave a very stable energetic signature. The 1:1 stoichiometric binding is endothermic and entropy-driven (K_1= 9.25 x 10^4 M^{-1}, ΔH_1 = +2.3 kcal mol^{-1}, ΔS_1 = +29.5 e.u.) whilst the 2:1 association is highly exothermic and entropically neutral (K_2= 1.59 x 10^4 M^{-1}, ΔH_2 = -5.9 kcal mol^{-1}, ΔS_2 = -0.6 e.u.).





125

+

153

Figure 30. ITC-traces of the titration of the macrocycle **125** (1.4 mM) into the D-Boc-glutamate (0.172 mM) solution in DMSO at 298 K.

The macrocycle **125** shows high affinity (K_{ass} in the range of 10^4-10^6 M^{-1}) with all the guests were probed in the highly competitive solvent DMSO. The complexation with the guest resembles strong ion pairing with high entropies of association.

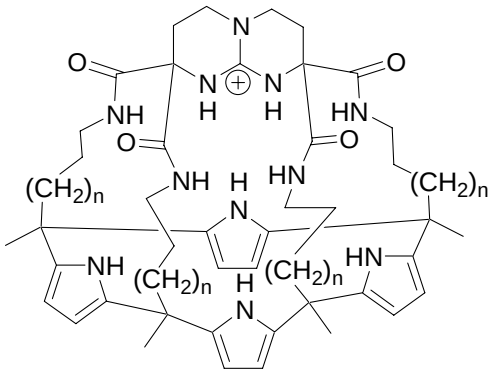
In conclusion, the detailed dissection of the Gibbs free energy of the complexes of the hosts **101-103** with different oxoanions (**80-87**) into the enthalpy and entropy components disclosed a very important feature. The supplementation of additional binding functions to the parent guanidinium moiety resulted in high affinity but at the same time offered more sites for binding to the incoming guest making it to wiggle around in the available binding sites that showed up in the positive entropy of association. Thus, the supplementation of additional binding functions should be done in a proper framework so that the guest will possess only one unique mode of interaction to form a more precisely structured host-guest complex. This result points toward restricting binding modes and one way to do this is by building a macrocyclic host with a cavity inside for the guest molecule.

These results gave the platform to design and successfully synthesize macrocycle **125** which has shown high affinity with favored enthalpy towards a variety of carboxylic oxoanions both in acetonitrile and DMSO. Specially, the chiral discrimination ability towards the carboxylate dianions (tartrate **93, 94** and aspartate **149,150**). There are only few examples of anion receptors with chiral discrimination towards simple carboxylates based on the bicyclic guanidinium anchor groups known.[112] The first anion receptor **28**, based on guanidinium moiety showing chiral recognition for carboxylate is reported by Lehn and de Mendoza.[58] They incorporated a naphthoyl side arm to the bicyclic guanidinium core to provide π-stacking interactions but a quantative study was not performed on the chiral recognition. More recently enantioselective recognition of phenylalanine zwitterions at the air-water interface was achieved by using calixarene derivative **35** bearing chiral bicyclic guanidinium moieties.[113]

Although macrocycle **125** showed high affinity and favorable enthalpic outcome there also occurred higher order bindings. This indicates that macrocycle also offers more binding sites which will definitely not form a precise structure of the host-guest complex and renders the macrocycle less important form the point of selectivity for oxoanions. This can well be attributed to the presence of the urea linkage which has the tendency to interact with each other and such interaction would destroy the main frame of binding. As a corollary one may assume that it is necessary to use less polar groups as linkers in constructing macrocyclic hosts.

Based on the results from the macrocycle complexation studies the compounds **101-103** have a better prospective in the future. The tetraester **104,** precursor in the hosts **101-103** synthesis, can be used to form a cage like receptor **154** based on the pyrrole units linked to bicyclic guanidinium core through amide linkages. This receptor is expected to interact with the guest, apart form the

electrostatic and hydrogen bonding interactions, by additional π-stacking from the pyrrole units. The receptor **154** is in preparation.



154

5. Experimental part

5.1 General methods and materials:

All the experiments in organic solvents were performed under nitrogen atmosphere and monitored by HPLC. The HPLC-analysis were performed on Merck-Hitachi instrument L 6200A or 655 A-11 pump connected to Knauer L 4250 UV detector or a EUROSEP DDL-3l light scattering detector and Kipps&Zonen two channel recorder. The columns that were used in HPLC analysis are

- Phenomenex, Aqua C_{18}, 250×4.60 mm, 5µ column
- Nucleodure-100-5 C_8 ec column
- Nucleodure-100-5 CN-RP column

The solvents were purchased in pure analytical grade and distilled before use except for DMF which was purchased in anhydrous quality from Aldrich and acetonitrile which was purchased in HPLC-quality from Baker. THF was dried and purified by distillation under nitrogen from benzophenone and sodium. Aqueous solutions were prepared from deionised, glass distilled water. The solvents (CH_2Cl_2, CH_3CN) were passed for drying through a small column of activated alumina directly into the reaction vessel. All other chemicals were purchased in reagent quality from commercial sources and used as received.

The column chromatography was performed on either silica gel 100 (0.063-0.200 mm) or activated alumina (neutral, activity grade-I).

Flash-Chromatography (MPLC) was performed using a C_8 or CN-modified silica gel in Michel-Miller columns connected to the Knauer HPLC-pump 364, and Knauer UV detector.

Purification of compounds by Solid Phase Extraction (SPE) was performed using commercially available SPE columns from Alltech (high capacity C_{18}).

Electrolysis was conducted in homemade thermostated cell taking mercury layer as cathode, a Pt-net anode separated from the cathode compartment by a D4 frit, and a calomel reference electrode connected to the cathodic compartment by a salt bridge.

^{1}H and ^{13}C-NMR spectra were recorded on a Brucker AM 250 or AC 360 (MHz) instruments and were referenced with respect to the residual solvent peak for the deuterated solvent.

All the melting points were measured in open capillary tubes using a Fisher-Jones apparatus.

Mass spectra were obtained on Finnigan LQC: Electrospray-Ionization (ESI, HPLC-MS).

Elemental analyses were made by the microanalytical laboratory of the TU Muenchen.

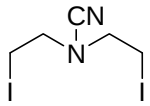
Calorimetric titrations were performed on the Isothermal Titration Calorimeter MCS-ITC from Microcal Inc., Northampton, Massachusetts, USA.

Molecular modeling were performed on Pentium-PCs using HyperChem 7 molecular modeling software from hypercube, Inc.

X-ray crystal structure analyses were made by Inorganic Chemistry Department of TU Muenchen.

5.2 Synthetic Procedures:

Bis(2-iodoethyl)cyanamide 105.



105

Bis(2-chloroethyl)amine hydrochloride (17.9 g ,100 mmol) was dissolved in a mixture of water(50 mL) and dichloromethane (100 mL). The mixture was cooled to 0 ^{o}C and 5 mL of 4N NaOH was added. Then a solution of cyanogen bromide (13.1 g, 125 mmol) in dichloromethane to make 30 mL solution and 30 mL of 4N NaOH were added alternately in portions with vigorous stirring while keeping the temperature below 5 ^{o}C. After the addition, the reaction mixture was stirred for another 30 min at room temperature and then the two phases were separated. The organic phase was washed first with dilute acetic acid, then with brine, dried over magnesium sulfate and the solvent was evaporated in vacuo to obtain a liquid residue which was distilled in the Kugelrohr apparatus {130 °C/ 0.05 torr}to afford bis(2-chloroethyl)cyanamide (11.65 g, 70%) as a colourless viscous liquid.

The liquid obtained above (11.65 g, 70 mmol) was dissolved in acetone (50 mL) to which finely powdered NaI (30 g, 200mmol) was added and the mixture was refluxed for 3 days. After complete conversion to the bisiodo compound **105** as seen from the HPLC analysis, the mixture was evaporated in vacuo and the residue obtained was taken up in toluene, filtered, and the filtrate was passed through a pad of alumina and finally concentrated, dried under high vacuo to afford the bisiodo compound **105** (18.13 g, 74%) as a viscous colourless liquid.

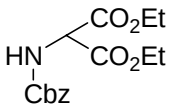
105: $C_5H_8I_2N_2$ (MW 349.9).

HPLC analysis: R_v = 10 ml, Nucleodure-100-5 C_8 ec column, UV_{220}, flow = 1 ml/min, gradient from 10% CH_3OH to 50% CH_3OH in 10 min and then 50% CH_3OH to 90% CH_3OH in next 10 min, 0.1% TFA as a buffer.

^{1}H-NMR (360 MHz; $CDCl_3$): δ= 3.45 (t, J= 7.0 Hz, 4H, -N-C**H$_2$**); 3.29 (t, J= 7.0 Hz, 4H, -C**H$_2$**I).

^{13}C-NMR (90.56 MHz; $CDCl_3$): δ= 115.12 (-**C**N); 53.75 (-N-**C**H_2); -0.65 (-**C**H_2I).

Benzyloxycarbonylamino malonic acid diethylester 106.



106

Diethyl 2-aminomalonate hydrochloride (21.2 g, 100 mmol) and bis(trimethylsilyl) acetamide (BTSA) (24.8 mL, 100 mmol) were taken in anhydrous diethylether (200 mL) under inert atmosphere. The reaction mixture was cooled to 0 ^{o}C in an ice bath when benzylchloroformate (14.4 mL, 100 mmol) was rapidly added to this reaction mixture. After the addition was complete, another batch of BTSA (24.8 mL, 100 mmol) was added. The resultant cloudy reaction mixture was then allowed to warm slowly to room temperature and stirred for 30 min. On completion of the reaction as monitored by HPLC analysis the reaction mixture was washed with 0.1 N HCl (100 mL), dried over magnesium sulfate and then evaporated by rotatory evaporation to get a gummy residue which was recrystallized from hexane/ether (1:1) to afford **106** (26.5 g , 85%) as a white solid.

106: $C_{15}H_{19}NO_6$ (MW 309.3).

mp: 30-32 ^{o}C (hexane/ether), (Lit.[114] mp 32-33 ^{o}C).

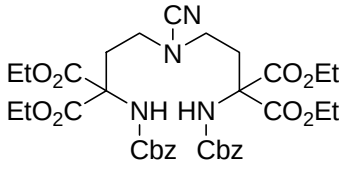
HPLC analysis: R_v= 13 ml, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5µ column, UV_{220}, flow = 1 ml/min, gradient from 50% CH_3OH to 90% CH_3OH in 10 min and then 90% CH_3OH for 5 min more, 0.1% TFA as a buffer.

MS-ESI: *m/z* = 310.2 $[(M+H)^+$, 100%].

^{1}H-NMR (360 MHz; $CDCl_3$): δ=7.36 (s, 5H, aromatic-**H**); 5.85 (d, J= 6.8 Hz, 1H, -N**H**); 5.14 (s, 2H, PhC**H$_2$**O-); 5.03 (d, J= 7.4 Hz, -C**H**-N); 4.29 (q, J= 6.5 Hz, 2H, -OC**H$_2$**CH_3); 1.32 (t, J= 7.0 Hz, -OCH_2C**H$_3$**).

^{13}C-NMR (90.56 MHz; $CDCl_3$): δ= 166.26 (-**C**O, ester); 155.37 (-N**C**O-); 135.90, 128.48, 128.21, 128.07 (aromatic carbons); 67.33 (Ph**C**H_2O-); 62.55 (-O**C**H_2CH_3); 57.74 (-**C**H-); 13.91 (-OCH_2**C**H_3).

N,N-Bis (3-Benzyloxycarbonylamino-3,3-diethoxycarbonyl) propyl-1-cyanamide 107.



107

To a suspension of NaH (3.59 g, 90 mmol) (60% dispersion in mineral oil) in dry DMF (20mL) cooled to 5 ^{0}C, was added carbamate **106** (26.48 g, 85.7 mmol) in dry DMF (60 mL). The rate of addition was such that the temperature of the reaction mixture was maintained below 12 ^{o}C. (Increase in temperature results in self ester condensation). When the evolutions of H_2 seizes and the reaction mixture becomes clear. The solution of bis-iodoethyl cyanamide **105** (10 g, 28.5 mmol) in DMF (30 mL) was added in two portions, half of the solution was added by syringe pump over a period of 6 hours and the remaining half was added after stirring for 24 hours (it is important to maintain the temperature of the reaction mixture below 20 ^{o}C throughout the reaction time to avoid formation of the unwanted elimination product **134**). After complete addition of **105**, the stirring was continued for 3 days, then the reaction mixture was poured into the cold aqueous solution containing 1 mL of acetic acid and the aqueous layer was extracted with dichloromethane. The organic phase was washed several times with water, dried over magnesium sulfate and concentrated in vacuo to get a gummy substance, which was further dried using high vacuum to remove residual DMF. Finally recrystallization from ether/Hexane (4:2) afforded **107** (14.2 g, 72%) as a white crystalline compound.

107: $C_{35}H_{44}N_4O_{12}$ (MW 712.7).

mp: 72-73 ^{o}C (ether/hexane).

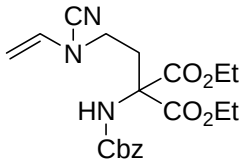
HPLC analysis: R_v= 16.4 ml, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5µ column, UV_{220}, flow = 1 ml/min, gradient from 50% CH_3OH to 90% CH_3OH in 10 min and then 90% CH_3OH for 5 min more, 0.1% TFA as a buffer.

