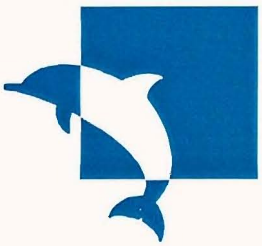

University of Southampton

FACULTY OF ENGINEERING, SCIENCE AND MATHEMATICS
SCHOOL OF CHEMISTRY

RATIONALLY DESIGNED RECEPTORS FOR CARBOXYLATES AND PEPTIDES

PhD Thesis

December 2004

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UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF ENGINEERING, SCIENCE AND MATHEMATICS
SCHOOL OF CHEMISTRY

Doctor of Philosophy

RATIONALLY DESIGNED RECEPTORS FOR CARBOXYLATES AND PEPTIDES

By Sandra Bartoli

This thesis entails the synthesis of a series of guanidinium based macrocycles, open chain analogues and tweezer receptors and their ability to bind amino acid derivatives (particularly glutamate) and peptides. It concerns also the synthesis of a novel protecting group for the guanidinium functionality.

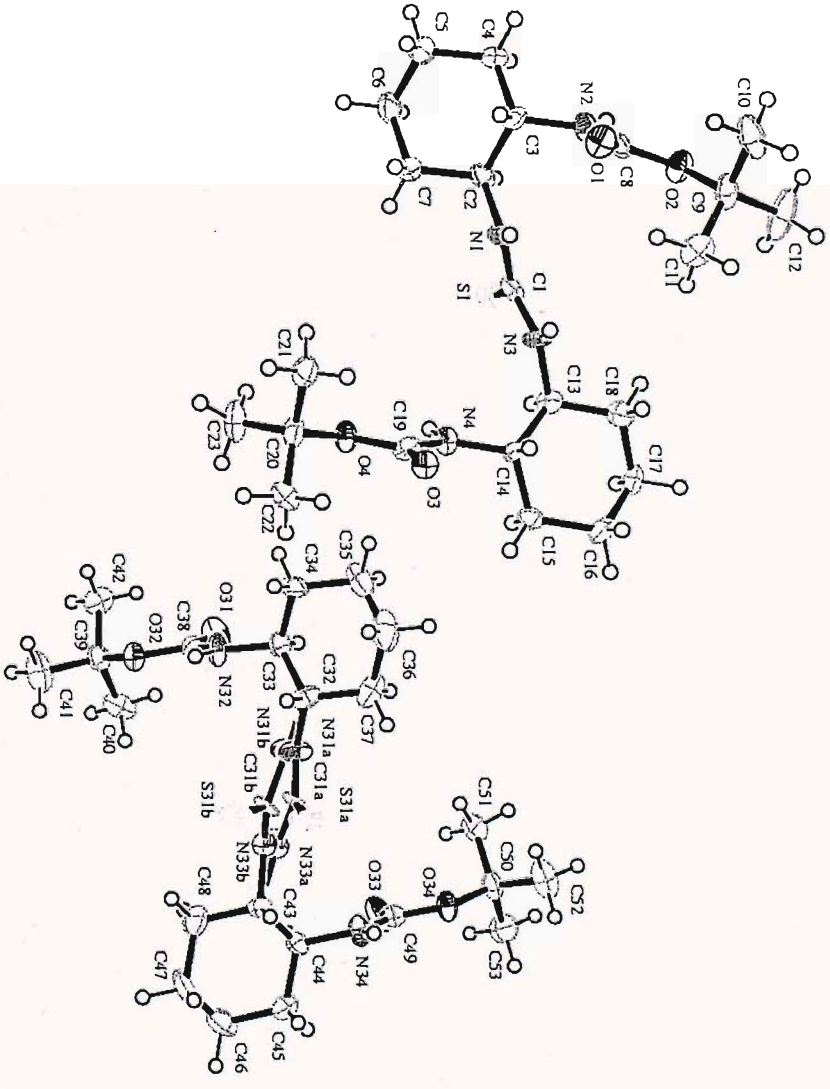
Chapter two discusses the synthesis of a series of bisguanidinium-based macrocyclic receptors and their ability to bind to *N*-Boc protected glutamate. Traditional NMR titration studies and isothermal calorimetry were employed as tools to study the complexation process in DMSO-d_6 and water/DMSO mixtures.

Chapter three discusses the synthesis and binding properties of a series of guanidinium based tweezer receptors. It also concerns the development of a novel protecting group for the guanidinium moiety, the trifluoroacetyl.

Preface

The research described in this thesis was carried out under the supervision of Professor Jeremy D. Kilburn at the University of Southampton between January 2001 and February 2004. No part of this thesis has been previously submitted at this or any other university.

Despite all the efforts to obtain nice crystals of the receptors described in this thesis, I was not able to do it... but... I did obtain the crystal structure of a thiourea I synthesised, which is not reported in this thesis experimental... I thought I could put it here...



Acknowledgements

I wish to thank Prof. Kilburn for being a very good supervisor throughout my PhD. I think I made a great decision when I decided to start my PhD in his group.

Many thanks to Mark Bradley for his help and for correcting my reports.

I am also grateful to Dr. Phil Gale for being my "last minute" advisor and to all the Southampton University staff.

I would also like to thank the Kilburn group, all of it, past and present, for the pleasant days in and outside the lab.. Thanks particularly to Jon and Oscar. Thanks to Tom, Kim, Rossella, Richard, Sara, Andrea, Sarah, Pla, Anawat. Thanks to Nittaya for her kindness and friendship. Thanks to Mariangela, whose help has been fundamental during my first year. Thanks to Suvi for her friendship which resists despite the distance. Thanks to Allison, Suad and Srini, with whom I had the pleasure to spend my final months of PhD.

Thanks to Loredana for being always there and for coming with me to visit so many cool places; thanks to Patrick, Cristophe and Carol for being very nice housemates. Thanks to the Bradleys for being very good neighbours.

Thanks to all the volleyball friends for making my Sundays and Wednesdays really enjoyable.

Thanks to all the others, they are really too many to be mentioned.

Thanks to my family. Without their support and understanding I could not have made it.

Abbreviations

Aloc	Allyloxycarbonyl
Bn	Benzyl
Boc	*tert*-butoxycarbonyl
CBS	Carboxylate Binding Site
Cbz	Benzyloxycarbonyl
CPK	Corey, Pauling and Kultan
δ	Chemical Shift
DBU	Diazabicycloundecene
DCC	*N,N'*-Dicyclohexylcarbodiimide
DCU	Dicyclohexylurea
DCM	Dichloromethane
Ddpe	1-(4,4-dimethyl-2,6-dioxocyclohexylidene)phenylethyl
DIC	Diisopropylcarbodiimide
DIPEA	*N,N'*-Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMSO	Dimethylsulfoxide
DNS	Dansyl
EDC	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
ES^+ MS	Positive electrospray mass spectrometry
ES^- MS	Negative electrospray mass spectrometry
FC	Flash chromatography
Fmoc	9-Fluorenylmethoxycarbonyl
HBTU	2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluroniumhexafluosphate
HOBt	1-Hydroxybenzotriazole
IR	Infra Red
HRMS	High Resolution Mass Spectroscopy
LRMS	Low Resolution Mass Spectroscopy
MeOH	Methanol
m.p.	Melting point
NMR	Nuclear Magnetic Resonance

n.O. e	Nuclear Overhauser effect
PyBOP	Benzotriazol-1-yloxy-tris-pyrrolidinophosphoniumhexafluorophosphate
ppm	parts per milion
r.t.	Room Temperature
THF	Tetrahydrofuran
TFA	Trifluoroacetic acid
TLC	Thin Layer Chromatography

Amino acids

Ala	Alanine
Asn	Asparagine
Asp	Aspartic acid
Gln	Glutamine
Glu	Glutamic acid
Gly	Glycine
His	Histidine
Lys	Lysine
Pro	Proline
Ser	Serine
Thr	Threonine
Trp	Tryptophan
Val	Valine

To my family

Contents

INTRODUCTION

1.1 From molecular to supramolecular chemistry

In 1906 Ehrlich[1] stated that "Corpora non agunt nisi fixata" (molecules do not act if they are not bound). That preannounced the advent of a totally new field, supramolecular chemistry. The term "übermoleküle", i.e. supermolecules, was introduced in the mid-1930's by Wolf and coworkers[2] to describe entities of higher organization resulting from the association of coordinatively saturated species.
Supramolecular chemistry, as it is now defined, is a discipline dating back to the '70s when Lehn introduced the concept of supramolecular chemistry[3] and in 1987, along with Cram and Pedersen, he received the Nobel Prize for chemistry for having given birth to a new, very fertile, area of chemistry. "Supramolecular chemistry is the chemistry of the intermolecular bond, covering the structures and functions of the entities formed by association of two or more chemical species"; it is the "chemistry beyond the molecule".[4]

1.2 Molecular recognition

Molecular recognition, i.e. a specific noncovalent interaction between a host and a guest molecule, is an extremely important and widespread phenomenon throughout all of biochemistry. To name a few obvious examples, the immune system of higher organisms depends on recognition of cell surface proteins to tell invading micro-organisms from the organism's own tissue and molecular recognition of substrates by enzymes enables specific chemical transformations of the substrates to be carried out despite the presence of thousands of other compounds. Furthermore the recognition of specific structures by macromolecules is fundamental to many biochemical processes, such as protein assembly and genetic code construction. Probably the most famous example for supramolecular chemistry in nature is the construction of the entire genetic code in DNA with only four nucleic acids, which always occur in the same distinct base-pairs.[5] The two single strands of DNA are held together by a number of hydrogen bonds, involving acidic hydrogen atoms (hydrogen bonding donor), oxygen atoms (hydrogen bonding acceptor), and nitrogen atoms (hydrogen bonding acceptor) of the

purine and pyrimidine bases, in order to maintain the double helical structure (Figure 1.1a). In this double helix guanine (G) forms triple hydrogen bonds with cytosine (C) and adenine (A) forms double hydrogen bonds with thymine (T) (Figure 1.1b). Guanine selectively interacts with cytosine because the G-C complex is much more stable than G-T complex which would form only one hydrogen bond. Similarly, adenine exclusively complexes with thymine because it would form no hydrogen bonds with cytosine. The X-ray diffraction studies revealed that the hydrogen bonds holding G-C and A-T complexes are about the same length (2.9 ± 0.1 Å).

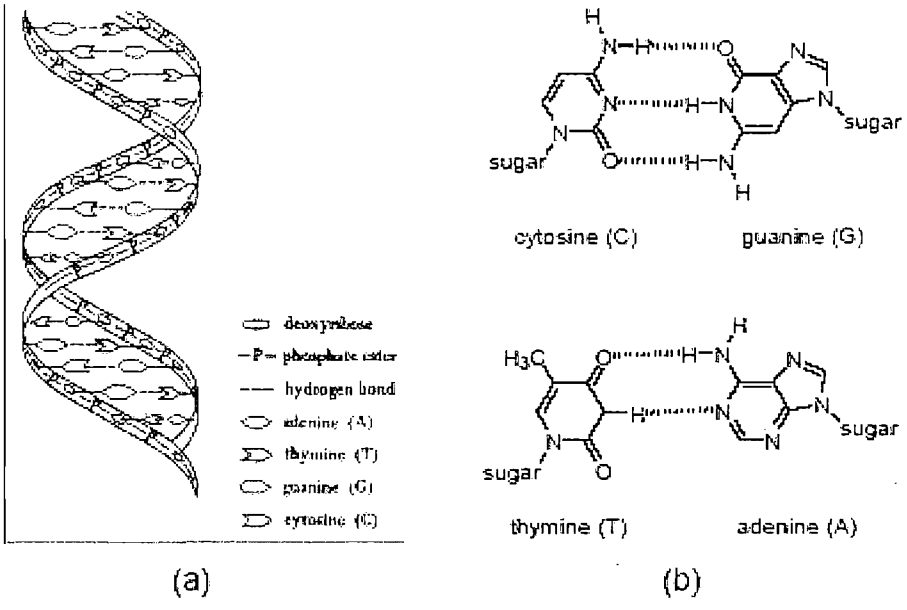


Figure 1.1 *a) Complementary base pairing in DNA helical structure and b) base pairing in DNA (guanine and cytosine form triple hydrogen bonds; adenine and thymine form double hydrogen bonds).*

The emergence of supramolecular chemistry has had a profound effect on how efficiently chemists prepare structures of different sizes and shapes with dimension in the range of 1 to 100 nm using spontaneous secondary noncovalent interactions such as hydrogen-bonding,[6] electrostatic, Van der Waals, hydrophobic, π-stacking, cation-π, which are in fact the principal interactions involved in the formation of molecular complexes. Although these interactions, when considered individually, are weak in nature compared to covalent bonds, the simultaneous action of several of these bonds often leads to very stable complexes. Understanding the basis of these interactions and developing synthetic molecules able to mimic the action of natural compounds with a

high degree of specificity has been a desirable goal since the birth of supramolecular chemistry, that can be traced back to the pioneering work of Charles Pedersen[7].

1.3 Complementarity of receptor and substrate

By the later half of the 19th century, molecular recognition began to emerge, *e.g.* through the van der Waals' studies of interactions between atoms in the gaseous state. In 1894 Fischer[8] presented his famous "lock and key" analogy of the way a substrate interacts with an enzyme. In this prophetic statement, an enzyme, which is large compared to the substrate, has clefts and depressions on its surface complementary to those of the substrate. Thus the substrate fits like a key into the lock of the enzyme active site.

Fischer's principle asserts that there must be steric (receptor and substrate need to have geometrically compatible domains in size and shape) and electronic complementarity (they must interact in attractive manner) between the substrate and the receptor in order to maximise the number of complementary interactions. In fact the complementarity relationship between receptor and substrate is crucial to recognition. All synthetic receptors are based on a similar design, consisting of a cavity, which approximates the size and shape of the potential guest molecule and incorporates some chemical features complementary to those displayed on the guest (Figure 1.2).

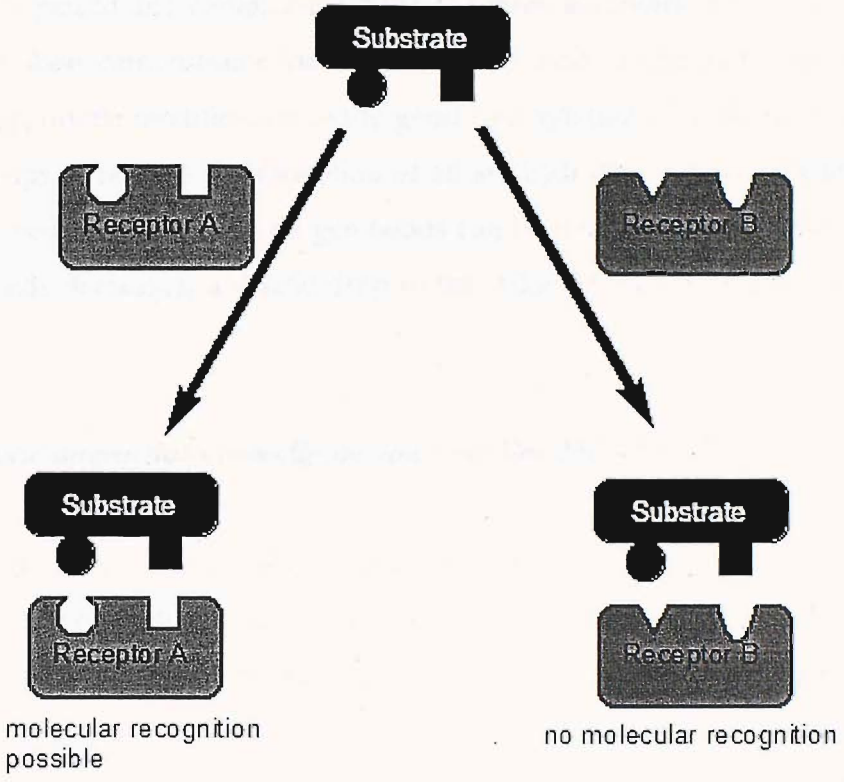


Figure 1.2 *Schematic representation of the complementarity principle*

Nanoscale structures in biological systems are specifically put together from two or more small molecular components by means of secondary interactions. The precision and specificity are indicative of control and directionality displayed by secondary interactions between complementary components in biological systems. Obviously, the challenge is to create synthetically nanostructures with such precision and specificity as seen in biological systems by incorporating complementary recognition sites in the molecular components for secondary interactions. This challenge is only met by first understanding how molecular self-assembly in biological systems operates to generate well-defined aggregates and then transferring the knowledge learned from biological systems to chemical synthesis.

1.4 Nature of supramolecular interactions

The term "noncovalent" encompasses an enormous range of attractive and repulsive forces. Herein, only the most important are explained. When considering a supramolecular system it is vital to consider the interplay of all of these interactions

acceptors ideally placed and complementary to the three acceptors and two donors of the guest. Hamilton demonstrates the importance of each single hydrogen bonding interaction by opportune modification of the guest (methylation of a nitrogen, removal of carbonyl groups to prevent the formation of all six hydrogen bonds). The strongest binding is observed when all six hydrogen bonds can be formed and when the number of hydrogen bonds decreases, a drastic drop in the value of the binding constant was observed.

1.4.2 Electrostatic interactions (ion-dipole, ion-ion; 100-250 kJ mol^{-1})

Electrostatic interactions play an essential role both in biological and synthetic systems, as they allow strong binding. The binding of an ion, such as Na^+, with a polar molecule, such as water, is an example of ion-dipole interaction. A supramolecular analogue is readily apparent in the structures of complexes of alkali metal cations with macrocyclic ethers called crown ethers, in which the oxygen atoms play the same role as the water molecules (Figure 1.4). The oxygen lone pairs are attracted to the cation positive charge.

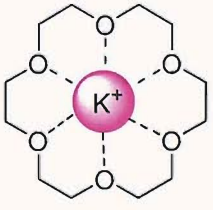


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Figure 1.4 *K^+ complex with [18]crown-6*

1.4.3 π-π Stacking interactions (0-50 kJ mol^{-1})

This weak electrostatic interaction occurs between the π-electrons of different species, most commonly aromatic rings, often in situations where one is relatively electron rich and the other one is electron poor. There are two general types of π-stacking: face-to-face and edge-to-face (Figure 1.5), although a wide variety of intermediate geometries are known.

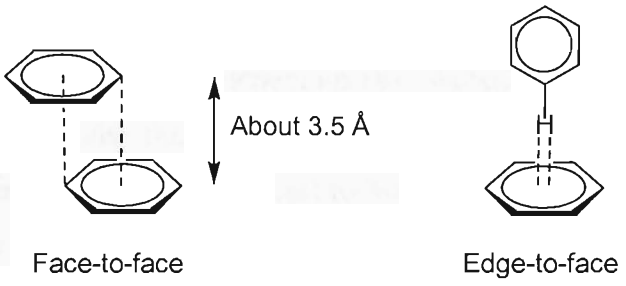

Figure 1.5 *π-π stacking interaction modes*

Face-to-face π-stacking interactions are responsible for the slippery feel of graphite and its useful lubricant properties. Similar π-stacking interactions between aryl rings help stabilising the DNA double helix. Edge-to-face interactions may be regarded as weak forms of hydrogen bonds between the slightly electron deficient hydrogen atoms of one aromatic ring and the electron rich π-cloud of another. Edge-to-face interactions are responsible for the characteristic herringbone packing in the crystal structures of a range of small aromatic hydrocarbons including benzene. There are in literature a few beautiful examples of how π-π stacking interactions can act together with hydrogen bonds to enhance the recognition process. Hunter[11] and co-workers reported an example of a receptor that binds benzoquinone (Figure 1.6).

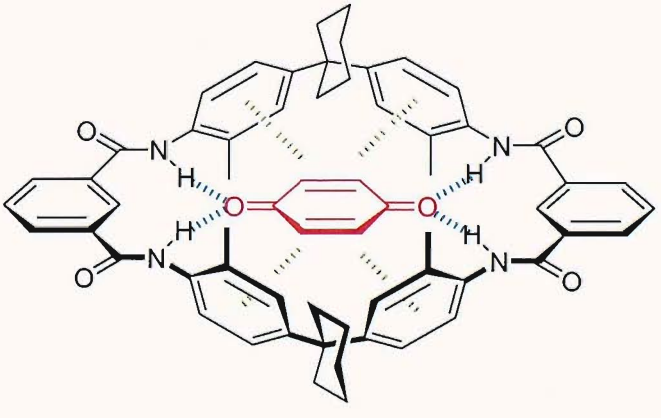

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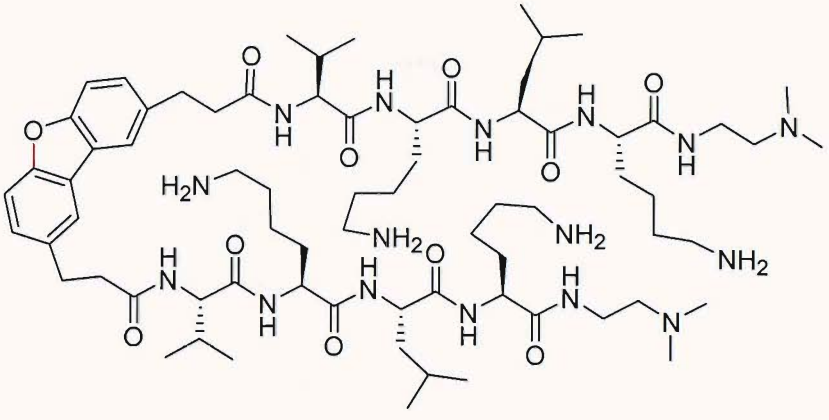
Figure 1.6 *Hunter's benzoquinone receptor*

The π-π stacking interactions do not merely assist in binding: they act cooperatively with the hydrogen bonds to polarise the guest, modifying its electronic properties. The receptor though functions in noncompetitive solvents, such as chloroform, which do not interfere with the recognition process. Another example, this time of an open chain

receptor, is given by Kelly's tweezer receptor **4**.[12] The tweezer structure incorporates a dibenzofuran moiety for additional interactions (π-π stacking) between the receptor and the guest, which provides also the right separation between the two covalently attached peptide strands. This allows a peptide guest to bind between the strands through anti-parallel β-sheet interactions (Figure 1.7).



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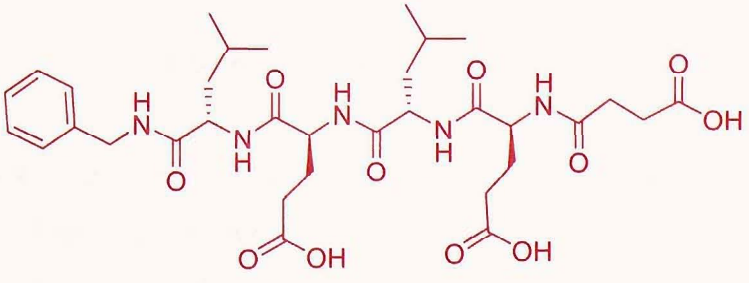
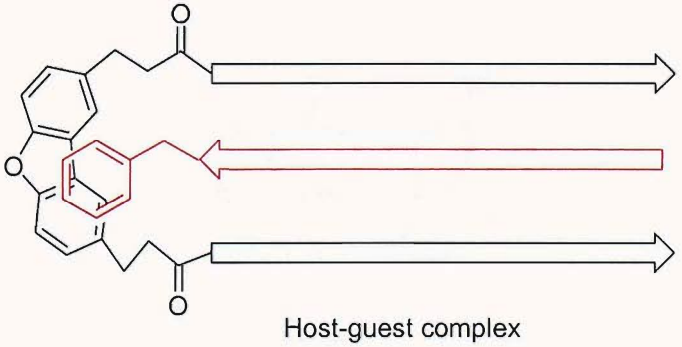


Guest



Host-guest complex

***Figure 1.7** Kelly's tweezer receptor*

1.4.4 Cation- π interactions (5-80 kJ mol^{-1})

The interaction of metallic cations such as RNH_3^+ with π systems[13] (i.e. double bonds or aromatic rings) may be thought as a form of X-H···π hydrogen bond. An example is given in Figure 1.8, where a tetramethylammonium is bound weakly inside the cavity of a cyclophanic receptor only exploiting cation- π interactions.[14]

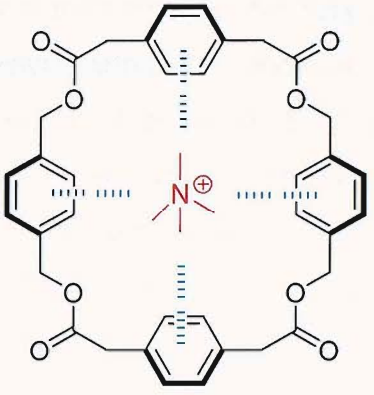


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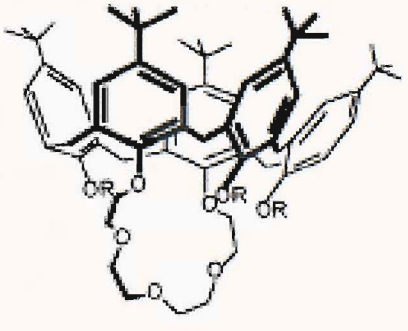
Figure 1.8 *Cation-π interaction*

Recently it was shown by Mandolini[15a] that the cavity of calix[5]-arene **6**, fixed in the cone conformation by the presence of a polyoxyethylene bridge between the phenolic units, is suitable to host a large variety of quats (tetramethylammonium, acetylcholine, N-methylpyridinium salts), with medium to high affinities, by cation-π interactions (Figure 1.9).



6

Figure 1.9 *Mandolini's calixarene*

1.4.5 Van der Waals interactions (<5 kJ mol^{-1})

Van der Waals forces are short range, nondirectional interactions which arise from the polarisation of an electron cloud by the proximity of an adjacent nucleus, resulting in a weak electrostatic interaction. They are believed to provide additional enthalpic stabilisation to the coordination of a hydrophobic guest into a hydrophobic cavity. They are though nondirectional, therefore they possess only limited scope in the design of specific hosts for selective complexation as it is very difficult to take full advantage of them. They provide a general attractive interaction for most "soft" (polarizable) species and they are the forces involved in interactions between the noble gases. In supramolecular chemistry, they are most important in formation of inclusion compounds, in which small, typically organic molecules are loosely incorporated within crystalline lattices or molecular cavities. One example is the inclusion of toluene within the molecular cavity of *p-tert*-butylcalix[4]arene (Figure 1.10).[15b]

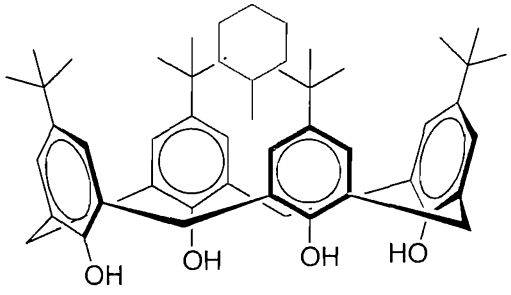


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Figure 1.10 *Inclusion of toluene in the cavity of p-tert-butylcalix[4]arene*

1.4.6 The hydrophobic effect. The role of solvent.

A key feature in the binding process is the role of solvent. The interactions between solvent and guest, and between solvent and host, are often of the same type as those between host and guest. In order to bind a guest a receptor must shed both its own solvation and that of the guest. In other words, if the solvent strongly solvates either host, guest or complex, or if the solvent interacts strongly with itself, this can have significant effect on the binding equilibrium. The host-guest complexation free energy thus represents the enthalpic energy gains resulting from favourable host-guest

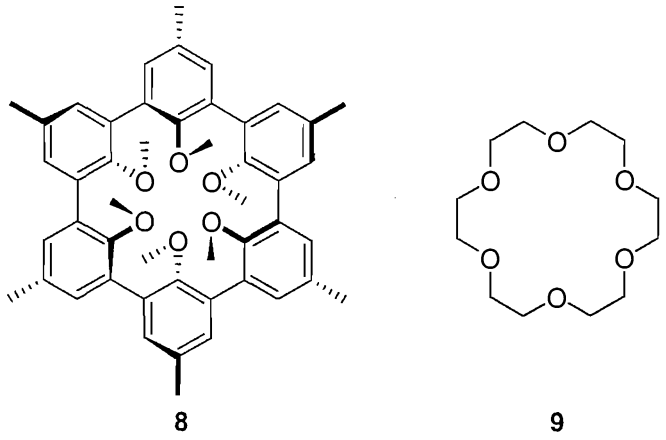
interactions and the entropic gain from release of ordered solvent molecules less the enthalpic loss from the desolvation of host and guest.

The hydrophobic effect is the driving force for the "exclusion" from polar solvents, particularly water, of particles which are weakly solvated. Water molecules ordered around apolar surfaces of a hydrophobic cavity are released upon complexation of a guest and become disordered. The result is a favourable entropic contribution. In addition there is an enthalpic contribution which involves the stabilisation of water molecules that are driven out from a host cavity upon guest binding. Because host cavities are often hydrophobic, intracavity water does not interact strongly with the host walls and is therefore of high energy. Upon release into the bulk solvent it is therefore stabilised by the interaction with other molecules of solvent, giving a favourable enthalpic contribution. Hydrophobic interactions are of crucial importance in the binding of organic guests in aqueous solution by cyclophanes and cyclodextrines, which are naturally occurring macrocycles composed by sugar units.

1.5 Conformational reorganisation and preorganisation

If a host molecule does not undergo significant conformational changes upon guest binding it is said to be preorganised. Host preorganisation[16] represents a major (and in some cases decisive) enhancement to the overall free energy of guest complexation. Neglecting the effects of solvation, the binding process may be divided in two stages. First, the activation stage, in which the host undergoes conformational rearrangement in order to arrange its binding sites in the fashion more complementary to the guest and minimising at the same time unfavourable interactions between different sites on the host. This is energetically unfavourable, and, because the host has to adopt the conformation for the rest of the life of the host-guest complex, it is never paid back. Following readjustment, binding can occur, and that is energetically favourable thank to the enthalpically stabilising attraction between mutually complementary sites on the host and on the guest. The overall free energy of complexation is the difference between the unfavourable reorganisation energy and the favourable binding energy. Therefore if the host is preorganised the rearrangement energy is small and the overall free energy increases.

We can see better the effects of preorganisation considering an example: the comparison between the preorganised spherand **8** and the conformationally mobile crown ether **9** (Figure 1.11).



8 **9**

Figure 1.11 *Spherand and crown ether*

The rigid spherand binds Na^+ and Li^+ much more strongly than the flexible crown ether. In addition the spherand is completely unable to bind cations larger than sodium (better selectivity).

1.6 Anion coordination

In comparison to cation coordination chemistry, the idea of constructing hosts to bind specific negatively charged guests is a more recent development. In the last twenty years, the coordination chemistry of anions by synthetic receptors has become a well-established subset of supramolecular chemistry. There are a number of reasons for this sudden growth in this new area of coordination chemistry. Anions are ubiquitous throughout biological systems. They carry genetic information (DNA is a polyanion containing phosphate esters along its ribose backbone) and the majority of enzyme substrates and co-factors are anionic. A well-known example is carboxypeptidase A (CPA), an enzyme that coordinates to the C-terminal carboxylate group of polypeptides by the formation of an arginine-aspartate salt bridge and catalyses the hydrolysis of this residue. The salt-bridge binding motif is also observed in zinc finger DNA complexes and RNA stem loop-protein interactions (Figure 1.12).

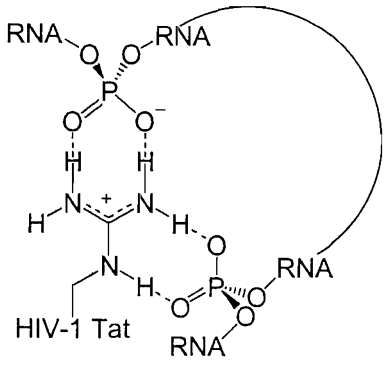


Figure 1.12 *Arginine-fork binding motif in the HIV-1Tat protein*

Anion receptors may be useful for phase-transfer catalysis, separations,[17] and anion selective electrodes. The design of anion receptors is particularly challenging.[18] There are a number or reasons for this. Anions are larger than their isoelectronic cations and therefore have a lower charge to radius ratio. This means that electrostatic binding interactions are less effective than they would be for the smaller cation. Being large they also require receptors of considerably greater size than cations. Additionally, anions like carboxylates, phosphates, sulphates, may be sensitive to pH and exist only in a relatively narrow pH window (becoming protonated at low pH and so losing their negative charge). Thus receptors must function within the pH window of their target anion (for instance receptors based on polyammonium salts may not be fully protonated in the pH region in which the anion is present in the desired form). Anionic species exhibit a wide range of shapes and geometries and therefore a higher degree of design may be required to make receptors complementary to their anionic guest.

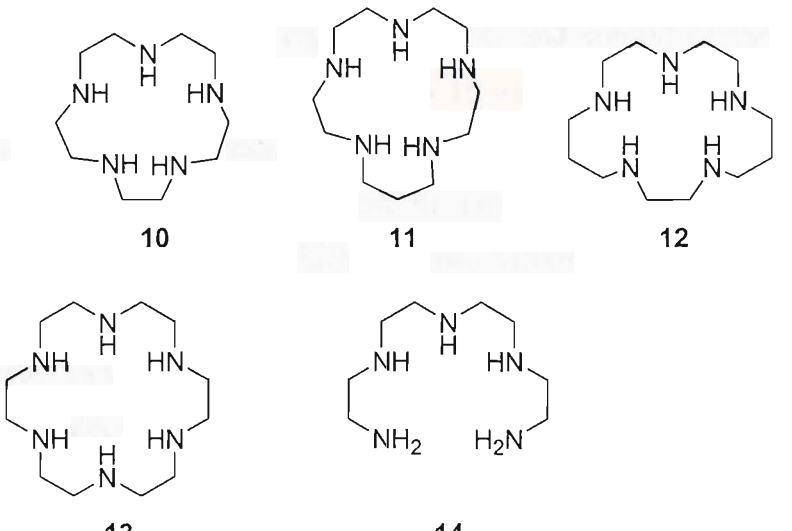
1.7 Synthetic Receptors for carboxylates

To gain a basic understanding of molecular recognition, supramolecular chemists have been interested in synthesising receptor molecules with a high selectivity for a particular guest molecule. This has been one of the driving forces for the design of synthetic receptors, and has resulted in a fairly good understanding of many molecular recognition phenomena. Another reason for the pursuit of new receptors is the potential to use them to construct devices that transform a property of interest, (*e.g.* the concentration of an analyte), to a measurable signal, (*e.g.* an increase in fluorescence). Applications for such synthetic receptors can be imagined in a number of areas, such as medical analysis, detection of toxic compounds in the environment, separation of mixtures of compounds, including enantiomer separation.[19] The crucial role of anionic substrates in biological processes is largely responsible for the recent and rapidly expanding field of anion recognition. Amino acids, and their N-Acyl derivatives, are attractive substrates because of their biological significance and practical importance.[20] Carboxylates in general are a particularly common functional group in biological and synthetic organic molecules and have inspired the development of a number of different approaches for their recognition. Binding sites have been developed both for simple carboxylic acids and for incorporation into sophisticated receptors for more complex carboxylic acid derivatives, both in polar and non-polar solvents.
This introduction on receptors for carboxylates is far from being exhaustive. It is only a selection of examples for each class of receptors.

1.7.1 Charged receptors

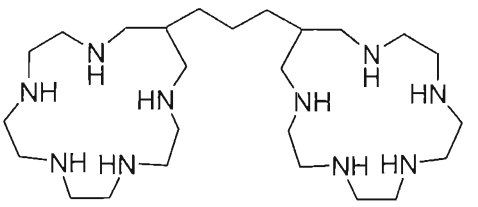
1.7.1.1 Ammonium salts

Cationic hosts capable of forming ion pairs with anions in solution are most easily prepared by protonation of suitable basic compounds. The interaction between a carboxylate and a protonated amine is conceptually the simplest method for binding a carboxylate anion. Kimura and Lehn were among the first to exploit this interaction to create carboxylate receptors. Kimura produced a series of macrocyclic pentamines **10-12** and hexamine **13**.[21]



10 11 12

13 14



15

Guest	$K\ (M^{-1})$					
	10	**11**	**12**	**13**	**14**	**15**
Citrate	55	250	100	24	30	480
Succinate	-	120	92	18	-	-
Malonate	-	66	25	33	-	-
Malate	-	50	26	15	-	-
Maleate	-	76	-	29	-	-
Fumarate	-	-	-	-	-	-

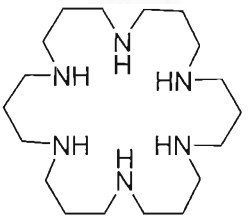
Figure 1.13 *Kimura's polyaza macrocycles*

There is a general observation for macrocycles of type **10-13** in which the nitrogen atoms are separated by only two or three carbon atoms. Protonation of all nitrogen atoms results in severe repulsion between the NH_2^+ groups and dramatically lowers the pK_a values compared to acyclic analogues, such as **14**, which can adopt a linear, all-anti, conformation when fully protonated, to minimise the repulsion between the cationic groups. One way to overcome the problem could be to use longer alkyl spacers between the nitrogen atoms, so that their positive charges do not repel one another so strongly. This stabilises the protonated form of the host and that means that it exists and can bind in less acidic solutions.

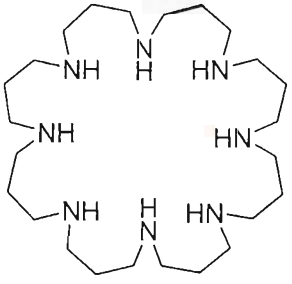
At neutral pH the macrocycles were all triply protonated and formed strong complexes with triscarboxylates such as citrate. The effect of the cycle dimensions on binding was significant. The protonated macrocycles **11–13** bound in fact biscarboxylates with little separation between the two carboxylate moieties in a 1:1 fashion. Biscarboxylates with a larger separation and monocarboxylates were instead not bound. In contrast the protonated acyclic pentamine **14** was a poor receptor and only bound citrate,[21,22]

probably because of its higher flexibility and conformational freedom. Kimura also prepared bis(polyazacrowns) such as **15** which, when quadruply protonated, formed a presumed sandwich complex with citrate.[23]

In order to avoid the problem of increasing difficulty in fully protonating the macrocycles like **10-14**, which have two carbon bridges between the nitrogen atoms, a number of alternatives have been prepared. Lehn synthesised larger polyaza macrocycles **16-18** *via* stepwise alkylation, reduction and subsequent ring closure of appropriately substituted tosylamides.[24]



16



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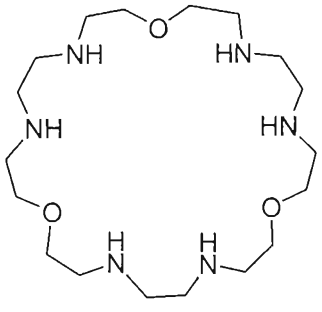


18

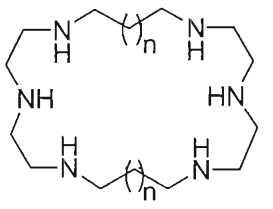
Guest	K (M^{-1})		
	16-6H$^+$	**17-8H$^+$**	**18-6H$^+$**
Oxalate	$6.3 \cdot 10^3$	$6.3 \cdot 10^3$	$50 \cdot 10^3$
Malonate	$2.0 \cdot 10^3$	$5.0 \cdot 10^3$	$6.3 \cdot 10^3$
Succinate	$2.5 \cdot 10^2$	$7.9 \cdot 10^3$	$6.3 \cdot 10^2$
Tartrate	$3.2 \cdot 10^2$	-	$7.9 \cdot 10^2$
Maleate	$5.0 \cdot 10^3$	$12.5 \cdot 10^3$	$10 \cdot 10^3$
Fumarate	$1.6 \cdot 10^2$	$7.9 \cdot 10^2$	$4.0 \cdot 10^2$
Citrate	$50 \cdot 10^3$	$40 \cdot 10^6$	$6.3 \cdot 10^5$
1,3,5-benzene-trycarboxylate	$3.2 \cdot 10^3$	$1.3 \cdot 10^6$	$6.3 \cdot 10^3$

***Figure 1.14** Lehn's polyaza macrocycles*

Macrocycles **16** and **17** allow protonated nitrogens to separate further (propyl spacers), and **18**, which is less conformationally flexible, uses ether groups to space out the positive charges. This strategy was successful, as the fully protonated species of **16-18** all exhibit pK_a values above 7. All three fully protonated compounds **16**-6H$^+$, **17**-8H$^+$ and **18**-6H$^+$ formed strong complexes with organic polyanions in water, but no complexation of monoanions was observed.

As with Kimura's receptors, electrostatic interactions were found to play a major role in both the strength and selectivity of anion binding. Thus the anions most strongly complexed were usually the smallest, with binding selectivity for biscarboxylates: oxalate > malonate > succinate > maleate > fumarate, although large polyanions such as citrate and 1,3,5-benzenetriscarboxylate formed very strong complexes with the large and highly charged **17**-$8H^+$.

The binding sites of polyaza macrocycles can also be sterically tuned. The ability of monocyclic azacrown type hosts to recognise anions on a size and shape fit basis has been investigated further by the preparation of receptors **19-21** which have two individual binding sites (the 1,4,7-triazaheptane moiety) separated by various-length alkyl chain spacers.[25]



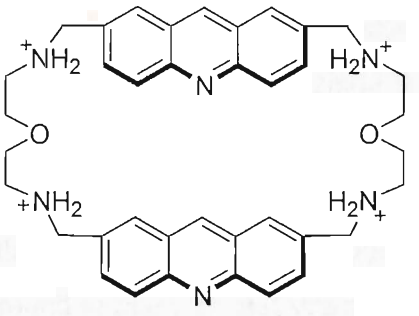
19 n= 3, **20** n = 7, **21** n = 10

	$logK_a$	
m	**20**	**21**
1	3.8	4.05
2	4.3	3.15
3	4.4	3.3
4	3.2	3.2
5	3.1	4.4
6		4.25
7		3.6
8		3.5

Figure 1.15 *Lehn's further macrocycles*

In neutral aqueous solution macrocycles **20** and **21** exist as hexaprotonated cations. The fully protonated forms of hexaaza macrocycles **20** and **21** were found to complex biscarboxylate substrates in H_2O. The strongest binding was observed with the dicarboxylate anions whose alkyl chain length fits better in the cavity. In fact it can be noted that receptor **20** (n = 7) binds shorter chain dicarboxyates (m = 2, 3) more strongly, while receptor **21** (n = 10) preferentially binds those with longer alkyl chains (m = 5, 6).

A modification of receptors **20-21**, incorporating two planar subunits, instead of the more flexible alkyl chains, and two oxygens in place of the nitrogens, is the acridine derived receptor **22** (Figure 1.16).



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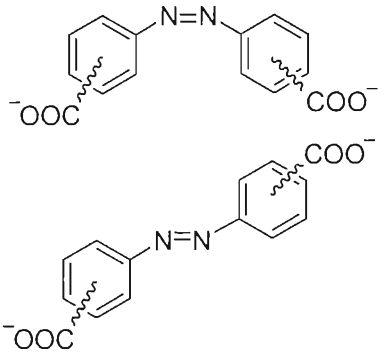


azobenzene dicarboxylates

Guest	Log K_a
trans-3,3'-azobenzene dicarboxylate	5.4
cis-3,3'-azobenzene dicarboxylate	4.0
trans-2,2'-azobenzene dicarboxylate	3.1
cis-2,2'-azobenzene dicarboxylate	2.7

Figure 1.16 *Lehn's acridine receptor*

Receptor **22** exploits, together with strong electrostatic interactions, additional π-stacking ones and it displays very pronounced selectivity with respect to both the pattern of substitution and the *cis* and *trans* configuration of the guest in D_2O.[26]

1.7.1.2 Guanidinium based receptors

Guanidinium cation has become a very popular motif in the design of carboxylate binding receptors. Its popularity is mostly due to the fact that it is part of the arginine residues in naturally occurring binding hosts (*i.e.* active site of carboxypeptidase A) and it is also involved in the stabilisation of protein tertiary structure *via* internal salt bridges with carboxylate functions. The guanidinium cation is in fact very useful as it is stabilized by resonance and charge delocalisation. A feature which makes the guanidinium moiety an attractive anchor group in artificial receptors[27] is the extremely high basicity of guanidine (pK_a = 13.6), which makes the guanidinium cation approximately three orders of magnitude more stable than a protonated secondary amine (pk_a = 10.5). Guanidinium remains protonated up to high pH values; it is

therefore ideal for extending the pH range over which anion receptors operate. In fact in biological systems, the arginine amino acid side chain is extensively used for anion binding. Initial studies by Lehn and co-workers[28] showed that anion recognition did indeed occur over a wide pH range. The binding of carboxylate salts combines an electrostatic interaction with a bidentate hydrogen-bonding pattern (Figure 1.17), a structural motif that can be found in many crystal structures of enzyme complexes with oxoanionic substrates as well as in simple guanidinium salts.[29]

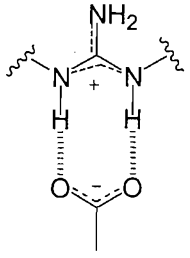
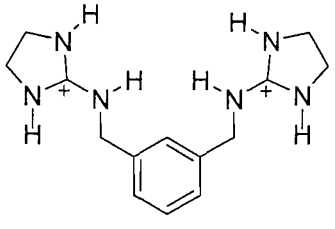


Figure 1.17 *Guanidinium-carboxylate binding pattern*

Herein a few examples are described, with particular attention to bisguanidinium hosts. In 1993, simple bisguanidinium compound **23** (Figure 1.18) was found to bind strongly to the dicarboxylate fumarate ($K > 10^4$ in DMSO).[30]



23

Figure 1.18 *Bisguanidinium receptor*

Improved complexation can be expected from more preorganised hosts.[31] Enhanced preorganisation was employed by Hamilton in the design of **24** (Figure 1.19). The receptor contains two guanidinium groups separated by 4-5 Å by a rigid bicyclo[3.3.0]octane spacer. He used a rigid scaffold to orient the two guanidinium groups for interaction with two carboxylates in a spatially fixed arrangement. The 16-

mer peptides with two aspartate groups located at different positions along the chain were tested for binding to **24**, and quite strong binding in methanol/water was found. Moreover, a noticeable preference for binding to the peptide with three amino acids between the aspartates was observed, indicating that the peptide most likely formed a helical secondary structure (Figure 1.19), theory which was also supported by CD measurements.

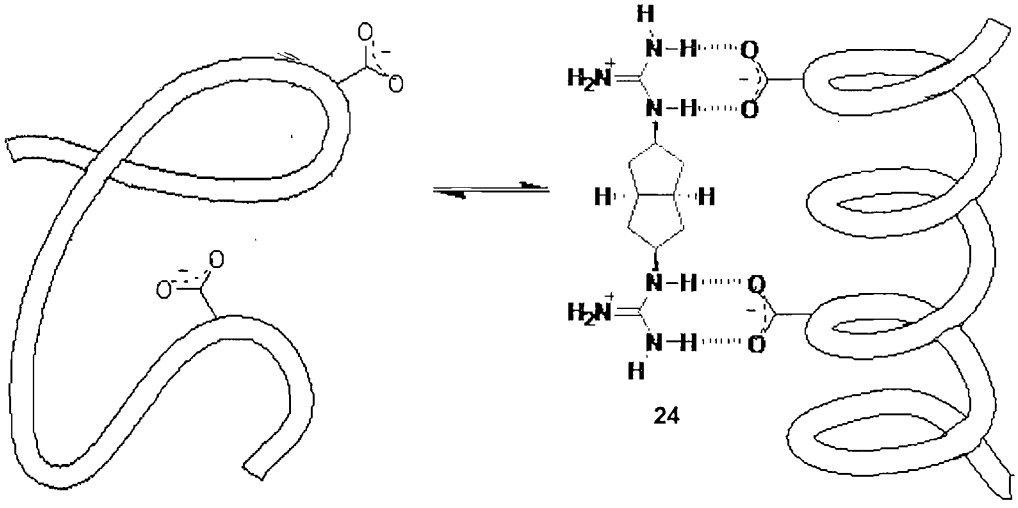
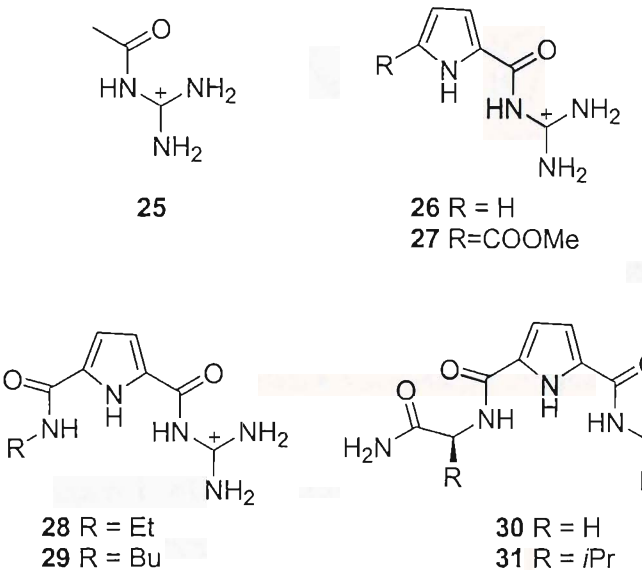


Figure 1.19 *Hamilton's receptor*

Schmuck prepared a series of guanidiniocarboxycarbonyl pyrrole receptors (**25-31**) which bind carboxylates by ion pairing in combination with multiple hydrogen bonds (Figure 1.20).[32]



HOST	K_a (M^{-1})
25	50
26	130
27	940
28	770
29	690
30	680
31	1610
Guanidinium chloride	<10

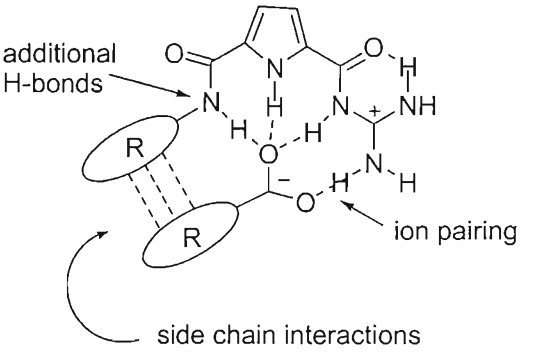


Figure 1.20 *Schmuck's first generation receptors with schematic binding pattern*

The receptors were shown to bind various carboxylates in 40% water/DMSO 30 times more strongly than the simple guanidinium chloride, but with modest selectivity. The results shown in the table were obtained with Ac-L-AlaO$^-$.

Very recently[33] Schmuck published some preliminary results on a new generation of receptors which seems to promise a better selectivity (Figure 1.21).

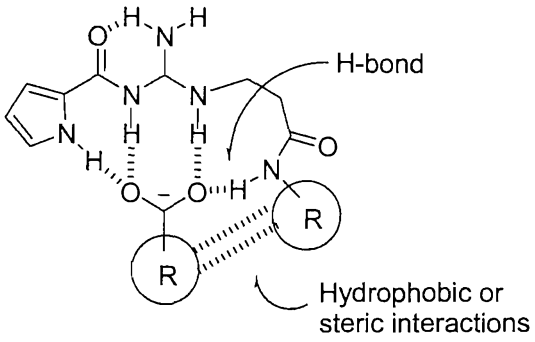
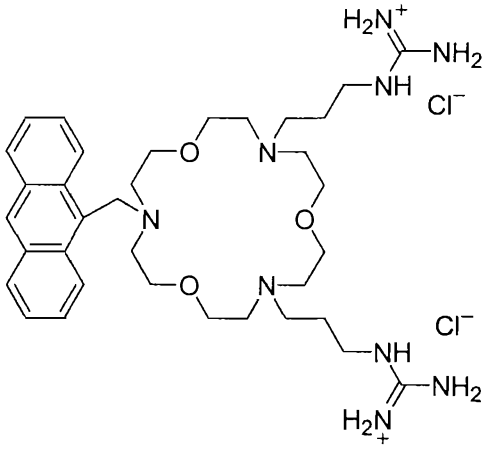


Figure 1.21 *Schmuck's second generation receptors (schematic representation)*

An example of a practical application of the guanidinium moiety, is given by a recent work published by Suzuki *et al.*[34] who developed fluororeceptor **32** (Figure 1.22), based on triaza-18-crown-6 ether combined with two guanidinium groups, for binding of zwitterionic amino acids in aqueous methanol solution.



32

Figure 1.22 *Suzuki's fluororeceptor for switterionic amino acids*

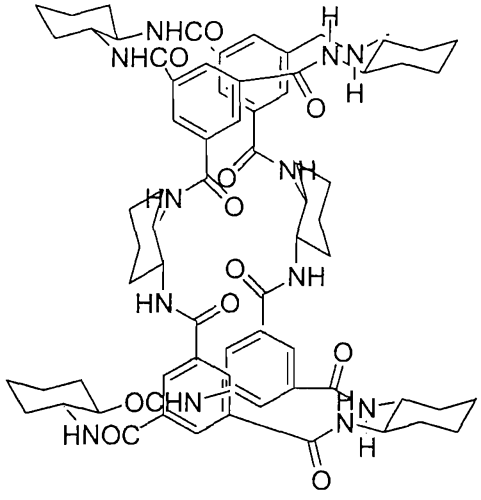
1.7.2 Neutral receptors

Ammonium salts described in the previous section possess a formal positive charge, therefore they bind carboxylates through the formation of nondirectional electrostatic interactions (although they can also form hydrogen bonds). Despite the strong binding by charged hosts, there are two disadvantages in the use of cations as complexing agents. First of all, the nondirectional nature of electrostatic interactions allows all anions to be bound with some strength, and that reduces anion selectivity dramatically.

The selectivity achieved in some cases, must be the result of some other additional interactions (i.e. hydrogen bonds), which anyway only contribute in small part to the overall binding and can be swamped by the electrostatic forces. Also, being positively charged, cationic hosts must have a counterion. This counterion has to be opportunely selected in order not to interfere with the binding (large counteranion with very small charge to radius ratio, as PF_6^-, are usually the best choice).
This problems have brought about significant research on neutral anion binding hosts.[35]

1.7.2.1 Amides

Still *et al.* showed that even relatively small receptor molecules could bind short peptide sequences with remarkable selectivity in $CDCl_3$. In 1993[36] they developed synthetic receptor **33**, whose binding selectivity properties approached those of biological receptors (Figure 1.23).

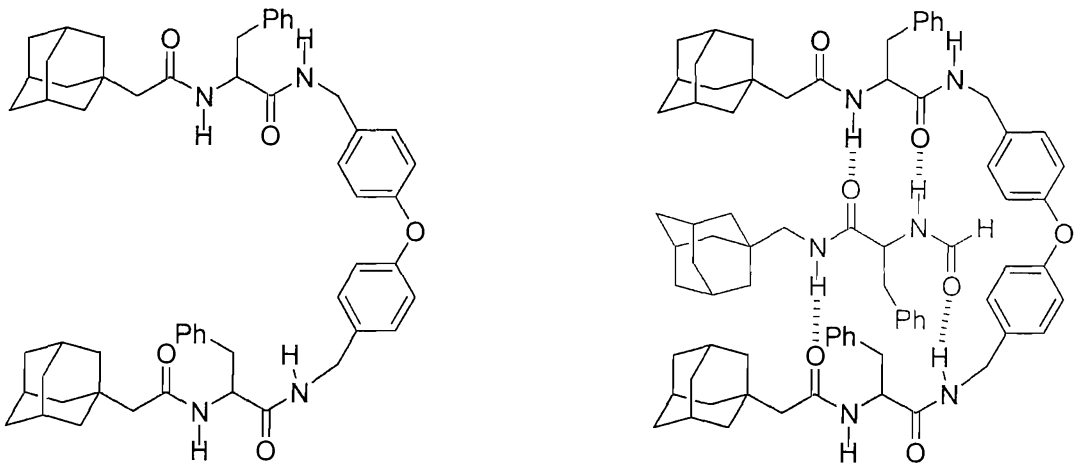


33

***Figure 1.23** Still's receptor for small peptides*

This molecule could recognise dipeptides, and even a tripeptide sequence was tightly bound in $CDCl_3$. In most of the cases (except when the side chains of the aminoacids forming the guest were too bulky) enantioselectivities of > 99% ee were observed, with the L amino acids being more strongly bound.
Amide NHs though can also be used as hydrogen bonding donors to bind carboxylates. The stability of bisamide-biscarboxylate complexes has been studied in detail by

Schneider.[37] Using computer aided molecular modelling Schneider synthesised host **34**, composed by two peptide strands coupled *via* the *para* positions to a diphenyl ether spacer (Figure 1.24). Such receptor could bind a single strand peptide in the fashion of an antiparallel β-sheet, *e.g.* with two hydrogen bonds per amino acid at each side of the guest molecule.

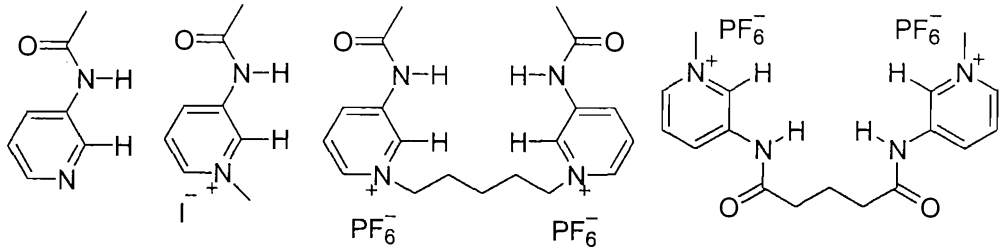


34

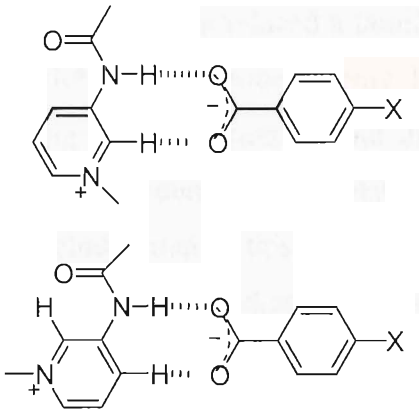
Figure 1.24 *Schneider's receptor and antiparallel β-sheet structure of the host-guest complex*

Modification of the biscarboxylate spacer and the length of the alkyl spacer in the bisamide allowed a study of the effect of the number of single bonds on complex stability (n = 2 - 8, K_a = 0.3 - 120 M^{-1} in $CDCl_3$).

Very simple amide based receptors **35-38** have been described by Jeong.[38]



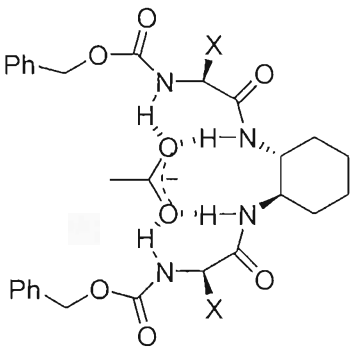
35 **36** **37** **38**



Host	Guest	K_a (M^{-1})
35	benzoate	16
36	benzoate	300
	p-nitrobenzoate	110
	p-chlorobenzoate	205
	p-dimethylaminobenzoate	430
37	adipate	2170
38	adipate	3090

Figure 1.25 *Jeong's receptors. Possible binding modes for* ***36*** *and association constants in DMSO-d_6 for* ***35*** *and* ***36****, and in 10 % D_2O/DMSO-d_6 for* ***37*** *and* ***38***

3-Acetylaminopyridine **35** was found to bind weakly to TBA-benzoate. Methylation of **35** gave the pyridinium salt **36** which bound TBA-benzoate significantly more strongly and downfield shifts of the pyridinium protons H^o and H^p ($\Delta\delta_{max}$ = 0.3 - 0.6 ppm) suggest that binding of the carboxylate involves hydrogen bonding to both the NH and one of the pyridinium CH's (Figure 1.25). Alkylation of the pyridine increases the hydrogen bond donor ability of these protons as well as providing electrostatic complementarity. Bispyridinium salts **37** and **38** were also prepared and bound the biscarboxylate adipate quite strongly in a polar solvent mixture.



39 X = H
40 X = CH_2OH
41 X = CH_3

Host	Log K_a
39	2.1×10^2
40	2.7×10^5
41	3.4×10^2

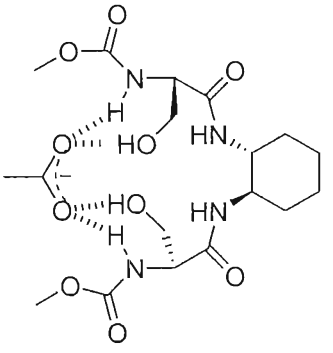
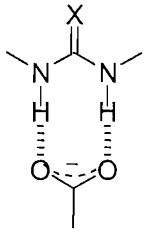


Figure 1.26 *Hamilton's amide receptors and table of association constants for TBA acetate in CD_3CN*

Hamilton also produced a family of structurally simple amide derivatives derived from cyclohexane diamine (Figure 1.26).[39] Tetra amide **41** bound TBA-acetate in CD_3CN using a combination of four amide donor hydrogen bonds. The serine derivative **40** however bound TBA-acetate significantly more strongly in CD_3CN and it was concluded that in this case two of the amide NH's and the two serine OH's provide a tighter binding pocket for the carboxylate.

1.7.2.2 Thioureas

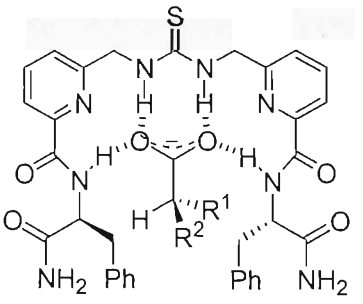
Thiourea derivatives have been proven particularly useful in the construction of neutral hydrogen bonding receptors.[40]



X = O pK_a = 26.9
X = S pK_a = 21.0

***Figure 1.27** Hydrogen-bonding motif*

The relatively acidic thiourea NH protons[41], with a strong hydrogen-bond donor capability, can establish multipoint hydrogen bonded patterns with complementary acceptor groups in a specific and predictable manner. Moreover, the diffusiveness of the electronic charge in the lone pairs of sulfur, leads to the thiocarbonyl group being a weak hydrogen-bonding acceptor, unlikely to interfere in conformational or complexing studies involving other stronger acceptor centers.[42] In general, thiourea derivatives show stronger binding than the corresponding ureas,[30] indeed because of their higher acidity. For instance, Umezawa *et al.* found a significant increase of binding when using thiourea groups with acidity enhancing substituents.[43] In some cases though, weaker anion binding of thiourea-based receptors has been observed, due to the competing intramolecular hydrogen bonding of thiourea groups.[44]
Kilburn has recently described the enantioselective binding of *N*-protected amino acids by a pyridyl thiourea receptor.[45]



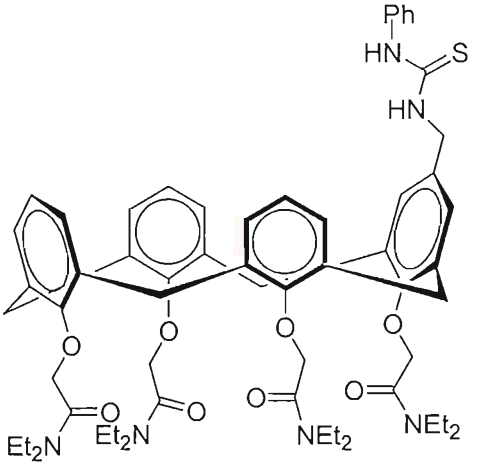
42

Guest	$K_a(M^{-1})$
N-Ac-L-Gln-COOTBA	9000
N-Ac-D-Gln-COOTBA	4520
N-Ac-L-Trp-COOTBA	12400
N-Ac-D-Trp-COOTBA	14800

Figure 1.28 *Kilburn's open-chain thiourea receptor. Binding constants in $CDCl_3$*

Receptor **42** was titrated with a range of amino acid carboxylates (tetrabutylammonium salts) and exhibited some selectivity particularly for amino acids with electron rich aromatic side chains *e.g, N*-Ac-D-Trp in $CDCl_3$ (Figure 1.28). It was modestly enantioselective with a general preference for L-amino acids *e.g.* for *N*-Ac-Gln-CO_2^- (K_a^L:K_a^D ~ 2:1).

Ungaro has used calixarene supported thiourea to create ditopic receptors like **43** (Figure 1.29), capable of recognition of a carboxylate at the upper rim of the calixarene and binding a counterion in the cation binding pocket appended to the lower rim.[46]

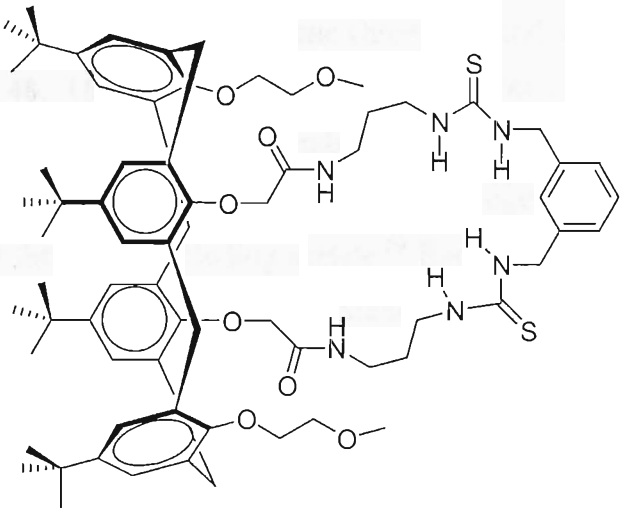


43

Figure 1.29 *Ungaro's calixarene receptor*

Solid-liquid extraction of stoichiometric amounts of sodium acetate with receptor **43** in $CDCl_3$ revealed that the ^1H NMR signals for both the thiourea protons and protons α to the amide groups were substantially shifted, indicating the ditopic nature of the complexation. The binding of carboxylates by related tetrathiourea calix[4]arenes has

also been reported.[47] Very recently a calix[4]arene derived ditopic receptor has been reported (Figure 1.30).[48]

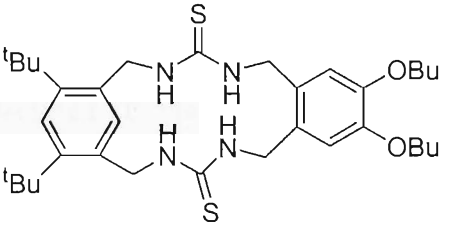


44

Figure 1.30 *Ditopic calixarene-based receptor*

In the absence of Na^+ the receptor binds acetate in preference to diphenyl phosphate (as the TBA salt), but in the presence of Na^+, the selectivity is reversed and the receptor, instead, binds diphenyl phosphate, and not acetate, which preferentially forms a salt ion-pair in free solution.

Sasaki *et al.*[49] reported the synthesis of thiourea derivatives having three different types of cyclophane structure, ortho-meta, **45**, meta-meta, **46**, and a lariat type thiourea, **47** (Fiure 1.31).



