UNIVERSITY OF SOUTHAMPTON

SOLID PHASE AND COMBINATORIAL SYNTHESIS OF RECEPTORS FOR SMALL PEPTIDES

by

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ABSTRACT

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This thesis entails the synthesis and investigation of the properties of novel receptors for small peptides. In chapter I recent developments in host-guest chemistry are reviewed, with particular reference to receptors for carboxylic acids and small peptides. The application of solid phase and combinatorial chemistry to the synthesis of receptors is also described.

Chapter II describes the synthesis of a combinatorial library of diamidopyridine derived tweezer receptors with peptidic side-arms. The library was screened with different labelled peptide guests. Screening experiments with dye-labelled tripeptide L-Glu-L-Ser-L-Val-OH showed excellent selectivity and led to the identification of a tweezer receptor structure which was shown to bind the tripeptide with good selectivity over related tripeptide sequences. The results are discussed in relation to previous screening experiments, using the same tripeptide guests, with related tweezer receptor libraries.

Chapter III describes the development of enantioselective receptors for separation of racemic mixtures carried out within an European Network (Milan, Bristol, Madrid).

Synthesis, conformational studies and binding properties of novel 2,6-diamidopyridine derived receptors bearing sulfonamidopeptide side-arms are described. The synthesis and binding properties of guanidinium based receptors for (*S*)-Naproxen are also described.

Chapter IV describes preliminary investigations towards dynamic combinatorial libraries of guanidinium and 2,6-diamidopyridine based tweezer receptors. The interest focused on dynamic combinatorial systems based on catalysed alkene metathesis and imine formation for selective binding of C-terminal peptides. Although promising results were obtained further investigations are required.

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Preface

The research described in this Thesis was carried out under the supervision of Prof. Jeremy D. Kilburn at the University of Southampton between September 1999 and September 2002. No part of this Thesis has been previously submitted at this or any other University.

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A special thanks to Dr Gwen Hickin, Dr Ray Boffey and Dr Phillip Nelson for proof reading part of this thesis.

Finally I wish to thank my whole family for their love and support.

Abbreviations

AA	Amino acid
Ac	Acetyl
Alloc	Allyloxycarbonyl
Ar	Aryl
Bn	Benzyl
Boc	*tert*-butyloxycarbonyl
BSA	Bis(trimethylsilyl)acetamide
But, ^{t}Bu	*ter*-Butyl
Bz	Benzyl
CBS	Carboxylic Binding Site
Cbz, Z	Benzyloxycarbonyl
δ	Chemical Shift
d	doublet
Da	Dalton
DIBAH	Diisobutylaluminium hydride
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	*N,N'*-Dicyclohexylcarbodiimide
1,2-DCE	1,2-dichloroethane
DCLs	Dynamic Combinatorial Libraries
Ddpe	2-(1-Hydroxy-2-phenyl-ethylidene)-5,5-dimethyl-cyclohexane-1,3-dione
DIC	1,3-Diisopropylcarbodiimide
DIEA, DIPEA	Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMSO	Dimethyl sulfoxide
DNS	5-Dimethylaminonaphthalene-1-sulfonyl
EDC	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
ELSD	Evaporative light scattering detection

ES^+	Positive electrospray
ESI-MS	electrospray ionization-MS
FAB	Fast atom bombardment
Fmoc	9-Fluorenylmethoxycarbonyl
h	hours
HBTU	2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HOBt	1-Hydroxybenzotriazole
HPLC	High Performace Liquid Chromatography
IR	Infra Red
ITC	Isothermal Titration Calorimetry
J	Coupling costant
lit.	Literature
m	multiplet
MALDI-TOF	matrix-assisted laser desorption and ionization-time of flight
MAS NMR	magic angle spinning NMR
min	minutes
MIP	Molecular Imprinting Polymer
mp	Melting point
MS	Mass spectroscopy
MTDA	Methyl trimethylsilyl dimethylketene acetal
NMM	*N*-Methylmorpholine
NMR	Nuclear Magnetic Resonance
NOESY	nuclear overhauser effect spectroscopy
PEG	Polyethylene glycol
PG	Protecting Group
Ph	Phenyl
ppm	parts per million
Py	Pyridine
PyBOP	Benzotriazol-1-yloxy-tris-pyrrolidinophosphonium hexafluorophosphate
PyBrOP	Bromo-tris-pyrrolidinophosphonium hexafluorophosphate

ROESY	Rotating frame nuclear overhauser effect spectroscopy
ROMP	Ring opening metathesis polymerisation
rt	room temperature
s	singlet
SPPS	Solid Phase Peptide Synthesis
t	triplet
TBA	Tetrabutylammonium aetate
TBAF	Tetra-n-butylammonium fluoride
TEA	Triethylamine
TEEA	Triethylammonium acetate
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
Trt	Trityl
Z	Benzyloxycarbonyl
UV	Ultra violet

Amino acids are abbreviated using the standard 3-letter code as follow:

Ala	Alanine
Arg	Arginine
Asn	Asparagine
Asp	Aspartic Acid
Cys	Cysteine
Gln	Glutamine
Glu	Glutamic acid
Gly	Glycine
His	Histidine
Ile	Isoleucine
Leu	Leucine
Lys	Lysine
Met	Methionine

Phe	Phenylalanine
Pro	Proline
Ser	Serine
Thr	Threonine
Trp	Tryptophan
Tyr	Tyrosine
Val	Valine

Chapter I

I.1 Supramolecular and Host-Guest Chemistry

Supramolecular Chemistry was first defined by Jean-Marie Lehn, one of its major exponents, as the 'chemistry of molecular assemblies and of the intermolecular bond'. For his work in this field he was awarded the Nobel Prize in Chemistry in 1987. [1]

J. M. Lehn stated: "Supramolecular chemistry is the chemistry of the intermolecular bond, covering the structures and functions of the entities formed by the association of two or more chemical species". [2]

The field of supramolecular chemistry describes molecular systems in which the individual components are held together by reversible non-covalent intermolecular forces. It is a relatively young discipline dating back to the late 1960s and early 1970s. However its concept and origins may be dated to the beginnings of modern chemistry itself.

Supramolecular chemistry has been explored since the publication of the discovery of crown ethers and their affinity for metals. [3,4] This led to the emergence of molecular recognition as a new area of research that covered the chemical, physical and biological features of chemical species held together by non-covalent bonding interactions. Molecular recognition is defined as a process involving both binding and selection of specific substrate. [1]

A landmark discovery in supramolecular chemistry was Fischer's 'lock-and-key' concept. [5] Emil Fischer introduced the concept of complementarity, an example of which is the specificity of enzymatic action. He proposed a model for how a substrate fits into the active site of an enzyme.

In this field of research it is the specific molecular interactions that are involved in such complexation events, which are of greatest interest. A new terminology was introduced to describe supramolecular systems. Generally, the host is defined as the entity showing convergent binding sites (the receptor molecule), an example of which is an enzyme in

biological chemistry. The substrate that the receptor molecule binds is identified as guest- the component showing divergent binding sites.

Highly specific chemical processes that occur in biology rely on molecular interactions. Substrate binding to an enzyme or a receptor, the self-assembling of protein complexes, nucleic acid synthesis, signal induction by neurotransmitters and immunological antigen-antibody association are only a few examples of biological processes that are dependent on a substrate binding to a receptor inducing a biological response.

A representative example of specific recognition is provided by carboxypeptidase-A, an enzyme that selectively catalyses the hydrolysis of the C-terminal amino-acid residues of proteins.

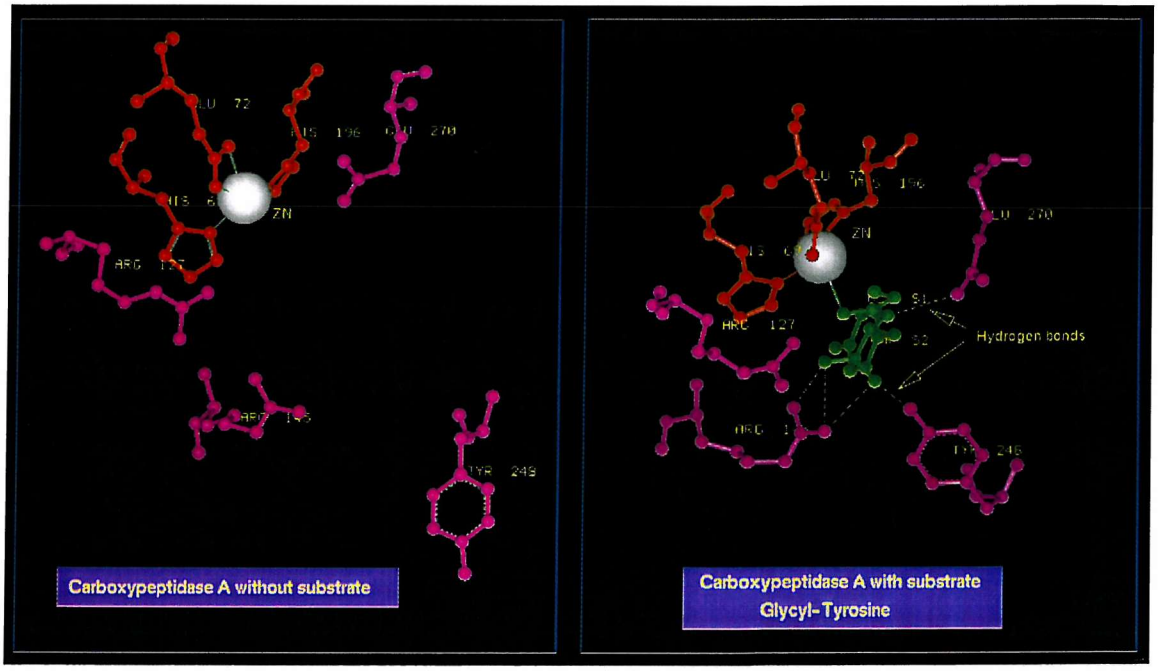


Figure I-1 The active carboxypeptidase-A

In order to understand the process of molecular interaction and the mechanisms of enzymatic catalysis, chemists have attempted to devise artificial hosts that can model these biological events.

The selective recognition of complex biological structures under physiological conditions is currently one of biggest challenges in supramolecular chemistry. The DNA recognition by hairpin polyamide is a very impressive example of such a process (Figure I-2). [6-9]

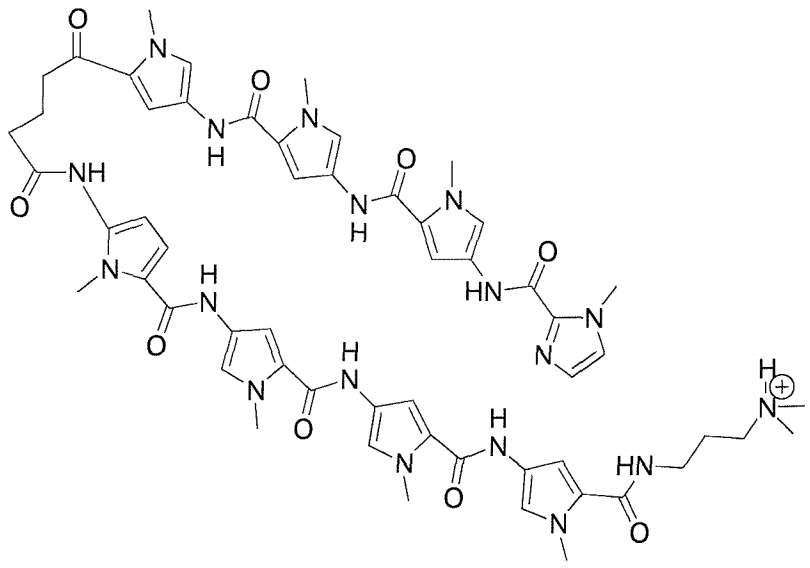


Figure I-2 Dervan's hairpin polyamide

The design of such ligands can be the basis of new gene-based drugs used in controlling gene expression. The increasing interest in artificial hosts is not only due to their direct relevance in biological systems, but also their potential to act as new therapeutics, biosensors and catalysts. [4,10]

A large number of synthetic receptors have been produced for the purpose of binding substrates with a great diversity. In the last two decades there has been an extensive literature describing the synthesis of nucleotide bases, amino acids, peptides and many other chemical entities.

I.2 General Features of Synthetic Receptors

The emphasis in the design of host molecules is the construction of a stable host-guest complex based on non-covalent interactions. This construction is only possible if the stabilisation gained by a set of small synergic interactions results in a significant binding energy and hence complex stability.

The thermodynamic stability of a host-guest complex in a given solvent is expressed by measurement of the binding constant K, which represents the thermodynamic equilibrium constant for the binding process.

$$[H] + [G] \overset{K}{\rightleftharpoons} [HG]$$

$$K = \frac{[HG]}{[H]\,[G]}$$

Thus, a large binding constant corresponds to a high concentration of host-guest complexes and hence a high complex stability. Binding constants are thermodynamic parameters and therefore related to the free energy of the association process. According to the Gibbs free energy equation the general affinity of a host for a guest under specific conditions (solvent, temperature, etc.) can be expressed in terms of K or ? G:

$$? G = -RT \ln K$$
$$K = e^{(-? G/RT)}$$

where R is the gas constant, T is the temperature in Kelvin and ? G is the free energy change. A thermodynamically favourable binding process is indicated by a negative value of ? G, which is the result of an enthalpic (? H) and entropic contribution (? S).

$$? G = ? H - T ? S$$

During a successful binding process the energetically unfavourable reorganisation step (host pre-organisation) is compensated for by the thermodynamically favourable energy of binding. During pre-organisation the host molecule undergoes a conformational modification to maximise the probability of binding with the guest.

In general, mechanically rigid host structures exhibit slow binding kinetics due to the high energy associated with movement to the complexation transition state. Flexible hosts are capable of rapid changes of conformation and therefore complexation and decomplexation are rapid processes.

I.3 Experimental Techniques for Measuring Binding Constants

Binding constants may be determined by experimental techniques that are able to give information regarding the concentration of a host-guest complex. Methods generally used are nuclear magnetic resonance titration, calorimetric titration and extraction experiments. If the host is rigid enough to reduce the rate of exchange of a complexed and uncomplexed guest on NMR time scale then the binding constant can be determined by integration of the signals for bound and unbound host or guest. However, most host-guest equilibria are fast on the NMR time scale.

Generally, a typical titration experiment is performed by addition of small aliquots of guest to a solution of host of known concentration. The chemical shift for a particular resonance is monitored as a function of guest concentration. Finally, the shape of the titration curve gives quantitative information about the binding constant.