MS-ESI: *m/z* = 735 [$(M+Na)^+$, 100%].

^{1}H-NMR (360 MHz; $CDCl_3$): δ= 7.34 (s, 10H, aromatic); 6.18 (s, 2H, -N**H**); 5.09 (s, 4H, PhC**H$_2$**O-); 4.24 (m, 8H, -OC**H$_2$**CH$_3$); 2.93 (t, J= 6.5 Hz, 4H, -N-C**H$_2$**-); 2.63 (t, J= 7.2 Hz, 4H, -CC**H$_2$**-); 1.24 (t, J= 7.0 Hz, 12H, -OCH$_2$C**H$_3$**).

^{13}C-NMR (90.56 MHz; CDCL3): δ= 167.23 (-**C**O, esters); 154.49 (-N**C**O-); 135.87, 128.49, 128.25, 128.12 (aromatic carbons); 116.19 (-CN); 67.17 (Ph**C**H_2O-); 64.73 (quaternary carbons); 63.00 (-O**C**H_2CH_3); 47.42 (-N-**C**H_2); 30.84 (-C**C**H_2-); 13.79 (-OCH_2**C**H_3).

Benzyl 1,1-di(ethoxycarbonyl)-3-(N-cyano-N-vinylamino)propylcarbamate 134.



134

134: $C_{20}H_{25}N_3O_6$ (MW 403.4).

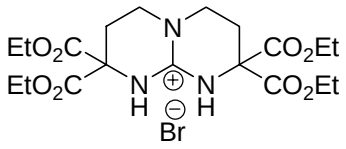
HPLC analysis R_v= 14 ml, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5µ column, UV_{220}, flow = 1 ml/min, gradient from 50% CH_3OH to 90% CH_3OH in 10 min and then 90% CH_3OH for 5 min more, 0.1% TFA as a buffer.

MS-ESI: *m/z* = 404.3 [$(M+H)^+$, 25%].

^{1}H-NMR (360 MHz; $CDCl_3$): δ= 7.34 (s, 5H, aromatic); 6.24 (s, 1H, -N**H**); 5.88 (dd, 1H,CH_2=C**H**-); 5.10 (s, 2H, PhC**H$_2$**O-); 4.63 (dd, 1H, C**H**$_2$=CH-); 4.44 (dd, 1H, C**H**$_2$=CH-); 4.21 (m, 4H, -OC**H$_2$**CH_3); 3.32 (t, J= 6.8 Hz, 4H, -N-C**H$_2$**); 2.72 (t, J= 7.2 Hz, 4H, -CC**H$_2$**-); 1.23 (t, J= 7.02 Hz, 12H, -OCH_2C**H$_3$**).

^{13}C-NMR (90.56 MHz; CDCL3): δ= 168.62 (-**C**O, esters); 156.11 (-N**C**O-); 137.37 (CH_2=**C**H-); 135.40, 130.03, 129.66, 129.55 (aromatic carbons); 114.07 (-**C**N); 96.56 (**C**H_2=CH-); 68.75 (Ph**C**H_2O-); 66.18(quaternary carbons); 64.65 (-O**C**H_2CH_3); 47.83 (-N-**C**H_2); 32.73 (-C**C**H_2-); 15.32 (-OCH_2**C**H_3).

2,2,8,8-Tetraethoxycarbonyl-3,4,6,7,8,9-hexahydro-2H-pyrimido[1,2-a]pyrimidine Hydrobromide 104.



104

To a solution of **107** (10 g, 14 mmol) in absolute ethanol (250 mL), was added a saturated solution of HCl in ethanol (10 mL). The reaction mixture was stirred overnight at 45 ^{o}C under nitrogen atmosphere. After cooling to room temperature, 15% Pd-C (500 mg) was added and the reaction mixture was stirred for 2-3 hours under H_2 atmosphere. The mixture was then filtered through a pad of celite and concentrated to get **109** as a solid residue (confirmed by MS-ESI).

The solid **109** was redissolved in absolute ethanol (250 mL) and to this solution, triethylamine (2.5 mL, 18.2 mmol) (**Caution**: Avoid use of excess of base which led to the formation of monocyclic guanidinium compound **130**) was added and the reaction mixture was stirred at 50 ^{o}C for two hours. After cooling to room temperature, the solvent was evaporated in vacuo and the residue was taken up in dichloromethane, washed with aqueous ammonium bromide solution (three times). The organic layer was dried and concentrated to get a slightly brown colored solid compound **104** which was recrystallized from ethyl acetate/dichloromethane (9:1) to afford 5.1 g of white crystalline solid **104** in an overall yield of 74% in four successive steps.

104: $C_{19}H_{29}N_3O_8 \cdot HBr$ (MW 508.4).

mp: 136 ^{o}C (ethyl acetate/dichloromethane).

HPLC analysis: R_v= 20 ml, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5µ column, $UV_{220,}$ flow = 1 ml/min, gradient from 10% CH_3OH to 50% CH_3OH in 10 min and then to 90% CH_3OH, in next 10 min more, 0.1% TFA as a buffer.

MS-ESI: *m/z* = 428 [$(M+H)^+$, 100%]

Elemental analysis: (%): Calc. C, 44.89; H, 5.95; N, 8.27; Br, 15.72, Found C, 45.03; H, 5.77; N, 8.23; Br, 15.87.

^{1}H-NMR (360 MHz; CD_3CN): δ= 9.64 (s, 2H, guanidinium-**H**); 4.26 (q, J= 7.05 Hz, 8H, -OC**H$_2$**CH_3); 3.38 (t, J= 6.1 Hz, 4H, -N-C**H$_2$**); 2.41 (t, J=6.1 Hz, 4H, -CC**H$_2$-**); 1.28 (t, J=7.02 Hz, 12H, -OCH_2C**H$_3$**).

^{13}C-NMR (90.56 MHz; CD_3CN): δ= 167.65 (-**C**O, esters); 151.09 (guanidinium carbon); 64.06 (-O**C**H_2CH_3); 63.13 (quaternary carbons); 44.37 (-N-**C**H_2); 26.09 (**-C**CH2-); 14.16 (-OCH_2**C**H_3).

108: $C_{37}H_{50}N_4O_{13} \cdot H^+$ (MW 759.8).

HPLC analysis: R_v= 15 ml, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5µ column, UV_{220}, flow = 1 ml/min, gradient from 50% CH_3OH to 90% CH_3OH in 10 min and then 90% CH_3OH for 5 min more, 0.1% TFA as a buffer.

MS-ESI: *m/z* = 759.5 [$(M+H)^+$, 100%].

109: $C_{21}H_{38}N_4O_9 \cdot H^+$ (MW 491.6)

HPLC analysis: R_v= 17 ml, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5µ column, UV detection at 220 nm, flow = 1 ml/min, gradient from 10% CH_3OH to 50% CH_3OH in 10 min and then to 90% CH_3OH, in next 10 min more, 0.1% TFA as a buffer.

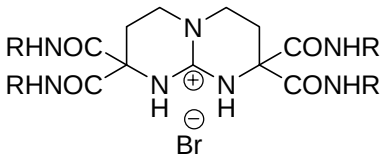
MS-ESI: *m/z* = 491.2 [$(M+H)^+$, 100%]

110: $C_{21}H_{35}N_3O_9 \cdot H^+$ (MW 474.5)

HPLC analysis: Rv= 18 ml, Phenomenex, Aqua C_{18}, 250x4.60 mm, 5µ column, UV_{220}, flow = 1 ml/min, gradient from 10% CH_3OH to 50% CH_3OH in 10 min and then to 90% CH_3OH, in next 10 min more, 0.1% TFA as a buffer.

MS-ESI: *m/z* = 474.2 [$(M+H)^+$, 100%]

2,2,8,8-Tetra(propylcarbamoyl)-3,4,6,7,8,9-hexahydro-2H-pyrimido[1,2-a]pyrimidine Hydrobromide 101.



101 R= n-C_3H_7

To a solution of n-propylamine (0.493 mL, 6 mmol) in dry dichloromethane (8 mL) under nitrogen atmosphere, was added dropwise a solution of trimethylaluminium (3.5mL, 7 mmol, 2M solution in toluene). After stirring at room temperature for 40 min, a solution of tetra-substituted

bicyclic guanidinium compound **104** (508 mg, 1 mmol) in dichloromethane (7mL) was added dropwise and then the reaction mixture was refluxed for 12 hrs. After cooling, the reaction mixture was quenched by addition of aqueous HBr (47%) solution and extracted with dichloromethane. The dichloromethane layer was washed with water, the aqueous layer was reextracted with dichloromethane, the combined organic layers were dried over magnesium sulfate and concentrated in vacuo to get white solid which was recrystallized from acetonitrile/ ether to afford **101** (475 mg, 85%) as white micro crystals.

101. $C_{23}\ H_{41}N_7O_4 \cdot HBr$ (MW 560).

mp.: 222 ^oC (ether/acetonitrile).

HPLC analysis: R_v= 16 ml, Nucleodure-100-5 C_8 ec column, UV_{220}, flow = 1 ml/min, gradient from 10% CH_3OH to 50% CH_3OH in 10 min and then 50% CH_3OH to 90% CH_3OH in next 10 min, 0.1% TFA as a buffer.

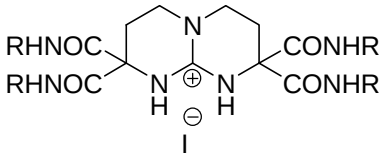
MS-ESI: *m/z* = 480.5 $[M+H)^+$, 100%].

Elemental analysis as an iodide salt: (%): Calc. C, 45.47; H, 6.97; N, 16.14; I, 20.89, Found C, 45.47; H, 6.53; N, 15.83; I, 19.94.

^{1}H-NMR (360 MHz; CD_3OD): δ= 8.41 (bs, guanidinium-**H**); 8.27 (t, J= 5.67 Hz, amide protons); 3.38 (t, J= 5.6 Hz, 4H, -N-C**H$_2$**); 3.21 (t, J= 7.03 Hz, 8H, -NC**H$_2$**CH_2CH_3); 2.47 (t, J= 6.1 Hz, 4H, -CC**H$_2$-**); 1.55 (h, J= 7.2 Hz, 8H, -NCH_2C**H$_2$**CH_3); 0.88 (t, J=7.49 Hz, 12H, -NCH_2CH_2**CH_3**).

^{13}C-NMR (90.56 MHz; CD_3OD): δ= 169.17 (-**C**O, amides); 150.80 (guanidinium carbon); 64.88 (-**C**CH_2-); 45.52 (-N-**C**H_2); 43.03 (-N**C**$H_2CH_2CH_3$); 28.58 (-C**C**H_2-); 23.47(-NCH_2**C**H_2CH_3); 11.57 (-NCH_2CH_2**C**H_3).

2,2,8,8-Tetra(phenylcarbamoyl)-3,4,6,7,8,9-hexahydro-2H-pyrimido[1,2-a]pyrimidine Hydroiodide 102.



102 R= Ph

To a solution of aniline (0.546 mL, 6 mmol) in dry dichloromethane (8 mL) under inert atmosphere, was added dropwise a solution of trimethylaluminium (3.5 mL,7 mmol, 2 M solution

in toluene). After stirring at room temperature for 40 min, a solution of tetra substituted bicyclic guanidinium compound **104** (508 mg, 1 mmol) in dichloromethane (7 mL) was added dropwise. Then the resultant mixture was refluxed for 5 hrs. After cooling, the reaction was cautiously quenched by addition of aqueous HBr (47%) solution. The dichloromethane layer was washed with water, the aqueous layer was reextracted with dichloromethane, the combined organic layers were washed with aqueous sodium iodide solution (3x10 mL), dried over magnesium sulfate and concentrated in vacuo to get a white solid. Recrystallization from acetonitrile/ether afforded **102** (572 mg, 77%) as a white crystalline substance.

102. $C_{35}H_{33}N_7O_4 \cdot HI$ (MW 743.6).

mp: 178-180 ^oC (acetonitrile/ether).

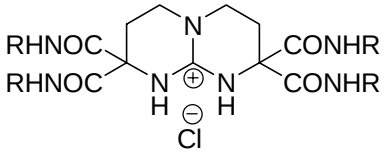
HPLC analysis: R_v= 20.2 ml, Nucleodure-100-5 C_8 ec column, UV_{220}, flow = 1 ml/min, gradient from 10% CH_3OH to 50% CH_3OH in 10 min and then 50% CH_3OH to 90% CH_3OH in next 10 min, 0.1% TFA as a buffer.

MS-ESI: *m/z* = 616.4 $[(M+H)^+$, 100%]

^{1}H-NMR (360 MHz; CD_3CN): δ= 9.19 (s, 4H, amide protons); 8.43 (bs, 2H, guanidinium-**H**); 7.66 (d, J= 8.1 Hz, 8H, aromatic); 7.32 (t, J= 7.9 Hz, 8H, aromatic); 7.15 (t, J= 7.37 Hz, 4H, aromatic); 3.42 (t, J=5.90 Hz, 4H, N-C**H$_2$**-); 2.67 (t, J= 6.1 Hz, 4H, -C**H$_2$-**).

^{13}C-NMR (90.56 MHz; CD_3CN): δ= 166.52 (-**C**O, amide); 149.80 (guanidinium carbon); 138.21, 129.73, 126.10, 122.03 (aromatic carbons); 65.41 (-**C**CH_2-); 45.50 (-N-**C**H_2); 27.88 (-C**C**H_2-).