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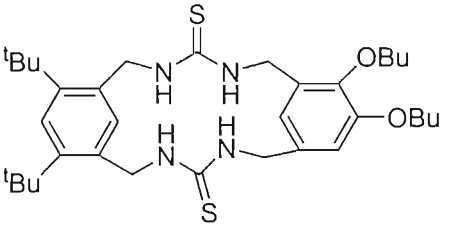

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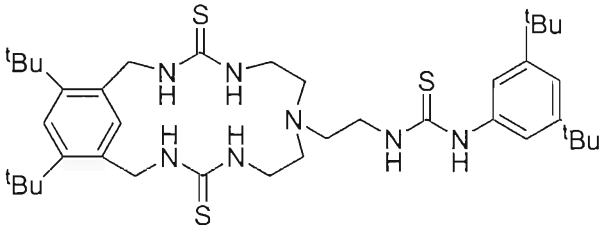

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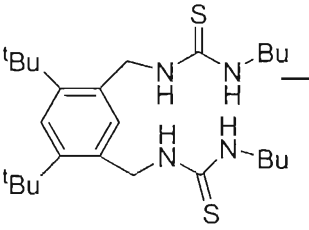


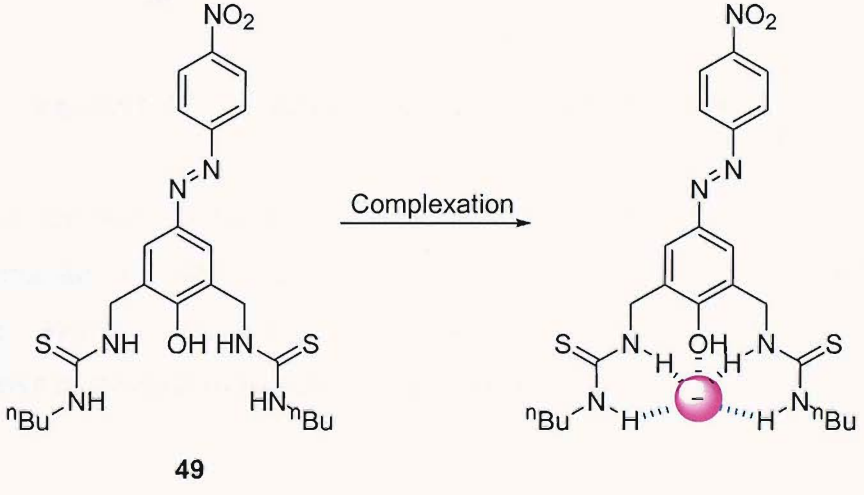
48

Host	$K_a(M^{-1})$
45	2200
46	390
47	8300
48	110

Figure 1.31 *Sasaki's thiourea-based cyclophanes. Association constants for acetate in DMSO-d_6.*

The macrocyclic framework should provide the preorganisation of the binding sites, therefore increasing the binding. All the cyclic thioureas bind acetate more strongly than acyclic thiourea **48**. The lariat-type receptor **47** exhibit even stronger acetate-binding ability than the simple cyclic compounds.

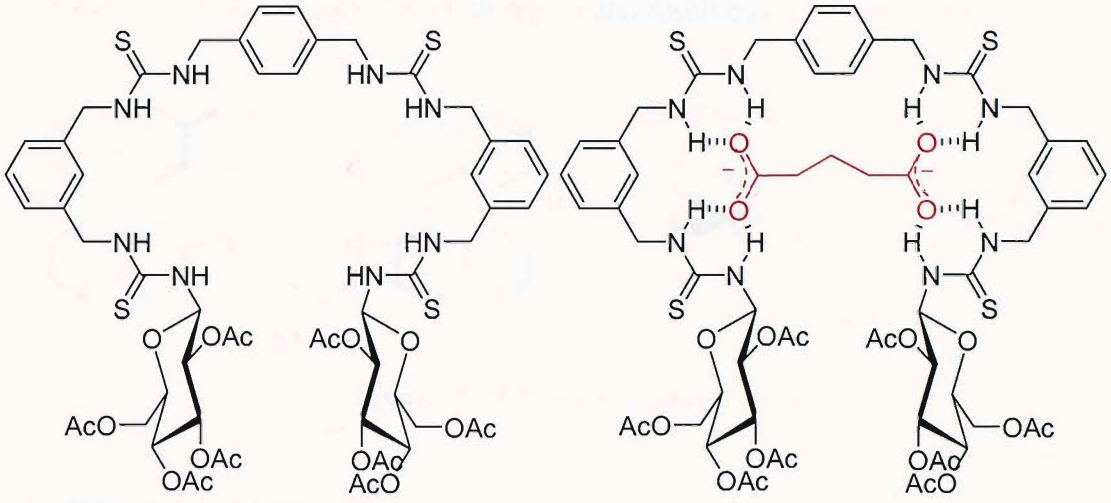
Hong has used a bisthiourea binding site to create chromogenic azophenol-thiourea-based anion sensor **49** for anions including acetate.[50] Receptor **49** bound TBA-acetate (K_a = 1.9 x 10^4 M^{-1}) and binding led to a pronounced red shift in the UV spectrum (Figure 1.32).



49

Figure 1.32 *Hong's thiourea-based anion sensor*

Finally, a series of carbohydrate derived multiple thiourea receptors, like **50** have been described for binding of biscarboxylates (Figure 1.33).[51]



50

Figure 1.33 *Carbohydrate-based multiple thiourea receptors*

The sugar moieties allowed conformational control of the receptor: **50** was effective in binding glutarate in DMSO-d_6, with a variety of different binding stoichiometries including 1:1 stoichiometry as with complex shown, which were found to be dependent on the relative disposition of recognition elements in the host molecule.

1.8 Enantioselective recognition

Enantioselective recognition, i.e. the ability of a receptor to discriminate between enantiomers of a molecule, is a particularly important, yet difficult task facing supramolecular chemists.[52] Many biologically and medically relevant molecules exist as enantiomers; as a result there is a great deal of interest in the exploitation of chiral supramolecular compounds as enzyme mimics as well as in abiotic chiral catalysis, which might find application in both the synthesis and the separation of pharmaceuticals. Enantioselective phase transfer is particularly attractive, raising the possibility of 'catalytic' resolutions based on the transport of substrates through otherwise impermeable barriers.[53] Designing a receptor for that purpose is highly demanding: it needs to be chiral and enantiomerically pure and also to display peculiar features, perfectly complementary to the ones of the guest. Hydrogen bonds are ideal interactions for this kind of recognition, in virtue of their high directionability and controllability.

As early as 1978, Peacock[54] *et al.* designed and resolved chiral corand **51** (Figure 1.34).

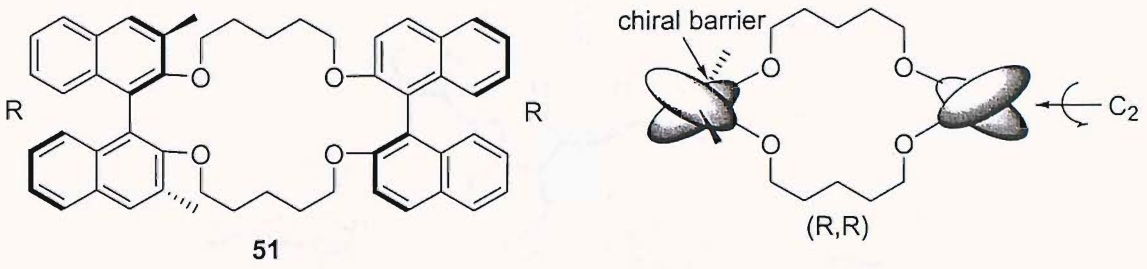


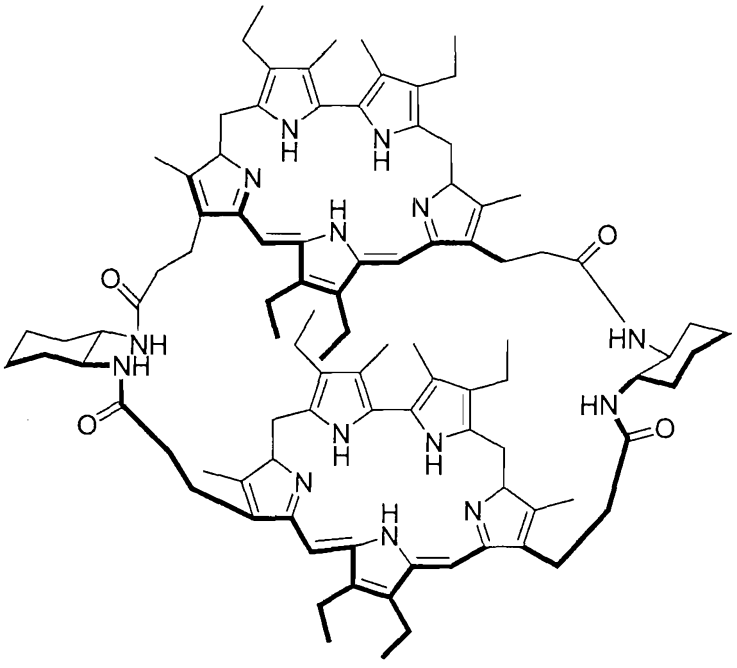
Figure 1.34 *Peacock's chiral corand*

Even though compound **51** does not possess any specific asymmetric carbon atoms, the relative orientaton of the two binaphtyl units gives chirality to the molecule. In fact the presence of the two methyl substituents prevents rotation around the bonds which connect the two naphtalene moieties on the left hand side of the binding site, determining a twisted conformation and a chiral barrier (Figure 1.34). The receptor has therefore a C_2 axis, which makes both binding faces equivalent. This means that enantioselective recognition must take place regardless of which side of the host the guest binds. The discrimination properties were tested utilising two phase liquid-liquid extraction experiments of racemic mixtures of amino acid and ester guests, resulting in selective extraction of the D-enantiomers in every case.

From 1978 onwards the field of chiral recognition has developed and progressed but it is still a very challenging and open area of research. Herein we will describe a few recent examples from many groups all over the world.

1.8.1 Enantioselective binding of carboxylates and dicarboxylates

Sessler and coworkers[55] developed a sapphyrin-based receptor for binding of dicarboxylates, such as Cbz-glutamate and Cbz-aspartate (Figure 1.35).

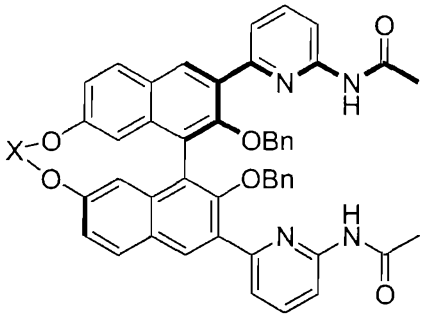


52

Figure 1.35 *Sessler's sapphyrin-based receptor*

The receptor interacts weakly in competitive solvents (methanol), but forms a strong 1:1 complex with both guests in less competitive solvents (dichloromethane containing 5 % methanol). It shows also excellent chiral discrimination ($-\Delta\Delta G° = 0.84$ kcal mol^{-1} for a pair of glutamate enantiomers).

Diederich has synthesised a highly enantioselective amidopyridine receptor, also for Cbz-aspartate, based around a 1,1-binaphthalene scaffold (Figure 1.36).[56]

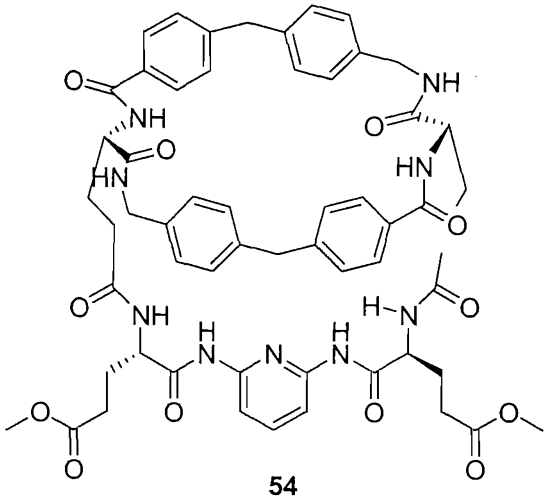


53

Figure 1.36 *Diederich's amidopyridine receptor*

Receptor **53** was found to bind aspartate with good enantioselectivity when the binaphthalene groups were locked in an appropriate conformation by a spacer X. The highest enantioselectivity was observed when X = $-(CH_2)_2-N(Me)-(CH_2)_2-$ with a 15-fold higher binding constant observed for Cbz-L-aspartate (K_a = 8.7 x 10^4 M^{-1}) over Cbz-D-aspartate (K_a = 5.6 x 10^3 M^{-1}) in $CDCl_3$.

Kilburn[57] also exploited the amidopirydine moiety potentiality for binding carboxylic acids in a series of receptors (one example is shown in Figure 1.37) featuring a specific binding site (diamidopyridine) for the carboxylic acid terminus of peptide guests at the base of a bowl shaped cavity.[58]

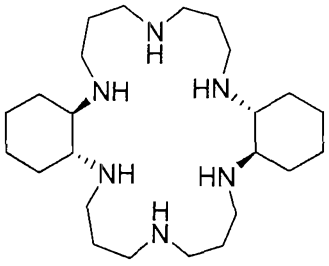


54

***Figure 1.37** Kilburn's bowl-shaped receptor*

Receptor **54** was found to be a sequence selective receptor for *N*-protected dipeptides with a free carboxylic acid terminus in non-competitive media ($CDCl_3$). **54** bound Cbz-L-Ala-L-Ala-OH (K_a = 3.3 x 10^4 M^{-1}) with ~ 8:1 selectivity over Cbz-D-Ala-D-Ala-OH (K_a = 4.5 x 10^3 M^{-1}).

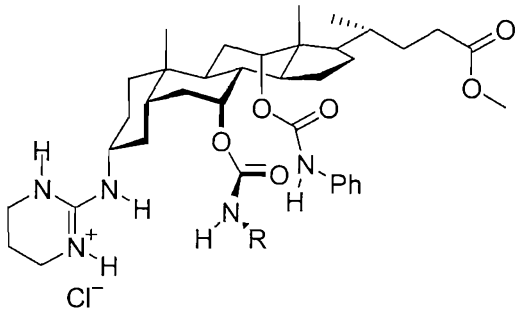
Gotor[59] has reported that incorporation of *trans*-cyclohexane-1,2-diamines into tetraaza macrocycles, such as **55**, gives receptors with enantioselective binding properties for tartrate, maleate and aspartate derivatives (Figure 1.38).



55

Figure 1.38 *Gotor's azamacrocycle*

Davis[60] recently reported the synthesis of a family of receptors derived from cholic acid, bearing guanidinium, carbamate and other functional groups for extraction of chiral carboxylate anions from aqueous buffer into chloroform with significant enantioselectivities. Two of those are shown in Figure 1.39.

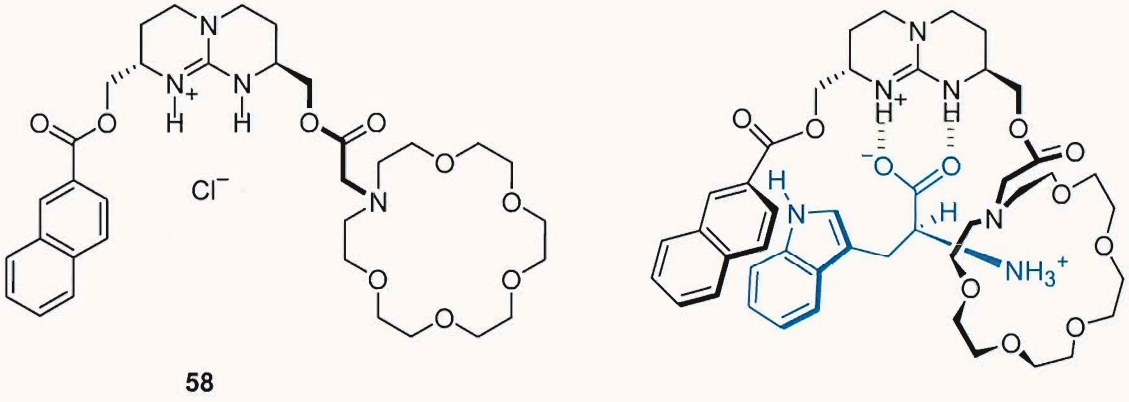


56 R = Ph
57 R = 2,6-dichlorophenyl

Figure 1.39 *Davis' steroidal guanidine*

Receptor **56** proved remarkably consistent in its ability to differentiate between enantiomers of the *N*-Ac-α-amino acids (L:D = 7:1) in all cases, irrespective of side-chain bulk. Receptor **57** showed generally higher extraction abilities (74-93 mol%) possibly due to the greater acidity of the dichlorophenylcarbamoyl NH, and was more sensitive to side-chain structure with L:D selectivites between 5:2 and 9:1, the greatest selectivity being observed with phenylalanine and methionine side chains. Perhaps surprisingly, the substrate with the most sterically hindered asymmetric centre *N*-Ac-*tert*-leucine gave the lowest selectivity. ^{1}H NMR spectroscopy and molecular modelling both suggested plausible models for the binding geometries.

De Mendoza described in 1992[61] a very clever piece of molecular design, receptor **58**, for enantioselective recognition of "zwitterionic" aminoacids under neutral conditions (Figure 1.40). That was the first exploitation of the chirality of bicyclic guanidines for enantiomeric discrimination.



58

Figure 1.40 *De Mendoza's receptor for zwitterionic amino acids*

The receptor incorporates non-self-complementary binding sites for carboxylate (guanidinium) and ammonium (crown ether), an aromatic planar surface for additional π-π stacking interactions with the side chain of aromatic aminoacids, and a chiral structure for enantioselective recognition. Molecular modelling studies indicate that the guanidinium group contributes about half the total binding energy, with a further third from the binding of the NH_3^+ group by the crown ether and the remainder from π-π stacking interactions.

The affinity of **58** toward amino acids was again determined by liquid-liquid single-extraction experiments: the receptor was completely selective for the L enantiomers and it showed high selectivity for amino acids with aromatic side chains, such as phenylalanine and tryptophan.

1.9 Aims of the project

The principal aim of this project is the development of receptors for amino acids and peptides (as carboxylates), exploiting the guanidinium moiety, extensively discussed above, covering also enantioselective recognition. The stability constants for each host-guest complex will be measured using ^{1}H NMR titrations and, in some cases, by

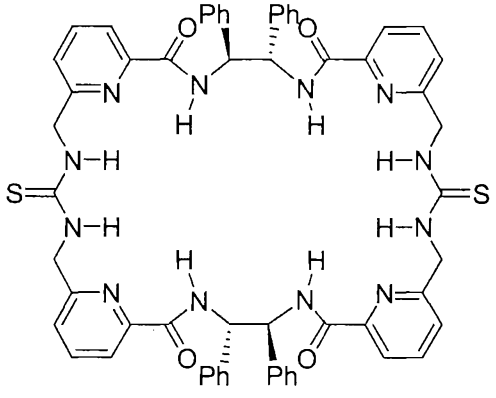
isothermal calorimetry. Due to the large variety of subjects treated, each chapter will have a brief independent introduction.

CHAPTER II

2.1 Introduction and background

Building on results obtained for tweezer **32**, described in the introduction of this thesis, Kilburn synthesised a bisthiourea macrocyclic receptor for enantioselective binding of dicarboxylates.[62] Receptor **59** features two thiourea moieties flanked by carboxypyridines and separated by a chiral diamine.

As the detection of glutamic acid in biological systems is of considerable interest to biologists, the receptor was specifically designed to produce a chiral pocket for enantioselective binding of *N*-Boc-glutamate through an array of eight hydrogen bonds with the pyridine unit to help preorganise[63] the receptor, as shown in figure 2.1.



59

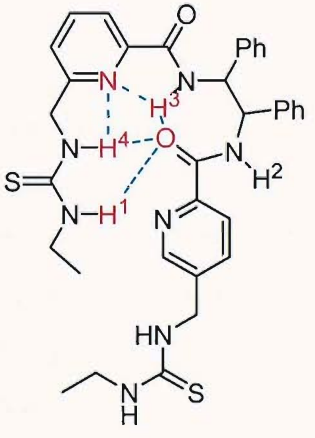
Figure 2.1 *Bisthiourea receptor for binding of Boc glutamate*

Binding studies with chiral bisthiourea receptor **59** and a range of amino acid dicarboxylates were carried out in different solvents, both by isothermal calorimetry and NMR titration (Table 2.1).

Solvent	Guest	$K_{1:1}$ (M^{-1})	$K_{1:2}$ (M^{-2})
CD_3CN	Boc-L-Glu	2.8×10^4	
	Boc-D-Glu	40	4.9×10^4
DMSO-d_6	Boc-L-Glu	2.3×10^3	
	Boc-D-Glu		m.e.
CD_3CN	Boc-L-Asp	2.1×10^5	
	Boc-D-Asp		2.0×10^5
DMSO-d_6	Boc-L-Asp	1.2×10^3	
	Boc-D-Asp		75

Table 2.1 *Association constants for receptor* ***59****; m.e. = multiple equilibria*

Binding measurements indicate that macrocycle **59** exhibits high 1:1 enantioselective binding of *N*-Boc-L-GluOTBA in relatively polar solvents (CH_3CN, DMSO) and that complexation involves a large favourable entropic contribution. Remarkably no binding was observed in chloroform, a less polar, less demanding solvent. This may be attributed to the wrapped conformation adopted by the macrocycle in chloroform, which involves multiple intramolecular hydrogen-bonding interactions (Figure 2.2).



NH^1 δ = 9.0 ppm NH^3 δ = 10.1 ppm
NH^4 δ = 8.1 ppm NH^2 δ = 7.9 ppm

Figure 2.2 *Conformation of macrocycle* ***59*** *in $CDCl_3$ as established using torsion angle dynamics with NOE and scalar coupling constant constraints*

2.2 Bisguanidinium receptors for dicarboxylates

A guanidinium version of macrocyclic bisthiourea **59** was envisaged (Figure 2.3). The guanidinium moiety was chosen to replace the thiourea and provide the primary binding interaction with the carboxylate guest, as, in theory, it should be able to interact more strongly with carboxylates in polar solvents.

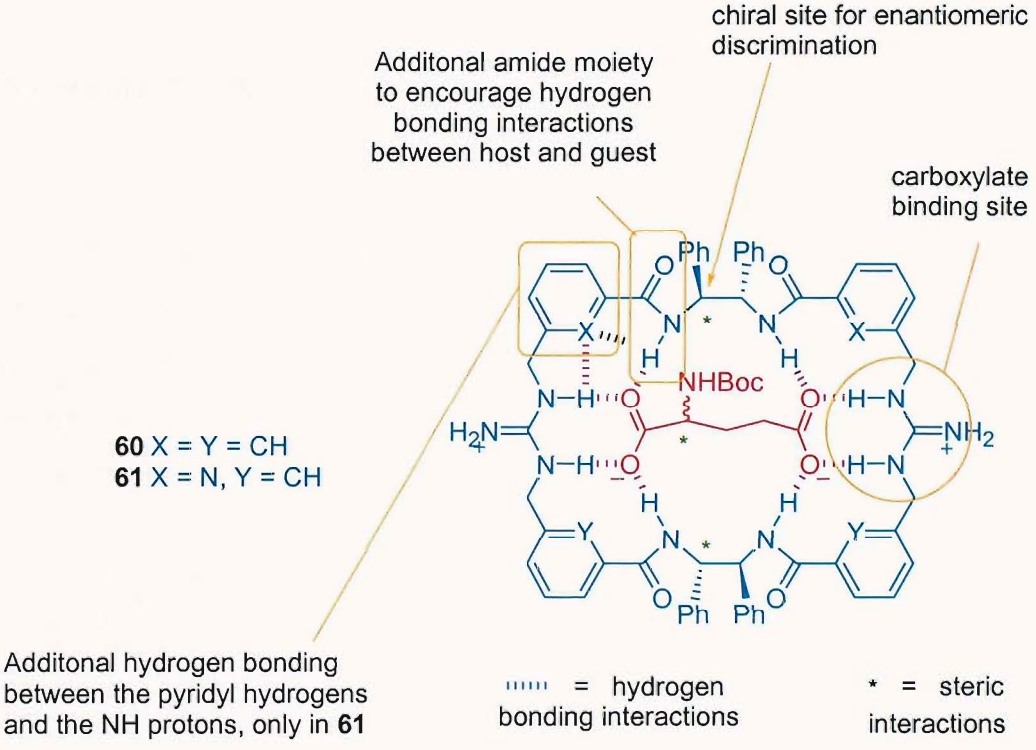
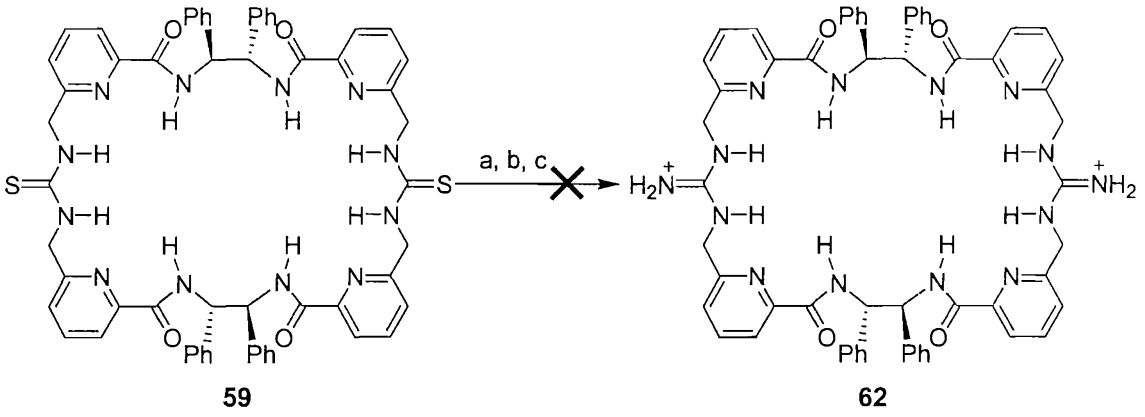


Figure 2.3 *Design concept of guanidinium-based macrocyclic receptor for glutamate*

The amide hydrogens were also intended to act as hydrogen-bonding donors to the anti lone pairs of the carboxylate guest oxygens. To encourage chiral recognition, sterically demanding chiral groups were incorporated in the structure of the receptor to interact with the amino acid Boc functionality.

2.2.1 Synthesis of bisguanidinium macrocycles 60 and 61

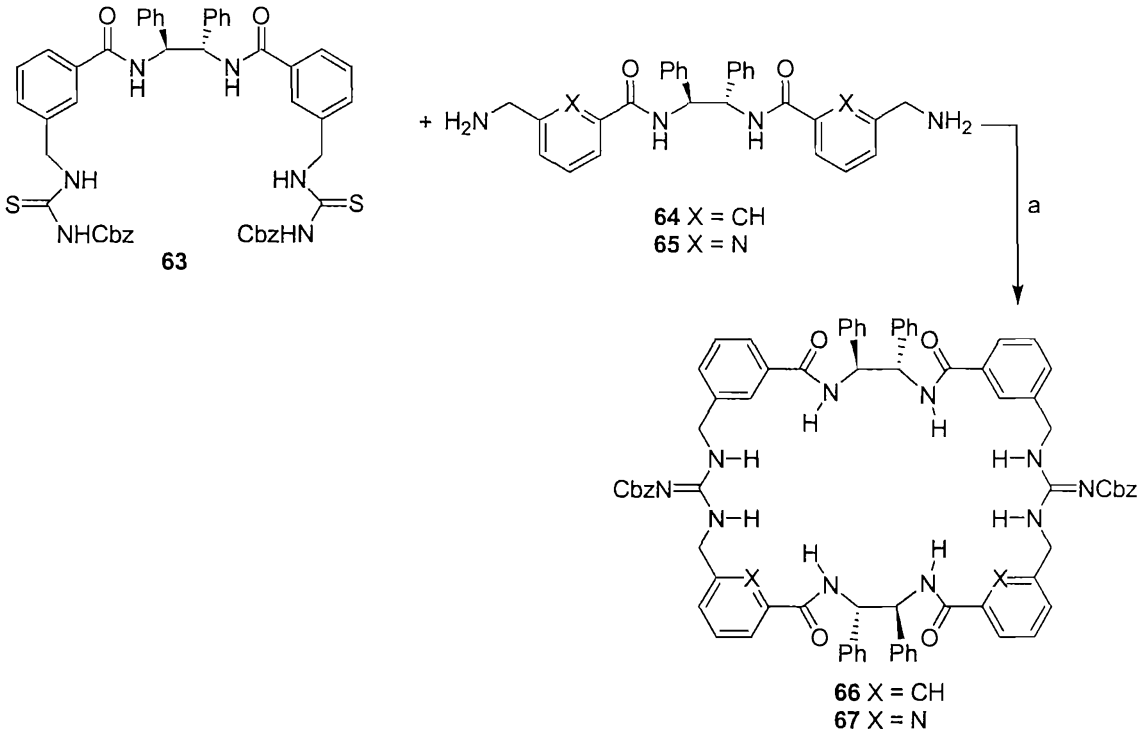
To develop a suitable synthesis for macrocycles **60** and **61** was not easy. The first route considered was the direct activation of the thioureas in macrocycle **59** and subsequent reaction with ammonia (Scheme 2.1).



Scheme 2.1 *Reagents and conditions: a) CH_3I, acetone; b) NH_4PF_6, CH_2Cl_2/CH_3OH; c) NH_3, CH_3OH*

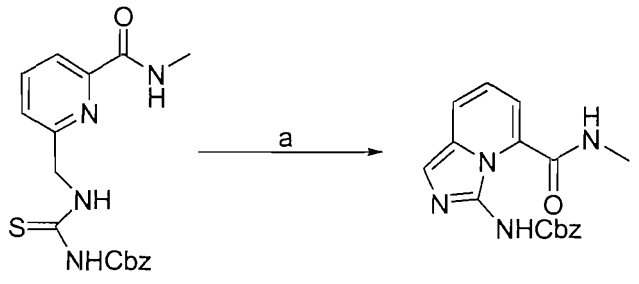
Unfortunately the reaction gave a mixture of products (monoguanidinylated, starting material, diguanidinylated) which could not be separated due to their similar polarities and it was clear that the yield of the desired product via this route was very poor.

Another route was envisaged, which would not allow the synthesis of the "full pyrido" macrocycle **62**, but would permit the synthesis of both **60** and **61** (Scheme 2.2).



Scheme 2.2 *Reagents and conditions: a) DMAP, EDC, DCM/DMF*

This route could not be used to synthesise **62** because, as extensively documented,[64] the reaction of compound **68** with EDC, leads to the formation of **69**. The addition of EDC to **68** activates the thiourea moiety which undergoes nucleophilic attack by the pyridine nitrogen, leading ultimately to the formation of **69** and preventing coupling of **68** with other amines (Scheme 2.3).



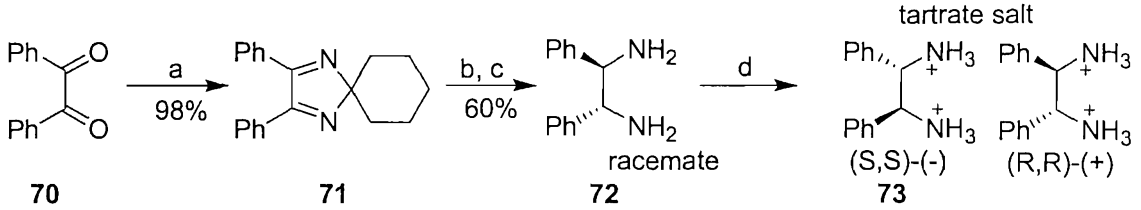
68 **69**

Scheme 2.3 *Reagents and conditions: a) DMAP, EDC, DCM/DMF*

The synthetic pathway is presented as different sections corresponding to the components of the macrocycle.

2.2.1.1 Synthesis of (1S, 2S)-1,2-diphenylethylene diamine

The synthesis of chiral diamine **73** was obtained following procedures published by Corey[65] (Scheme 2.4). Commercially available benzil **70** was converted to the corresponding spirocyclohexane imidazole **71** in nearly quantitative yield. Birch reduction of **71** was stereospecific for the *trans* product and the yield of **72** following reduction and acidic hydrolysis was also reasonable (60 %). The resolution was accomplished satisfactorily using tartaric acid for salt formation and separation of diasteroisomers by a series of recrystallisations in hot water-ethanol solution.

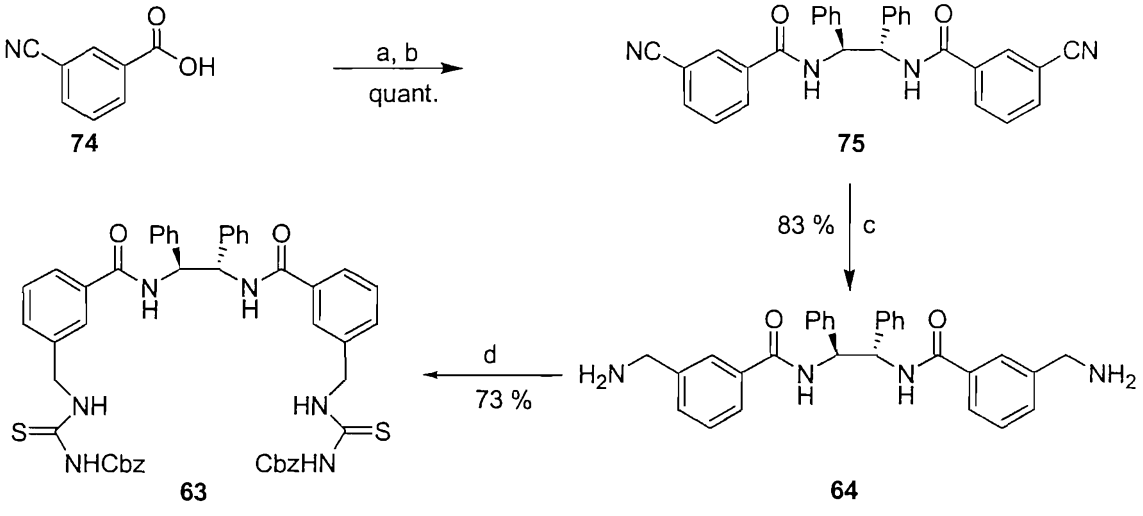


70 **71** **72** **73**

Scheme 2.4 *Reagents and conditions: a) NH_4OAc, AcOH, cyclohexanone; b) Li, THF-NH_3, EtOH; c) HCl; d) (L)-(+)-tartaric acid*

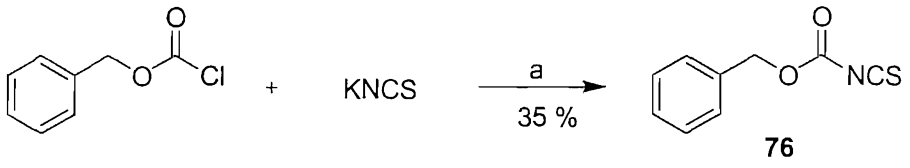
2.2.1.2 Synthesis of bisthiourea 63

Commercially available 3-cyanobenzoic acid **74** was activated with diphenylchlorophosphate and was coupled with (S, S)-1,2 diphenylethylene diamine **73** in a biphasic reaction (H_2O/CH_2Cl_2). The *bis*cyanobenzamide **75** was reduced to the corresponding diamine using $Pd/C/H_2$ in the presence of excess of trifluoroacetic acid (Scheme 2.5). Trifluoroacetic acid was necessary to prevent dimerisation by-products. This procedure proved to work better than Caddick's procedure[66] which uses sodium borohydride, nickel(II) chloride and Boc anhydride, and it also saved one step (Boc deprotection). Diamine **64** was obtained by dissolving the *bis*trifluoroacetate salt in methanol: after addition of triethylamine the methanol insoluble amine precipitated and could be isolated by filtration. The *bis*amine could also be purified by flash chromatography (2% NH_3 saturated methanol in dichloromethane), to obtain pure **64** in 83 % yield.



Scheme 2.5 *Reagents and conditions: a) $(PhO)_2P(O)Cl$, NEt_3, DCM; b) 1,2-(S,S)-diphenylethylene diamine, K_2CO_3, H_2O; c) H_2, Pd/C, TFA/Dioxane/MeOH, then NEt_3; d) CbzNCS **76**, DMF/DCM*

Benzyloxycarbonyl isothiocyanate **76** was synthesised from commercially available benzyl chloroformate and potassium isothiocyanate (Scheme 2.6) following Hamilton's procedure.[67]

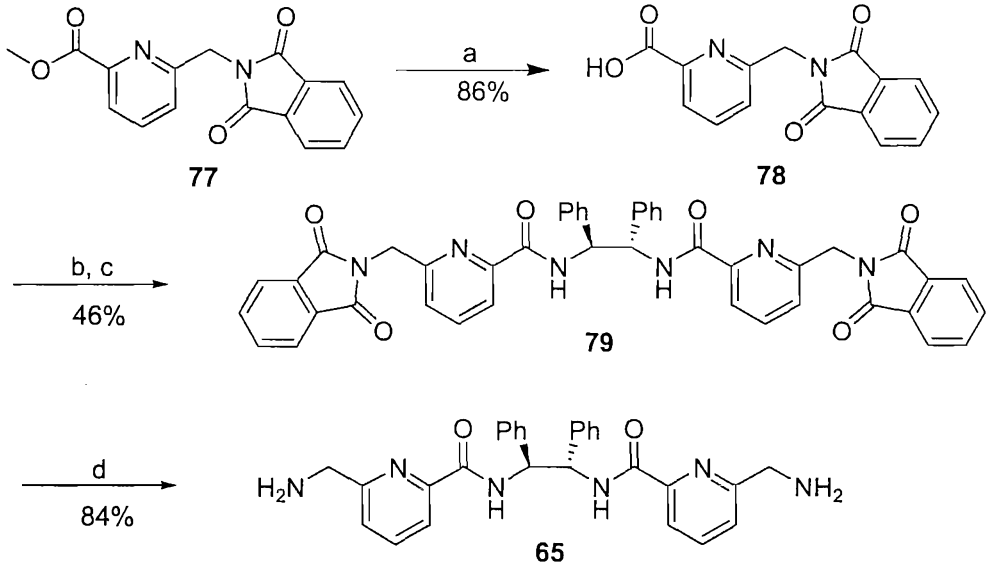


Scheme 2.6 *Reagents and conditions: a) CH_3CN 20 % in toluene, 48 h.*

*Bis*thiourea **63** was synthesised by coupling of *bis*amine **64** with **76** in a mixture of dimethylformamide (5-10 %, for complete solubilization of **64**) and dichloromethane. The presence of an electron-withdrawing group (carbamate) increases the reactivity of isothiocyanate **76**, permitting the formation of **63**.

2.2.1.3 Synthesis of bisamine 65

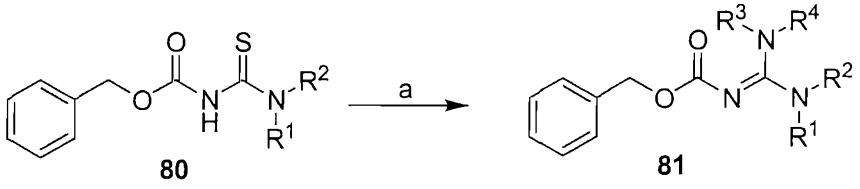
Ester **77**[68] was hydrolysed to the corresponding carboxylic acid by modifying a procedure from Olah.[69] Previous studies carried out in the group[70], found that the use of lithium iodide in place of sodium iodide increases the yield from 35% to 84%, under the same experimental conditions. Acid **78** was activated with diphenylchlorophosphate and coupled to diamine **73** following the procedure described above (Scheme 2.5). Diphthalimide **79** was deprotected using hydrazine hydrate to give diamine **65** in 84 % yield (Scheme 2.7). Again the yields were reproducible and reliable and reactions could be performed on a large scale.



Scheme 2.7 *Reagents and conditions: a) Me_3SiCl, LiI b) $(PhO)_2P(O)Cl$, NEt_3,DCM/H_2O; c) 1,2-(S,S)-diphenylethylene diamine; d) NH_2NH_2, EtOH.*

2.2.1.4 Synthesis of macrocycles 60 and 61

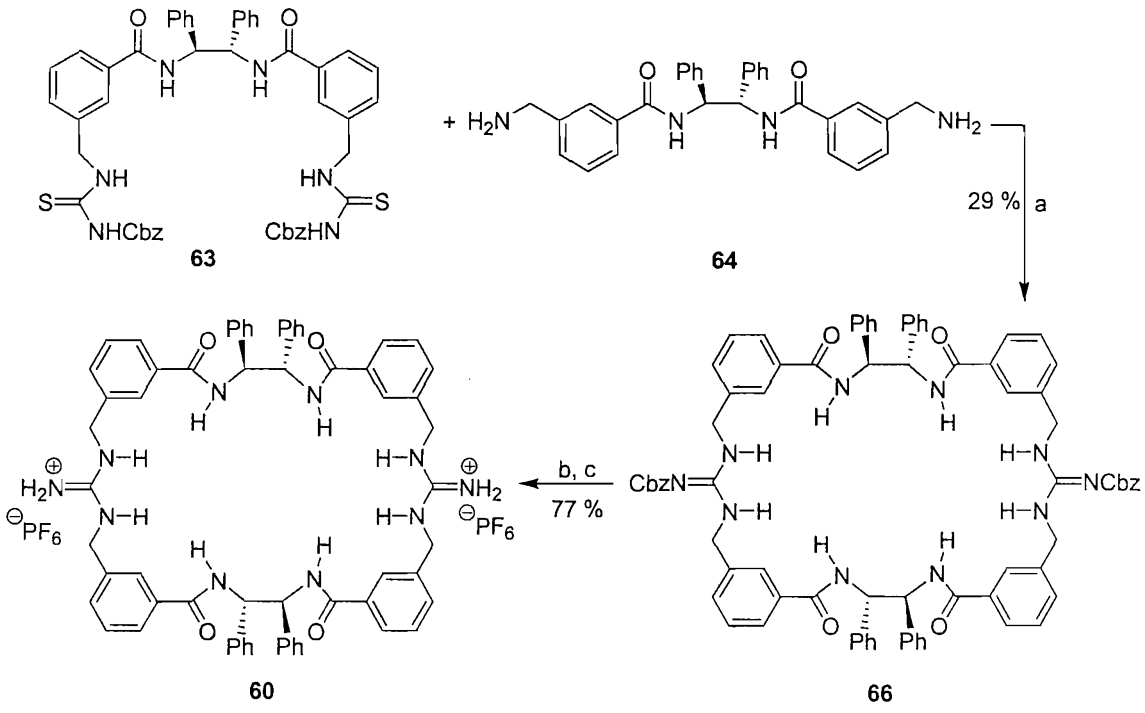
The coupling of bisamine **64** and bisthiourea **63** was the most challenging step of the planned synthesis of the macrocycles. Hamilton[67] devised the synthesis of Cbz protected guanidines from condensation of amines onto thioureas (Scheme 2.8), but this reaction was always performed in excess of amine, and, therefore, had never been exploited to achieve a macrocyclisation.



Scheme 2.8 *Reagents and conditions: a) R^3R^4NH, EDC, DIPEA, DCM*

The challenge was taken up and the first attempt of macrocyclisation (Scheme 2.9) was tried in high dilution conditions *via* syringe pump over six hours with DIPEA as a base (Scheme 2.9). Under these conditions no macrocycle **66** was formed even after four days.

The reaction was then performed by batchwise addition of a ~0.05 M solution of amine **64** in DMF/DCM to a vigorously stirred ~0.05 M solution of the *bis*thiourea **63**, EDC and DIPEA in DCM/DMF. After 24 h, some macrocycle **66** had formed, but the yield was a very poor 3%.



Scheme 2.9 *Reagents and conditions: a) Base, EDC, DCM/DMF, b) H_2, Pd/C, MeOH/DMF; c) HPF_6, DCM/MeOH*

Such a low yield was probably due to the fact that with stoichiometric amine, the reaction was very slow and that would explain also why under high dilution conditions, the reaction did not work at all.

Changing the solvent was not an option as **63** was only soluble in DMF/DCM mixture. Instead DMAP was chosen, as an alternative base, well known to help in coupling reactions.

Using catalytic DMAP (~5 mol %) did not improve the yield, but increasing the amount of DMAP to a maximum of 2 equivalents gave significantly improved yields (29 %). Subsequent Cbz deprotection and addition of HPF_6 gave **60** in ~80% yield.

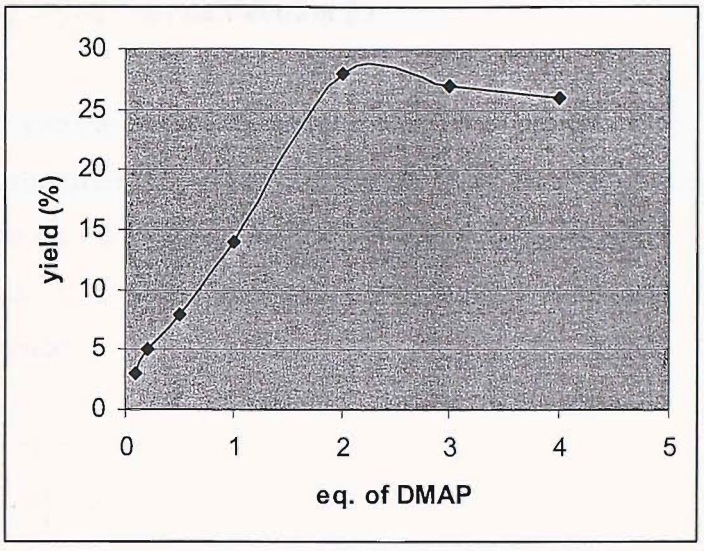
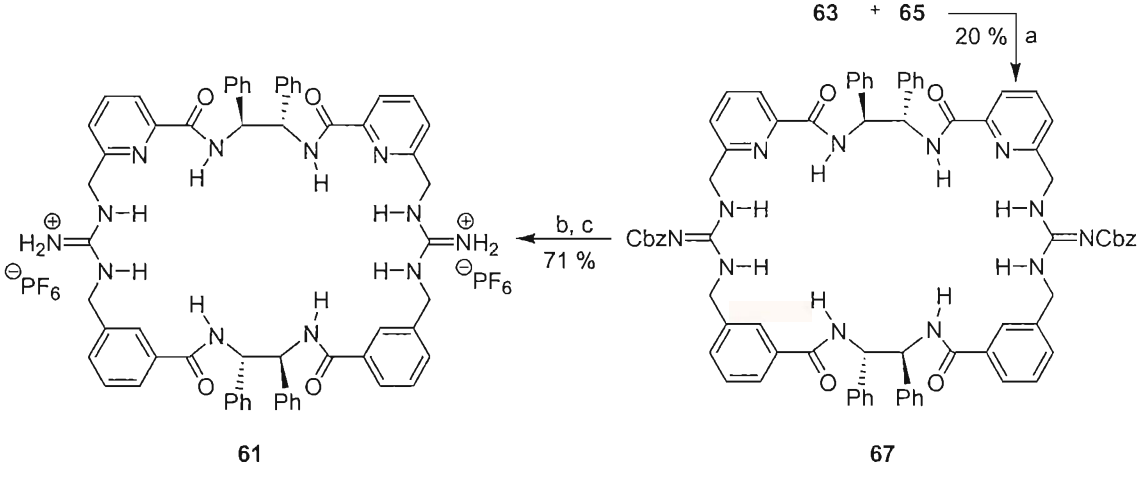


Figure 2.4 *Yield of formation of* ***66*** *vs eq. of DMAP added*

Synthesis of pyridine-containing macrocycle **61** was achieved *via* the same route, reacting *bis*thiourea **63** with *bis*amine **65** and gave **67** in 20% yield, using 2 equivalents of DMAP (Scheme 2.10).

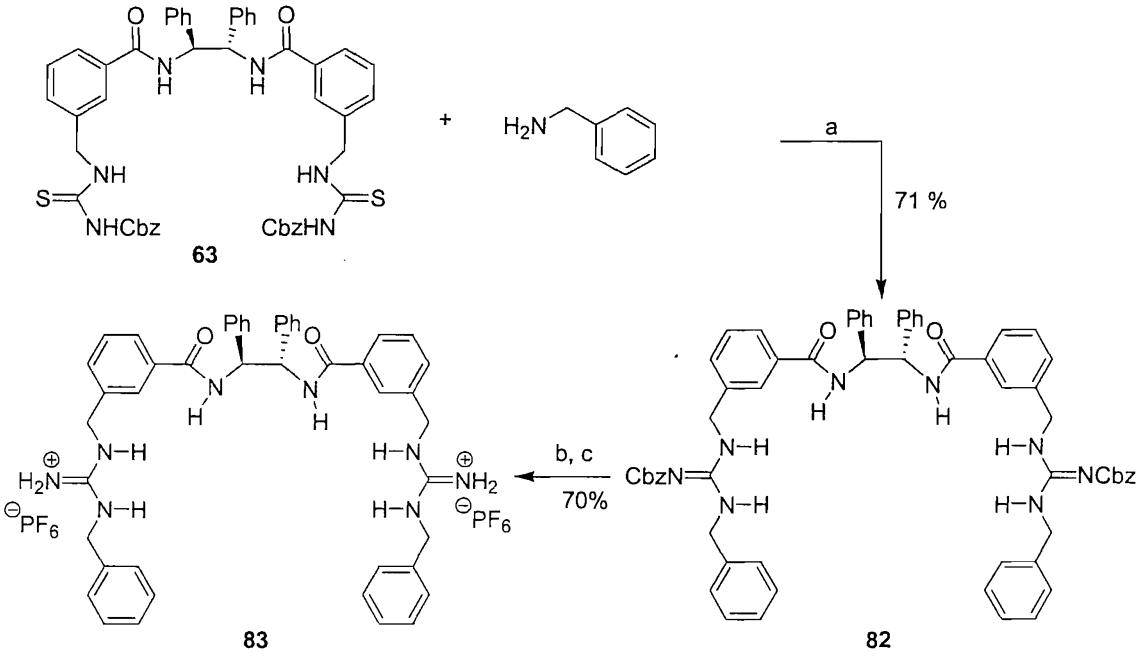


Scheme 2.10 *Reagents and conditions: a) DMAP, EDC, DCM/DMF; b) H_2, Pd/C, MeOH/DMF; c) HPF_6, DCM/MeOH*

Cbz deprotection and addition of HPF_6 gave **61** in 70-80 % yield.

2.2.2 Synthesis of acyclic bisguanidinium 83

To have a direct comparison of the results obtained with macrocycle **60**, an acyclic analogue, *bis*guanidinium **83**, was synthesised (scheme 2.11). Bisthiourea **63** was reacted with large excess of benzylamine following the protocol described above and **82** was obtained in 71 % yield. Deprotection and addition of hexafluorophosphoric acid gave **83** in 70 % yield.



Scheme 2.11 *Reagents and conditions: a) DMAP or TEA, EDC, DCM/DMF; b) H_2, Pd/C, MeOH/DMF; c) HPF_6, DCM/MeOH*

The conversion of bisthiourea **63** to the Cbz protected bisguanidine **82**, was unaffected, not only in efficiency, but also in reaction time, by the choice of base (triethylamine, DIPEA). This suggests that DMAP does not form an activated intermediate (EDC is already a powerful coupling reagent) and it does not function as a catalyst (a stoichiometric amount with respect to the thiourea functionalities is needed for the macrocyclisation to give maximum yield).

Even with a large excess of benzylamine (5 equivalents) the reaction was slow for the open chain analogue of our macrocycle. A possible explanation could be that DMAP acts as a templating agent for the macrocyclisation. It could bind through hydrogen bonds to the macrocyclisation intermediate, so that, after the conversion of the first

thiourea functionality to the guanidine, the two reaction sites (second thiourea carbon and amine NH_2) are held close together to give a better alignment in order to close the ring. ^1H NMR, performed in $CDCl_3$, showed that after addition of 0.5 equivalents of macrocycle **60**, the aromatic CH's of DMAP ortho to the nitrogen undergo a downfield shift ($\Delta\delta$ = 0.15 ppm) which could indicate the presence of an interaction, but no change to the signals of the macrocycle could be detected.

2.3 Conformational and binding properties (NMR binding studies)

2.3.1. Conformation

Bisguanidinum macrocycle **60** was found to be soluble in DMSO-d_6 and the ^1H NMR spectrum is shown in Figure 2.5.

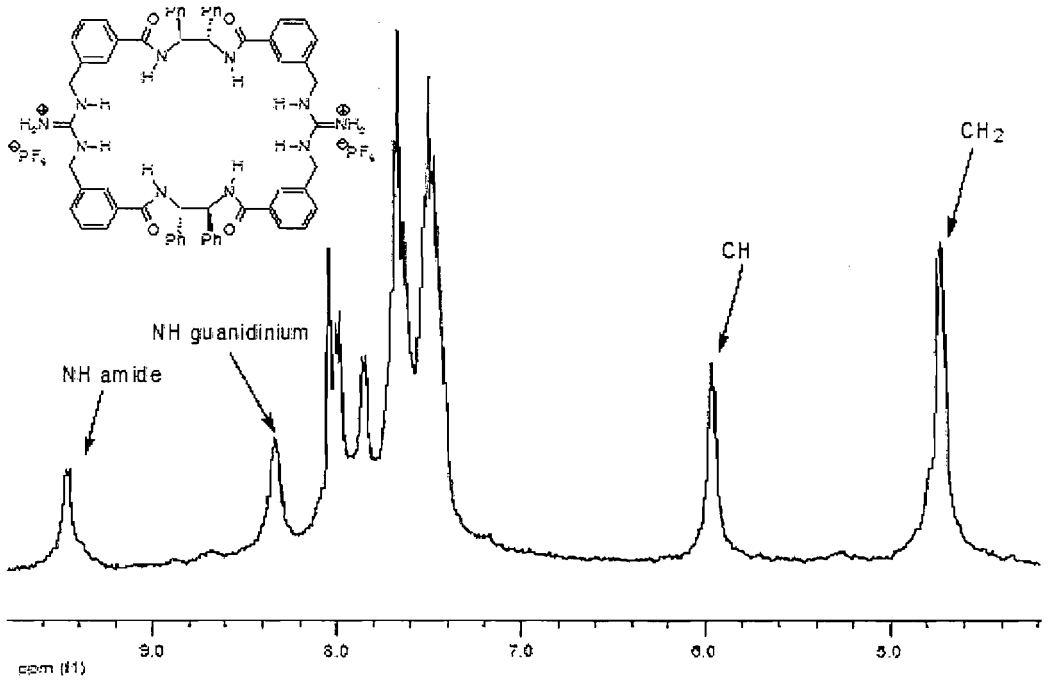


***Figure 2.5** ^1H NMR spectrum of macrocycle **60** in DMSO-d_6*

The spectrum appears to be consistent with the expected 4-fold symmetry. In $CDCl_3$, in which bisthiourea receptor **59** adopts a wrapped conformation that does not allow the binding of the guest, macrocycle **60** proved to be insoluble. The spectrum in 5% CD_3OD in $CDCl_3$ does, however show the same 4-fold symmetry.

Spectrum of macrocycle **61** (Figure 2.6) shows the expected 2-fold symmetry in DMSO-d_6. The chemical shifts of the protons are not significantly different to those of macrocycle **60**.

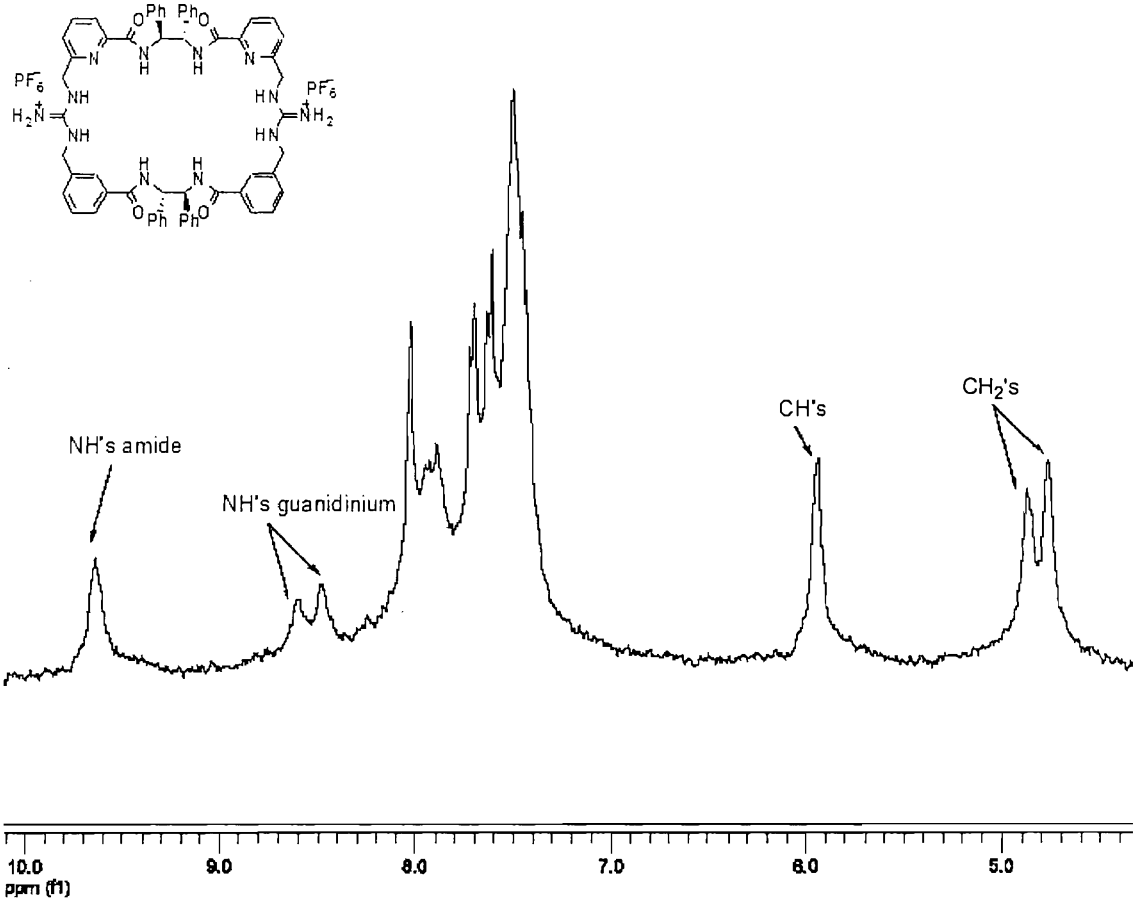


Figure 2.6 *^{1}H NMR spectrum of macrocycle **61** in DMSO-d_6*

Acyclic *bis*guanidinium **83** (Figure 2.7) shows the expected 2-fold symmetry and the relative shifts are practically the same as those of macrocycle **60**.

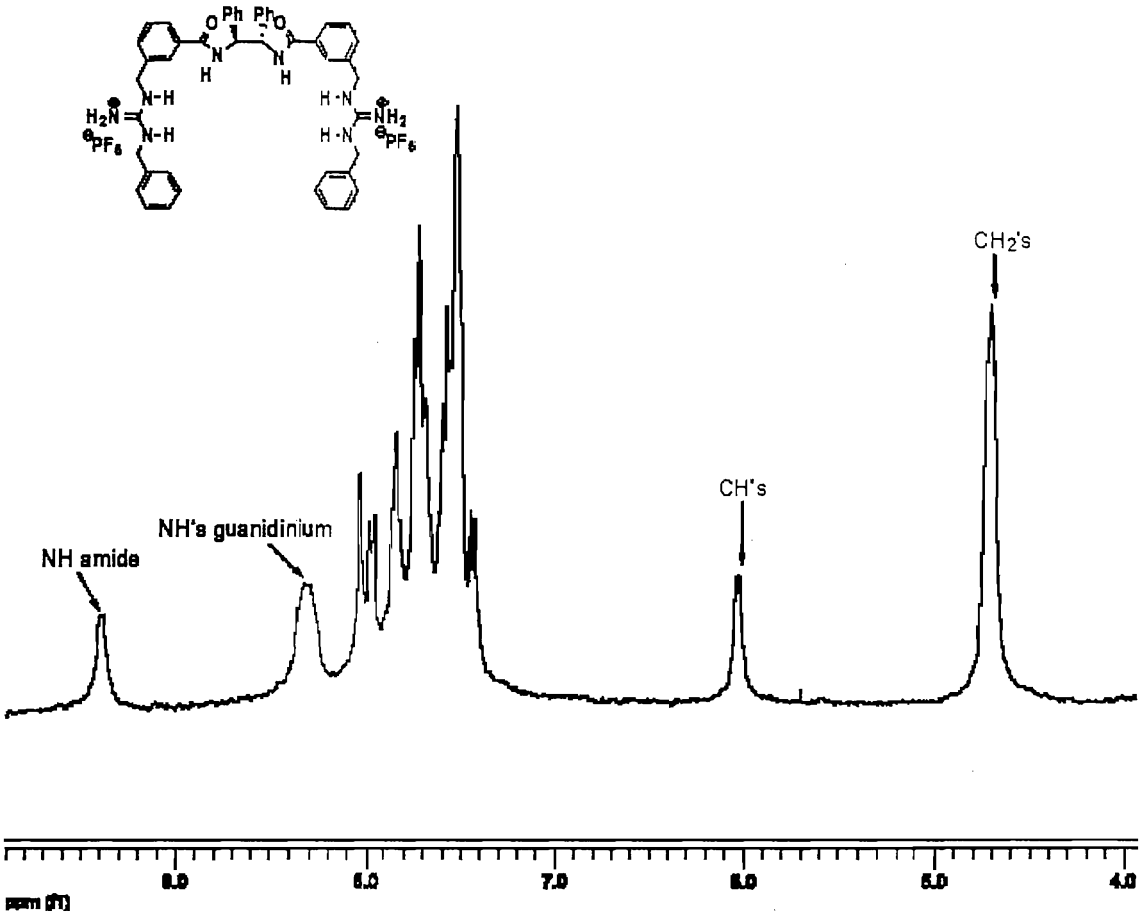


Figure 2.7 *^{1}H NMR spectrum of bisguanidinium **83** in DMSO-d_6*

2.3.2 NMR Binding studies

Binding studies of macrocycles **66**, **67**, **60**, **61**, *bis*guanidinium **83** and *bis*thiourea **63** with *N*-Boc-L- Glu, *N*-Boc-D-Glu, and of macrocycles **60** and **61** with *N*-Boc-L-Asp, as their tetrabutylammonium salts, were carried out in DMSO-d_6 and, when possible, in $CDCl_3$ by ^{1}H NMR titration, using the procedure outlined in appendix 1 and following, in most cases, the shift of the amide NH protons.

Particular attention was paid to estimating the selectivity and enantioselectivity of the receptor with the guests. Also, it was hoped that **60** would give a tighter binding than with bisthiourea macrocycle **59** in polar solvents such as DMSO.

N-Boc-L-Asp was investigated in order to probe the possible influence of the chain length between the two carboxylate groups on the binding properties of the host.

2.3.2.1 Cbz-protected macrocycles

Binding studies with Cbz protected macrocycle **66** were conducted in both $CDCl_3$ and DMSO-d_6 with *N*-Boc-L-Glu.

All the signals (CH, CH_2, NH's) of the macrocycle shifted downfield upon addition of *N*-Boc-L-Glu (more significantly in $CDCl_3$) showing a neat sigmoidal effect (Figure 2.8), similar to that observed by Wilcox for the more weakly bound guest when titrating a mixture of two guests.[71]

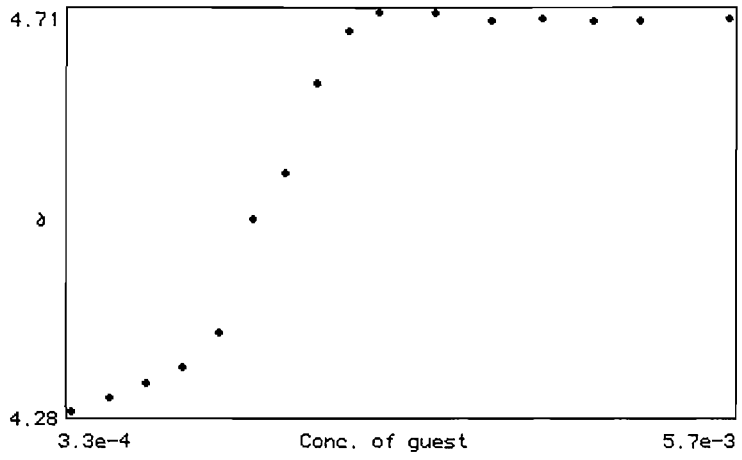


Figure 2.8 *Sigmoidal curve ($CDCl_3$)*

It was noted that during the titration the signal of the water, observed even in dry $CDCl_3$ in the NMR spectrum of the host, was significantly moving downfield and then upfield (after addition of more then 0.3 equivalents of *N*-Boc-L-Glu). That could indicate that water is bound inside the receptor cavity and that the effect competes with the binding of the guest. This is also confirmed by the elemental analysis which is consistent with the presence of two water molecules in the receptor solid structure. However, reliable data could not be obtained and the phenomenon has not been fully understood.

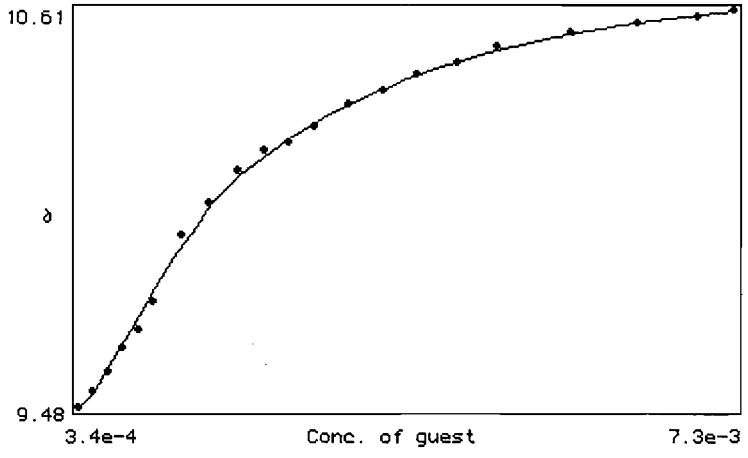
Cbz protected macrocycle **67** instead gave no significant shifts of the CH and CH_2 protons and broadening of the NH signals on addition of 0.2 equivalents.

2.3.2.2 Guanidinium macrocycles

Macrocycle **61** showed no evidence of binding of *N*-Boc-L-Glu in DMSO-d_6. Only small shifts of the NH signals were observed ($\Delta\delta \sim 0.1$ ppm for the amide NH) and the data were not reliable enough to be fitted to any binding models. A plausible explanation could be that strong intramolecular hydrogen bonds force the macrocycle to adopt a conformation unsuitable for binding.