In isothermal calorimetric titrations, experiments involve the measurement of the heat of complexation (a thermodynamic quantity) as a function of guest or host concentration. [11] As the two elements interact, heat is released or absorbed in direct proportion to the amount of binding that occurs.

I.4 Factors Affecting the Association of Host-Guest Molecules

The stability of a host-guest complex is derived from the synergic contribution of non-covalent intermolecular interactions. The design of a viable synthetic receptor should therefore maximise all of the relevant interactions and effects of both the host and the guest.

Common interactions in synthetic host-guest complexes are: electrostatic interactions (dipole-dipole and dipole-induced dipole interactions), hydrogen bonds, van der Waals interactions or London dispersion forces, π–π stacking interactions and charge transfer interactions. Hydrophobic and solvent effects also play an important role in the formation of the host-guest complex and will be briefly described in this section.

I.4.1 Electrostatic Interactions

Electrostatic interactions result from the attraction of two charged species. The force of attraction, F, is given by Coulomb`s law:

$$F = \frac{q_1 q_2}{r^2 D}$$

where q_1 and q_2 are the charges on the two groups, r is the distance between them and D is the dielectric constant of the solvent. The optimal distance between the charged groups has been shown to be 2.8 Å. [12]

Ion-ion interactions are non-directional, however for an ion-dipole interaction to give optimum binding the groups must be suitably aligned. (Figure I-3)

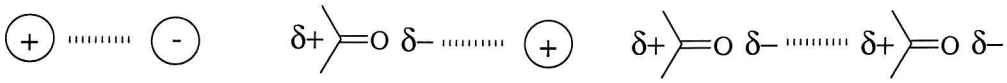


Figure I-3 Electrostatic interactions (ion-ion, ion-dipole, dipole-dipole)

The application of electrostatic interactions is exemplified by the binding displayed by crown ethers with cations. A representative example of such crown ether is given by the cyclic polyethers reported by Pedersen [4] (Figure I-4).

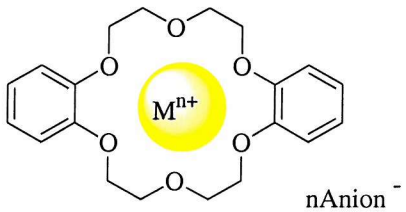


Figure I-4 Pedersen's Cyclic polyether dibenzo [18]crown-6

A great number of polyethers capable of binding a large range of alkali cations have been described. These polyethers were derived from aromatic vicinal diols and the complexes formed by ion-dipole interactions between the alkali metal cation and the oxygen donor atoms in the polyether ring. There exists a relationship between cavity

size, cation radius and stability of the complex and as such a stronger complex is formed as a result of a better fit of the cation into the crown (optimal spatial fit).

Before this work it had been discovered that ionophores such as valinomycin and nonactin were able to incorporate alkali metals (Na^+, K^+ etc.) into their cavity. Valinomycin (Figure I-5) is a cyclic depsipeptide which has been extensively studied for its ion binding capacity, conformational properties and its ability to transport ions across membranes. [13,14]

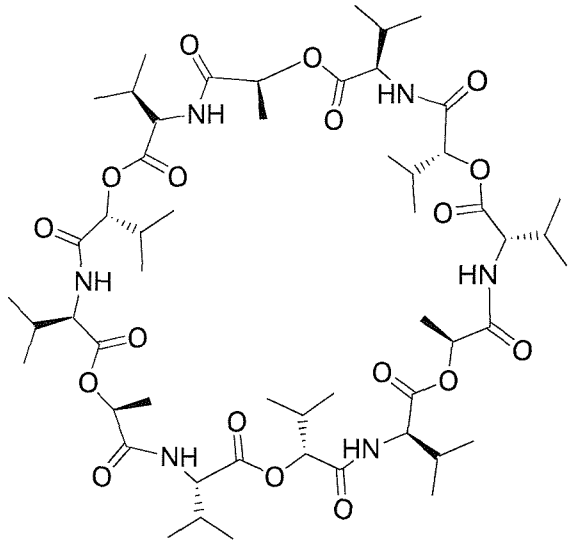


Figure I-5 The natural-product ionophore Valinomycin

I.4.2 Van der Waals Forces

Molecules attract each other at moderate distances and repel each other at close range. The attractive interactions are collectively called 'van der Waals forces'. The forces operate only when molecules are very close to each other. When two uncharged particles approach one another, the electron clouds of the competing particles undergo a deformation that leads to an induced dipole moment and weak attraction between the particles. The sum of the repulsive and attractive interactions given by equation I-1 is called the Lennard-Jones potential (Figure I-6). [15]

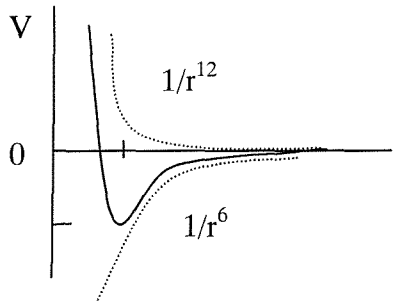


Figure I-6 Lennard-Jones potential and relation of the parameters to the features of the curve.

$$V_{LJ} = 4\varepsilon\left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6}\right]$$

Equation I-1

The Lennard-Jones potential is mildly attractive as two uncharged molecules or atoms approach one another from a distance, but strongly repulsive when they become too close. At equilibrium, the pair of atoms or molecules tends to move toward a separation corresponding to a minimum in the Lennard-Jones potential.

Although these forces are weak making individual energies low, the interactions are additive and can make significant contributions to binding when summed over the entire system.

I.4.3 Hydrogen Bonds

The use of hydrogen bonds to confer binding strength and selectivity is a dominant theme in host-guest complexation.

Hydrogen bonds are generally established between an electronegative heteroatom with a lone pair of electrons, the hydrogen acceptor, and a hydrogen atom that has a formal bond to another atom, the hydrogen bond donor (Figure I-7-8).

Hydrogen bonds can be formed between charged and uncharged molecules; the higher the charge on the interacting atoms the shorter and stronger the hydrogen bond.

The strength of a hydrogen bond is much weaker than that of a covalent bond (50-100 kcal/mol) but much stronger than van der Waals interactions (0.05-0.5 kcal/mol). [16]

Figure I-7 Strong hydrogen bonds

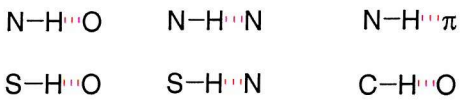
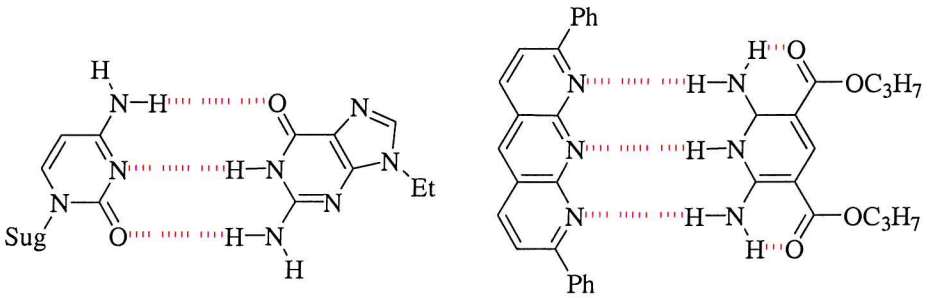


Figure I-8 Weak hydrogen bonds

Typically, the length of a hydrogen bond in a system such as NH---O=C is between 1.7 and 2.0 Å, however larger distances have been reported. [17]

Hydrogen bonds are ubiquitous in supramolecular chemistry, in particular they are responsible for the overall shape of many proteins, recognition of substrates by numerous enzymes and for the double helix structure of DNA.

The triply hydrogen bonded complex between guanine and cytosine (Figure I-9 a) is essential to the structure of nucleic acids. The analysis of triply hydrogen bonded systems was reported by Jorgensen and Zimmerman [18,19] in which the strength of the interaction was identified by a K value of ~ 10^4-10^5. The third hydrogen bond gave an additional K of 40-130 M^{-1} compared to complexes with 2 hydrogen bonds. [20]

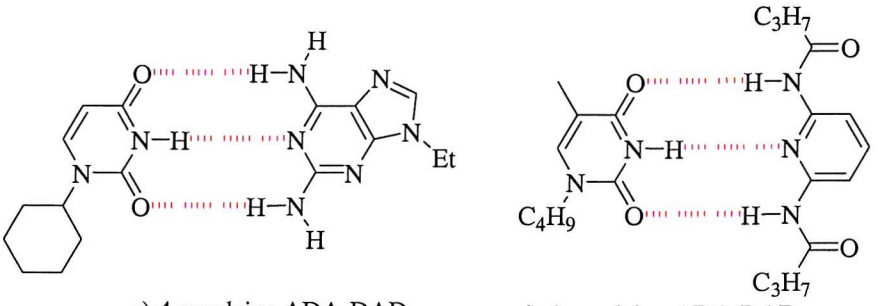


a) 2 repulsive +2 attractive DAA-ADD
K_a= 10^4-10^5 M^{-1}
in Chloroform

b) 4 attractive AAA-DDD
K_a= > 10^5 M^{-1}
in Chloroform

Figure I-9 Triply hydrogen bonded complexes

When other triply hydrogen bonded systems were considered (Figure I-10) [18] the origin of the reduced binding compared to the complexes reported in Figure I-9 [18,19] was not obvious.



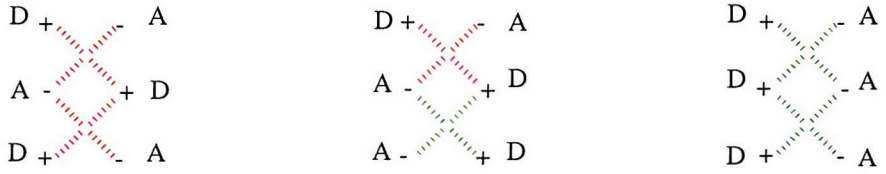
c) 4 repulsive ADA-DAD
K_a= 170 M^{-1} in
Chloroform

d) 4 repulsive ADA-DAD
K_a= 90 M^{-1}
in Chloroform

Figure I-10 Hydrogen bonded complexes

Jorgensen pointed out that hydrogen bonds have considerable electrostatic character because they are formed between an electropositive hydrogen atom and an electronegative heteroatom. This means that diagonal interactions (also called secondary electrostatic interactions) in an array are important in the determination of binding strength.

The three possible arrangements of the partially charged sites for the triply hydrogen bonded systems are shown below (Figure I-11).



4 repulsive interactions

2 repulsive
2 attractive

4 attractive interactions

Figure I-11 Arrangements of the partially charged sites for triply hydrogen bonded systems

Jorgensen observed that two complexes in which hydrogen bond donor (D) and acceptor (A) groups alternated (ADA-DAD) had lower K values than complexes that are (DDA-AAD) or (DDD-AAA). [19] This demonstrated how multiple hydrogen bonds could be used to confer a high level of selectivity on the binding of a host to its substrate. An example of a hydrogen bonding receptor was synthesised by Hamilton and co-workers. [21] The receptor had two 2,6-diaminopyridine groups that were capable of forming six hydrogen bonds with a complementary guest such as a barbiturate in a non-

competitive solvent. Hamilton proved the importance of hydrogen bonding by measuring the value of the binding constant with several guests that were strategically modified.

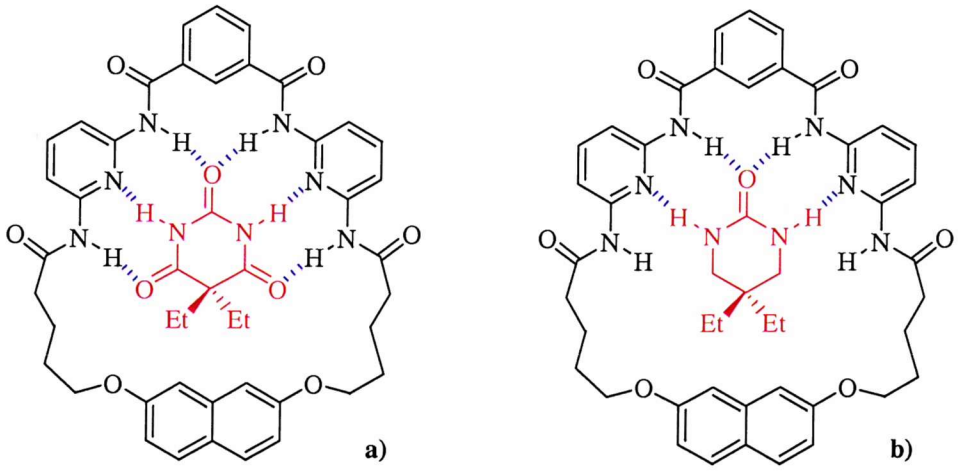


Figure I-12 Hamilton's barbiturate receptor: a) barbital guest b) cyclic urea guest

Cyclic urea (Figure I-12 **b**) lacks two of the six H-bonding sites in barbital binding (Figure I-12 **a**). As the number of hydrogen bonds formed between host and guest decreased a corresponding drop in the value of the binding constant was observed (Table I-1).

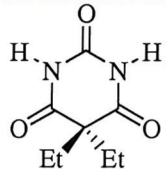
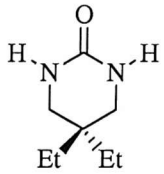
Guest	$K(M^{-1})$
	250000
	400

Table I-1 Binding data forHamilton's barbital receptor

I.4.4 Aromatic-Aromatic Interactions

Intermolecular interactions involving aromatic rings are essential in chemical and biological recognition, particularly for rational drug design and lead optimisation in medicinal chemistry. [22] An example of non-covalent interactions involving aromatics rings is given by protein-ligand recognition. Most of the structures of protein complexes with small molecules show interactions involving aromatic amino acid side chains of the receptor and aromatic and heteroaromatic rings of the ligand.