2,2,8,8-Tetra(2-methoxyethylcarbamoyl)-3,4,6,7,8,9-hexahydro-2H-pyrimido[1,2-a]pyrimidine Hydrochloride 103.



103- R- CH_2CH_2OMe

To a solution of 2-methoxyethyleamine (0.522 mL, 6 mmol) in dry dichloromethane (8 mL) under nitrogen atmosphere, was added dropwise a solution of trimethylaluminium (3.5mL, 7 mmol, 2M solution in toluene). After stirring at room temperature for 40 min, a solution of tetra-

substituted bicyclic guanidinium compound **104** (508 mg, 1 mmol) in dichloromethane (7 mL) was added slowly and then the mixture was refluxed for 12 hrs. After cooling, the reaction was cautiously quenched by adding aqueous 6 N HCl solution. The dichloromethane layer was washed with water, the aqueous layer was reextracted with dichloromethane, the combined organic layers were again washed with water, brine, and dried with magnesium sulfate. The evaporation of the organic layer left a crude solid which was recrystallized from acetonitrile/ether to yield **103** (435 mg, 75%) as a white crystalline solid.

103: $C_{23}H_{41}N_7O_8 \cdot HCl$ (MW 580.1)

mp: 178-179 ^oC (acetonitrile/ether).

HPLC analysis: R_v= 10.8 ml, Nucleodure-100-5 C_8 ec column, UV_{220}, flow = 1 ml/min, gradient from 10% CH_3OH to 50% CH_3OH in 10 min and then 50% CH_3OH to 90% CH_3OH in next 10 min, 0.1% TFA as a buffer.

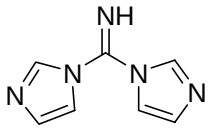
MS-ESI: *m/z* = 544.5 [$(M+H)^+$, 100%)]

Elemental analysis as an iodide salt: (%): Calc. C, 41.14; H, 6.30; N, 14.60; I, 18.90. Found C, 41.26; H, 6.03; N, 14.62; I, 18.51.

^{1}H-NMR (360 MHz; CD_3OD): δ= 3.35-3.44 (m, 28H); 3.29 (s, 12H, -$CH_2OC\mathbf{H}_3$); 2.41 (t, J=5.01 Hz, 4H, -$CC\mathbf{H}_2$-).

^{13}C-NMR (90.56 MHz; CD_3OD): δ= 169.33 (-**C**O, amide); 151.05 (guanidinium carbon), 71.53 (-$CH_2\mathbf{C}H_2OCH_3$); 64.8 (-$\mathbf{C}CH_2$-); 58.95 (-$CH_2CH_2O\mathbf{C}H_3$); 45.38 (-$N\mathbf{C}H_2$-); 40.86 (-$\mathbf{C}H_2CH_2OCH_3$), 29.20 (-$C\mathbf{C}H_2$-).

Di(1H-imidazol-1-yl)methanimine 111.[102]



111

To a solution of imidazole **75** (6.8 g, 100 mmol) in dry dichloromethane (500 mL) under inert atmosphere, was added a solution of cyanogen bromide (3.7 g, 33 mmol) in dichloromethane (20mL) and the resultant reaction mixture was heated at reflux temperature for 30 min. The mixture was then cooled to room temperature, and the obtained white precipitate was removed by

filtration, the filtrate was further concentrated to 50 mL and cooled to 0 ^{o}C for 2 days to get a crystalline solid. This solid was filtered, washed with cold dichloromethane and dried to afford **111** (3.8 g, 70%) as a white crystalline substance.

111: $C_7H_7N_5$ (MW 161.2)

HPLC analysis: R_v= 3ml, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5µ column, UV_{254}, flow = 1 ml/min, gradient from 80% CH_3OH to 90% CH_3OH in 5 min and then 90% CH_3OH for 10 min more, 0.1% TFA as a buffer.

mp: 102-103 ^{o}C (dichloromethane), (Lit.[115] mp: 103^{o}C).

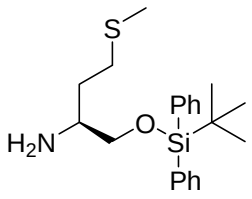
MS-ESI: *m/z* = 162.1 [$(M+H)^+$, 100%].

Elemental analysis: Calc. C, 52.17; H, 4.38; N, 43.45. Found**:** C, 51.91; H, 4.24; N, 43.96.

^{1}H-NMR (360 MHz; DMSO-d_6): δ= 10.19 (s, 1H, -N**H**); 8.09 (d, J=14.8 Hz, 1H, C**H**); 7.60 (d, J= 22.7 Hz, 1H, C**H**); 7.11 (s, 1H, C**H**).

^{13}C-NMR (90.56 MHz; DMSO-d_6): δ= 140.91; 137.37; 129.63; 118.93.

(2*S*)-2-Amino-1-[(tert-butyldiphenylsilyl)oxy]-5-thiahexane 112.[99]



112

To a well-stirred suspension of L-methionine (29.8 g, 200 mmol) in dry THF (500 mL) was added borane dimethylsulfide complex (40 mL, 400 mmol). After stirring for an hour, the reaction mixture was refluxed for a period of 16 hrs to give clear solution. After cooling the reaction mixture 10% aqueous HCl solution (150 mL) was added and then the mixture was further refluxed for 30 min. Evaporation of solvent in vacuo left an oily residue, which was taken up in water (100 mL) and made basic by addition of 4N NaOH (70 mL). The resultant emulsion formed was extracted with dichloromethane (3×150 mL). The combined organic layers were dried using magnesium sulfate, concentrated and distilled at 100 ^{o}C/ 13 Pa to give amino alcohol (23 g, 85%) as a colorless oil.

The amino alcohol obtained above (23 g, 170 mmol) and imidazole (23 g, 340 mmol) were taken up in dry CH_3CN (190 mL) under nitrogen. To this mixture, a solution of *tert*-butyldiphenylchlorosilane (60.8 g, 220 mmol) in CH_3CN (30 mL) was added while maintaining the temperature at 20 ^oC. After standing overnight, the solvent was evaporated and the residue was distributed at 50 ^oC between 1N NaOH (600 mL) and hexane (500 mL), the aqueous phase was extracted again with hexane (200 mL), and the combined organic phases were washed with water (3 × 200 mL).

Separation from the silanol byproduct was achieved by extraction of the hexane layer with $CH_3CN/H_2O/CH_3COOH$ (40:60:2) (300 mL, 3×100 mL). The combined aqueous phases were made basic by addition of anhydrous Na_2CO_3 (25 g), and concentrated to half of its volume in vacuo. This aqueous phase was extracted with hexane (6x150 mL) and the combined phases were dried over magnesium sulfate. Evaporation in vacuo furnished **112** (58 g, 78%) as a colorless viscous oil.

112: $C_{21}H_{31}NOSSi$ (MW 373.6).

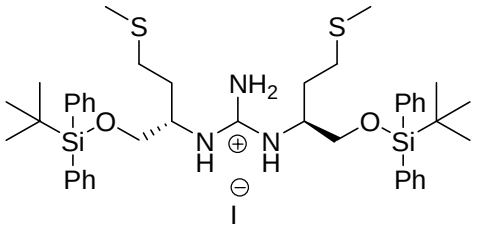
HPLC analysis: R_v= 7ml, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5µ column, UV_{254}, flow = 1 ml/min, gradient from 80% CH_3OH to 90% CH_3OH in 5 min and then 90% CH_3OH for 10 min more, 0.1% TFA as a buffer.

MS-ESI: *m/z* = 374.3 $[(M+H)^+$, 100%].

^{1}H-NMR (360 MHz; $CDCl_3$): δ= 7.66-7.69 (m. 4H, aromatic); 7.38-7.41 (m, 6H, aromatic); 3.59-3.63 (dd, J= 4.3Hz, 1H, -C**H**$_2$OSi-); 3.45-3.49 (dd, J= 6.5 Hz, 1H, -C**H**$_2$OSi-); 2.92-3.01(m, 1H, H_2NC**H**-); 2.54-2.58 (m, 2H, -C**H$_2$**SCH_3); 2.07 (s, 3H, -SC**H$_3$**); 1.68-1.78 (m, 1H; -CHC**H**$_2$-); 1.52-1.60 (m, 1H, -CHC**H**$_2$-); 1.09 (s, 9H, t-butyl C**H$_3$**).

^{13}C-NMR (90.56 MHz; $CDCl_3$): δ= 135.43, 133.33, 129.57, 127.58 (aromatic carbons); 68.84 (-**C**H_2OSi-); 51.97 (H_2N**C**H-); 33.03 (-**C**H_2SCH_3); 30.91 (-CH**C**H_2-); 26.77 (t-butyl **C**H_3); 19.14 (quaternary carbon); 15.31 (-S**C**H_3).

1,3-Bis[(*S*)-1-(tert-butyldiphenylsilyl)oxy-4-(methylthio)butan-2-yl]guanidine Hydroiodide 113.



113

Four reaction vials each containing a mixture of guanidylating reagent **111** (80.5 mg, 0.5 mmol), amino reagent **112** (392 mg, 1.05 mmol) and trifluoroacetic acid (30 µL) were placed and stirred in an oil bath at 105°C. After stirring the reaction mixture for 4 hours, the reaction mixture was cooled and a gummy substance obtained from each vial was dissolved in 3 mL of DMSO. To the combined DMSO solutions, acetic acid (40 µL) was added. This solution was then washed with ether/isooctane (3:1) (4 x 8 mL) to remove excess of **112**. The DMSO layer was diluted with water, and the emulsion obtained was extracted with dichloromethane (3x10 mL). The combined organic phases were washed with a nearly saturated aqueous NaI solution (2x20 mL), dried over magnesium sulfate, and concentrated in vacuo to give a gummy compound **113** (1.4 g, 77%) which was used in the next step without any further purification.

113: $C_{43}H_{61}N_3O_2Si_2 \cdot HI$ (MW 900.2).

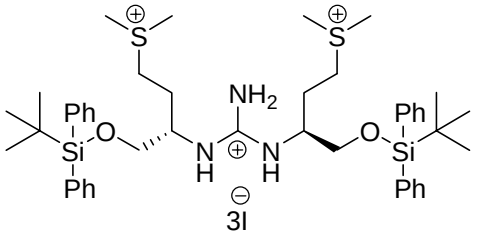
HPLC analysis: R_v= 16 ml, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5µ column, UV_{254}, flow = 1 ml/min, gradient from 80% CH_3OH to 90% CH_3OH in 5 min and then 90% CH_3OH for 10 min more, 0.1% TFA as a buffer.

MS-ESI *m/z* = 772.6 $[(M+H)^+$, 100%].

^{1}H-NMR (360 MHz; $CDCl_3$): δ= 9.04 (bs guanidinium-1**H**); 8.57 (bs, guanidinium-1H); 7.51-7.57 (m, 8H, aromatic); 7.38-7.41 (m, 12H, aromatic); 6.7 (bs guanidinium, 2H); 3.66-3.72 (m, 6H, -C**H₂**OSi-+-C**H**CH_2-); 2.41-2.58 (m, 4H, -C**H₂**SCH_3); 2.08 (s, 6H, -SC**H₃**); 1.75-1.84 (m, 2H, -CHC**H₂**-); 1.52-1.68 (m, 2H, -CHC**H₂**-); 1.04 (s, 18H, *t*-butyl-C**H₃**).

^{13}C-NMR (90.56 MHz; $CDCl_3$): δ= 158 .64 (guanidinium carbon); 135.38; 131.64; 130.33; 128.10 (aromatic carbons); 69.04 (-**C**H_2OSi-); 55.11 :(-**C**HCH_2); 30.23 (-**C**H_2SCH_3); 29.10 (-CH**C**H_2-); 26.90 (t-butyl-**C**H_3); 19.05 (quaternary carbon); 15.13 (-S**C**H_3).

1,3-Bis[(*S*)-1-(tert-butyldiphenylsilyl)oxy-4-(dimethylsulfonium)butan-2-yl]guanidine Trishydroiodide 114.



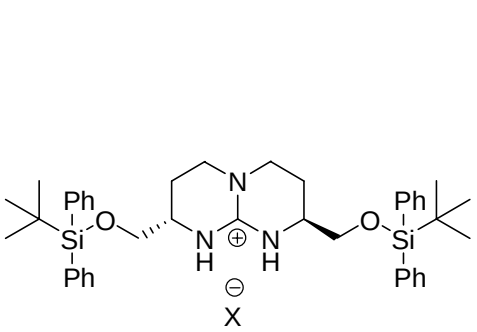
114

The guanidinium compound **113** (1.4 g, 1.55 mmol) was dissolved in methyl iodide (5 mL) and the brown mixture was stirred overnight at room temperature under inert atmosphere, where complete conversion of guanidinium compound **113** to bissulfonium compound **114** was achieved (as indicated by HPLC analysis). The excess of methyl iodide was removed by jet of nitrogen leaving a brownish residue which was again dissolved in dichloromethane. Finally, the evaporation of dichloromethane left a brown colored solid residue containing bissulfonium salt **114** (1.87 g, 98%) which was directly used in to the next reaction without any further purification.

113: $C_{45}H_{68}N_3O_2Si_2SI_3$ (MW 1184.1).