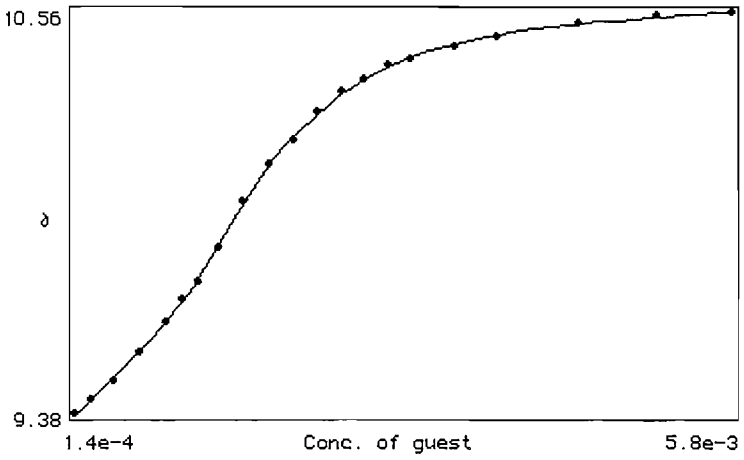
Macrocycle **60** instead, showed evidence of strong binding both with *N*-Boc-L-Glu and *N*-Boc-D-Glu.

After addition of one equivalent of guest both the guanidinium NH ($\Delta\delta \sim 1.2$ ppm) and the amide NH ($\Delta\delta \sim 1.0$ ppm) showed a dramatic downfield shift. The curves were fitted to a 1:2 association model (Figure 2.9 and 2.10), which assumes both 1:1 and 1:2 complexation, giving a very large K_1K_2 value ($K_1K_2 > 10^8$ M^{-2}). The relative values of the two constants ($K_{1:1}$ and $K_{1:2}$) could not be determined reliably, as the values given by the fitting program were highly dependent upon the chosen input values (due to the numbers of parameters being adjusted). The indication given by the curve fit though is that $K_{1:1}$ and $K_{1:2}$ are very large both in the case of *N*-Boc-L-Glu and in the case of *N*-Boc-D-Glu.



N-Boc-L-Glu

***Figure 2.9** Association curves for macrocycle **60** with N-Boc-L-Glu bis TBA salt in DMSO-d_6*



N-Boc-D-Glu

Figure 2.10 *Association curves for macrocycle **60** with N-Boc-D-Glu bis TBA salt in DMSO-d_6*

No shift of the CH or CH_2 signals was noted, indicating that the receptor did not undergo major rearrangements upon complexation of both the guests in DMSO-d_6. In both cases an upfield shift of the aromatic protons of the aryl ring was however observed with the one *ortho* to the CO group and *para* to the other substituent of the ring undergoing the larger shift ($\Delta\delta$ = -0.2 ppm).

Solubility problems did not allow us to test the binding in other solvents, such as CD_3CN or $CDCl_3$.

N-Boc-L-Asp was also found to be bound. However the curve (Figure 2.11) could not be fitted reliably, suggesting the presence of multiple binding equilibria (1:1, 1:2 and possibly 2:2).

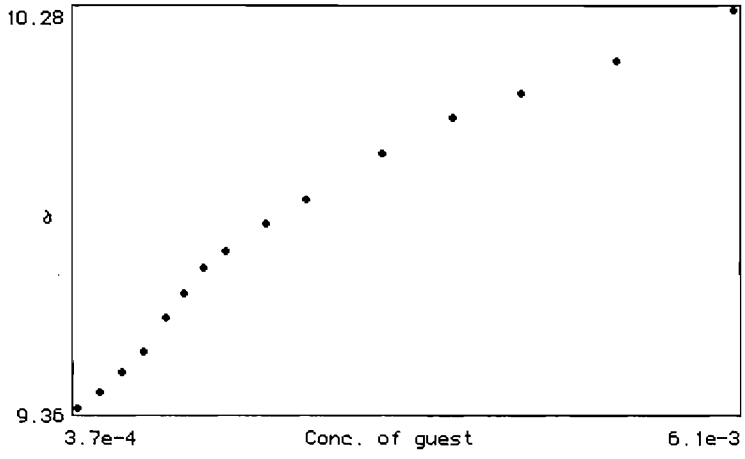


Figure 2.11 *Association curves for macrocycle **60** with N-Boc-L-Asp bis TBA salt in DMSO-d_6*

2.3.2.3 Acyclic bisguanidinium 83 and bisthiourea 63

Binding studies in DMSO-d_6 for acyclic *bis*guanidinium **83** and *bis*thiourea **63** were carried out. Bisguanidinium **83** showed much weaker binding than macrocycle **60** ($K_1K_2 > 10^5$ M^{-2}; K_1 ~1000 M^{-1}, K_2 ~ 500 M^{-1} for the L enantiomer and K_1 ~ 600 M^{-1}, K_2 ~ 2000 M^{-1} for the D enantiomer) and a very similar behaviour for the two enantiomers of the *N*-Boc-Glu guest (Figure 2.12 and 2.13). Saturation is not reached even after addition of more than three equivalents of guest.

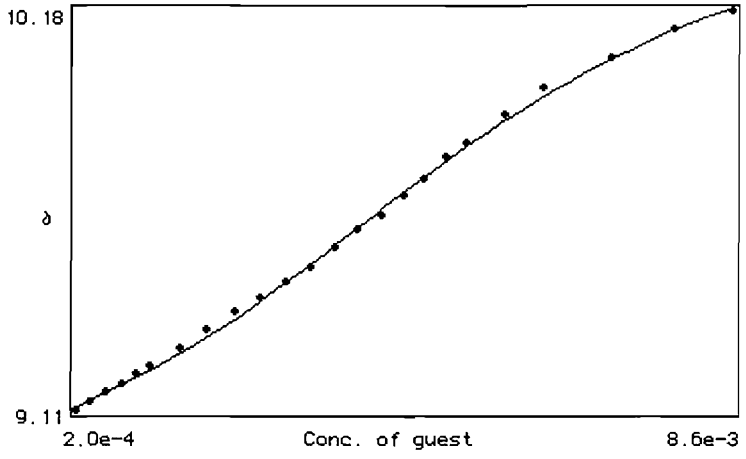


Figure 2.12 *Association curves for bisguanidinium **83** with N-Boc-L-Glu bis TBA salt in DMSO-d_6*

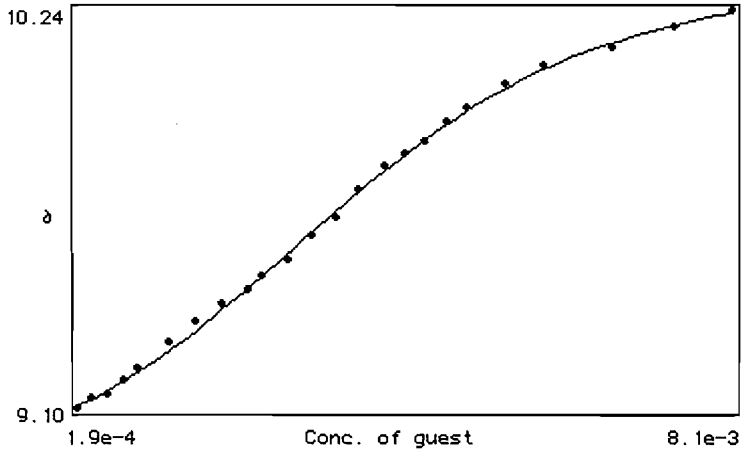


Figure 2.13 *Association curves for bisguanidinium **83** with N-Boc-D-Glu bis TBA salt in DMSO-d_6*

Acyclic *bis*thiourea **63** shows a very weak 1:1 binding (K = 100 M^{-1}) of *N*-Boc-L-Glu (Figure 2.14).

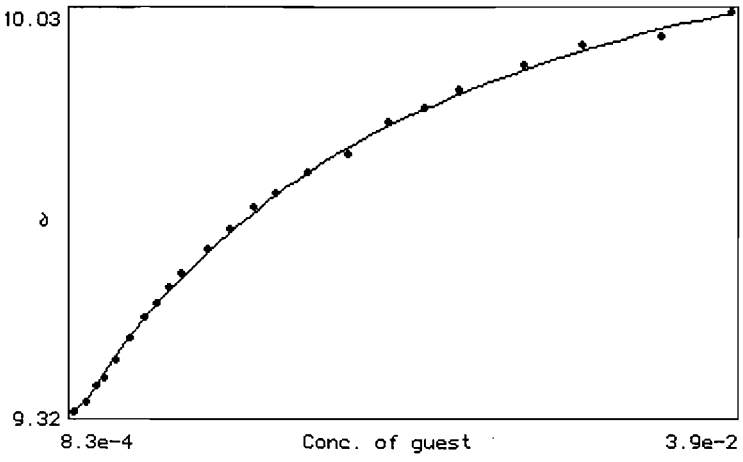


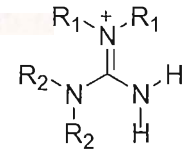
Figure 2.14 *Association curve for bisthiourea* ***63*** *with N-Boc-L-Glu bis TBA salt in DMSO-d_6*

2.4 Application of calorimetry to supramolecular chemistry

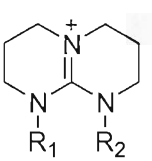
Thanks to recent advances in instrument sensitivity, isothermal calorimetry (ITC) is becoming increasingly important as a tool for probing the thermodynamics of binding processes. By directly measuring the heat evolved or absorbed as a function of time, ITC can determine, in one experiment, all the thermodynamic parameters involved in a chemical process. In a single experiment the binding constant (K_a), the stoichiometry (n) and the enthalpy (ΔH) of the process investigated are also determined. From the association constant the free energy and entropy of binding are determined. Recently chemists have started to apply the ITC tool to gain an insight into the thermodynamic parameters that govern the complexation of a guest by a synthetic host.

2.4.1 Thermodynamic aspects of molecular recognition of dicarboxylates

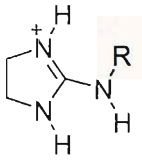
Hamilton *et al.*[72] applied ITC to investigate the association between a series of simple guanidinium derivatives and tetrabutylammonium (TBA) acetate in DMSO, **84-91**, showed in Figure 2.15.



84 $R_1 = R_2 = H$
85 R_1 = Me, $R_2 = H$
86 $R_1 = R_2$ = Me



87 $R_1 = R_2 = H$
88 R_1 = Me, $R_2 = H$
89 $R_1 = R_2$ = Me



90 R = H
91 R = Bn

HOST	K (M^{-1})
84	7.9×10^3
85	3.4×10^3
86	1.1×10^2
87	5.6×10^3
88	-
89	-
90	8.7×10^3
91	7.2×10^3

Figure 2.15 *Hamilton's guanidinium receptors for acetate and table of the association constants with TBAAc*

Various guanidinium salts were studied, acyclic (**84-86**), bicyclic (**87-89**) and monocyclic (**90, 91**) and their association constants were determined.

In the acyclic systems, sequential removal of hydrogen bond donors (substitution of a hydrogen with a methyl) resulted in a drastic decrease in the association constant. Similarly, sequential methylation of the corresponding bicyclic guanidiniums **87-89** resulted in a loss of binding affinity for TBA acetate. Derivatives **88** and **89** produced no signal during the calorimetric titration resulting in a horizontal isotherm. Monocyclic guanidiniums **90** and **91** displayed a high affinity for acetate. All the association constants were confirmed by traditional ^{1}H NMR titrations. Even though in most cases the receptors formed a 1:1 complex with the guest, two receptors (**84** and **90**) were able to complex an additional equivalent of TBA acetate to form a weak 2:1 complex, thanks to the availability of extra hydrogen bond donors. In all cases, both the association enthalpy and entropy were favourable, indicating that the complexation process was driven both by hydrogen bond formation and by the liberation of bound solvent molecules into the bulk solvent.

Building on this work, Hamilton[73] extended his studies to more complicated host-guest systems. He studied the solvent participation in the binding process: in fact, although it had been previously documented in lipophilic cyclophane hosts, this effect remained to be established for receptors that rely on hydrogen bonding interactions. The work focused indeed on association in increasingly competitive solvents, from DMSO to methanol and water. One approach to study the process was to create a series of synthetic receptors that provide the same presentation of hydrogen bonding sites but

differ vastly in their binding strength. Simple artificial receptors **92-95** were synthesised (Figure 2.16).

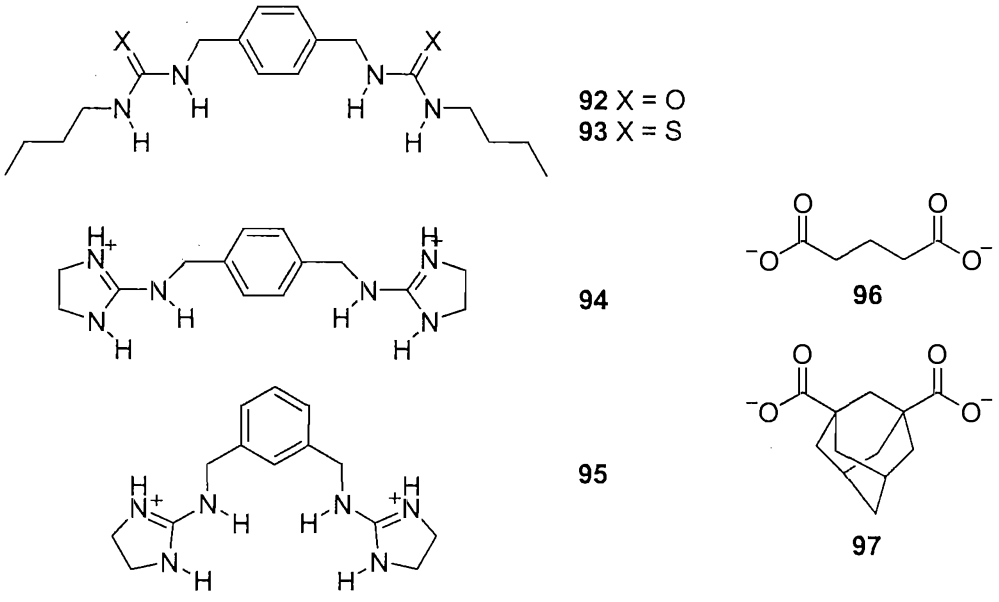


Figure 2.16 *Hamilton's receptors for dicarboxylates*

Three functional groups, ureas, thioureas and guanidiniums, with increasing bond donor acidity (pK_a = 26.9, 21.0, 13.6 respectively) and correspondingly increasing association strength, were investigated. The association was measured by ITC and confirmed by NMR titration. In less polar solvents such as DMSO, Hamilton found that the complex formation is enthalpically driven. In methanol and methanol/water mixtures, association becomes instead endothermic, with favourable entropy providing the associative force. This is thought to indicate a change from association that is promoted primarily by hydrogen bond formation to association that is driven by solvent liberation. Qualitative ITC indicated also the presence of multiple binding equilibria in complexes of *bis*guanidiniums and dicarboxylates.

2.4.2 ITC titrations for macrocycle 60 with N-Boc-L,D-glutamate in DMSO

Isothermal calorimetry was used to investigate and possibly determine the association constant of macrocycle **60** with both *N*-Boc-L-glutamate and *N*-Boc-D-glutamate. The ITC titration with *N*-Boc-L-glutamate in DMSO was repeated three times without giving reproducible results.

ITC calorimetry showed instead the presence of multiple binding equilibria when the guest is *N*-Boc-D-glutamate (Figure 2.17).

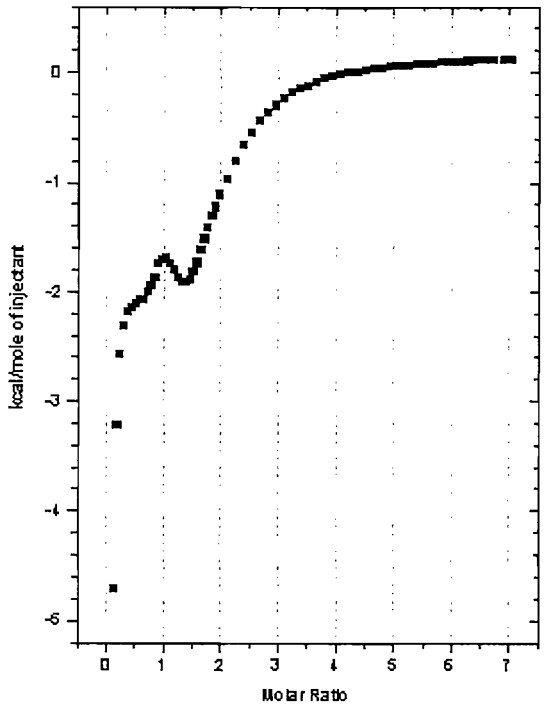


***Figure 2.17** ITC titration curve for N-Boc-D-Glu*

It was not possible to fit the ITC curve with either the one or the two sites model, therefore the data for the *N*-Boc-D-Glu in DMSO could only provide a qualitative analysis of the interactions occurring. The experiment, reproducible for three times, displayed several exothermic and endothermic events occurring at both low and high concentrations. Presumably, these are due to multiple binding equilibria such as 1:1, 2:1, 1:2 and 2:2 complexes, but the absence of transitions at distinct molar equivalents, suggested nondiscrete aggregation rather than formation of a distinct complex.

2.4.3 ITC titrations for macrocycle 60 with N-Boc-L,D-glutamate in DMSO/water mixtures.

The ITC curve obtained for *N*-Boc-D-Glu in DMSO suggested a very strong binding and the presence of multiple equilibria. It was thought that a more polar solvent system could suppress the weaker equilibria and leave the stronger ones. Titrations performed in 10% H_2O/DMSO showed again multiple binding equilibria. Again, the fit was not reliable and only a qualitative estimation could be made. This time though, the titration

curve (figure 2.18) showed only two main binding equilibria: an exothermic binding (presumably for the 1:1 binding) and an endothermic one (presumably for the 1:2).

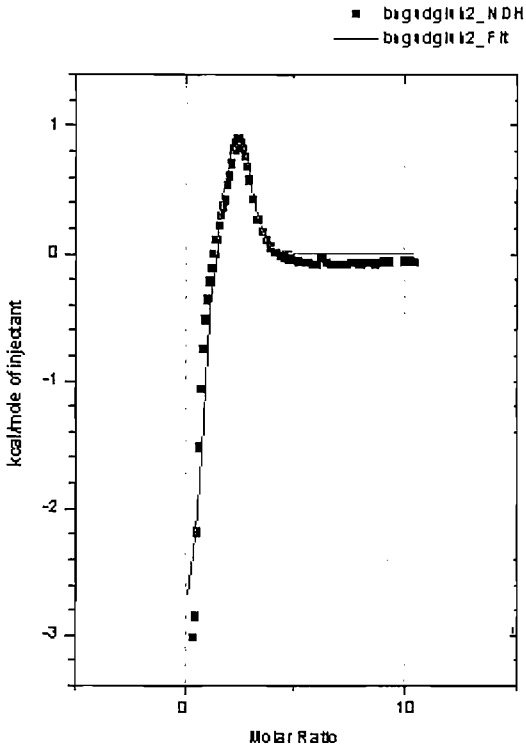


Figure 2.18 *ITC titration curve for N-Boc-D-Glu in 10% H_2O/DMSO*

Finally, with great satisfaction, titrations performed in 50 % H_2O/DMSO showed the presence of one equilibrium only (1:1 binding equilibrium). The association becomes endothermic ($\Delta H = 1.309 \pm 0.0165$ kJ mol^{-1}) and entropically driven ($T\Delta S = 20.31$ kJ mol^{-1}) with a binding constant, $K = (3010 \pm 151.8)$ M^{-1} (Figure 2.19).

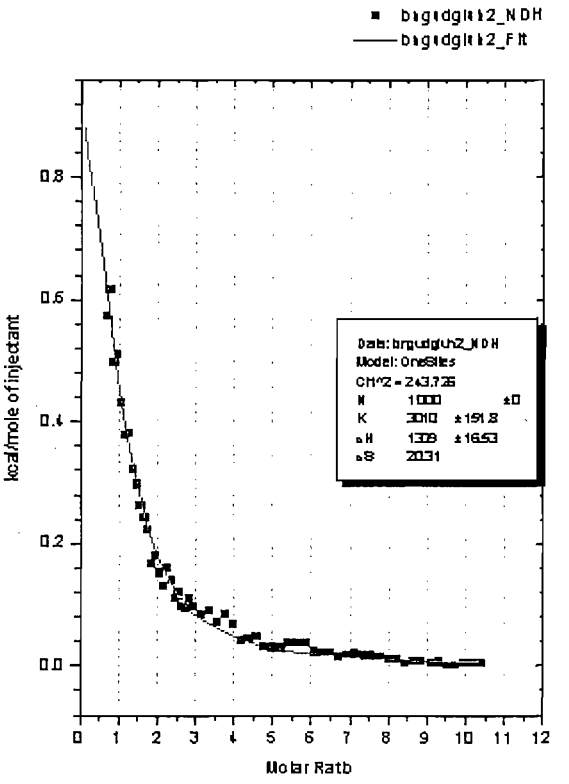


Figure 2.19 *ITC titration curve for N-Boc-D-Glu in 50% H_2O/DMSO*

Titrations for *N*-Boc-L-Glu gave again a curve which could not be reproduced. Investigations are being continued in the group at present.

2.5 Conclusions

An interesting, DMAP-aided synthesis of macrocycle **60** was developed.

Macrocycle **60** gave evidence of a strong binding of *N*-Boc-D-Glu in DMSO, much stronger than bisthiourea macrocycle **59**, showing the presence of multiple binding equilibria. ITC titrations showed a remarkable endothermic 1:1 binding of *N*-Boc-D-Glu in the very polar solvent system 50% H_2O/DMSO, result which is very promising in order to achieve binding of the zwitterion in neat water. *N*-Boc-L-glutamate seems to be strongly bound but both calorimetry and ^1H NMR experiments showed the presence of multiple equilibria in DMSO and no reproducible results could be obtained in 50% H_2O/DMSO.

CHAPTER III

3.1 Introduction and background

3.1.1 Tweezer receptors with non-specific hinges

Many "tweezer" receptors have been synthesised by host-guest chemists, which have been screened against combinatorial libraries of resin-bound peptides, allowing a rapid evaluation of their binding properties.[74] These non-macrocyclic hosts are so named because they contain particular structural features, which give them the appearance and the mode of binding of a pincer. Despite their inherent flexibility, they have proven to be highly selective for certain peptide sequences in both nonpolar[75] and aqueous[76] solvent systems.

Liskamp *et al.*[77] showed that an open and flexible receptor was able to bind tripeptide sequences with high selectivity and affinity (Figure 3.1).

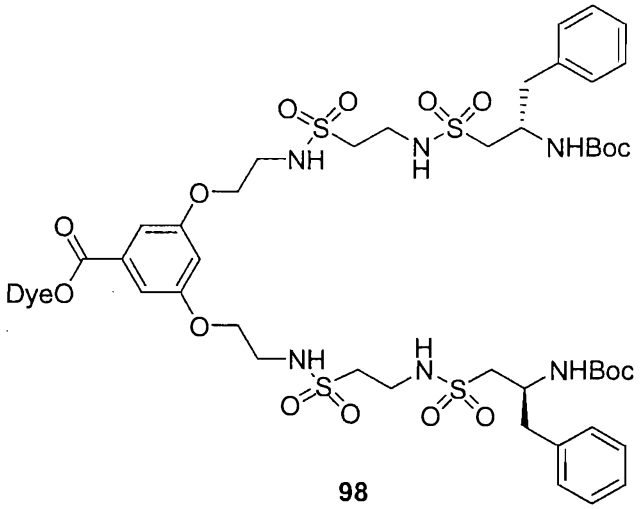


Figure 3.1 *Liskamp's peptidosulfonamide tweezer receptors*

Receptors **98** and **99** were both screened against a 24389-member side chain-deprotected tripeptide library, and they displayed high selectivity for the peptide sequence D-Ala-L-Asp-D-Ser in $CDCl_3$, with receptor **99** showing a higher binding affinity. The binding constant decreased in CD_2Cl_2, and was very sensitive to traces of a protic solvent such as methanol. The presence of only 1% of methanol was sufficient to reduce the binding to almost zero in both chloroform and dichloromethane. In fact, in polar (especially aqueous) media, selective binding of polar substrates, such as peptides, has proven to be much more demanding.[78, 79]

Still[80] described a class of simple tweezer receptors (Figure 3.2) for binding of peptides.

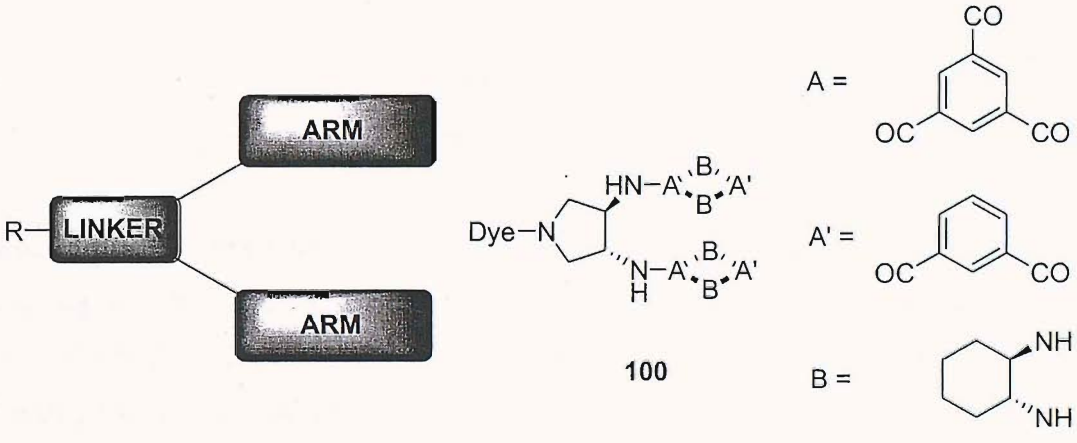
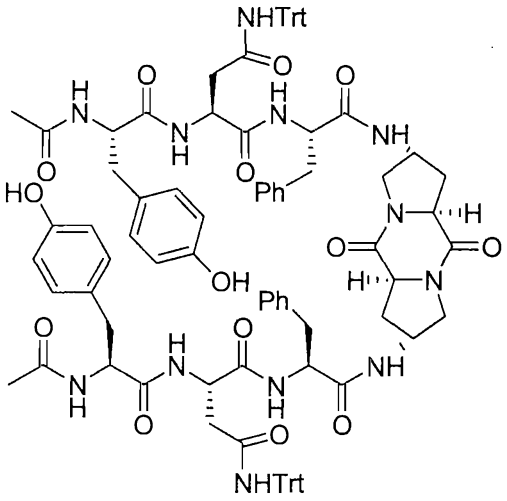


***Figure 3.2** Still's "two-armed" receptor*

Receptor **100** is an example of what has been described as a "two-armed receptor", whose general structure is sketched in Figure 3.2. The linker is typically a conformationally restricted moiety covalently attached to two functionalised, substrate-binding arms, which are directed towards one another to form a binding cleft. In receptor **100** the arms are macrocyclic oligomers of isophthalic acid (A, A') and *trans*-1,2-diaminocyclohexane (B). The Dye is used as a label to allow direct observation of the binding of **100** to peptides attached to the solid support.

After screening, receptor **100** was found to bind tightly only with two sequences, D-Pro-L-Val-D-Gln and L-Lys-L-Val-D-Pro, out of a 3375-member tripeptide library in chloroform.

Wennemers *et al.*[81] developed a class of receptors consisting of a diketopiperazine backbone and peptidic side arms (Figure 3.3).



101

Figure 3.3 *Two-armed diketopiperazine receptor*

Tweezer **101** has been found to bind, in $CHCl_3$, only peptides which contain D-His following two hydrophobic D-amino acids and to have a strong preference for Ac-D-Val-D-Val-D-His (K_a = 1420 M^{-1}). Small modifications of the ligand peptide such as inverting the stereochemistry of the central D-Val led to a considerable decrease of the binding constant (K_a = 260 M^{-1}).

3.1.2 Tweezer receptors with specific head groups

All the tweezer receptors described above use a "hinge" with no specific binding properties. To achieve stronger binding in polar solvents "hinges" capable of giving strong interactions with the substrate can be used, so that the binding affinity can be increased while maintaining the selectivity.

A large part of this project has been focused on the synthesis of "tweezer" receptors for small peptides with a carboxylate terminus. The basic design of our tweezer receptors incorporates a "head" group or "hinge" capable of binding the carboxylate terminus and bearing two side arms that contain appropriate functionality for binding with the backbone of suitable peptide substrates (Figure 3.4).

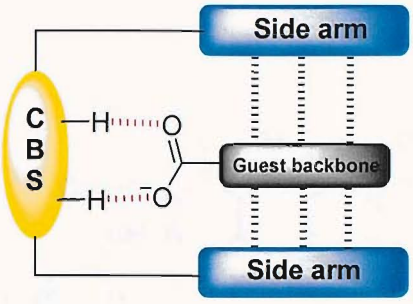


Figure 3.4 *Schematic representation of a tweezer receptor*

Typically the side arms are peptides. Recognition of peptide guests depends therefore on β-sheetlike hydrogen bonding interactions between peptide backbone amide bonds and the receptor, coupled with side chain specific interactions so that different peptides can be discriminated. Incorporation of a head group (hinge) with a specific recognition site for the C-terminus of a peptide (carboxylic binding site, CBS) should significantly increase the binding affinity.

3.1.2.1 Diamidopyridine hinges

Amidopyridines[82] provide an excellent structural motif for binding carboxylic acids with the ability to form two complementary hydrogen bonds from the carboxylic acid hydrogen to the pyridine nitrogen and the carboxylic acid carbonyl to the amide hydrogen as shown in Figure 3.5.

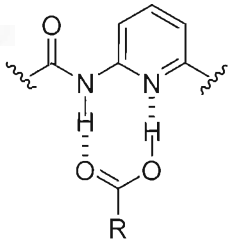
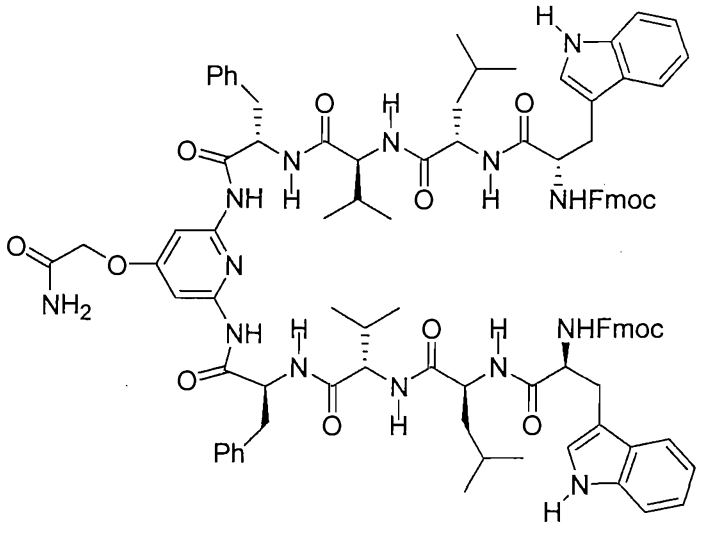


Figure 3.5 *Amido pyridine binding motif*

Kilburn has incorporated diamidopyridines in tweezer receptors and used a solid phase approach to synthesise libraries of receptors that could be screened for selective binding of peptides with a carboxylic acid terminus in organic media.[83]



102

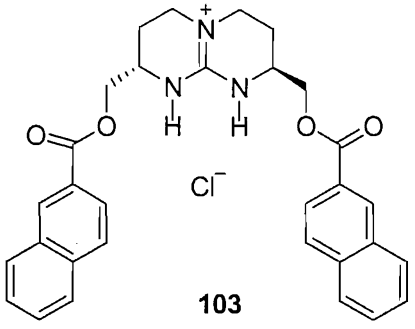
Figure 3.6 *Kilburn's diamidopyridine tweezer*

Using this approach, tweezer **102** (Figure 3.6) was identified as a receptor for the protected tripeptide DNS-L-Glu(O^{t}Bu)-L-Ser(O^{t}Bu)-L-Val-OH (K_a = 2.6 x 10^5 M^{-1}) in 2% DMSO/$CHCl_3$ by UV titration. It was concluded that, as anticipated, the incorporation of a specific binding site for the carboxylic acid terminus of peptide guests into a tweezer structure provided a considerably higher affinity than with a non-specific head group.

In the binding of carboxylic acids with diamidopyridines though, unfavourable secondary interactions arise, particularly between the relatively electropositive carboxylic acid and the amide protons, which make amidopyridines a less potent binding site for carboxylic acids than ureas and thioureas for carboxylates. Thus, amidopyridines are generally only effective in relatively non-polar solvents.

3.1.2.2 Guanidinium hinges

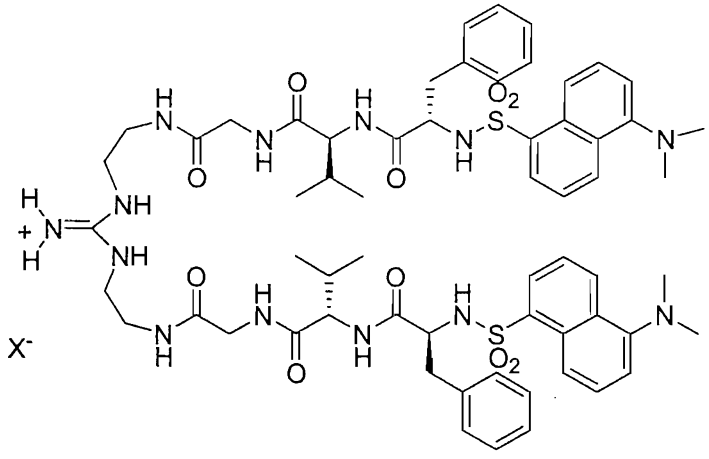
The guanidinium functionality has been extensively discussed in Chapter I. Schmidtchen,[84] De Mendoza[85] and Davis[86] have developed routes to chiral bicyclic guanidinium salts. Receptor **103** (Figure 3.7) was found to bind *p*-nitrobenzoate, using a combination of carboxylate-guanidinium salt interaction and π-π stacking (K_a = 1609 M^{-1} in $CDCl_3$).



103

Figure 3.7 *De Mendoza's bicyclic guanidinium receptor*

A guanidinium-based tweezer receptor was synthesised[87] in the Kilburn group (Figure 3.8). Receptor **104** is designed to bind peptides with a carboxylate terminus and contains a guanidinium salt to provide the primary binding interaction for the carboxylate, and peptidic arms with a potential to form both hydrophobic and β-sheet like hydrogen bonding interactions with the backbone of the peptide substrate.



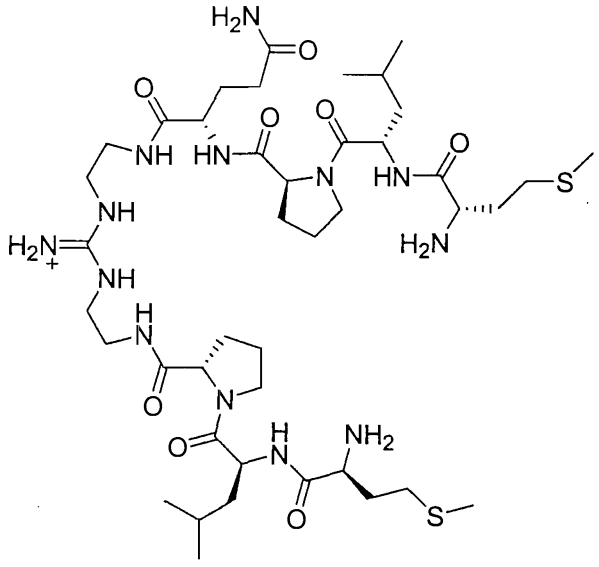
104

Figure 3.8 *Kilburn's fluorescently labelled tweezer receptor*

Fluorescently labelled tweezer receptor **104** was screened against a TentaGel supported library of tripeptides with free carboxylic terminus in a buffered aqueous medium. **104** was found to bind to approximately 3% of the library members and following sequencing of 20 beads, showed 95% selectivity for L-Val-OH at the carboxy terminus of the tripeptides and 40% selectivity for L-Glu(O^{t}Bu) at the amino terminus. The binding constant (K_a = 4 x 10^5 M^{-1} in 17% DMSO/H_2O, pH = 9.2) for one of the

peptides selected from the screening experiments (Cbz-L-Glu(O^{t}Bu)-L-Ser(O^{t}Bu)-L-Val-OH) was measured using titration calorimetry. Even in aqueous media, the guanidinium moiety has been proven to work as a binding site for the carboxylate as the primary binding interaction.

Tweezer receptor **105** (Figure 3.9) proved to be highly selective for peptide guest **106** over the side chain deprotected equivalent guest (>100:1) and enantioselective with an approximate ratio L:D of 10:1.



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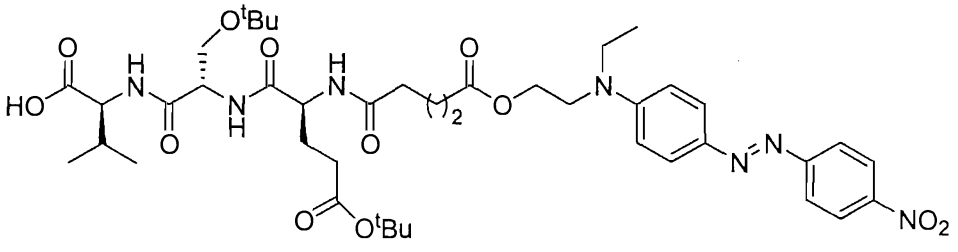


106

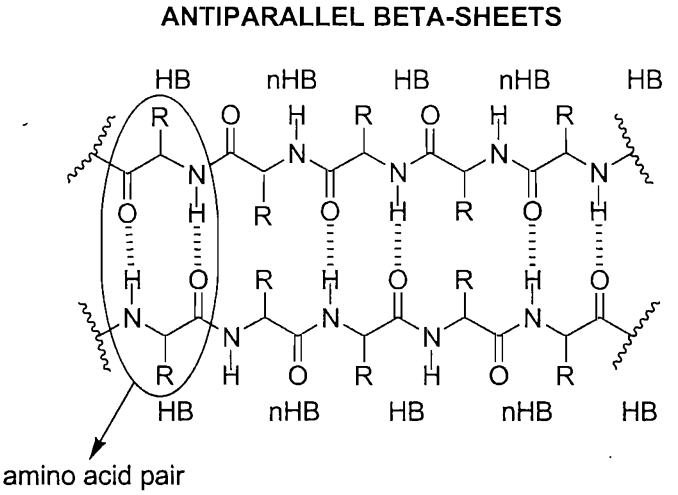
Figure 3.9 *Kilburn's guanidinium tweezer receptor*

UV titrations showed a good fit for the presumed 1:1 binding and allowed an estimate of the binding constant ($K_a = 8.2 \times 10^4\ M^{-1}$).[83a]

3.2 Design concept of guanidinium based tweezer receptors

Selective binding by tweezer receptors is believed to occur through a combination of the primary carboxylate-guanidinium interaction and hydrophobic and hydrogen-bonding interactions, typically β-sheets.

The β-sheet structure is one of the major secondary structures of proteins. β-sheets commonly observed in proteins are composed of nearly fully extended polypeptide chains (β-strands) which interact with each other through hydrophobic and electrostatic forces including van der Waals forces and hydrogen bonding involving the backbone carbonyl and amide proton functionalities on neighbouring strands. The understanding of the β-sheet propensity is essential in conformational studies of proteins but is also important for drug design (for example, proteolitic enzymes such as aspartic acid and serine proteases bind both substrate and inhibitors as β-pleated sheets). In 1951 Pauling and Corey proposed detailed structures for both parallel and anti-parallel sheets in which the backbones of the β-strands adopted a pleated conformation (Figure 3.10).[88] The correct hydrogen bonding pattern for both types of sheets was also proposed at that time. β-sheets come in two basic varieties, parallel (the strands all run in one direction) and anti-parallel (they run in opposite directions).



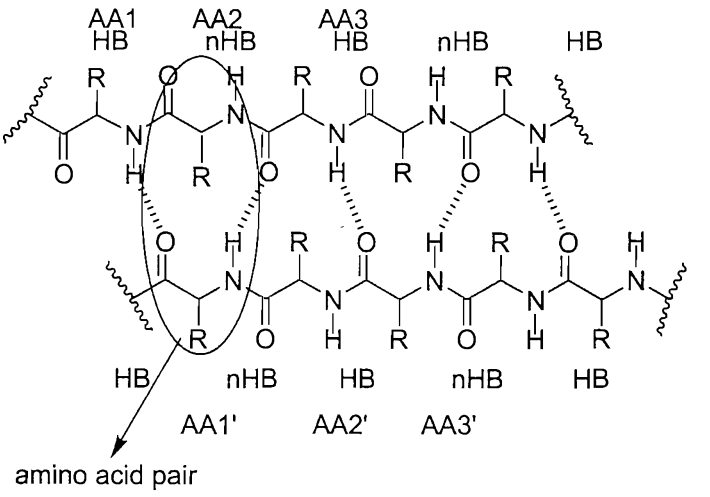


Figure 3.10 *Parallel and antiparallel β-sheets*

Anti-parallel β-sheets are characterised by adjacent antiparallel peptide strands that form an alternating series of 10- and 14-membered hydrogen-bonded rings (each amino acid residue forms two hydrogen bonds with a single residue of an adjacent peptide strand with which it is aligned) (Figure 3.10). Parallel β-sheets are characterised by adjacent parallel peptide strands that form a series of 12-membered hydrogen-bonded rings. Residues (AA1') are not hydrogen bonded to the aligned residues (AA2) of adjacent strands. Instead they form a single hydrogen bond to each neighbour (AA1 and AA3) of the aligned residue (Figure 3.10). Thus, considering only two strands, AA1' is a hydrogen bonding residue (HB) whilst AA2 is a non hydrogen bonding residue (nHB).

β-sheets generally have several strands with approximately six residues per strand. Parallel β-sheets are proposed to be less stable than antiparallel sheets because they are generally composed of five or more strands (anti-parallel can be composed of as few as two β-strands), and are usually buried within the hydrophobic interior of proteins.[89] However, the validity of this assumption is not clear. Computational studies suggest that the nature of the amino acids may determine the relative stability of the two types of sheets.[90]

Studies started by Levitt in 1978[91] showed that the β-branched, the aromatic and cystein side chains (Val, Ile, Phe, Tyr, Trp, Thr, Cys) have the highest tendency to form β-sheets, and glutamine, asparagine, aspartic acid and proline the lowest. The

derived statistical preferences of β-strand forming for the 20 amino acids are consistent with most experimental scales of β-strand propensities in host-guest studies.[92]

Recent studies from Nowick[93] indicate that sequence-selective molecular recognition of antiparallel β-sheets can be achieved through side chain interactions and that patterning valine and threonine residues across the non-hydrogen bonded rings formed at the interface between antiparallel β-sheets can impart dramatic sequence selectivity.

Studies carried out by groups in Sussex and Reading[94] suggest that Thr (nHB)-Asn (HB) and Asn (nHB)-Asn (HB) pairs could be particularly stable in parallel β-sheet formation. In fact when Asn is at the HB position and Thr or Asn are at the nHB position, hydrogen bonds can form between the two pairing residues when the side chains of both residues are orientated into their most preferred conformations,[95] hence enabling a stable interaction. *i.e.* the residues are not only forming a hydrogen bond between themselves, but are also both orientated into their most preferred conformations; therefore two factors contribute to the formation of highly stabilised parallel β-sheets. Thus a tweezer (Figure 3.11, compound **107**) containing alternating asparagine and threonine residues should be ideal for the binding of an all asparagine peptide (Figure 3.11, compound **108**).

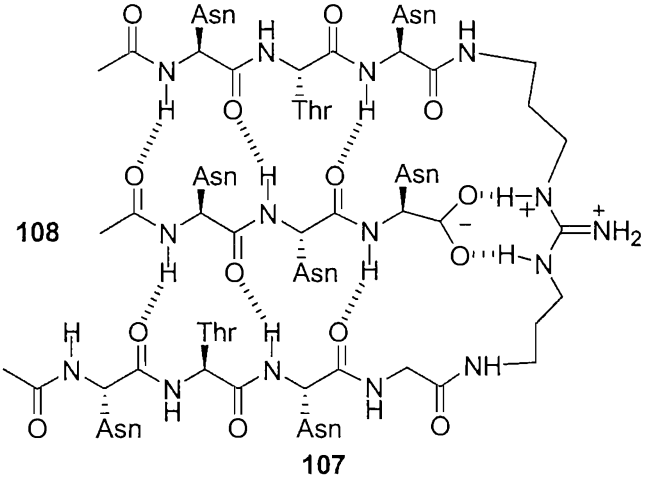


Figure 3.11 *Host-guest complex. Side chains are omitted for clarity*

The side chain interactions between two aligned residues of two different strands should be all favourable as they are exclusively of Asn (HB)-Thr (nHB) or Asn (HB)-Asn (nHB) type (Figure 3.12).

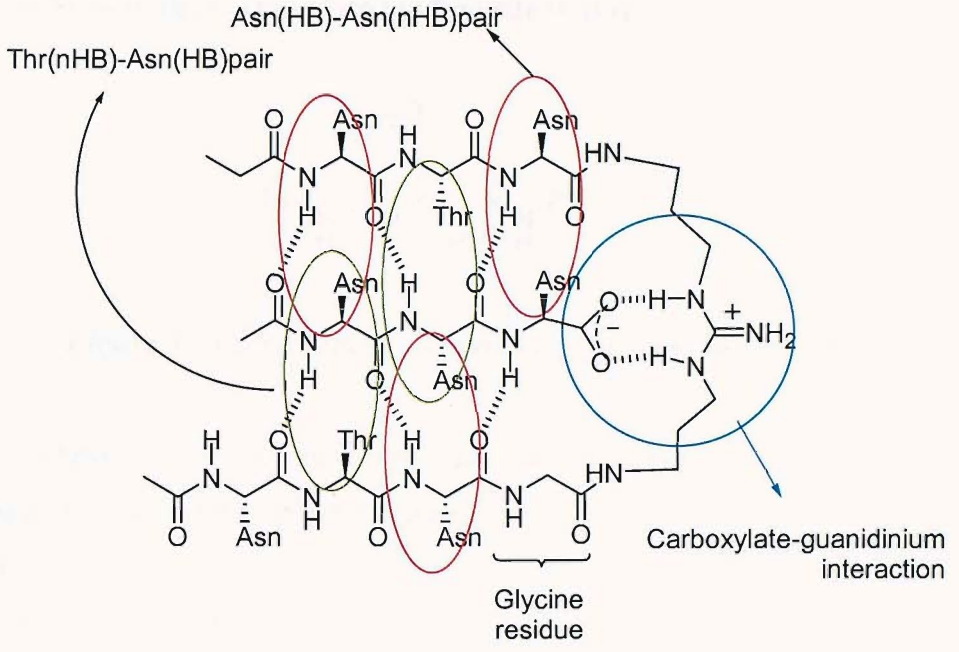
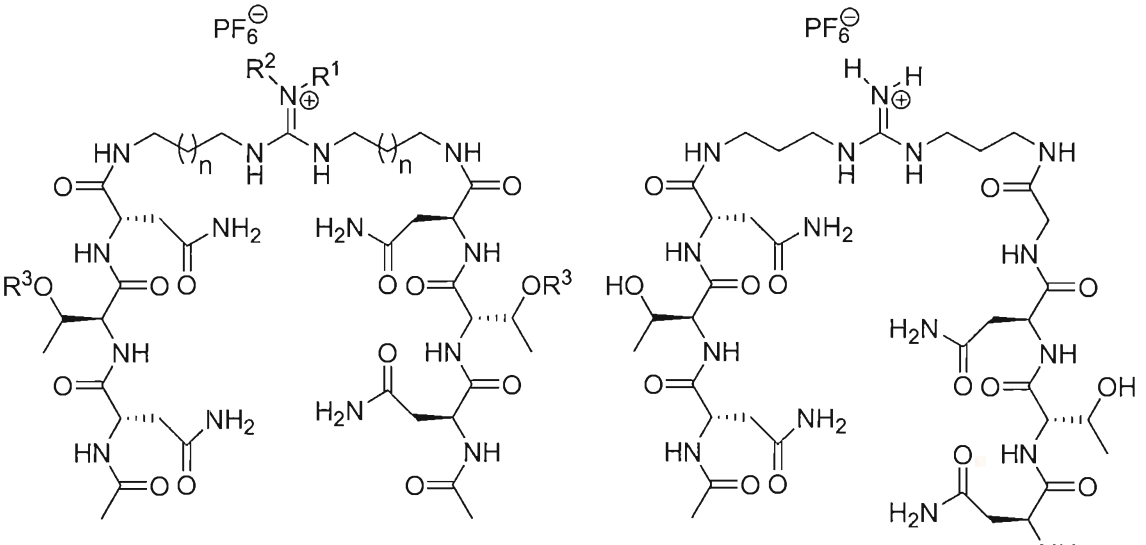


Figure 3.12 *Host-guest complex showing the favourable interactions. The side chains of the amino acids are omitted for clarity*

A family of guanidinium based tweezer receptors (Figure 3.13) with the same or similar structure to that described above were therefore to be synthesised, all containing asparagine and threonine residues.



109 R^1, R^2 = H, R^3 = H, n = 1
110 R^1, R^2 = Me, R^3 = Ac, n = 1
111 R^1, R^2 = Me, R^3 = H, n = 1
112 R^1 = H, R^2 = Me, R^3 = OAc, n =1
113 R^1, R^2 = H, R^3 = H, n = 0

107

Figure 3.13 *Synthesised tweezer receptors*

3.3 Synthesis of the carboxylate binding site (CBS)

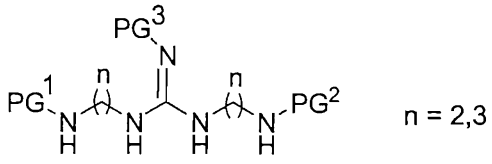


Figure 3.14 *Schematic representation of a guanidinium-based CBS*

The synthesis of tweezer receptors such as the ones in Figure 3.13 requires the preparation of a suitably protected guanidine precursor (the carboxylate binding site, CBS).

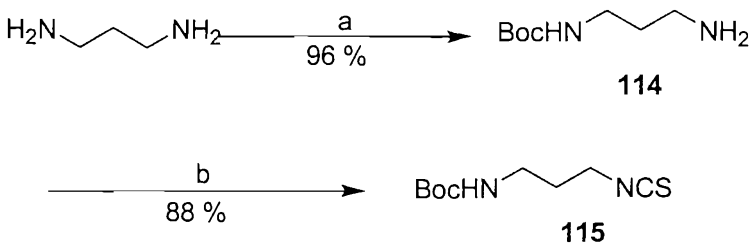
The synthesis of the guanidinium based carboxylate binding site requires the employment of three orthogonal protecting groups (Figure 3.14). In fact an orthogonal protecting strategy enables the synthesis of a symmetrical as well as an unsymmetrical tweezer receptor system. This concept has been previously realized in the Kilburn group by using the Fmoc-Aloc pair of protecting groups for PG^1 and PG^2 [96] but it was found that the deprotection of the Aloc group on solid support was not straightforward. We therefore investigated the use of other protecting groups for the preparation of a suitable CBS.

The tosyl group has been used so far as a protecting group for the guanidinium moiety; its cleavage though requires rather harsh conditions (liquid HF).

3.3.1 Synthesis of the carboxylate binding site using Cbz group

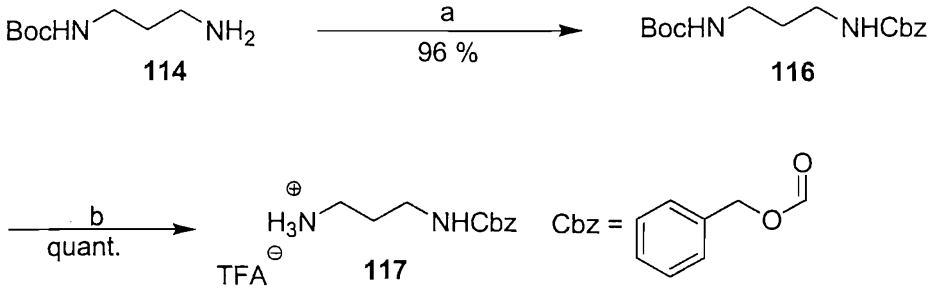
The synthesis of a suitable CBS for the solution phase synthesis of the tweezer receptor was achieved in eight steps with an overall yield of 50% starting from propylenediamine.

The mono-Boc-protection of the propylene diamine was achieved in one simple step, using high dilution conditions. The synthesis of **114** and the subsequent nucleophilic addition to the thiophosgene ("cold" isothiocyanation) following established literature procedure,[106b] afforded **115** in 85% overall yield of the crude product, which has not been purified as the ^{1}H-NMR did not reveal notable impurities (Scheme 3.1).



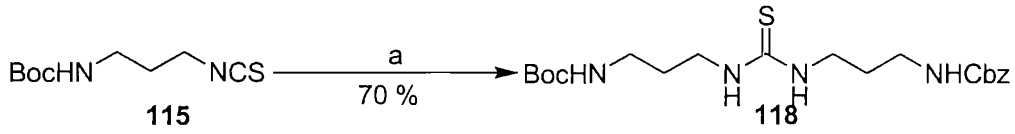
Scheme 3.1 *Reagents and conditions: a) Boc_2O, CH_2Cl_2; b) $CSCl_2$, NEt_3, $CHCl_3$*

As a simple mono-Cbz protection of the diamine in one step did not provide clean products and only modest yields were recorded, a mono-Boc protection followed by Cbz protection of the remaining amino function and subsequent Boc deprotection was employed to give **117** (Scheme 3.2).



Scheme 3.2 *Reagents and conditions: a) CbzCl, sat. aq. $NaHCO_3$, $CHCl_3$; b) TFA, DCM*

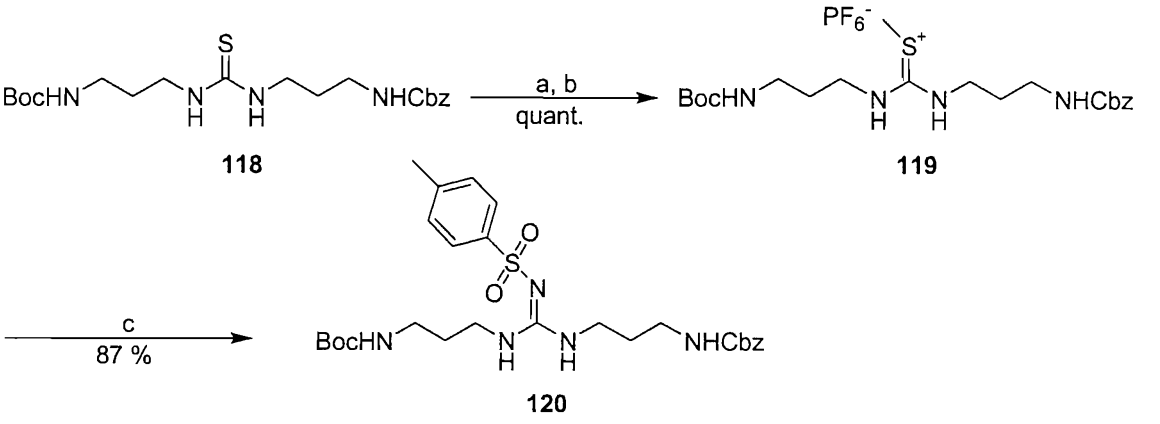
Orthogonally protected thiourea **118** was prepared by reaction of the isothiocyanate **115** with the previously synthesised TFA salt **117**, in presence of a base (Scheme 3.3).



Scheme 3.3 *Reagents and conditions: a) **117**, $CSCl_2$, NEt_3, $CHCl_3$*

Formation of the guanidine moiety was achieved using the classical strategy of activating the thiourea sulphur by alkylation (quantitative yields) and subsequent addition/elimination with an amine. Herein the tosyl amide was used as an amine equivalent to have the suitable tosyl protecting group directly in place, obtaining **120** in

87% yield. Furthermore, DBU was utilised as strong non-nucleophilic base to enhance the addition/elimination step to generate the guanidine system (Scheme 3.4).

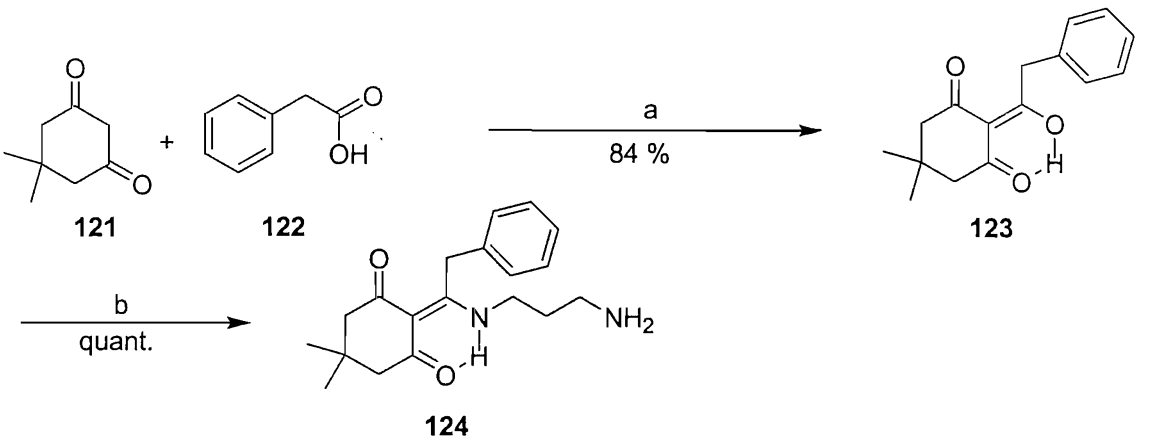


Scheme 3.4 *Reagents and conditions: a) CH_3I, acetone; b) NH_4PF_6, DCM/MeOH; c) $TosNH_2$, DBU, toluene/$CHCl_3$ 4:1*

3.3.2 Synthesis of the carboxylate binding site using Ddpe protecting group

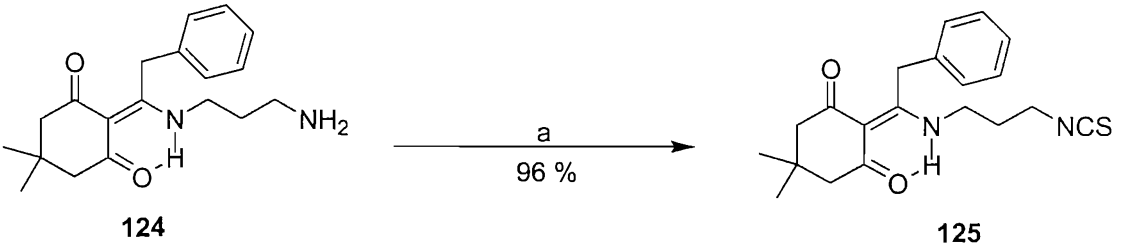
3.3.2.1 Synthesis of the CBS starting from propylene diamine

The solution phase synthesis of a suitable CBS to attach on solid phase and make a library of tweezer receptors was achieved in nine steps with an overall yield of 20 % starting from propylenediamine. The synthesis is similar to that of **120**, but uses Ddpe in place of Cbz as a protecting group as the latter cannot be easily removed on the solid phase, while Ddpe can be removed with 2 % hydrazine. The preparation of the Ddpe protected propylenediamine **124** was achieved in two easy steps with an 84% overall yield of the pure product (about 2 % impurities from ^{1}H-NMR) using the procedure described by Bycroft *et al*[97] for the first step and partially modifying the established procedure[98] for the second. The mono protection was carried out in high dilution conditions, to avoid the formation of the di-protected diamine (Scheme 3.5).



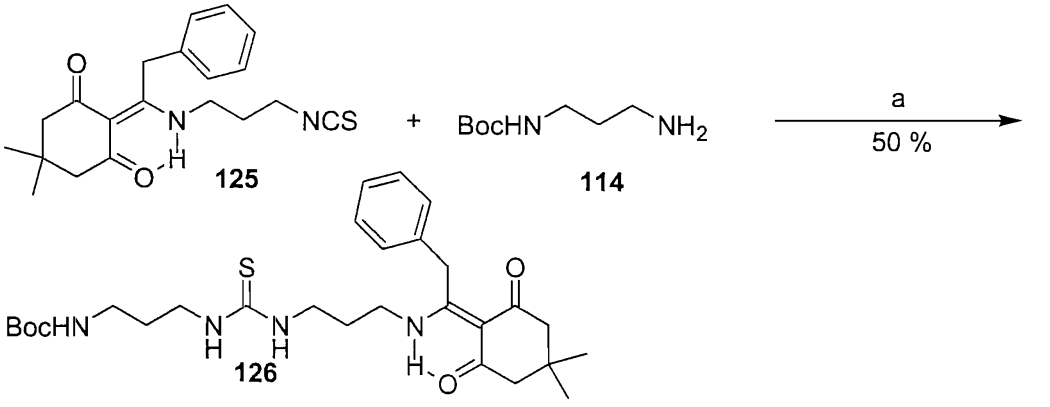
Scheme 3.5 *Reagents and conditions: a) DCC, DMAP, DMF; b) propylene diamine, DCM*

Isothiocyanate **125** was obtained as previously described ("cold" isothiocyanation) (Scheme 3.6).



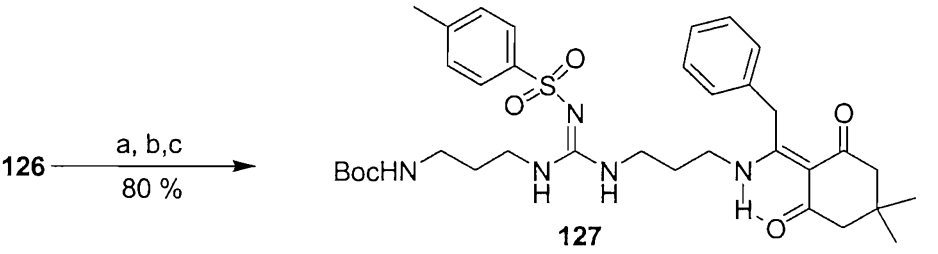
Scheme 3.6 *Reagents and conditions: a) $CSCl_2$, sat. aq. $NaHCO_3$, CH_2Cl_2*

The mono-Boc-protection of the propylene diamine was achieved as previously described and orthogonally protected thiourea **126** was prepared by reaction of **125** with the previously synthesised Boc protected amine **114** (Scheme 3.7).



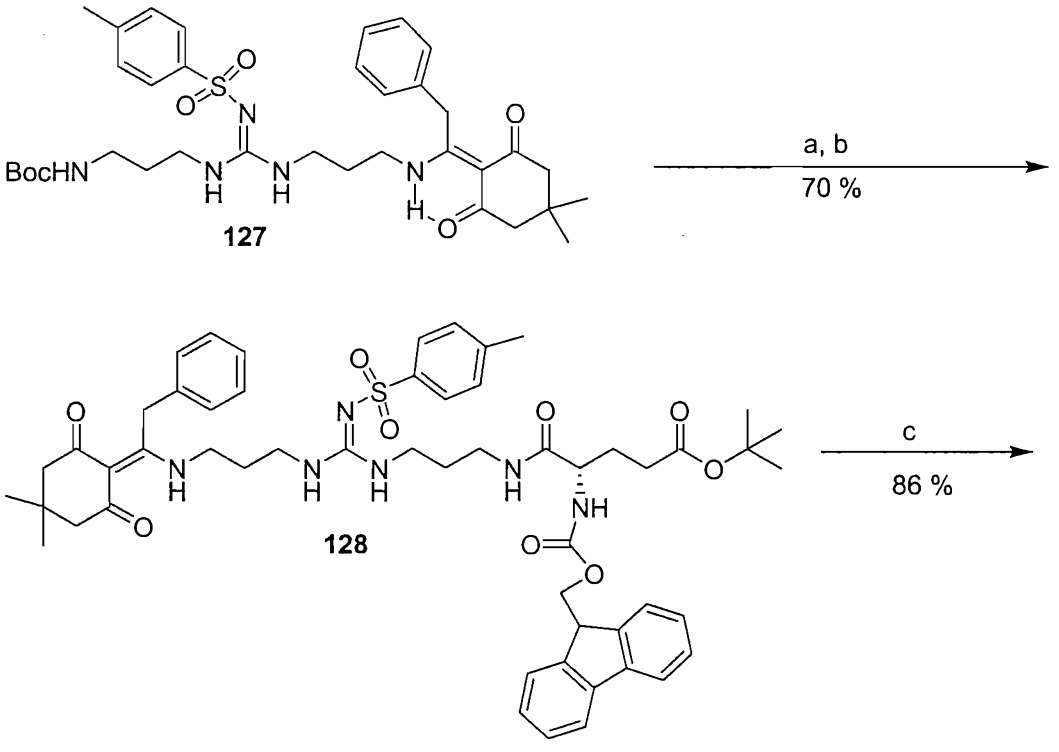
Scheme 3.7 *Reagents and conditions: a) $CHCl_3$, reflux*

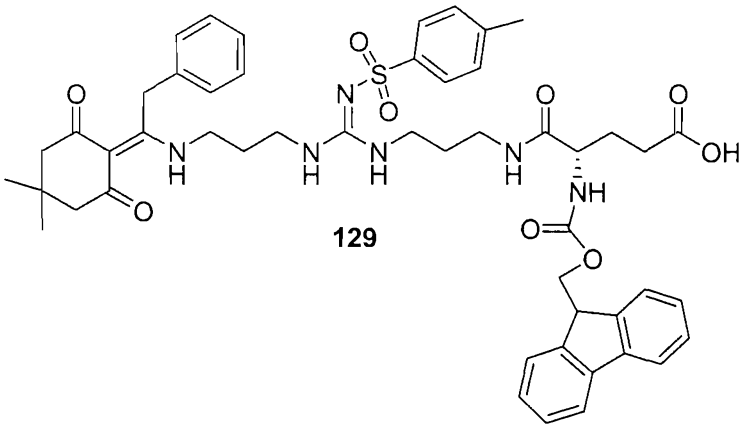
Although it does not give a very good yield (50%) this reaction is straightforward and well suited for large scale preparations; the pretty low yield is due to the fact that the free amine partially cleaves the Ddpe group leading to the formation of DdpeNH$(CH_2)_3$NHBoc. Formation of the guanidine moiety was achieved as previously described obtaining **127** in 80% yield (Scheme 3.8).



Scheme 3.8 *Reagents and conditions: a) CH_3I, acetone; b) NH_4PF_6, DCM/MeOH; c) $TosNH_2$, DBU, toluene/$CHCl_3$ 4:1*

In order to attach the CBS on the solid support, activated *N*-α-Fmoc-L-glutamic acid γ-*tert*-butyl ester was coupled to Boc-deprotected **127**, obtaining ester **128** in good yield (70%). Subsequent deprotection of the γ-acid function gave **129** in very good yield (86 %) (Scheme 3.9).