An interesting example is given by the complex (Figure I-13) of the enzyme acetylcholinesterase (AchE) and a drug developed to treat symptoms of Alzheimer's disease (E2020 Aricept). [23,24]

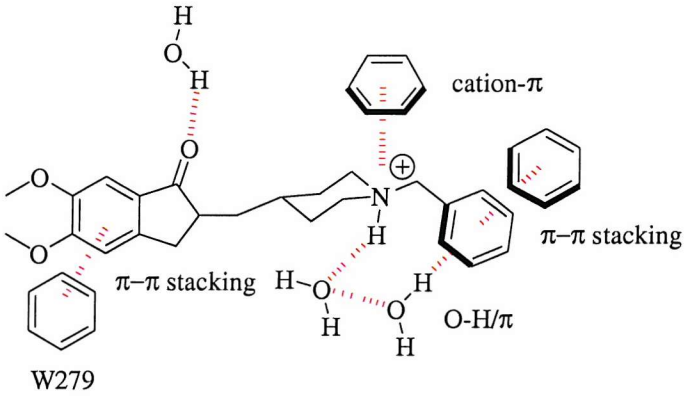
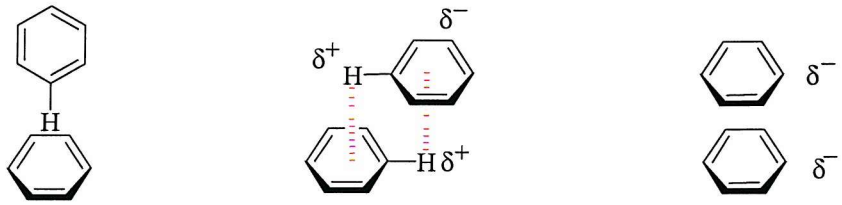


Figure I-13 Binding mode of the anti-Alzheimer drug E2020 within the acetylcholinesterase

The analysis of the X-ray crystal structure revealed the diversity of these interactions: π–π stacking, O-H/π and cation- π interactions. π–π stacking interaction occurs between aromatic rings when one is relatively electron rich and one is electron poor.

Although a variety of intermediate geometries are involved in π–π interactions, the predominant arrangement was found to be the T-shaped 'edge to face' and the parallel-displaced 'face to face' (Figure I-14)



T-shaped "edge-to-face" parallel-displaced "face-to-face" "face-to-face"

Figure I-14 Proposed lowest energy structures of the benzene dimer

Hunter and Sanders [25] used a simple model of the charge distribution in a π-system to explain the strong geometrical requirement for interactions between aromatic molecules. The model considers π electrons and the σ-bonding system separately, and reveals that π–π repulsion and π–σ attractions are the factors governing interaction between two aromatic molecules.

The positively polarised aromatic hydrogen atoms are directed towards the regions of high electron density, namely the π-electron cloud of another ring. This gives rise to the off-set stacking geometries observed. For benzene rings, the 'edge-to-face' geometry with an interplanar angle of 90° has been calculated to be more stable than the "face to face" geometry by approximately 6.3 $kJmol^{-1}$. [26] Face to face stacking is not favoured as this results in regions of negative charge being in close proximity to one another. Prediction of such aromatic interactions in host-guest systems is complicated. This is because the interactions are often between heterocyclic species. The magnitude of the π-π stacking interaction was investigated by Wilcox [27] using a molecular torsion balance to measure an intramolecular 'edge-to-face' π-π interaction: this value was reported as 1 $kJmol^{-1}$. More recently, Hunter [28] employed a double mutant approach to estimate the value of an 'edge-to-face' π-π interaction to be -1.4 kJ mol^{-1} although Schneider [29] has questioned the validity of this measurement.

Although π–π interactions are accepted as weak, they have been recognised to play an important role in the folding [30] and thermal stability of proteins. [31] Burley and Petsko [32,33] demonstrated in a study involving 34 protein structures that on average 60 % of aromatic side chains (Phe, Trp, Tyr) are involved in π–π interactions where the predominant arrangement was found to be the T-shaped edge-to-face structure.

I.4.5 Solvent Effects

The solvent plays an important role on supramolecular equilibria. It exerts a great influence on the stabilisation of the host-guest complex. [34]

The understanding of macroscopic (bulk) physical solvent properties does not lead to a satisfactory quantitative interpretation of solvent effects. Generally, solute-solvent interactions take into account several contributions: non-specific electrostatic interactions (ion-ion, ion-dipole, dipole-dipole, induced dipoles etc.), specific electron or proton donor-acceptor interactions and solvophobic interactions. In the last decades however, progress in the theoretical calculation of solvation free energies has been significant. Host-guest complexes are favoured by solvents which either weakly solvate the uncomplexed components of the system or strongly solvate the molecular complex.

In the last two decades inclusion complexation of aromatic solutes by cyclophanes in water has been extensively reviewed. [35] Diederich [22] reported calorimetric studies conducted with cyclophanes investigating the nature of the driving force for inclusion complexation of –para-disubstituted derivatives (Figure I-15).

	X	Y
1	COOMe	COOMe
2	Me	NO_2
3	OH	NO_2
4	MeO	MeO
5	Me	Me

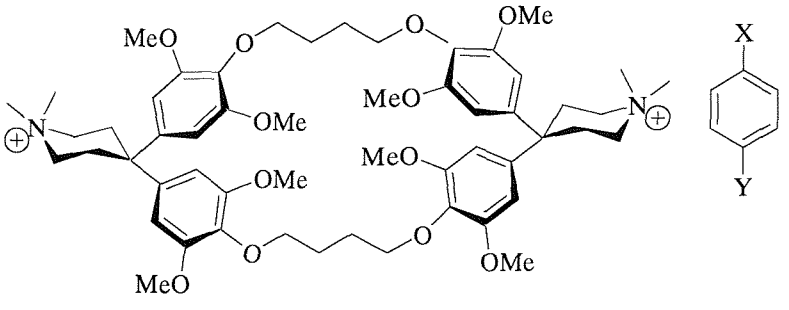


Figure I-15 Diederich's cyclophanes

The binding of aromatic guest molecules to water soluble cyclophanes with hydrophobic cavities was used to demonstrate the relationship between binding strength and the solvent's dipole moment, cohesion and polarisability. The studies demonstrated that the binding constants decrease for this system when moving from a polar protic solvent, which is directly related to solvation. [36]

Hydrophobic interactions influence many important processes, such as aggregation of surfactants to micelles, folding of proteins, stabilization of protein-protein or protein-ligand complexes and supramolecular complexation of guests with non-polar moieties. The hydrophobic interactions arise from the fact that non-polar molecules tend to avoid water, that is, they prefer to be associated with non-polar molecules. The stronger van der Waals interactions between hydrocarbons in comparison with the interactions between hydrocarbons and water is the driving force for hydrocarbon association.

I.5 Synthetic Receptors

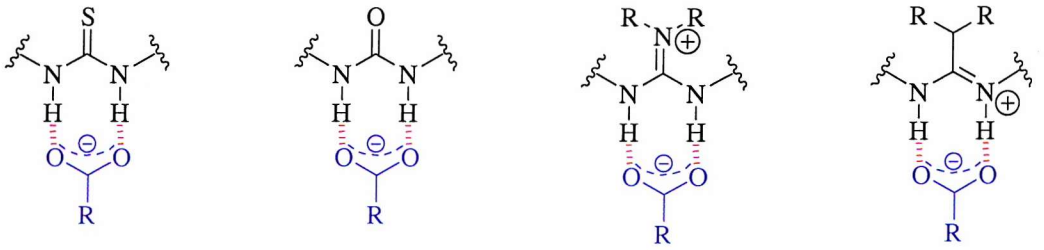
Supramolecular chemistry is highly interdisciplinary and covers the chemical, physical and biological features of chemical species held together by non-covalent binding interactions. Given the enormity of the topic, this review can only be approached in an incomplete way. The following section attempts to provide a number of examples, focusing on receptors that bind peptides and related guests. A more extensive review can be obtained in the literature. [37,34,38-41]

I.6 Synthetic Receptors Designed to Bind Carboxylic Acids and Carboxylates

A range of structural alternatives exists which are capable of binding the carboxylate terminus of peptides. Examples of binding sites for carboxylates and carboxylic acid functionalities include urea, thiuorea, guanidinium, amidine, diamidopyridine.

I.6.1 Receptor for Carboxylates

The carboxylic binding site (CBS) functionality is the site within the receptor to which the carboxylate or carboxylic acid group is bound. Carboxylates can be bound to a bidendate hydrogen bonding system such as a guanidinium, urea, thiourea or amidine (Figure I-16).



Thiourea **Urea** **Guanidinium** **Amidine**

Figure I-16 Carboxylates binding sites (CBS)

Nature uses guanidinium moieties to coordinate different anionic groups. Excellent reviews of the chemical and biophysical properties of natural and synthetic guanidine compounds has been reported in the literature. [42]

The guanidinium group possesses peculiar features. It remains protonated over a wider pH range than the ammonium group and therefore is capable of binding carboxylates over a considerable pH range. It forms characteristic strong zwitterionic hydrogen bonds ($N\text{-}H^{+\cdots}O^{-}$) with carboxylates and phosphates. [43]

Lehn was the first to synthesise a series of structurally different guanidinium species in investigating the role of the guanidinium group in the complexation of anions (Figure I-17). Both of these systems demonstrated only weak complexation of PO_4^{3-} (log K_s = 1.7 and 2.4 in methanol/water), which were governed by electrostatic interactions. [44,45]

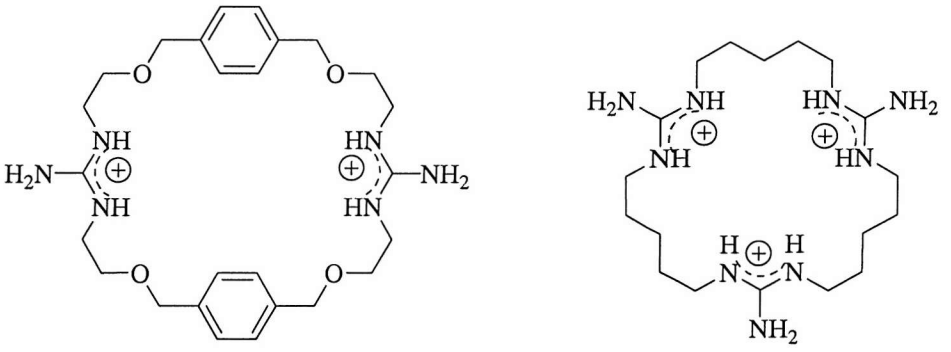
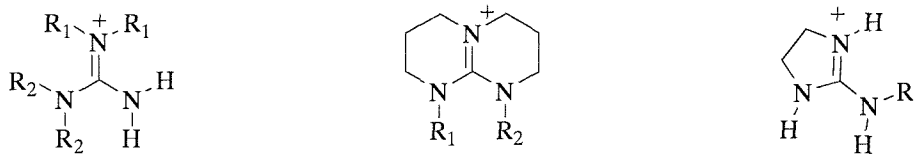


Figure I-17 The first examples of guanidinium-based macrocyclic hosts

Hamilton reported studies on the association of simple guanidinum derivatives **1-8** with tetrabutylammonium (TBA) acetate in DMSO (Figure I-18). [46]



1 $R_1 = R_2 = H$
2 $R_1 = Me, R_2 = H$
3 $R_1 = R_2 = Me$

4 $R_1 = R_2 = H$
5 $R_1 = Me, R_2 = H$
6 $R_1 = R_2 = Me$

7 R = H
8 R = Bn

Figure I-18 Hamilton's guanidinium receptors for acetate

Systems studied included acyclic, monocyclic and bicyclic guanidinium salts. Within the acyclic systems **1-3**, it was observed that sequential removal of hydrogen bond donors resulted in a drastic decrease in the association constant ($K_{1:1}$ = 7.9 x 10^3 M^{-1}, $K_{1:1}$ = 3.4 x 10^3 M^{-1}, $K_{1:1}$ = 1.1 x 10^2 M^{-1}). Similarly, sequential methylation of the corresponding bicyclic guanidiniums **4-6** resulted in a loss of binding affinity for TBA acetate ($K_{1:1}$ = 5.6x10^3 M^{-1}, **4**). Monocyclic guanidiniums **7** and **8** displayed a high affinity for acetate ($K_{1:1}$ = 8.7x10^3 M^{-1}, $K_{1:1}$ = 7.2x10^3 M^{-1} respectively). The association constants were confirmed by traditional ^1H NMR titrations. In most cases, the receptors formed 1:1 complexes with the guest, however two receptors (**1** and **7**) were proven to complex an additional equivalent of TBA acetate to form a weak 2:1 complex due 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. Building on this work, Hamilton moved to more complicated host-guest systems. [47]

In order to improve its binding strength, the guanidinium group can be incorporated into a bicyclic framework. As a result, the hydration of the cation by accumulation of hydrophobic hydrocarbon residues is reduced and conformational freedom is restricted. Forming part of a bicyclodecane, the guanidinium cation becomes a perfect complement for carboxylate anions, with both protons acting as docking sites for the two syn- lone pairs of the carboxylate. [48,49]

Lehn and de Mendoza [48] described the synthesis of a receptor for chiral recognition of aromatic carboxylate anions containing a rigid bicyclic guanidine and a bis-naphthoyl unit (Figure I-19).

Sodium p-nitrobenzoate was extracted from water by receptor **9** in chloroform. The binding constant was determined by ^{1}H-NMR titration ($K = 1.6 \times 10^3\ M^{-1}$). The data supported the formation of a complex involving a double recognition of the guest by the guanidinium cation (zwitterionic hydrogen bonds with the carboxylate function) and the naphthoyl side arms (π–π stacking).

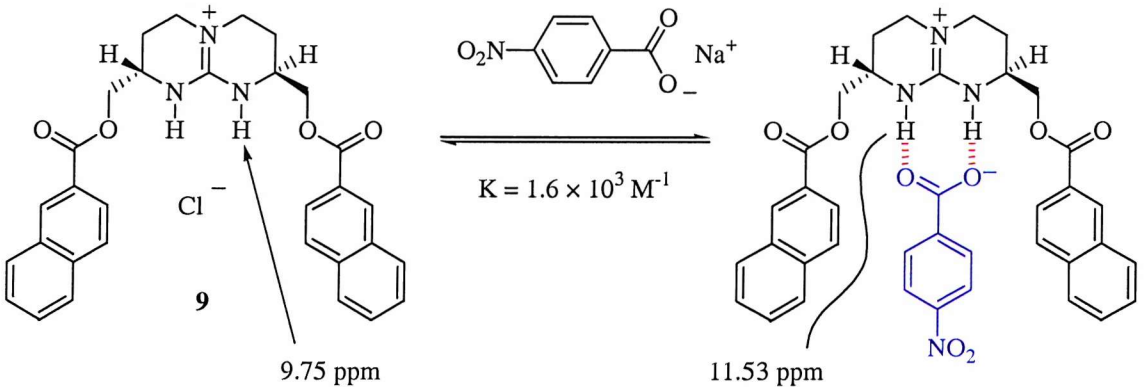


Figure I-19 Lehn and Mendoza's chiral receptor containing a rigid bicyclic guanidine

Further investigations focused on overcoming the problem related to the binding of amino acids in zwitterionic form.

De Mendoza designed a modified receptor featuring three different recognition sites: a guanidinium as binding site for carboxylate; a crown ether to bind the ammonium ion; and an aromatic planar surface (the naphthalene ring) for an additional selective stacking interaction with the side chain of the aromatic amino acids.