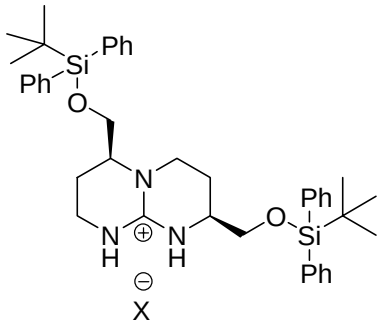
HPLC analysis: Rv= 10 ml, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5μ column, UV_{254}, flow = 1 ml/min, gradient from 80% CH_3OH to 90% CH_3OH in 5 min and then 90% CH_3OH for 10 min more, 0.1% TFA as a buffer.

[2*S*,8*S*]-2,8-Di[[(tert-butyldiphenylsilyl)oxy]methyl]-3,4,6,7,8,9-hexahydro-2H-pyrimido[1,2-a]pyrimidine Hydroiodide 115.



115, X= $I^{\ominus}$

143, X= $ClO_4^{\ominus}$



116, X= $I^{\ominus}$

44, X= $ClO_4^{\ominus}$

To a solution of the bissulfonium salt **114** (1.87 g, 1.5 mmol) in dry dichloromethane (50 mL) cooled to less than 5 ^{0}C, was added DBU (1.61 mL, 9 mmol) and then the reaction mixture was stirred allowing it to warm to room temperature. After 4 hrs of stirring, HPLC analysis showed the completion of reaction yielding a 65:35 mixture of bicyclic guanidinium **115** to its structural isomer **116**. The reaction mixture was quenched by adding acetic acid (1 mL) and then the dichloromethane layer was washed with 1N acetic acid (40 mL), water (40 mL) and finally by nearly saturated aqueous NaI (2x40 mL) solution. The organic layer was dried over magnesium sulfate and the evaporation of solvent left 940 mg of crude gummy mixture of isomers **115** and **116**. The partial purification of the isomers was achieved by crystallization from acetonitrile at -18 °C where the desired bicyclic guanidinium compound **115** crystallizes preferentially to afford 300 mg of slightly brownish colored crystalline solid.

The remaining 1:1 mixture of isomers were separated by reversed phase MPLC, in 80 % methanol/ water containing 30 mmol $NaClO_4$ as elutant to yield bicyclic guanidinium compound **143** (240 mg) and isomer **44** (245 mg). Thus the overall yield of bicyclic guanidinium compound (**115**+**143**) in two steps was 45 % along with 20% structural isomer **44**.

115: $C_{41}H_{53}N_3O_2Si_2 \cdot HI$ (MW 804.0).

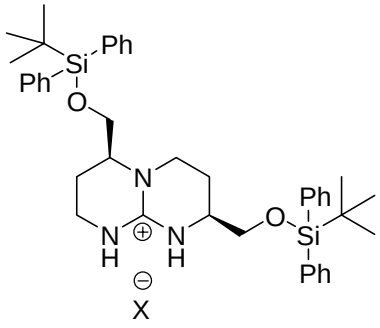
HPLC analysis: R_v= 14ml, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5µ column, UV_{254}, flow = 1 ml/min, gradient from 80% CH_3OH to 90% CH_3OH in 5 min and then 90% CH_3OH for 10 min more, 0.1% TFA as a buffer.

MS-ESI *m/z* = 676.6 $[(M+H)^+$, 100%].

^{1}H-NMR (360 MHz; $CDCl_3$): δ= 8.07 (s, 2H, guanidinium-**H**); 7.61-7.64 (m, 8H, aromatic); 7.36-7.43 (m, 12H, aromatic); 3.77-3.81 (m, 2H, -C**H**CH_2-); 3.58-3.62 (m, 4H, -C**H$_2$**OSi-); 3.11-3.24 (m, 4H, -NC**H$_2$**-); 1.89-1.99 (m, 4H, -CHC**H$_2$**-); 1.06 (s 18H, t-butyl-C**H$_3$**).

^{13}C-NMR (62.9 MHz; $CDCl_3$): δ= 151.15 (guanidinium carbon); 135.62, 132.42, 130.00, 130.16, 127.95 (aromatic. carbons); 65.31 (-**C**H_2OSi-); 49.45 (-**C**HCH_2-); 44.96 (N-**C**H_2); 26.90 (t-butyl-**C**H_3); 22.69 (-CH**C**H_2-) 19.19 (quaternary carbons)

[2*S*,6*R*]-2,6-Di[[(tert-butyldiphenylsilyl)oxy]methyl]-3,4,6,7,8,9-hexahydro-2H-pyrimido[1,2-a]pyrimidine Hydroperchlorate 44.



116, X= $I^\ominus$

44, X= $ClO_4^\ominus$

44: $C_{41}H_{53}N_3O_2Si_2 \cdot HClO_4$ (MW 776.51).

HPLC analysis: R_v= 16ml, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5µ column, UV254 nm, flow = 1 ml/min, gradient from 80% CH_3OH to 90% CH_3OH in 5 min and then 90% CH_3OH for 10 min more, 0.1% TFA as a buffer.

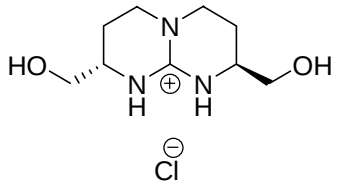
MS-ESI, *m/z* = 676.6 $[(M+H)^+$, 100%].

^{1}H-NMR (360 MHz; $CDCl_3$): δ= 7.59-7.62 (m, 8H, aromatic); 7.34-7.48 (m, 12H, aromatic); 6.98 (d, 1H, guanidinium); 6.72 (bs, 1H, guanidinium); 3.49-3.69 (m, 5H, -C**H$_2$**OSi-+-C**H**CH_2-);

3.42 (m, 1H, -C**H**CH_2-); 3.07-3.30 (m, 4H, -NC**H_2**-); 1.70-1.97 (m, 4H, -CHC**H_2**-); 1.05 (2s, 18H, t-butyl-C**H_3**).

^{13}C-NMR (90.56 MHz; $CDCl_3$): δ= 150.68 (guanidinium carbon); 135.46, 135.42, 132.60, 132.30, 130.19, 130.16, 127.97, 127.79 (aromatic. carbons); 65.23, 63.00 (-**C**H_2OSi-); 56.95, 50.02 (-**C**HCH_2-); 44.28, 35.10 (-N**C**H_2-); 26.74, 26.72 (t-butyl-**C**H_3); 22.69, 21.45 (-CH**C**H_2-); 19.04, 18.97 (quaternary carbons).

[2*S*,8*S*]-2,8-Dihydroxymethyl-3,4,6,7,8,9-hexahydro-2H-pyrimido[1,2-a]pyrimidine Hydrochloride 117.



117

To a solution of the bicyclic guanidinium iodide **115** (1.0 g, 1.24 mmol) in dry THF (25 mL), was added triethylamine hydrochloride (513 mg, 3.73 mmol) and fluoride (1.25 g, 3.73 mmol) supported on polymer (Fluka cat.nr. 47060). The suspension was stirred at room temperature. In the initial 2 hrs of stirring the reaction mixture became a clear solution with polymer bits in it. After continued stirring overnight, a precipitation occurred in the reaction mixture. The precipitate was filtered and the residue was taken up in methanol (15 mL) and stirred vigorously for 15 min. Filtration separated the product solution from the polymer which was repeatedly washed with methanol. The combined filtrates and washings were evaporated in vacuo to afford a white solid compound **117** (400 mg) (containing triethylamine hydrochloride salt). A small sample was purified for analysis using SPE, and the remainder was used as is in the further reaction without any purification.

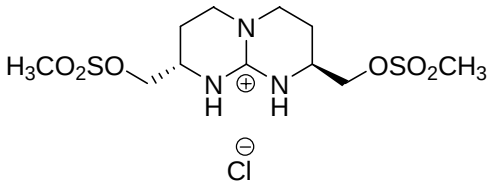
117: $C_9H_{17}N_3O_2 \cdot HCl$ (MW 235.7)

HPLC analysis: R_v= 7ml, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5µ column, UV_{254}, flow = 1 ml/min, gradient from 10% CH_3OH to 50% CH_3OH in 10 min and then to 90% CH_3OH in next 10 min, 0.1% TFA as a buffer.

^{1}H-NMR (360 MHz; CD_3OD): δ= 9.2 (bs, 2H, guanidinium-**H**); 3.72 (dd, 2H, -C**H**CH_2-); 3.5-3.6 (m, 4H, -**C**H_2OH); 3.36-3.41(m, 4H, -NC**H$_2$**-); 1.97-2.04 (m, 2H, -CHC**H$_2$**-); 1.8-1.9 (m, 2H, -CHC**H$_2$**-).

^{13}C-NMR (90.56 MHz; CD_3OD): δ= 151.01 (guanidinium carbon); 63.5 (-**C**H_2OH); 50.21 (-**C**HCH_2); 45.4 (N-**C**H_2-); 22.6 (-CH**C**H_2-).

[2*S*,8*S*]-2,8-Dimethylsulfonyloxymethyl-3,4,6,7,8,9-hexahydro-2H-pyrimido[1,2-a]pyrimidine Hydrochloride 118.



118

The above reaction residue containing dihydroxymethylguanidinium chloride **117** (400 mg, 1.24 mmol) was suspended in dry dichloromethane (20 mL) under nitrogen atmosphere. The mixture was cooled to 0 ^{o}C in an ice bath and triethylamine (0.520 mL, 3.72 mmol) was added. Then the solution of methanesulfonylchloride (0.560 mL, 7.44 mmol) in dichloromethane (4 mL) was added dropwise. The initial suspension disappeared to give a clear solution and then product started precipitating out from the reaction mixture. After stirring for 30 min, the reaction mixture was evaporated to dryness by a jet of nitrogen and the colorless residue was used in further reaction without any purification.

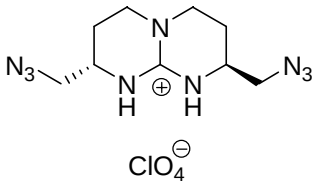
118: $C_{11}H_{21}N_3O_6S_2 \cdot HCl$ (MW 391.9)

HPLC analysis: R_v= 10ml, Phenomenex, Aqua C_{18}, 250×4.60 mm, UV_{254}, flow = 1 ml/min, gradient from 10% CH_3OH to 50% CH_3OH in 10 min and then to 90% CH_3OH in next 10 min, 0.1% TFA as a buffer.

^{1}H-NMR (360 MHz; CD_3CN): δ= 9.03 (bs, 2H, guanidinium); 4.27 (dd, 2H, -C**H$_2$**O-); 4.12 (dd, 2H, -C**H$_2$**O-); 3.74-3.80 (m, 2H, -C**H**CH_2-); 3.26-3.37 (m, 4H, -NC**H$_2$**-); 3.21 (s, 6H, -SC**H$_3$**); 2.01-2.08 (m, 2H, -CHC**H$_2$**-); 1.78-1.86 (m, 2H, -CHC**H$_2$**-).

^{13}C-NMR (90.56 MHz; CD_3CN): δ= 152.65 (guanidinium carbon); 71.5 (-**C**H_2O-); 48.27 (-**C**HCH_2-); 45.54 (-N**C**H_2-); 37.93 (-S**C**H_3); 22.75 (-CH**C**H_2-).

[2*S*,8*S*]-2,8-Diazidomethyl-3,4,6,7,8,9-hexahydro-2H-pyrimido[1,2-a]pyrimidine Hydroperchlorate 119.



119

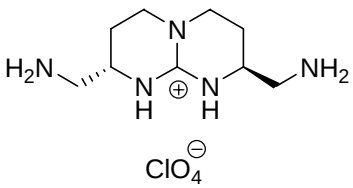
The reaction residue obtained above containing the bicyclic guanidinium compound **118** (1.24 mmol) was dissolved in absolute DMF (20 mL) and finely powdered sodium azide (1.5 g, 23 mmol) was added. The mixture was stirred in an oil bath at 90 ^{o}C. After stirring overnight, the reaction mixture was cooled to room temperature and then DMF was removed under reduced pressure. The solid residue obtained was taken up in dichloromethane (20 mL), the insoluble part was filtered and washed with dichloromethane (2x10 mL). The combined dichloromethane layers were washed with nearly saturated aqueous $NaClO_4$ solution (2x30 mL), dried over magnesium sulfate and evaporation in vacuo left gummy substance which was recrystallized from methanol/water to furnish bisazide **119** (260 mg, 60% over three steps) as a colorless crystalline compound.

119: $C_9H_{15}N_9 \cdot HClO_4$ (MW 349.7)

HPLC analysis: R_v= 12ml, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5µ column, UV_{254}, flow = 1 ml/min, gradient from 10% CH_3OH to 50% CH_3OH in 10 min and then to 90% CH_3OH in next 10 min, 0.1% TFA as a buffer.

IR: (methanol) ν: 2109 cm^{-1} (N_3).

[2*S*,8*S*]-2,8-Diaminomethyl-3,4,6,7,8,9-hexahydro-2H-pyrimido[1,2-a]pyrimidine Hydroperchlorate 120.



120

To a solution of bisazide **119** (260 mg, 0.74 mmol) dissolved in methanol (25 mL), was added 15% Pd-C (50 mg).The suspension was stirred at room temperature under the atmosphere of H_2. After stirring for 3 hrs, the reaction mixture was filtered through a pad of celite and the evaporation of filtrate gave bisaminoguanidinium compound **120** (211 mg, 95%) as a gummy substance which solidifies on standing.