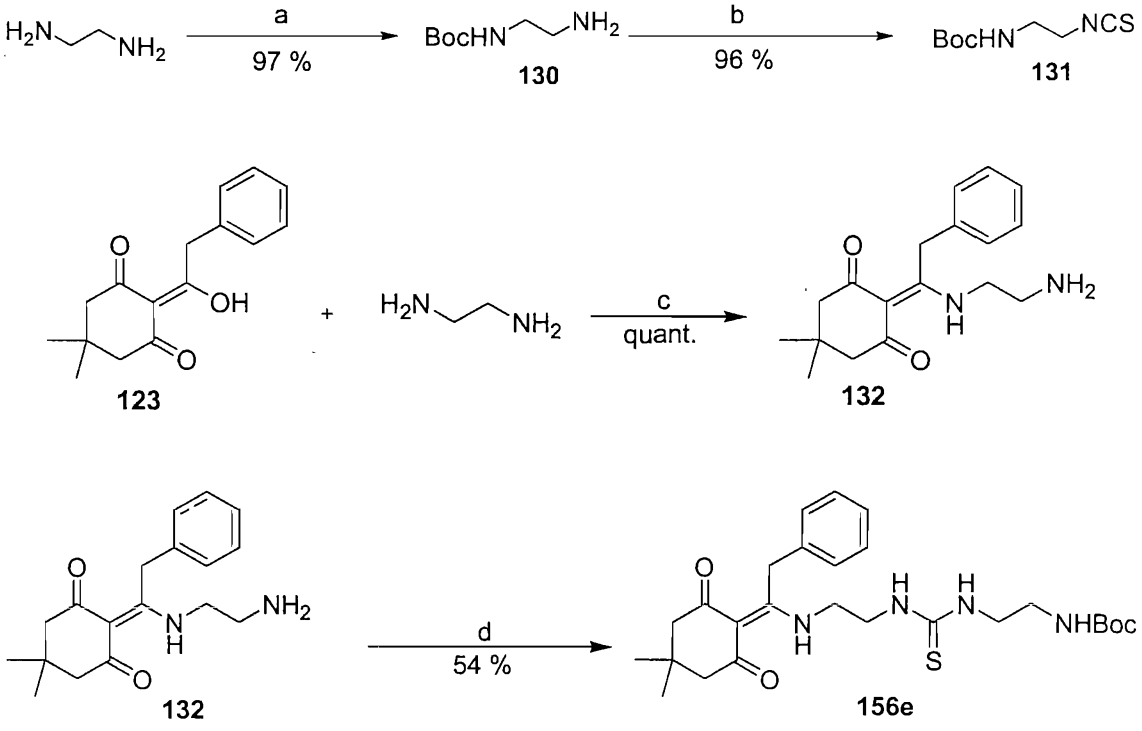


129

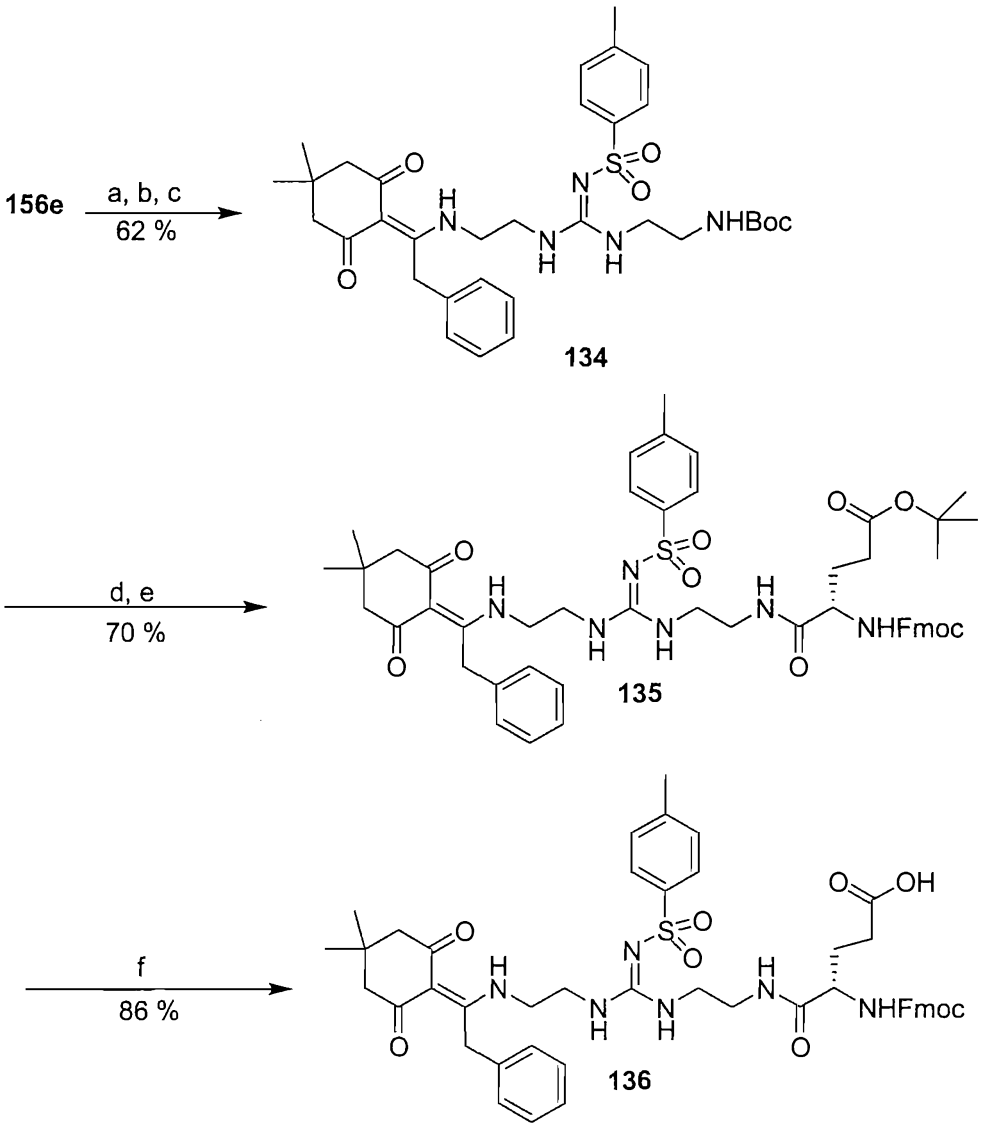
Scheme 3.9 *Reagents and conditions: a) TFA, DCM; b) N-L-Fmoc-Glu-OH, HOBt, PyBOP, DIPEA, DCM; c) TFA, DCM*

3.3.2.2 Synthesis of the CBS starting from ethylene diamine

The same strategy has been used to synthesise an analogous CBS using ethylene instead of propylene diamine to be able to compare the results obtained with two different spacers (Schemes 3.10 and 3.11).



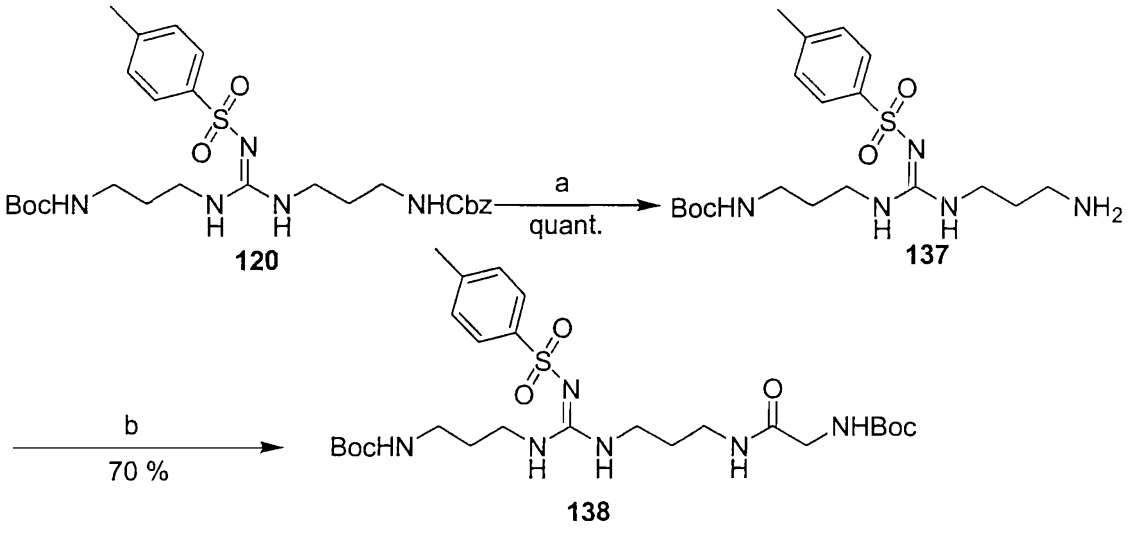
Scheme 3.10 *Reagents and conditions: a) Boc_2O, DCM; b) $CSCl_2$, NEt_3, $CHCl_3$; c) DCM; d)* ***131****, $CHCl_3$, reflux*



Scheme 3.11 *Reagents and conditions: a) CH_3I, acetone; b) NH_4PF_6, DCM/MeOH; c) $TosNH_2$, DBU, toluene/$CHCl_3$ 4:1; d) TFA, DCM; e) N-L-Fmoc-Glu-OH, HOBt, PyBOP, DIPEA, DCM; f) TFA, DCM*

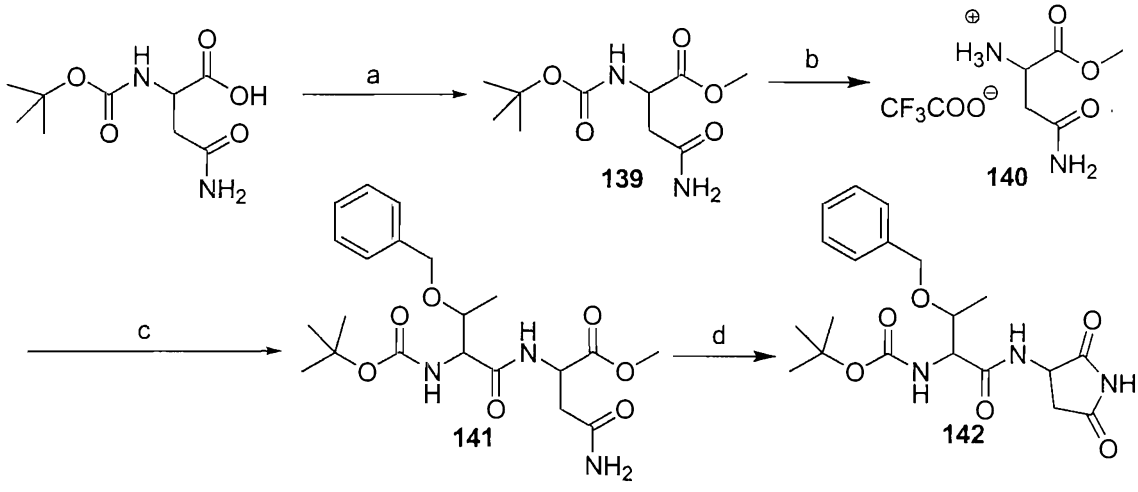
3.4 Synthesis of tweezer receptor 149

The deprotection of Cbz group by hydrogenolysis on activated palladium was straightforward and gave **137** in quantitative yields. Moreover the product was clean and did not need any further purification. The following step, the coupling of the *N*-L-Boc-Gly-OH, gave **138** in 70 % yield after purification by column chromatography (Scheme 3.12).



Scheme 3.12 *Reagents and conditions: a) Pd/C, H_2, MeOH; b) N-Boc-Gly-OH, EDC, HOBt, DIPEA, THF/DMF*

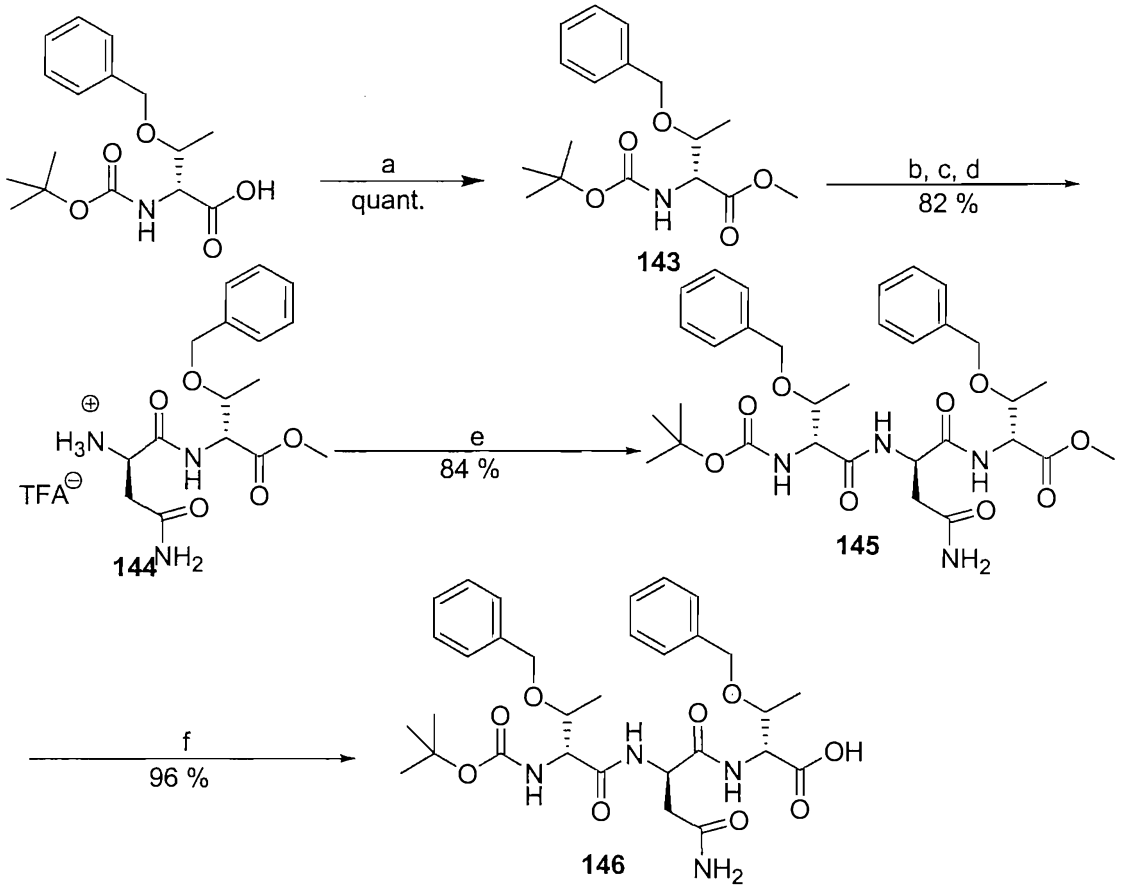
The synthesis of the tweezer receptor **148** was achieved by coupling the amino acids to the CBS using a Boc strategy. This approach is the most suitable for solution phase chemistry since the TFA salt (derived from the Boc deprotection with TFA) can be purified by simple trituration with ether. Since the two side arms contain the same amino acids Asn-Thr-Asn-Thr (apart from glycine), it was decided to couple the CBS to the pre-synthesised tetrapeptide.



Scheme 3.13 *Reagents and conditions: a)K_2CO_3, MeI, DMF; b) TFA, DCM; c) N-Boc-L-Thr-OH, EDC, HOBt, DIPEA, DCM; d) LiOH, H_2O/MeOH*

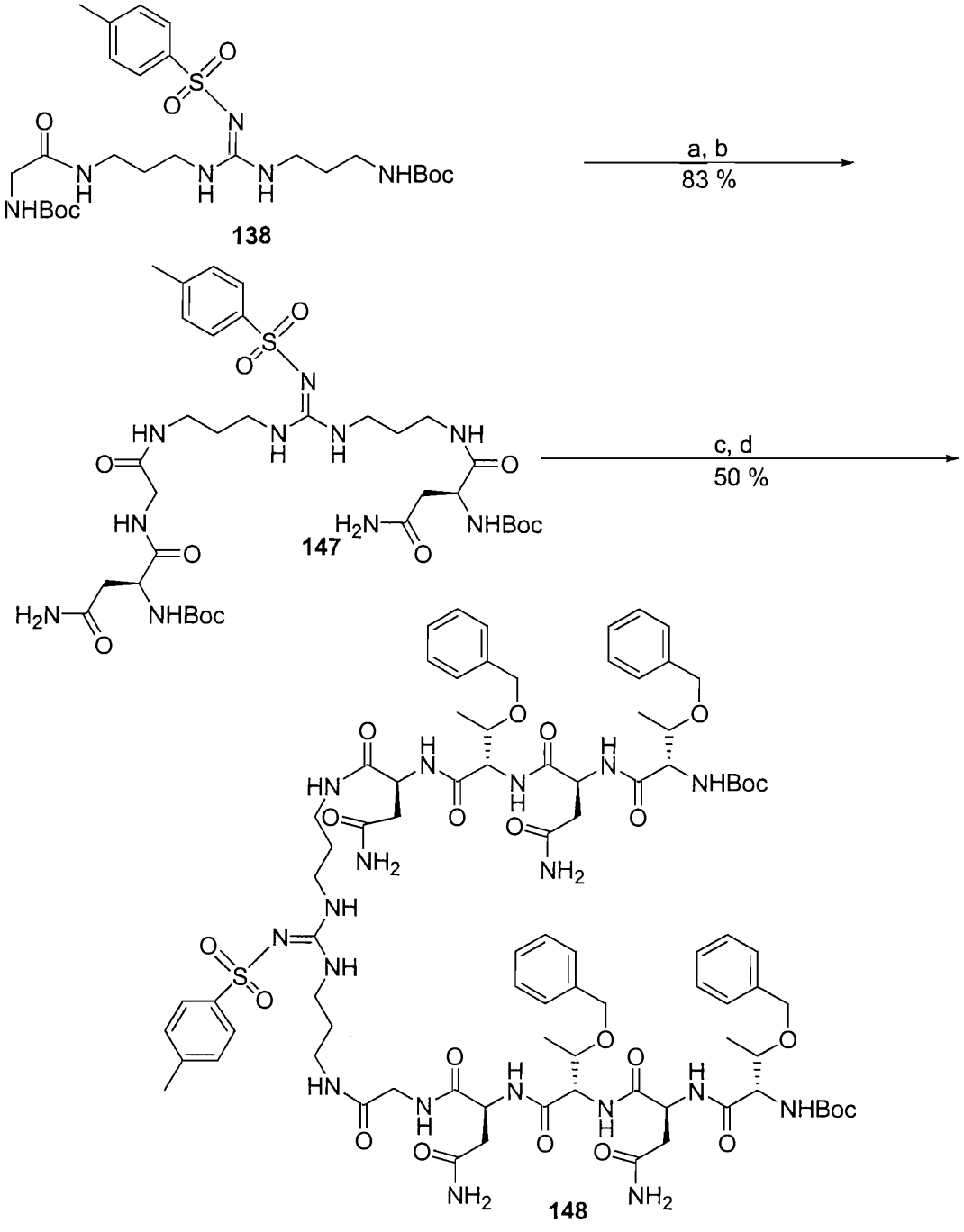
Unfortunately, the deprotection of Asn methyl ester **141** under basic conditions gave problems (Scheme 3.13). In fact, the cyclisation of Asn in basic conditions is very straightforward and cannot be avoided.[99] For that reason tripeptide **146** was synthesised instead of the tetrapeptide (Scheme 3.14), using standard Boc strategy in solution with EDC and HOBt as coupling reagents. The yield of each single amino acid coupling was about 80 %.

Other methods of coupling were tried but gave worse yields because other coupling reagents such as HBTU, PyBOP or carbodiimides alone can cause the dehydration of the side-chain carboxamide (Asn) to the corresponding nitrile.[100] Fortunately this problem is completely avoided when using carbodiimides in combination with additives like HOBt, that is also useful to prevent racemization.



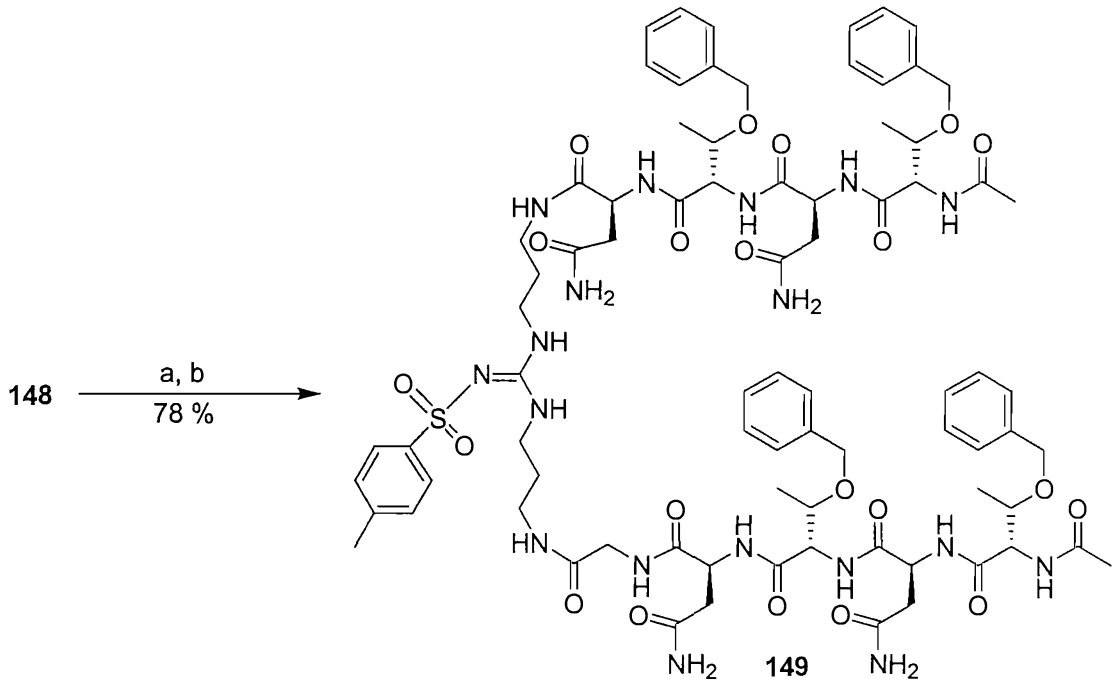
Scheme 3.14 *Reagents and conditions: a) $NaHCO_3$, MeI, DMF; b) TFA, DCM; c) N-Boc-L-Asn-OH, EDC, HOBt, DIPEA, THF/DMF; d) TFA, DCM; e) N-Boc-L-Thr(OBn)-OH, EDC, HOBt, DIPEA, THF/DMF; f) LiOH, dioxane/H_2O*

Tripeptide **146** was coupled to the CBS after having coupled glycine on one arm and Asn on both arms to give the desired tweezer **148** (Scheme 3.15).



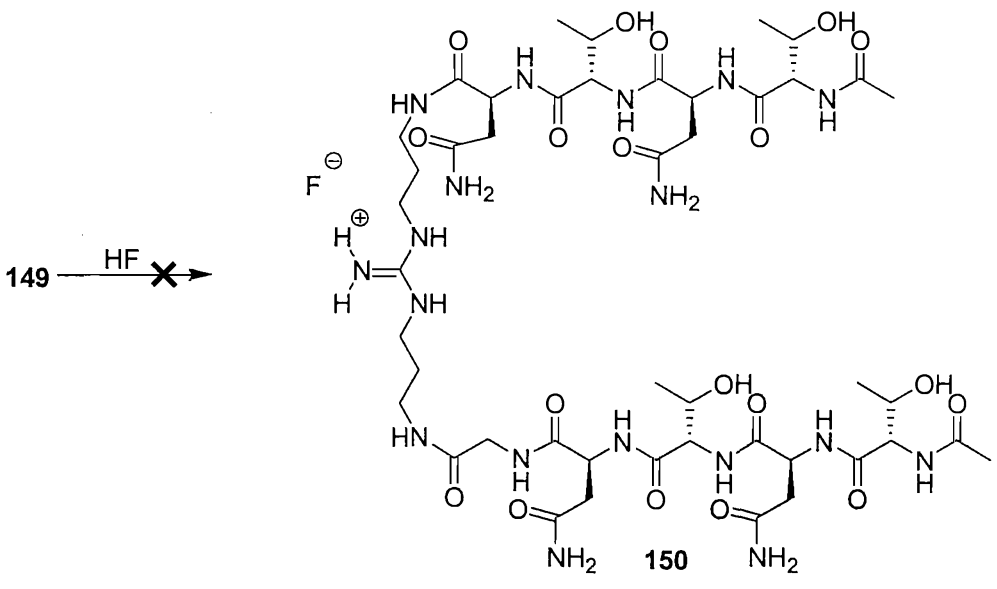
Scheme 3.15 *Reagents and conditions: a) TFA, DCM; b) N-Boc-L-Asn-OH, EDC, HOBt, DIPEA, THF/DMF; c) TFA, DCM; d)* ***146****, EDC, HOBt, DIPEA, THF/DMF*

Subsequent Boc deprotection and acetylation of **148** in dimethylsulfoxide/chloroform (dimethylsulfoxide was the only solvent in which the receptor is soluble) afforded the acetylated product **149** (Scheme 3.16).



Scheme 3.16 *Reagents and conditions: a) TFA, DCM; b) AcCl, NEt_3, DMSO*

Treatment of **149** with liquid HF should have given the fully deprotected tweezer **150** which would have allowed us to carry out binding studies. Unfortunately, the HF treatment was unsuccessful (Scheme 3.17).



Scheme 3.17

This gave us the motivation to develop another protecting strategy for the guanidinium group, which is the subject of the next section.

3.5 Conclusions

A strategy for the synthesis of a single tweezer receptor was developed, but the final deprotection of the tweezer was unsuccessful. However, two CBS's incorporating the Ddpe protecting group have been successfully synthesised for the further synthesis of libraries on solid phase.

The failure of the tosyl as a protecting group for the guanidinium led to the development of another protecting strategy.

3.6 Development of a novel protecting group for the guanidinium moiety

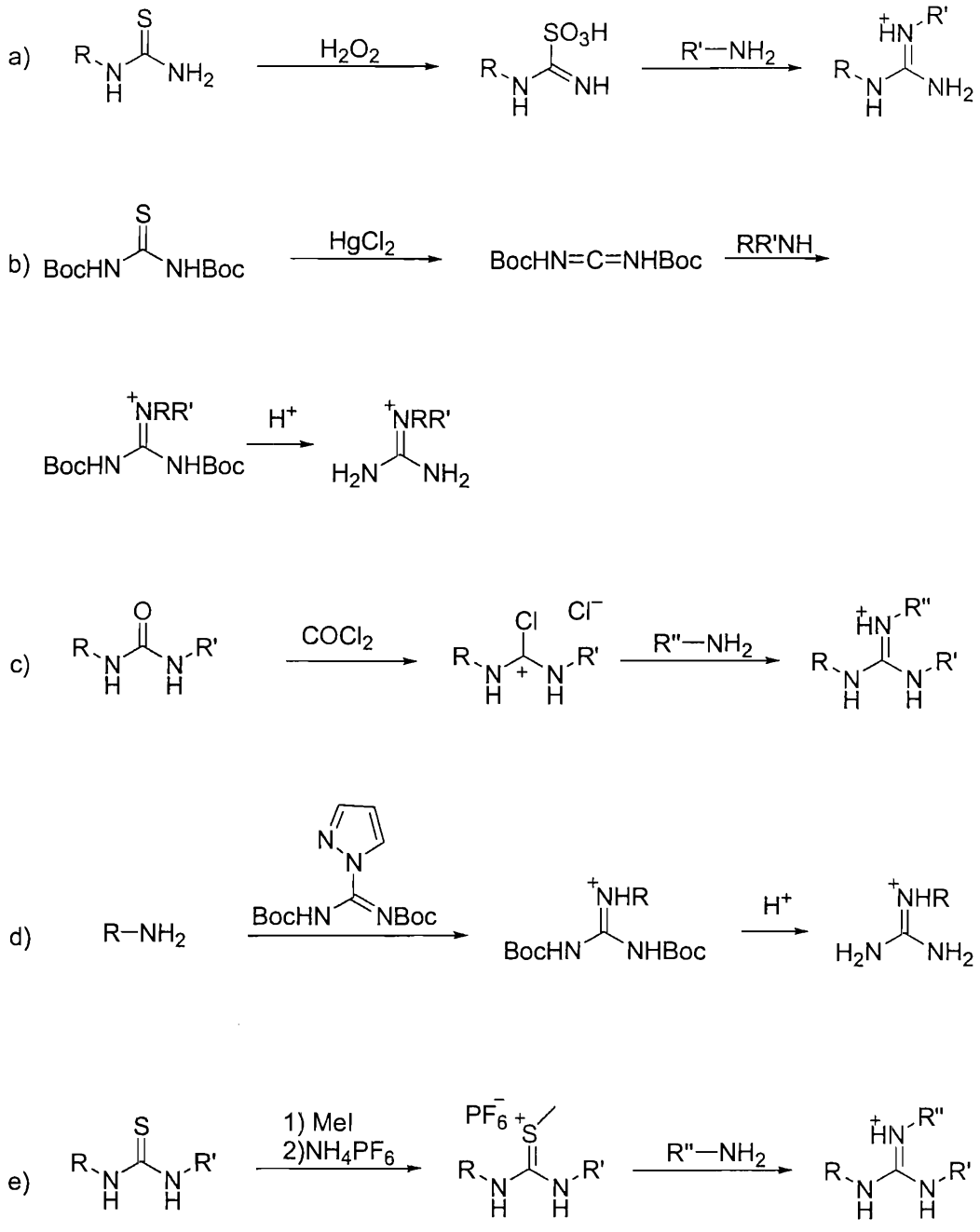
3.6.1 Synthesis of guanidines in solution

Various methods exist for the solution synthesis of guanidines from different starting materials and reagents (Scheme 3.18).[101]

Thioureas have been for example activated by oxidation with hydrogen peroxide or peracetic acid (Scheme 3.18a).[102] Inorganic thiophiles such as mercuric chloride, mercuric oxide and lead oxide have been used to eliminate hydrogen sulfide from thioureas to form carbodiimides, which then react with amines (Scheme 3.18b).[103]

Ureas have been used to prepare guanidiniums using both phosgene and Burgess' reagent (Scheme 3.18c).[104] Other non-thiourea methods for synthesising guanidiniums include the use of cyanamides and reagents based on 1-H-pyazole-1-carboxamidine hydrochloride (Scheme 3.18d).[105]

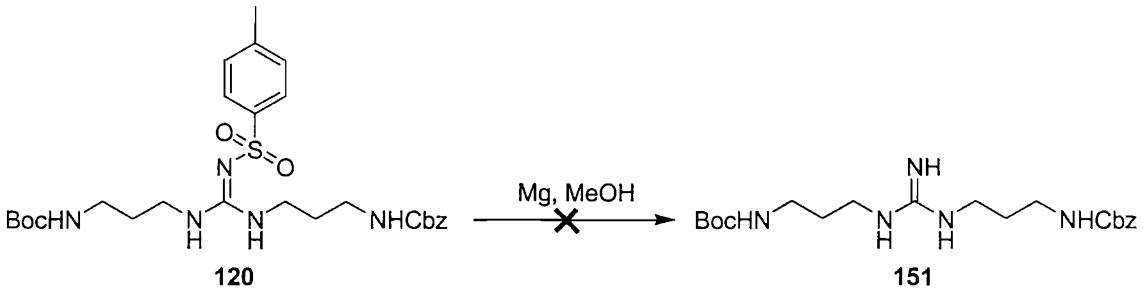
Probably the most common method involves the activation of a thiourea *via* conversion to its thiouronium salt before guanidinylation (Scheme 3.18e).[106, 87]



Scheme 3.18

3.6.2 Trifluoroacetyl as a novel protecting group for the guanidinium moiety

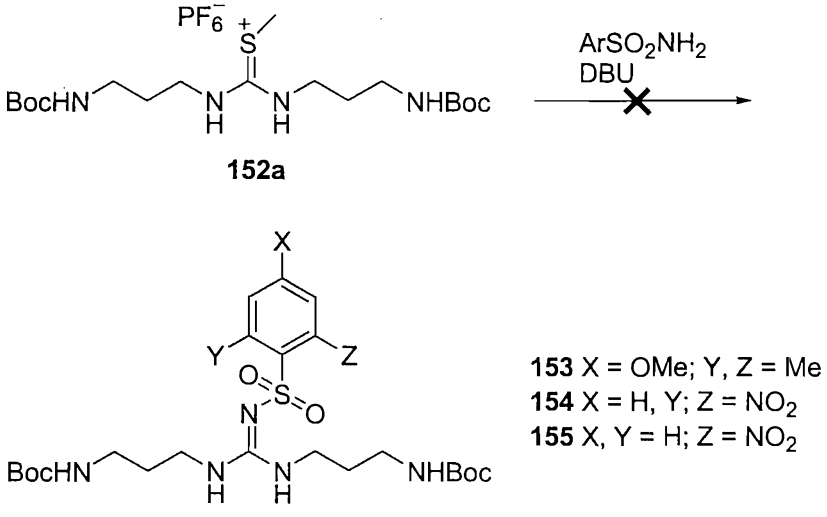
Guanidinylation of a thiouronium worked well using tosylamide for the synthesis of, for example, **148**, but problems arose in the deprotection step using HF. An alternative method for the cleavage of the tosyl group was tried, which had been previously reported to work for amines (Mg powder in MeOH and sonication of the mixture).[107]



Scheme 3.19

Unfortunately this method was not successful and even after two days, the unaltered starting material was recovered (Scheme 3.19).

A series of other feasible protecting groups for our guanidinium-based CBS were then examined, in particular a range of sulfonamides, which, once introduced as protecting groups for the guanidine functionality, could be cleaved under much milder conditions than the tosyl group (Scheme 3.20).



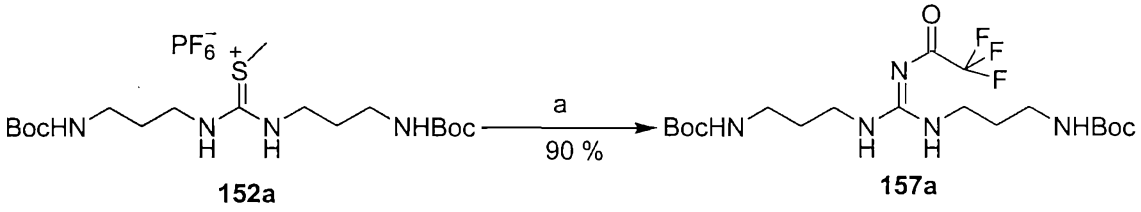
Scheme 3.20

4-Methoxy-2,6-dimethyl-benzenesulfonyl group can be cleaved, on the guanidine functionality of a protected arginine with TFA and 5 % thioanisole,[108] while 2-nitro- and 2,4-dinitro- benzensulfonyl groups were deprotected, on an amine, using thiophenol and potassium carbonate.[109]

The guanidinylation step did not work for compound **153** (Scheme 3.20), and it was thought that the reason could be the less acidic nature of those specific sulfonamide

protons (presence of a strong electron-donating group on the aromatic ring) compared to the ones of the tosyl. Therefore two nitrosulfonamides, featuring electron withdrawing groups on the aromatic ring (NO_2), were also investigated. Surprisingly enough, once again the reaction did not work. A range of solvents and solvent mixtures ($CHCl_3$, Toluene/$CHCl_3$ 1:1 to 4:1, CH_3CN) and several different bases (DIPEA, DBU, DMAP, NaH) were tried, but unreacted starting material was always recovered. The reason might be the steric bulk caused by the ortho substituent on the ring, and also the fact that the amide anion can be stabilised by the nitro groups on the aromatic ring, making the species less reactive.

A carboxylic amide could be used in the guanidinylation step in place of the sulfonamide, but that amide needed to have fairly acidic amide protons. Our attention focused then on the trifluoroacetyl group, which is a well-known protecting group for amine functionality and is easily cleaved[110] but had not been used before as a protecting group for guanidines. The guanidinylation step with trifluoroacetamide worked very well with the bisBoc protected thiouronium **152a** (Scheme 3.21), even better than with the tosylamide, so it was decided to investigate the scope and limitation of the trifluoroacetyl group as a protecting group for guanidines.

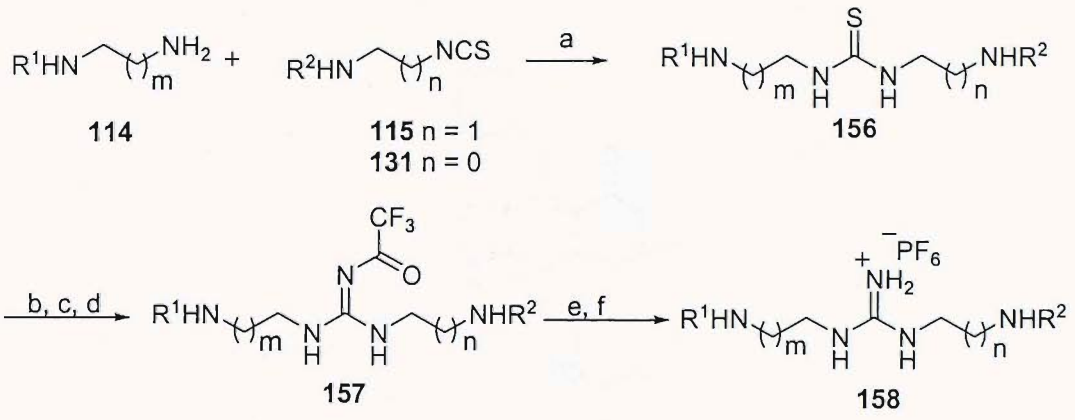


152a **157a**

Scheme 3.21 *Reagents and conditions: a) CF_3CONH_2, DBU, toluene/$CHCl_3$ 4:1*

To gauge the applicability of the trifluoroacetamide guanidinylation procedure, thioureas **156a-f** with different protecting groups were prepared and conversion to the corresponding guanidines was investigated. Thioureas **156a-f** were synthesized from the corresponding amine **114a-f** and isothiocyanates **115** and **131** (Scheme 3.22).

Alkylation of all the thioureas **156a-f** with methyl iodide and counterion exchange gave the thiouronium hexafluorophosphate, which was guanidinylated in the presence of DBU and trifluoroacetamide to give the protected guanidine **157a-f** (Scheme 3.22).

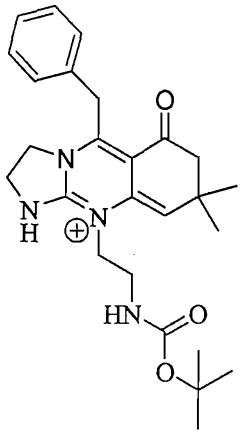


Scheme 3.22 *Reagents and conditions: a) MeOH/DCM, 20°C; b) CH_3I, acetone; c)NH_4PF_6, MeOH/DCM; d) CF_3CONH_2, DBU, toluene/$CHCl_3$, reflux; e) K_2CO_3, MeOH/H_2O; f) NH_4PF_6, MeOH*

Entry	**156/157/158**	R^1	R^2	m	n	**156** [%]	**157** [%]	**158** [%]
1	**a**	Boc	Boc	2	2	75	86	90
2	**b**	Boc	Boc	1	1	75	84	88
3	**c**	Boc	Aloc	1	1	72	92	83
4	**d**	Cbz	Boc	2	1	72	85	90
5	**e**	Ddpe	Boc	1	1	69	45	75
6	**f**	Ddpe	Boc	2	1	78	75	76

Table 3.1

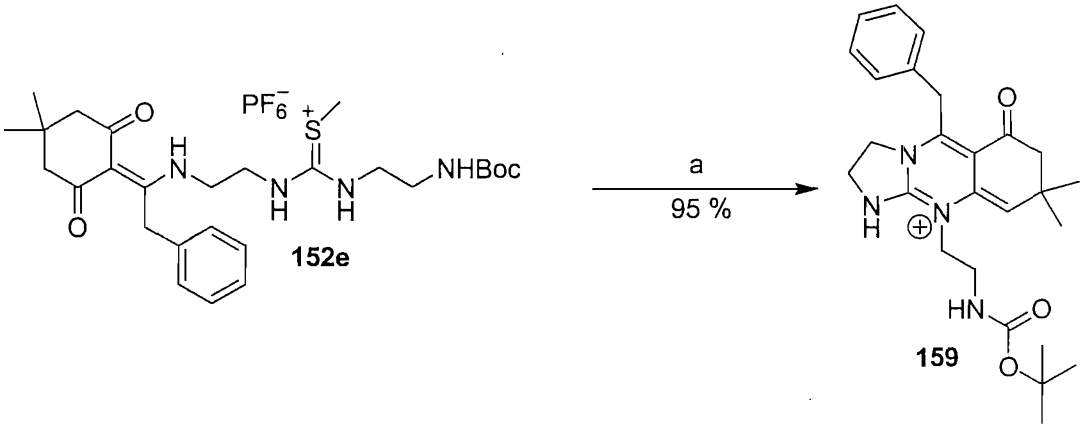
Guanidinylation of thiouroniums proceeded with very good yields for **157a-d** and **157f** (Table 3.1, entry 1-4 and 6), whereas a moderate yield was obtained in the case of **157e** (Table 3.1, entry 5). The reduced yield of **157e** is due to both the partial cleavage of the Ddpe protecting group (R^1) and the formation of a cyclic by-product with presumed structure **159** (Figure 3.15), formed *via* nucleophilic attack of the Ddpe nitrogen onto the thiocarbonyl.



159

Figure 3.15 *Byproduct of the guanidinylation step with Ddpe*

Several reaction conditions were tried reducing the quantity of base and using mercuric acetate in the reaction mixture, but this lead only to 95% yield of compound **159** (Scheme 3.23).

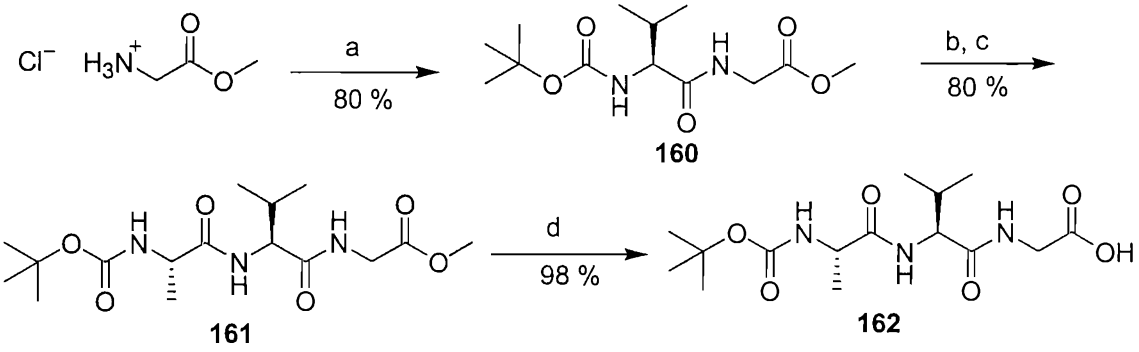


Scheme 3.23 *Reagents and conditions: a) CF_3CONH_2, DBU, $Hg(CH_3COO)_2$, toluene/$CHCl_3$ 4:1*

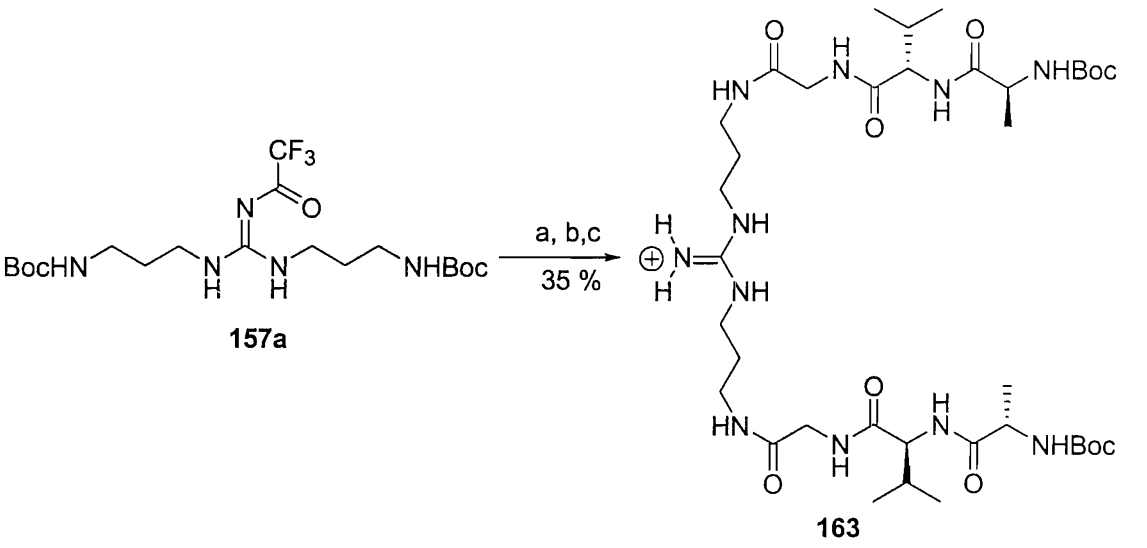
The corresponding by-product is not formed when the longer propylene spacer is used to separate the Ddpe and the guanidine moieties, and consequently **157f** is formed in a much better yield, although some cleavage of the Ddpe protecting group was observed. The guanidinylation was not compatible with the Fmoc group which was cleaved under the basic conditions used, and Fmoc protected variants of **157** could not be prepared directly by this route.

The resulting guanidines **157** could in each case be converted to the corresponding guanidinium hexafluorophosphate **158** (Scheme 3.22) when treated with potassium carbonate in a mixture of methanol and water, without loss of any of the accompanying protecting groups, except, again, in the case of the Fmoc group. However, all of the amine protecting groups, including the Fmoc protecting group, could be cleaved with standard methods (Aloc: $Pd(PPh)_3$, tributyl tin hydride 1.2 eq.,[111] Boc: 20% TFA, dichloromethane, Ddpe: aqueous hydrazine 5%,[97,98] Fmoc: piperidine, dichloromethane) giving quantitative yields of the correspondent free amines and without affecting the trifluoroacetyl protecting group.

In order to show the utility of the new protecting group in peptide chemistry, it was decided to synthesize two tweezers, in which the guanidine has been incorporated. The peptide arms of the tweezer could either be synthesised in solution using a Boc-coupling strategy (Scheme 3.24) or on solid phase using Fmoc strategy (the Fmoc group can be cleaved without affecting the trifluoroacetyl). For the peptide coupling in solution, guanidine **157a** was used as starting material. After Boc deprotection of **157a** and coupling with previously synthesised *N*-Boc-Gly-L-Val-L-Ala-OH **162** (Scheme 3.24) using EDC/HOBt, the trifluoroacetyl guanidine tweezer was obtained in 45% yield. Cleavage of the trifluoroacetyl protecting group afforded the hydrogen carbonate salt of the guanidinium tweezer **163** as a white powder in quantitative yield (Scheme 3.25).



Scheme 3.24 *Reagents and conditions: a) EDC, HOBt, N-Boc-L-Val-OH, DIPEA, DMF/THF; b) TFA, DCM; c) EDC, HOBt, N-Boc-L-Ala-OH, DIPEA, DMF/THF; d) LiOH, dioxane/H_2O*



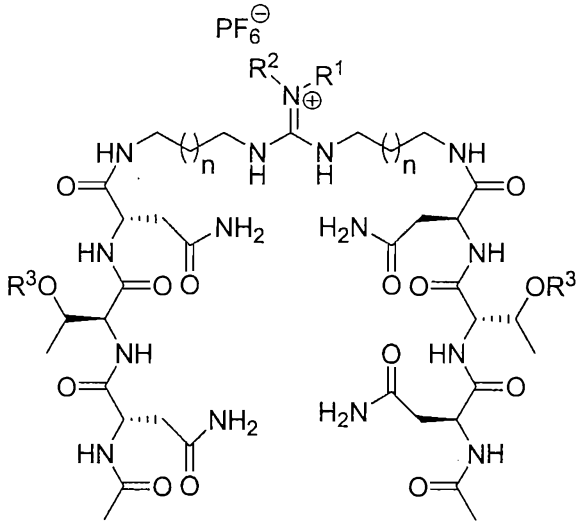
Scheme 3.25 *Reagents and conditions: a) 30% TFA, DCM; b) N-Boc-Gly-L-Val-L-Ala-OH, EDC, HOBt, DIPEA, DMF (45%); c) K_2CO_3, MeOH/H_2O.*

3.7 Conclusions

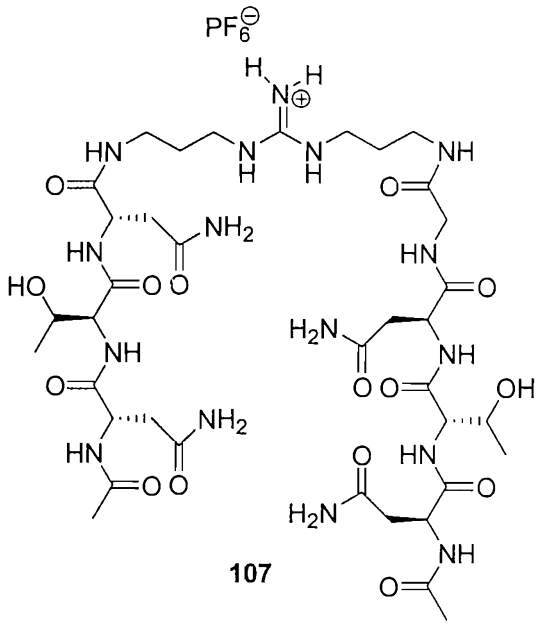
The trifluoroacetyl moiety has been used as a new protecting group for guanidine functionality. The protecting group is orthogonal to acid cleavable protecting groups as well as Cbz and Ddpe, and is easily cleaved under mild basic conditions. The utility of the protecting group for the guanidine moiety is shown with examples from peptide synthesis in solution and experiments have been carried out also on solid support (all the presented results have been published in Bartoli, S.; Jensen, K. B.; Kilburn, J. D. *J. Org. Chem.*, **2003**, *68*, 9416-9422). This protecting group may find widespread use in the synthesis of both simple and highly complex guanidine-containing compounds.[112]

3.8 Synthesis of a family of tweezer receptors using the trifluoroacetyl as a protecting group for the guanidinium.

The trifluoroacetyl was used instead of the tosyl, which revealed unsuccessful, to synthesise the family of tweezers previously shown (Figure 3.13).



109 R^1, R^2 = H, R^3 = H, n = 1
110 R^1, R^2 = Me, R^3 = Ac, n = 1
111 R^1, R^2 = Me, R^3 = H, n = 1
112 R^1 = H, R^2 = Me, R^3 = OAc, n =1
113 R^1, R^2 = H, R^3 = H, n = 0



107

Making slightly different tweezer structures incorporating the same amino acid residues would allow the hypothesis according to which asparagine-threonine and asparagine-asparagine residues should interact strongly to form parallel β-sheet structures (discussed above) to be tested. However the length of the strands was limited by solubility problems, and formation of β-sheets is in general not observed for strands with less than six amino acids.[113]

The first class of receptors in Figure 3.13 is "symmetrical", with the two side arms incorporating the same sequence of three amino acids (asparagine, threonine, asparagine). Dimethyl guanidinium based equivalent of tweezer **109**, with protected and unprotected threonine (**110-111**) were synthesised to investigate the effect on the binding of the presence of methyl groups on the guanidinium and to prevent the possible binding of the guest to the external face of the tweezer receptor (Figure 3.16).

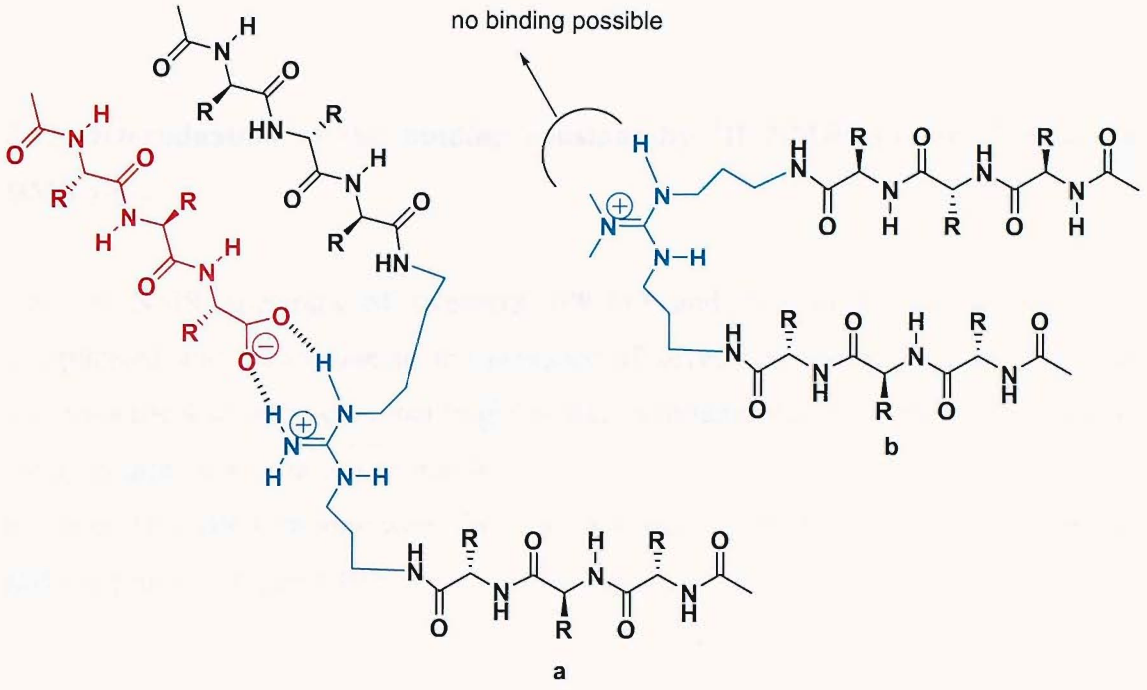


Figure 3.16 *(a) Possible binding on the external face of the tweezer with the guanidinium tweezer; (b) no possible binding on the external face of the tweezer with the dimethyl guanidinium tweezer*

Monomethyl guanidinium tweezer **112** was also synthesised, as it could give more information about the mode of binding. Ethylene diamine has been used as a spacer instead of propylene to synthesise tweezer **113**. In fact the length of the spacer could play an important role in the binding process. For this reason, receptor **107** incorporates a glycine residue on one of the side arms. CPK modelling suggested that an additional Gly residue on one of the side arms, could allow a better alignment of the arms in order to achieve effective side chain interaction in parallel β-sheet formation.

3.9 Guanidinium carboxylate binding sites

3.9.1 Synthesis of symmetrical guanidinium CBS

The synthesis of carboxylate binding sites **157a** and **157b** (Figure 3.17) has been already described previously.

Pages 93 - 97 missing

3.12 Determination of the binding constant by ^{1}H NMR reverse titration in DMSO-d_6.

The ^{1}H NMR spectrum of tweezers **109-113** and **107** in DMSO-d_6 was very complicated and broad, due to the presence of several amino acid residues and an unsymmetrical conformation that might be due to intramolecular interactions involving the guanidinium and the amide bonds.

Reverse ^{1}H NMR titrations were therefore performed, following the shift of the NH and CH protons of guest **108**, whose spectrum is shown in Figure 3.18.

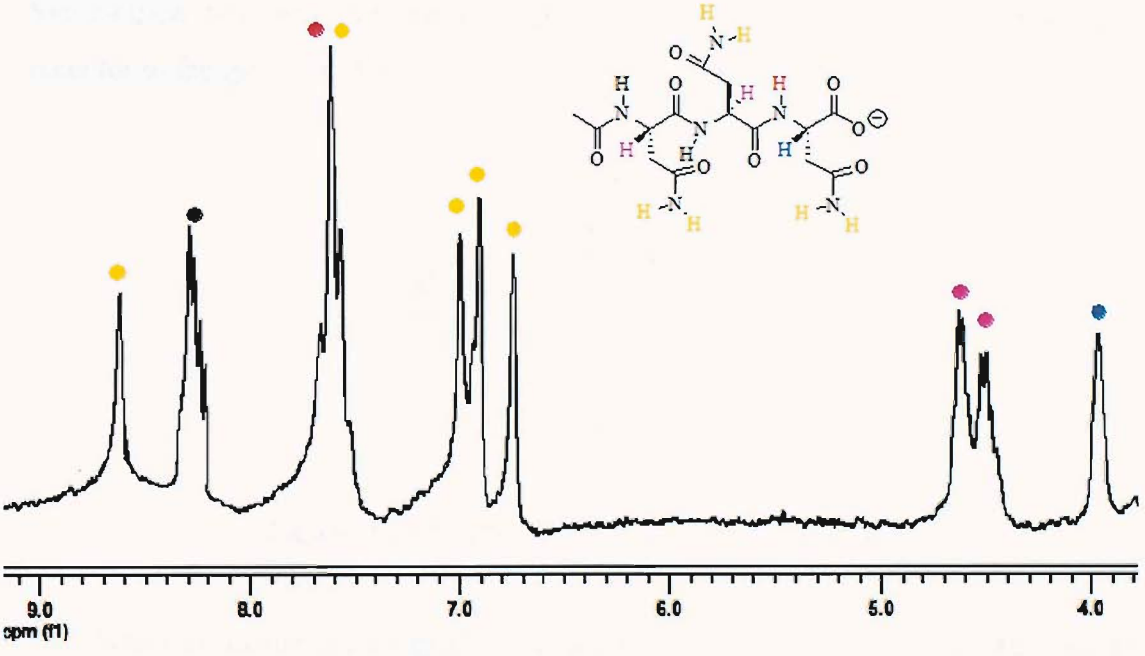


Figure 3.18 *Spectrum of guest* ***108*** *in DMSO-d_6*

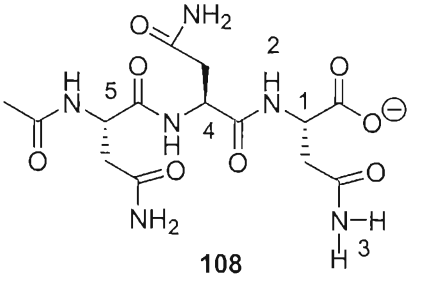
Association constants in DMSO-d_6 for tweezers **109-113** and **107** with guest **108** and for **107** with *N*-Ac-L-Ala-L-Ala-OTBA are reported in table 3.2.

HOST	GUEST	K (M^{-1})
109	*N*-Ac-L-Asn-Asn-Asn-OTBA	3900
110	*N*-Ac-L-Asn-Asn-Asn-OTBA	380
111	*N*-Ac-L-Asn-Asn-Asn-OTBA	370
112	*N*-Ac-L-Asn-Asn-Asn-OTBA	610
113	*N*-Ac-L-Asn-Asn-Asn-OTBA	3600
107	*N*-Ac-L-Asn-Asn-Asn-OTBA	26400
107	*N*-Ac-L-Ala-Ala-OTBA	1300

Table 3.2 *Association constants for tweezer receptors **109-113** and **107***

Addition of 1 equivalent of host to guest **108** led to significant changes in the ^{1}H NMR spectrum of the tripeptide.

Symmetrical tweezers **109** and **113** showed a similar behaviour. On addition of the receptor to the guest, all the signals but one shifted downfield.



108

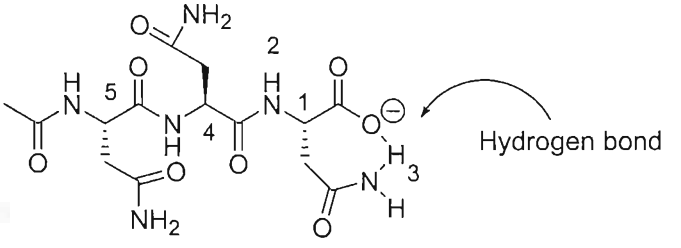
Figure 3.19 *N-Ac-L-Asn-L-Asn-L-Asn-OTBA guest*

The shifts ($\Delta\delta$) of the guest signals on addition of one equivalent of hosts **109** and **113** are listed in table 3.3.

signal	Δδ (ppm)
CH^1	**0.5**
NH^2	**0.4**
CH^4	**0.15**
NH^3	**-1.0**
Other NH's and CH's	**0.1**

Table 3.3 *Δδ of the signals of the guest on addition of one equivalent of hosts **109** and **113***

CH^1 for instance shifted ~0.5 ppm downfield after addition of 1 equivalent of host and NH^2 ~0.4 ppm. The other NH and CH signals all shifted less significantly (0.1-0.2 ppm). One of the NH's of the side chain of the asparagines, which is believed to be the one at the carboxylate terminus (NH^3), shifted a long way upfield (~1.0 ppm), indicating the breaking of a possible intramolecular hydrogen bond (Figure 3.20) in order to bind to the receptor.



108

Figure 3.20 *Possible H-bonding interaction between the carboxylate terminus and NH^3*

The titration curves for NH^2 of peptide **108** on addition of hosts **109** and **113** are reported in Figure 3.21. The data were fitted to a 1:1 binding model.

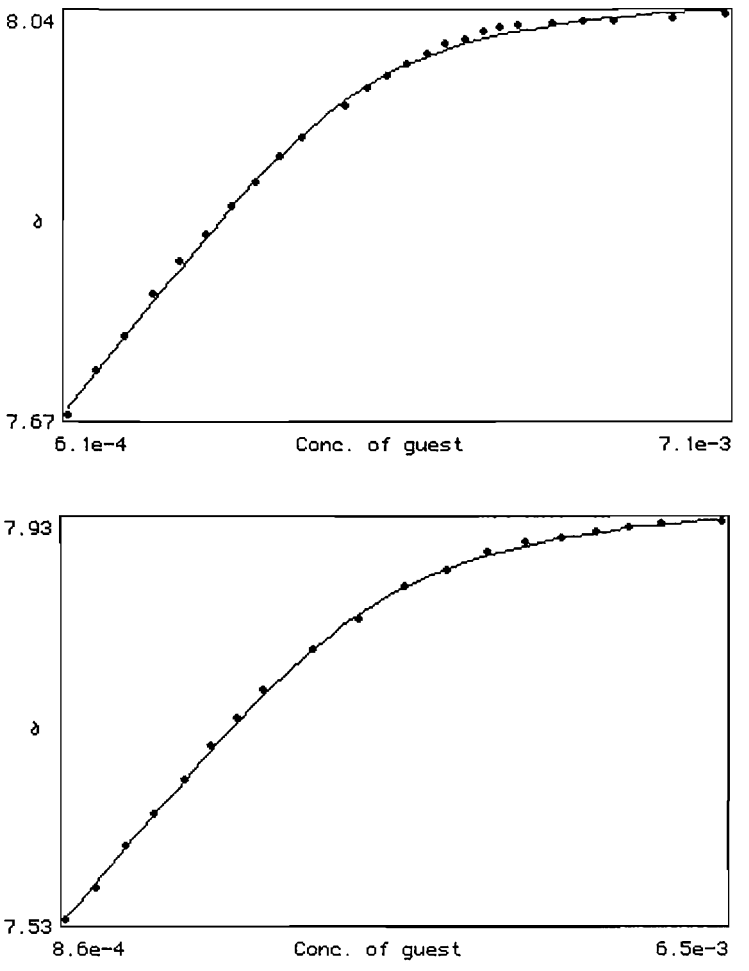


Figure 3.21 *Titration curves for NH[2] of peptide* ***108*** *on addition of receptors* ***109*** *and* ***113***

The binding constants for the two tweezers are K_a = ~ 4000 M^{-1}, results confirmed by the curve obtained for the CH proton and the one obtained for NH[3], which shifts upfield. The presence of an extra methylene unit in the spacer does not have any effect on the binding, but there is evidence that the asparagine residues of the guest are interacting with the residues of the side arms of the tweezers (shift of the NH's) and that all the NH's are somehow taking part in the binding.

Binding of hosts **110-112** did not lead instead to such significant changes (Table 3.4).

signal	Δδ (ppm)
CH^1	**0.15**
NH^2	**0.09**
Other NH's and CH's	**<0.1**

Table 3.4 *Δδ of the signals of guest **108** on addition of one equivalent of hosts **110**, **111** and **112***

CH^1 proton shifted only ~ 0.15 ppm after addition of 1 eq., and NH^2 shifted only of 0.09 ppm. The curves, fitted to a 1:1 binding model, are reported in Figures 3.22-3.24. The binding constants for the monomethyl (K = 610 M^{-1}) and dimethyl (K = 370, 380 M^{-1}) receptors are significantly lower than the ones for the simple guanidinium equivalent.

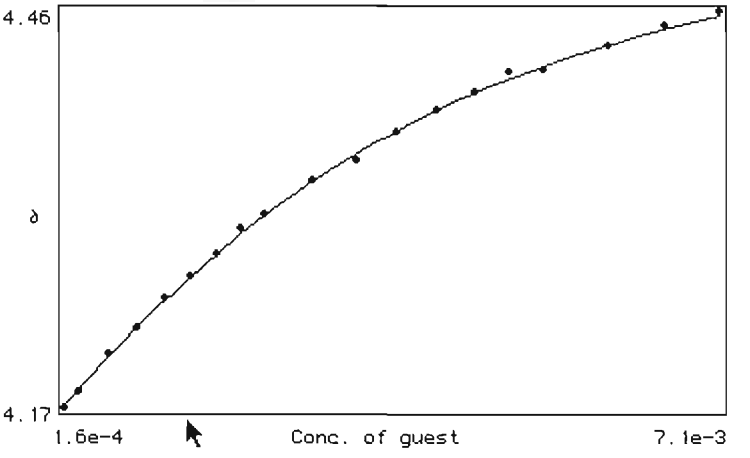


Figure 3.22 *Titration curve for CH^1 of peptide guest **108** on addition of receptor **110***

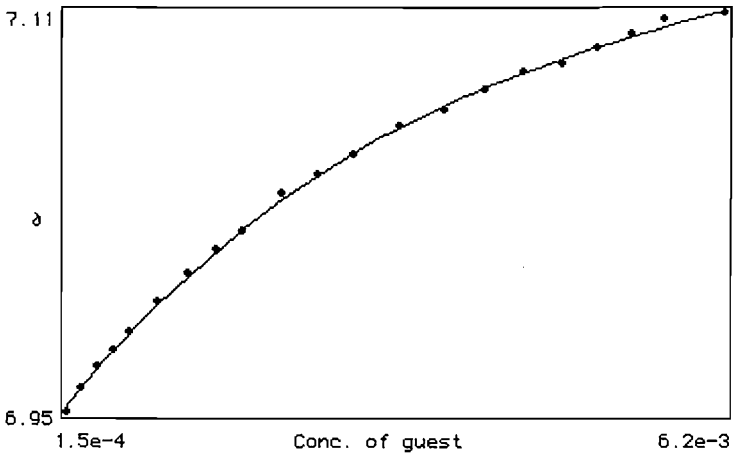


Figure 3.23 *Titration curve for NH^2 of peptide guest* ***108*** *on addition of receptor* ***111***

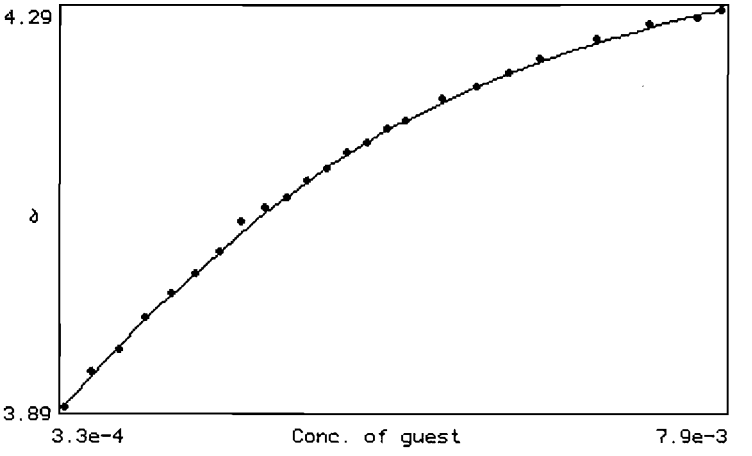


Figure 3.24 *Titration curve for CH^1 of peptide guest* ***108*** *on addition of receptor* ***112***

This result suggests that the dimethyl and monomethyl guanidiniums do not adopt a suitable conformation for binding. In fact it can be hypothesized that a steric clash between the methyl group and the spacer CH_2 forces the receptors in a conformation that does not allow the guest to reach the guanidinium functionality (Figure 3.24).