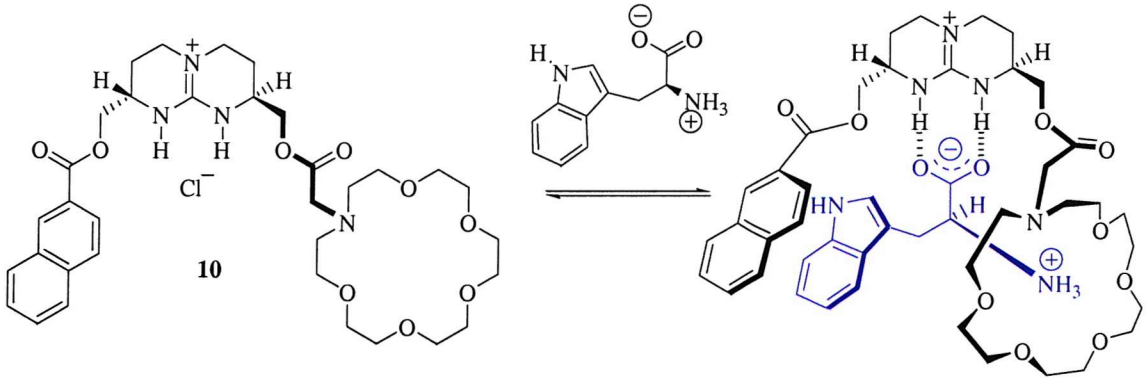
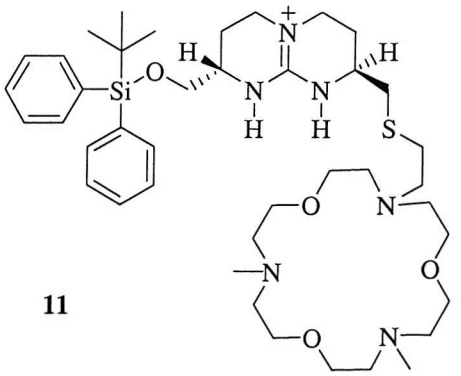


Figure I-20 (S,S) de Mendoza's receptor incorporating a crown ether moiety

Figure I-20 shows the proposed structure for a 1:1 complex between (*S,S*)-**10** and L-tryptophan. The affinity of **10** toward amino acids was determined by liquid-liquid single extraction experiments. It was found that the (*S,S*)-receptor could extract L-Trp and L-Phe from the corresponding racemic mixture in aqueous solutions into dichloromethane. Amino acids such as L-Val, without any aromatic side chain were not detected. The extraction efficiencies (i.e. the fraction of receptor molecules occupied by the substrate) in the organic phase as determined by NMR integration were around 40 %. A competition experiment with a mixture of all three amino acids resulted in 100:97:6 Phe/Trp/Val ratios. In comparison, it was interesting to observe that no Phe and Trp extraction was observed when the dinaphthoyl guanidinum **9** (Figure I-19) derivative was employed as receptor. The corresponding D-enantiomers were not extracted as confirmed by NMR. When the same experiment was carried out on the (*R,R*) enantiomer of **10** the D-enantiomers were extracted (D-Phe, D-Trp) but not the corresponding L-enantiomers. This high degree of chiral recognition is explained by the presence of three simultaneous non-covalent interactions of the substrate with the flexible and foldable receptor.

Following this principle, Schmidtchen [50,51] reported a similar receptor in a study on the extraction of ^{14}C-labeled amino acids.

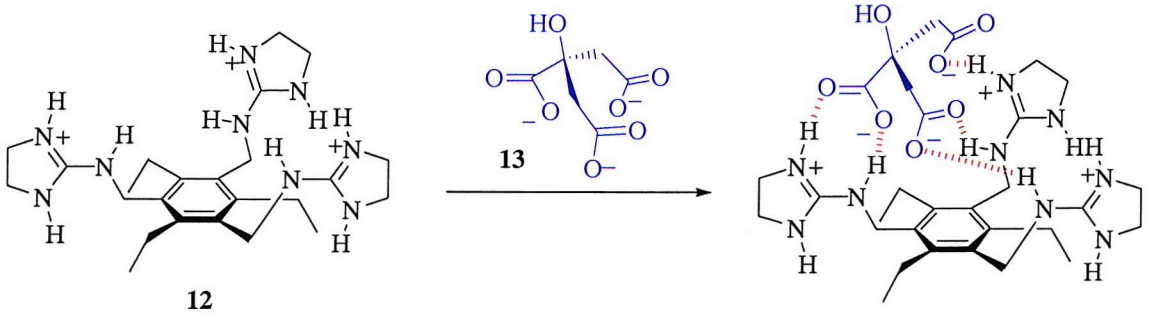
It was observed that even quite hydrophilic amino acids such as serine and glycine could be transferred to the organic phase with 1:1 host-guest stoichiometry. Maximum extractability was reached at pH 9, indicating that the amino acids were extracted in their zwitterionic forms. Although Schmidtchen's modified receptor **11** (Figure I-21) in principle could provide aromatic rings to an amino acid side chain for stacking and thus emulate a three-point binding mode as postulated for **10**, it was less enantioselective than **10** (40 % with Phe).



11

Figure I-21 Schmidtchen's modified receptor

Anslyn has synthesised receptor **12** (Figure I-22), which was found to be a chemosensor (a synthetic sensor coupled with a signalling element) for citrate **13** in beverages. The anionic fluorescent dye 5-carboxyfluorescein was used to signal anion complexation in solutions of methanol and water buffered at pH 7.4. [52-54]



12 13

Figure I-22 Anslyn's chemosensor for citrate in beverages

Trisguanidinium **12** was used to detect a specific compound in a multicomponent aqueous solution in a manner similar to that of antibody-based biosensors in immunoassays. Receptor **12** was selective for the recognition of citrate in water over di- and monocarboxylates, phosphates, sugars, and simple salts. The receptor consisted of three guanidinium groups for hydrogen bonding and charge pairing with citrate. The steric gearing imparted by ethyl groups on the 2-, 4- and 6-positions ensures that the guanidinium moieties are preorganised on the same face of the benzene ring. This conformation yields several hydrogen bonds and three sets of ionic interaction in the host-guest complex (Figure I-22), leading to good binding in water with a measured [54] K of 6.8×10^3 M^{-1}.

Recently, Anslyn described a modified citrate sensor based upon the scaffold **12** in which one guanidinium salt is replaced by an internal fluorescent probe ($K > 8.3 \times 10^6$ M^{-1}). [55]

Urea and thiourea provide a strong binding site for carboxylate using, as shown in the guanidinium, a bidentate bonding motif. Wilcox was the first to employ ureas and thioureas for carboxylate binding. [56] Studies were carried out on urea **14** (Figure I-23) which was found to bind TBA benzoate in chloroform with an association constant of 2.7×10^4 M^{-1}. ^1H NMR titrations showed large downfield shifts of the N-H protons, indicating strong hydrogen bonding between the urea hydrogens and carboxylate oxygen atoms.

14

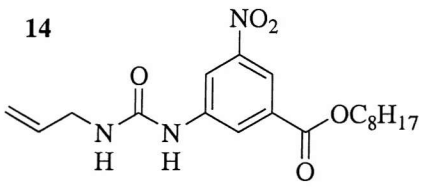


Figure I-23 Wilcox's urea carboxylate receptor

Morán developed neutral anion binding receptors with urea and amide functions to bind carboxylate anions (Figure I-24). [57]

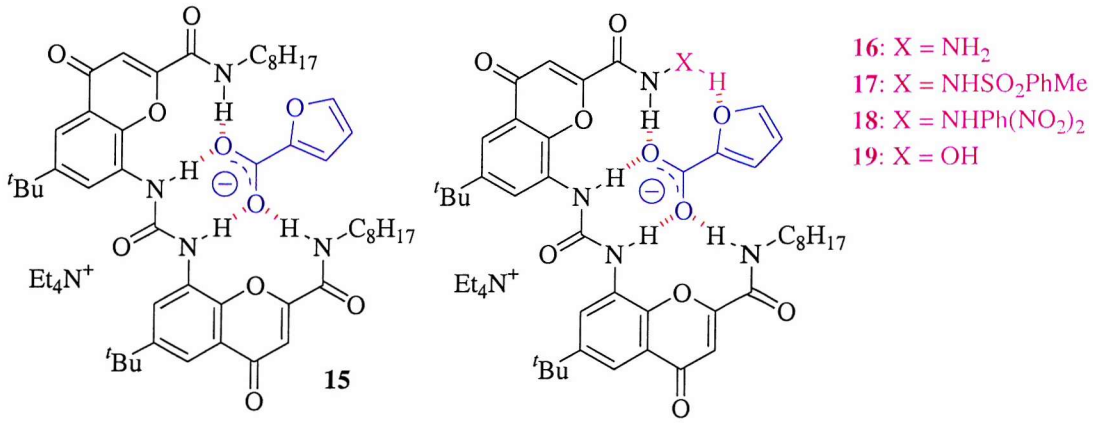


Figure I-24 Morán's abiotic receptor for complexing carboxylates[57]

These receptors presented a bis-chromenyl-urea backbone (Figure I-24 left) and demonstrated a binding pattern that occurred *via* four hydrogen bonds with an association constant $K > 10^4\ M^{-1}$ in DMSO-d_6.

The introduction of a single H-bond within the receptor led to a favourable increase in binding (Figure I-24 right). [57] In order to investigate the mode of binding of the new binding molecules with respect to the original receptor **15** (Figure I-24), competitive titration experiments were carried out on hosts **16-19**. For the study concerning receptors **16** and **15** a value for K_{rel} of 3 was obtained. This low value was due to the unfavourable electrostatic interactions between receptor **16** (non-bonding electron pairs of the nitrogen) and the oxygen atoms of the guest (Figure I-25).

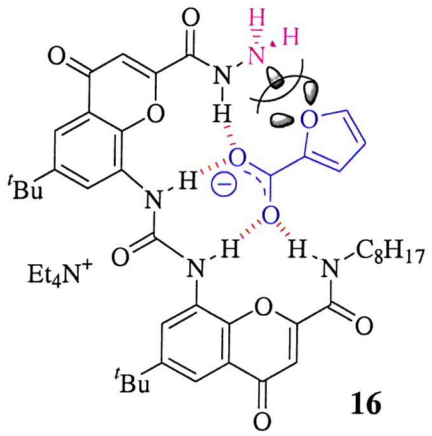
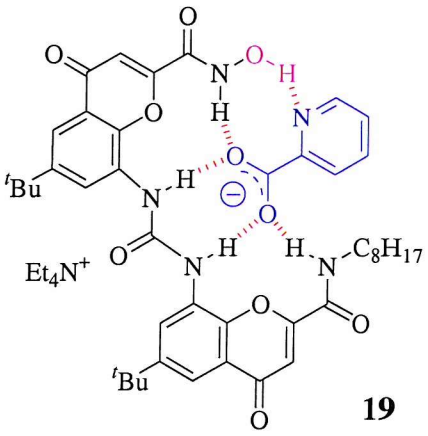


Figure I-25 Conformation of non-substituted acylhydrazides in solution 16

In order to increase the strength of the fifth H-bond the new receptors **17-19** (Figure I-24) were synthesised. Receptors **17** and **18** showed only a slightly improved value for K_{rel} of 4 and 4.7 respectively. A good solution was instead found with receptor **19**, which bore an hydroxamic group rather than a hydrazine function (Figure I-26).



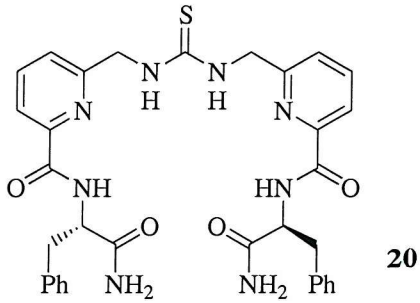
19

Figure I-26 Proposed complex between receptor 19 and the tetraethylammonium salt of fusaric acid by five hydrogen bonds

A 36-fold improvement was observed for the association constant of **19** with respect to **15** using the tetraethylammonium salt of fusaric acid as the guest. This result confirmed that an increase in association could be promoted by introducing an additional hydrogen bond.

Recently Kilburn described the synthesis of a pyridyl thiourea receptor and its enantioselective binding of *N*-protected amino acids. [58,38,59,60]

Receptor **20** (Figure I-27) was titrated with a range of amino acid carboxylates (TBA salts) and exhibited some selectivity, particularly for amino acids with electron rich aromatic side chains such as *N*-Ac-D-Trp ($K = 1.5 \times 10^4\ M^{-1}$) in chloroform.

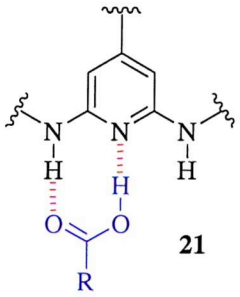


20

Figure I-27 Kilburn's receptor containing a thiourea

I.6.2 Receptors for Carboxylic Acids

Carboxylic acids can be bound using a different binding pattern. Amidopyridines provide an excellent structural motif for binding carboxylic acids. Complex **21** (Figure I-28) shows the ability of the amidopyridine moiety to form two complementary hydrogen bonds from the carboxylic acid hydrogen to the pyridine nitrogen and the carboxylic acid carbonyl to the amide hydrogen.

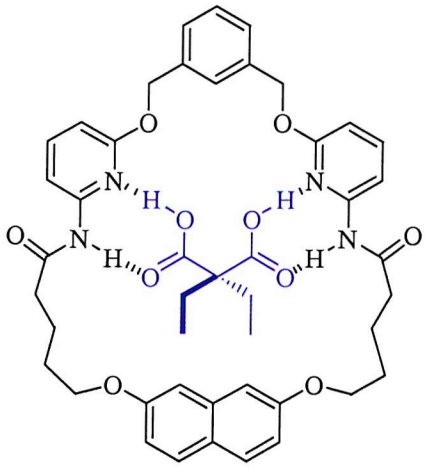


21

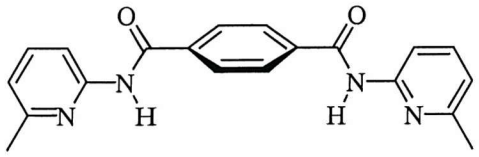
Figure I-28 2,6 diamidopyridine carboxylic acid binding site (CBS)

Amidopyridines are generally only effective in relatively non-polar solvents. Unfavourable secondary interactions, particularly between the relatively electropositive carboxylic acid and amide protons make the amidopyridines a less potent binding site for carboxylic acids than ureas and thioureas are for carboxylates. [38]

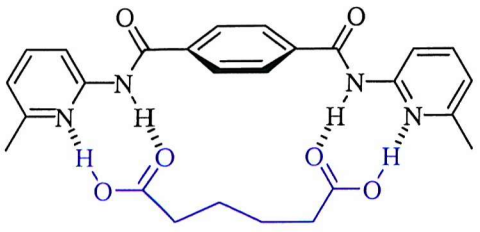
Hamilton used two amidopyridine units in macrocycle **22** to bind diethylmalonic acid using four hydrogen bonds (Figure I-29). [61]



complex 22



receptor 23



complex 24

Figure I-29 Hamilton's diacid receptors 22 and 23

Titration of **22** in chloroform with diethylmalonic acid gave large downfield shifts of the amide protons which is consistent with a tetra-hydrogen-bonded complex.