120: $C_9H_{19}N_5 \cdot HClO_4$ (MW 297.7)

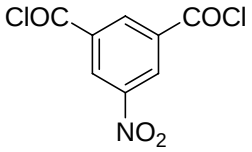
MS-ESI *m/z* = 198.3 [$(M+H)^+$, 100%].

HPLC analysis: R_v= 4 ml, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5µ column, UV_{254}, flow = 1 ml/min, gradient from 10% CH_3OH to 50% CH_3OH in 10 min and then to 90% CH_3OH in next 10 min, 0.1% TFA as a buffer.

^{1}H-NMR (360 MHz; CD_3OD): δ= 3.33-3.42 (m, 6H, -C**H**CH_2-,-C**H$_2$**NH_2); 2.68-2.83 (m, 4H, -NC**H$_2$**-); 2.05-2.10 (m, 2H, -CHC**H$_2$**-); 1.80-1.89 (m, 2H, -CHC**H$_2$**-).

^{13}C-NMR (90.56 MHz; CD_3OD): δ= 152.31 (guanidinium carbon); 51.77 (-**C**HCH_2-); 46.33 (-**C**H_2NH_2); 46.16 (-N**C**H_2); 24.68 (-CH**C**H_2-).

5-Nitroisophthalic acid dichloride 122.



122

To a suspension of 5-nitroisophthalic acid **121** (20g, 95 mmol) in of dry dichloromethane (120 mL) under nitrogen, was added dropwise oxalyl chloride (75 mL, 393 mmol). Then DMF (0.5 mL) was added cautiously to the reaction mixture. After stirring the reaction mixture for 22 hrs at room temperature, a clear solution was obtained. The evaporation of excess oxalyl chloride and solvent left a solid residue, which was recrystallized, from carbon tetrachloride to afford **122** (12 g, 51%) as colorless crystalline substance.

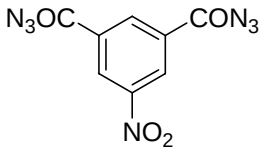
121: $C_8H_3Cl_2NO_4$ (MW. 248.0)

mp: 60^{o}C (CCl_4), (Lit.[116] mp, 59-61 ^{o}C).

^{1}H-NMR (360 MHz; $CDCl_3$): δ= 9.20 (d, J= 1.52 Hz, 2H); 9.10 (t, J= 1.52 Hz, 1H).

^{13}C-NMR (90.56 MHz; $CDCl_3$): δ= 165.75 (**C**O); 149.02, 137.55, 136.16, 131.01 (aromatic carbons).

5-Nitroisophthalic acid azide 123.[117-119]



123

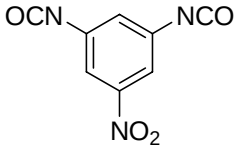
To a solution of acid chloride **122** (5 g, 20 mmol) dissolved in dichloromethane (100 mL) under nitrogen, was added trimethylsilyl azide (12 mL, 80 mmol). After stirring for 16 hrs at room temperature, the excess of trimethylsilyl azide and solvent was removed under reduced pressure to obtain the acid bisazide **123** (4.8 g, 92%) as colorless residue which was pure enough to be used in the next step without any purification.

123: $C_8H_3N_7O_4$ (MW. 261.1).

^{1}H-NMR (360 MHz; $CDCl_3$): δ= 9.05 (d, J= 1.1 Hz, 2H); 8.94 (t, J= 1.1 Hz, 1H).

^{13}C-NMR (90.56 MHz; $CDCl_3$): δ= 169.58 (**C**O); 148.72, 134.96, 133.17, 128.74 (aromatic carbons)

1,3-Diisocyanato-5-nitrobenzene 24.[105,117]



124

The solution of bisazido compound **123** (4.8 g, 18.4 mmol) in dry toluene (100 mL), was slowly added to an empty round bottom flask preheated in an oil bath at 110 ^{o}C. The evolution of nitrogen was observed which seized after 1 hour of addition. The reaction mixture was then cooled to room temperature and solvent was removed under reduced pressure to yield a yellowish solid residue. The residue was recrystallized from petroleum ether at -20 ^{0}C to afford white

crystals of **124** (2.6 g, 70 %), which turn slightly yellowish on standing. This solid can be sublimed at 80°C/ 0.1 Torr to get white crystalline compound.

124: $C_8H_3N_3O_4$ (MW. 205.13)

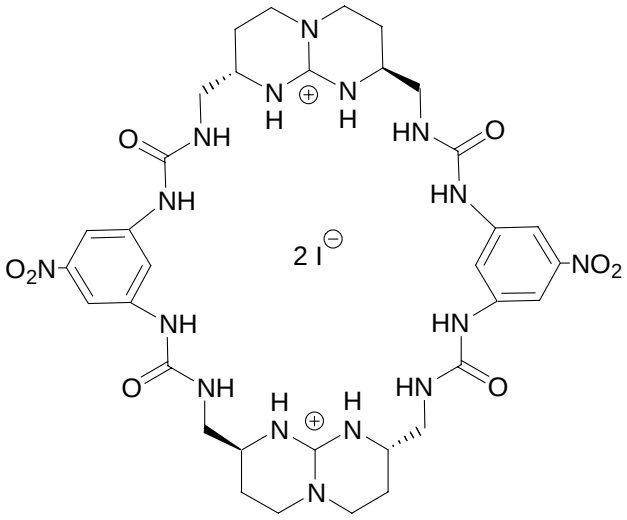
mp = 80-81 °C (pet. ether).

IR: (CCl_4) ν: 2255 cm^{-1} (NCO).

^{1}H-NMR (360 MHz; $CDCl_3$): δ= 7.79 (d, J= 1.97 Hz, 2H); 7.14 (t, J= 1.97 Hz, 1H).

^{13}C-NMR (90.56 MHz; $CDCl_3$): δ= 149.41 (**C**O); 136.02, 126.50, 125.81, 117.21 (aromatic carbons).

Macrocycle 125.



125

To the mixture of bisaminoguanidinium compound **120** (105 mg, 0.35 mmol) and EDIPA (0.25 mL, 1.5 mmol) in DMF (25 mL) under nitrogen atmosphere cooled in an ice salt bath, was added the solution of freshly sublimed isocyanato reagent **124** (75 mg, 0.36 mmol) dissolved in dry dichloromethane (7 mL) over a period of 1.5 hrs using a syringe pump. After complete addition of reagent, the reaction mixture was allowed to warm up slowly to room temperature and then quenched by addition of TFA (0.25 mL). The removal of solvent under reduced pressure left a yellowish residue. The residue was taken up in CH_3CN (10 mL) and filtered to remove most of the polymeric impurities. The filtrate was concentrated to half of its volume and then dilution with water resulted in precipitation of the product. The precipitate was filtered, washed with

water (5 mL) and dried to get yellow powder (90 mg), which was then subjected to reversed phase MPLC using 30 % acetonitrile/water along with 0.5% TFA. The fractions containing the product were pooled and concentrated to get a yellow solid (34 mg, 10%). This solid was dissolved in 70% acetonitrile/water mixture and passed through an anion exchange resin (AG® 4-x4) containing iodide as a counter ion. The evaporation of solvent gave **125** as a yellow solid**.**

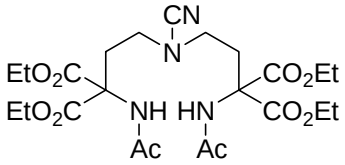
125: $C_{34}H_{44}N_{16}O_8 \cdot 2HI$ (MW 1060.6)

MS–ESI: *m/z* = 919.1 [$(M+TFA)^+$, 100%]; 805.3 (M^+, 75%); 403.3 (M^{2+}, 50%).

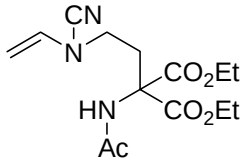
HPLC analysis: R_v= 13 ml, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5µ column, UV_{254}, flow = 1 ml/min, gradient from 10% CH_3CN to 50% CH_3CN in 10 min and then to 90% CH_3CN in next 10 min, 0.1% TFA as a buffer.

^{13}C-NMR for **125** (90.56 MHz; DMSO-d_6): δ= 155.32 (-**C**O-); 150.52 (guanidinium carbons); 148.44, 141.63, 111.68, 104.85 (aromatic carbons); 48.10 (-**C**HCH_2-); 44.97 (-N**C**H_2); 43.22 (-CH**C**H_2NH-) 24.68 (-CH**C**H_2-).

N,N-Bis[(3-acetylamino-3,3-diethoxycarbonyl)propyl] cyanamide 126.



126



127

To a suspension of NaH (820 mg, 20.5 mmol) (60 % dispersion in mineral oil) in DMF (5 mL), was added dropwise a solution of diethyl acetamidomalonate **45** (4.34 g, 20 mmol) in DMF (10 mL). When the evolution of H_2 seized, the reaction mixture was heated in an oil bath at 50°C. Then the solution of bis-iodoethylcyanamide **105** (700 mg, 2 mmol) in DMF (5 mL) was added over a period of 20 min using a syringe pump. After stirring for 3 hrs, the reaction mixture was cooled to room temperature, poured into the aqueous 0.1 N $NaHCO_3$ solution (15 mL) and the aqueous layer was extracted with diethyl ether (3x15 mL). The combined organic layers were washed with water and dried over magnesium sulfate. Evaporation of the organic phase left a crude solid material containing **126** and **127**. The crude material was purified by column chromatography over aluminium oxide (50 % ethyl acetate/hexane) to yield crystalline **126** (350 mg, 40%) and the elimination product **127** (150 mg).

126: $C_{23}H_{36}N_2O_{10}$ (MW 528.5)

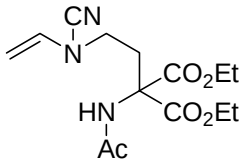
MS-ESI: *m/z* = 529.3 [$(M+H)^+$, 100%].

HPLC analysis: R_v= 17 ml, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5µ column, UV_{220}, flow = 1 ml/min, gradient from 20% CH_3CN to 80% CH_3CN in 20 min., 0.1% TFA as a buffer.

^{1}H-NMR (360 MHz; $CDCl_3$): δ= 6.81 (s, 2H, -N**H**); 4.22-4.30 (m, 8H, -OC**H₂**CH_3), 2.97 (t, J= 6.8 Hz, 4H, -NC**H₂**); 2.63 (t, J= 7.7 Hz, 4H, -CC**H₂**-); 2.06 (s, 6H, -COC**H₃**); 1.27 (t, J= 7.0 Hz, 12H, -OCH_2C**H₃**).

^{13}C-NMR (90.56 MHz; CDCl3): δ= 169.69 (-N**C**OCH_3); 167.48 (-**C**O, ester); 116.6 (-**C**N); 64.7 (quaternary carbons); 63.06 (-O**C**H_2CH_3), 47.42 (-N**C**H_2-), 30.77 (-CCH_2-) 13.94 (-OCH_2**C**H_3).

N-Vinyl-N-[(3-acetylamino-3,3-diethoxycarbonyl) propyl cyanamide 127.



127

127: $C_{14}H_{21}N_2O_5$ (MW 311.3)

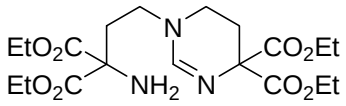
MS-ESI: *m/z* = 312.2 [$(M+H)^+$, 100%].

HPLC analysis: R_v= 16 ml, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5µ column, UV_{220}, flow = 1 ml/min, gradient from 20% CH_3CN to 80% CH_3CN in 20 min., 0.1% TFA as a buffer.

^{1}H-NMR (250 MHz; $CDCl_3$): δ= 6.88 (s, 1H, -N**H**); 5.89 (dd, J= 8.5 Hz, 1H, CH_2=C**H**-); 4.66 (dd, J_1= 2.3 Hz, J_2=2.4 Hz, 1H, C**H₂**=CH-); 4.45 (dd, J= 2.1 Hz,1H, C**H₂**=CH-); 4.21-4.31 (m, 4H, -OC**H₂**CH_3); 3.33 (t, J= 10 Hz, 2H, -NC**H₂**); 2.92 (t, J= 10.1, Hz, 2H, -CC**H₂**-); 2.04 (s, 3H, -COC**H₃**); 1.23 (t, J= 10.1 Hz, 12H, -OCH_2C**H₃**).

^{13}C-NMR (62.9 MHz; CDCl3): δ= 169.64 (-N**C**OCH_3); 167.35 (-**C**O, ester); 116.6 (-**C**N); 64.7(quaternary carbons), 63.03 (-O**C**H_2CH_3); 47.25 (-N**C**H_2-); 30.59 (-CCH_2-); 22.91 (-CO**C**H_3); 13.83 (-OCH_2**C**H_3).

3-(3-Amino-3,3-diethoxycarbonyl)propyl-6,6-diethoxycarbonyl-3,4,5,6-tetrahydropyrimidine 128.



128

To a solution of bis-carbamate **107** (1 g, 1.4 mmol) in absolute ethanol (25 mL) was added 15% Pd-C (150 mg). The suspension was stirred in an atmosphere of H_2 for 2 hrs. Then the reaction mixture was filtered through a pad of celite and the filtrate on evaporation under vacuo left a gummy substance, which was recrystallized from ether/hexane to afford **128** (600 mg, 99%) as a colourless crystalline substance.

128: $C_{19}H_{31}N_3O_8$ (MW 429.5)

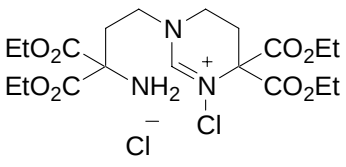
MS-ESI: *m/z* = 430.4 $[(M+H)^+$, 100%].