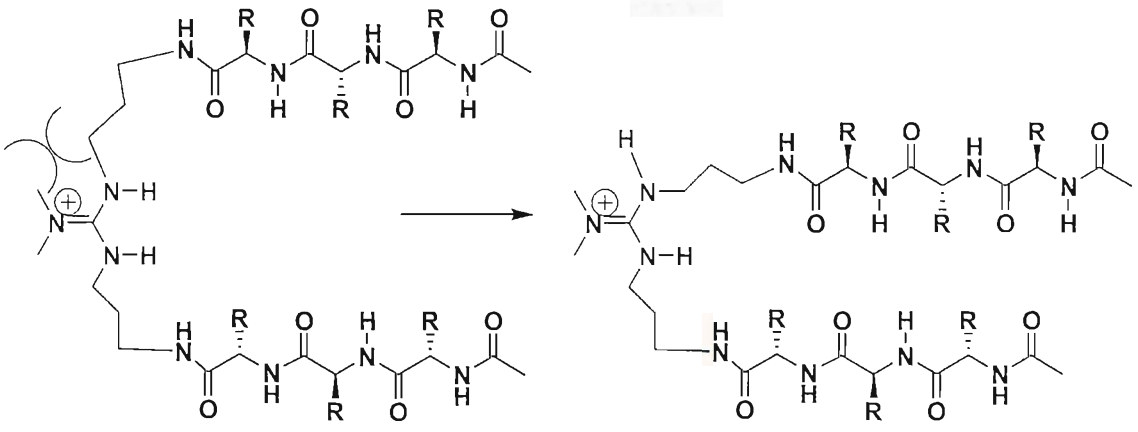


Figure 3.25 *Possible steric clash between the methyl group and the CH_2 of the spacer in methylated guanidiniums*

In fact, the binding constant observed for binding of acetate to guanidinium chloride (K = 1300 M^{-1}) is higher than that found for **110**, **111** and **112** with tripeptide **108**. Receptor **107**, as suggested by preliminary CPK modelling, indeed appeared to be the best choice. The $\Delta\delta$'s of the guest signals are reported in Table 3.5.

signal	$\Delta\delta$ (ppm)
CH^1	**0.6**
NH^2	**0.45**
CH^4	**0.2**
NH^3	**-1.0**
Other NH's and CH's	**0.1-0.2**

Table 3.5 *$\Delta\delta$ of the signals of the guest **108** on addition of one equivalent of hosts **107***

Addition of one equivalent of host led to significant shifts of all the protons of the guest, including CH^4 and CH^5 ($\Delta\delta$ = 0.15 ppm). Both the CH^1 ($\Delta\delta \sim 0.6$ ppm) and NH^2 ($\Delta\delta \sim 0.4$ ppm) curves, fitted to a 1:1 binding model are very sharp, giving a binding constant of 26400 M^{-1}, seven times higher than the one found for **121** and **125**. The curve for NH^2 is shown in Figure 3.26.

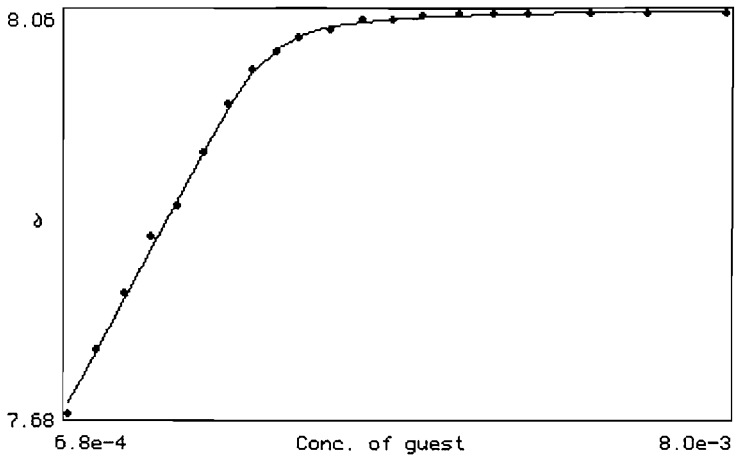
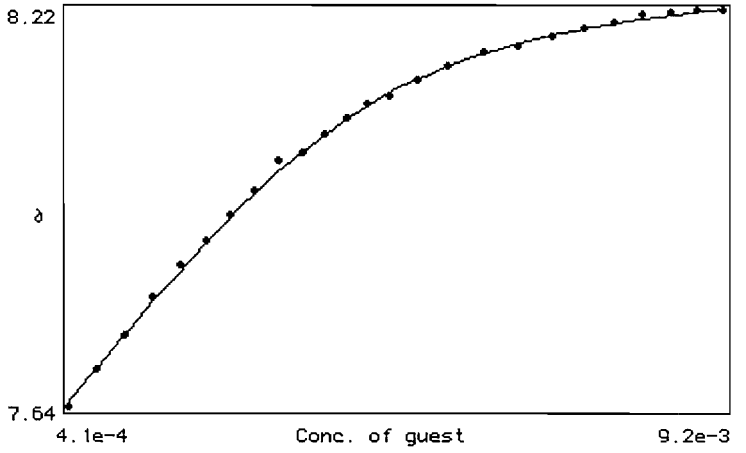
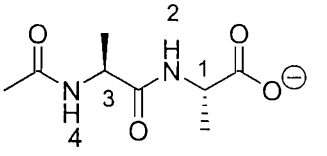


Figure 3.26 *Titration curve for NH^2 of peptide guest **108** on addition of receptor **107***

NH^3 also shifted a long way upfield ($\Delta\delta \sim -1$ ppm).

The extra glycine spacer seems to play an important role providing a better alignment for the additional Asn-Asn and Asn-Thr side chain interactions. To confirm this it was decided to take a model dipeptide, *N*-L-Ala-L-AlaOTBA and to measure the binding constant with **107**. The association curve for NH^2 is reported in Figure 3.27. The association constant is 1300 M^{-1}.





174

NH^2 $\Delta\delta \sim 0.35$ ppm

NH^4 $\Delta\delta \sim -0.1$ ppm

CH^1 $\Delta\delta \sim 0.4$ ppm

CH^3 $\Delta\delta \sim 0.08$ ppm

Figure 3.27 *Titration curve for NH^2 of peptide guest **174** on addition of receptor **107**; list of the $\Delta\delta$ of the signals of **174** on addition of 1 equivalent of **107***

The result seems to suggest that there is a strong selectivity (20 times stronger) of **107** towards **108**, and that additional side chain interactions are crucial for binding.

3.13 Conclusions

Tweezer receptor **107** was found to be, as predicted by CPK modelling, the most selective for the chosen guest. Its binding constant with guest **108** is 20 times higher than the one with the guest *N*-Ac-L-Ala-L-Ala-OTBA (**174**), indicating that some stabilising side-chain interactions are occurring with the chosen guest. The existence of these interactions is confirmed by the fact that all the signals of the guest are moving notably on addition of the host.

EXPERIMENTAL

4.1 General Experimental and Instrumentation

4.1.1 General Experimental

All the reactions described were carried out in solvents of commercial grade. Petroleum ether for column purification was distilled collecting the fraction boiling between 40 and 60 °C. TLC was done on foil backed sheets coated with silica gel (0.25 mm) which contained the fluorescent indicator UV_{254}.

4.1.2 Instrumentation

^{1}H-NMR spectra were obtained at 300 MHz on Brüker AC 300 and Brüker AM 300 spectrometers, and at 400 MHz on a Brüker DPX 400 spectrometer. ^{13}C NMR spectra were obtained at 75.5 MHz on a Brüker AC 300 and at 100 MHz on a Brüker DPX 400 spectrometer. Spectra were referenced with respect to the residual peak for the deuterated solvent.

Infrared spectra were obtained on a Golden Gate FT-IR.

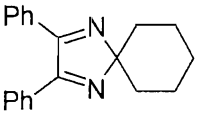
Electrospray mass spectra were obtained on a micromass platform with a quadrupole mass analyser.

All melting points were measured in open capillary tubes using a Gallenkamp Electrothermal Melting Point Apparatus.

4.2 Experimental Procedures

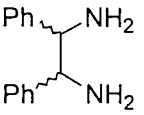
Experimental for chapter 2

2,2-spirocyclohexane-4,5-diphenyl-2H-imidazole,[65] 71



Following a procedure from Corey,[65] a 2 l three-necked round-bottomed flask, equipped with a mechanical stirrer and a reflux condenser, was charged with benzil (90.3 g, 0.43 mol), glacial acetic acid (570 ml), ammonium acetate (229 g, 2.97 mol) and cyclohexanone (46 ml). The mixture was refluxed for 90 minutes and then, while hot, poured into 2 l of vigorously stirred water. The mixture was left to cool down to room temperature. The crystals were collected by filtration, washed three times with water (3 × 100 ml), crushed in a mortar and dried to give compound **71** as yellowish-green powder (122.1 g, 98%): m.p. 103-104°C (lit. 105-106°C); IR (neat) ν_{max} = 2924 (m), 2844 (w), 1555 (w), 1442 (m), 980 (m), 690 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.53-7.33 (m, 10H, ArH), 2.00-1.95 (m, 4H, CH_2C), 1.92-1.65 (m, 6H, CH_2); ^{13}C NMR (400 MHz, $CDCl_3$) δ 164.0 (C), 133.3 (C), 130.1 (CH), 129.0 (CH), 128.3 (CH), 104.2 (C), 34.8 (CH_2), 25.8 (CH_2), 24.2 (CH_2); *m/z* (ES+) 289 $[M+H]^+$.
Data are consistent with those reported by Corey.[114]

(±)-1,2-diphenyl-1,2-ethylenediamine,[65] 72

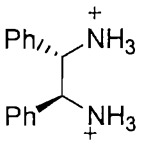


A 2 l four-necked round bottomed flask was equipped with a mechanical stirrer, thermometer, dry ice condenser and compound **71** (70 g, 0.24 mol). The flask was flushed with argon and dry THF (400 ml) was added. The mixture was stirred until all solids dissolved, then cooled to -78°C (dry ice / acetone bath) and treated with a stream

of gaseous NH_3 until the volume increased of 400 ml. Lithium (lithium wire in mineral oil, 6.75 g, 0.97 mol) was slowly added by cutting the wire with scissors (the mineral oil was wiped off with a paper towel). The rate of lithium addition was such that the temperature did not rise above -65°C. The mixture was stirred for 30 minutes and EtOH (30 ml) was slowly added. The mixture was stirred for 20 minutes and NH_4Cl (68 g, 1.24 mol) was added. The cooling bath was removed and the mixture allowed to warm to 0°C. Water (400 ml) was carefully added and the phases separated. The aqueous phase was extracted with ether (3 × 300 ml) and the combined organic layers washed with brine (100 ml), dried over magnesium sulphate and concentrated to about 200 ml. The solution was then transferred to a 1 l round-bottomed flask with a mechanical stirrer, cooled to 0°C and 2 M HCl added (300 ml). The mixture was stirred at room temperature for 1 h, water (500 ml) was added and the phases separated. The organic phase was washed with water (150 ml) and the aqueous layer washed with ether (300 ml). The aqueous phase was then carefully treated with 2 M NaOH (300 ml) and extracted with CH_2Cl_2 (150 ml). The combined organic extracts were washed with brine (100 ml) and dried over magnesium sulphate. The solvent was evaporated under reduced pressure to afford the racemic diamine **72** as a pale yellow solid (29.7 g, 60%): m.p. 81-82°C (lit. 82°C); IR (neat) ν_{max} = 3386 (w), 3354 (w), 3268 (b) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.30-7.20 (m, 10H, PhH), 4.10 (s, 2H, CH), 1.59 (s, 4H, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.0 (C), 127.7 (CH), 127.4 (CH), 126.3 (CH), 62.6 (CH); *m/z* (ES+) 213 $[M+H]^+$.

Data consistent with those reported by Corey.[114]

(1S,2S)-(-)-1,2-diphenyl-1,2-ethylenediamine,[65] 73

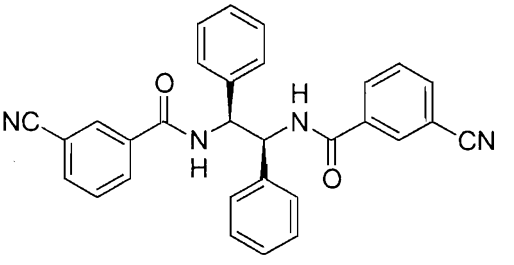


tartrate salt

A 1 l, round-bottomed flask was equipped with a mechanical stirrer and charged with racemic diamine **72** (29.7 g, 0.14 mol), and EtOH (161 ml). The solids were dissolved by heating the mixture to 70°C whereupon a hot solution of (L)-(+)-tartaric acid (20.8

g, 0.14 mol) in EtOH (161 ml) was added. The tartrate salt precipitated while the mixture was cooled to room temperature. The crystals were collected by filtration, washed with EtOH (30 ml) and dried. The solids were then dissolved in boiling water (161 ml), EtOH (161 ml) was added and the solution allowed to cool slowly to room temperature. The crystals were collected by filtration, washed with EtOH (30 ml) and dried. The recrystallization procedure was repeated twice with the same volumes of solvents to give chiral diamine **73** as a tartrate salt (white solid, 22 g, 40%): $[\alpha]_D^{21}$ = -10° (c = 1, DMSO); ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 7.20 (bs, 10H, ArH), 6.11 (bs, 6H, NH_3^+), 4.32 (s, 2H, CHPh), 4.00 (s, 2H, C*H*OH); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 174.5 (C), 138.8 (C), 128.2 (CH), 127.8 (CH), 127.7 (CH), 71.9 (CH), 59.5 (CH). Data consistent with those reported from Corey.[114]

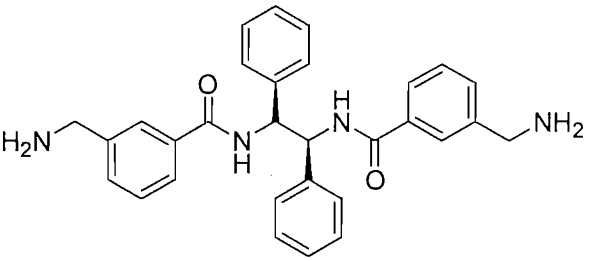
_N,N'_-(1S,2S)-diphenyl-ethyl-1,2-diylbis-(3-cyanobenzamide), 75



Commercially available 3-cyanobenzoic acid (3 g, 0.02 mol) was suspended in CH_2Cl_2 (20 ml) and cooled to 0°C. Triethylamine (3.1 ml, 0.022 mol) was slowly added to obtain a clear solution. Diphenylchlorophosphate (4.15 ml, 0.02 mol) was added and the mixture stirred for 1 h at 0°C. (1*S*,2*S*)-(-)-1,2-Diphenylethylenediamine-L-tartrate salt (3.6 g, 0.01 mol) was suspended in water (15 ml) and K_2CO_3 (4.5 g, 0.03 mol) added. A clear solution was obtained. The diamine solution was added to the mixed anhydride suspension at 0°C. The resulting mixture was stirred for 3 hours (0°C to room temperature). The mixture was poured into a separating funnel and CH_2Cl_2 (100 ml) and water (50 ml) added. The organic phase was separated, washed with saturated aqueous $NaHCO_3$ (50 ml) and 2 M HCl (50 ml). As a gel started to precipitate, the organic layer was not dried over magnesium sulphate. The solvent was evaporated under reduced pressure to give a yellowish solid that was suspended in a saturated $NaHCO_3$ aqueous solution (50 ml), filtered and washed with water (2×20 ml) and

diethyl ether (2×20 ml) to afford **75** as a white solid (5.61 g, 100%): R_f = 0.8 (5% methanol in dichloromethane); m.p. = 210-211 °C; $[\alpha]_D^{23}$ = -102.7° (c = 0.84, DMSO); IR (neat) ν_{max} = 3287 (b), 2981 (w), 2927 (w), 1696 (s), 1631 (s) cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ 9.33 (d, *J* = 8 Hz, 2H, NH), 8.17 (s, 2H, ArH), 8.06 (d, *J* = 8 Hz, 2H, ArH), 7.99 (d, *J* = 8 Hz, 2H, ArH), 7.67 (t, *J* = 8 Hz, 2H, ArH), 7.31-7.10 (m, 10H, PhH), 5.58 (d, *J* = 8 Hz, 2H, NHC*H*); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 164.6 (C), 140.2 (C), 135.6 (C), 134.9 (C), 132.2 (CH), 130.9 (CH), 129.9 (CH), 128.1 (CH), 127.7 (CH), 127.0 (CH), 118.3 (C), 111.5 (CH); 57.9 (CH); *m/z* (ES+) 471 $[M+H]^+$, 963 $[2M+Na]^+$; HRMS (ES+) Calcd. for $C_{60}H_{44}N_8NaO_4^+$ 963.3377. Found 963.3369.

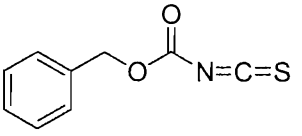
_N,N'_-(1S,2S)-diphenyl-ethyl-1,2-diylbis-3-aminomethylbenzamide, 64



Compound **75** (3 g, 4.33 mmol) was dissolved in a mixture of DMF (20 ml), TFA (8 ml) and methanol (10 ml). Pd/C (300 mg) was then added and the mixture stirred for 48 hours under hydrogen atmosphere, filtered though celite and the solvents evaporated to give a white solid. The compound was then dissolved in MeOH (20 ml) and triethylamine (1.2 ml, 8.7 mmol) was added. A precipitate was formed which was filtered and purified by column chromatography (2% NH_3 saturated MeOH / CH_2Cl_2) to yield compound **64** as a white solid (2.5 g, 83%): R_f = 0.15 (2% NH_3 saturated MeOH / CH_2Cl_2); m.p. = 282-283 °C; $[\alpha]_D^{22}$ = -89.3° (c = 0.7, DMSO); IR (neat) ν_{max} = 3298 (w), 1633 (s) cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ 9.10 (d, *J* = 6.5 Hz, 2H, N*H*CH), 7.56 (s, 2H, ArH), 7.50-7.10 (m, 16H, ArH), 5.67 (d, *J* = 6.5 Hz, 2H, CHPh), 3.89 (s, 4H, NH_2CH_2), 3.00 (br s, 4H, NH_2); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 167.2 (C), 144.6 (C), 141.2 (C), 135.2 (C), 130.3 (CH), 128.4 (CH), 128.3 (CH), 127.8 (CH), 127.3 (CH), 126.5 (CH), 125.6 (CH), 57.8 (CH), 45.8 (CH_2).

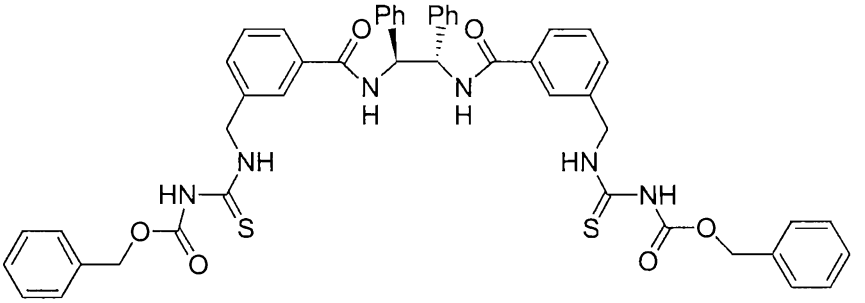
All data according to previously reported.[70]

Benzyloxycarbonyl isothiocyanate, 76



NaNCS (7.0 g, 89 mmol) was suspended in a 20 % mixture of toluene/acetonitrile (100 ml) and benzyl chloroformate (4.2 ml, 29.7 mmol) was added under vigorous stirring. After 2 days the suspension was filtered and the solid washed on the filter with toluene (20 ml). The solution was concentrated and the crude product purified by FC (20 % DCM in petrol ether) to yield 2.0 g of **76** as a yellow oil (35 %): R_f = 0.30 (20% DCM in petrol ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 (s, 5H, PhH), 5.22 (s, 2H, CH_2). According to literature.[67]

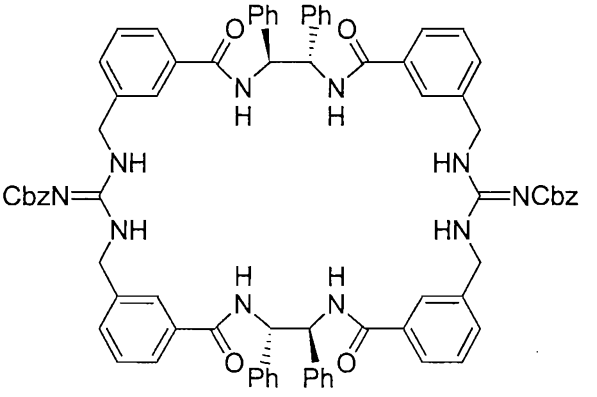
***N,N'*-(1S,2S)-diphenyl-ethyl-1,2-diylbis-[3-(N'-Benzyl-N''-carbobenzyloxy-thioureido)-benzamide, 63**



Amine **64** (250 mg, 0.52 mmol) was dissolved in a mixture of DCM (10 ml) and DMF (1 ml, necessary to dissolve the solid). CbzNCS **76** (252 mg, 1.3 mmol) was added and the mixture stirred for 8 h. A precipitate was formed. The solvents were evaporated under reduced pressure and the residue resuspended in neat DCM. The solid was filtered, washed with DCM (2 × 5 ml) and ether (2 × 5 ml) and dried to yield 330 mg of **63** as a white solid (73 % yield): R_f = 0.25 (3% MeOH in DCM); m.p. = 155-156 °C; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 11.23 (s, 2H, NHCbz), 10.24 (bs, 2H, N*H*CH_2), 9.04 (d, *J* = 7 Hz, 2H, N*H*CH), 7.63 (s, 2H, ArH), 7.50-7.10 (m, 26H, ArH), 5.65 (d, *J* = 6.5 Hz, 2H, CH), 5.17 (s, 4H, CH_2O), 4.30 (br s, 4H, C*H*$_2$NH); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 180.1 (C), 166.6 (C), 153.4 (C), 140.8 (C), 138.2 (C), 135.7 (C), 135.2

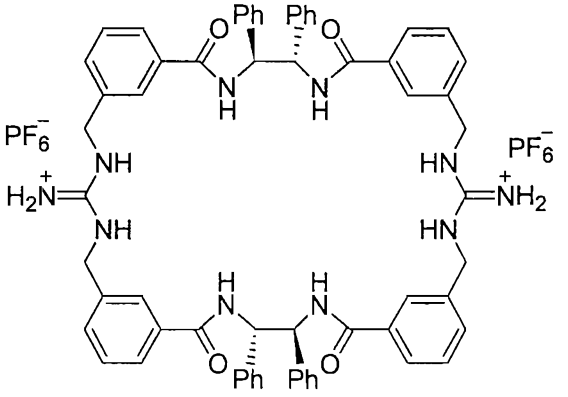
(C), 130.4 (CH), 128.6 (CH), 128.5 (CH), 128.4 (CH), 128.0 (CH), 127.4 (CH), 127.0 (CH), 126.7 (CH), 125.9 (CH), 67.0 (CH_2), 57.5 (CH), 47.8 (CH_2); *m/z* (ES+): 865 (90) $[M+H]^+$, 887 (85) $[M + Na]^+$, 1752 (5) $[2M + Na]^+$.

Macrocycle 66



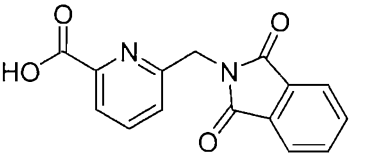
Dithiourea **63** (60 mg, 0.069 mmol) was dissolved in a mixture of DCM (10 ml) and DMF (10 ml) and EDC (53 mg, 0.276 mmol) and DMAP (15 mg, 0.14 mmol) were added. To the vigorously stirred solution, a solution of amine **64** (34 mg, 0.071) in DCM/DMF (10ml/5ml) was added and the mixture stirred for 36 h at room temperature. The solvents were evaporated and the residue purified twice by F.C. (gradient, CH_2Cl_2 to AcOEt/ CH_2Cl_2 5:1; 2 % MeOH in CH_2Cl_2). **66** was obtained as a white solid (28 mg, 29 % yield): m.p. = 171-172 °C; R_f = 0.25 (AcOEt/DCM 1:5); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-7.20 (m, 46H, ArH), 7.12 (bs, 4H, NHCN), 7.04 (d, *J* = 7 Hz, 4H, NHCO), 5.64 (br s, 4H, CHPh), 5.09 (s, 4H, CH_2O), 4.24 (br s, 8H, CH_2NH); ^{13}C NMR (100 MHz, 5% CD_3OD in $CDCl_3$) δ 168.5 (C), 163.9 (C), 159.9 (C), 138.7 (C), 137.1 (C), 134.8 (C), 130.4 (C), 128.7 (CH), 128.5 (CH), 128.3 (CH), 127.9 (CH), 127.8 (CH), 127.7 (CH), 127.6 (CH), 126.0 (CH), 66.8 (CH_2), 59.3 (CH), 44.5 (CH_2); *m/z* (ES+): 639 (100) $[M+2H]^{2+}$; 1276 (30) $[M+H]^+$; Anal. Calcd. for $C_{78}H_{70}N_{10}O_8 \times 2H_2O$: C, 71.43; H, 5.69; N, 10.68. Found C, 71.70; H, 5.54; N, 10.57.

Macrocycle 60



Macrocycle **66** (17 mg, 0.013 mmol) was dissolved in a mixture of MeOH and DMF (1:1, 2 ml). Pd/C (3 mg) was added and the mixture stirred under hydrogen atmosphere for 14 h. The Pd/C was then filtered off through celite and the solvents evaporated. The residue was redissolved in a mixture of DCM (5 ml) and MeOH (1 ml) and 60 % HPF_6 solution in water (10 μl, 0.04 mmol) was added. The solvents were evaporated and the residue suspended in water, filtered and dried to yield **60** as a white solid (13 mg , 77 % yield): m.p. = 183-185 °C; ^{1}H NMR (300 MHz, CD_3SO_2) δ 9.16 (br s, 4H, NHCO), 8.03 (br s, 4H, N*H*CH_2), 7.80-7.00 (m, 40 H, d, ArH + NH_2^+), 5.66 (br s, 4H, CHPh), 4.43 (d, J = 4 Hz, 8H, C*H*$_2$NH); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$) δ 171.5 (C), 141.5 (C), 138.9 (C), 137.5 (C), 133.3 (C), 131.9 (CH), 131.4 (CH), 130.6 (CH), 130.4 (CH), 129.9 (CH), 128.9 (CH), 126.2 (CH), 61.8 (CH), 47.5 (CH_2); *m/z* (ES+): 504 (93) $[M+2H]^{2+}$; 526 (100) $[M + 2Na]^{2+}$; 1008 (30) $[M+H]^+$; Anal. Calcd. for $C_{62}H_{60}F_{12}N_{10}O_4P_2 \times 2H_2O$: C, 55.77; H, 4.83; N, 10.89. Found C, 55.55; H, 4.90; N, 10.77.

6-[(1,3-dioxo-2,3-dihydro-1*H*-2isoindolyl)methyl]-2-pyridine carboxylic acid[115], 78

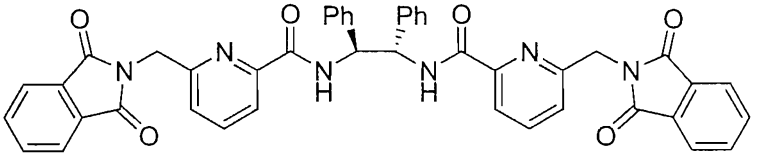


This procedure is modified from that of Olah.[115] To a solution of compound **77**[68] (8.5 g, 0.027 mol) and LiI (16.5 g, 0.12 mol) in acetonitrile (46 ml) was slowly added

chlorotrimethylsilane (14 ml) at 0°C. The reaction was allowed to warm to room temperature and then refluxed for 4 days. After allowing to cool to room temperature, water (200 ml) and CH_2Cl_2 (100 ml) were added. The organic phase was washed again with water (100 ml) and aqueous sodium thiosulfate (10% wt, 150 ml) to remove inorganic salts and residual iodine. The solvent was then removed under reduced pressure yielding compound **78** as a pale yellow solid (6.52 g, 86%) without any further purification: m.p. 219-221°C; IR (neat) ν_{max} = 3327 (b), 3031 (w), 1752 (s), 1703 (s), 1370 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$ / 10% $(CD_3)_2SO$) δ 8.10-7.95 (m, 6H, Pht + Pyr H), 7.31 (t, 1H, J = 4 Hz, ArH), 5.07 (s, 2H, CH_2); ^{13}C NMR (100 MHz $(CD_3)_2SO$) δ 170.3 (C), 168.5 (C), 158.2 (C), 150.6 (C), 140.8 (C), 137.1 (CH), 134.2 (CH), 126.8 (CH), 125.9 (CH), 125.8 (CH), 44.8 (CH_2); *m/z* (ES+) 283 $[M+H]^+$, 305 $[M+Na]^+$.

Data in agreement with previously reported.[70]

_N_-2-(_1S,2S_)-2-[({6-[(1,3-dioxo-2,3-dihydro-1_H_-2-isoindolyl)methyl]-2-pyridyl} carbonyl)amino]-1,2-diphenylethyl}-6-[(1,3-dioxo-2,3-dihydro-1_H_-2-isoindolyl)methyl]-2-pyridine carboxamide, 79

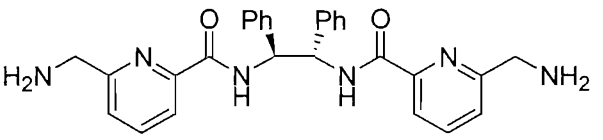


78 (1.84 g, 6.5 mmol) was suspended in CH_2Cl_2 (15 ml) and cooled to 0°C. Et_3N (1.0 ml, 7.15 mmol) was slowly added. A clear solution was obtained. Diphenylchlorophosphate (1.35 ml, 6.5 mmol) was added and the solution stirred for 1 h at 0°C. (*1S,2S*)-1,2-diphenyl-1,2-ethylenediamine-L-tartrate salt **73** (1.18 mg, 3.25 mmol) was suspended in water (7 ml) and K_2CO_3 (2.0 g, 10.7 mmol) added. After 30 minutes, the solution of diamine was added to the mixed anhydride solution at 0°C and the resulting mixture stirred for 2 hours at 0°C, then allowed to warm to room temperature. After 14 hours, the mixture was poured into a separating funnel and CH_2Cl_2 (10 ml) and water (10 ml) added. The organic phase was separated, washed with 2 M HCl (10 ml) and saturated aqueous $NaHCO_3$ (10 ml). The organic layer was dried over magnesium sulphate and the solvent evaporated under reduced pressure. The

crude material was purified by column chromatography (30% ethyl acetate / petrol ether up to 50% ethyl acetate / petrol ether) to afford compound **79** as a white solid (1.1 g, 46%): R_f = 0.3 (neat ethyl acetate); m.p. 106-107°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (d, J = 6 Hz, 2H, NH), 7.86 (d, J = 8 Hz, 2H, PyrH), 7.85-7.65 (m, 8H, Phth), 7.63 (t, J = 8 Hz, 2H, PyrH), 7.22 (d, J = 8 Hz, 2H, PyrH), 7.22 –6.90 (m, 10H, ArH), 5.30 (dd, J = 6, 2 Hz, 2H, C*H*NHPh), 4.97 (d, J = 16 Hz, 2H, PhthNCH^AH^B), 4.92 (d, J = 16 Hz, 2H, PhthNCH^AH^B); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.4 (C), 163.4 (C), 153.5 (C), 148.8 (C), 138.0 (C), 137.5 (C), 133.5 (CH), 131.6 (CH), 127.8 (CH), 127.0 (CH), 123.1 (CH), 123.0 (CH), 120.6 (CH), 58.4 (CH), 41.9 (CH_2); *m/z* (ES+) 741 $[M+H]^+$, 763 $[M+Na]^+$.

Data are consistent with those previously reported.[70]

N2-{(1S,2S)-2-({[6-(aminomethyl)-2-pyridyl]carbonyl}amino)-1,2-diphenylethyl]-6-(aminomethyl)-2-pyridine carboxamide, 65

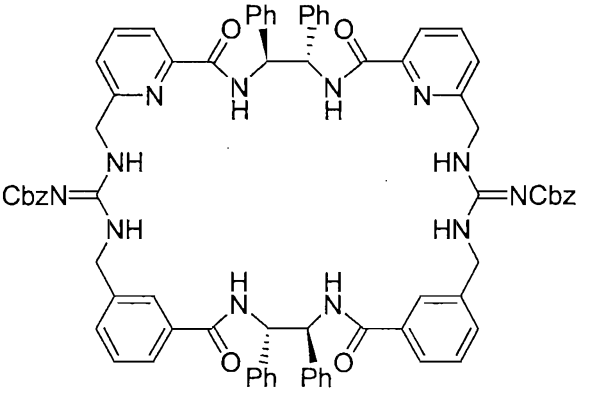


Hydrazine monohydrate (80 µl, 1.64 mmol) was added to a solution of compound **79** (600 mg, 0.82 mmol) in ethanol (5 ml) and the mixture heated at reflux for 8 h. After allowing to cool to room temperature, the solvent was removed under reduced pressure to afford a white solid to which 2 M HCl (5 ml) was added. The solution was refluxed for 30 minutes, after which the insoluble material was filtered off. The aqueous solution was then basified to pH=9 with 1 M NaOH. The precipitated diamine was extracted with CH_2Cl_2 (5 × 10ml). The organic layer was dried over $MgSO_4$ and the solvent evaporated under reduced pressure to afford compound **65** as white crispy foam (330 mg, 84%): IR (neat) ν_{max} = 2362 (w), 1659 (m), 1590 (w) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.17 (s, 2H, NHCO), 7.88 (d, J = 8 Hz, 2H, PyrH), 7.64 (t, J = 8 Hz, 2H, PyrH), 7.21 (d, J = 8 Hz, 2H, PyrH), 7.19-7.10 (m, 10H, ArH), 5.50 (dd, 4H, J = 7, 2 Hz, C*H*NH), 3.93 (d, J = 16 Hz, 2H, NHCH^AH^B), 3.85 (d, J = 16 Hz, 2H, NHCH^AH^B), 2.36 (s, 4H, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.9 (C), 160.4 (C),

149.0 (C), 138.9 (C), 137.8 (CH), 128.6 (CH), 127.9 (CH), 127.8 (CH), 124.0 (CH), 120.4 (CH), 59.5 (CH), 47.2 (CH_2).

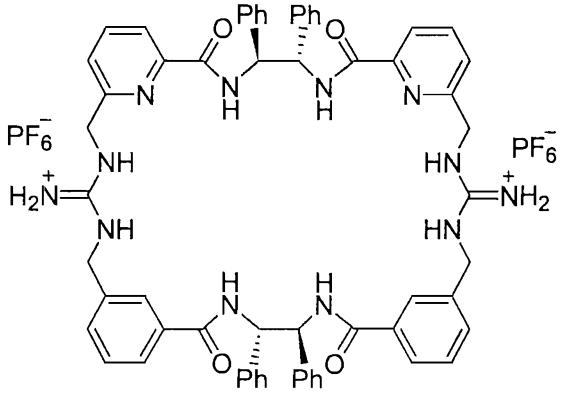
Data are in agreement with those previously reported.[70]

Macrocycle 67



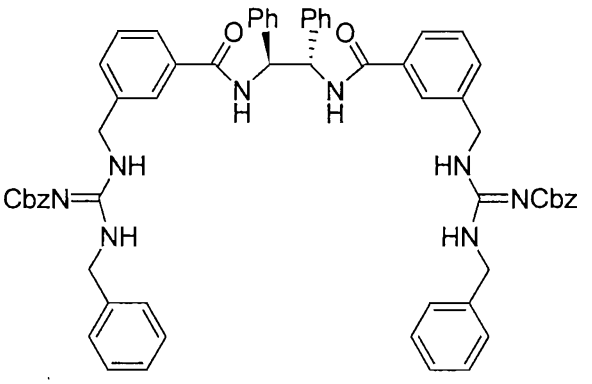
Dithiourea **63** (60 mg, 0.069 mmol) was dissolved in a mixture of DCM (10 ml) and DMF (10 ml) and EDC (59 mg, 0.307 mmol) and DMAP (15 mg, 0.14 mmol) were added. To the vigorously stirred solution a solution of amine **65** (37 mg, 0.077 mg) in DCM/DMF (10ml/5ml) was added and the mixture stirred for 36 h at room temperature. The solvents were evaporated and the residue purified twice by F.C. (gradient, CH_2Cl_2 to AcOEt/CH_2Cl_2 5:1; 2 % MeOH in CH_2Cl_2). **67** was obtained as a white solid (21 mg, 20 % yield): m.p. = 164-165 °C; R_f = 0.25 (AcOEt/DCM 1:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-7.20 (m, 44 H, ArH), 7.12 (bs, 4H, NHCS), 7.04 (d, *J* = 7 Hz, 4H, NHCO), 5.64 (br s, 4H, CHPh), 5.09 (s, 4H, CH_2O), 4.24 (br s, 8H, CH_2NH); ^{13}C NMR (100 MHz, CD_3SO_2) δ 167.5 (C), 164.0 (C), 159.2 (C), 149.0 (C), 139.7 (C), 136.5 (C), 136.3 (C), 134.8 (C), 130.4 (C), 128.6 (CH), 127.8 (CH), 126.7 (CH), 126.6 (CH), 126.4 (CH), 126.0 (CH), 125.8 (CH), 125.5 (CH), 124.4 (CH), 120.3 (CH), 64.9 (CH_2), 57.9 (CH), 43.6 (CH_2), 42.8 (CH_2); *m/z* (ES+): 639 $[M+2H]^{2+}$; 1277 $[M+H]^+$; 1299 $[M + Na]^+$; Anal. Calcd. for $C_{60}H_{56}N_{10}O_6 \times 3H_2O$: C, 68.56; H, 5.60; N, 12.62. Found C, 68.79; H, 5.41; N, 12.28.

Macrocycle 61



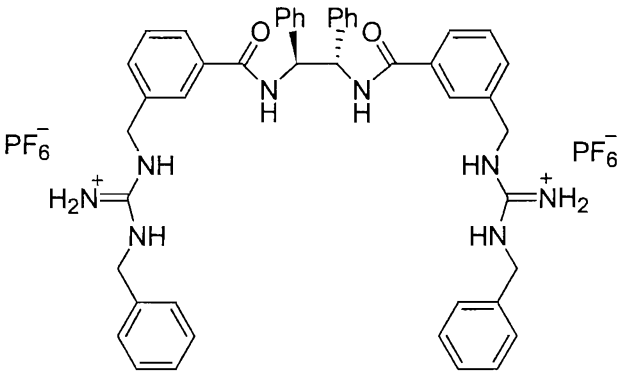
Macrocycle **67** (16 mg, 0.01 mmol) was dissolved in a mixture of MeOH and DMF Pd/C (3 mg) was added and the mixture stirred under hydrogen atmosphere for 14 h. The Pd/C was then filtered off through celite and the solvents evaporated. The residue was redissolved in a mixture of DCM (5 ml) and MeOH (1 ml) and 60 % HPF_6 solution in water (5 µl, 0.02 mmol) was added. The solvents were evaporated and the residue suspended in water, filtered and dried to yield compound **61** as a white solid (12 mg , 71 % yield): decomposes at 250 °C; ^{1}H NMR (300 MHz, CD_3SO_2) δ 9.40-9.30 (br m, 4H, NHCO), 8.31 (br s, 2H, N*H*CH_2), 8.18 (br s, 2H, N*H*CH_2), 7.80-7.00 (m, 38H, d, ArH + NH_2^+), 5.65 (br s, 4H, CHPh), 4.65-4.50 (br m, 4H, C*H*$_2$NH), 4.47 (br m, 4H, C*H*$_2$NH); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$) δ 169.5 (C), 157.1 (C), 149.0 (C), 141.5 (C), 138.9 (C), 137.5 (C), 133.3 (C), 132.1 (CH), 131.9 (CH), 131.2 (CH), 130.6 (CH), 130.4 (CH), 129.9 (CH), 128.9 (CH), 126.2 (CH), 61.8 (CH), 47.6 (CH_2); *m/z* (ES+): 506 (100) $[M+2H]^{2+}$.

_N,N'_-(1S,2S)-diphenyl-ethyl-1,2-diylbis-[3-(N'-Benzyl-N''-carbobenzyloxy-guanidinomethyl)-benzamide, 82



Dithiourea **63** (60 mg, 0.069 mmol) was dissolved in a mixture of DCM (1 ml) and DMF (1 ml) and EDC (53 mg, 0.28 mmol) and trietylamine (40 μl, 0.29 mmol) were added. To the vigorously stirred solution a solution of benzylamine (30 μl, 0.28 mmol) was added and the mixture stirred for 36 h at room temperature. The solvents were evaporated and the residue purified by F.C. (gradient, CH_2Cl_2 to AcOEt/ CH_2Cl_2 5:1). The product **82** was obtained as a white solid (50 mg, 71 % yield): R_f = 0.3 (AcOEt/CH_2Cl_2 3:1); m.p. = 155-156 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (br s, 2H, NHCO), 7.53 (br s, 2H, NH), 7.43 (br s, 2H, NH), 7.30-7.00 (m, 38H, ArH), 5.59 (br s, 2H, CHPh), 4.96 (s, 4H, CH_2O), 4.26 (br s, 4H, C*H*$_2$NH), 4.14 (br s, 4H, C*H*$_2$NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.1 (C), 159.4 (C), 139.1 (C), 134.6 (C), 130.4 (C), 129.0 (C), 128.8 (C), 128.6 (CH), 128.3 (CH), 128.1 (CH), 128.0 (CH), 127.8 (CH), 127.4 (CH), 126.5 (CH), 67.3 (CH_2), 60.0 (CH), 45.5 (CH_2), 44.9 (CH_2); *m/z* (ES+): 506 (100) $[M+2H]^{2+}$, 1011 (80) $[M+H]^+$, 1033 (25) $[M+Na]^+$; Anal. Calcd. for $C_{62}H_{58}N_8O_6$: C, 73.64; H, 5.78; N, 11.08. Found C, 73.27; H, 5.78; N, 10.92.

***N,N'*-(1S,2S)-diphenyl-ethyl-1,2-diylbis-[3-(N'-Benzyl- guanidinomethyl)-benzamide, *bis*hexafluorophosphate salt, 83**



82 (25 mg, 0.025 mmol) was dissolved in a mixture of MeOH and DMF Pd/C (3 mg) was added and the mixture stirred under hydrogen atmosphere for 14 h. The Pd/C was then filtered off through celite and the solvents evaporated. The residue was redissolved in a mixture of DCM (5 ml) and MeOH (1 ml) and 60 % HPF_6 solution in water (20 µl, 0.08 mmol) was added. The solvents were evaporated and the residue suspended in water, filtered and dried to yield compound **83** as a white solid (18 mg, 70%): m.p. = 185-186 °C; ^{1}H NMR (300 MHz, CD_3SO_2) δ 9.39 (br s, 2H, N*H*CH), 8.35-8.25 (br m, 4H, N*H*CH_2), 8.05-7.35 (m, 28 H, ArH), 6.03 (d, *J* = 6 Hz, 2H, CHPh), 4.73 (d, *J* = 6 Hz, 4H, C*H*$_2$NH), 4.69 (d, *J* = 6 Hz, 4H, C*H*$_2$NH); ^{13}C NMR (100 MHz, CD_3OD) δ 169.9 (C), 157.4 (C), 140.3 (C), 138.2 (C), 137.4 (C), 136.0 (C), 131.4 (CH), 129.9 (CH), 129.8 (CH), 129.4 (CH), 128.8 (CH), 128.7 (CH), 128.5 (CH), 128.1 (CH), 127.7 (CH), 127.3 (CH), 59.7 (CH), 45.9 (CH_2), 45.5 (CH_2); *m/z* (ES+): 372 (100) $[M+2H]^{2+}$.

Guests

Preparation of bis-tetrabutylammonium salts of *N*-Boc-L-(D)-Glutamic acid

A 1 M aqueous solution of tetrabutylammonium hydroxide (TBA, 0.74 ml) was added to a solution of commercially available *N*-Boc-L(D)-glutamic acid (100 mg, 0.39 mmol)

in methanol (2.5 ml). The solution was stirred for 2 hours at room temperature. The solvent was then removed under reduced pressure. Residual water was removed by freeze-drying on high vacuum over 16 hours. Compounds were obtained as a clear oils (456 mg, quantitative).

N-Boc-L-glutamate: $[\alpha]_D^{21}$ = 35.6° (c = 1, $CHCl_3$) ^1H NMR (300 MHz, $CDCl_3$) δ 3.91 (m, 1H, C*H*NH), 3.25 (t, *J* = 4.5 Hz, 16H, $TBACH_2N$), 2.30 (t, *J* = 7.0 Hz, 2H, CH_2CO), 1.65 (m, 18H, CH_2CHNH + TBAC$H_2CH_2CH_3$), 1.38 (m, 25H, CH_3 + TBACH_2CH_3), 0.98 (t, *J* = 7.0 Hz, 24H, $TBACH_3$).

N-Boc-D-glutamate: $[\alpha]_D^{21}$ = -35.6° (c = 1, $CHCl_3$); ^1H NMR same as with the one above

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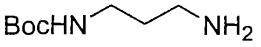
Preparation of bis-tetrabutylammonium salts of *N*-Boc-L-Aspartic acid

A 1 M aqueous solution of tetrabutylammonium hydroxide (TBA, 0.40 ml) was added to a solution of commercially available *N*-Boc-L(D)-aspartic acid (50 mg, 0.2 mmol) in methanol (2.5 ml). The solution was stirred for 2 hours at room temperature. The solvent was removed under reduced pressure. Residual water was removed by freeze-drying on high vacuum over 16 hours. The compound was obtained as a clear oil (210 mg, quantitative).

N-Boc-L-aspartate: $[\alpha]_D^{21}$ = 16.2° (c = 1, $CHCl_3$); ^1H NMR (300 MHz, DMSO) δ 3.65 (m, 1H, C*H*NH), 3.21 (t, *J* = 4.5 Hz, 16H, $TBACH_2N$), 2.30 (2H, t, *J* = 7.2 Hz, CH_2), 1.68 (16H, m, TBAC$H_2CH_2CH_3$), 1.45 (m, 25H, CH_3 + TBACH_2CH_3), 1.00 (t, *J* = 7.2 Hz, 24H, $TBACH_3$).

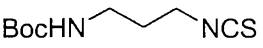
Experimental for chapter 3

tert-butyl N-(2-aminoethyl)carbamate, 114



Di-*tert*-butyl dicarbonate (4.0 g, 18.3 mmol) in dichloromethane (350 ml) was added dropwise to a solution of 1,3-diaminopropane (7.1 g, 95.8 mmol) in dichloromethane (30 ml) over a period of 10 h, with vigorous stirring. The stirring was continued for further 36 h at room temperature. After concentration in vacuo to an oily residue, the reaction mixture was dissolved in aqueous sodium carbonate (63.5 g in 300 ml of water) and extracted with dichloromethane (2 × 300 ml). The organic layer was dried over magnesium sulfate and the solvent evaporated under reduced pressure to afford **114** as a colourless viscous liquid (3.1 g, 96%): I.R. (film) ν_{max} = 3352 (w), 2974 (w), 2932 (w), 2863 (w), 1688 (vs), 1520 (s), 1364 (s), 1272 (s), 1250 (s); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.92 (br s, 1H, OCON*H*CH_2), 3.21 (q, *J* = 6 Hz, 2H, OCONHC*H*$_2$$CH_2CH_2NH_2$), 2.70 (t, 2H, *J* = 6 Hz, $OCONHCH_2CH_2CH_2NH_2$), 1.56 (quint, *J* = 6 Hz, 2H, $OCONHCH_2CH_2CH_2NH_2$), 1.49 (br. s, 2H, NH_2).
All data according to literature.[116]

tert-butyl N-(3-isothiocyanatopropyl) carbamate, 115

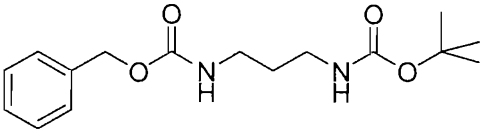


Thiophosgene (1 ml, 15 mmol) was added to a solution of 1-(tert-butyloxycarbonyl) propylene diamine **114** (1.8 g, 11.5 mmol) and trietylamine (2 ml, 14.3 mmol) in chloroform (200 ml) at 0 °C. The resulting reaction mixture was stirred at room temperature for 18 h. The solution was then washed with saturated aqueous sodium dicarbonate (100 ml) and water (2 × 100 ml). The organic layer was dried over magnesium sulfate and the solvent evaporated under reduced pressure to give **115** as a yellow solid (2.2 g, 88 % yield); m.p. = 76-78 °C; I.R. (neat) ν_{max} = 3377 (m), 2977 (m), 2933 (m), 2184 (m) 2139 (s), 2089 (s), 1671 (s), 1513 (s), 1365 (s), 1246 (s), 1158 (s) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.70 (br s, 1H, NH), 3.59 (t, *J* = 6.5 Hz, 2H,

CH_2NCS), 3.23 (q, J = 6.5 Hz, 2H, CH_2NH), 1.89 (quint, J = 6.5 Hz, 2H, $CH_2CH_2CH_2$), 1.44 (s, 9H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.8 (C), 155.9 (C), 79.9 (C), 42.6 (CH_2), 37.7 (CH_2), 30.3 (CH_2), 28.4 (CH_3).

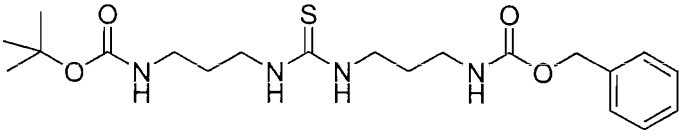
Unstable on stand. Although this product has already been synthesised by Davis *et al.*[117] It was not possible to find any spectral data.

Benzyl N-[3-(tert-butoxycarbonylamino)propyl] carbamate, 116



Benzyl chloroformate (1.6 ml, 11.4 mmol) was added to a solution of **2** (2 g, 11.4 mmol) in chloroform (100 ml) and saturated sodium bicarbonate aqueous solution (60 ml). The reaction was stirred for 12 h at room temperature. After separation of the organic layer the aqueous layer was extracted with chloroform (150ml). The combined organic layers were washed with water (80 ml), dried over magnesium sulfate and the solvent evaporated under reduced pressure to give **116** as a colourless oil (3.4 g, 96% yield): I.R. (neat) ν_{max} = 3337 (m), 2971 (m), 2932 (m), 1689 (s), 1511 (s), 1365 (s), 1245 (s), 1166 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.35 (m, 5H, Ph), 5.37 (br s, 1H, NH), 5.09 (s, 2H, $PhCH_2$), 4.91 (br s, 1H, NH), 3.23 (q, J = 6 Hz, 2H, CH_2NH), 3.16 (m, 2H, CH_2NH), 1.62 (quint, J = 6 Hz, 2H, $CH_2CH_2CH_2$), 1.43 (s, 9H, $(CH_3)_3C$). I.R. and ^1H NMR according to literature[118]

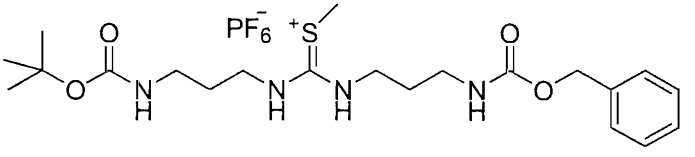
Benzyl-N-{3-[({[3-(tert-butyloxycarbonylamino)-propyl]amino}carbothioyl) amino]propyl} carbamate, 118



Dicarbamate **116** (3.0 g , 9.73 mmol) was stirred for 2 h in a 20 % solution of trifluoroacetic acid in dichloromethane. After addition of toluene (130 ml) the solvents

were removed under reduced pressure to give the corresponding TFA salt as a pale yellow oil. The residue was redissolved in chloroform (100 ml) and triethylamine (3.5 ml, 25.1 mmol) and isothiocyanate **115** (2.05 g, 9.5 mmol) were added to the solution. The mixture was refluxed for 8 h. The solvent was removed under reduced pressure to give a yellow foam. Further purification by column chromatography (ethyl acetate/petroleum ether 2:1) afforded carbamate **118** as a pale yellow foam (2.7 g, 70% yield): R_f = 0.29 (ethyl acetate/petroleum ether 2:1); I.R. (neat) ν_{max} = 3302 (m), 2961 (m), 2932 (m), 2883 (w) 1679 (s), 1521 (s), 1245 (s), 1161 (s) cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ 7.40-7.10 (m, 8H, Ph + NH), 6.82 (t, *J* = 7 Hz, 1H, NH), 5.01 (s, 2H, $PhCH_2$), 3.36 (br s, 4H, CH_2NH), 3.01 (q, *J* = 7 Hz, 2H, CH_2NH), 2.92 (q, *J* = 7 Hz, 2H, CH_2NH), 1.59 (quint, *J* = 7 Hz, 4H, $CH_2CH_2CH_2$), 1.42 (s, 9H, $(CH_3)_3C$); ^{13}C NMR (75 MHz, $(CD_3)_2SO$) δ 156.1 (C), 155.6 (C), 137.2 (C), 128.3 (CH), 127.7 (CH), 127.7 (CH), 77.5 (C), 65.1 (CH_2), 44.0 (CH_2), 37.9 (CH_2), 37.4 (CH_2), 29.2 (CH_2), 28.2 (CH_3); LRMS (ES^+): m/z (%) = 425 (8) $[M+H]^+$, 447 (100) $[M+Na]^+$, 871 (8) $[2M + Na]^+$; HRMS (ES^+): found 425.2215 Da $[M+H]^+$, calculated 425.2217 Da; found 447.2031 Da $[M+Na]^+$, calculated 447.2036 Da; found 463.1775 Da $[M+K]^+$, calculated 463.1776 Da.

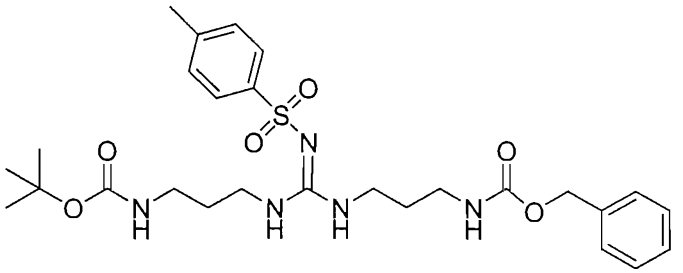
{(E)-1-{[3-(*tert*-butoxycarbonylamino)propyl]amino}propyl)amino] methylidene}(methyl) sulfonium hexafluorophosphate, 119



Iodomethane (700 µl, 10.8 mmol) was added to a stirred solution of thiourea **118** (2.3 g, 5.42 mmol) in acetone (100 ml) and the reaction mixture was stirred for 18h at room temperature. The solvent and all volatile compounds were removed under reduced pressure to give a slightly yellow foam. The foam was redissolved in a 1:1 mixture of dichloromethane (60 ml) and methanol (60 ml). Ammonium hexafluorophosphate (1.9 g, 11.6 mmol) was added and the resulting solution stirred for 5 h at room temperature. The solvents were evaporated and the yellow oily residue resuspended in dichloromethane (150 ml) and washed with water (100 ml). The organic solution was

dried over magnesium sulfate and the solvent removed under reduced pressure to afford hexafluorophosphate **119** as a pale yellow foam (3.2 g, 100 % yield): I.R. (neat) ν_{max} = 3302 (m), 2961 (m), 2932 (m), 2883 (w) 1679 (s), 1521 (s), 1245 (s), 1161 (s) cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ 9.00 (br s, 1H, NH) 8.61 (br s, 1H, NH) 7.35 (m, 5H, Ph), 6.88 (br s, 2H, NH), 5.02 (s, 2H, $PhCH_2$), 3.34 (br s, 4H, CH_2NH, under H_2O signal), 3.07 (m, 2H, CH_2NH), 2.98 (m, 2H, CH_2NH), 2.64 (s, 3H, CH_3S), 1.71 (m, 4H, $CH_2CH_2CH_2$), 1.38 (s, 9H, $(CH_3)_3C$); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 167.6 (C), 156.7 (C), 156.2 (C), 137.6 (C), 128.8 (CH), 128.3 (CH), 128.2 (CH), 78.2 (C), 65.8 (CH_2), 42.9 (CH_2), 41.8 (CH_2), 38.0 (CH_2), 37.5 (CH_2), 29.6 (CH_2), 28.7 (CH_3) 28.1 (CH_2), 14.3 (CH_3); LRMS (ES^+): m/z (%) = 439 (100) $[M\text{-}PF_6]^+$; LRMS (ES^-): m/z (%) = 145 $[PF_6]^-$.

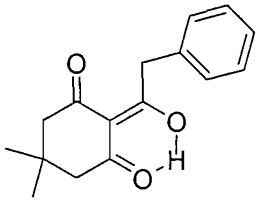
Benzyl-N-{3-[({[3-(tert-butoxycarbonylamino)propyl]amino}{[(4-methylphenyl)sulfonyl] imino}methyl)amino]propyl} carbamate, 120



Hexafluorophosphate **119** (2.7 g, 4.62 mmol) was redissolved in a 4:1 mixture of toluene (40 ml) and chloroform (10 ml) and neat DBU (4 ml, 9.36 mmol) and tosyl amide (3.2 g, 18.7 mmol) were added. The mixture was refluxed for 6 h under vigorous stirring. The solvents were removed under reduced pressure to give an orange oil. Further purification by gradient column chromatography (ethyl acetate/petroleum ether 2:1, pure ethyl acetate) afforded the carbamate **120** as a white foam (2.0 g, 87 %. yield): R_f = 0.25 (ethyl acetate/petroleum ether 2:1); I.R. (neat) ν_{max} = 3337 (br), 2927 (w), 1689 (s), 1571 (s), 1519 (s), 1415 (w), 1365 (m), 1248 (s), 1130 (s), 1079 (s) cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.77 (d, J = 8 Hz, 2H, ArH, o to SO_2), 7.45-7.30 (m, 5H, Ph), 7.31 (d, J = 8 Hz, 2H, ArH, m to SO_2), 5.11 (s, 2H, $PhCH_2$), 3.24 (br s, 4H, CH_2NH), 3.11 (t, J = 6.5 Hz, 2H, CH_2NH), 3.03 (t, J = 6.5 Hz, 2H, CH_2NH), 2.41 (s, 3H, CH_3Ar), 1.68 (m, 4H, $CH_2CH_2CH_2$), 1.47 (s, 9H, $(CH_3)_3C$); ^{13}C NMR (75 MHz,

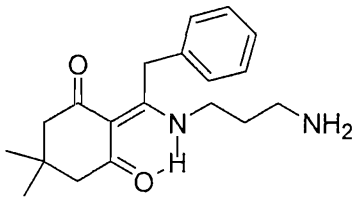
CD_3OD) δ 171.4 (C), 158.8 (C), 156.9 (C), 143.5 (C), 142.0 (C) 138.1 (C), 130.3, 129.5, 129.0, 128.8, 127.2 (CH Ph and Ar), 80.0 (C), 67.4 (CH_2), 39.6 (CH_2), 38.8 (CH_2), 38.4 (CH_2), 30.7 (CH_2), 28.7 (CH_3), 21.4 (CH_3); LRMS (ES^+): m/z (%) = 562 (43) $[M+H]^+$, 584 (100) $[M+Na]^+$, 1123 (4) $[2M + H]^+$, 1145 (11) $[2M+Na]^+$; HRMS (ES^+): found 562.2691 Da $[M+H]^+$, calculated 562.2694 Da; found 584.2511 Da $[M+Na]^+$, calculated 526.2513 Da, found 600.2260 Da $[M+K]^+$, calculated 600.2253 Da.

2-(1-hydroxy-2-phenyl-ethylidene)-5,5-dimethyl-cyclohexane-1,3-dione,[98] 123



Dimedone (3.0 g, 21.4 mmol), DCC (4.0 g, 19.4 mmol) and DMAP (2.4 g, 19.6 mmol) were added to phenylacetic acid (2.6 g, 19.4 mmol) in DMF (200 ml) and the mixture allowed to stir at room temperature for 60 h. Precipitated DCU was removed and the solution evaporated under reduced pressure. The yellow residue was redissolved in ethyl acetate (200 ml) and the organic solution washed with a 1M solution of potassium hydrogen sulfate (200 ml) and a saturated solution of sodium hydrogen carbonate. The organic layer was dried over magnesium sulfate and evaporated under reduced pressure to give a yellow solid which was crystallised from methanol to afford the pure product **123** as a white crystalline solid (4.2 g, 84%): m.p. = 102-104 °C; I.R. (film) ν_{max} = 2961 (w), 2880 (w), 1650 (s), 1556 (s), 1453 (m), 1429 (m), 1407 (m), 1036 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 14.15 (br s, 1H, OH), 7.24 (m, 5H, Ph), 4.33 (s, 2H, CH_2Ph), 2.51 (s, 2H, $COCH_2$), 2.30 (s, 2H, $COCH_2$), 1.02 (s, 6H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.4 (C), 195.3 (C) 174.1 (C), 134.7 (C), 130.0 (CH), 128.6 (CH), 127.1 (CH), 111.8 (C), 51.0 (CH_2), 46.8 (CH_2), 46.4 (CH_2), 30.9 (C), 28.3 (CH_3).
The compound fragments during ionisation process so it is not possible to see M or M+H cations.

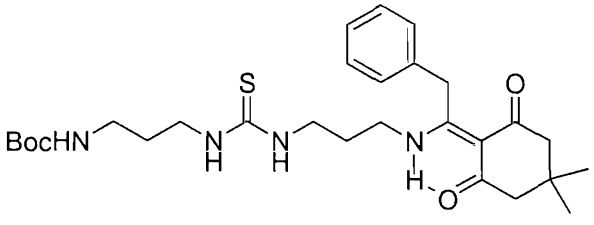
2-{1-[(2-aminopropyl)amino]-2-phenylethylidene}-5,5-dimethyl-1,3-cyclohexanedione, 124



2-(1-hydroxy-2-phenyl-ethylidene)-5,5-dimethyl-cyclohexane-1,3-dione **123** (2.0 g, 8.1 mmol) in dichloromethane (200 ml) was added dropwise to a solution of 1,3-diaminopropane (3.1 g, 41.9 mmol) and TFA (70 µl, 0.909 mmol) in dichloromethane (10 ml), over a period of 10 h, with vigorous stirring. The stirring was continued for further 36 h at room temperature. The solution was washed with a 2M solution of sodium carbonate (2 × 100 ml) and water (2 × 100 ml). The organic layer was dried over magnesium sulfate and the solvent evaporated under reduced pressure to afford **124** as a pale yellow oil (2.7 g, 100%): I.R. (film) ν_{max} = 2953 (w), 2867 (w) 2363 (w), 1636 (s), 1450(vs), 1417 (s), 1282 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.85 (br s, 1H, NH), 7.20 (m, 5H, Ph), 4.64 (s, 2H, $PhCH_2$), 3.44 (q, J = 6 Hz, 2H, CH_2NH), 2.75 (t, J = 6 Hz 2H, CH_2NH_2), 2.40 (m, 4H, CH_2CO), 1.68 (quint, J = 6 Hz, 2H, $CH_2CH_2CH_2$) 1.50 (br s, 2H, NH_2), 1.05 (s, 6H, CH_3); LRMS (ES^+): m/z (%) = 615 (13) $[2M+H]^+$.

Very Unstable on stand.

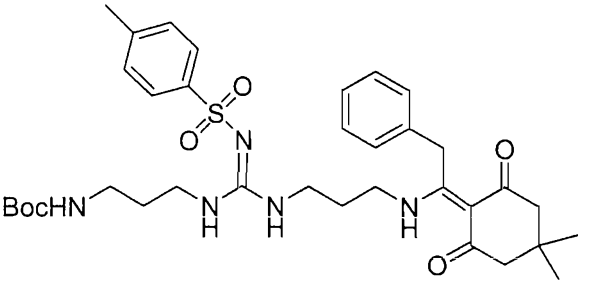
***tert*-Butyl-N-{2-({[(2-{[1-4,4-dimethyl-2,6-dioxocyclohexyliden)-2-phenylethyl]amino}-propyl)amino]carbothioyl}amino)propyl]carbamate, 126**



Thiophosgene (800 µl, 10.5 mmol) was added to a solution of **124** (2.4 g, 7.6 mmol) in chloroform (500 ml) and aqueous sodium hydrogen carbonate (4.1 g, 49 mmol in 200

ml of water) at 0 °C. The resulting reaction mixture was stirred at room temperature for 18 h. After separation of the organic layer the aqueous layer was extracted with chloroform (150 ml). The combined organic layers were dried over magnesium sulfate and the solvent evaporated under reduced pressure to give **125** as a yellowish solid (2.6 g, 96%). The isothiocyanate was added to a solution of amine **114** (1.22 g, 7.01 mmol) in chloroform (70 ml) and the mixture refluxed for 36 h. The solvent was removed under reduced pressure to give a yellow foam. Further purification by column chromatography (ethyl acetate /dichloromethane 3:2) afforded carbamate **126** as a pale yellow foam (1.85 g, 50%): R_f = 0.31 (ethyl acetate /dichloromethane 3:2); I.R. (neat) ν_{max} = 3288 (m), 2954 (m),2931 (m), 2869 (w) 1688 (m), 1630 (m), 1565 (s), 1496 (s), 1450 (s), 1341 (s), 1272 (s), 1248 (s), 1163 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.82 (br s, 1H, NH), 7.23 (m, 5H, Ph), 6.89 (br s, 1H, NH), 6.20 (br s, 1H, NH), 4.96 (br s, 1H, NH), 4.55 (s, 2H, $PhCH_2$), 3.55 (br s, 2H, CH_2NH), 3.42 (m, 4H, CH_2NH), 3.13 (m, 2H, CH_2NH), 2.39 (s, 4H, CH_2CO), 1.85 (m, 2H, $CH_2CH_2CH_2$), 1.66 (m, 2H, $CH_2CH_2CH_2$) 1.42 (s, 9H, $(CH_3)_3C$), 1.04 (s, 6H, $(CH_3)_2C$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.4 (C), 182.5 (C), 174.3 (C), 157.6 (C), 136.2 (C), 129.3 (CH), 128.5 (CH), 127.1 (CH), 108.6 (C), 80.3 (C), 60.8 (CH_2), 53.3 (CH_2), 41.3 (CH_2), 35.5 (CH_2), 30.2 (CH_2), 30.0 (CH_2), 29.2 (CH_2), 28.8 (CH_2), 28.3 (CH_3), 28.2 (CH_3); LRMS (ES^+): m/z (%) = 531 (8) $[M+H]^+$, 553 (25) $[M+Na]^+$.