The association constant for macrocycle **22** with diethylmalonic acid was $K = 1.1 \times 10^3$ M^{-1} and with ethylmalonic acid 7.2×10^3 M^{-1}. The relatively low association constant for macrocycle **22** reflects the unfavourable planar conformation required for binding into the macrocycle cavity. A wider separation of the two hydrogen bonding regions minimises carboxylic acid–carboxylic acid interactions and changes the binding specificity to favour longer dicarboxylic acid substrates. Addition of solid adipic acid to a solution in chloroform of receptor **23** led to a rapid dissolution of the normally insoluble substrate. ^1H NMR integration established a 1:1 stoichiometry for the hydrogen-bonded complex **24** ($K > 10^5$ M^{-1}).

An X-ray analysis of complex **24** confirmed the proposed solution structure and showed that no proton transfer to the carboxylate-pyridinium ion pair occurs.

The cis-trans isomerization (Figure I-30) of acylprolyl groups is a kinetically significant step in protein folding. In most acylprolines the s-trans rotamer is favoured over the s-cis due to the steric demands of the acyl group.

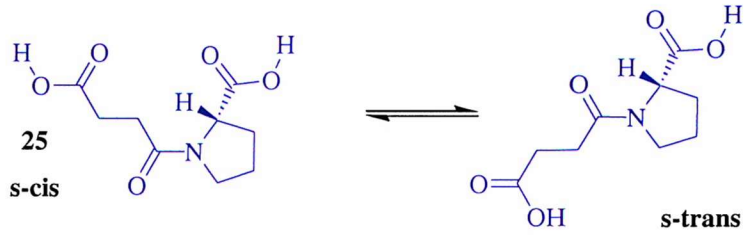


Figure I-30 s-cis and s-trans rotamer

Following the successful results obtained with receptor **23** (Figure I-29) Hamilton carried out further work on acyclic receptors **26-27** (Figure I-31).

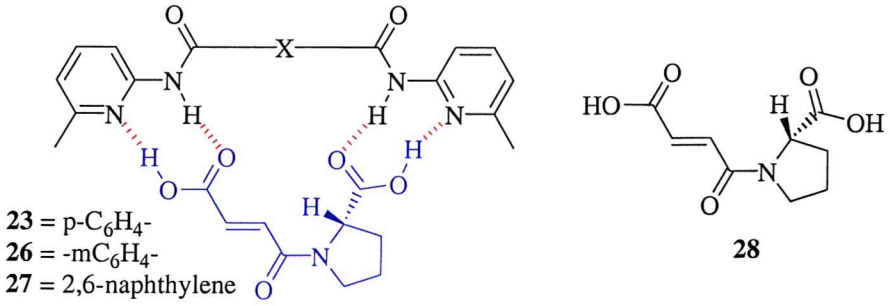


Figure I-31 Hamilton's receptors 25-27

Receptor **23** was shown to stabilise the s-cis rotamer of proline diacid **28** in preference to the s-trans rotamer, whereas the naphthalene derived bisamidopyridine **27** stabilised the s-trans rotamer (Figure I-32). [62]

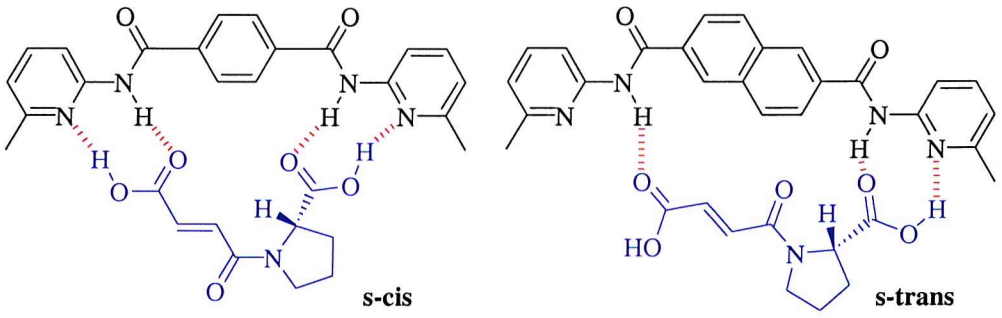


Figure I-32 Hamilton's receptors 23 and 27

Monoamidopyridine derivatives with an additional amide or urea functionality have been shown to bind both carboxylic acid and amide functionalities and therefore are effective receptors for acylated amino acids such as *N*-Ac-Pro-OH. Receptor **29** binds the maleimide acid forming complex **30** (K = 4.8 $\times 10^3$ M^{-1}) illustrated in Figure I-33. [63,47]

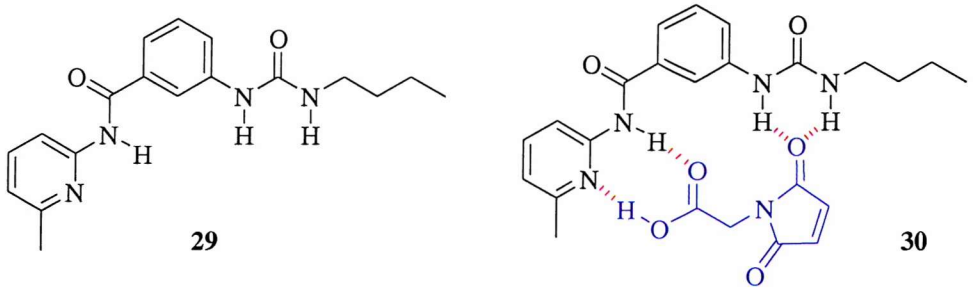


Figure I-33 Hamilton's receptor 29

Helmchen [64] reported the synthesis of chiral hosts for carboxylic acids and described their capacity for discrimination of enantiotopic nuclei. A series of sterically similar but electronically different hosts were prepared in order to understand better the interaction between R^1 and R^2. It was found that when R^1 = Ph or 1-naphthyl, complexation of aromatic carboxylic acid guests (e.g. naproxen, phenylacetic acid and hydratopic acid) led to upfield shifts of the signals for the α-Hs of the guests ($\Delta\delta_H$ = 0.283 ppm for naproxen). Helmchen attributed the higher $\Delta\delta_H$ for R^1 = 1-naphthyl than R^1 = Ph to the larger magnetic anisotropic effect of the naphthyl group. Interaction of hosts **31** and **32** (Figure I-34) with hydratropic acid and α-cyclohexylpropionic acid (R^2 = cyclohexyl) assessed the role of the aryl group for the $\Delta\delta_H$ of diastereotopic nuclei. To both hosts, hydratropic acid was bound stronger than α-cyclohexylpropionic acid; this confirmed the importance of the aryl group. Receptor **31** was also moderately enantioselective, binding the (*S*)-enantiomer of hydratropic acid (K = 1.1 × 10^3 M^{-1}) with a stronger association constant than for the (*R*) enantiomer (K = 700 M^{-1}). Receptor **32** instead produced the opposite result for (*R*)- and (*S*)- hydratropic acid. The (*R*)-enantiomer showed a stronger association (K = 1.12 × 10^3 M^{-1}) than the (*S*)-enantiomer (K = 860 M^{-1}) (Figure I-34).

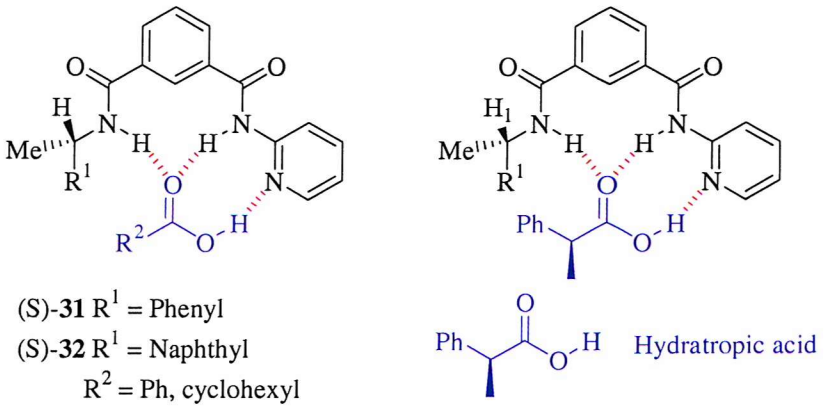


Figure I-34 Helmchen's receptors for carboxylic acids

Morán and co-workers [65] developed a series of receptors capable of chiral discrimination of lactic acid carbamoyl derivatives. These receptors were based on a chromenone-benzoxazole framework.

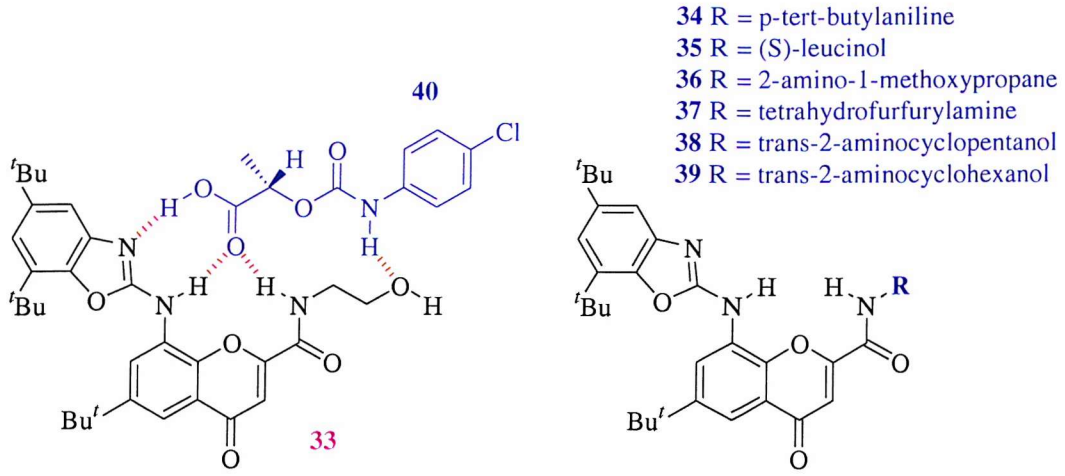


Figure I-35 Morán's receptors 33-39

The proposed structure for a complex of carbamoyllactic acid **40** and a generic structure **33** substituted with an aminoethanol unit are illustrated in Figure I-35. These receptors **35-39** (Figure I-35) display K_{rel} (defined as K_{SS}/ K_{RS}) values of between 1.2 and 9.0, depending on the aminoethanol employed (Table I-2).

receptor	K_{rel}	K_{SS} (M^{-1})	K_{RS} (M^{-1})
35	5.2	1.2×10^5	2.3×10^4
36	4.3	1.1×10^5	2.5×10^4
37	1.2	3.4×10^5	2.8×10^4
38	1.8	3.6×10^5	2.0×10^4
39	9.0	3.0×10^5	3.3×10^4

Table I-2 K_{rel} and K_{ass} between Receptors and Lactic Derivative 40

Diederich synthesised helicopodand **41** (Figure I-36) and incorporated two pyridinecarboxamides as the dicarboxylic acid binding site. [66]

Receptor **41** formed stable 1:1 complexes with α,ω–dicarboxylic acids and a diastereoselectivity in complexation of $\Delta\Delta G$ = 1.4 kcal mol^{-1} was measured for substrates **42** and **43** which differ only in the *E/Z* configuration at their double bond.

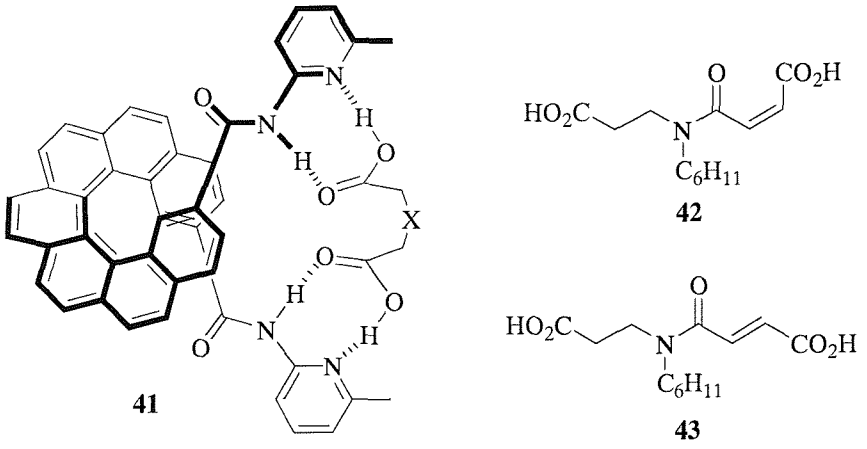


Figure I-36 Diederich's helicopodand receptor 41 for diacids

Molecular modelling suggested that only the *E* derivative possessed the correct geometry for a ditopic four-fold hydrogen bonding interaction between its two carboxylic acid residues and the two CONH(py) groups in **41** (Figure I-36).