HPLC analysis: R_v= 17 ml, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5µ column, UV_{220}, flow = 1 ml/min, gradient from 10% CH_3OH to 50% CH_3OH in 10 min and then to 90% CH_3OH, in next 10 min more, 0.1% TFA as a buffer.

^{1}H-NMR (250 MHz; $CDCl_3$): δ= 7.06 (s, 1H, -C**H**=N-); 4.04-4.15 (m, 8H, -OC**H$_2$**CH_3); 3.18 (t, J= 7.3 Hz, 2H, -NC**H$_2$**); 3.08 (t, J= 5.7 Hz, 2H, -NC**H$_2$**); 2.09 (t, J= 6.1 Hz, 2H, -CC**H$_2$**-); 1.99 (t, J= 7.1 Hz, 2H, -CC**H$_2$**-); 1.13 (t, J= 7.0 Hz, 12H, -OCH_2C**H$_3$**).

^{13}C-NMR (62.9 MHz; CDCl3): δ= 170.50, 169.93 (-**C**O, ester); 150.53 (-**C**H=N-); 64.60, 63.96 (quaternary carbons); 61.86, 61.41 (-O**C**H_2CH_3); 47.92, 39.66 (-N**C**H_2-); 33.70, 25.39 (-C**C**H_2-); 13.73, 13.66 (-OCH_2**C**H_3).

3-[(3-Amino-3,3-diethoxycarbonyl)propyl]-1-chloro-6,6-diethoxycarbonyl-3,4,5,6-tetrahydropyrimidin-1-ium chloride 129.



129

To a solution of **128** (100 mg, 0.23 mmol) dissolved in dry chloroform (5 mL) under nitrogen atmosphere, was added NCS (50 mg, 0.37 mmol) and the mixture was stirred at room temperature for 2 hrs. The obtained precipitate was filtered and the filtrate on evaporation left crude solid, which on characterization showed compound **129.**

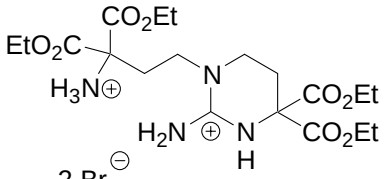
129: $C_{19}H_{31}N_3O_8Cl$ (MW 465.0)

MS-ESI: *m/z* = 464.3 (M^+, 100%); 466.2 (33%).

HPLC analysis R_v= 20 ml, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5µ column, UV_{220}, flow = 1 ml/min, gradient from 10% CH_3OH to 50% CH_3OH in 10 min and then to 90% CH_3OH, in next 10 min more, 0.1% TFA as a buffer.

^{13}C-NMR (90.6 MHz; $CDCl_3$): δ= 166.63, 166.58 (-**C**O, ester); 152.38 (-**C**H=N-, assigned on the basis of DEPT-135 spectrum); 64.6, 63.9 (quaternary carbons); 62.81, 62.35 (-COO**C**H_2CH_3); 49.31, 40.77 (-N**C**H_2-); 31.81, 24.31 (-C**C**H_2-); 13.65, 13.54 (-OCH_2**C**H_3).

1-[(3-Amino-3,3-diethoxycarbonyl)propyl]-2-amino-4,4-diethoxycarbonyl-1,4,5,6-tetrahydropyrimidine- Bishydrobromide 130.



130

To a solution of bis-carbamate **107** (40 mg, 0.056 mmol) dissolved in dry dichloromethane (10 mL) was added solution of 33% HBr in propionic acid (1 mL) and the mixture was stirred at room temperature for 1 hour. The excess of HBr was removed by jet of nitrogen and the residue was redissolved in acetonitrile (5 mL). The solvent was again removed by stream of nitrogen to strip of residual HBr. The residue was dried under high vacuo to get the monocyclic guanidinium compound **130** (32 mg, 95%) as a yellow powder.

130: $C_{19}H_{32}N_4O_8 \cdot 2$ HBr (MW 606.3)

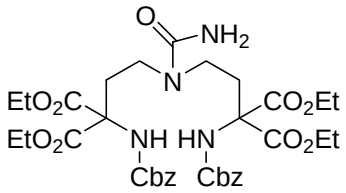
MS-ESI: *m/z* = 445.3 [$(M+H)^+$, 100%].

HPLC analysis: R_v= 16 ml, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5µ column, UV_{220}, flow = 1 ml/min, gradient from 10% CH_3OH to 50% CH_3OH in 10 min and then to 90% CH_3OH, in next 10 min more, 0.1% TFA as a buffer.

^{1}H-NMR (250 MHz; CD_3CN): δ= 7.96 (s, 1H, guanidinium-**H**); 7.49 (s, 2H, guanidinium-**H**); 4.21-4.38 (m, 8H, -OC**H$_2$**CH_3); 3.63-3.70 (m, 2H, -NC**H$_2$**); 3.42-3.51 (m, 2H, -NC**H$_2$**); 2.40-2.51 (m, 4H, -CC**H$_2$**-); 1.22-1.31 (m, 12H, -OCH_2C**H$_3$**).

^{13}C-NMR (62.9 MHz; CD_3CN): δ= 167.64, 165.20 (-**C**O, ester); 153.82 (guanidinium carbon); 65.62 (-O**C**H_2CH_3), 65.52, (quaternary carbon); 64.85 (-O**C**H_2CH_3); 63.17 (quaternary carbon); 46.80, 44.14 (-N**C**H_2-); 29.58, 26.24 (-CCH_2-); 14.18, 1409 (-OCH_2**C**H_3).

N,N-Bis[(3-benzyloxycarbonylamino-3,3-diethoxycarbonyl)propyl]urea 131.



131

To a solution of bis-carbamate **107** (2.29 g, 3.21 mmol) in CH_3CN (20 mL), was added aqueous 6N H_2SO_4 (9 mL) and the resulting mixture was stirred in an oil bath at 80 ^{o}C. After 30 min of stirring, the reaction mixture was cooled to room temperature, neutralized using aqueous 6N NaOH, concentrated to half its volume and freeze dried to get a colorless solid. The solid was taken up in acetonitrile (10 mL) and insoluble part was removed by filtration. The filtrate was then evaporated under vacuo to obtain urea **131** (2.3 g, 98%) as a gummy substance.

131: $C_{35}H_{46}N_4O_{13}$ (MW 730.8)

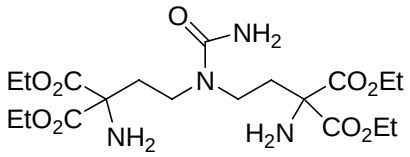
HPLC analysis: R_v= 14 ml, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5μ column, UV_{220}, flow = 1 ml/min, gradient from 50% CH_3OH to 90% CH_3OH in 10 min and then 90% CH_3OH extended for 5 min more, 0.1% TFA as a buffer.

MS-ESI: *m/z* = 731.3 [$(M+H)^+$, 100%].

^{1}H-NMR (360 MHz; $CDCl_3$): δ= 7.25-7.34 (m, 10H, aromatic); 6.29 (s, 2H, -N**H**); 5.74 (bs, 2H, -CON**H$_2$**); 5.07 (s, 4H, PhC**H$_2$**O-); 4.15- 4.23 (m, 8H, -OC**H$_2$**CH_3); 3.13 (t, J= 7.2 Hz, 4H, -NC**H$_2$**); 2.46 (t, J= 7.9 Hz, 4H,-CC**H$_2$**-); 1.19 (t, J= 7.1 Hz, 12H, -OCH_2C**H$_3$**).

^{13}C-NMR (62.9 MHz; $CDCl_3$): δ= 167.54 (-**C**O, ester); 158.39 (-**C**O-, urethane); 154.73 (-**C**ONH_2-); 135.99, 128.42 128.15, 128.99 (aromatic carbons); 66.99 (Ph**C**H_2-); 64.9 (quaternary carbons); 62.83 (-O**C**H_2CH_3); 42.55 (-N**C**H_2-); 31.84 (-C**C**H_2-); 13.76 (-OCH_2**C**H_3).

N,N-Bis[(3-amino-3,3-diethoxycarbonyl)propyl]urea 132.



132

To a solution of urea **131** (3.27g, 4.47 mmol) dissolved in absolute ethanol (35 mL), was added 15% Pd-C (250 mg). The obtained suspension was stirred under atmosphere of H_2 for 3 hrs. Then the reaction mixture was filtered through a pad of celite and the evaporation of filtrate under vacuo left gummy diamino compound **132** (2.04 g, 99%).

132: $C_{19}H_{34}N_4O_9$ (MW 462.5)

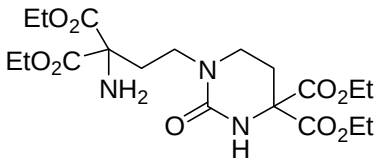
HPLC analysis R_v= 16 ml, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5µ column, UV_{220}, flow = 1 ml/min, gradient from 10% CH_3OH to 50% CH_3OH in 10 min and then to 90% CH_3OH, in next 10 min more, 0.1% TFA as a buffer.

MS-ESI: *m/z* = 463.2 [$(M+H)^+$, 100%].

^{1}H-NMR (250 MHz; CD_3CN): δ= 5.35 (bs, 2H, -CON**H$_2$**); 4.14 (q, J= 7.0 Hz, 8H, -OC**H$_2$**CH_3); 3.20 (t, J= 7.3 Hz, 4H, -NC**H$_2$**-); 2.3 (bs, 4H, -N**H$_2$**); 2.08 (t, J= 7.3 Hz, 4H,-CC**H$_2$**-); 1.20 (t, J= 6.9 Hz, 12H, -OCH_2C**H$_3$**).

^{13}C-NMR (90.56 MHz; CD_3CN): δ= 172.24 (-**C**O, ester); 159.99 (-**C**ONH_2); 65.23 (quaternary carbons); 62.78 (-O**C**H_2CH_3); 42.50 (-N**C**H_2-); 33.96 (-C**C**H_2-); 14.29 (-OCH_2**C**H_3).

1-[(3-Amino-3,3-diethoxycarbonyl)propyl]-2-oxo-4,4-diethoxycarbonyl-1,4,5,6-tetrahydropyrimidine 133.



133

The solution of the diamine compound **132** (80 mg, 0.17 mmol) in nitropropane (2 mL) was heated in an oil bath at 135 ^{o}C for 2 hrs. After cooling the reaction mixture to room temperature, the solvent was removed at reduced pressure and the residue was purified by column chromatography over silica gel (50% ethylacetate/hexane) to get monocyclic urea **133** (20 mg, 27%) as a gummy substance.

133: $C_{19}H_{31}N_3O_9$ (MW 445.5)

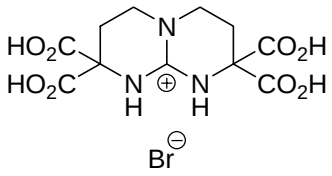
HPLC analysis: R_v= 16 ml, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5µ column, UV_{220}, flow = 1 ml/min, gradient from 10% CH_3OH to 50% CH_3OH in 10 min and then to 90% CH_3OH, in next 10 min more, 0.1% TFA as a buffer.

MS-ESI: *m/z* = 446.2 [$(M+H)^+$, 100%].

^{1}H-NMR (250 MHz; $CDCl_3$): δ=6.4 (s, 1H, -N**H**-); 5.1 (s, 2H, -N**H$_2$**); 4.2-4.3 (m, 8H, -OC**H$_2$**CH_3); 3.7-3.9 (m, 4H, -NC**H$_2$**-); 2.1-2.6 (m, 4H, -CC**H$_2$**-); 1.2-1.3 (m, 12H, -OCH_2C**H$_3$**).

^{13}C-NMR (62.9 MHz; $CDCl_3$): δ= 169.86, (-**C**O, ester); 169.69 (ring -**C**O); 65.29, (quaternary carbons); 62.54 (-O**C**H_2CH_3); 62.50 (-O**C**H_2CH_3); 45.14, 39.30 (-N**C**H_2-); 31.8, 31.16 (-CCH_2-); 13.9 (-OCH_2**C**H_3).

2,2,8,8-Tetracarboxy-3,4,6,7,8,9-hexahydro-2H-pyrimido[1,2-a]pyrimidine Hydrobromide 136.



136

To a solution of tetra-ester **104** (1g, 2 mmol) dissolved in ethanol (16 mL) and cooled to 0 ^{0}C was added the aqueous solution of 4N NaOH (16 mL). The resulting mixture was stirred at room temperature for 24 hrs. The reaction mixture was acidified to pH 1 with 47% HBr solution. Evaporation of solvent in vacuo left a colorless solid residue which was then taken up in isopropanol (15 mL) and stirred vigorously. After stirring for 15 min, the insoluble salt residue was removed by filtration and the filtrate was evaporated in vacuo to obtain a colorless residue, which was recrystallized from acetonitrile/H_2O to afford tetra-acid **136** (700 mg, 88%), as colorless crystalline substance.

136: $C_{11}H_{13}N_3O_8 \cdot HBr$ (MW 396.1)

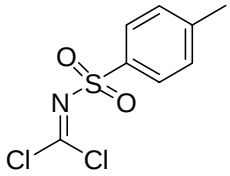
HPLC analysis: R_v= 2 ml, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5µ column, UV_{220}, flow = 1 ml/min, gradient from 10% CH_3OH to 50% CH_3OH in 10 min and then to 90% CH_3OH, in next 10 min more, 0.1% TFA as a buffer.