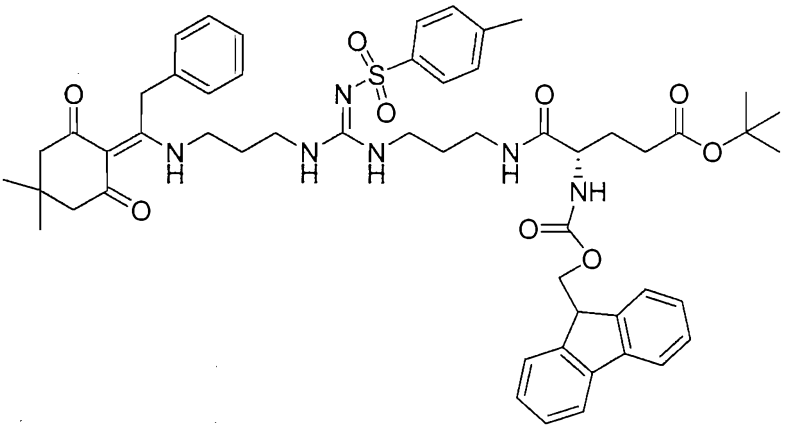
***tert*-Butyl-N-{2-({[(2-{[1-4,4-dimethyl-2,6-dioxocyclohexyliden)-2-phenylethyl]amino}-propyl)amino]{[(4-methylphenyl)sulfonyl] imino}methyl)amino]propyl} carbamate, 127**



Iodomethane (400 µl, 5.92 mmol) was added to a stirred solution of thiourea **126** (1.57 g, 2.96 mmol) in acetone (50 ml) and the reaction mixture was stirred for 18h at room temperature. The solvent and all volatile compounds were removed under reduced

pressure to give a slightly yellow foam. The foam was redissolved in a 1:1 mixture of dichloromethane (30 ml) and methanol (30 ml). Ammonium hexafluorophosphate (962 mg, 5.92 mmol) was added and the resulting solution stirred for 18 h at room temperature. The solvents were evaporated and the yellow oily residue redissolved in dichloromethane (100 ml) and washed with water (80 ml). The organic solution was dried over magnesium sulfate and the solvent removed under reduced pressure to afford the hexafluorophosphate as a white foam (1.93 g, 97 %). The hexafluorophosphate (1.93 g, 2.80 mmol) was redissolved in a 4:1 mixture of toluene (20 ml) and chloroform (5 ml) and neat DBU (868 mg, 5.7 mmol) and tosyl amide (1.46 g, 8.55 mmol) were added. The mixture was refluxed for 48 h under vigorous stirring. The solvents were removed under reduced pressure to give an orange oil (2.8 g). Further purification by column chromatography (ethyl acetate/petroleum ether 7:1) afforded the carbamate **127** as a white foam (1,4 g, 80 %. yield): R_f = 0.27 (ethyl acetate/petroleum ether 7:1); I.R. (neat) ν_{max} = 3328 (br), 2956 (w), 2931 (w), 2862 (w), 1692 (m), 1630 (m), 1564 (s), 1452 (m), 1417 (m), 1344 (m), 1250(m), 1130 (s), 1080 (s) cm^{-1}; ^{1}H-NMR (400 MHz, CD_3OD) δ 7.64 (d, J = 7.5 Hz, 2H, ArH, *o* to SO_2), 7.20 (m, 4H, Ph), 7.09 (m,1H, Ph), 6.98 (d, J = 7.5 Hz, 2H, ArH, *m* to SO_2), 4.73 (s, 2H, $PhCH_2$), 3.28 (m, 2H, CH_2NH), 3.15 (br s, 2H, CH_2NH), 3.05 (br s, 2H, CH_2NH), 2.90 (t, J = 6 Hz, 2H, CH_2NH), 2.29 (s, 4H, CH_2CO), 2.25 (s, 3H, CH_3Ar), 1.60 (quint, 2H, J = 6 Hz, $CH_2CH_2CH_2$), 1.49 (quint, J = 6 Hz, 2H, $CH_2CH_2CH_2$), 1.31 (s, 9H, $(CH_3)_3C$), 0.96 (s, 6H, $(CH_3)_2C$); ^{13}C NMR (100 MHz, CD_3OD) δ 200.4 (C), 175.9 (C), 173.4 (C), 159.1 (C), 157.3 (C), 144.0 (C), 142.6 (C), 137.4 (C), 130.8 (CH), 130.2 (CH), 129.6 (CH), 128.0 (CH), 127.5 (CH), 109.2 (C), 80.5 (C), 54.0 (CH_2), 42.3 (CH_2), 40.2 (CH_2), 40.0 (CH_2), 38.8 (CH_2), 31.4 (CH_2), 31.2 (CH_2), 30.7 (C) 29.2 (CH_3), 28.8 (CH_3), 21.8 (CH_3); LRMS (ES^+): m/z (%) = 668 (13) $[M+H]^+$, 690 (100) [M+Na]+, 1329 (11) $[2M+Na]^+$; HRMS (ES^+): found 668.3493 Da $[M+H]^+$, calculated 668.3477 Da; found 690.3298 Da $[M+Na]^+$, calculated 690.3296 Da.

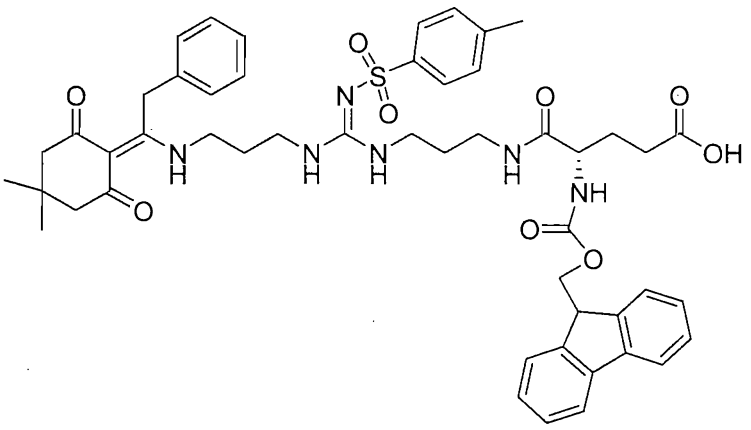
tert-Butyl-5-({3-[([(3-{[1-(4,4-dimethyl-2,6-dioxocyclohexyliden)-2-phenylethyl] amino}propyl)amino]{[(4-methylphenyl)sulfonyl]imino}methyl)amino]propyl} amino)-4-{[9H-9- fluorenylmethoxy)carbonyl]amino}-5-oxopentanoate, 128



Carbamate **127** (500 mg, 0.75 mmol) was stirred in a 20 % solution of trifluoroacetic acid in dichloromethane (25 ml) at room temperature for 3 h. After addition of toluene the solvents were removed under reduced pressure to give a yellow oil that was redissolved in dichloromethane (5 ml). A solution of *N*-α-Fmoc-L- glutamic acid-γ-*tert*-butyl ester (351 mg, 0.82 mmol), PyBop (352 mg, 0.68 mmol) and HOBt (104 mg, 0.68 mmol) in dichloromethane (15 ml) was stirred at room temperature for 15 minutes and added to the previously mentioned solution. After addition of DIPEA (300 µl, 1.72 mmol) the resulting reaction mixture was stirred for 36 h. Further dichloromethane (50 ml) was added and the mixture washed with water (60 ml). The organic layer was dried over magnesium sulphate and the solvent removed under reduced pressure to give a yellow solid. Purification by gradient column chromatography on silica gel (ethyl acetate/petroleum ether 8:1, ethyl acetate) afforded **128** as a white foam (510 mg, 70 % yield): R_f = 0.18 (ethyl acetate/petroleum ether 8:1); I.R. (neat) ν_{max} = 3322 (br), 2949 (w), 2926 (w), 1720 (m), 1561 (s), 1496 (w), 1450 (m), 1419 (w), 1365 (w), 1342 (m), 1245 (s), 1182 (w), 1131 (s), 1081 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 13.48 (br s, 1H, C=C-NH), 7.67 (d, *J* = 7.5 Hz, 2H, ArH Fmoc), 7.63 (d, *J* = 8 Hz, 2H, ArH Fmoc), 7.49 (d, *J* = 7.5 Hz, 2H, ArH *o* to SO_2), 7.30 (t, *J* = 7.5 Hz, 2H, ArH Fmoc), 7.25-7.15 (m, 4H, Ph), 7.12-7.07 (m, 3H, ArH Fmoc + Ph), 7.00 (d, *J* = 7.5 Hz, 2H, ArH *m* to SO_2), 6.75 (br s, 1H, NH), 6.15 (br s, 1H, NH), 4.41 (s, 2H, PhCH_2), 4.29 (m, 1H, C*H*H Fmoc), 4.24 (m, 1H, CH*H* Fmoc), 4.10 (t, *J* = 7.5 Hz, 1H, H_9 Fmoc), 4.06 (m, 1H, CH Glu), 3.16 (m, 8H, CH_2NH), 2.30 (s, 3H, CH_3Ar), 2.27 (s, 6H, CH_2CO + CH_2CO Glu),

1.97 (m, 1H, CH*H*CH Glu), 1.81 (m, 1H, C*H*HCH Glu), 1.67 (m, 2H, $CH_2CH_2CH_2$) 1.55 (m, 2H, $CH_2CH_2CH_2$) 1.36 (s, 9H, $(CH_3)_3C$), 0.94 (s, 6H, $(CH_3)_2C$); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 174.2 (C), 173.4 (C), 155.8 (C), 144.1 (C), 142.3 (C), 141.7 (C), 141.4 (C), 136.1 (C), 129.6 (CH), 129.2 (CH), 128.4 (CH), 128.2 (CH), 127.5 (CH), 127.5 (CH), 127.0 (CH), 126.3 (CH), 125.5 (CH), 120.4 (CH), 108.5 (C), 81.6 (C), 67.5 (CH_2), 55.5 (CH), 47.5 (CH), 40.9 (CH_2), 39.1 (CH_2), 35.3 (CH_2), 32.2 (CH_2), 30.4 (C), 30.1 (CH_2), 29.3 (CH_2), 28.7 (CH_3), 28.5 (CH_3), 27.9 (CH_2), 21.8 (CH_3); LRMS (ES^+): m/z (%) = 975 (10) $[M+H]^+$, 997(23) $[M+Na]^+$.

5-({3-[([(3-{[1-(4,4-dimethyl-2,6-dioxocyclohexyliden)-2-phenylethyl]amino} propyl)amino]{[(4-methylphenyl)sulfonyl]imino}methyl)amino]propyl}amino)-4-{[9H-9- fluorenylmethoxy)carbonyl]amino}-5-oxopentanoic acid, 129



To a solution of ester **128** (470 mg, 0.48 mmol) a ~60 % solution of trifluoroacetic acid in dichloromethane (11 ml) was added and the mixture stirred vigorously for 18 h at room temperature. Toluene (150 ml) was added and the solvents removed under reduced pressure to obtain a yellow oil. Further column chromathography on silica gel (10% methanol in dichloromethane) afforded **129** as a cream-coloured foam (380 mg, 86 %): R_f = 0.25 (10% methanol in dichloromethane); I.R. (neat) ν_{max} = 3319 (br), 2951 (w), 1705 (m), 1561 (s), 1496 (w), 1449 (m), 1341 (m), 1241 (m), 1181 (m), 1128 (s), 1079 (s) cm^{-1}; ^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ 13.46 (br s, 1H, NH), 8.04 (t, *J* = 8 Hz, 1H, NH), 7.95 (t, *J* = 7.5 Hz, 1H, NH), 7.89 (d, *J* = 8 Hz, 2H, ArH Fmoc), 7.74-7.70 (m, 2H, ArH Fmoc), 7.63 (d, *J* = 7.5 Hz, 2H, ArH, *o* to SO_2), 7.58 (d, *J* = 7.5 Hz, 1H, NH Glu), 7.45-7.00 (m, 10H, PhH + ArH Fmoc + ArH *m* to SO_2 + NH), 4.44 (s,

2H, $PhCH_2$), 4.22 (m, 3H, CH_2 + CH Fmoc), 3.95 (m, 1H, CH Glu), 3.21 (m, 2H, CH_2NH), 3.15-2.95 (m, 6H, CH_2NH), 2.32 (s, 2H, CH_2CO), 2.31 (s, 3H, CH_3Ar), 2.29 (s, 2H, CH_2CO), 2.24 (m, 2H, CH_2COOH Glu), 1.88 (m, 1H, $CHHCHNH$ Glu), 1.78 (m, 1H, $CHHCHNH$ Glu), 1.58 (m, 2H, $CH_2CH_2CH_2$), 1.48 (m, 2H, $CH_2CH_2CH_2$), 0.96 (s, 6H, $(CH_3)_2C$); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 197.3 (C), 173.1 (C), 172.0 (C), 156.4 (C), 155.3 (C), 144.3 (C), 144.2 (C), 143.0 (C), 141.9 (C), 141.6 (C), 137.9 (C), 136.6 (C), 129.5 (C), 129.4 (CH), 129.0 (CH), 128.4 (CH), 128.1 (CH), 127.7 (CH), 127.5 (CH), 126.0 (CH), 125.8 (CH), 120.5 (CH), 107.4 (C), 66.1 (CH_2), 54.7 (CH), 52.9 (CH_2), 47.1 (CH), 40.6 (CH_2), 38.7 (CH_2), 38.6 (CH_2), 36.2 (CH_2), 34.3 (CH_2), 30.1 (C), 29.5 (CH_2), 29.3 (CH_2), 28.3 (CH_3), 27.9 (CH_2), 21.3 (CH_3); LRMS (ES^+): m/z (%) = 941 (10) $[M+Na]^+$; HRMS (ES^+): found 919.4075 Da $[M+H]^+$, calculated 919.4059 Da.

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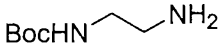
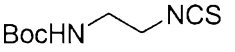
tert-butyl N-(2-aminoethyl)carbamate, 130



Di-_tert_-butyl dicarbonate (6.1 g, 28 mmol) in dichloromethane (400 ml) was added dropwise to a solution of ethylene diamine (10 g, 166.7 mmol) in dichloromethane (50 ml) over a period of 10 h, with vigorous stirring. The stirring was continued for further 18 h at room temperature. After concentration in vacuo to an oily residue, the reaction mixture was dissolved in aqueous sodium carbonate (63.5 g in 300 ml of water) and extracted with dichloromethane (2 × 300 ml). The organic layer was dried over magnesium sulfate and the solvent evaporated under reduced pressure to afford **130** as a colourless viscous liquid (4.35 g, 97%): I.R. (film) ν_{max} = 3352 (m), 2974 (m), 2931 (m), 2863 (w), 1688 (s), 1520 (s), 1250 (m), 1168 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.96 (br s, 1H, $OCONHCH_2$), 3.17 (q, J = 6.0 Hz, 2H, $OCONHCH_2CH_2NH_2$), 2.80 (t, J = 6.0 Hz, 2H, $OCONHCH_2CH_2NH_2$), 1.32 (br. s, 2H, NH_2); LRMS (ES^+): m/z (%) = 161 (28) $[M+H]^+$, 202 (8) $[M + H + CH_3CN]^+$, 321 (15) $[2M + H]^+$.

All data according to literature[119]

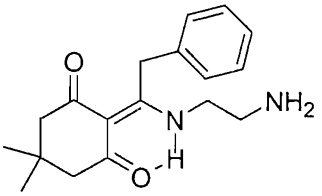
***tert*-butyl N-(2-isothiocyanatoethyl)carbamate, 131**



Thiophosgene (1.2 ml, 15 mmol) was added to a solution of 1-(tert-butyloxycarbonyl) ethyl diamine **130** (1.8 g, 11.2 mmol) in chloroform (500 ml) and aqueous sodium hydrogen carbonate (4.1 g, 49 mmol in 200 ml of water) at 0 °C. The resulting reaction mixture was stirred at room temperature for 18 h. After separation of the organic layer the aqueous layer was extracted with chloroform (150 ml). The combined organic layers were dried over magnesium sulfate and the solvent evaporated under reduced pressure to give **131** as a yellow solid (2.17 g, 96%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.88 (br s, 1H, NH), 3.62 (t, J = 6.0 Hz, 2H, CH_2NCS), 3.33 (q, J = 6.0 Hz, CH_2NH), 1.38 (s, 9H, CH_3).

Data according to literature.[106a]

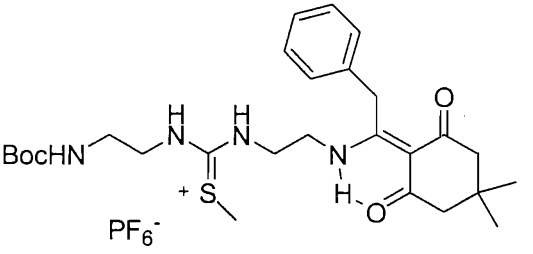
2-{1-[(2-aminoethyl)amino]-2-phenylethylidene}-5,5-dimethyl-1,3-cyclohexanedione, 132



2-(1-hydroxy-2-phenyl-ethylidene)-5,5-dimethyl-cyclohexane-1,3-dione **123** (2.3 g, 8.9 mmol) in dichloromethane (200 ml) was added dropwise to a solution of ethylene diamine (2.25 g, 37.4 mmol) and TFA (70 µl, 0.909 mmol) in dichloromethane (10 ml), over a period of 10 h, with vigorous stirring. The stirring was continued for further 36 h at room temperature. The solution was washed with a 2M solution of sodium carbonate (2 × 100 ml) and water (2 × 100 ml). The organic layer was dried over magnesium sulfate and the solvent evaporated under reduced pressure to afford **132** as a pale yellow oil (2.7 g, 100%): I.R. (film) ν_{max} = 2909 (w), 2250 (w), 1688 (s), 1604 (s), 1509 (s), 1390 (s) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.32 (br s, 1H, NH), 7.20 (m, 5H, Ph), 4.60 (s, 2H, $PhCH_2$), 3.39 (q, J = 6 Hz, 2H, CH_2NH), 2.89 (br m, 2H,

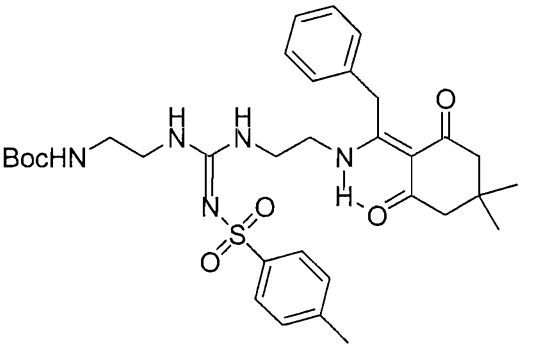
CH_2NH$_2$), 2.38 (m, 4H, CH$_2$CO), 1.50 (br s, 2H, NH$_2$), 1.05 (s, 6H, CH$_3$); LRMS (ES$^+$): m/z (%) = 601 (13) [2M+H]$^+$; sample very unstable on stand.

{(Z)-1-({2-*tert*-butoxycarbonyl)amino]ethyl}amino)-1-[(2-{[1-(4,4-dimethyl-2,6-dioxocyclohexyliden)-2-phenylethyl]amino}ethyl)amino]methylidene}(methyl) sulfonium hexafluorophosphate, 133



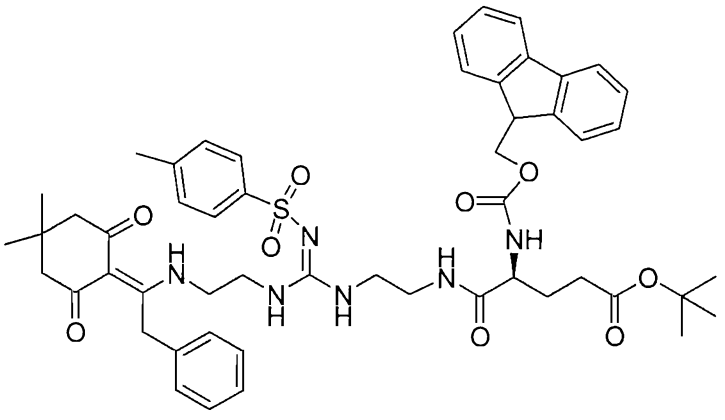
Iodomethane (300 µl, 4.82 mmol) was added to a stirred solution of thiourea **156e** (1.2 g, 2.39 mmol) in acetone (50 ml) and the reaction mixture was stirred for 18h at room temperature. The solvent and all volatile compounds were removed under reduced pressure to give a slightly yellow foam. The foam was redissolved in a 1:1 mixture of dichloromethane (30 ml) and methanol (30 ml). Ammonium hexafluorophosphate (780 mg, 4.78 mmol) was added and the resulting solution stirred for 18 h at room temperature. The solvents were evaporated and the yellow oily residue redissolved in dichloromethane (100 ml) and washed with water (80 ml). The organic solution was dried over magnesium sulfate and the solvent removed under reduced pressure to afford hexafluorophosphate **133** as a white foam (1.55 g, 98%): I.R. (neat) ν_{max} = 2340 (w), 2250 (w), 1650 (s), 1590 (m), 1476 (m), 1386 (s), 1188 (s) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 12.46 (br s, 1H, C=C-NH), 8.50 (br s, 2H, NHCS), 7.25 (m, 5H, Ph), 5.57 (t, 1H, NHBoc), 4.62 (s, 2H, PhCH_2), 3.65 (m, 2H, CH_2NH), 3.55 (m, 4H, CH_2NH), 3.45 (m, 2H, CH_2NH), 2.64 (s, 3H, SCH$_3$), 2.41 (s, 4H, CH$_2$CO), 1.44 (s, 9H, (CH$_3$)$_3$C), 1.06 (s, 6H, (CH$_3$)$_2$C); ^{13}C NMR (75 MHz, CDCl$_3$) δ 174.5 (C), 174.4 (C), 173.9 (C), 169.0 (C), 159.5 (C), 135.6 (C), 129.1 (CH), 128.2 (CH), 126.9 (CH), 108.8 (C), 81.7 (C), 46.7 (CH$_2$), 44.8 (CH$_2$), 41.7 (CH$_2$), 38.7 (CH$_2$), 34.8 (CH$_2$), 30.2 (C), 28.5 (CH$_3$), 13.7 (CH$_3$); LRMS (ES$^+$): m/z (%) = 517 (100) [M]$^+$.

***tert*-Butyl-N-{2-({[(2-{[1-4,4-dimethyl-2,6-dioxocyclohexyliden)-2-phenylethyl]amino}-ethyl)amino]{[(4-methylphenyl)sulfonyl] imino}methyl)amino]ethyl} carbamate, 134**



Neat DBU (575 mg, 3.78mmol) was added to a solution of tosyl amide (800 mg, 4.7 mmol) and hexafluorophosphate **133** (1.25 g, 1.89 mmol) in a 4:1 mixture of toluene and chloroform (125 ml). The mixture was refluxed for 48 h under vigorous stirring, then the solvents were removed under reduced pressure to give an orange oil (3.3 g). Further purification by column chromatography (ethyl acetate/petroleum ether 8:1) afforded the carbamate **134** as an orange/pink foam (760 mg, 63%): R_f = 0.28 (ethyl acetate/petroleum ether 8:1): I.R. (neat) ν_{max} = 3141 (br), 2945 (w), 2333 (w), 2250 (w), 1644 (s), 1590 (m), 1556 (w), 1392 (m), 1274 (m), 1105 (m) cm^{-1}; ^{1}H-NMR (400 MHz, CD_3CN) δ 11.99 (br s, 1H, C=C-NH), 7.64 (d, *J* = 7 Hz, 2H, ArH, *o* to SO_2), 7.20 (m, 5H, Ph), 7.08 (d, *J* = 7 Hz, 2H, ArH, *m* to SO_2), 5.67 (br s, 1H, NHBoc), 4.44 (s, 2H, $PhCH_2$), 3.43 (m, 2H, CH_2NH), 3.29 (m, 2H, CH_2NH), 3.12 (m, 4H, CH_2NH), 2.34 (s, 4H, CH_2CO), 1.38 (s, 9H, $(CH_3)_3C$), 1.10 (s, 6H, $(CH_3)_2C$); ^{13}C NMR (100 MHz, CD_3CN) δ 199.8 (C), 175.7 (C), 159.0 (C), 157.1 (C), 143.9 (C), 142.7 (C), 137.8 (C), 129.9 (CH), 129.3 (CH), 128.7 (CH), 127.0 (CH), 126.4 (CH), 108.9 (C), 79.9 (C), 53.6 (CH_2), 42.7 (CH_2), 41.0 (CH_2), 39.8 (CH_2), 30.3 (C), 28.3 (CH_3), 28.0 (CH_3), 21.2 (CH_3); LRMS (ES^+): m/z (%) = 640 (13) $[M+H]^+$, 662 (100) $[M+Na]^+$, 1301 (11) $[2M+Na]^+$; HRMS (ES^+): Found 662.2987 Da $[M+Na]^+$, calculated 662.2983 Da.

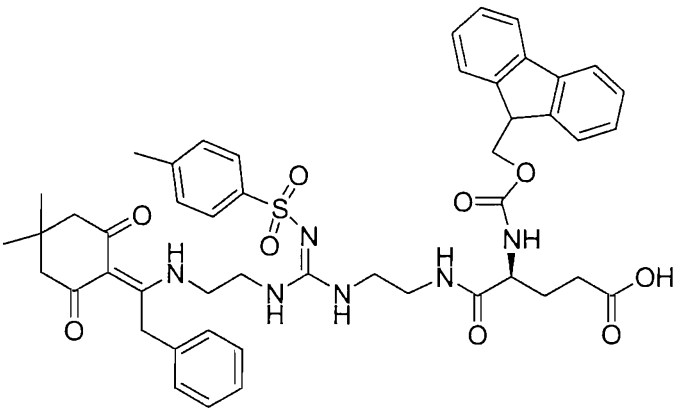
Tert-Butyl-5-({3-[([(3-{[1-(4,4-dimethyl-2,6-dioxocyclohexyliden)-2-phenylethyl] amino}propyl)amino]{[(4-methylphenyl)sulfonyl]imino}methyl)amino]propyl} amino)-4-{[9H-9- fluorenylmethoxy)carbonyl]amino}-5-oxopentanoate, 135



Carbamate **134** (500 mg, 0.75 mmol) was stirred in a 20 % solution of trifluoroacetic acid in dichloromethane (25 ml) at room temperature for 3 h. After addition of toluene the solvents were removed under reduced pressure to give a yellow oil that was redissolved in dichloromethane (5 ml). A solution of N-α-Fmoc-L-glutamic acid-γ-*tert*-butyl ester, EDC and HOBt in dichloromethane (15 ml) was stirred at room temperature for 15 minutes and added to the previously mentioned solution. After addition of DIPEA the resulting reaction mixture was stirred for 36 h. Further dichloromethane (50 ml) was added and the mixture washed with water (60 ml). The organic layer was dried over magnesium sulphate and the solvent removed under reduced pressure to give a yellow solid. Purification by gradient column chromatography on silica gel (ethyl acetate/petroleum ether 8:1, ethyl acetate) afforded **135** as a white foam (510 mg, 70 % yield): I.R. (neat) ν_{max} = 3322 (br), 2949 (w), 2926 (w), 1720 (m), 1561 (s), 1496 (w), 1450 (m), 1419 (w), 1365 (w), 1342 (m), 1245 (s), 1182 (w), 1131 (s), 1081 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 13.48 (br s, 1H, C=C-NH), 7.67 (d, J = 7.5 Hz, 2H, ArH Fmoc), 7.63 (d, J = 8 Hz, 2H, ArH Fmoc), 7.49 (d, J = 7.5 Hz, 2H, ArH *o* to SO_2), 7.35-7.05 (m, 12H, ArH Fmoc(4H) + PhH(5H) + NH(3H)), 6.99 (d, J = 7.5 Hz, 2H, ArH *m* to SO_2), 6.35 (br s, 1H, NH), 4.39 (m, 2H, PhCH_2, AB sistem), 4.28 (m, 2H, CH_2 Fmoc), 4.13-4.00 (m, 2H, H_9 Fmoc + CH Glu), 3.45 (m, 2H, CH_2NH), 3.30-3.10 (m, 6H, CH_2NH), 2.40-1.80 (m, 11H, CH_3Ar + CH_2CO + CH_2COO Glu + $CHCH_2$ Glu), 1.36 (s, 9H, $(CH_3)_3C$), 0.91 (s, 6H, $(CH_3)_2C$); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 198.1 (C), 174.2 (C), 174.0 (C), 173.0 (C), 156.6

(C), 155.6 (C), 143.8 (C), 142.1 (C), 141.4 (C), 141.1 (C), 135.8 (CH), 129.3 (CH), 128.9 (CH), 128.1 (CH), 127.9 (CH), 127.2 (CH), 126.8 (CH), 126.0 (CH), 125.2 (CH), 120.2 (CH), 108.4 (C), 81.3 (C), 67.2 (CH_2), 60.5 (CH_2), 54.6 (CH_2), 53.0 (CH), 47.3 (CH), 42.4 (CH_2), 40.9 (CH_2), 39.7 (CH_2), 35.0 (CH_2), 31.7 (CH_2), 30.1 (C), 28.4 (CH_3), 28.2 (CH_3), 27.5 (CH_2), 21.6 (CH_3); LRMS (ES^+): m/z (%) = 947 (100) $[M+H]^+$, 969 (23) $[M+Na]^+$

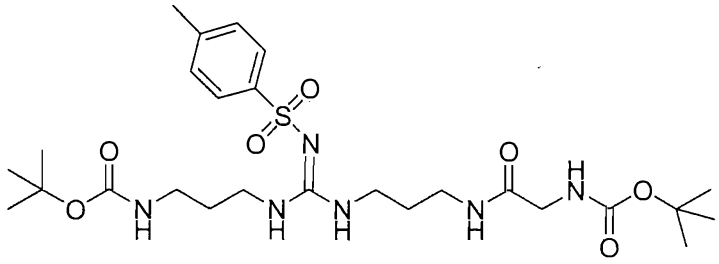
5-({3-[([(3-{[1-(4,4-dimethyl-2,6-dioxocyclohexyliden)-2-phenylethyl] amino}propyl)amino]{[(4-methylphenyl)sulfonyl]imino}methyl)amino]propyl} amino)-4-{[9H-9- fluorenylmethoxy)carbonyl]amino}-5-oxopentanoic acid, 136



To a solution of ester **135** (470 mg, 0.48 mmol) a ~60 % solution of trifluoroacetic acid in dichloromethane (11 ml) was added and the mixture stirred vigorously for 18 h at room temperature. Toluene (150 ml) was added and the solvents removed under reduced pressure to obtain a yellow oil. Further column chromathography on silica gel (10% methanol in dichloromethane) afforded **136** as a cream-coloured foam (380 mg, 86 %): I.R. (neat) ν_{max} = 3319 (br), 2951 (w), 1705 (m), 1561 (s), 1496 (w), 1449 (m), 1341 (m), 1241 (m), 1181 (m), 1128 (s), 1079 (s) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 13.49 (s, 1H, NH), 7.77 (d, J = 8.0 Hz, 2H, ArH), 6.49 (br s, 1H, NH), 7.73 (d, J = 8.0 Hz, 2H, ArH), 7.60-7.05 (m, 11H, ArH), 6.98 (d, J = 7.0 Hz, 2H, ArH, *m* to SO_2), 4.46-4.18 (m, 6H, $PhCH_2$+CH Fmoc+CH Glu+CH_2 Fmoc), 3.55-3.30 (m, 8H, CH_2NH), 2.45-2.20 (m, 9H, CH_2CO + CH_3Ar + CH_2COOH Glu), 2.15 (m, 1H, C*H*HCHNH), 2.03 (m, 1H, CH*H*CHNH Glu), 1.02 (s, 6H, $(CH_3)_2C$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.3 (C), 176.1 (C), 174.7 (C), 173.5 (C), 156.6 (C), 155.6 (C), 143.8 (C), 142.4 (C),

141.4 (C), 140.8 (C), 135.6 (C), 129.4 (CH), 129.0 (CH), 128.1 (CH), 127.9 (CH), 127.2 (CH), 126.8 (CH), 126.0 (CH), 125.2 (CH), 120.2 (CH), 108.3 (C), 67.4 (CH_2), 66.0 (CH_2) 54.2 (CH), 53.6 (CH_2), 52.8 (CH_2), 47.2 (CH), 42.7 (CH_2), 40.8 (CH_2), 38.7 (CH_2), 36.2 (CH_2), 35.0 (CH_2), 30.1 (C), 28.3 (CH_3) 27.8 (CH_2), 21.6 (CH_3); LRMS (ES^+): m/z (%) = 914 (100) [M+Na].

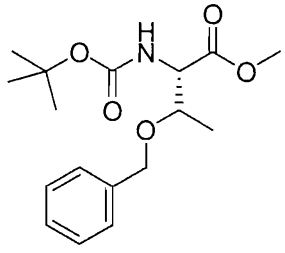
tert-butyl N-{3-[([(3-{[2-(_tert_-butyloxycarbonylamino)acetyl]amino}propyl) amino] {[4-methylphenyl)sulfonyl]imino}methyl)amino]propyl}carbamate, 138



Carbamate **120** (780 mg, 1.39 mmol) was dissolved in methanol (20 ml). Pd/C (10 %) was added (100 mg) and the mixture was stirred for 12 h under H_2 atmosphere. After having filtered the Pd off through celite, the solvent was removed under reduced pressure to afford **137** as a white foam (594 mg, 100 % yield); I.R. (neat) ν_{max} = 3337 (br), 2927 (w), 1689 (s), 1571 (s), 1519 (s), 1415 (w), 1365 (m), 1248 (s), 1130 (s), 1079 (s) cm^{-1}; ^{1}H-NMR (300 MHz, CD_3OD) δ 7.77 (d, J = 8 Hz, 2H, ArH, *o* to SO_2), 7.35 (d, J = 8 Hz, 2H, ArH, *m* to SO_2), 3.27 (br s, 4H, CH_2NH), 3.03 (t, J = 6.5 Hz, 2H, CH_2NH), 2.65 (t, J = 7 Hz, 2H, CH_2NH), 2.44 (s, 3H, CH_3Ar), 1.67 (m, 4H, $CH_2CH_2CH_2$), 1.48 (s, 9H, $(CH_3)_3C$). Amine **137** (550 mg, 1.29 mmol) was dissolved in a mixture of tetrahydrofuran (10 ml) and dimethylformamide (5 ml). The solution was cooled to 0° C and *N*-Boc-L-Gly-OH (123 mg, 1.63 mmol), HOBt·H_2O (220 mg, 1.63 mmol), DIPEA (426 µl, 2.44 mmol) and EDC (312 mg, 1.63 mmol) were added[120]. The mixture was stirred for 24 h (0° C to room temperature). Tetrahydrofuran was removed and the residue was diluted with dichloromethane (150 ml) and washed with 1M sodium hydrogen carbonate, saturated brine and water, and dried over magnesium sulfate. The solvent was evaporated under reduced pressure to give a yellow oil. Further purification by column chromatography (ethyl acetate) afforded **138**

as a white foam (520 mg, 70 % yield): R_f = 0.25 (ethyl acetate); I.R. (neat) ν_{max} = 3323 (m), 2978 (w), 2928 (w), 1680, 1572 (s), 1518 (m), 1360 (m), 1241 (s), 1158 (s), 1123 (s), 1084 (s); ^{1}H-NMR (400 MHz, CD_3CN) δ 7.74 (d, J = 8 Hz, 2H, ArH, *o* to SO_2), 7.31 (d, J = 8 Hz, 2H, ArH, *m* to SO_2), 6.91 (br s, 2H, NH), 5.72 (br s, 1H, NH), 5.51 (br s, 1H, NH), 3.64 (d, J = 6.0 Hz, 2H, CH_2CO), 3.19 (m, 6H, CH_2NH), 2.99 (q, J = 6.5 Hz, 2H, CH_2NH), 2.40 (s, 3H, CH_3Ar), 1.61 (br s, 4H, $CH_2CH_2CH_2$), 1.43 (s, 18H, $(CH_3)_3C$); ^{13}C NMR (100 MHz, CD_3CN) δ 183.0 (C), 180.3 (C), 155.3 (C), 171.3 (C), 141.6 (C), 141.4 (C), 128.9 (CH), 125.5 (CH), 78.7 (C), 78.0 (C), 43.7 (CH_2), 38.1 (CH_2), 37.3 (CH_2), 36.8 (CH_2), 35.2 (CH_2), 29.2 (CH_2), 28.8 (CH_2), 27.4 (CH_3), 27.3 (CH_3), 20.1 (CH_3); LRMS (ES^+): m/z (%) = 585 (100) $[M+H]^+$; HRMS (ES^+): found 585.3063 Da $[M+H]^+$, calculated 585.3065 Da; found 607.2883 Da $[M+Na]^+$, calculated 607.2884 Da, found 623.2631 Da $[M+K]^+$, calculated 623.2624 Da.

Methyl 3-(benzyloxy)-2-[(*tert*-butoxycarbonyl)amino]butanoate, 143

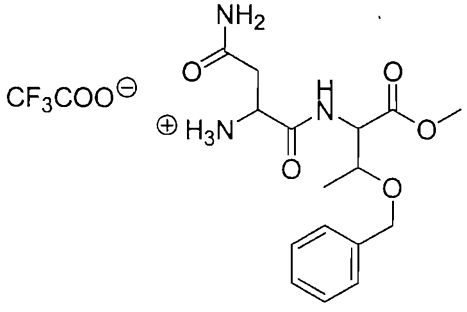


To a solution of *N*-Boc-L-Thr(OBn)-OH (1.05 g, 3.39 mmol) in dimethylformamide (20 ml) were added solid sodium bicarbonate (570 mg, 6.78 mmol) and methyl iodide (840 µl, 13.5 mmol) following established literature[121] procedures and the mixture was stirred at room temperature for 24 h. Following dilution with water (100 ml) the crude product was extracted with ethyl acetate (3 × 80 ml) and the organic extracts were dried over magnesium sulfate and evaporated to give **143** as a pale yellow oil (1.1 g, 100 %): I.R. (neat) ν_{max} = 3449 (w), 2978 (w), 2933 (w), 1751 (m), 1715 (s), 1677 (s), 1500 (s), 1386 (m), 1367 (m), 1166 (s) cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.29 (m, 5H, Ph) 5.30 (d, J = 10 Hz, 1H, NH), 4.56 (d, J = 11 Hz, 1H C*H*HPh), 4.37 (d, J = 11 Hz, 1H CH*H*Ph), 4.31 (dd, J = 10, 2.5 Hz, 1H, HNC*H*CO), 4.11 (qd, 1H, J = 6.5, 2.5 Hz, CH_3CHO), 3.68 (s, 3H, CH_3O), 1.46 (s, 9H, $(CH_3)_3CO$), 1.26 (d, J = 6.5 Hz, 3H, CH_3CH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8 (C), 156.3 (C), 138.0 (C), 128.5 (CH),

127.9 (CH), 80.0 (C), 74.5 (CH_2), 70.9 (CH_3), 58.4 (CH), 52.4 (CH), 28.5 (CH_3), 16.3 (CH_3); LRMS (ES^+): m/z (%) = 324 (15) $[M+H]^+$, 346 (100) $[M+Na]^+$, 669 (22) $[M+Na]^+$.

All data according to literature.[122]

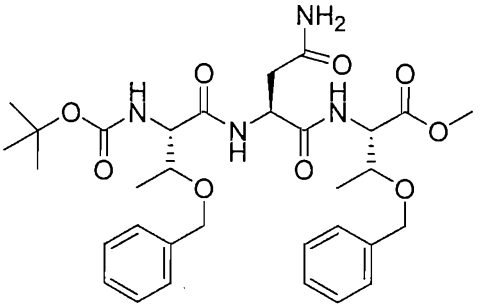
[3-amino-1-{[2-(benzyloxy)-1-(methoxycarbonyl)propyl]amino}carbonyl)-3-oxopropyl]ammonium 2,2,2-trifluoroacetate, 144



Ester **143** (1 g, 3.09 mmol) was stirred in a 20 % solution of trifluoroacetic acid in dichloromethane (10 ml of TFA in 40 ml of DCM) at room temperature for 3 h. After addition of toluene (130 ml) the solvents were removed under reduced pressure to give the corresponding TFA salt as a pale yellow oil. The residue was dissolved in a mixture of tetrahydrofuran (25 ml) and dimethylformamide (15 ml). The solution was cooled to 0° C and N-Boc-L-Asn-OH (836 mg, 3.67 mmol), HOBt·H_2O (626 mg, 4.63 mmol), DIPEA (1 ml, 3.67 mmol) and EDC (704 mg, 3.67 mmol) were added.[120] The mixture was stirred for 24 h (0° C to room temperature). Tetrahydrofuran was removed and the residue was diluted with dichloromethane (150 ml) and washed with 1M sodium hydrogensulfate (80 ml), 1M sodium hydrogencarbonate (80 ml), saturated brine (80 ml) and water (80 ml), and dried over magnesium sulfate. After removing the solvent, the pale yellow oil obtained was treated with a 20% solution of trifluoroacetic acid in dichloromethane (10 ml of TFA in 40 ml of DCM) for 2h. After evaporating the solvents, the residue was triturated with ether to give **144** as a white solid (1.1 g 82%): m.p. = 136-137 °C; I.R. (neat) ν_{max} = 3345 (w), 3133 (w), 1743 (m), 1673 (s), 1539 (m), 1493 (m), 1434 (m), 1344 (w), 1260 (m), 1202 (s), 1176 (s), 1118 (s); ^{1}H-NMR (300 MHz, $(CD_3)_2SO$) δ 8.79 (d, J = 9 Hz, 1H, NH), 8.16 (br s, 3H, NH_3^+), 7.78 (s, 2H, $CONH_2$), 7.30 (m, 5H, Ph), 4.59 (d, J = 12 Hz, 1H, C*H*HPh), 4.54 (dd, J = 9, 3 Hz,

1H, CH Asn), 4.39 (d, J = 12 Hz, 1H, CH*H*Ph), 4.28 (dd, J = 8.5, 4 Hz, 1H, CHN Thr), 4.09 (m, 1H, CH_3CHO Thr), 3.61 (s, 3H, CH_3OCO), 2.75 (dd, J = 17, 3 Hz, 1H, C*H*H$CONH_2$), 2.55 (dd, J = 17, 9 Hz, 1H, CH*H*$CONH_2$), 1.17 (d, J = 6 Hz, 3H, CH_3CH); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 171.1 (C), 170.3 (C), 169.3 (C), 168.9 (C), 138.2 (C), 128.3 (CH), 127.8 (CH), 127.6 (CH), 73.5 (CH_2), 70.0 (CH_3), 56.8 (CH), 52.2 (CH), 49.0 (CH), 35.5 (CH_2CO), 15.8 (CH_3); LRMS (ES^+): m/z (%) = 338 (100) $[M\text{-}TFA+H]^+$, 360 (6) $[M\text{-}TFA+Na]^+$, 675 (12) $[2(M\text{-}TFA)]^+$.

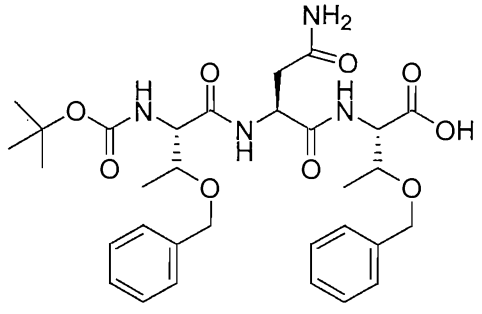
Methyl 2-{[4-amino-2-({3-(benzyloxy)-2-[(*tert*-butoxycarbonyl)amino]butanoyl} amino)-4-oxobutanoyl]amino}-3-(benzyloxy)butanoate, 145



TFA salt **144** (1.0 g, 2.3 mmol) was dissolved in a mixture of tetrahydrofuran (25 ml) and dimethylformamide (15 ml) and cooled to 0°C. Into the solution were added Boc-L-Thr(OBn)OH (817 mg, 2.6 mmol), HOBt·H_2O (446 mg, 3.3 mmol), DIPEA (800 µl, 4.6 mmol) and EDC (506 mg, 2.6 mmol). The mixture was stirred for 18 h (0°C to room temperature). Tetrahydrofuran was removed and the residue was diluted with dichloromethane (150 ml) and washed with 1M sodium bisulfate (80 ml), 1M sodium bicarbonate (80 ml) and water (2 × 80 ml). The organic layer was separated and dried over magnesium sulfate. After removing the solvent, crystallisation with ether/petroleum ether (3:1) gave **145** as a white solid (1.2 g, 84 %); m.p.= 139-140 °C; I.R. (neat) ν_{max} = 3425 (w), 3306 (m), 2972 (w), 2932 (w), 1741 (m), 1691 (s), 1657 (vs), 1643 (vs), 1523 (s), 1443 (m), 1388 (m), 1305 (m), 1247 (m), 1210 (w), 1165 (s), 1113 (s), 1095 (s); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.92 (d, J = 7.5 Hz, 1H, NH), 7.60 (d, J = 9 Hz, 1H, NH), 7.26 (m, 10H, Ph), 5.97 (br s, 1H, NH), 5.71 (br s, 1H, NH), 5.48 (d, J= 7.5 Hz, 1H, NH), 4.89 (m, 1H, CH Asn), 4.47 (m, 6H, CH_2Ph + C*H*CH_3 Thr), 4.09 (m, 2H, αCH Thr), 3.64 (s, 3H, CH_3OCO), 2.87 (dd, J = 16, 3.5 Hz, 1H,

C*H*HCONH$_2$), 2.53 (dd, J = 16, 6 Hz, 1H, CH*H*CONH$_2$), 1.44 (s, 9H, (CH$_3$)$_3$C), 1.17 (d, J = 6.5 Hz, 3H, C*H*$_3$CH), 1.16 (d, J = 6.5 Hz, 3H, C*H*$_3$CH); ^{13}C NMR (75 MHz, CDCl$_3$) δ 173.3 (C), 171.3 (C), 170.8 (C), 170.3 (C), 155.8 (C), 138.2 (C), 138.0 (C), 128.5 (CH), 128.4 (CH), 127.9 (CH), 127.8 (CH), 80.3 (C), 74.8 (CH), 73.8 (CH), 71.5 (CH$_2$), 70.7 (CH$_3$), 58.1 (CH), 57.2 (CH), 49.8 (CH), 37.1 (CH$_2$), 28.4 (CH$_3$), 16.1 (CH$_3$), 15.7 (CH$_3$); LRMS (ES$^+$): m/z (%) = 629 (65) [M+H]$^+$, 646 (100) [M+Na]$^+$.

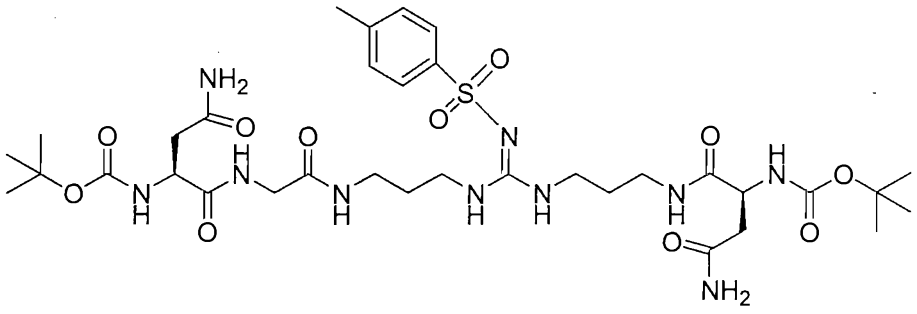
2-{[4-amino-2-({3-(benzyloxy)-2-[(*tert*-butoxycarbonyl)amino]butanoyl}amino)-4-oxobutanoyl]amino}-3-(benzyloxy)butanoic acid, 146



Ester **145** (500 mg, 0.81 mmol) was dissolved in a mixture of dioxane (6 ml) and 1M aqueous lithium hydroxide solution (2 ml). The mixture was stirred at room temperature for two hours and acidified till pH 5 with a 1M solution of hydrochloric acid. The precipitate obtained was filtered, washed with water (10 ml) and diethyl ether (3×10 ml) to give **146** as a white solid (470 mg, 96 %): m.p. = 208-210 °C; I.R. (neat) ν_{max} = 3450 (w), 3307 (m), 3275 (m), 2970 (w), 1715 (m), 1685 (s), 1642 (vs), 1530 (s), 1452 (w), 1386 (m), 1304 (m), 1248 (m), 1163 (s), 1113 (s); ^{1}H-NMR (400 MHz, (CD$_3$)$_2$SO) δ 8.36 (d, J = 7.5 Hz, 1H, NH Asn), 7.92 (d, J = 8.5 Hz, 1H, NH Thr), 7.49 (br s, 1H, N*H*H), 7.40 (m, 10H, Ph), 7.04 (br s, 1H, NH*H*), 6.54 (d, J = 9 Hz, 1H, NH Thr), 4.89 (m, 1H, C*H*CH$_2$CONH$_2$), 4.57 (m, 6H, CH$_2$Ph and CH$_3$C*H*O), 4.18 (m, 2H, C*H*CH$_3$), 3.93 (m, 1H, CH$_3$C*H*O), 1.49 (s, 9H, (CH$_3$)$_3$C), 1.18 (m, 6H, CH$_3$CH); Asn signals are covered by the DMSO signal; ^{13}C NMR (75 MHz, (CD$_3$)$_2$SO) δ 172.0 (C), 171.7 (C), 170.3 (C), 155.7 (C), 139.2 (C), 138.9 (C), 128.5 (CH), 128.5 (CH), 127.9 (CH), 127.8 (CH), 127.7 (CH), 127.6 (CH), 78.8 (C), 75.6 (CH), 74.8 (CH), 70.8 (CH$_2$), 70.5 (CH$_2$), 58.7 (CH), 56.7 (CH), 49.8 (CH), 37.7 (CH$_2$), 28.6 (CH$_3$), 17.0 (CH$_3$), 16.4 (CH$_3$); LRMS (ES$^+$): m/z (%) = 615 (65) [M+H]$^+$, 637 (100) [M+Na]$^+$;

HRMS (ES^+): found 615.3033 Da $[M+H]^+$, calculated 615.3025 Da; found 637.2856 Da $[M+Na]^+$, calculated 637.2844 Da.

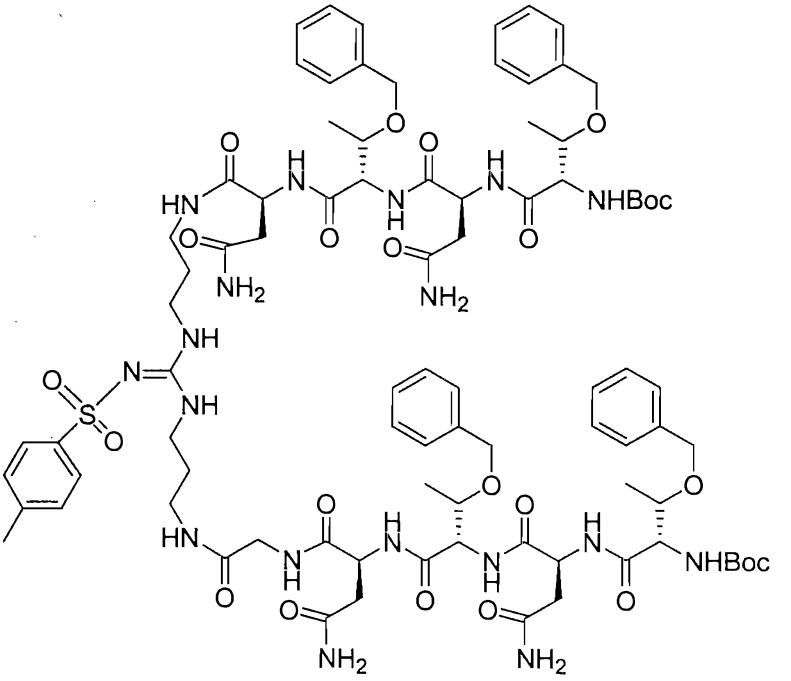
***tert*-butyl N-[1-({[3-({({3-[(2-{[2-(tertbutyloxyamino)-4-amino-4-oxobutanoyl] amino}acetyl)amino)[(phenylsulfonyl)imino]methyl}amino)propyl]amino}carbony l)3-amino-3-oxopropyl]} carbamate, 147**



Dicarbamate **138** (470 mg, 0.78 mmol) was stirred in a 20% solution of trifluoroacetic acid in dichloromethane (5 ml of TFA in 20 ml of DCM) at room temperature for 3 h. After evaporation of the solvents the residue was triturated with ether to give a white solid that was redissolved in a mixture of tetrahydrofuran (10 ml) and dimethylformamide (5 ml). The solution was cooled to 0° C and *N*-Boc-L-Asn-OH (500 mg, 2.2 mmol), HOBt·H_2O (290 mg, 2.1 mmol), DIPEA (550 µl, 3.2 mmol) and EDC (389 mg, 2.0 mmol) were added. The mixture was stirred for 18 h (0° C to room temperature). Tetrahydrofuran was removed and the residue was suspended in ether (60 ml). The solution was decanted and the yellow oil obtained was purified by column chromatography (20 % ethyl acetate in dichloromethane) to give **147** as a white foam (500 mg, 83 % yield); R_f = 0.28 (20 % ethyl acetate in DCM); I.R. (neat) ν_{max} = 3323 (m), 2978 (w), 2928 (w), 1680, 1572 (s), 1518 (m), 1360 (m), 1241 (s), 1158 (s), 1123 (s), 1084 (s); ^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ 8.15 (m, 1H, NH), 7.82 (m, 1H, NH), 7.75 (m, 1H, NH), 7.65 (d, *J* = 8 Hz, 2H, ArH, *o* to SO_2), 7.43 (br s, 1H, NH), 7.28 (d, *J* = 8 Hz, 3H, ArH, *m* to SO_2+ NH), 7.07 (br s, 2H, NH), 6.94 (m, 2H, NH), 6.87 (br s, 1H, NH), 6.83 (d, *J* = 7.5 Hz, 1H, NH), 4.19 (m, 2H, CH Asn), 3.64 (m, 2H, CH_2 Gly), 3.10 (m, 4H, C*H*$_2$NH), 3.00 (m, 4H, C*H*$_2$NH), 2.60-2.35 (m, 4H, CH_2CO Asn), 2.34 (s, 3H, CH_3Ar), 1.51 (m, 4H, CH_2C*H*$_2$$CH_2$), 1.38 (s, 18H, $(CH_3)_3$C); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 172.4 (C), 172.1 (C), 172.0 (C), 155.7 (C), 155.5 (C), 155.3 (C), 142.0

(C), 141.5 (C), 129.5 (CH), 126.1 (CH), 78.9 (C), 78.6 (C), 52.0 (CH), 51.8 (CH), 42.9 (CH_2), 38.7 (CH_2), 38.5 (CH_2), 37.8 (CH_2), 37.6 (CH_2), 36.1 (CH_2), 31.1 (CH_2), 29.5 (CH_2), 28.6 (CH_3), 21.3 (CH_3); LRMS (ES^+): m/z (%) = 835 (8) $[M+Na]^+$; HRMS (ES^+): found 813.3943 Da $[M+H]^+$, calculated 813.3924 Da; found 835.3767 Da $[M+Na]^+$, calculated 835.3743 Da, found 851.3505 Da $[M+K]^+$, calculated 851.3483 Da.

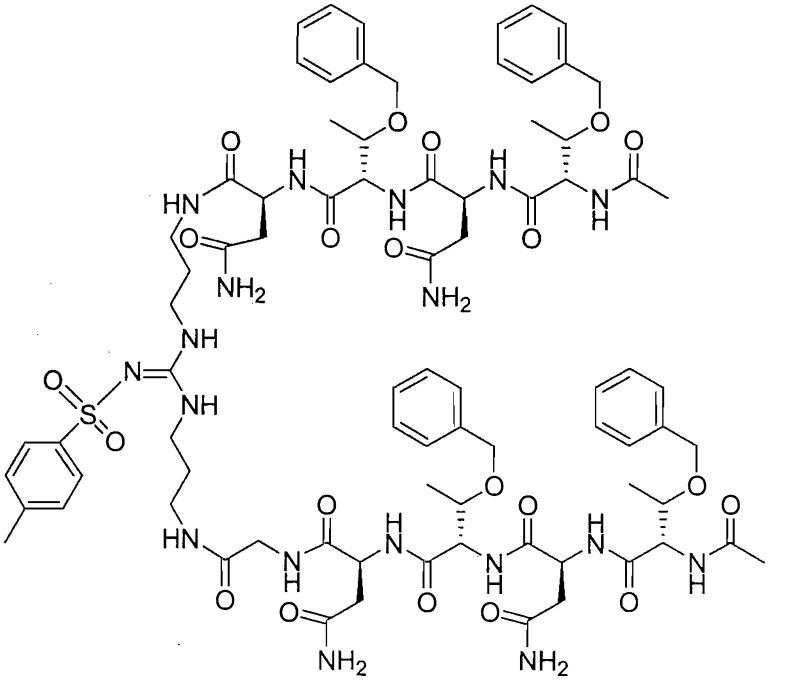
Boc-protected tweezer, 148



Dicarbamate **147** (180 mg, 0.22 mmol) was stirred in a 25% solution of trifluoroacetic acid in dichloromethane (20 ml) at room temperature for 3 h. After evaporation of the solvents the residue was triturated with ether to give a white solid that was redissolved in a mixture of tetrahydrofuran (10 ml) and dimethylformamide (5 ml). The solution was cooled to 0° C and tripeptide **146** (300 mg, 0.48 mmol), HOBt·H_2O (59 mg, 0.43 mmol), DIPEA (110 µl, mmol) and EDC (84 mg, 0.44 mmol) were added. The mixture was stirred for 24 h (0° C to room temperature). Tetrahydrofuran was removed and ether (5 ml) and DCM (10 ml) were added to the solution. The precipitate (a gel) was centrifuged and washed with ether, methanol and dichloromethane, to give **148** as a white solid (200 mg, 50% yield): m.p. = 222-224 °C; I.R. (neat) ν_{max} = 3425 (w), 3306 (m), 2972 (w), 2932 (w), 1741 (m), 1691 (s), 1657 (vs), 1643 (vs), 1523 (s), 1443

(m), 1388 (m), 1305 (m), 1247 (m), 1210 (w), 1165 (s), 1113 (s), 1095 (s); ^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ 8.32 (m, 3H, NH Asn), 8.25 (d, *J* = 5.5 Hz, 1H, NH Asn), 8.18 (m, 2H, NH Thr + NH Gly) 8.06 (d, *J* = 8 Hz, 1H, NH Thr), 7.83 (t, *J* = 5.5 Hz, 1H, NH), 7.76 (d, *J* = 8 Hz, 2H, ArH, o to SO_2), 7.60 (m, 5H, $CONH_2$ + NH), 7.38 (m, 2H + 20H, ArH, m to SO_2 + Ph), 7.10 (m, 5H, $CONH_2$ + NH), 7.00 (br s, 1H, NH), 6.57 (d, *J* = 6 Hz, 2H, NH Thr), 4.86 (m, 2H, CHN Asn), 4.67 (m, 2H, CHN Asn), 4.53 (m, 8H, $PhCH_2O$), 4.46 (dd, *J* = 4.5, 2 Hz, 1H, CHN Thr), 4.39 (dd, *J* = 4.5, 2 Hz, 1H, CHN Thr), 4.20 (dd, *J* = 6 Hz, *J* = 5.5 Hz, 2H, CH_3CHCHN Thr), 4.08 (m, 2H, CH_3CHO Thr), 3.91 (m, 2H, CH_3CHO Thr), 3.77 (dd, *J* = 17, 5.5 Hz, 1H, NCHHCO Gly), 3.63 (dd, *J* = 17, 5.5 Hz, 1H, NCHHCO Gly), 3.18 (m, 6H, CH_2NH), 3.09 (m, 2H, CH_2NH), 2.75 (m, 2H, CH_2CO) 2.43 (s, 3H, CH_3Ar), 1.61 (m, 2H, $CH_2CH_2CH_2$), 1.54 (m, 2H, $CH_2CH_2CH_2$) 1.48 (s, 9H, $(CH_3)_3C$), 1.16 (m, 6H, CH_3CH); part of Asn signals are covered by the DMSO signal; ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 173.6 (C), 173.5 (C), 173.4 (C), 173.0 (C), 173.0 (C), 172.6 (C), 171.6 (C), 171.3 (C), 170.4 (C), 156.5 (C), 143.1 (C), 142.8 (C), 140.4 (C), 140.2 (C), 140.1 (C), 130.7 (CH), 129.8 (CH), 129.7 (CH), 129.2 (CH), 128.9 (CH), 128.9 (CH), 127.3 (CH), 123.2 (CH), 80.0 (C), 72.2 (CH), 72.1 (CH), 72.0 (CH), 52.0 (CH), 51.2 (CH), 50.5 (CH), 50.3 (CH), 50.3 (CH), 32.3 (CH_2), 29.8 (CH_3), 22.5 (CH_3), 18.2 (CH_3), 18.1 (CH_3), 18.0 (CH_3); MS (MALDI-TOF): m/z (%) = 1828 (92) $[M+Na]^+$, 1844 (82) $[M+K]^+$; Anal. Calcd for $C_{86}H_{120}N_{18}O_{23}S \times 5\ H_2O$: C, 54.48; H, 6.91; N, 13.30. Found: C, 54.54; H, 6.68; N 13.26.

Acetyl-protected tweezer, 149

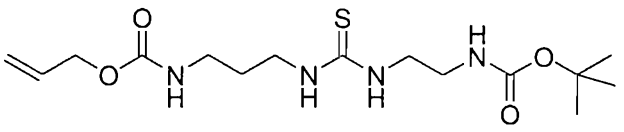


Compound **148** (35 mg, 0.019 mmol) was stirred in a ~25% solution of trifluoroacetic acid in dichloromethane (10 ml) for 3h at room temperature. After removing the solvent the residue was triturated with diethyl ether (10 ml) and redissolved in a 1:1 mixture of dimethylsulfoxide (1 ml) and chloroform (1 ml). The solution was cooled to 0°C, and triethylamine (25 µl, 0.14 mmol) and acetyl chloride (10 µl, 0.14 mmol) were added dropwise. The mixture was stirred for 1 h (0°C to room temperature). Chloroform was then removed and ether (2 ml) and dichloromethane (3 ml) were added. The jelly precipitate obtained was centrifuged, dried and washed with chloroform and methanol to give **149** as a white grey solid (25 mg, 78 % yield): m.p. = 236-238 ° C; I.R. (neat) ν_{max} = 3425 (w), 3306 (m), 2972 (w), 2932 (w), 1741 (m), 1691 (s), 1657 (vs), 1643 (vs), 1523 (s), 1443 (m), 1388 (m), 1305 (m), 1247 (m), 1210 (w), 1165 (s), 1113 (s), 1095 (s); ^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ 8.37 (m, 2H, NH Asn), 8.30 (d, J = 7 Hz, 1H, NH Asn), 8.23 (d, J= 7.5 Hz, 1H, NH Asn), 8.19 (m, 1H, NH Gly), 8.10 (d, J = 7.5 Hz, 1H, NH Thr), 8.04 (m, 2H, NH Thr), 8.00 (d, J = 8 Hz, 1H, NH Thr), 7.83 (t, J = 5.5 Hz, 1H, NH), 7.76 (d, J = 8 Hz, 2H, ArH, *o* to SO_2), 7.60 (m, 4H, $CONH_2$), 7.38 (m, 2H + 20H, ArH *m* to SO_2 + PhH), 7.10 (m, 6H, $CONH_2$+NH), 7.00 (br s, 1H, NH), 4.84 (m, 2H, CHN Asn), 4.66 (m, 2H, CHN Asn), 4.60-4.45 (m, (8+2)H, PhCH_2O + CHN Thr), 4.47 (dd, J = 5, 1 Hz, 1H, CHN Thr), 4.40 (dd, J = 5, 1 Hz, 1H, CHN Thr), 4.07 (m, 2H, CH_3CHO Thr), 3.95 (m, 2H, CH_3CHO Thr), 3.77 (dd,

J = 17, 6 Hz, 1H, NC*H*HCO Gly), 3.64 (dd, J = 17, 5.5 Hz, 1H, NCH*H*CO Gly), 3.18 (m, 6H, C*H*$_2$NH), 3.09 (m, 2H, C*H*$_2$NH), 2.80-2.50 (m, CH_2CO, part under DMSO signal) 2.43 (s, 3H, CH_3Ar), 2.00 (s, 6H, CH_3CO), 1.61 (m, 2H, $CH_2CH_2CH_2$), 1.55 (m, 2H, $CH_2CH_2CH_2$), 1.16 (m, 6H, C*H*$_3$CH); part of Asn signals are covered by the DMSO signal; ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 176.2 (C), 172.5 (C), 170.3 (C), 170.1 (C), 155.3 (C), 142.0 (C), 139.2 (C), 139.0 (C), 138.9 (C), 129.5 (CH), 128.5 (CH), 128.0 (CH), 127.9 (CH), 127.7 (CH), 126.0 (CH), 75.5 (CH), 70.9 (CH), 57.1 (CH), 50.1 (CH_2), 36.8 (CH_2), 28.8 (CH_2), 21.3 (CH_3), 20.8 (CH_2), 17.0 (CH_3), 16.8 (CH_3), 16.8 (CH_3); MS (MALDI-TOF): m/z (%) = 1713 (100) $[M+Na]^+$, 1728 (60) $[M+K]^+$.

Procedure A for the Synthesis of Thiourea 156c, 156d, 156e and 156f. Mono-protected diamine **158** (7.6 mmol) was added to isothiocyanate **159** (6.9 mmol) in a 1:1 solvent mixture of methanol (10 mL) and dichloromethane (10 mL). After stirring at room temperature for 48 h the solvent was removed under reduced pressure. The crude product was purified by FC on silica gel (EtOAc/dichloromethane 50:50 for **156c** and **156f**, 30:70 for **156d** and EtOAc/Pet. Ether 80:20 for **156e**).