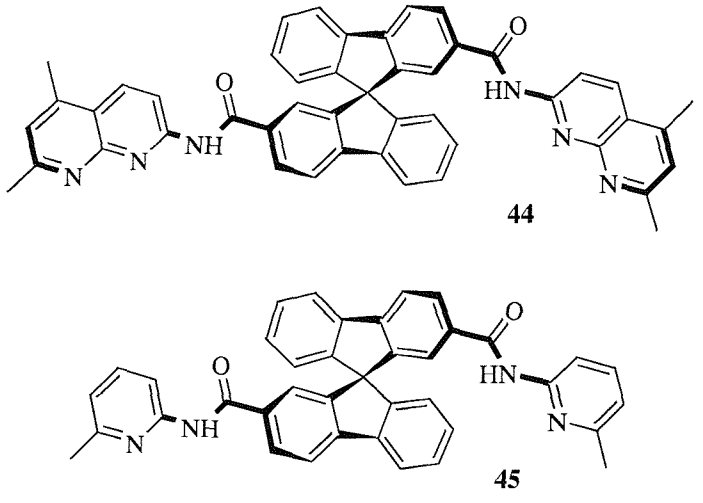
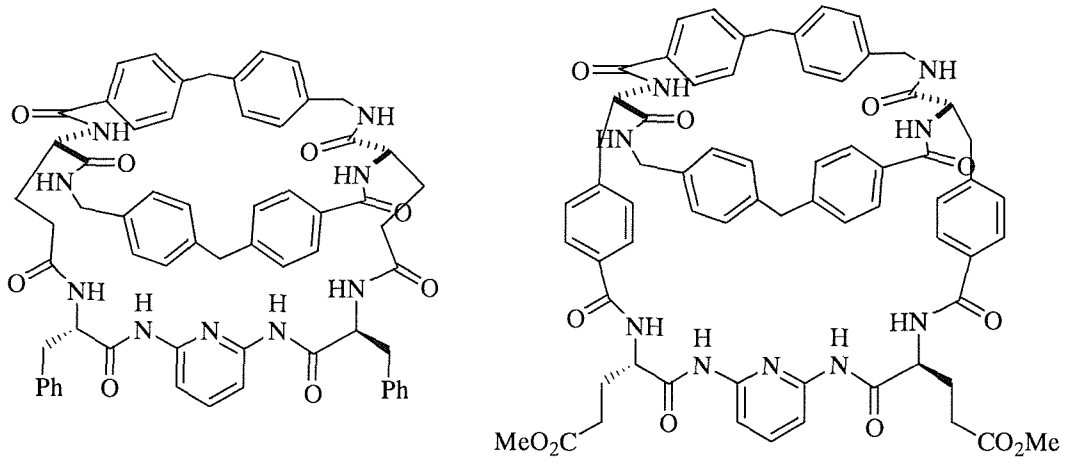


Figure I-37 Diederich's spirobi[fluorene] based receptors 44 and 45

Diederich also prepared optically active molecular cleft **44** and **45**, which incorporated a spirobi[fluorene] spacer and two naphthyridinecarboxamide moieties as H-bonding sites. [67]

Solution binding studies in chloroform showed **44** was able to complex optically active dicarboxylic acids with a diastereoselectivity in complexation ? ? G of between 0.5 and 1.6 kcal mol^{-1}. By covalently binding a closely related structure to silica gel a chiral

stationary phase was generated. HPLC separations of racemic diacids in different solvents suggested that the attractive interactions between solute and immobilised chiral selector are a combination of hydrogen bonding, which prevails in apolar eluents and aromatic p-p stacking, which dominates in polar eluents. Changing the hydrogen bonding sites from naphthyridinecarboxamide in **44** to pyridinecarboxamide in **45** did not significantly alter the free enthalpy and enantioselectivity of complexation. This initially surprising observation was rationalised by two compensating effects. Naphthyridine N-atoms are weaker H-bond acceptors than pyridine N-atoms as the pK_a value is 1.84 lower for naphthyridine. On the other hand binding to **44** should be strengthened as a result of a more favourable DAA/AD H-bonding pattern (as opposed to DA/AD in **45**), which should enable the formation of a bifurcated H-bond between the naphthyridine donor site and the COOH protons (Figure I-37).



46 **47**

Figure I-38 Kilburn's diamidopyridine receptors for peptides 46 and 47.

In the last decade Kilburn has synthesised a series of complex macrocyclic receptors incorporating the 2,6 diaminopyridine unit. [68-71]

Receptors **46** and **47** (Figure I-38) were found to be sequence selective receptors for *N*-protected dipeptides with a free carboxylic acid terminus in non-competitive media (chloroform). Receptor **47** showed the most significant selectivity which bound Cbz-L-Ala-L-Ala-OH ($K = 3.3 \times 10^4\ M^{-1}$) with ~ 8:1 selectivity over its D-enantiomer ($K = 4.5 \times 10^3\ M^{-1}$).

I.7 Molecular Tweezers

Molecular tweezers are an important class of receptors. Synthetic receptors with molecular pockets or cavities can be used as models for complicated biological systems, for example, for protein folding or recognition of substrates by enzymes. The structure of these receptors generally consists of a head group or hinge which is typically a conformationally restricted moiety that directs two functionalised substrate-binding arms. [72,73]

Klärner and co-workers [74,75,76] described the synthesis and properties of two peculiar molecular tweezers **48** and **49** (Figure I-39).

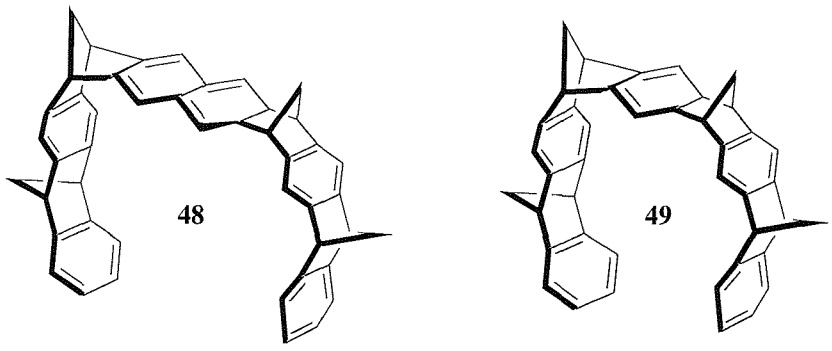


Figure I-39 Klärner's molecular tweezers 48 and 49

The naphthalene tweezer **48** and the benzene tweezer **49** selectively bind electron-deficient aromatic and aliphatic substrates as well as organic cations. Single-crystal structure analyses suggested that the naphthalene-spaced receptor **48** presents the ideal geometrical topology for the complexation of benzene derivatives, whereas the benzene-spaced receptor **49** requires an expansion of the tweezer's tips. These investigations confirmed that the geometric topology as well as electronic structure is important for the binding properties of the receptor molecules.

Recently Hayes [77] reported the synthesis of a new macroporous stationary phase bearing tweezer receptors that are specific for cholesterol (Figure I-40). The novel tweezer monomer bearing cholesterol receptor arms was co-polymerised to afford a diverse set of macroporous materials. The selectivity and efficacy for cholesterol binding was determined by a chromatographic screening process. It was found that the polymer

composition was selective in binding cholesterol over unrelated steroidal structures and it was thus used to construct monolithic solid phase extraction cartridges.

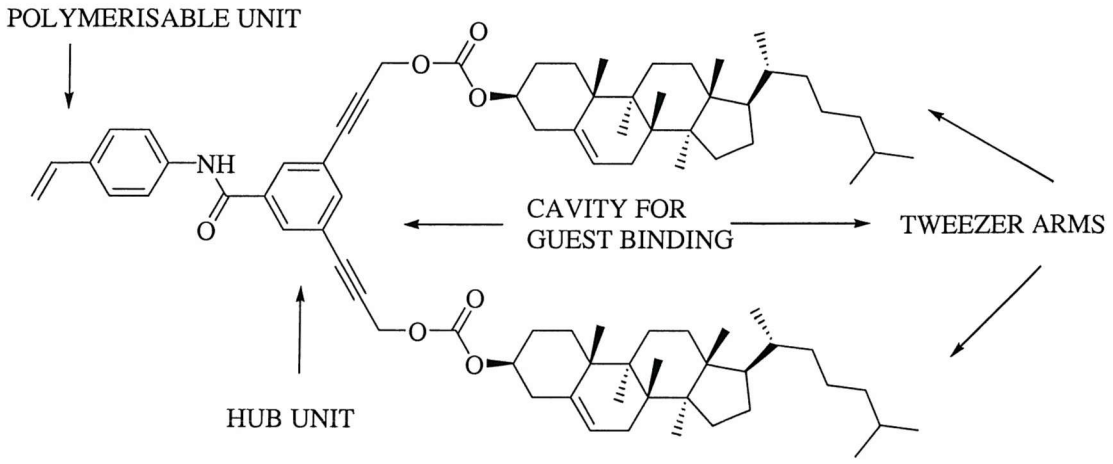


Figure I-40 The cholesterol-based molecular tweezer system [77]

Tweezer receptors are also an important class of receptors for small peptides and simple amino acids. The basic design of tweezer receptors for peptides incorporates a 'head' group bearing two side arms that incorporate appropriate functionality for binding the backbone of a suitable peptide substrate (Figure I-41).

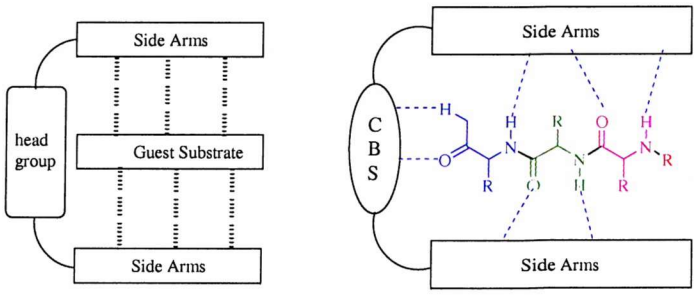


Figure I-41 Design of tweezer receptors

Incorporation of a 'head group' or anchor point, in addition to the binding interactions from the tweezer side arms, increases the binding affinity of such tweezer receptors. It was anticipated that the peptidic arms might form parallel β-sheet-like interactions with the backbone of the peptidic guest. [78] Despite their conformational flexibility, such structures are capable of selective peptide recognition, both in organic and aqueous

solvents. The advantage of using such tweezer structures as receptors for peptides is that they allow the use of a combinatorial strategy for the generation of a large number of compounds.

I.8 Peptide Receptors

The most ubiquitous mode for controlling and modulating cellular function, intercellular communication, immune response and information-trasduction pathways is through peptide-protein non-covalent interactions. The discovery of small molecules that regulate protein-protein interactions is of great structural interest and practical importance. The development of artificial receptors that recognise small peptides is one of the central challenges of supramolecular chemistry. The many degrees of freedom of a simple di- or tripeptide make the rational design of a sequence-selective receptor an extremely difficult task. [40]

Still [79,80] described the synthesis and binding properties of a family of receptors that bind peptides sequence selectively in chloroform (Figure I-42, **50**) and in water (Figure I-42, **51**). Receptor **50** is based on polycyclic oligomers of trimesic acid ($\mathbf{A}(OH)_3$) and 1,2-diamines (e.g. $\mathbf{B}H_2$). The D_2-symmetric receptor **50**, incorporates a large, hydrophobic binding cavity and shows selectivity in binding certain amino acid sequences containing L-Val. Receptor **51** includes the hydrophilic ammonium groups and is a water-soluble analogue of **50**.

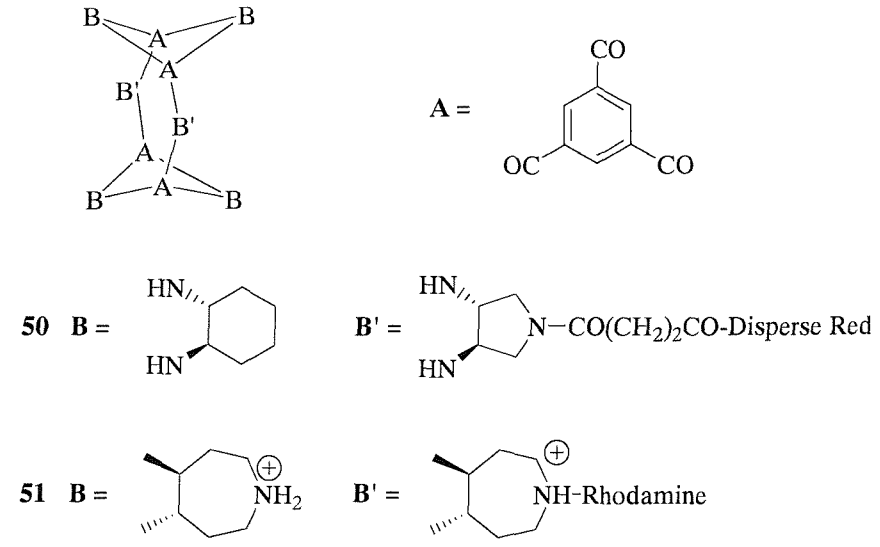


Figure I-42 Still's polycyclic receptors 50 and 51

In order to assay the binding properties of receptor **51** in water, libraries of tripeptides (side chain protected and deprotected) were prepared on tentagel resin. The receptor-library binding assay was conducted at pH 4 in water. It was found that with the protected tripeptide library receptor **51** binds with high selectivity several dipeptide sequences that were very similar to those preferentially bound by receptor **50** in chloroform. Both receptors bound hydrophobic amino acids of similar size as Leu or Val adjacent to amide-substituted amino acid having the opposite chirality (Gln, Asn). With the deprotected tripeptide library the sequences bound by **51** in water were different from those bound by **50** in chloroform. These results demonstrate that highly sequence-selective synthetic receptors for peptides in water can be constructed from information obtained from receptors developed for binding in organic solvents.

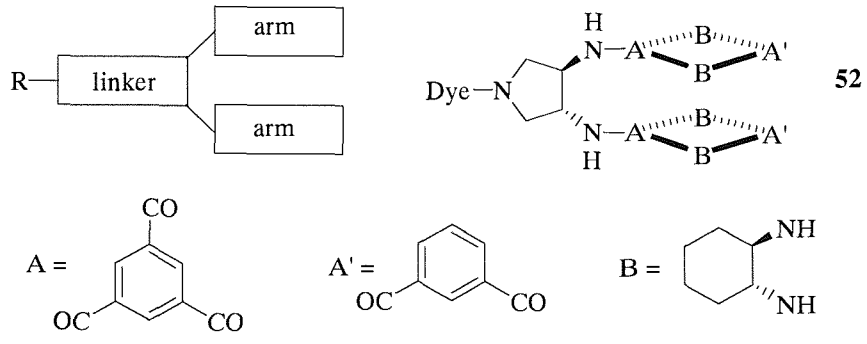


Figure I-43 Still's simple synthetic receptor for peptides 52

Following the success of the synthesis of complex, cage-like structures (Figure I-42), Still [81] described simpler receptors that also had highly sequence-selective peptide-binding properties (Figure I-43). As mentioned earlier one class of receptor structures described as 'two-armed receptors' consists of a core or linker, usually a conformationally restricted moiety, which covalently bonds and directs two functionalised arms. Still described a two-armed receptor **52** (Figure I-43) where the arms were consisting of macrocyclic oligomers of isophthalic acids (A,A`) and trans-1,2-diaminocyclohexane (B). It was found that tweezer **52** (Figure I-43) binds only two sequences between 3375 members of a peptide library in chloroform: D-Pro-L-Val-D-Gln and L-Lys-L-Val-D-Pro.