MS-ESI: *m/z* = 228.3 [$(M\text{-}2xCO_2)^+$, 100%], 316.2 [$(M+H)^+$, 25 %].

^{1}H-NMR (250 MHz, D_2O): δ= 3.5 (t, J= 5.5 Hz, 4H, -NC**H$_2$**-); 2.51 (t, J=5.4 Hz, 4H, -CC**H$_2$**-).

^{13}C-NMR (62.9 MHz; D_2O): δ= 176.01 (**C**O, acid); 152.10 (guanidinium carbon); 67.23 (quaternary carbons); 47.46 (-N**C**H_2-), 29.56 (-C**C**H_2-).

N-Tosylphosgeneimine 137.



137

To a solution of p-toluenesulfonylimidodithiocarbonate **46** (2.75 g, 1 mmol) suspended in CCl_4 (30 mL) under nitrogen and cooled to –10 ^{0}C, was bubbled chlorine gas. The reaction mixture acquires yellowish colour. After stirring for 15 min, the excess of chlorine and solvent were removed by a jet of nitrogen. The solid was sublimed to get bis-chloro **137** (2.13 g, 85%) as a colourless solid.

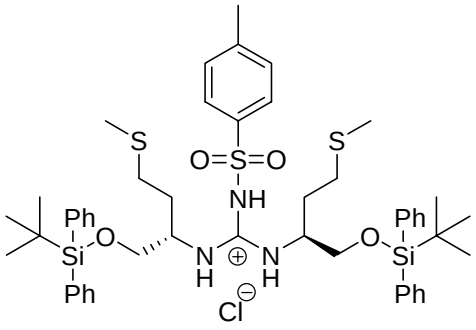
137: $C_8H_7NO_2Cl_2S$ (MW 252.1).

mp: 80 ^{o}C.

^{1}H-NMR (250 MHz; $CDCl_3$): δ= 7.86 (d, J= 8.2 Hz, 2H, aromatic); 7.38 (d, J= 8.2 Hz, 2H, aromatic); 2.45 (s, 3H, -C**H$_3$**).

^{13}C-NMR (62.9 MHz; CD_3Cl): δ= 145.56 (-N=**C**-); 142.30, 135.37, 129.82, 127.81 (aromatic carbons); 21.61(-**C**H_3).

N,N$^{'}$-Bis[(*S*)-1-(tert-butyldiphenylsilyl)oxy-4-(methylthio)butan-2-yl]-N$^{''}$-(4-methylphenyl-sulfonyl)guanidine Hydrochloride 139.



139

To the mixture of amino compound **112** (3.92 g, 10.5 mmol), triethylamine (2.8 mL, 20 mmol) in acetonitrile (25 mL) cooled to 0 ^{o}C was added a solution of **137** (1.2 g, 5 mmol) in acetonitrile

(15 mL) over a period of 15 min. After stirring for 1 hr at room temperature, solvent was evaporated and the residue was taken up in dichloromethane (30 mL). The organic phase washed with water (3x20 mL), dried over magnesium sulfate and concentrated to get a gummy substance which was recrystallized from methanol yielding **139** (3.5 g, 76%) as white crystalline solid.

139: $C_{50}H_{67}N_3O_4S_3Si_2 \cdot HCl$ (MW 962.9).

mp: 127-129 ^oC (methanol).

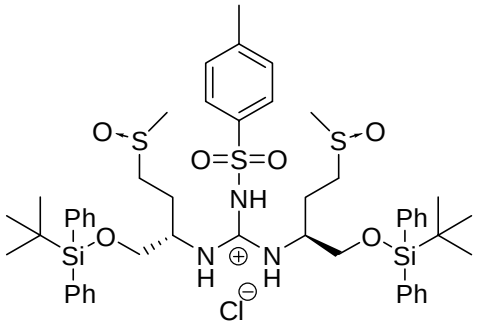
HPLC analysis: R_v= 18 ml, Nucleodure-100-5 C_8 ec column, UV_{254}, flow = 1 ml/min, gradient from 50% CH_3OH to 90% CH_3OH in 5 min and then 90% CH_3OH extended to next 15 min, 0.1% TFA as a buffer.

MS-ESI *m/z* = 926.5 $[(M+H)^+$, 100%].

^{1}H-NMR (360 MHz; $CDCl_3$): δ= 7.75 (d, 2H, J= 8.1 Hz, aromatic); 7.58-7.62 (m, 8H, aromatic);7.34-7.41 (m, 12H, aromatic); 7.11 (d, J= 7.96, 2H, aromatic); 4.25 (m, 1H, -C**H**CH_2-); 3.44-3.62 (m, 5H, -C**H$_2$**OSi-+-C**H**CH_2-); 2.38 (m, 4H, -C**H$_2$**SCH_3); 2.34 (s, 3H, tosyl-C**H$_3$**); 2.0 (s, 6H, -SC**H$_3$**); 1.75-1.88 (m, 4H, -CHC**H$_2$**-); 1.04 (s, 18H, t-butyl-C**H$_3$**).

^{13}C-NMR (90.56 MHz; $CDCl_3$): δ= 154 .50 (guanidinium carbon); 141.45, 140.97, 135.41, 132.53, 129.83, 128.92, 127.74, 125.87 (aromatic carbons); 65.35, 65.02 (-**C**H_2OSi-); 51.76, :(-**C**HCH_2-); 30.78, (-**C**H_2SCH_3); 30.32 (-CH**C**H_2-); 26.72 (t-butyl -**C**H_3); 21.28 (tosyl-**C**H_3); 19.05 (quaternary carbon); 15.18 (-S**C**H_3).

N,N'-Bis[(*S*)-1-(tert-butyldiphenylsilyl)oxy-(methylsulfinyl)butan-2-yl]-N"-4-methylphenylsulfonyl-guanidine Hydroiodide 140.



140

In a thermostated (20 ^oC) electrochemical cell which was divided into two compartments by D4-frit to separate anodic and cathodic solutions was introduced 0.1 M NH_4Br in methanol (10 mL)

as the catholyte and electrolyzed between a mercury cathode and Pt net anode at -1.8 mV against calomel electrode as a reference electrode connected by a salt bridge close to the cathode surface. The solution of 500 mg of tosyl protected guanidinium compound **139** 0.1 M NH_4Br in methanol (1 mL) was added. After 5 hrs, the catholyte was concentrated in vacuo and the residue was extracted with water (10 mL) to remove inorganic salts. The NMR of the remaining solid residue still showed the presence of a tosyl group and the compound was found to be **140** in almost quantitative yield.

140: $C_{50}H_{67}N_3O_6S_3Si_2 \cdot HCl$ (MW 994.9).

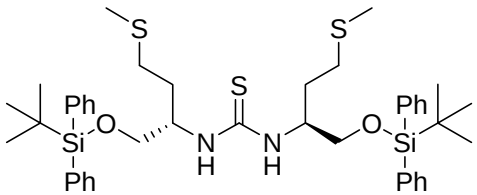
HPLC analysis: R_v= 16 ml, Nucleodure-100-5 C_8 ec column, UV_{254}, flow = 1 ml/min, gradient from 50% CH_3OH to 90% CH_3OH in 5 min and then 90% CH_3OH extended to next 15 min, 0.1% TFA as a buffer.

MS-ESI *m/z* = 958.38 $[(M+H)^+$, 100%].

^{1}H-NMR (250 MHz; $CDCl_3$): δ= 7.00-7.85 (m, 24H, aromatic); 4.28 (m, 1H, -C**H**CH_2-); 3.34-3.82 (m, 5H, -C**H$_2$**OSi-+-C**H**CH_2-); 2.41-3.10 (m, 10H, -C**H$_2$**SCH_3, -SOC**H$_3$**); 2.30 (s, 3H, tosyl-C**H$_3$**); 1.75-2.21 (m, 4H, -CHC**H$_2$**-); 0.90-1.09 (2s, 18H, t-butyl-C**H$_3$**).

^{13}C-NMR (62.9 MHz; $CDCl_3$): δ= 154 .91, 154.48 (guanidinium carbon); 141.47, 141.35, 140.95, 140.85, 135.42, 135.35, 129.82, 128.97, 128.92, 127.72, 125.82 (aromatic carbons); 65.08, 64.76 (-**C**H_2OSi-); 51.81, 51.56 :(-**C**HCH_2-); 47.45 (-**C**H_2SOCH_3); 26.72 (t-butyl -**C**H_3); 25.23, 24.33 (-CH**C**H_2-); 22.74 (tosyl-**C**H_3); 19.06 (quaternary carbon).

N,N'-Bis-[1-(-tert-butyldiphenyl-silyloxy)-4-(methylthio)butan-2-yl]-thiourea. 138.



138

To a solution of amino compound **112** (2.24g, 6 mmol) and triethylamine (0.97 mL, 6 mmol) in dry dichloromethane (15 mL) under nitrogen was added a solution of thiophosgene **47** (0.35 mL, 3 mmol) dropwise and the reaction mixture was stirred at room temperature. After stirring overnight, the solvent was evaporated and the residue was dissolved in dichloromethane (20 mL)

and washed with 0.1 N HCl (15 mL). The organic layer was dried over magnesium sulfate and the evaporation of the organic layer gave thiourea **138** (1.85 g, 78%) as a gummy substance.

138: $C_{43}H_{60}N_2O_2S_3Si_2$ (MW 789.3)

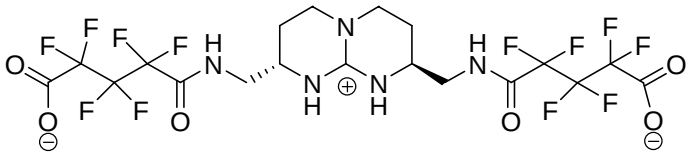
HPLC analysis: R_v = 18 ml , Nucleodure-100-5 CN-RP column, UV_{254}, flow = 1 ml/min, gradient from 10% CH_3OH to 50% CH_3OH in 5 min, then 50% CH_3OH to 90% CH_3OH in next 10 min and 90% CH_3OH extended for next 5 min, 0.1% TFA as a buffer.

MS-ESI: *m/z* = 789.5 $[(M+H)^+$, 100%].

^{1}H-NMR (360 MHz; $CDCl_3$): δ= 7.59-7.64 (m, 8H, aromatic); 7.35-7.41 (m, 12H, aromatic); 6.11 (bs, 2H, -N**H**-); 3.69-3.72.(m, 6H, -C**H$_2$**O-, -C**H**-); 2.44-2.52 (m, 4H, -C**H$_2$**S-); 2.08 (s, 6H, -SC**H$_3$**); 1.92-1.94 (m, 4H, -CHC**H$_2$**-); 1.09 (s, 18H, t-butyl-C**H$_3$**).

^{13}C-NMR (90.56 MHz; $CDCl_3$): δ = 181.27 (-**C**=S); 135.55; 132.79; 129.93; 127.82 (aromatic carbons); 64.93 (-**C**H_2O-); 58.57 (-**C**HCH_2-); 31.31(-**C**H_2SCH_3-); 30.43 (-CH**C**H_2-); 26.90 (t-butyl-**C**H_3); 19.25 (quaternary carbons); 15.38 (-S**C**H_3).

Triethylammonium [2*S*,8*S*]-2,8-bis[(2,2,3,3,4,4-hexafluoro-glutaroylamino)-methyl]-3,4,6,7,8,9-hexahydro-2H-pyrimido[1,2-a]pyrimidinium chloride (141).



141 $Et_3\overset{\oplus}{N}H$

To a well stirred solution of diamine **120** (90 mg, 0.3 mmol) and triethylamine (0.14 mL, 1 mmol) in absolute CH_3CN (2 mL) under nitrogen, cooled to –10 ^{0}C was added a solution of hexafluoroglutaric anhydride **48** (0.25 mL, 2 mmol) in absolute CH_3CN (0.5 mL) dropwise. Then the reaction mixture was stirred overnight at room temperature. The residue obtained after evaporating the solvent under reduced pressure was dissolved in CH_3CN (5 mL), acidified to pH less than 1 using conc. HCl, and concentrated to obtain crude **141**. The purification was further achieved by SPE (30% CH_3CN/H_2O) to yield **141** (125 mg, 62%) as a gummy substance.

141: $C_{25}H_{34}N_6O_6F_{12}$ (MW 742.5)

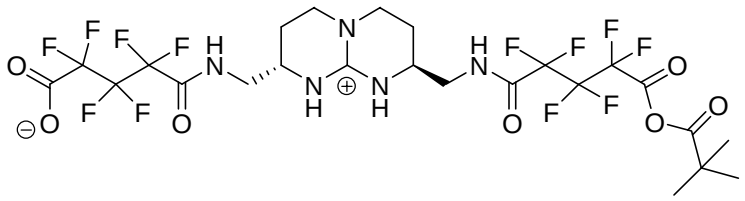
MS-ESI *m/z* = 642.5 $[(M+H)^+$, 100%]; 1283.1 $[(2M+H)^+$, 50 %].

HPLC analysis: R_v= 8.0 ml, Nucleodur-100-5 C_8 ec column, UV_{220}, flow = 1 ml/min, gradient from 10% CH_3CN to 50% CH_3CN in 10 min and then 50% CH_3CN to 90% CH_3CN in next 10 min, 0.1% TFA as a buffer.