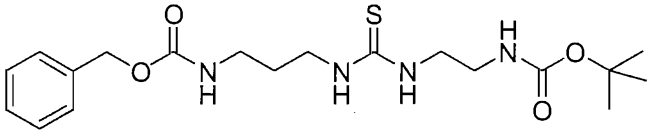
{2-[3-(2-Vinyloxycarbonylamino-ethyl)-thioureido]-ethyl}-carbamic acid *tert*-butyl ester, 156c



Synthesised according to procedure A on a 6.9 mmol scale: yield (1.72 g, 72%): pale yellow oil; R_f = 0.16 (EtOAc/dichloromethane 50:50); I.R. (neat) ν_{max} = 3300 (m), 2964 (m), 2930 (m), 2883 (w) 1669 (s), 1521 (s), 1245 (s), 1160 (s) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.83 (br s, 2H, NHCS), 5.90 (m, 1H, CH_2=C*H*CH_2O), 5.45 (br s, 1H, NHCO), 5.30 (dd, J = 17.0, 1.5 Hz, 1H, CH*H*=$CHCH_2O$), 5.22 (dd, J = 10.5, 1.5 Hz, 1H, C*H*H=$CHCH_2O$), 5.09 (br s, 1H, NHCO), 4.57 (d, J = 5.0 Hz, 2H, CH_2=$CHCH_2O$), 3.62 (br s, 2H, $NHCH_2CH_2NHCS$), 3.54 (br s, 2H,

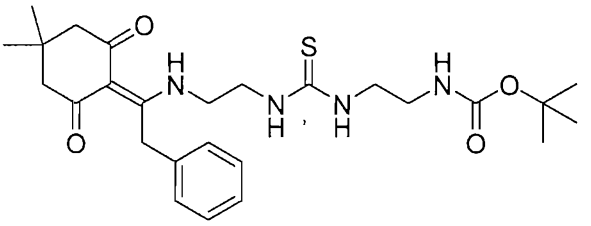
$NHCH_2CH_2NHCS$), 3.42 (q, J = 6.0 Hz, 2H, $NHCH_2CH_2NHCS$), 3.31 (q, J = 6.0 Hz, 2H, $NHCH_2CH_2NHCS$), 1.45 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.3 (C), 157.3 (C), 132.7 (CH), 117.9 (CH_2), 80.4 (C), 65.9 (CH_2), 45.2 (CH_2), 44.9 (CH_2), 40.3 (CH_2), 39.7 (CH_2), 28.4 (CH_3); MS (ES^+): *m/z* (%) = 347 (5) $[M+H]^+$, 693 (5) $[2M+H]^+$, 715 (27) $[2M+Na]^+$. All structural assignments were in agreement with MS, ^1H and ^{13}C data available from the literature.[96]

{3-[3-(2-*tert*-Butoxycarbonylamino-ethyl)-thioureido]-propyl}-carbamic acid benzyl ester, 156d



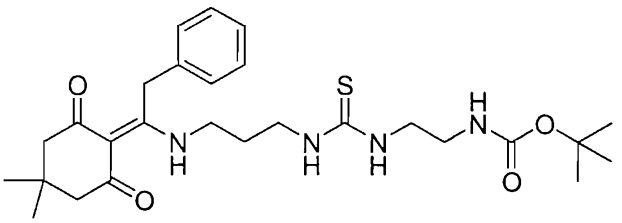
Synthesised according to procedure A on a 2.53 mmol scale: yield (750 mg, 72%); white foam; R_f = 0.19 ($EtOAc/CH_2Cl_2$ 30:70); I.R. (neat) ν_{max} = 3302 (m), 2961 (m), 2932 (m), 2883 (w) 1679 (s), 1521 (s), 1245 (s), 1161 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.27 (m, 5H, Ph), 6.76 (br s, 2H, NHCS), 5.32 (br s, 1H, NHCO), 5.02 (s, 3H, $PhCH_2$ + NH), 3.45 (br s, 4H, CH_2NHCS), 3.19 (m, 4H, CH_2NHCO), 1.70 (quint, J = 6 Hz, 2H, $CH_2CH_2CH_2$), 1.35 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.3 (C), 157.6 (C), 157.5 (C), 136.9 (C), 128.9 (CH), 128.6 (CH), 128.4 (CH), 80.6 (C), 67.2 (CH_2), 41.5 (CH_2), 40.2 (CH_2), 38.2 (CH_2), 29.8 (CH_2), 28.8 (CH_3); MS (ES^+): m/z (%) = 411 (20) $[M+H]^+$, 433 (100) $[M+Na]^+$, 449 (40) $[M+K]^+$, 843 (25) $[2M+Na]^+$.

[2-(3-{2-[1-(4,4-Dimethyl-2,6-dioxo-cyclohexylidene)-2-phenyl-ethylamino]-ethyl}-thioureido)-ethyl]-carbamic acid tert-butyl ester, 156e



Synthesised according to procedure A on a 8.3 mmol scale: yield (3.0 g, 69%); white foam; R_f = 0.18 (EtOAc/Pet. Ether 80:20); I.R. (neat) ν_{max} = 3118 (m), 2909 (w), 2250 (w), 1659 (s), 1596 (s), 1475 (s), 1406 (s), 1304 (m), 1185 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 12.46 (br s, 1H, C=C-NH), 7.19 (m, 5H, Ph), 4.59 (s, 2H, PhCH_2), 3.70 (br s, 4H, CH_2NH), 3.54 (br s, 2H, CH_2NH), 3.29 (m, 2H, CH_2NH), 2.40 (s, 4H, CH_2CO), 1.43 (s, 9H, $(CH_3)_3C$), 1.04 (s, 6H, $(CH_3)_2C$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 198.4 (C), 179.3 (CS), 166.1 (C), 158.9 (C), 141.9 (C), 128.9 (CH), 128.3 (CH), 127.7 (CH), 108.5 (C), 81.0 (C), 53.0 (CH_2), 44.0 (CH_2), 42.5 (CH_2), 39.1 (CH_2), 30.2 (C), 28.5 (CH_3), 28.4 (CH_3); LRMS (ES^+): m/z (%) = 503 (79) $[M+H]^+$, 1005 (20) $[2M+H]^+$; HRMS (ES^+): m/z (%) = found 503.2685 Da $[M+H]^+$, calculated 503.2687 Da; found 525.2502 Da $[M+Na]^+$, calculated 525.2506 Da.

[2-(3-{3-[1-(2,6-Dioxo-cyclohexilidene)-2-phenyl-ethylamino]-propyl}-thioureido)-ethyl]-carbamic acid *tert*-butyl ester, 156f



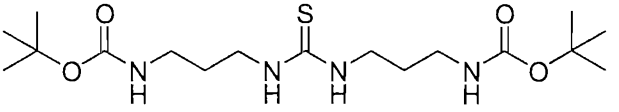
Synthesised according to procedure A on a 1 mmol scale: yield (400 mg, 78%); white foam; R_f = 0.20 (EtOAc/Dichlorometane 50:50); I.R. (neat) ν_{max} = 3288 (m), 2954 (m),2931 (m), 2869 (w) 1688 (m), 1630 (m), 1565 (s), 1496 (s), 1450 (s), 1341 (s), 1272 (s), 1248 (s), 1163 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 13.53 (br s, 1H, C=C-

NH), 7.15 (m, 5H, Ph), 4.50 (s, 2H, $PhCH_2$), 3.43 (br s, 4H, CH_2NH), 3.38 (q, J = 7 Hz, 2H, CH_2NH), 3.19 (t, J = 6 Hz, 2H, CH_2NH), 2.34 (s, 4H, CH_2CO), 1.82 (quint, J = 7 Hz, 2H, $CH_2CH_2CH_2$), 1.36 (s, 9H, $(CH_3)_3C$), 0.98 (s, 6H, $(CH_3)_2C$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.8 (C), 182.5 (CS), 174.3 (C), 157.9 (C), 136.2 (C), 128.8 (CH), 128.0 (CH), 126.5 (CH), 108.0 (C), 80.2 (C), 52.8 (CH_2), 40.8 (CH_2), 39.7 (CH_2), 38.3 (CH_2), 35.0 (CH_2), 29.9 (C), 28.5 (CH_2), 28.2 (CH_3), 28.1 (CH_3); MS (ES^+): m/z (%) = 515 (79) $[M+H]^+$.

Procedure B for the Synthesis of Thiourea 156a and 156b.

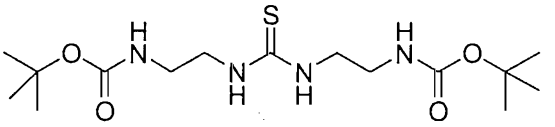
1-(*tert*-Butyloxycarbonyl)propyldiamine (1.2 g, 6.9 mmol) was dissolved in chloroform (50 ml) and triethylamine (0.9 ml, 6.8 mmol) was added to the solution that was cooled to 0°C. Thiophosgene (264 µl, 3.4 mmol) was added dropwise and the solution allowed to stir at room temperature for 36 h. The solvents were evaporated under reduced pressure to give a yellow oil. Further purification by column chromatography (EtOAc/CH_2Cl_2 25:75) afforded the pure products as white foams.

{3-[3-(3-*tert*-Butoxycarbonylamino-propyl)-thioureido]-propyl}-carbamic acid *tert*-butyl ester, 156a



Synthesised according to procedure B in a 3.4 mmol scale: yield (1.00 g, 75 %); R_f = 0.28 (EtOAc/CH_2Cl_2 25:75); I.R. (neat) ν_{max} = 3295 (m), 2978 (m), 2935 (m), 1686 (m), 1625 (s), 1520 (s), 1438 (m), 1367 (m), 1276 (m), 1251 (s), 1163 (s), 1139 (s), 1051 (m), 946 (w), 904 (m), 841 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.70 (br s, 2H, NH), 4.87 (br s, 2H, NHBoc), 3.49 (br s, 4H, CH_2NHCS), 3.13 (q, J = 6 Hz, 4H, CH_2NHCO), 1.68 (quint, J = 6 Hz, 4H, $CH_2CH_2CH_2$), 1.37 (s, 18H, $(CH_3)_3C$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.5 (C), 155.9 (C), 78.7 (C), 39.8 (CH_2), 36.3 (CH_2), 28.7 (CH_2), 27.4 (CH_3); (ES^+): m/z (%) = 391 (100) $[M+H]^+$, 413 (20) $[M+Na]^+$.

{3-[3-(3-*tert*-Butoxycarbonylamino-ethyl)-thioureido]-ethyl}-carbamic acid *tert*-butyl ester, 156b

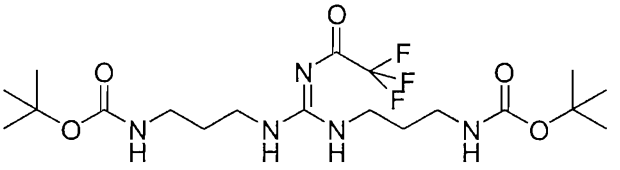


Synthesised according to procedure B in a 3.0 mmol scale: yield (821 mg, 75 %); R_f = 0.25 (EtOAc/CH_2Cl_2 25:75); I.R. (neat) ν_{max} = 3334 (m), 3256 (m), 2974 (m), 2935 (m), 1676 (m), 1624 (s), 1532 (s), 1437 (m), 1365 (m), 1275 (m), 1237 (s), 1160 (s), 1134 (s), 891 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.82 (br s, 2H, NHCS), 5.09 (br s, 2H, NHCO), 3.48 (br s, 4H, CH_2NHCS), 3.26 (q, J = 6 Hz, 4H, CH_2NHCO), 1.37 (s, 18H, $(CH_3)_3C$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.5 (C), 157.5 (C), 80.5 (C), 40.1 (CH_2), 36.3 (CH_2), 28.8 (CH_2); (ES^+): m/z (%) = 367 (18) $[M+H]^+$, 399 (100) $[M+Na]^+$.

General Procedure for the Synthesis of Guanidine 157. Iodomethane (200 µl, 2.1 mmol) was added to a stirred solution of thiourea **156** (1.3 mmol) in acetone (20 ml) and the reaction mixture was stirred for 18 h at room temperature. The solvent and all volatile compounds were removed under reduced pressure to give a slightly yellow foam. The foam was redissolved in a 1:1 mixture of dichloromethane (15 ml) and methanol (15 ml). Ammonium hexafluorophosphate (424 mg, 2.6 mmol) was added and the resulting solution stirred for 18 h at room temperature. The solvents were evaporated and the yellow oily residue redissolved in dichloromethane (100 ml) and washed with water (80 ml). The organic solution was dried over magnesium sulphate and the solvent removed under reduced pressure to afford the methylthiouronium hexafluorophosphate as a white foam in a quantitative yield. The hexafluorophosphate was redissolved in a 4:1 mixture of toluene (20 ml) and chloroform (5 ml) and neat DBU (450 µl, 3.0 mmol) and trifluoroacetamide (542 mg, 4.8 mmol) were added. The mixture was refluxed for 8-18 h under vigorous stirring. The solvents were removed under reduced pressure to give a oil. The crude product was purified by FC on silica gel using the eluent mixtures EtOAc/CH_2Cl_2.

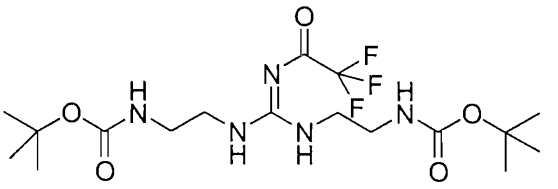
All the NMR spectra show that the trifluoroacetyl guanidines exist in solution in more than one conformer; ^{13}C NMR are messy and they show a lot more signal than they should.

{3-[*N'*-(3-*tert*-Butoxycarbonylamino-propyl)-*N''*-(2,2,2-trifluoro-acetyl)-guanidino]-propyl}-carbamic acid *tert*-butyl ester, 157a



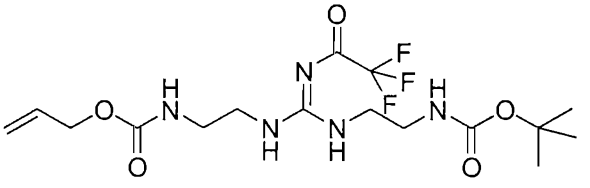
Synthesised according to the general procedure on a 1.3 mmol scale: yield (579 mg, 86%); white foam; R_f = 0.28 (EtOAc/CH_2Cl_2 40:60); I.R. (neat) ν_{max} = 3328 (m), 2975 (m), 2939 (m), 1692 (m), 1629 (s), 1520 (s), 1438 (m), 1371 (m), 1257 (m), 1163 (s), 1171 (s), 1139 (m), 849 (w), 795 (m) cm^{-1}; ^1H NMR (400 MHz, CD_3CN) δ 9.29 (br s, 1H, NH), 6.37 (br s, 1H, NH), 5.46 (br s, 1H, NHCO), 5.37 (br s, 1H, NHCO), 3.35 (m, 2H, CH_2NH), 3.15 (m, 2H, CH_2NH), 3.03 (m, 4H, CH_2NH), 1.67 (br s, 2H, $CH_2CH_2CH_2$), 1.55 (br s, 2H, $CH_2CH_2CH_2$), 1.34 (s, 18H, $(CH_3)_3C$); ^{13}C NMR (100 MHz, CD_3CN) δ 165.9 (q, J = 35 Hz, $COCF_3$), 160.2 (C), 156.2 (C), 156.1 (C), 117.0 (q, J = 285 Hz, CF_3), 79.1 (C), 39.1 (CH_2), 38.4 (CH_2), 38.1 (CH_2), 37.3 (CH_2), 30.5 (CH_2), 29.6 (CH_2), 28.3 (CH_3); MS (ES^+): m/z (%) = 527 (21) $[M+H]^+$, 549 (60) $[M+Na]^+$, 1075 (5) $[2M+Na]^+$; Anal. Calcd for $C_{19}H_{34}F_3N_5O_5$: C, 48.61; H, 7.30; N, 14.92. Found: C, 48.38; H, 7.41; N 14.80.

{3-[*N'*-(3-*tert*-Butoxycarbonylamino-ethyl)-*N''*-(2,2,2-trifluoro-acetyl)-guanidino]-ethyl}-carbamic acid *tert*-butyl ester , 157b



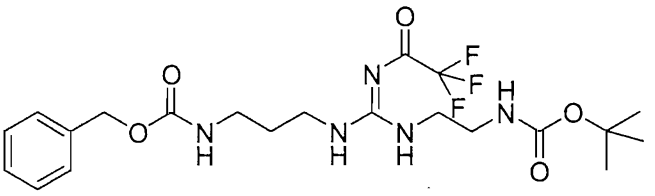
Synthesised according to the general procedure on a 0.56 mmol scale: yield (74 mg, 92%); pale yellow oil; R_f = 0.16 (EtOAc:CH_2Cl_2 40:60); I.R. (neat) ν_{max} = 3328 (m), 2975 (m), 2939 (m), 1692 (m), 1629 (s), 1520 (s), 1438 (m), 1371 (m), 1257 (m), 1163 (s), 1171 (s), 1139 (m), 849 (w), 795 (m) cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ 9.21 (br s, 1H, NH), 7.65 (br s, 1H, NH), 7.07 (br s, 1H, NH), 6.93 (br s, 1H, NH), 3.10-3.60 (m, 8H, C*H*$_2$NH), 1.43 (s, 18H, $(CH_3)_3C$); ^{13}C NMR (100 MHz, CD_3CN) δ 165.2 (q, *J* = 32 Hz, *C*OCF_3), 160.9 (C), 156.4 (C), 116.8 (q, *J* = 285 Hz, CF_3), 78.8 (C), 78.1 (C), 41.8 (CH_2), 40.5 (CH_2), 39.9 (CH_2), 38.2 (CH_2), 27.3 (CH_3); MS (ES^+): m/z (%) = 527 (21) $[M+H]^+$, 549 (60) $[M+Na]^+$, 1075 (5) $[2M+Na]^+$; Anal. Calcd for $C_{17}H_{30}F_3N_5O_5$: C, 46.25; H, 6.85; N, 15.86. Found: C, 45.98; H, 7.03; N 15.60.

{2-[*N*'-(2-Allyloxycarbonylamino-ethyl)-*N*''-(2,2,2-trifluoro-acetyl)-guanidino]-ethyl}-carbamic acid tert-butyl ester, 157c



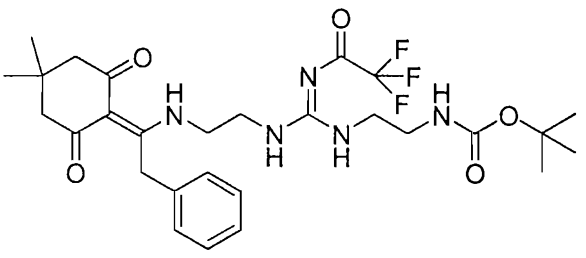
Synthesised according to the general procedure on a 0.19 mmol scale: yield (74 mg, 92%); pale yellow oil; R_f = 0.16 (EtOAc:CH_2Cl_2 40:60); ^1H NMR (400 MHz, $CDCl_3$) δ 9.32 (br s, NH), 6.96 (br s, NH), 6.64 (br s, NH), 6.47 (br s, NH), 6.07 (br s, NHCO), 5.93 (m, 1H, CH_2=C*H*CH_2O), 5.68 (br s, NHCO), 5.30 (d, *J* = 17.5 Hz, 1H, CH*H*=$CHCH_2O$), 5.20 (d, *J* = 8.0 Hz, 1H, C*H*H=$CHCH_2O$), 4.54 (d, *J* = 5.5 Hz, 2H, CH_2=$CHCH_2O$), 3.47 (br s, 2H, C*H*$_2$NH), 3.26 (br s, 6H, C*H*$_2$NH), 1.42 (s, 9H, $(CH_3)_3C$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.2 (q, *J* = 35.0 Hz, $COCF_3$), 160.7 (C), 157.0 (C), 156.6 (C), 133.0 (CH), 116.5 (CH_2), 115.8 (q, *J* = 285 Hz, CF_3), 79.2 (C), 65.1 (CH_2), 38.0-41.7 (br, CH_2), 27.3 (CH_3); MS (ES^+): *m/z* (%): 426 (95) $[M+H]^+$, 448 (100) $[M+Na]^+$; Anal. Calcd for $C_{16}H_{26}F_3N_5O_5$: C, 45.17; H, 6.16; N, 16.45. Found: C, 45.22; H, 6.30; N 16.32

{3-[N-(2-*tert*-Butoxycarbonylamino-ethyl)-N'-(2,2,2-trifluoro-acetyl)-guanidino]-propyl}-carbamic acid benzyl ester , 157d



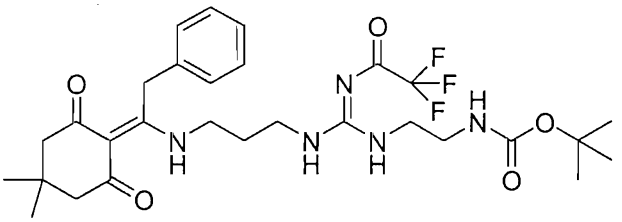
Synthesised according to to the general procedure on a 1.22 mmol scale: yield (500 mg, 85%); white foam; R_f = 0.28 (EtOAc);); I.R. (neat) ν_{max} = 3329 (m), 2973 (m), 2939 (m), 1690 (m), 1629 (s), 1438 (m), 1371 (m), 1257 (m), 1163 (s), 1171 (s), 1139 (m), 849 (w), 795 (m) cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ 9.45 (br s, 0.5H, NH), 9.04 (br s, 0.5H, NH), 9.01 (br s, 0.5H, NH), 7.48 (br s, 1H, NH), 7.27 (m, 5H, Ph), 7.16 (br s, 0.5H, NH), 6.93 (br s, 0.5H, NH), 6.82 (br s, 0.5H, NH), 4.98 (s, 2H, $PhCH_2$), 3.20 (m, 4H, CH_2NH), 3.03 (m, 4H, CH_2NH), 1.67 (br s, 2H, $CH_2CH_2CH_2$), 1.59 (m, 2H, $CH_2CH_2CH_2$), 1.32 (s, 9H, $(CH_3)_3C$); ^{13}C NMR (100 MHz, CD_3CN) δ 165.1 (q, J = 35.0 Hz, $COCF_3$), 160.5 (C), 156.4 (C), 137.1 (C), 128.1 (CH), 127.5 (CH), 127.4 (CH), 117.0 (q, J = 286 Hz, CF_3), 78.8 (C), 78.3 (C), 65.6 (CH_2), 41.8 (CH_2), 40.4 (CH_2), 39.8 (CH_2), 38.3 (CH_2), 38.1 (CH_2), 37.7 (CH_2), 37.5 (CH_2), 37.0 (CH_2), 29.1 (CH_2), 28.4 (CH_2), 27.2 (CH_3); MS (ES^+): m/z (%) = 490 (100) $[M+H]^+$, 512 (35) $[M+Na]^+$, 979 (10) $[2M+H]^+$, 1001 (5) $[2M+Na]^+$; Anal. Calcd for $C_{21}H_{30}F_3N_5O_5$: C, 51.53; H, 6.18; N, 14.31. Found: C, 51.29; H, 6.43; N 14.08

{2-[N'-{3-[1-(2,6-Dioxo-cyclohexylidene)-2-phenyl-ethylamino]-ethyl}-N''-(2,2,2-trifluoro-acetyl)-guanidino]-ethyl}-carbamic acid tert-butyl ester, 157e



Synthesised according to the general procedure on a 0.12 mmol scale: yield (36 mg, 50%); white foam; R_f = 0.25 (EtOAc/CH_2Cl_2 75:25); I.R. (neat) ν_{max} = 3636 (m), 3568 (m), 2982 (w), 1625 (m), 1566 (s), 1520 (s), 1367 (m), 1190 (m), 1140 (s), 825 (s) cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ 9.29 (br s, NH), 7.60-7.40 (m, NH), 7.16 (br s, NH), 6.30-6.00 (m, NH), 3.63 (d, J = 5.5 Hz, 2H, CH_2), 3.36 (m, 2H, CH_2NH), 3.21 (m, 4H, CH_2NH), 3.08 (m, 2H, CH_2NH), 1.37 (s, 9H, CH_3); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 162.5 (C), 138.4 (C), 130.7 (CH), 130.2 (CH), 128.5 (CH), 110.0 (C), 80.2 (C), 54.5 (CH_2), 36.3 (CH_2), 30.4 (CH_3), 30.1 (CH_3); MS (ES^+): m/z (%) = 582 (55) $[M+H]^+$, 604 (90) $[M+Na]^+$, 1185 (20) $[2M+Na]^+$; HRMS calcd for $C_{28}H_{39}N_5O_5F_3$ $[M+H]^+$ 582.2887, found 582.2898.

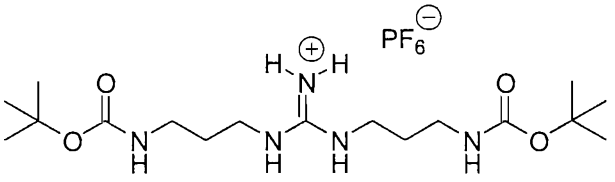
{2-[N'-{3-[1-(2,6-Dioxo-cyclohexylidene)-2-phenyl-ethylamino]-propyl}-N''-(2,2,2-trifluoro-acetyl)-guanidino]-ethyl}-carbamic acid tert-butyl ester (157f)



Synthesised according to the general procedure on a 0.72 mmol scale: yield (321 mg, 75%); white foam; R_f = 0.28 (EtOAc/CH_2Cl_2 70:30); I.R. (neat) ν_{max} = 3637 (m), 3569 (m), 2982 (w), 1625 (m), 1566 (s), 1520 (s), 1367 (m), 1190 (m), 1140 (s), 825 (s) cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ 9.12 (br s, NH), 7.60 (br s, NH), 7.38 (m, 2H, Ph), 7.30 (m, 1H, Ph), 7.21 (m, 2H, Ph), 7.06 (br s, NH), 6.93 (br s, NH), 4.60 (s, 2H, $PhCH_2$), 3.30 (m, 4H, CH_2NH), 3.18 (m, 2H, CH_2NH), 3.13 (br s, 2H, CH_2NH), 2.37 (s, 4H, CH_2CO), 1.82 (m, 2H, $CH_2CH_2CH_2$), 1.42 (s, 9H, $(CH_3)_3C$), 0.98 (s, 6H, $(CH_3)_2C$); ^{13}C NMR (100 MHz, CD_3CN) δ 173.1 (C), 160.5 (C), 156.6 (C), 156.1 (C), 136.1 (C), 128.3 (CH), 127.7 (CH), 126.1 (CH), 117.0 (q, J = 286 Hz, CF_3), 107.2 (C), 79.2 (C), 52.2 (CH_2), 40.4 (CH_2), 40.2 (CH_2), 38.4 (CH_2), 38.3 (CH_2), 34.2 (CH_2), 29.3 (CH_2), 28.6 (C), 27.3 (CH_3), 27.0 (CH_3), $COCF_3$ not visible; MS (ES^+): m/z (%) = 596 (100) $[M+H]^+$, 1181 (10) $[2M+H]^+$; Anal. Calcd for $C_{29}H_{40}N_5O_5F_3$: C, 58.48; H 6.77; N 11.76. Found: C, 58.23; H, 6.96; N, 11.54.

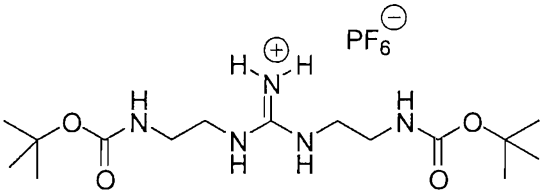
General procedure for the deprotection of the trifluoroacetamide and exchange of the counterion to the hexafluorophosphate 158. Dicarbamate **157** (0.092 mmol) was dissolved in a mixture of methanol and water 5:2 (3.5 ml) and potassium carbonate (100 mg, 0.725 mmol) was added. The mixture was stirred for 3h at room temperature.[123] The solvents were then removed under reduced pressure and the residue suspended in ethyl acetate (3 × 10 ml) and filtered. The organic layer was concentrated and dissolved in methanol (5 ml). Ammonium hexafluorophosfate was added and the solution stirred for 3h. The solvent was removed under reduced pressure and the residue dissolved in ethyl acetate (30 ml) and washed with water (10 ml). The solution was dried over magnesium sulfate and the solvent removed to give the crude product, that was purified by column chromatography (5% methanol in dichlorometane) to afford 33 mg of the pure product (70 %).

[di (3-[(*tert*-butoxycarbonyl)amino] propylamino) methylene] ammonium hexafluorophosphate, 158a.



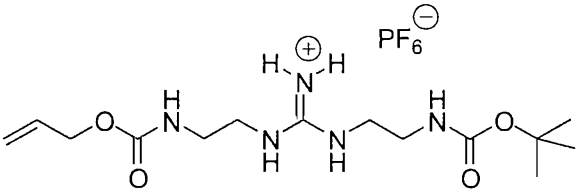
Synthesised according to the general procedure on a 42 mg (0.090 mmol) scale: yield (42 mg, 90% yield); white foam; R_f = 0.19 (MeOH 5 % in methanol); I.R. (neat) ν_{max} = 3411 (m), 2976 (m), 2936 (m), 1658 (s), 1635 (s), 1518 (s), 1367 (s), 1277 (m), 1252 (m), 1161 (s) cm^{-1}; ^1H NMR (400 MHz, CD_3CN) δ 6.70 (br s, 1H, NH), 4.87 (br s, 2H, NHBoc), 3.49 (br s, 4H, CH_2NH), 3.13 (q, J = 6 Hz, 4H, CH_2NH), 1.68 (quint, J = 6 Hz, 4H, $CH_2CH_2CH_2$), 1.37 (s, 18H, $(CH_3)_3C$); ^{13}C NMR (100 MHz, CD_3CN) δ 156.6 (C), 155.5 (C), 78.5 (C), 38.6 (CH_2), 36.6 (CH_2), 28.8 (CH_2), 27.3 (CH_3); (ES^+): m/z (%) = 374 $[M+H]^+$ (100); Anal. calcd for $C_{17}H_{34}N_5O_4PF_6*2H_2O$: C, 36.76; H, 7.26; N, 12.61. Found: C, 36.61; H, 7.31; N, 12.40.

[di (3-[(*tert*-butoxycarbonyl)amino] ethylamino) methylene] ammonium hexafluorophosphate, 158b.



Synthesised according to the general procedure on a 20 mg (0.045 mmol) scale: yield (20 mg, 88% yield); white foam; R_f = 0.17 (MeOH 5 % in methanol); I.R. (neat) ν_{max} = 3409 (m), 2966 (m), 2934 (m), 1658 (s), 1635 (s), 1516 (s), 1360 (s), 1275 (m), 1252 (m), 1161 (s) cm^{-1}; ^1H NMR (400 MHz, CD_3CN) δ 6.62 (br s, 2H, NH), 6.26 (br s, 2H, NH), 5.70 (br s, 2H, NHBoc), 3.20-3.22 (m, 8H, CH_2NH), 1.43 (s, 18H, $(CH_3)_3C$); ^{13}C NMR (100 MHz, CD_3CN) δ 157.2 (C), 155.9 (C), 79.1 (C), 41.7 (CH_2), 38.5 (CH_2), 27.3 (CH_3); (ES^+): m/z (%) = 350 $[M+H]^+$ (100); Anal. Calcd for $C_{15}H_{32}N_5O_4PF_6$*$2H_2O$: C, 34.16; H, 6.88; N, 13.28. Found: C, 34.25; H, 6.60; N, 13.32.

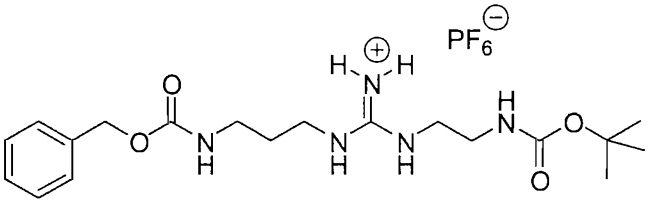
([(2-{[(allyloxy)carbonyl)amino}ethyl)amino]({2-[(*tert*-butoxycarbonyl)amino] ethyl}amino)methylene] ammonium hexafluorophosphate, 158c



Synthesised according to the general procedure on a 20 mg (0.047 mmol) scale: yield (18 mg, 83% yield); white foam; R_f = 0.17 (MeOH 5 % in methanol); I.R. (neat) ν_{max} = 3414 (m), 2975 (m), 2937 (m), 1659 (s), 1634 (s), 1517 (s), 1367 (s), 1249 (m), 1249 (m), 1159 (s) cm^{-1}; ^1H NMR (400 MHz, CD_3CN) δ 6.47 (m, 3H, NH), 6.15 (br s, 2H, NH), 5.85 (ddt, J = 17, 11, 6 Hz, 1H, $CH{=}CH_2$), 5.59 (br s, 1H, NH), 5.12 (dd, J_{trans} = 17, 1 Hz, 1H, CH*H*=), 5.12 (dd, J_{cis} = 11 Hz, 1 Hz, 1H, C*H*H=), 4.45 (d, J = 6.0 Hz, 2H, CH_2O), 3.16-3.20 (m, 4H, CH_2NH), 3.09-3.16 (m, 4H, CH_2NH), 1.34 (s, 9H, $(CH_3)_3C$); ^{13}C NMR (100 MHz, CD_3CN) δ 157.1 (C), 155.9 (C), 133.0 (CH), 116.3 ($=CH_2$), 79.2 (C), 65.0 (CH_2), 41.6 (CH_2), 41.5 (CH_2), 38.9 (CH_2), 38.5 (CH_2), 27.3

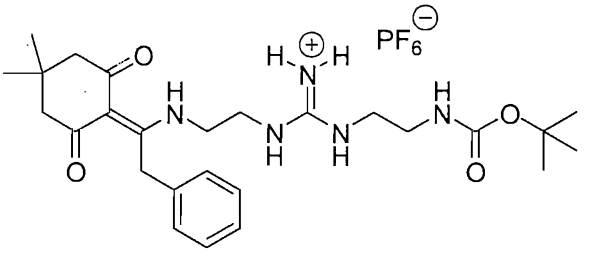
(CH_3); (ES^+): m/z (%) = 330 $[M+H]^+$ (100); Anal. Calcd for $C_{14}H_{27}N_5O_4PF_6*H_2O$: C, 34.08; H, 6.13; N, 14.19. Found: C, 34.29; H, 6.06; N, 13.92.

{3-[N'-(2-tert-Butoxycarbonylamino-ethyl)-guanidinium hexafluorophosphate]-propyl}-carbamic acid benzyl ester, 158d.



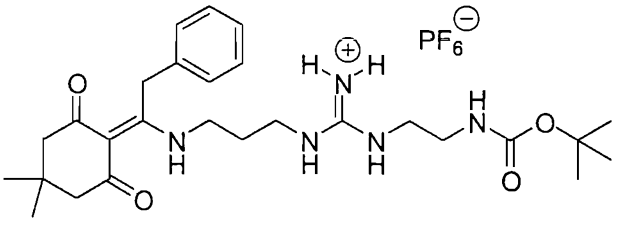
Synthesised according to the general procedure on a 45 mg (0.092 mmol) scale: yield (45 mg, 90% yield); white foam; R_f = 0.17 (MeOH 5 % in methanol); I.R. (neat) ν_{max} = 3417 (m), 2975 (m), 2938 (m), 1659 (s), 1633 (s), 1516 (s), 1453 (m), 1367 (m), 1247 (s), 1158 (s) cm^{-1}; ^1H NMR (400 MHz, CD_3CN) δ 7.28 (m, 5H, PhH), 6.40 (br s, 2H, NH), 6.07 (br s, 2H, NH), 5.71 (br s, 1H, NH), 5.59 (br s, 1H, NH), 4.98 (s, 2H, $PhCH_2$), 3.12-3.07 (m, 8H, CH_2NH), 1.64 (quint, J = 7.0 Hz, 2H, $CH_2CH_2CH_2$), 1.33 (s, 9H, $(CH_3)_3C$); ^{13}C NMR (100 MHz, CD_3CN) δ 156.9 (C), 156.8 (C), 155.7 (C), 137.0 (C), 128.2 (CH), 127.7 (CH), 127.4 (CH), 79.2 (C), 65.8 (CH_2), 41.7 (CH_2), 38.6 (CH_2), 37.2 (CH_2), 28.4 (CH_2), 27.3 (CH_3); MA: $C_{19}H_{32}N_5O_4PF_6*H_2O$: calculated: C 40.94, H 6.15, N 12.56; found: C 41.05, H 6.01, N 12.23; (ES^+): m/z (%) = 394 $[M+H]^+$ (100); Anal. Calcd for $C_{19}H_{32}N_5O_4PF_6*H_2O$: C, 40.94; H, 6.15; N, 12.56. Found: C, 41.05; H, 6.01; N, 12.23.

[2-(N'-{3-[1-(4,4-Dimethyl-2,6-dioxo-cyclohexylidene)-2-phenyl-ethylamino]-ethyl}-guanidinium hexafluorophosphate)-ethyl]-carbamic acid tert-butyl ester, 158e



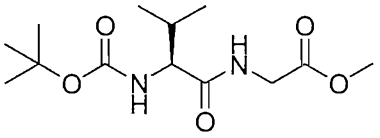
Synthesised according to the general procedure on a 20 mg (0.034 mmol) scale: yield (16 mg, 75% yield); white foam; R_f = 0.15 (MeOH 5 % in methanol); I.R. (neat) ν_{max} = 2969 (m), 2884 (m), 1629 (s), 1560 (s), 1517 (s), 1449 (s), 1282 (m), 1247 (m), 1158 (s), 1025 (m), 1001 (m) cm^{-1}; ^1H NMR (400 MHz, CD_3CN) δ 13.67 (br s, 1H, NH), 7.33 (t, *J* = 7.5 Hz, 2H, PhH), 7.26 (t, *J* = 7.5 Hz, 1H, PhH), 7.17 (t, *J* = 7.5 Hz, 2H, PhH), 6.40-6.60 (m, 2H, NH), 6.16 (br s, 2H, NH), 5.77 (br s, 2H, NHBoc), 4.58 (s, 2H, CH_2Ph), 3.54 (q, *J* = 6.0 Hz, 2H, CH_2NH), 3.31 (q, *J* = 6.0 Hz, 2H, CH_2NH), 3.18-3.20 (m, 4H, CH_2NH), 2.32 (s, 4H, CH_2CO), 1.43 (s, 9H, $(CH_3)_3C$), 1.04 (s, 6H, $(CH_3)_2C$); ^{13}C NMR (100 MHz, CD_3CN) δ 173.2 (C), 155.7 (C), 155.6 (C), 136.1 (C), 128.3 (CH), 127.7 (CH), 126.1 (CH), 107.3 (C), 79.3 (C), 52.2 (CH_2), 41.7 (CH_2), 40.0 (CH_2), 38.6 (CH_2), 34.2 (CH_2), 29.3 (CH_2), 27.6 (CH_2), 27.3 (CH_3), 27.0 (CH_3); (ES^+): m/z (%) = 488 $[M+H]^+$ (100); Anal. Calcd for $C_{26}H_{40}N_5O_4PF_6*2H_2O$: C, 46.77; H, 6.64; N, 10.49. Found: C, 46.69; H, 6.34; N, 10.23

[2-(N'-{3-[1-(4,4-Dimethyl-2,6-dioxo-cyclohexylidene)-2-phenyl-ethylamino]-propyl}-guanidinium hexafluorophosphate)-ethyl]-carbamic acid tert-butyl ester, 158f



Synthesised according to the general procedure on a 20 mg (0.034 mmol) scale: yield (17 mg, 76% yield); white foam; R_f = 0.16 (MeOH 5 % in methanol); ^{1}H NMR (400 MHz, CD_3CN) δ 13.64 (br s, 1H, NH), 7.29 (t, *J* = 7.5 Hz, 2H, PhH), 7.23 (t, *J* = 7.0 Hz, 1H, PhH), 7.16 (d, *J* = 7.0 Hz, 2H, PhH), 6.40-6.60 (m, 2H, NH), 6.15 (br s, 2H, NH), 5.72 (br s, 1H, NHBoc), 4.56 (s, 2H, CH_2Ph), 3.54 (q, *J* = 7.0 Hz, 2H, C*H*$_2$NH), 3.41 (q, *J* = 6.0 Hz, 2H, C*H*$_2$NH), 3.09-3.17 (m, 6H, C*H*$_2$NH), 2.35 (s, 4H, CH_2CO), 1.81 (quint, *J* = 7.0 Hz, 2H, $CH_2CH_2CH_2$), 1.40 (s, 9H, $(CH_3)_3C$), 1.02 (s, 6H, $(CH_3)_2C$), ; ^{13}C NMR (100 MHz, CD_3CN) δ 173.2 (C), 155.7 (C), 155.6 (C), 136.1 (C), 128.3 (CH), 127.7 (CH), 126.1 (CH), 107.3 (C), 79.3 (C), 52.2 (CH_2), 41.7 (CH_2), 40.0 (CH_2), 38.6 (CH_2), 34.2 (CH_2), 29.3 (CH_2), 27.6 (CH_2), 27.3 (CH_3), 27.0 (CH_3); (ES^+): m/z (%) = 500 $[M+H]^+$ (100); MA: $C_{27}H_{44}N_5O_5PF_6*H_2O$: calculated: C 48.87, H 6.68, N 10.55; found: C 48.69, H 6.69, N 10.20

(2-tert-Butoxycarbonylamino-3-methyl-butyrylamino)-acetic acid methyl ester, 160

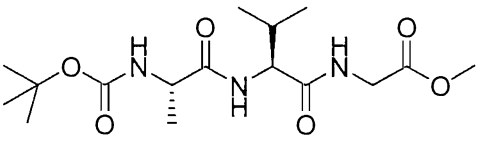


Glycine methyl ester hydrochloride (1 g, 8 mmol) was redissolved in a mixture of THF (25 ml) and DMF (10 ml) and cooled to 0 °C. To the solution were added *N*-Boc-Val-OH (2.4 g, 10 mmol), HOBt (1.8 g, 13 mmol), DIPEA (4.5 ml, 26 mmol) and EDC (2.2 g, 10 mmol) and the mixture was stirred for 18 h (0°C to room temperature). THF was removed and the residue diluted with DCM (150 ml) and washed with a 1M solution of sodium hydrogencarbonate (80 ml), 1M solution of sodium hydrogensulfate (80 ml) and water (80 ml). The organic layer was dried over magnesium sulfate and after removing the solvent the residue was dissolved in the minimum amount of DCM (1 ml) and precipitated with a mixture of ether and petroleum ether 2:1 (30 ml) to yield 1.9 g of **160** as a white solid (80 %): m.p. = 110-112 °C (lit.[124] 111-112 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.51 (br s, 1H, NH Gly), 5.03 (d, *J*= 8 Hz, 1H, NH Val), 4.07 (d, *J* = 5 Hz, 1H, CH_2 Gly), 4.06 (d, *J* = 5 Hz, 1H, CH_2 Gly), 4.00 (dd, *J* = 8 Hz, *J* = 5.5 Hz,1H, CH Val), 3.77 (s, 3H, CH_3OCO), 2.20 (octet, *J* = 6 Hz, 1H, C*H*$(CH_3)_2$ Val), 1.45 (s,

9H, $(CH_3)_3C$), 0.99 (d, J = 6.5 Hz, 3H, CH_3 Val), 0.94 (d, J = 6.5 Hz, 3H, CH_3 Val); LRMS (ES^+): m/z (%) = 311 (100) $[M+Na]^+$

All data according to literature.[125]

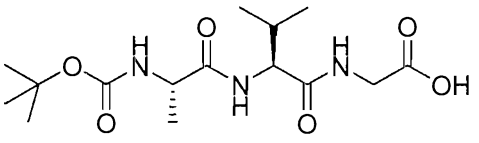
[2-(2-*tert*-Butoxycarbonylamino-propionylamino)-3-methyl-butyrylamino]-acetic acid methyl ester, 161



Dipeptide **7** (1.9 g, 6.6 mmol) was stirred in a 20 % solution of TFA in DCM (20 ml) for 3h. After addition of toluene (80 ml) the solvents were removedunder reduced pressure and the oily residue was redissolved in a mixture of THF (20 ml) and DMF (15 ml) and cooled to 0 °C. To the solution were added *N*-Boc-Ala-OH (436 mg, 1.25 mmol), HOBt (192 mg, 1.42 mmol), DIPEA (400 µl, 2.3 mmol) and EDC (240 mg, 1.25 mmol) and the mixture was stirred for 18 h (0°C to room temperature). THF was removed and the residue dissolved in DCM (150 ml) and washed with a 1M solution of sodium hydrogencarbonate (80 ml), 1M solution of sodium hydrogensulfate (80 ml) and water (80 ml). The organic layer was dried over magnesium sulfate and after removing the solvent the residue was dissolved in the minimum amount of DCM (1 ml) and precipitated and precipitated with a mixture of ether and petroleum ether 10:1 (30 ml) to yield 1.9 g of **161** as a white solid (80 %): ^1H NMR (300 MHz, $CDCl_3$) δ 6.82 (br s, 1H, NH Gly), 6.72 (d, J = 9 Hz, 1H, NH Val), 4.99 (br s, 1H, NH Ala), 4.34 (dd, J = 8.5, 5.5 Hz, 1H, CH Val), 4.16 (m, 1H, CH Ala), 4.03 (m, 2H, CH_2 Gly), 3.74 (s, 3H, CH_3OCO), 2.31 (m, 1H, $CH(CH_3)_2$ Val), 1.45 (s, 9H, $(CH_3)_3C$), 1.38 (d, J = 7 Hz, 3H, CH_3 Ala), 0.97 (d, J = 7 Hz, 3H, CH_3 Val), 0.95 (d, J = 7 Hz, 3H, CH_3 Val); LRMS (ES^+): m/z (%) = 360 (8) $[M+H]^+$, 382 (100) $[M+Na]^+$, 741 (35) $[2M+Na]^+$

All data according to literature.[125]

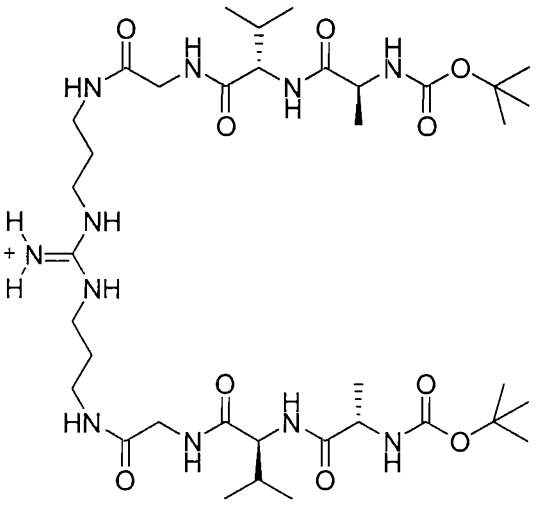
[2-(2-*tert*-butoxycarbonylamino-propionylamino)-3-methyl-butyrylamino]-acetic acid, 162



Ester **161** (1.7 g, 4.7 mmol) was dissolved in a mixture of dioxane (6 ml) and 1M aqueous lithium hydroxide solution (4 ml). The mixture was stirred at room temperature for two hours and acidified till pH 5 with a 1M solution of hydrochloric acid. The water layer was then extracted with ethyl acetate (3 × 100 ml). The combined organic layer was dried over magnesium sulfate and the solvent evaporated to give **162** as a white solid (1.6 g, 98 %): m.p. = 208-210 °C; I.R. (neat) ν_{max} = 3450 (w), 3307 (m), 3275 (m), 2970 (w), 1715 (m), 1685 (s), 1642 (vs), 1530 (s), 1452 (w), 1386 (m), 1304 (m), 1248 (m), 1163 (s), 1113 (s); ^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ 6.82 (br s, 1H, NH Gly), 6.72 (d, J = 9 Hz, 1H, NH Val), 4.99 (br s, 1H, NH Ala), 4.34 (dd, J = 8.5, 5.5 Hz, 1H, CH Val), 4.30 (quint, 2H, J = 7.5 Hz, CH Ala), 3.84 (m, 2H, CH_2 Gly), 2.06 (m, 1H, C*H*$(CH_3)_2$ Val), 1.47 (s, 9H, $(CH_3)_3C$), 1.25 (d, J = 7 Hz, 3H, CH_3 Ala), 0.96 (d, J = 6.5 Hz, 3H, CH_3 Val), 0.94 (d, J = 6.5 Hz, 3H, CH_3 Val); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 172.7 (C), 171.4 (C), 171.3 (C), 155.4 (C), 78.4 (C), 57.2 (CH), 50.0 (CH_2), 40.7 (CH), 31.3 (CH), 28.4 (CH_3), 19.3 (CH_3), 18.0 (CH_3); LRMS (ES^+): m/z (%) = 368 (13) $[M+Na]^+$, 382 (11) $[M+MeOH+Li]^+$, 713 (11) $[2M+Na]^+$; LRMS (ES^-): m/z (%) = 363 (5) $[M+OH]^-$, 458 (25) $[M+TFA]^-$.

All data according to literature.[125]

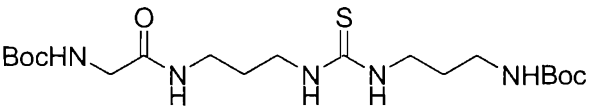
Tweezer receptor GVA, 163



Compound **157a** (220 mg, 0.57 mmol) was stirred in a 20 % solution of TFA in DCM (20 ml) for 3h. After addition of toluene (80 ml) the solvents were removed under reduced pressure to give a yellow oil. The residue was redissolved in a mixture of THF (5 ml) and DMF (5 ml) and cooled to 0 °C. To the solution were added tripeptide **162** (436 mg, 1.25 mmol), HOBt (192 mg, 1.42 mmol), DIPEA (400 µl, 2.3 mmol) and EDC (240 mg, 1.25 mmol) and the mixture was stirredfor 18 h (0°C to room temperature). THF was removed and the residue diluted with DCM (100 ml) and washed with a 1M solution of sodium hydrogencarbonate (50 ml), 1M solution of sodium hydrogensulfate (50 ml) and brine (50 ml). The organic layer was dried over magnesium sulfate and after removing the solvent the residue was washed with ether, dissolved in the minimum amount of DCM (1 ml) and precipitated with ether (5 ml) to yield the trifluoroacetyl protected tweezer as a cream-coloured solid (170 mg, 35 %): I.R. (neat) ν_{max} = 3284 (m), 2975 (m), 2934 (m), 1686 (m), 1633 (s), 1529 (s), 1443 (m), 1371 (m), 1248 (m), 1230 (s), 1171 (s), 1148 (s), 1076 (m), 849 (s) cm^{-1}; ^1H NMR (400 MHz, CD_3CN) δ 4.20-4.00 (m, 4H, CH Val + CH Ala), 3.77 (m, 4H, CH_2 Gly), 3.38 (m, 2H, CH_2NH), 3.23 (m, 6H, CH_2NH), 2.07 (m, 2H, CH Val), 1.76 (m, 4H, $CH_2CH_2CH_2$), 1.64 (m, 2H, $CH_2CH_2CH_2$), 1.39 (s, 18H, CH_3 Boc), 1.21 (d, J = 7 Hz, 3H, CH_3 Ala), 0.91 (d, J = 6 Hz, 6H, CH_3 Val); ^{13}C NMR (100 MHz, CD_3CN) δ 175.3 (C), 172.8 (C), 171.2 (C), 80.4 (C), 60.5 (CH), 60.2 (CH), 51.6 (CH_2), 43.6 (CH_2), 43.3 (CH_2), 39.6 (CH_2), 38.8 (CH_2), 37.3 (CH_2), 36.8 (CH_2), 31.1 (CH), 30.1 (CH_2), 29.5 (CH_2), 28.6 (CH_3), 19.6 (CH_3), 18.5 (CH_3), 17.9 (CH_3), LRMS (ES^+): m/z (%) = 845 (30) $[M+H]^+$, 867 (30) $[M+Na]^+$. The Trifluoroacetyl tweezer (25 mg, 0.03 mmol) was

dissolved in a mixture of methanol and water 5:2[124] (3.5 ml) and potassium carbonate (100 mg, 0.725 mmol) was added. The mixture was stirred for 3 h at room temperature. The solvents were then removed under reduced pressure and the residue extracted with ethyl acetate (3 × 20 ml) and chloroform (2 × 20 ml) . The combined organic layer was then concentrated to afford **163** as a white solid (23 mg, 96 % yield); m.p. = 119-120 °C; $[\alpha]^{rt}_D$ = -15 (c = 3.0 mg/mL, l = 2.0 dm, $(CH_3)OH$); I.R. (neat) ν_{max} = 3288 (m), 2975 (m), 2930 (m), 1647 (s), 1525 (s), 1371 (m), 1248 (m), 1166 (s), 1135 (s), 1071 (m), 1026 (w), 845 (s) cm^{-1}; ^1H NMR (400 MHz, CD_3CN) δ 7.23 (d, *J* = 8.0 Hz, 2H, NH), 7.11 (br s, 2H, NH), 6.98 (br s, 2H, NH), 5.95 (br s, 2H, NH), 4.10-3.90 (m, 4H, αCH Val + CH Ala), 3.74 (d, *J* = 8.0 Hz, 4H, CH_2 Gly), 3.25-3.00 (m, 8H, CH_2NH), 2.10 (m, 2H, βCH Val), 1.70 (m, 4H, $CH_2CH_2CH_2$), 1.39 (s, 18H, $(CH_3)_3C$), 1.26 (d, *J* = 7 Hz, 6H, CH_3 Ala), 0.92 (d, *J* = 7 Hz, 12H, CH_3 Val); ^{13}C NMR (100 MHz, CD_3CN) δ 174.2 (C), 171.9 (C), 169.3 (C), 160.8 (C), 155.9 (C), 79.1 (C), 59.4 (CH), 50.4 (CH), 42.5 (CH_2), 38.4 (CH_2), 35.7 (CH_2), 29.6 (CH_2), 28.1 (CH), 27.3 (CH_3), 18.3 (CH_3), 17.2 (CH_3), 16.5 (CH_3); MS (ES^+): m/z (%) = 828 (100) $[M]^+$; Analytical data: HPLC 3.50 min (S5OD, gradient 60 to 45 % MeCN, analytical).

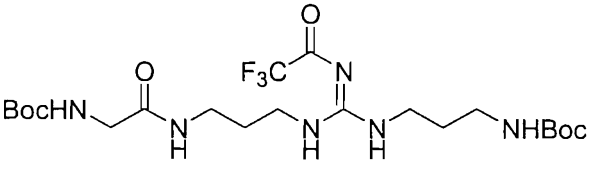
(3-{3-[3-(2-*tert*-Butoxycarbonylamino-acetylamino)-propyl]-thioureido}-propyl)-carbamic acid *tert*-butyl ester, 165



A solution of **115** (950 mg, 4.4 mmol) in toluene (100 ml) was added dropwise to a solution of propylene diamine (2 ml, 24 mmol) in toluene (8 ml) over a period of 6 h, with vigorous stirring. The stirring was continued for further 18 h at room temperature. The solution was decanted and the oily precipitate washed with toluene (20 ml) and petrol ether (20 ml) to give 928 mg (72 %) of compound **164** as an orange oil: ^1H NMR (300 MHz, $CDCl_3$) δ 4.93 (br s, 1H, NH), 3.60 (br s, CH_2NH_2), 3.59 (t, *J* = 6.5 Hz, 2H, CH_2NH), 3.48 (q, *J* = 7 Hz, 2H, CH_2NH), 3.19 (q, *J* = 6.5 Hz, 2H, CH_2NH), 2.86 (t, *J* = 6.5 Hz, 2H, CH_2NH), 2.79 (t, *J* = 7 Hz, 2H, CH_2NH_2), 1.72 (m, 4H, $CH_2CH_2CH_2$), 1.44 (s, 9H, CH_3); LRMS (ES^+): m/z (%) = 291 (100) $[M+H]^+$; Unstable on stand. Compound **164** (1.2 g, 4.1 mmol) was dissolved in a mixture of tetrahydrofuran (10

ml) and dimethylformamide (10 ml). The solution was cooled to 0° C and *N*-Boc-Gly-OH (1.1 g, 6.2 mmol), HOBt·H_2O (839 mg, 6.2 mmol), DIPEA (1.4 ml, 8.0 mmol) and EDC (952 mg, 5.0 mmol) were added.[121] The mixture was stirred for 36 h (0° C to room temperature). Tetrahydrofuran was removed and the residue was diluted with dichloromethane (200 ml) and washed with 1M sodium hydrogen carbonate (100 ml), brine (100 ml) and water (100 ml), and dried over magnesium sulfate. The solvent was evaporated under reduced pressure to give a yellow oil. Further purification by column chromatography (5 % methanol in DCM) afforded thiourea **165** as a white foam (1.0 g, 60 % yield): R_f = 0.25 (5 % methanol in DCM); I.R. (neat) ν_{max} = 3295 (m), 2974 (m), 2931 (m), 1656 (s), 1509 (s), 1438 (m), 1390 (m), 1364 (s), 1273 (s), 1247 (s), 1160 (s), 1050 (m), 944 (m), 861 (m) cm^{-1}; ^1H NMR (400 MHz, CD_3CN) δ 6.98 (br s, 1H, NH), 6.70 (br s, 1H, NH), 6.63 (br s, 1H, NH), 5.71 (br s, 1H, NH), 5.59 (br s, 1H, NH), 3.60 (d, *J* = 6 Hz, 2H, CH_2 Gly), 3.44 (br s, 4H, CH_2NHCS), 3.20 (q, *J* = 6.5 Hz, 2H, CH_2NH), 3.05 (q, *J* = 6.5 Hz, 2H, CH_2NH), 1.64 (m, 4H, $CH_2CH_2CH_2$), 1.41 (s, 9H, CH_3), 1.40 (s, 9H, CH_3); ^{13}C NMR (100 MHz, CD_3CN) δ 171.4 (C), 157.8 (C), 80.1 (C), 79.3 (C), 44.8 (CH_2), 41.8 (CH_2), 38.2 (CH_2), 36.9 (CH_2), 30.4 (CH_2), 30.0 (CH_2), 28.7 (CH_3), 28.6 (CH_3); LRMS (ES^+): m/z (%) = 448 (12) $[M+H]^+$, 470 (8) $[M+Na]^+$, 917 (3) $[2M+Na]^+$.

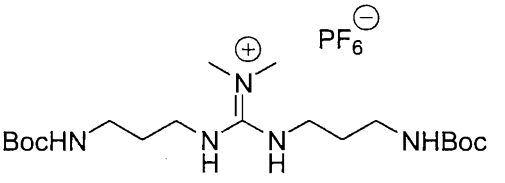
{3-[*N'*-[3-(2-*tert*-Butoxycarbonylamino-acetylamino)-propyl]-*N''*-(2,2,2-trifluoro-acetyl)-guanidino]-propyl}-carbamic acid *tert*-butyl ester, 166



Iodomethane (150 µl, 2.4 mmol) was added to a stirred solution of thiourea **165** (550 mg, 1.23 mmol) in acetone (20 ml) and the reaction mixture was stirred for 18 h at room temperature. The solvent and all volatile compounds were removed under reduced pressure to give a slightly yellow foam. The foam was redissolved in a 1:1 mixture of dichloromethane (15 ml) and methanol (15 ml). Ammonium hexafluorophosphate (330 mg, 2.0 mmol) was added and the resulting solution stirred for 18 h at room temperature. The solvents were evaporated and the yellow oily residue

redissolved in dichloromethane (100 ml) and washed with water (80 ml). The organic solution was dried over magnesium sulfate and the solvent removed under reduced pressure to afford methylsulfonium hexafluorophosphate as a white foam (746 mg, 100 %). The hexafluorophosphate (450 mg, 0.74 mmol) was redissolved in a 4:1 mixture of toluene (20 ml) and chloroform (5 ml) and neat DBU (280 µl, 1.9 mmol) and trifluoroacetamide (339 mg, 3.0 mmol) were added. The mixture was refluxed for 48 h under vigorous stirring. The solvents were removed under reduced pressure to give a brown oil. Further purification by column chromatography (ethyl acetate) afforded the carbamate **166** as a white foam (380 mg, 96 %. yield); R_f = 0.28 (ethyl acetate); I.R. (neat) ν_{max} = 3295 (m), 2978 (m), 2935 (m), 1686 (m), 1625 (s), 1520 (s), 1438 (m), 1367 (m), 1276 (m), 1251 (s), 1163 (s), 1139 (s), 1051 (m), 946 (w), 904 (m), 841 (s) cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ 9.29 (br s, NH), 7.50 (m, NH), 7.16 (m, NH), 6.21 (br s, NH), 6.15 (br s, NH), 6.05 (br s, NH), 3.63 (d, J = 5.5 Hz, 2H, CH_2 Gly), 3.36 (m, 2H, CH_2NH), 3.21 (m, 4H, CH_2NH), 3.08 (m, 2H, CH_2NH), 1.72 (m, 2H, $CH_2CH_2CH_2$), 1.60 (m, 2H, $CH_2CH_2CH_2$), 1.37 (s, 18H, CH_3); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 169.9 (C), 160.2 (C), 156.2 (C), 156.1 (C), 78.5 (C), 78.0 (C), 43.8 (CH_2), 37.7 (CH_2), 36.3 (CH_2), 30.2 (CH_2), 29.9 (CH_2), 28.7 (CH_3), 28.6 (CH_3); MS (ES^+): m/z (%) = 527 (21) $[M+H]^+$, 549 (60) $[M+Na]^+$, 1075 (5) $[2M+Na]^+$.

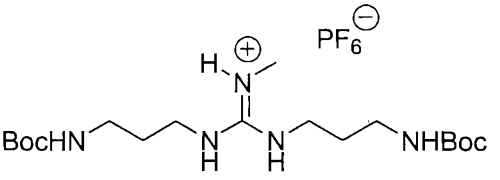
[di (3-[(*tert*-butoxycarbonyl)amino] propylamino) methylene] (dimethyl)ammonium hexafluorophosphate, 168



Iodomethane (300 µl, 3.2 mmol) was added to a stirred solution of thiourea **156a** (585 mg, 1.6 mmol) in acetone (20 ml) and the reaction mixture was stirred for 18 h at room temperature. The solvent and all volatile compounds were removed under reduced pressure to give a slightly yellow foam. The foam was redissolved in a 1:1 mixture of dichloromethane (15 ml) and methanol (15 ml). Ammonium hexafluorophosphate (522 mg, 3.2 mmol) was added and the resulting solution stirred for 18 h at room temperature. The solvents were evaporated and the yellow oily residue redissolved in dichloromethane (100 ml) and washed with water (80 ml). The organic solution was

dried over magnesium sulphate and the solvent removed under reduced pressure to afford the methylthiouronium hexafluorophosphate as a white foam in a quantitative yield. The hexafluorophosphate was redissolved in a mixture of acetonitrile (25 ml) and dimethylamine 70 % solution in water (5 ml) and DMAP (cat.) was added. The mixture was refluxed for 8 h under vigorous stirring. The solvents were removed under reduced pressure to give an oil. The crude product was purified by FC on silica gel using the eluent mixture EtOAc/CH_2Cl_2 50:50, obtaining 612 mg (70%) of **168** as a white foam: R_f = 0.20 (EtOAc/CH_2Cl_2 50:50); I.R. (neat) ν_{max} = 3409 (m), 2974 (m), 2936 (m), 1659 (s), 1632 (s), 1518 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.62 (br s, 2H, NH), 4.93 (t, J = 6.0 Hz, 2H, NHBoc), 3.34 (q, J = 6 Hz, 4H, CH_2NHCN), 3.23 (q, J = 6 Hz, 4H, CH_2NHBoc), 3.04 (s, 6H, $(CH_3)_2N$), 1.80 (quint, J = 6 Hz, 4H, $CH_2CH_2CH_2$), 1.42 (s, 18H, $(CH_3)_3C$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.1 (C), 157.9 (C), 80.4 (C), 41.9 (CH_2), 39.7 (CH_3), 37.3 (CH_2), 30.6 (CH_2), 28.7 (CH_3); (ES^+): m/z (%) = 402 $[M+H]^+$ (100).

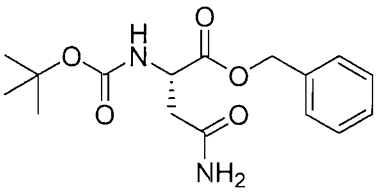
[di (3-[(*tert*-butoxycarbonyl)amino] propylamino) methylene] (dimethyl)ammonium hexafluorophosphate, 167



Iodomethane (150 µl, 1.6 mmol) was added to a stirred solution of thiourea **156a** (293 mg, 0.8 mmol) in acetone (20 ml) and the reaction mixture was stirred for 18 h at room temperature. The solvent and all volatile compounds were removed under reduced pressure to give a slightly yellow foam. The foam was redissolved in a 1:1 mixture of dichloromethane (15 ml) and methanol (15 ml). Ammonium hexafluorophosphate (261 mg, 1.6 mmol) was added and the resulting solution stirred for 18 h at room temperature. The solvents were evaporated and the yellow oily residue redissolved in dichloromethane (80 ml) and washed with water (40 ml). The organic solution was dried over magnesium sulphate and the solvent removed under reduced pressure to afford the methylthiouronium hexafluorophosphate as a white foam in a quantitative yield. The hexafluorophosphate (200 mg, 0.36 mmol) was redissolved in a mixture of

acetonitrile (7 ml) and methylamine (70 % solution in water, 2.0 ml) and DMAP (cat.) was added. The mixture was refluxed for 8 h under vigorous stirring. The solvents were removed under reduced pressure to give an oil. The crude product was purified by FC on silica gel using the eluent mixture (5 % MeOH in DCM) obtaining 160 mg (83 %) of **167** as a white foam: R_f = 0.20 (5 % MeOH in DCM); I.R. (neat) ν_{max} = 3410 (m), 2976 (m), 2935 (m), 1656 (s), 1635 (s), 1518 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.71 (br s, 2H, NH), 6.28 (br s, 1H, NH), 4.98 (br s, 2H, NHBoc), 3.25 (q, *J* = 6 Hz, 4H, C*H*$_2$NHCN), 3.15 (br m, 4H, C*H*$_2$NHBoc), 2.90 (d, *J* = 6 Hz, 3H, $(CH_3)_2N$), 1.73 (quint, *J* = 6 Hz, 4H, $CH_2CH_2CH_2$), 1.39 (s, 18H, $(CH_3)_3C$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.2 (C), 155.3 (C), 80.6 (C), 41.9 (CH_2), 38.8 (CH_3), 37.4 (CH_2), 29.8 (CH_2), 28.7 (CH_3); (ES^+): m/z (%) = 388 $[M+H]^+$ (100).

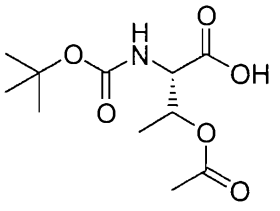
2-*tert*-Butoxycarbonylamino-succinamic acid benzyl ester, 170



To a solution of *N*-Boc-L-Asn-OH (1.0 g, 4.3 mmol) in dimethylformamide (20 ml) were added solid sodium bicarbonate (3.6 g, 43 mmol) and benzyl bromide (560 µl, 4.7 mmol) following established literature[122] procedures and the mixture was stirred at room temperature for 24 h. Following dilution with water (100 ml) the crude product was extracted with ethyl acetate (3 × 80 ml) and the organic extracts were dried over magnesium sulfate and evaporated to give a pale yellow oil that was dissolved in DCM and treated with a mixture of ether and petroleum ether 1:10, to give **170** as a white solid (1 g, 72 %); ^1H-NMR (300 MHz, $CDCl_3$) δ 7.25 (m, 5H, Ph), 4.99 (s, 2H, CH_2Ph), 4.53 (m, 1H, CH Asn), 2.32 (dd, *J* = 15, 4.0 Hz, 1H, C*H*HCO), 2.22 (dd, *J* = 15, 6.5 Hz, 1H, C*H*HCO), 1.46 (s, 9H, $(CH_3)_3C$).