Several tweezer receptors that bind peptides sequence-selectively in aqueous solution have been synthesised.

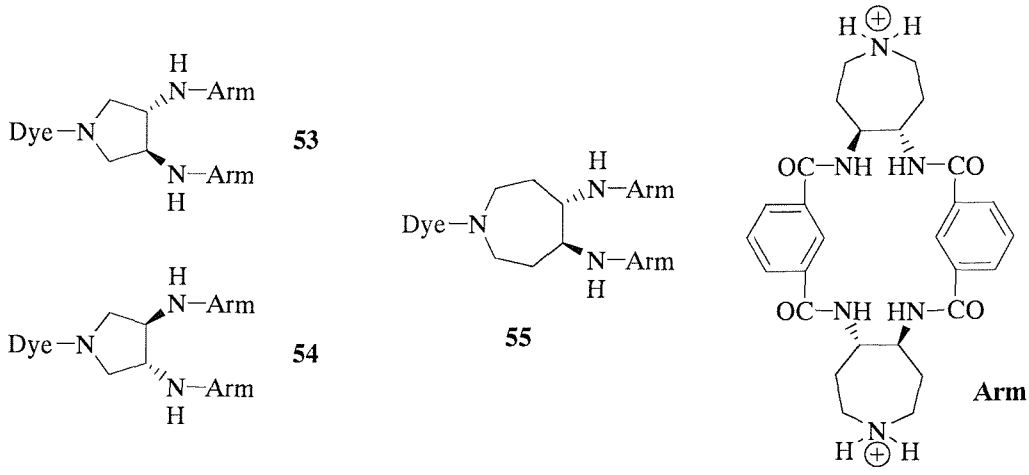


Figure I-44 Still's synthetic receptors that bind peptides in water

In receptors **53-55** the hydrophobic unit B (Figure I-43) was substituted by the hydrophilic azepinoid unit (Figure I-44). With a side chain protected tripeptide library these receptors showed high preference for binding peptides having carboxamide-bearing amino acids (frequently L-Gln) followed by D-Leu. An interesting point was that these preferred sequences were related to the L-Val-D-Gln dipeptide sequence found to bind to **52** (Figure I-43) in organic solvents. Still proved that two-armed receptors can bind peptides in water with significant sequence-selectivity. Furthermore

the selectivities observed were closely related to those found for similar receptors in organic solvents.

Hossain and Schneider [82] described receptors that incorporate functionalities to complement both side chains and the termini of an extented peptide. The receptors presented recognition sites for the zwitterionic form of unprotected peptides. The 18-crown-6 unit could associate with the N-terminus of a tripeptide and a peralkylammonium group could associate with the C-terminus (Figure I-45). It was found that receptor **57** showed the strongest association for tripeptides where the second amino acid contained an aromatic side chain to interact with the dansyl group. NMR titration in water of receptor **57** with the tripeptides H_2N-Phe-Gly-Gly-OH (K = 220 M^{-1}), and H_2N-Gly-Gly-Phe-OH (K = 215 M^{-1}) gave binding constants similar to the base tripeptide H_2N-Gly-Gly-Gly-OH (K = 210 M^{-1}). While tripeptides H_2N-Gly-Phe-Gly-OH (K = 1.7 × 10^3 M^{-1}) and H_2N-Gly-Trp-Gly-OH (K = 2.2 × 10^3 M^{-1}) bound more strongly. The key feature of this work was the recognition, in one system, of multiple functionalities.

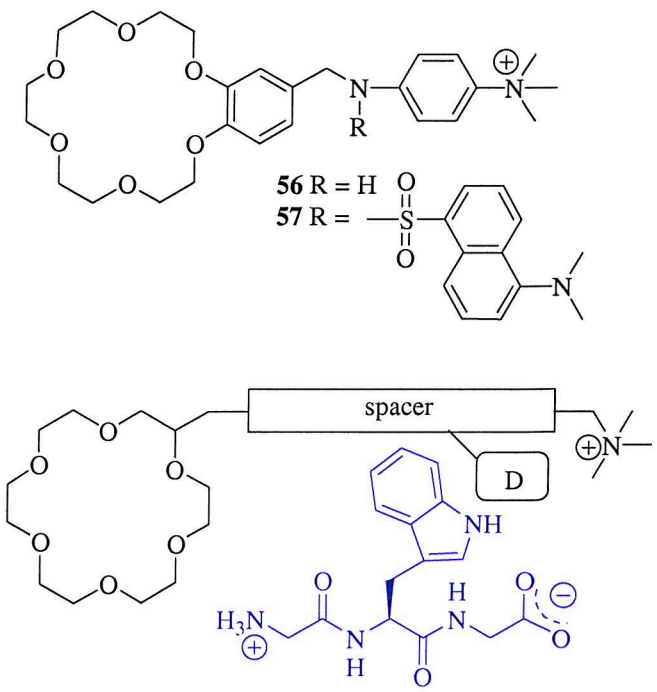


Figure I-45 Schneider's receptors 56 and 57. The dansyl functionalised receptor 57 and the unprotected tripeptide Gly-Trp-Gly

Hamilton [83] was interested in the design of small molecules capable of influencing protein-protein interactions by complexing with protein surfaces. It was expected that short peptides would have a substantial degree of conformational flexibility in solution and that would result in a heterogeneous mixture of target conformers. However if the target adopted an α-helical conformation, the carboxylates could assume positions that allowed recognition.

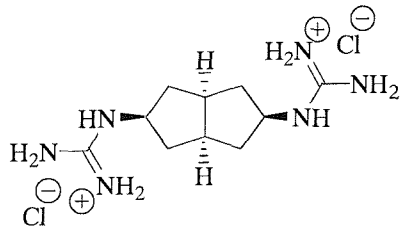
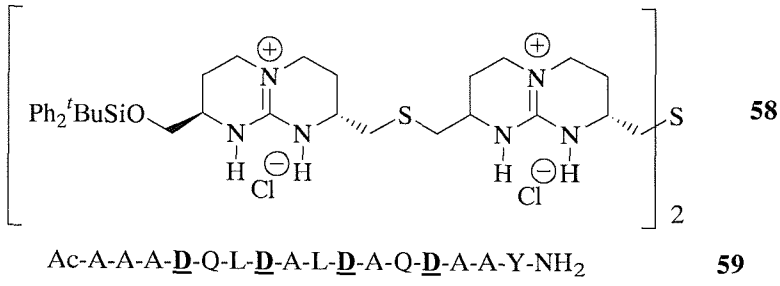


Figure I-46 Hamilton's receptor for recognition of aspartate pairs in an α–helical conformation [83]

Hamilton [83] targeted aspartate residues separated by a variable number of alanine or glutamine residues. The receptors were based on two guanidinium groups separated by a rigid bicyclo[3.3.0]octane spacer (Figure I-46). The results obtained suggested that the bicyclo-octane spacer serves as a rigid scaffold that preorganizes the guanidinium groups in a complementary manner to a specific aspartate arrangement in the peptide strand.

Further work conducted in collaboration with de Mendoza [84,85] described the synthesis of tetraguanidinium-based receptor **58**. It was found to bind strongly to a peptide with four aspartate residues **59** (K ~ 10^5 M^{-1}) and to stabilise it in an α-helical conformation (Figure I-47).

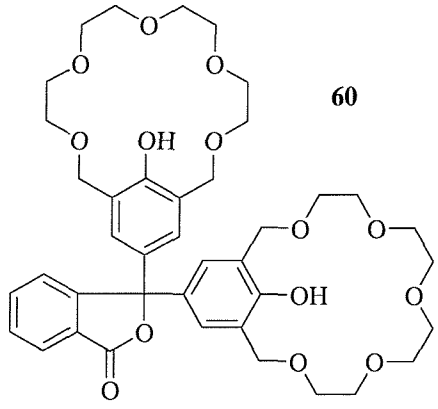


58

Ac-A-A-A-**D**-Q-L-**D**-A-L-**D**-A-Q-**D**-A-A-Y-NH_2 **59**

Figure I-47 Hamilton and de Mendoza peptide receptors 58 and 59

Tsubaki [86] reported the synthesis of receptor **60** (Figure I-48) that recognise the chain lengths of α,? -diamines and linear triamines and reveal binding through the use of colour. Receptor **60** consisted of phenolphtalein, commonly used as pH indicator, and two crown ether moieties. The recognition was based on the distance between an *N*-terminal amino group and the basic functional group in the side chains of peptides. The recognition, translated in colour changes occurred only if the appropriate distance existed between the two amino groups. Therefore receptor **60** bound dipeptide **61** while no colour changes were observed with the reversed sequence **62**. They also devised a system that could be used for the visual recognition of proteins with a (Gly, Ala, Ser, Thr, Arg, or Lys)-Lys sequence at their N-terminus.



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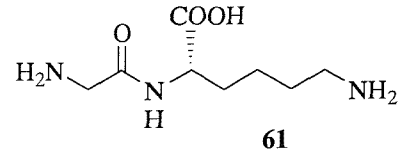

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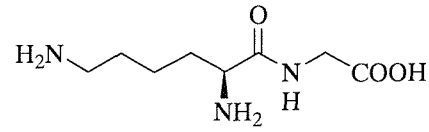


62

Figure I-48 Tsubaki's sequence-selective visual recognition

I.9 Combinatorial Chemistry

Combinatorial chemistry is a relatively young but intensely popular branch of science. In the last two decades combinatorial chemistry has radically changed the theory and practice of designing and preparing new substances in pharmaceutical research and other areas. [87,88]

It allows the simultaneous synthesis and rapid testing for a desired property of large numbers of chemically related compounds (libraries). The fundamental idea for the synthesis of libraries is to achieve diversity rather than purity.

Combinatorial chemistry has its earliest origins in solid phase peptide synthesis [89] however combinatorial processes have since expanded to other areas of chemistry including catalysis and materials science.

I.9.1 Split and Mix Strategy

One of the first and most revolutionary combinatorial synthetic methods was the 'portioning-mixing' technique introduced by Furka and co-workers. [90,91] The utility of this strategy has been further investigated by Houghten [92] and Lam [93] in their respective 'divide-couple-recombine' and 'split and mix' processes. The split and mix process has been exemplified predominantly for the synthesis of the library compounds on small resin beads. The procedure consists of dividing the resin into equal portions with subsequent individual reaction of each of these portions with a different monomeric starting material. Thus, the synthesis of a library of n^x components could be generated by repeating the protocol for a total of x cycles with n monomers for each cycle. The split and mix process for the synthesis of a library of 27 trimer combinations is illustrated in Figure I-49.

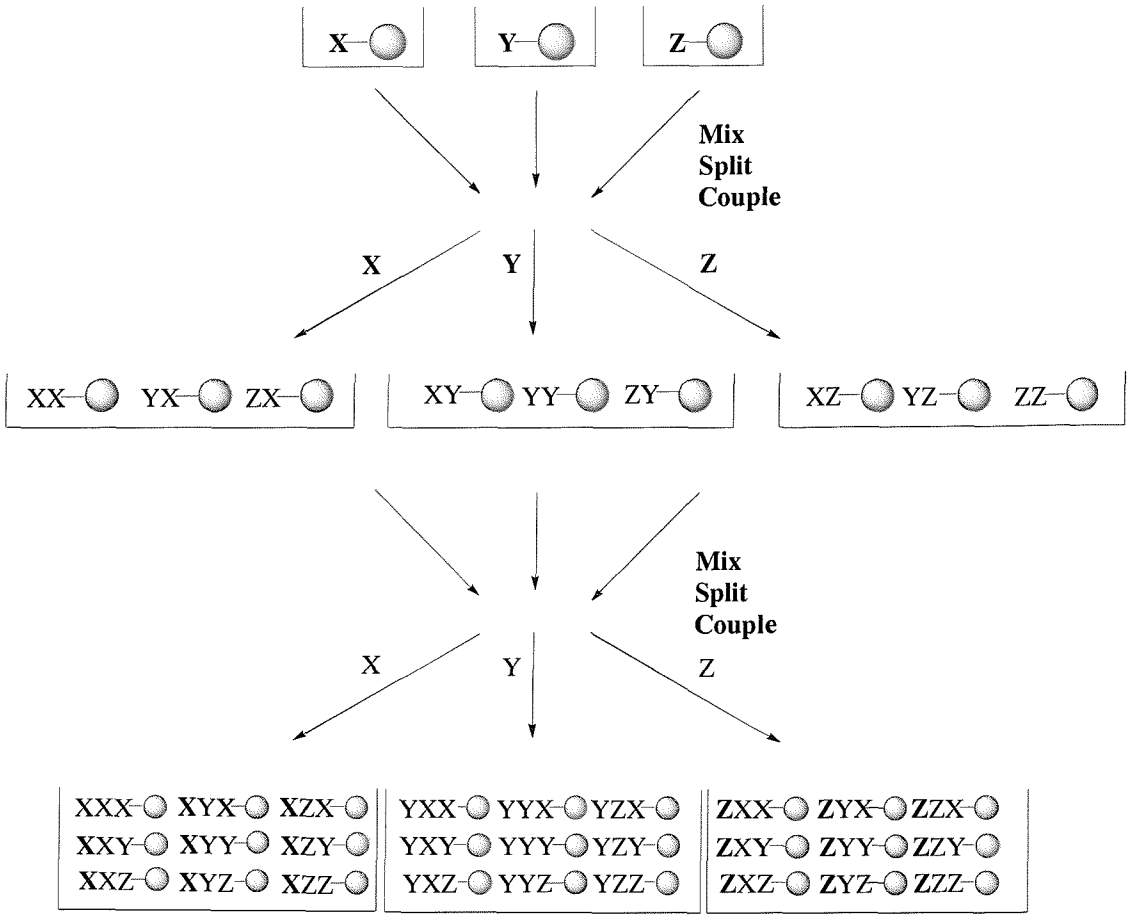


Figure I-49 The split and mix synthetic scheme for the synthesis of a 27 member library

An important observation is that the products synthesised do not necessarily need to be linear. Suitable templates with different functional groups allow the production of combinatorial libraries of branched products using a variety of solid phase chemistries (Figure I-50) [94].