^{1}H-NMR (250 MHz; CD_3CN): δ= 8.08 (bs, 2H, guanidinium-**H**); 7.63 (bs, N**H**); 3.10-3.65 (m, 10H, -C**H**CH_2-,-C**H_2**NH-, -NC**H_2**-); 2.92-3.12 (m, triethylammonium-C**H_2**-); 1.95-2.20 (m, 2H, -CHC**H_2**-); 1.61-1.89 (m, 2H, -CHC**H_2**-); 1.23 (t, triethylammonium-C**H_3**).

^{13}C-NMR (62.9 MHz; CD_3CN): δ= 162.95, 162.55, 162.13 (-CO-); 151.9 (guanidinium carbon); 48.92 (-**C**HCH_2-); 47.70 (triethylammonium-**C**H_2-); 45.61 (-**C**H_2NH_2); 44.12 (-N**C**H_2); 24.21 (-CH**C**H_2-); 9.09 (triethylammonium-**C**H_3).

142:



142

To a solution of bis-acid **141** (24 mg, 0.04 mmol) and triethylamine (0.025 mL, 0.16 mmol) in absolute CH_3CN (2 mL) under inert nitrogen atmosphere, was added pivalylchloride (0.04 mL, 0.32 mmol). Then the reaction mixture was stirred at room temperature for 2 hrs. Removal of excess pivalylchloride and solvent under reduced pressure gave **142**.

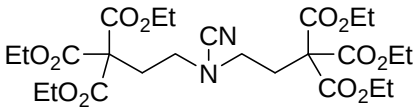
142: $C_{24}H_{27}N_5O_7F_{12}$ (MW 725.5)

MS-ESI *m/z* = 726.4 [$(M+H)^+$, 100%]; 827.2 [$(M+TFA)^+$, 25%].

HPLC analysis: R_v= 10.5. ml, Nucleodure-100-5 C_8 ec column, UV_{220}, flow = 1 ml/min, gradient from 10% CH_3CN to 50% CH_3CN in 10 min and then 50% CH_3CN to 90% CH_3CN in next 10 min, 0.1% TFA as a buffer.

^{13}C-NMR (62.9 MHz; CD_3CN): δ= 180.05, 170.50, 162.55, 162.13 (-CO-); 152.01 (guanidinium carbon); 48.97 (-**C**HCH_2-); 45.65 (-**C**H_2NH_2); 43.92 (-N**C**H_2); 38.85 (quaternary carbon); 27.41 (t-butyl-**C**H_3); 24.29 (-CH**C**H_2-).

N,N-Bis[(3,3,3-triethoxycarbonyl)propyl] cyanamide 57.



57

To a suspension of NaH (2.04 g, 51 mmol) (60 % dispersion in mineral oil) in DMF (30 mL), was added dropwise a solution of diethyl (ethoxycarbonyl)malonate **56** (11.61 g, 50 mmol) in DMF (30 mL). When the evolution of H_2 seized, the reaction mixture was heated in an oil bath at 80°C. Then the solution of bis-iodoethylcyanamide **105** (7 g, 20 mmol) in DMF (15 mL) was added over a period of 30 min using a syringe pump. After stirring overnight the reaction mixture was cooled to room temperature, poured in to ether (300 mL) and filtered to remove inorganic salt. Then the ether layer was washed with water (3x200 mL), dried over magnesium sulfate and evaporation in vacuo left a slightly yellowish solid which was recrystallized from hexane/ether to afford a colorless crystalline compound **57** (10.5 g, 95%).

57: $C_{25}H_{38}N_2O_{12}$ (MW 558.6)

MS-ESI: *m/z* = 559.4 $[(M+H)^+$, 30%]; 581.3 $[(M+Na)^+$, 100%].

HPLC analysis: R_v= 16 ml, Phenomenex, Aqua C_{18}, 250×4.60 mm, 5µ column, UV_{220}, flow = 1 ml/min, gradient from 50% CH_3CN to 90% CH_3CN in 20 min., 0.1% TFA as a buffer.

^{1}H-NMR (360 MHz; $CDCl_3$): δ= 4.28 (q, 12H, J= 7.2 Hz, -OC**H$_2$**CH_3), 3.30-3.27 (m, 4H, -NC**H$_2$**); 2.46-2.42 (m, 4H, -CC**H$_2$**-); 1.30 (t, J= 7.2 Hz, 18H, -OCH_2C**H$_3$**).

^{13}C-NMR (90.56 MHz; CDCl3): δ= 166.27 (-**C**O, ester); 116.82 (-**C**N); 63.90 (quaternary carbons); 62.50 (-O**C**H_2CH_3), 48.09 (-N**C**H_2-), 31.00 (-CCH_2-) 13.81 (-OCH_2**C**H_3).

A general method for NMR titrations:

To labeled (from 1-11) NMR tubes were added aliquots of a solution of host (1mM) in deuterated solvent varying from 500 µl in 1st to 0 µl in 11th NMR tube. From the stock solution of guest (60 mM) in deuterated solvent a standard (3 mM) solution was prepared by using (1mM) solution of host for dilution. Then varying amounts of the guest solution prepared above were added to the NMR tubes to make 500 µl solution in each tube. After 1 hour ^{1}H-NMR spectra ware measured and the changes in chemical shifts were noted. A NMR titration curve was calculated by non-linear regression using Origin-5 software from Origin lab.

A general method for the preparation of guests for complexation with prepared hosts:

The oxoanionic guests were prepared by the titration of the commercially available corresponding acids with either tetrabutylammonim hydroxide or tetraethylammonium hydroxide (40% solution in water) to a pH jump and then the solution was freeze dried to get a residue which was either recrystallized or used as it was for preparing stock solutions for ITC-measurements.

5.3 A general discussion on the ITC Titrations and curve fitting.

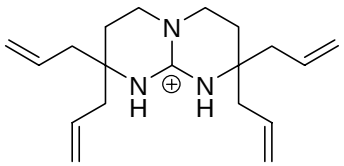
In the present study standard isothermal titration calorimetry (ITC) is used for the complexation studies of prepared hosts **101-103, 125** with the guest anions. The ITC is the powerful analytical tool since in a single run it gives information of thermodynamic parameters, enthalpy, entropy and free energy which are essential to understand the host-guest complex structures. Moreover, it is a very sensitive method such that one can use micro molar concentrations for measurements. There are several important factors that are needs to be taken into account. For instance, the concentrations have to be adjusted such that there is a measurable heat effect yet, it must not saturate the intrinsic temperature difference of the cell pair used for measurement. Once the raw data was recorded, it is fitted to a curve by using predefined models provided by the software. In the present study Origin 5 was used for analysis of the ITC data. The curve fitting can be done using different models like one-site-model, two-site-model, sequential-binding-site model and so

on. In the one-site-binding model it is assumed that all interacting sites have the same strength and will produce the same heat effect. The situation is different when the host has two sets of sites with different affinities, then the model with two sets of sites must be used.

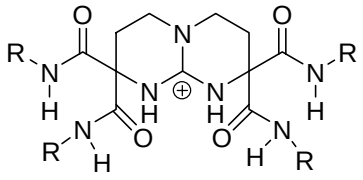
For the proper analysis of the general case it is very important which partner is loaded in the cell and which one in the syringe. Only if the complexation equilibrium is symmetrical as in the 1:1 stoichiometric interaction this condition is relieved. The situation is a little more complicated if the host has more than one site. For instance assume that it has two fairly strong sites with different affinity for the ligand. If the host is contained in the cell and ligand in the syringe, then the stronger will form the complex in the early injections and the weaker of the two in the subsequent injections giving the clear picture of the processes going on during titration. However, if the ligand is loaded in the cell and the host into the syringe, then the situation is different, since the ligand will be in excess in the early injections, so both sites will have the chance to form a complex . This being the case, then all of the ligand will be in the 1:1 complex when the molar ratio reaches 1 and further injections of the host give zero heat effect. So choosing right conditions is important to get the interpretable results. The models discussed above address a scenario involving independent binding sites. In many host guest systems the binding of a ligand to one site will influence an other site. In such cases an interacting-site-model is used to determine the cooperativity between the sites. If the sites are non-identical in the first place, then it is difficult to determine cooperativity by binding studies alone. On the other hand if the sites within a molecule are known to be identical, then it is sometime possible to determine the cooperativity. In any case the number of interacting binding sites must be obtained before and included as a supplementory input for data evaluation in the sequential model.

6. Summary

The present study describes the design, synthesis and complexation of the open chain and macrocyclic hosts for oxoanions based on the bicyclic guanidinium anchor group. The hosts **101-103** were designed by the incorporation of additional hydrogen bonding functions, the secondary carboxyamido groups, to the parent bicyclic guanidinium moiety. These groups are supposed to additionally interact with the guest anion, by means of the array of hydrogen bonds along with the preferential mode of binding to the guanidinium anchor to form a well-structured 1:1 stoichiometry complex. Such a complex formation should lead to selectivity and high affinity towards oxoanions.



79



101 R= n-C_3H_7

102 R= Ph

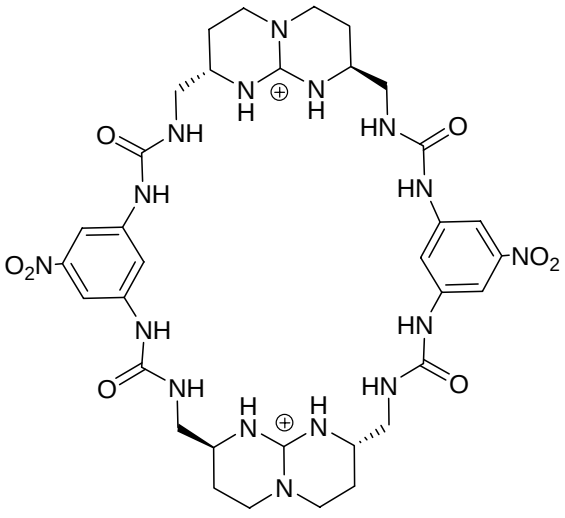
103 R= CH_2CH_2OMe

The novel hosts **101-103** are synthesized by the very efficient synthetic strategy in five steps process giving an overall yield of 70 % from the commercially available starting materials. Isothermal titration calorimetry (ITC) was used to study the association properties of hosts towards oxoanionic guests. Using this technique one can obtain in a single experiment the thermodynamic parameters of association like affinity, stoichiometry of binding, enthalpy and entropy.

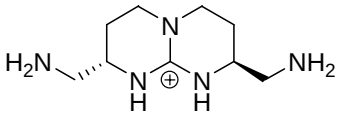
The complexation of hosts **101-103** with various oxoanions (**80-87**) were tested in acetonitrile. The host **101-103** showed high affinity (K_{ass}= 10^5-10^6 M^{-1}) towards a variety of oxoanions, but at the same time indicated the formation of higher order complexes reflecting the fuzziness in the structure of these complexes. The comparison of the binding energetics with that of the known host **79** which lacks carboxyamido functions showed a quite unexpected but important feature. The high affinity of the hosts **101-103** in the series of oxoanions examined arises exclusively from the more positive entropy of association. The enthalpic interactions reflecting the net

attraction of the binding partner was smaller for the hosts **101-103** containing carboxyamido functions. In the conclusion, it was shown qualitatively by correlation of interface area of host and guest with the entropy output that desolvation cannot be the only cause for the observed highly positive entropy of association. The presence of higher order complexes and positive entropies of association support the fact that the observed enhanced affinity in guest binding by hosts **101-103** results from weakened structural definition of the host-guest associated ensembles. The introduction of several hydrogen bonding functions into the parent bicyclic guanidinium core counteracted rather than promoted the formation of well-structured host-guest complexes. These results conveyed the message that in designing receptors not only the mere addition of more functional groups is important but the essential part is the proper placement in the framework that would restrict the binding modes possible to the guest molecule resulting in the unique mode of interaction.

With the purpose of restricting binding modes, that emerged as a conclusion from the binding studies involving hosts **101-103**, the new host **125** was designed in which the two guanidinium moieties are connected by a spacer unit need to form a macrocyclic framework. The urea function was used as connecting bridge aiming at the addition of favorable hydrogen bonding interactions. The known chiral bicyclic guanidinium building block **120** required in the construction of the macrocycle **125** was prepared by a different and much shortened synthetic strategy than the already established one and then used to prepare a novel macrocycle **125.**



125



120

The macrocycle **125** showed high affinity towards various carboxylate dianions (**89-96**) as measured by ITC in acetonitrile. The affinities were in the range of 10^5 to 10^6 M^{-1}. In most cases the complexation was enthalpy-driven reflecting the formation of well-structured complexes. The saturated dicarboxylic guests (**97-100, 145**) showed very small enthalpic interactions.

The macrocycle displayed enantiomeric recognition of the D(-)/ L(+) tartrate dianion (**93-94**) and this was clearly reflected in all of the three energetic parameters of association. The enantiomeric recognition of aspartate dianions (**149, 150**) by macrocycle **125** is significantly reflected in the counteracting entropies of association.

The macrocycle **125** displace high affinity (K_{ass} = 10^4-10^6 M^{-1}) with all of the guest that were probed (**80, 89,95, 98, 152, 153**) in a more polar solvent DMSO. It also showed high affinity to the biologically important ions like ADP^{2-} **151** and folic acid dianion **154.**

The dissection of the free energy of association ΔG into its enthalpy and entropy components in these studies of host-guest bindings to the host **101**-**103** and **125** enables as a prerequisite to understand supramolecular binding principles in solution. As such it lays the foundation for rational design in artificial receptors.

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