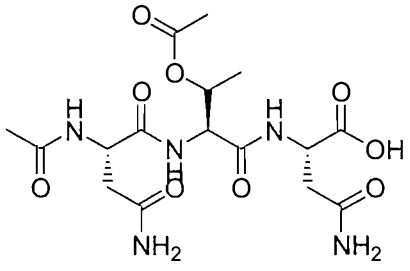
^1H NMR according to literature.[126]

3-Acetoxy-2-*tert*-butoxycarbonylamino-butyric acid, 169



N-Boc-L-Thr-OH (500 mg, 2.28 mmol) and triethylamine (465 µl, 3.50 mmol) were dissolved in chloroform (50 ml) and the solution cooled to 0 °C. Acetyl chloride was added dropwise to the solution under vigorous stirring and the stirring was continued for further 1 h at room temperature. The mixture was diluted with chloroform (100 ml) and washed with a 1M solution of sodium hydrogencarbonate (80 ml), brine (80 ml) and dried over magnesium sulphate. The solvent was evaporated under reduced pressure to give **169** as a colourless oil (522 mg, 87 %); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 5.45 (q, J = 6.5 Hz, 1H, αCH Thr), 5.25 (d, J = 8 Hz, 1H, NH), 4.41 (m, 1H, βCH Thr), 2.04 (s, 3H, CH_3CO), 1.47 (s, 9H, $(CH_3)_3C$), 1.32 (d, J = 6.5 Hz, 3H, CH_3CH).
^{1}H NMR according to literature.[127]

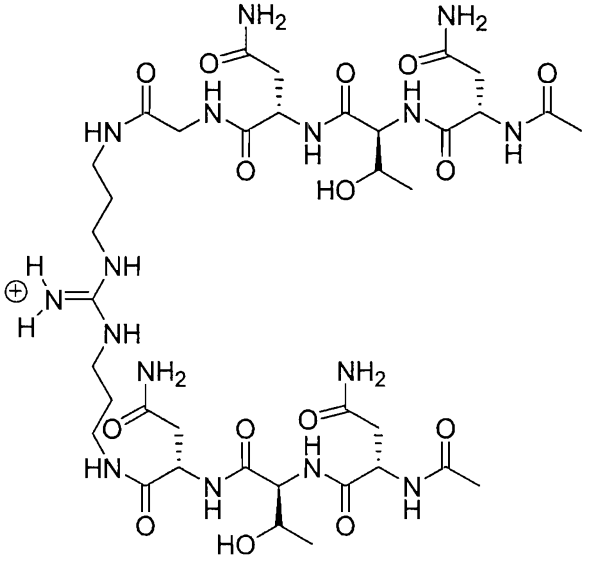
2-[3-Acetoxy-2-(2-acetylamino-3-carbamoyl-propionylamino)-butyrylamino]-succinamic acid, 173



N-Boc-L-Asn-OBn **170** (400 mg, 1.23 mmol) was stirred in a 20% solution of TFA in DCM (20 ml) for 3 h. After removing the solvents under reduced pressure, the residue was redissolved in DMF (10 ml) and cooled to 0 °C. To the solution were added *N*-Boc-L-Thr(Ac)-OH (400 mg, 1.53 mmol), HOBt (250 mg, 1.85 mmol), DIPEA (0.53 ml, 3.88 mmol) and EDC (283 mg, 1.47 mmol) and the mixture was stirred for 18 h (0°C to room temperature). The solution was diluted with DCM (150 ml) and washed

with a 1M solution of sodium hydrogencarbonate (80 ml), 1M solution of sodium hydrogensulfate (80 ml) and water (80 ml). The organic layer was dried over magnesium sulfate and, after removing the solvent, the residue was dissolved in the minimum amount of DCM (1 ml) and precipitated with a mixture of ether and petroleum ether 1:1 (30 ml) to give **171** as a white solid (480 mg, 81%). **171** was dissolved in a 20 % solution of TFA (20 ml) and stirred for 3 h. After removing the solvents under reduced pressure, the residue was redissolved in DMF (10 ml) and cooled to 0 °C. To the solution *N*-Ac-L-Asn-OH (226 mg, 1.3 mmol), HOBt (203 mg, 1.5 mmol), DIPEA (400 µl, 2.9 mmol) and EDC (250 mg, 1.3 mmol) were added and the mixture stirred for 18 h (0°C to room temperature). A white jelly precipitate was present. DCM (10 ml) was added and the mixture filtered to yield 400 mg of the benzyl ester **172** as a white solid (74 %). **172** was dissolved in DMF and Pd/C (40 mg) was added. The mixture was stirred for 18 h under hydrogen. The Pd/C was filtered off through celite and washed twice with methanol. The solvents were concentrated and ether (10 ml) was added to the residue to precipitate acid **173** as a white solid (310 mg, 93 %); m.p. = 110-112 °C (lit.[124] 111-112 °C); I.R. (neat) ν_{max} = 3279 (m), 3201 (m), 1727 (m), 1630 (s), 1538 (s), 1368 (s), 1231 (s) cm^{-1}; ^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ 8.34 (d, *J* = 7 Hz, 1H, NH Asn), 8.25 (d, *J* = 7 Hz, 1H, NH Asn), 7.92 (d, *J* = 7 Hz, 1H, NH Thr), 7.46 (s, 1H, N*H*H Asn), 7.44 (s, 1H, NH*H* Asn), 6.98 (s, 2H, NH_2 Asn), 5.23 (q, *J* = 7 Hz, 1H, CHO Thr), 4.70 (q, *J* = 7 Hz, 1H, CH Asn), 4.60-4.50 (m, 2H, CH Asn + CH Thr), 2.70-2.50 (m, 4H, CH_2CO Asn), 2.14 (s, 3H CH_3COO), 1.98 (s, 3H CH_3CON), 1.24 (d, *J* = 7 Hz, 3H, CH_3 Thr); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 172.9 (C), 172.0 (C), 171.8 (C), 171.7 (C), 170.0 (C), 169.9 (C), 168.7 (C), 70.3 (CH), 55.9 (CH), 50.2 (CH), 49.5 (CH), 37.3 (CH_2), 37.0 (CH_2), 22.9 (CH_3), 21.3 (CH_3), 16.8 (CH_3); LRMS (ES^+): m/z (%) = 453 (100) $[M+Na]^+$; HPLC 2.15 min (Isocratic 50% water 50% MeCN, gradient , analytical).

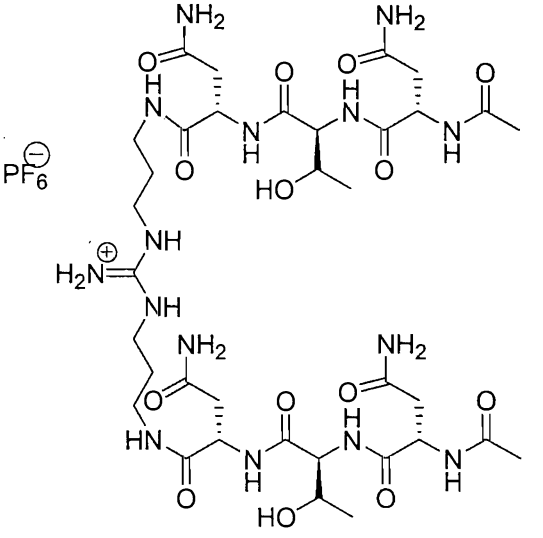
Tweezer receptor 107



Compound **166** (106 mg, 0.2 mmol) was stirred in a 20 % solution of TFA in DCM (20 ml) for 3h. After addition of toluene (80 ml) the solvents were removed under reduced pressure to give a yellow oil. The residue was redissolved in DMF (5 ml) and cooled to 0 °C. To the solution were added tripeptide **173** (220 mg, 0.5 mmol), HOBt (81 mg, 0.6 mmol), DIPEA (160 µl, 0.92 mmol) and EDC (96 mg, 0.5 mmol) and the mixture was stirred for 18 h (0°C to room temperature). DMF was concentrated and to the residue was added a mixture of ether/DCM 1:1 (20 ml). The white solid precipitated was filtered and washed with DCM, ether and acetonitrile to yield 110 mg of a cream-coloured solid (trifluoroacetyl protected tweezer, 50 %). The trifluoroacetyl tweezer (50 mg, 0.04 mmol) was dissolved in a mixture of methanol and water 5:2[123] (3.5 ml) and potassium carbonate (28 mg, 0.16 mmol) was added. The mixture was stirred for 3h at room temperature. The solvents were then removed under reduced pressure and the residue diluted with methanol and filtered. To the filtrate HPF_6 (60% solution in water, 2 drops, pH = 5). was added and the solution stirred for 20'. The solvent was evaporated and a mixture 1:1 acetonitrile/DCM added. The precipitate was collected and washed with DCM, acetonitrile and methanol/acetonitrile 10:1, to yield 34 mg of tweezer **107** as a white solid (80 %); m.p. = 211-212 °C; I.R. (neat) ν_{max} = 3323 (m), 3211 (m), 1645 (s), 1518 (m), 1407 (m) cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 4.38-4.21 (m, 4H, αCH Thr + CH_3CH Thr), 3.85 (s, 2H, CH_2 Gly), 3.30-3.20 (m, 4H, CH_2NH), 3.20-3.10 (m, 4H, CH_2NH), 2.90-2.65 (m, 8H, CH_2 Asn), 2.04 (s, 6H, CH_3CO), 1.76 (m, 4H, $CH_2CH_2CH_2$), 1.17 (d, J = 6 Hz, 6H, CH_3 Thr); Asn CH's

signals are covered by the H_2O signal; ^{13}C NMR (100 MHz, D_2O) δ 174.1 (C), 173.5 (C), 173.0 (C), 172.3 (C), 171.3 (C), 171.7 (C), 170.1 (C), 156.9 (C), 69.8 (CH), 57.0 (CH), 50.6 (CH), 50.1 (CH), 41.0 (CH_2), 38.4 (CH_3), 36.1 (CH_2), 35.9 (CH_2), 35.8 (CH_2), 28.0 (CH_2), 22.1 (CH_3), 19.1 (CH_3); MALDI-TOF: m/z (%) = 973.6 (100) $[M+H]^+$; 995.6 (60) $[M+Na]^+$; 1011.6 (80) $[M+K^+]$; Analytical data: HPLC 1.37 min (S15PROD, gradient 90 to 10 % water/TFA in MeCN/TFA, analytical); HPLC 1.21 min (Isocratic 50 % water 50 % MeCN, analytical); Anal. Calcd for $C_{37}H_{65}F_6N_{16}O_{15}P \times 2H_2O$: C, 38.48; H, 6.02; N, 19.40. Found: C, 38.29; H, 5.99; N 19.14.

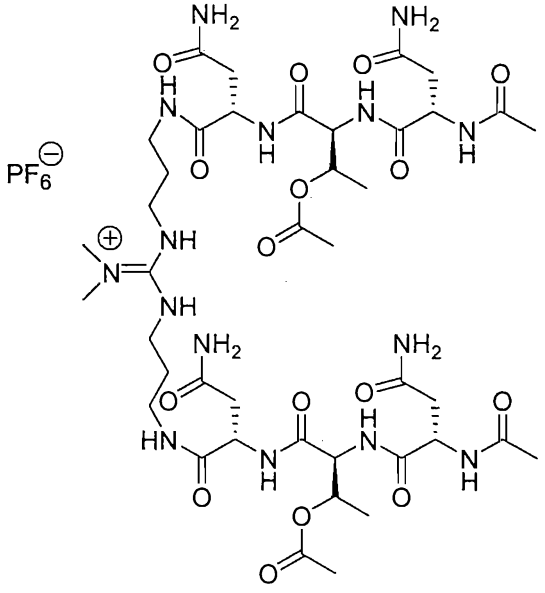
Tweezer receptor 109



Compound **157a** (53 mg, 0.11 mmol) was stirred in a 20 % solution of TFA in DCM (20 ml) for 3h. After addition of toluene (80 ml) the solvents were removedunder reduced pressure to give a yellow oil. The residue was redissolved in DMF (3 ml) and cooled to 0 °C. To the solution were added tripeptide **173** (55 mg, 0.25 mmol), HOBt (40 mg, 0.30 mmol), DIPEA (160 µl, 0.92 mmol) and EDC (48 mg, 0.25 mmol) and the mixture was stirred for 18 h (0°C to room temperature). DMF was concentrated and to the residue was added a mixture of ether/DCM 1:1 (20 ml). The white solid precipitated was filtered and washed with DCM, ether and acetonitrile to yield 52 mg of a cream-coloured solid (Trifluoroacetyl tweezer, 47 %). The solid (52 mg, 0.05 mmol) was dissolved in a mixture of methanol and water 5:2 (3.5 ml) and potassium carbonate (28 mg, 0.16 mmol) was added. The mixture was stirred for 3h at room temperature. The solvents were then removed under reduced pressure and the residue

suspended in methanol and filtered. To the filtrate HPF_6 (60% solution in water, 2 drops, pH = 5) was added and the solution stirred for 20'. The solvents were evaporated and a mixture 1:1 acetonitrile/DCM added. The precipitate was collected and washed with DCM, acetonitrile and methanol/acetonitrile 1:10, to yield 42 mg of tweezer **109** as a white solid (80 %): I.R. (neat) ν_{max} = 3320 (m), 3209 (m), 1650 (s), 1510 (m), 1407 (m) cm^{-1}; ^{1}H-NMR (400 MHz, D_2O) δ 4.65-4.55 (m, 1H, CH Asn), 4.45-4.40 (m, 1H, CH Asn), 4.30-4.20 (m, 2H, CH Asn), 4.20-4.10 (m, 4H, CH Thr (α+β)), 3.50-3.20 (m, 8H, CH_2N), 3.10-2.70 (m, 8H, CH_2CO Asn), 1.95 (s, 6H, CH_3CON), 1.74 (m, 4H, $CH_2CH_2CH_2$), 1.18 (d, J = 6.5 Hz, 6H, CH_3 Thr); ^{13}C NMR (100 MHz, D_2O) δ 176.3 (C), 176.2 (C), 172.8 (C), 172.6 (C), 171.9 (C), 171.7 (C), 154.2 (C), 66.1 (CH), 58.1 (CH), 51.3 (CH), 50.9 (CH), 37.8 (CH_2), 37.7 (CH_2), 35.8 (CH_2), 35.7 (CH_2), 27.0 (CH_2), 20.9 (CH_3), 17.6 (CH_3); MS(ES^+): m/z (%) = 916 (100) $[M+H]^+$; HPLC 1.19 min (Isocratic 50% water 50% MeCN, analytical).

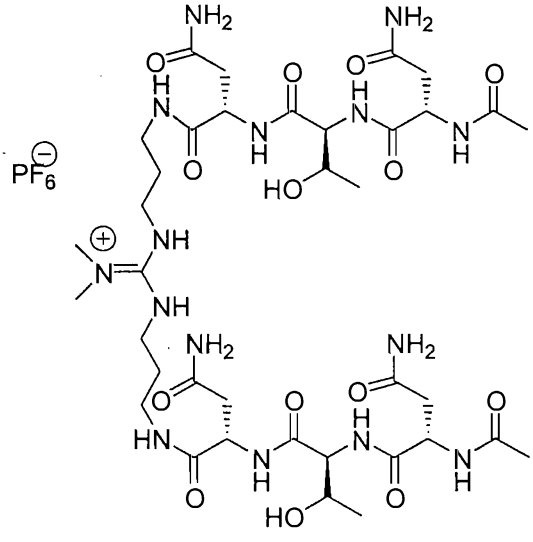
Tweezer 110



Compound **168** (100 mg, 0.18 mmol) was stirred in a 20 % solution of TFA in DCM (20 ml) for 3h. After addition of toluene (80 ml) the solvents were removed under reduced pressure to give a yellow oil. The residue was redissolved in DMF (5 ml) and cooled to 0 °C. To the solution were added tripeptide **173** (138 mg, 0.4 mmol), HOBt (73 mg, 0.55 mmol), DIPEA (125 µl, 0.72 mmol) and EDC (77 mg, 0.4 mmol) and the

mixture was stirred for 18 h (0°C to room temperature). DMF was removed and the residue suspended in methanol and filtered. The solvent was evaporated and the residue suspended in DCM (10 ml) and filtered to give tweezer **110** as a white solid (150 mg, 71 %): I.R. (neat) ν_{max} = 3215 (m), 1645 (s), 1520 (m), 1410 (m) cm^{-1}; ^1H NMR (400 MHz, D_2O) δ 5.35 (m, 2H, CHOCO Thr), 4.80-4.60 (m, 4H, CH Asn), 4.57 (dd, J = 8, 6 Hz, 2H, αCH Thr), 3.40-3.20 (m, 8H, CH_2N), 2.94 (s, 6H, $(CH_3)_2N$), 2.90-2.60 (m, 8H, CH_2CO Asn), 2.03 (s, 6H, CH_3COO), 1.99 (s, 6H, CH_3CON), 1.76 (quint, J = 7 Hz, 4H, $CH_2CH_2CH_2$), 1.22 (d, J = 6.5 Hz, 6H, CH_3 Thr); ^{13}C NMR (100 MHz, D_2O) δ 174.1 (C), 174.0 (C), 173.8 (C), 173.4 (C), 172.6 (C), 171.7 (C), 170.4 (C), 159.0 (C), 69.8 (CH), 57.0 (CH), 50.6 (CH), 50.1 (CH), 41.0 (CH_2), 38.4 (CH_3), 36.1 (CH_2), 35.9 (CH_2), 35.8 (CH_2), 28.4 (CH_2), 21.4 (CH_3), 20.1 (CH_3), 15.7 (CH_3); MS (MALDI): m/z (%) = 1028 (100) $[M+H]^+$; HPLC 1.24 min (Isocratic 50 % water 50 % MeCN, analytical); Anal. Calcd for $C_{41}H_{70}F_6N_{15}O_{16}P \times H_2O$: C, 41.31; H, 6.09; N, 17.62. Found: C, 41.38; H, 6.19; N 17.48.

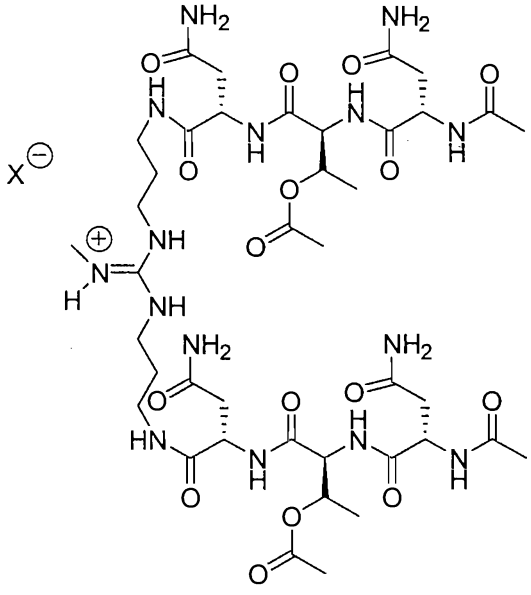
Tweezer 111



Tweezer **110** (25 mg, 0.021 mmol) was dissolved in a mixture of methanol and water 5:2 (3.5 ml) and potassium carbonate (100 mg, 0.725 mmol) was added. The mixture was stirred for 3h at room temperature. The solvents were then removed under reduced pressure and the residue suspended in methanol (5 ml) and filtered. The solution was treated with HPF_6 (60% solution in water, 1 drop, pH = 5). The solvents were

evaporated and the residue was redissolved in methanol (1 ml) and precipitated with a 1:1 mixture of chloroform and acetonitrile (4 ml) to give **111** as a white solid (19 mg, 82 % yield): I.R. (neat) ν_{max} = 3328 (br), 3216 (m), 1640 (s), 1520 (m), 1410 (m) cm^{-1}; ^{1}H-NMR (400 MHz, D_2O) δ 4.70-4.60 (m, 2H, CH Asn), 4.57 (m, 4H, αCH+CHO Thr), 3.40-3.20 (m, 8H, CH_2N), 2.94 (s, 6H, $(CH_3)_2N$), 2.90-2.60 (m, 8H, CH_2CO Asn), 1.99 (s, 6H, CH_3CON), 1.76 (quint, J = 7 Hz, 4H, $CH_2CH_2CH_2$), 1.19 (d, J = 6.5 Hz, 6H, CH_3 Thr); 2 CH of the Asn are underneath the water signal; ^{13}C NMR (100 MHz, D_2O) δ 173.7 (C), 173.6 (C), 173.0 (C), 171.4 (C), 171.1 (C), 158.8 (C), 65.9 (CH), 57.0 (CH), 50.1 (CH), 49.8 (CH), 40.5 (CH_2), 37.9 (CH_2), 35.4 (CH_2), 35.3 (CH_3), 33.9 (CH_2), 27.9 (CH_2), 20.9 (CH_3), 17.9 (CH_3); MS (ES^+): m/z (%) = 944 (60) $[M+H]^+$; HPLC 1.20 min (Isocratic 50 % water 50 % MeCN, analytical).

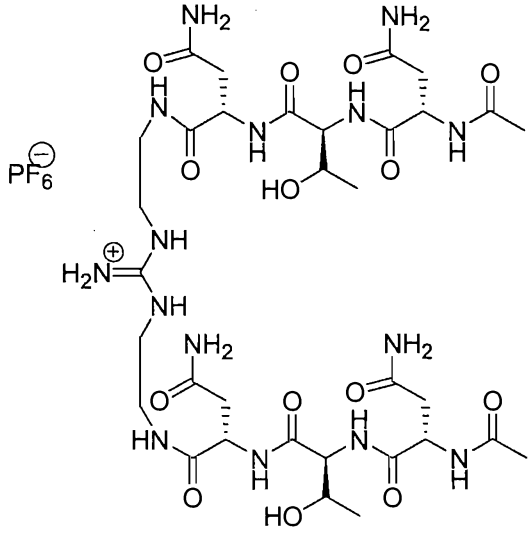
Tweezer 112



Compound **167** (40 mg, 0.075 mmol) was stirred in a 20 % solution of TFA in DCM (10 ml) for 3h. After addition of toluene (80 ml) the solvents were removed under reduced pressure to give a yellow oil. The residue was redissolved in DMF (5 ml) and cooled to 0 °C. To the solution were added tripeptide **173** (77 mg, 0.16 mmol), HOBt (30 mg, 0.22 mmol), DIPEA (70 µl, 0.40 mmol) and EDC (33 mg, 0.17 mmol) and the mixture was stirred for 18 h (0°C to room temperature). DMF was removed and the residue treated with DCM (20 ml) to give tweezer **112** as a white solid (60 mg, 65 %):

I.R. (neat) ν_{max} = 3215 (m), 1645 (s), 1520 (m), 1410 (m) cm^{-1}; ^1H NMR (400 MHz, D_2O) δ 5.35 (m, 2H, CHOCO Thr), 4.71 (q, J = 7 Hz, 2H, CH Asn), 4.57 (dd, J = 8, 6 Hz, 2H, αCH Thr), 3.30-3.10 (m, 8H, CH_2N), 2.80-2.60 (m, 8H, CH_2CO Asn), 2.94 (s, 3H, CH_3N), 1.99 (s, 6H, CH_3COO), 1.95 (s, 6H, CH_3CON), 1.70 (quint, J = 7 Hz, 4H, $CH_2CH_2CH_2$), 1.18 (d, J = 6.5 Hz, 6H, CH_3 Thr), 2 Asn CH's under H_2O signal; ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 172.3 (C), 171.8 (C), 171.2 (C), 170.3 (C), 170.1 (C), 170.0 (C), 169.0 (C), 154.9 (C), 69.9 (CH), 56.8 (CH), 50.7 (CH), 50.2 (CH), 38.7 (CH_2), 37.3 (CH_3), 36.3 (CH_2), 34.5 (CH_3), 29.0 (CH_2), 28.5 (CH_2), 22.9 (CH_3), 21.3 (CH_3), 16.9 (CH_3); MS (ES+): m/z (%) = 1014 (100) $[M+H]^+$, 1036 (60) $[M+Na]^+$; HPLC 1.22 min (Isocratic 50 % water 50 % MeCN, analytical); Anal. Calcd for $C_{40}H_{68}F_6N_{15}O_{15}P \times H_2O$: C, 40.78; H, 5.99; N, 17.83. Found: C, 40.56; H, 6.08; N 17.65.

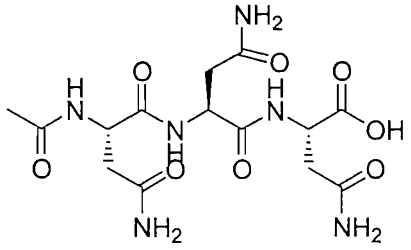
Tweezer receptor 113.



Synthesised according to the procedure used for compound **107** starting from **157b** on a 0.2 mmol scale; 80 mg (40 % overall yield); m.p. = 151-152 °C; I.R. (neat) ν_{max} = 3304 (w), 2960 (m), 1653 (s), 1428 (w), 1417 (m) cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 4.65-4.55 (m, 1H, CH Asn), 4.50-4.30 (m, 3H, CH Asn), 4.30-4.10 (m, 4H, CH Thr), 3.35-3.20 (m, 4H, CH_2NH), 3.20-3.10 (m, 4H, CH_2NH), 2.75 (m, 8H, CH_2 Asn), 2.04 (s, 6H, CH_3CO), 1.76 (m, 4H, $CH_2CH_2CH_2$), 1.17 (d, J = 6 Hz, 6H, CH_3 Thr); part of the signals are covered by the H_2O signal; ^{13}C NMR (100 MHz, D_2O) δ 176.0 (C),

176.1 (C), 172.8 (C), 172.7 (C), 172.0 (C), 172.1 (C), 154.0 (C), 66.2 (CH), 58.2 (CH), 51.3 (CH), 50.3 (CH), 39.7 (CH_2), 37.3 (CH_2), 37.2 (CH_2), 35.3 (CH_2), 20.9 (CH_3), 17.7 (CH_3); MS(ES^+): m/z (%) = 889 (100) $[M+H]^+$; HPLC 1.18 min (Isocratic 50 % water 50 % MeCN, analytical).

2-[3-Acetoxy-2-(2-acetylamino-3-carbamoyl-propionylamino)-butyrylamino]-succinamic acid, 108



N-Boc-L-Asn-OBn **170** (400 mg, 1.23 mmol) was stirred in a 20% solution of TFA in DCM (20 ml) for 3 h. After removing the solvents under reduced pressure, the residue was redissolved in DMF (10 ml) and cooled to 0 °C. To the solution were added *N*-Boc-L-Asn-OH (400 mg, 1.53 mmol), HOBt (250 mg, 1.85 mmol), DIPEA (0.53 ml, 3.88 mmol) and EDC (283 mg, 1.47 mmol) and the mixture was stirred for 18 h (0°C to room temperature). The solution was diluted with DCM (150 ml) and washed with a 1M solution of sodium hydrogencarbonate (80 ml), 1M solution of sodium hydrogensulfate (80 ml) and water (80 ml). The organic layer was dried over magnesium sulfate and after removing the solvent the residue was dissolved in the minimum amount of DCM (1 ml) and precipitated with a mixture of ether and petroleum ether 1:1 (30 ml) to yield g of a white solid (480 mg, 81%). The solid was dissolved in a 20 % solution of TFA (20 ml) and stirred for 3 h. After removing the solvents under reduced pressure, the residue was redissolved in DMF (10 ml) and cooled to 0 °C. To the solution *N*-L-Ac-Asn-OH (175 mg, 1.2 mmol), HOBt (202 mg, 1.5 mmol), DIPEA (405 µl, 3 mmol) and EDC (193 mg, 1.2 mmol) were added and the mixture stirred for 18 h (0°C to room temperature). A white jelly precipitate was present. DCM was added and the mixture filtered to yield the benzyl ester of the tripeptide as a white solid (369 mg, 75 %). The ester was dissolved in water/DMSO 10:1, Pd/C (50 mg) was added and the mixture stirred for 18 h under hydrogen at 40

°C. The Pd/C was filtered off through celite and washed twice with water. The solvents were concentrated and acetonitrile (15 ml) was added to the residue to precipitate **108** as a white solid (302 mg, 100 %): m.p. = 120-122 °C; I.R. (neat) ν_{max} = 3284 (m), 3201 (m), 1630 (s), 1523 (s), 1411 (m), 1372 (m), 1188 (m) cm^{-1}; ^{1}H-NMR (400 MHz, $(CD_3)_2SO$) δ 8.23 (m, 2H, NH), 8.05 (m, 1H, NH), 7.50 (m, 3H, NH_2CO), 6.98 (m, 3H, NH_2CO), 4.66 (m, 3H, CH Asn), 2.60 (m, 6H, CH_2CO Asn), 1.94 (s, 3H CH_3CON); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 173.0 (C), 172.2 (C), 172.1 (C), 171.8 (C), 171.4 (C), 171.1 (C), 169.9 (C), 50.3 (CH), 49.9 (CH), 49.5 (CH), 37.6 (CH_2), 37.3 (CH_2), 37.2 (CH_2), 23.0 (CH_3); LRMS (ES^+): m/z (%) = 425 (100) $[M+Na]^+$; HPLC 1.52 min (Isocratic 50 % water 50 % MeCN, analytical);

GUEST

2-[3-Acetoxy-2-(2-acetylamino-3-carbamoyl-propionylamino)-butyrylamino]-succinamate tetrabutylamonium salt, 108a

A 1 M solution of tetrabutylammonium hydroxide in methanol (TBA, 0.74 ml) was added to a solution of **108** (0.75 mmol) in methanol (2.5 ml). The solution was stirred for 2 hours at room temperature. The solvent was then removed *in vacuo*. Residual water was removed by freeze-drying on high vacuum over 16 hours. The reaction was quantitative; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 8.56 (s, 1H, N*H*H), 8.20 (d, *J* = 7 Hz, 1H, NH), 8.14 (d, *J* = 7 Hz, 1H, NH), 7.60-7.40 (m, 3H, NH+NH_2), 6.92 (s, 1H, N*H*H), 6.83 (s, 1H, N*H*H), 6.67 (s, 1H, N*H*H), 4.60-4.45 (m, 1H, CH), 4.00-3.80 (m, 1H, $CHCOO^-$), 3.20-3.10 (m, 8H, CH_2N), 2.60-2.00 (m, 6H, CH_2CO), 2.09 (s, 3H, CH_3), 1.81 (m, 8H, $CH_2CH_2CH_2$), 1.30 (q, *J* = 6 Hz, 8H, CH_2CH_3), 0.92 (t, *J* = 6 Hz, 12H, CH_3).

APPENDIX 1

Experimental for binding studies

Obtaining association constants by ^{1}H NMR titration experiments involves titration of a solution of host with a specific guest and recording a ^{1}H NMR spectrum after each addition. Upon complexation, protons in the host or guest may undergo a change in chemical shifts. In particular, protons involved in hydrogen bonding undergo a dramatic shift and therefore are used to determine association constants. After the data from the titration experiment have been acquired, curve fitting software is employed to determine the association constant. Free host and guest are in equilibrium with the host-guest complex. As association and dissociation is fast on the NMR time scale, only a time averaged spectrum of the host (or guest) and the host-guest complex is observed. Therefore, any observed chemical shift (δ_{obs}) is the mole fraction weighted average of the shifts observed in the free (δ_{free}) and complexed (δ_{bound}) molecule. During the curve fitting procedure, after an initial estimate for K_a and $\Delta\delta$, the theoretical δ_{obs} is obtained for each point. The theoretical values are then compared with the experimentally observed ones and the sum of the difference between each point is determined by the following equation: .

$$\text{Sum of differences} = \Sigma\,(\delta_{obs\,(experimental)} - \delta_{obs(theoretical)})$$

If the sum of differences is positive (or negative), the K_a is increased (or decreased) and the value $\Delta\delta$ recalculated and the whole calculation repeated until the values converge. A detailed explanation of the theoretical basis to the above discussion has been published by Wilcox.[128] A more recent review on the determination of association constants from solution NMR data has been published by Fielding. [129]

Method Used for obtaining binding constants

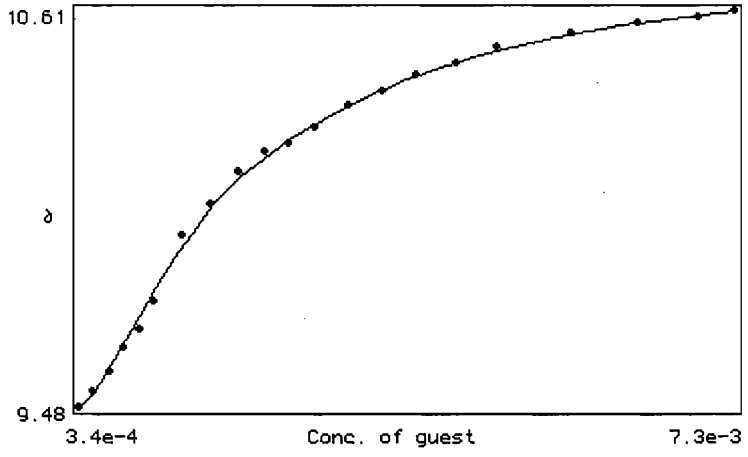
All ^{1}H NMR titration experiments were conducted on either a Brüker AM 300 or Brüker DPX 400 spectrometer at 298 K. All $CDCl_3$ was passed over a pad of basic alumina prior to use and collected over molecular sieves (4Å). A sample of host was dissolved in the deuterated solvent. A portion of this solution was used as the host

NMR sample and the remainder used to dissolve a sample of the guest, so that the concentration of the host remained constant throughout the titration. Guest (host) stock solutions were typically prepared such that the concentration of guest was at least ten times the concentration of the host (the "reverse titration" method used for the tweezer receptors is essentially the same, with the host in place of the guest and *viceversa*). Successive aliquots of the guest solution were added to the host NMR sample and ^{1}H NMR sample recorded after each addition. The hydrogens monitored during binding studies were the amide protons in the host or in the guest (reverse titration) molecule unless otherwise stated. The changes in chemical shifts of all the hosts signals as a function of guest concentration were analysed with purpose-written software, kindly provided by C.A. Hunter, where a 1:1 binding mode or a 1:2 mode was assumed. These programs fit the data to the appropriate binding model to yield the association constant, the bound chemical shift and the free chemical shift.

*Macrocycle **60** with N-Boc-L-Glu in DMSO-d_6*

```
 V   = 6.00000000000e+2
[H]  = 1.75000005402e-3
[G]  = 2.07000000000e-2
```

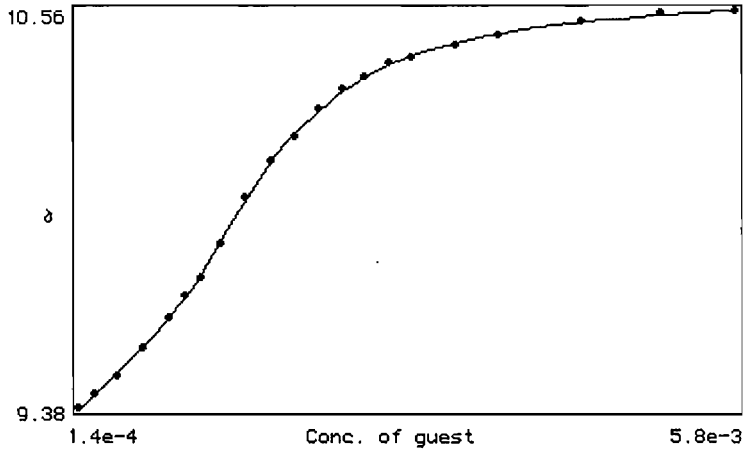
µl	δ
1.00000000000e+1	9.47970000000e+0
1.50000000000e+1	9.52300000000e+0
2.00000000000e+1	9.57780000000e+0
2.50000000000e+1	9.64810000000e+0
3.00000000000e+1	9.70130000000e+0
3.50000000000e+1	9.78350000000e+0
4.50000000000e+1	9.96860000000e+0
5.50000000000e+1	1.00600000000e+1
6.50000000000e+1	1.01520000000e+1
7.50000000000e+1	1.02100000000e+1
8.50000000000e+1	1.02320000000e+1
9.50000000000e+1	1.02820000000e+1
1.10000000000e+2	1.03420000000e+1
1.25000000000e+2	1.03840000000e+1
1.40000000000e+2	1.04290000000e+1
1.60000000000e+2	1.04620000000e+1
1.80000000000e+2	1.05070000000e+1
2.20000000000e+2	1.05490000000e+1
2.60000000000e+2	1.05760000000e+1
3.00000000000e+2	1.05970000000e+1
3.25000000000e+2	1.06150000000e+1



*Macrocycle **60** with N-Boc-D-Glu in DMSO-d_6*

```
 V   = 6.00000000000e+2
[H]  = 1.30000005402e-3
[G]  = 2.90000000000e-2
```

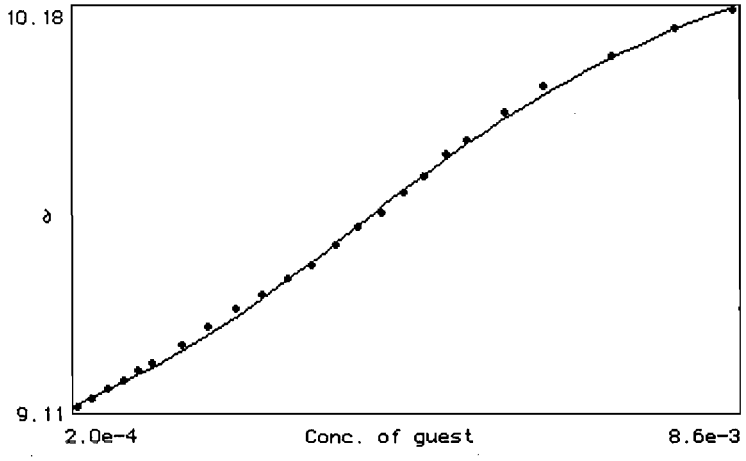
µl	δ
3.00000000000e+0	9.37740039825e+0
6.00000000000e+0	9.41450023651e+0
1.00000000000e+1	9.47290039062e+0
1.50000000000e+1	9.55189990997e+0
2.00000000000e+1	9.64529991150e+0
2.30000000000e+1	9.71000003815e+0
2.60000000000e+1	9.76399993896e+0
3.00000000000e+1	9.86289978027e+0
3.50000000000e+1	1.00009997635e+1
4.00000000000e+1	1.01115999222e+1
4.50000000000e+1	1.01829996109e+1
5.00000000000e+1	1.02630995941e+1
5.50000000000e+1	1.03230995941e+1
6.00000000000e+1	1.03630995941e+1
6.50000000000e+1	1.04000995941e+1
7.00000000000e+1	1.04209995270e+1
8.00000000000e+1	1.04530995941e+1
9.00000000000e+1	1.04872999191e+1
1.10000000000e+2	1.05288000107e+1
1.30000000000e+2	1.05495996475e+1
1.50000000000e+2	1.05605996475e+1



*Bisguanidinium **83** with N-Boc-L-Glu bis TBA salt in DMSO-d_6*

```
 V   = 6.00000000000e+2
[H]  = 2.95000000000e-3
[G]  = 2.47000000000e-2
```

µl	δ
5.00000000000e+0	9.11260000000e+0
1.00000000000e+1	9.13330000000e+0
1.50000000000e+1	9.15750000000e+0
2.00000000000e+1	9.18020000000e+0
2.50000000000e+1	9.20600000000e+0
3.00000000000e+1	9.22900000000e+0
4.00000000000e+1	9.27630000000e+0
5.00000000000e+1	9.32510000000e+0
6.00000000000e+1	9.37440000000e+0
7.00000000000e+1	9.41290000000e+0
8.00000000000e+1	9.45620000000e+0
9.00000000000e+1	9.49590000000e+0
1.00000000000e+2	9.54910000000e+0
1.10000000000e+2	9.59600000000e+0
1.20000000000e+2	9.63450000000e+0
1.30000000000e+2	9.68820000000e+0
1.40000000000e+2	9.73300000000e+0
1.50000000000e+2	9.79060000000e+0
1.60000000000e+2	9.82840000000e+0
1.80000000000e+2	9.90580000000e+0
2.00000000000e+2	9.97850000000e+0
2.40000000000e+2	1.00587000000e+1
2.80000000000e+2	1.01358000000e+1
3.20000000000e+2	1.01846000000e+1



*Bisguanidinium **83** with N-Boc-D-Glu bis TBA salt in DMSO-d_6*

```
 V   = 6.00000000000e+2
[H]  = 2.33999988995e-3
[G]  = 2.32999995351e-2
```

µl	δ
5.00000000000e+0	9.10350036621e+0
1.00000000000e+1	9.12650012970e+0
1.50000000000e+1	9.14319992065e+0
2.00000000000e+1	9.18140029907e+0
2.50000000000e+1	9.21389961243e+0
3.60000000000e+1	9.29220008850e+0
4.50000000000e+1	9.34549999237e+0
5.50000000000e+1	9.39739990234e+0
6.50000000000e+1	9.44229984283e+0
7.00000000000e+1	9.47920036316e+0
8.00000000000e+1	9.52690029144e+0
9.00000000000e+1	9.59720039368e+0
1.00000000000e+2	9.64760017395e+0
1.10000000000e+2	9.72949981689e+0
1.21000000000e+2	9.79539966583e+0
1.30000000000e+2	9.82989978790e+0
1.40000000000e+2	9.86569976807e+0
1.50000000000e+2	9.92370033264e+0
1.60000000000e+2	9.96259975433e+0
1.80000000000e+2	1.00289001465e+1
2.00000000000e+2	1.00825996399e+1
2.40000000000e+2	1.01361999512e+1
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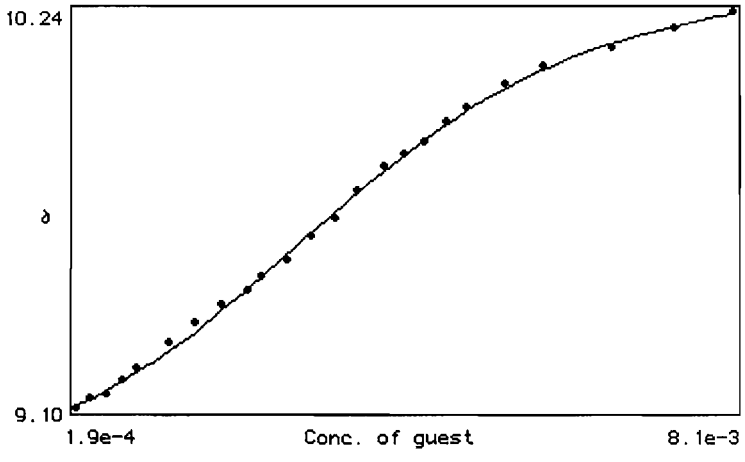
3.20000000000e+2	1.02413997650e+1



*Tweezer receptor **107** with guest **108** in DMSO-d_6*

```
 V   = 6.00000000000e+2
[H]  = 3.3000000000e-3
[G]  = 2.100000232e-2
```

µl	δ
2.00000000000e+1	7.68400000000e+0
3.00000000000e+1	7.74300000000e+0
4.00000000000e+1	7.79800000000e+0
5.00000000000e+1	7.85000000000e+0
6.00000000000e+1	7.87900000000e+0
7.00000000000e+1	7.92750000000e+0
8.00000000000e+1	7.97450000000e+0
9.00000000000e+1	8.00550000000e+0
1.00000000000e+2	8.02300000000e+0
1.10000000000e+2	8.03600000000e+0
1.25000000000e+2	8.04500000000e+0
1.40000000000e+2	8.05330000000e+0
1.55000000000e+2	8.05450000000e+0
1.70000000000e+2	8.05850000000e+0
1.90000000000e+2	8.06000000000e+0
2.10000000000e+2	8.06000000000e+0
2.30000000000e+2	8.06000000000e+0
2.70000000000e+2	8.06050000000e+0
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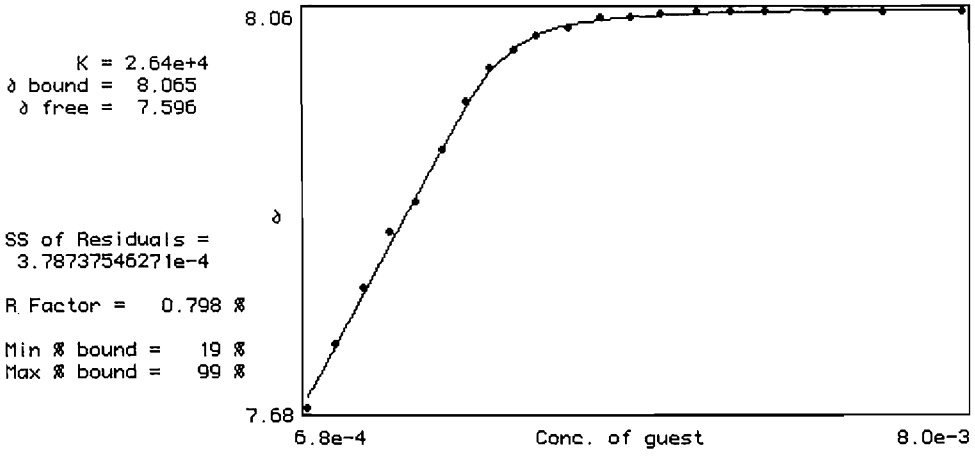
3.70000000000e+2	8.06050000000e+0



*Tweezer receptor **107** with guest **174** in DMSO-d_6*

```
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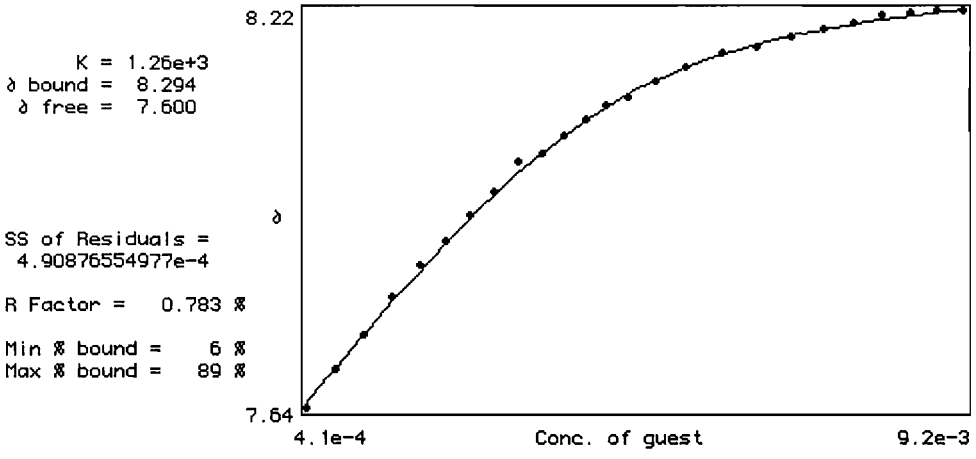
[H]  = 4.70000000000e-3
[G]  = 2.50000002325e-2
```

µl	δ
1.00000000000e+1	7.64450000000e+0
2.00000000000e+1	7.69840000000e+0
3.00000000000e+1	7.74660000000e+0
4.00000000000e+1	7.80290000000e+0
5.00000000000e+1	7.84940000000e+0
6.00000000000e+1	7.88200000000e+0
7.00000000000e+1	7.92070000000e+0
8.00000000000e+1	7.95640000000e+0
9.00000000000e+1	7.99780000000e+0
1.00000000000e+2	8.01100000000e+0
1.10000000000e+2	8.03610000000e+0
1.20000000000e+2	8.06000000000e+0
1.30000000000e+2	8.08010000000e+0
1.40000000000e+2	8.09220000000e+0
1.55000000000e+2	8.11310000000e+0
1.70000000000e+2	8.13320000000e+0
1.90000000000e+2	8.15350000000e+0
2.10000000000e+2	8.16460000000e+0
2.30000000000e+2	8.17870000000e+0
2.50000000000e+2	8.19000000000e+0
2.70000000000e+2	8.19920000000e+0
2.90000000000e+2	8.20830000000e+0
3.10000000000e+2	8.21210000000e+0
3.30000000000e+2	8.21670000000e+0
3.50000000000e+2	8.21670000000e+0



*Tweezer receptor **109** with guest **108** in DMSO-d_6*

```
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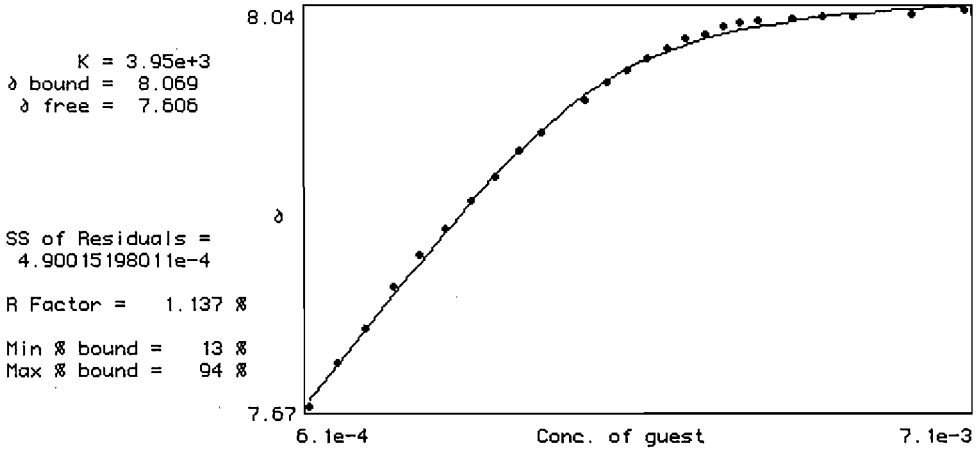
[H]  = 4.00000000000e-3
[G]  = 1.90000002325e-2
```

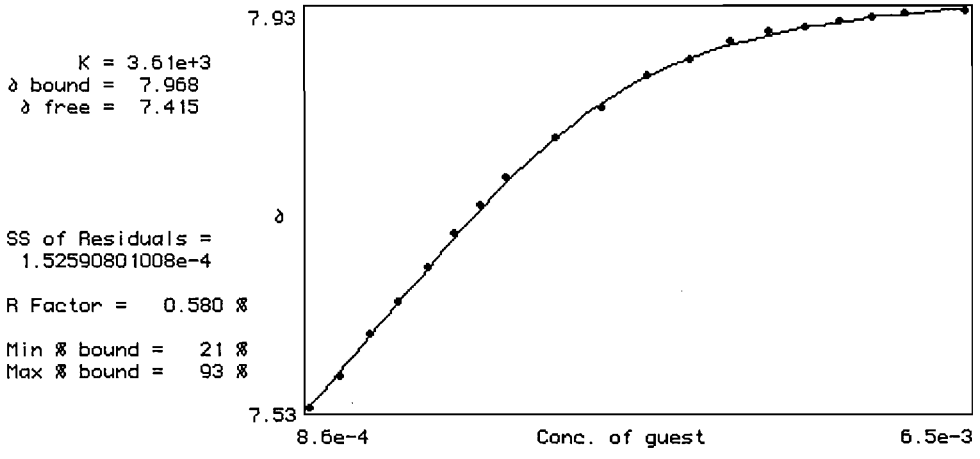
µl	δ
2.00000000000e+1	7.66650000000e+0
3.00000000000e+1	7.70700000000e+0
4.00000000000e+1	7.73850000000e+0
5.00000000000e+1	7.77800000000e+0
6.00000000000e+1	7.80850000000e+0
7.00000000000e+1	7.83450000000e+0
8.00000000000e+1	7.86000000000e+0
9.00000000000e+1	7.88350000000e+0
1.00000000000e+2	7.90650000000e+0
1.10000000000e+2	7.92350000000e+0
1.30000000000e+2	7.95500000000e+0
1.40000000000e+2	7.97200000000e+0
1.50000000000e+2	7.98250000000e+0
1.60000000000e+2	7.99500000000e+0
1.70000000000e+2	8.00350000000e+0
1.80000000000e+2	8.01350000000e+0
1.90000000000e+2	8.01750000000e+0
2.00000000000e+2	8.02500000000e+0
2.10000000000e+2	8.02800000000e+0
2.20000000000e+2	8.03030000000e+0
2.40000000000e+2	8.03150000000e+0
2.60000000000e+2	8.03330000000e+0
2.80000000000e+2	8.03330000000e+0
3.20000000000e+2	8.03580000000e+0
3.60000000000e+2	8.04040000000e+0



Tweezer receptor ***113*** *with guest* ***108*** *in DMSO-d_6*

```
 V   = 6.00000000000e+2
[H]  = 4.00000000000e-3
[G]  = 1.80000002325e-2
```

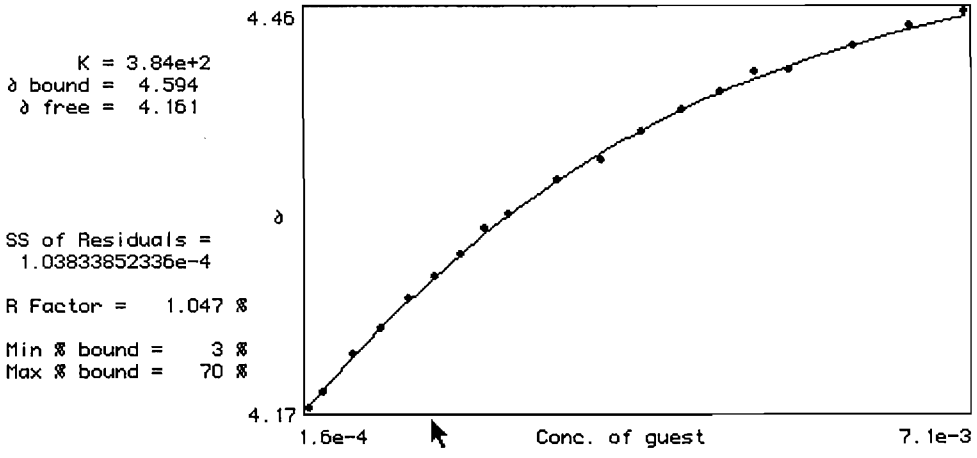
µl	δ
3.00000000000e+1	7.52800000000e+0
4.00000000000e+1	7.56000000000e+0
5.00000000000e+1	7.60200000000e+0
6.00000000000e+1	7.63500000000e+0
7.00000000000e+1	7.67000000000e+0
8.00000000000e+1	7.70400000000e+0
9.00000000000e+1	7.73300000000e+0
1.00000000000e+2	7.76200000000e+0
1.20000000000e+2	7.80100000000e+0
1.40000000000e+2	7.83300000000e+0
1.60000000000e+2	7.86400000000e+0
1.80000000000e+2	7.88200000000e+0
2.00000000000e+2	7.90000000000e+0
2.20000000000e+2	7.90900000000e+0
2.40000000000e+2	7.91400000000e+0
2.60000000000e+2	7.91900000000e+0
2.80000000000e+2	7.92400000000e+0
3.00000000000e+2	7.92700000000e+0
3.40000000000e+2	7.93100000000e+0



*Tweezer receptor **110** with guest **108** in DMSO-d_6*

```
 V   = 6.00000000000e+2
[H]  = 2.71999998949e-3
[G]  = 1.96000002325e-2
```

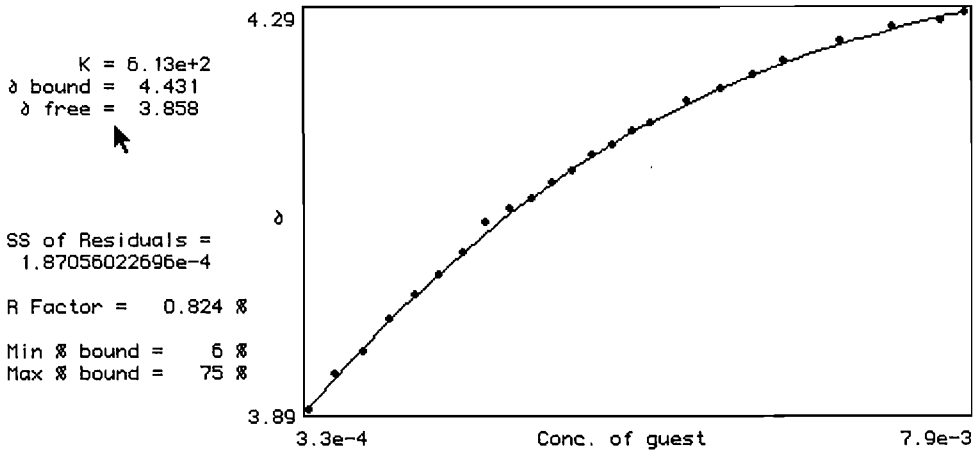
µl	δ
5.00000000000e+0	4.17339992523e+0
1.00000000000e+1	4.18450021744e+0
2.00000000000e+1	4.21229982376e+0
3.00000000000e+1	4.23180007935e+0
4.00000000000e+1	4.25320005417e+0
5.00000000000e+1	4.27029991150e+0
6.00000000000e+1	4.28620004654e+0
7.00000000000e+1	4.30439996719e+0
8.00000000000e+1	4.31440019608e+0
1.00000000000e+2	4.34060001373e+0
1.20000000000e+2	4.35410022736e+0
1.40000000000e+2	4.37510013580e+0
1.60000000000e+2	4.39139986038e+0
1.80000000000e+2	4.40450000763e+0
2.00000000000e+2	4.41919994354e+0
2.20000000000e+2	4.42140007019e+0
2.60000000000e+2	4.43790006638e+0
3.00000000000e+2	4.45260000229e+0
3.40000000000e+2	4.46409988403e+0



*Tweezer receptor **112** with guest **108** in DMSO-d_6*

```
 V   = 6.00000000000e+2
[H]  = 4.00000000000e-3
[G]  = 2.04000002325e-2
```

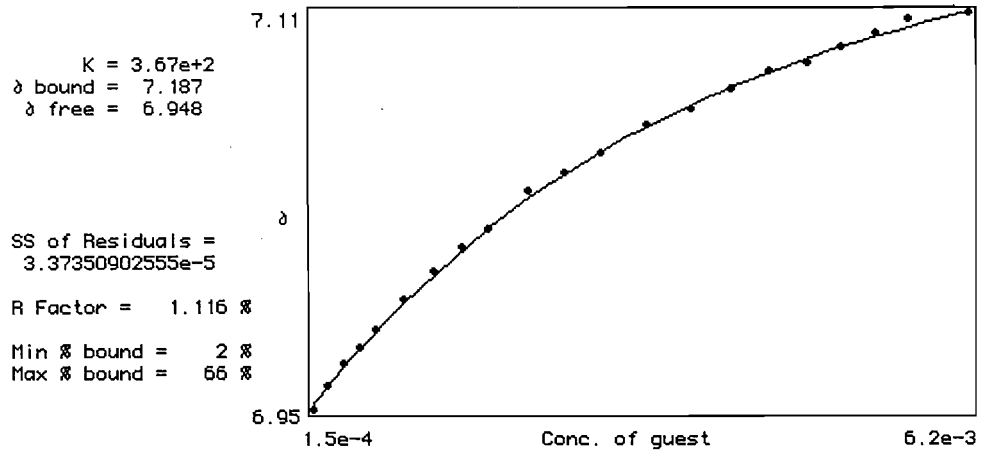
µl	δ
1.00000000000e+1	3.89010000000e+0
2.00000000000e+1	3.92500000000e+0
3.00000000000e+1	3.94780000000e+0
4.00000000000e+1	3.97960000000e+0
5.00000000000e+1	4.00390000000e+0
6.00000000000e+1	4.02290000000e+0
7.00000000000e+1	4.04490000000e+0
8.00000000000e+1	4.07520000000e+0
9.00000000000e+1	4.09000000000e+0
1.00000000000e+2	4.10030000000e+0
1.10000000000e+2	4.11550000000e+0
1.20000000000e+2	4.12760000000e+0
1.30000000000e+2	4.14380000000e+0
1.40000000000e+2	4.15340000000e+0
1.50000000000e+2	4.16780000000e+0
1.60000000000e+2	4.17620000000e+0
1.80000000000e+2	4.19740000000e+0
2.00000000000e+2	4.21030000000e+0
2.20000000000e+2	4.22470000000e+0
2.40000000000e+2	4.23910000000e+0
2.80000000000e+2	4.25810000000e+0
3.20000000000e+2	4.27180000000e+0
3.60000000000e+2	4.27940000000e+0
3.80000000000e+2	4.28770000000e+0



*Tweezer receptor **111** with guest **108** in DMSO-d_6*

```
 V = 6.00000000000e+2
[H] = 1.84000004083e-3
[G] = 1.83000005782e-2
```

µl	δ
5.00000000000e+0	6.95319986343e+0
1.00000000000e+1	6.96239995956e+0
1.50000000000e+1	6.97030019760e+0
2.00000000000e+1	6.97700023651e+0
2.50000000000e+1	6.98339986801e+0
3.50000000000e+1	6.99569988251e+0
4.50000000000e+1	7.00649976730e+0
5.50000000000e+1	7.01520013809e+0
6.50000000000e+1	7.02269983292e+0
8.00000000000e+1	7.03700017929e+0
9.50000000000e+1	7.04419994354e+0
1.10000000000e+2	7.05170011520e+0
1.30000000000e+2	7.06250000000e+0
1.50000000000e+2	7.06920003891e+0
1.70000000000e+2	7.07679986954e+0
1.90000000000e+2	7.08389997482e+0
2.10000000000e+2	7.08710002899e+0
2.30000000000e+2	7.09259986877e+0
2.50000000000e+2	7.09819984436e+0
2.70000000000e+2	7.10379981995e+0
3.10000000000e+2	7.10650014877e+0



APPENDIX 2

Experimental for calorimetric studies (ITC)

By directly measuring the heat evolved or absorbed as a function of time ITC can determine, in one stroke, all the thermodynamic parameters involved in a chemical process. In a single experiment the binding constant (K_a), the stoichiometry (n) and the enthalpy (ΔH) of the process investigated are determined. From the association constant, it is possible to determine the free energy and entropy of binding for the interaction. A typical ITC titration begins with a known concentration of macrocycle dissolved in solution to which controlled aliquots of the guest species under test are added through a syringe. As the complexation takes place, an endothermic or exothermic signal, depending on the nature of the complexation, is observed. As the guest concentration increases and the supply of macrocycle available for binding is exhausted, we reach a plateau in terms of the amount of heat evolved or absorbed on further addition of guest.

Method for obtaining calorimetric data

All binding experiments were performed on an isothermal titration calorimeter from Microcal Inc. (Northampton, MA). In the described experiment a 0.6 mM receptor solution is added to the calorimetric cell. A 40 mM solution of tetrabutylammonium salts of N-Boc-Glu is introduced in 65 injections (30 of 2.5 μL and 35 of 5 μL), for a total of 250 μL of added guest. Such high concentration of guests are important to generate sharp curves, necessary for acceptable curve fitting. The solution is continuosly stirred to ensure rapid mixing and kept at 25°C, through the combination of an external cooling bath and an internal heater. Dilution effects are determined by performing a blank experiment by adding the same guest solution into the pure solvent and subtracting this from the raw titration to produce the final binding curve. Association parameters are found by applying either one-site or two-sites models, using the Origin software provided. These methods rely on standard nonlinear least-squares regression (Levenberg-Marquard method) to fit the curves, taking into account the change in volume that occurs during the calorimetric titration.

*Calorimetric data for N-Boc-D-Glu-OTBA with macrocycle **60***

Solvent: 50 % DMSO/H_2O

Concentration of host solution: 0.6 mM

Concentration of guest solution: 40 mM

$K_{1:1}$: $(3.01 \pm 0.15) \times 10^3\ M^{-1}$

ΔG: $-19.0 \pm 0.5\ kJ\ mol^{-1}$

ΔH: $1.31 \pm 0.17\ kJ\ mol^{-1}$

$T\Delta S$: $20.31\ kJ\ mol^{-1}$

0.09616	--
0.19249	--
0.28898	--
0.38563	--
0.48245	--
0.57944	--
0.67659	571.13199
0.7739	614.18549
0.87137	495.55087
0.96902	510.59915
1.06682	430.49387
1.16479	377.82222
1.26293	380.69336
1.36122	320.42935
1.45969	297.17218
1.55831	262.12551
1.65711	243.1369
1.75606	223.05858
1.85518	165.08444
1.95447	179.73071
2.05392	151.42512
2.15353	129.25917
2.25331	159.54491
2.35325	139.8526
2.45336	109.86127
2.55363	118.78552
2.65406	97.10466
2.75466	92.70758
2.85543	109.31449
2.95636	97.03225
3.15871	83.33908
3.36171	90.06733
3.56538	70.06027
3.7697	85.20039
3.97468	66.54042
4.18032	41.39236
4.38661	43.29154
4.59356	46.05398
4.80117	30.85669
5.00944	29.59239
5.21837	29.26322
5.42795	38.76473
5.63819	36.14123

```
5.84908     38.04734
6.06064     22.26972
6.27285     20.26048
6.48572     20.20565
6.69925     14.70082
6.91343     17.4424
7.12827     22.1759
7.34377     16.77812
7.55993     17.76789
7.77674     15.59061
7.99421     10.03947
8.21234     11.65579
8.43113     4.12631
8.65057     8.43869
8.87067     8.89952
9.09143     5.59201
9.31285     6.1662
9.53492     0.34367
9.75765     1.1868
9.98104     2.97747
10.20509    2.96198
10.42979    3.20543
--
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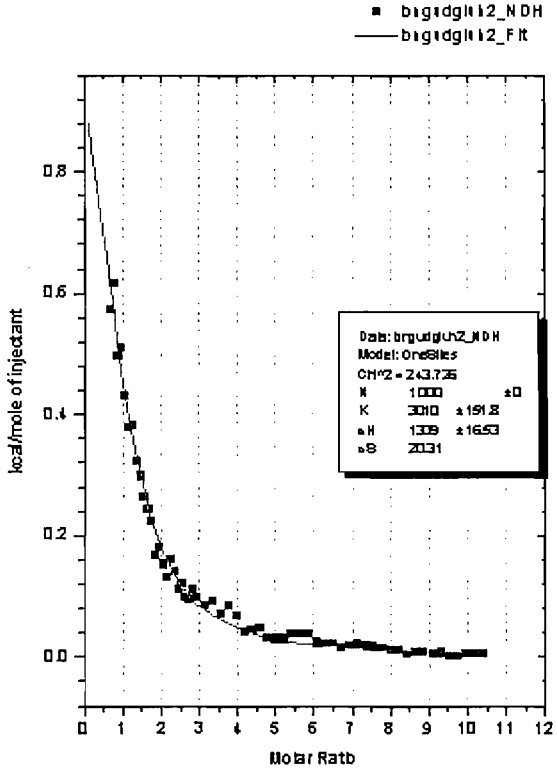

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