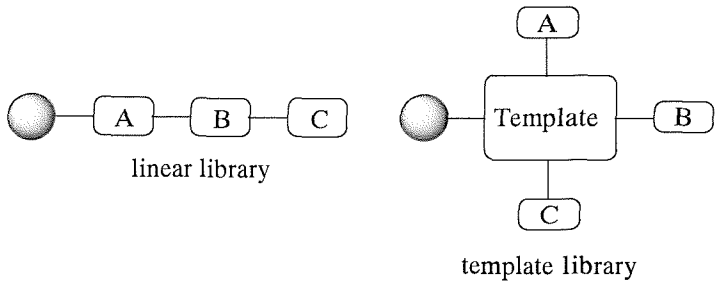


Figure I-50 Linear combinatorial library and branched products

It is important to realise that each bead of a library carries one compound only (the "one bead one compound" principle). The 'one-bead-one-compound' concept was first recognised by Lam. [93] It is based on the fact that combinatorial bead libraries prepared *via* a 'split synthesis' approach contain single beads displaying only one type of compound although there may be several copies of the same compound. [95]

I.9.2 Screening

All combinatorial library methods involve three main steps: 1) preparation of the library, 2) screening of the library components and, 3) determination of the chemical structures of active compounds. The screening process involves the identification of chemical structures of molecules isolated from just one resin bead. A number of different techniques exist for this purpose: bead tagging [96,97] (a technique that labels each individual library member with a unique array of readily analysable molecules called tags), deconvolution [98-100] (the process of synthesising and screening a series of sub-libraries which are constructed in such a way that the active components of the parent library can be identified) and multiple release systems [101,102] (compounds are attached to the solid support *via* different linkers which can be cleaved individually). As with oligonucleotides, [103] methods exist for the rapid sequencing of peptides. It was demonstrated that peptide libraries prepared from naturally occurring amino acids could be encoded by Edman degradation. [104]

Repetition of the Edman-degradation cycle allows the stepwise identification of all amino residues of the peptide.

I.10 Combinatorial Approach in the Synthesis of Libraries of Receptors

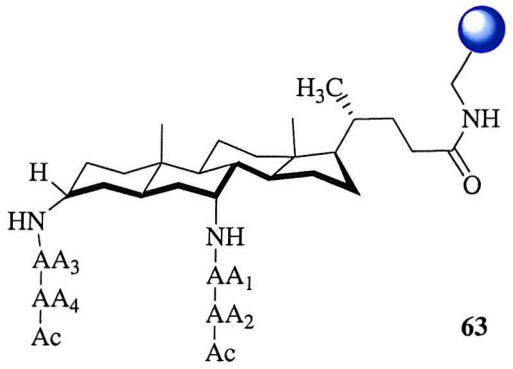
In the 1990s, an alternative to the iterative approach to the identification of peptide receptors was pioneered by Still and co-workers. Mirroring developments in medicinal chemistry, the combinatorial approach was used to prepare large libraries of possible receptor structures, which could then be screened to identify a receptor for a given substrate.

Still [105,106] used a steroidal core to attach two variable peptide strands to produce libraries which were then screened to identify receptors for enkephalin-like peptides. Since these original publications by Still there have been a few reports of the approach being used by other research groups. However the reverse process — i.e. screening a single receptor with a library of peptide guests to identify possible substrates for the receptor – has been used quite extensively.

In the original work [107] Still described a receptor library based on a A,B-cis-cholic acid core with two variable tripeptidic arms

Further investigations produced a receptor library **63** designed around an A,B-trans-steroidal core with the dipeptidic arms substituted at the C_3 and C_7 axial positions (Figure I-51). This new receptor was less flexible than the previous peptidosteroid built around an A,B-cis-steroidal diol core. The use of encoded split bead synthesis allowed the generation of a library containing 10^4 variants (Figure I-51). [108]

Screening experiment showed that only a small fraction (0.1 %) of this library binds the pentapeptide Leu enkephalin(Figure I-51). Upon collecting 37 of the active beads and reading their tags only eight different receptor library members were found. Remarkably all 37 beads had one peptidic arm AA_1-AA_3 = (L)Asn(N-trityl), (D)Asn(N-trityl) and (D)Phe. These results supported the theory that receptor libraries are a source of highly selective receptor molecules. It was also proved that decreased receptor flexibility result in increased receptor selectivity.



63

Leu enkephalin: dye-CO(CH_2)$_2$CO-L-Tyr-Gly-Gly-L-Phe-L-Leu-OMe

Figure I-51 Still's library of peptide receptors based on A,B-trans-steroidal core

The latter strategy has been applied particularly to the identification of peptide substrates for acyclic 'tweezer' receptors or 'two-armed' receptors. The advantage of such structures is that they are relatively easy to synthesise (in comparison with more conformationally restricted macrocyclic structures) and the work of several groups has demonstrated that, despite their conformational flexibility, such structures are capable of peptide recognition, both in organic and aqueous solvents.

For example, Liskamp [109] synthesised peptidosulponamide tweezer receptor **64** and **65** (Figure I-52).

Peptides are attractive targets for drug discovery becase of their affinities and specificities toward biological receptors. However, the poor stability and bioavailability of peptides *in vivo* have generally limited their therapeutic application. One approach to overcome this obstacle has been for example the development of non-natural biopolymer scaffolds. The peptidosulfonamide peptidomimetics [110] are more resistant to degradation by proteases, more flexible than the amide bond, and the more acidic hydrogen may give rise to stronger hydrogen bonds.

Liskamp demonstrated that by varying the 'hinge' part of a tweezer-like receptor molecule it was possible to increase the binding affinity while maintaining the selectivity. Receptors **64** and **65** (Figure I-52) were screened for binding against a library of side chain-deprotected tripeptides of 24389 members. Both receptors showed high selectivity for the peptide sequence D-Ala-L-Asp-D-Ser. The binding affinity of the original receptor **64** (K = 320 M^{-1}) was increased more than ten fold compared to receptor **65** (K = 4100 M^{-1}).

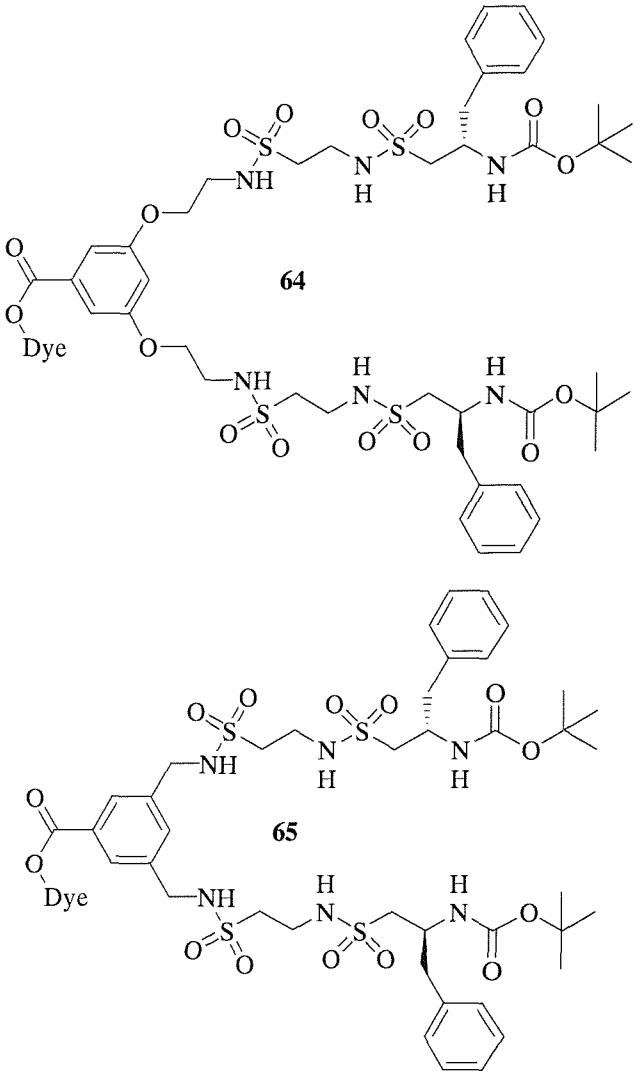


Figure I-52 Liskamp's peptidosulfonamide tweezer receptors 64 and 65

Liskamp [111] has recently reported the synthesis of and screening of libraries against synthetic tripodal receptors (Figure I-53). The receptor was based on a triazacyclophane scaffold and split-and-mix synthesis was used to generate three different peptidic arms. A library of 27000 members was screened and a remarkable selectivity of some library members for Fe^{3+} was observed. An interesting point was that Edman degradation of their structures revealed consensus sequences with structural resemblance to non-heme iron proteins.

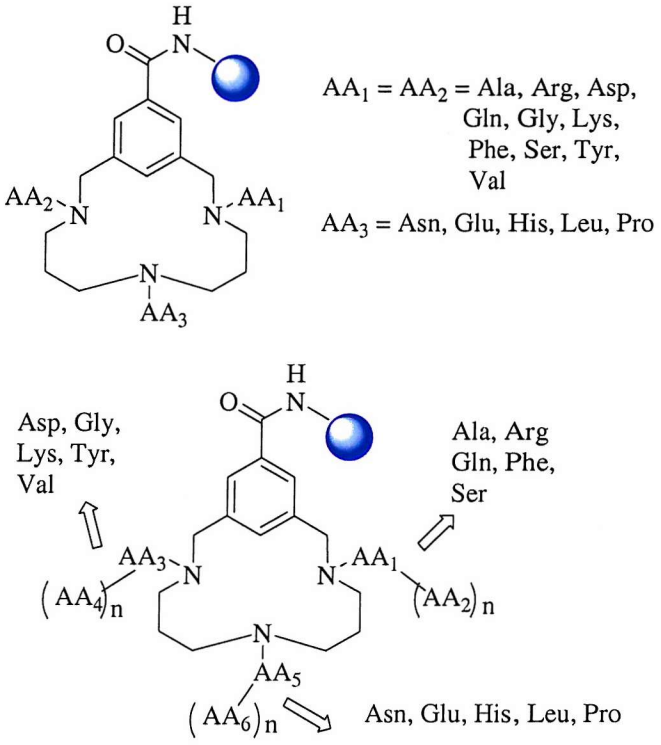


Figure I-53 Liskamp's tripodal receptor: identification of iron binders [111]

Kilburn [112] has synthesised a tweezer receptor **66** for peptides with a guanidinium carboxylate terminus, using guanidinium for the peptide carboxylate as a specific binding site (Figure I-54).

A guanidinium based CBS (carboxylic acid binding site) can bind to the deprotonated carboxylic terminus of peptides via hydrogen bonds as shown in structure **67**. The synthesis of this receptor was carried out using an appropriately functionalised guanidine which was attached to the solid phase. The guanidine unit was linked to the aminomethylpolystyrene resin via an arylsulphonamide. The linker was providing an effective protecting group for the basic guanidine functionality and at the same time would allow the cleavage of the tweezer receptor from the resin with strong acid. The guanidine functionalised resin allowed subsequent coupling of amino acids to give the peptidic arms.

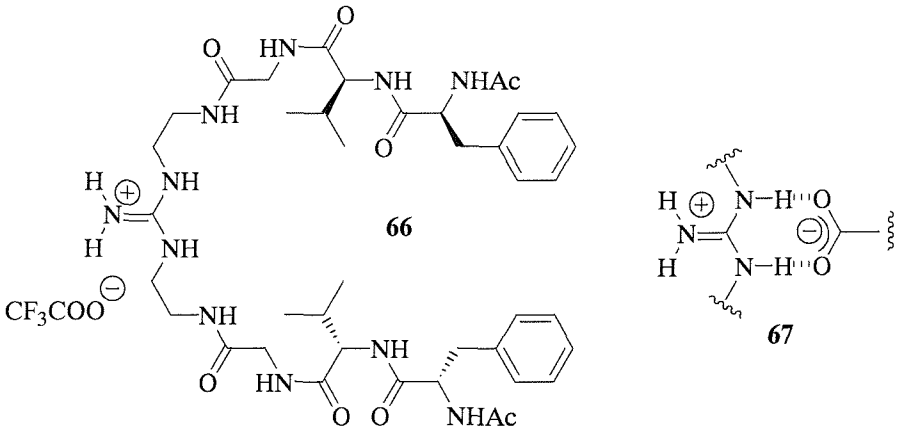


Figure I-54 Kilburn's Guanidinium based tweezer receptor.

Tweezer receptor **66** was modified with dansyl chloride (DNSCl) to give a fluorescently labeled tweezer receptor **68** [113] (Figure I-55). The fluorescently labeled tweezer receptor was screened against a library of peptides attached to Tentagel resin via the amino terminus in a buffered aqueous medium. Such structures can indeed bind selectively to peptides with a carboxy terminus in an aqueous medium, through a combination of carboxylate-guanidinium interactions and hydrophobic and hydrogen-bonding interactions. Tweezer receptor **68** was found to bind to approximately 3% of the library members. Sequencing by Edman degradation of 20 active beads, showed 95% selectivity for valine at the carboxy terminus of the tripeptides and 40% selectivity for Glu(O^{t}Bu) at the amino terminus.

The limited solubility of tweezer receptor **68** in predominantly aqueous solutions has precluded a NMR investigation of the binding properties of the tweezer. However a binding constant for the binding of **68** with Z-L-Glu(O^{t}Bu)-L-Ser(O^{t}Bu)-L-Val-OH was estimated using isothermal titration calorimetry. The data obtained from these experiments could be fitted to a simple 1:1 binding model and gave an apparent association constant of $K = 4 \times 10^5\ M^{-1} \pm 5 \times 10^4\ M^{-1}$.

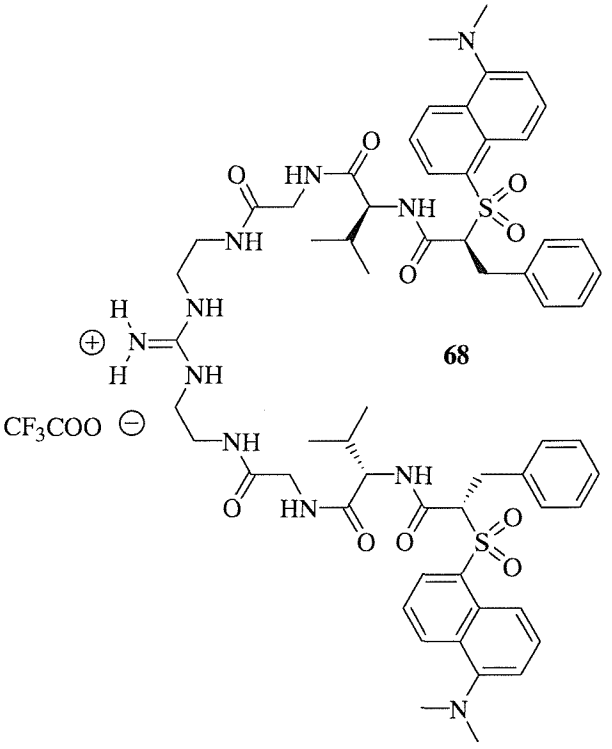


Figure I-55 Fluorescently labeled tweezer receptor 68

Having successfully demonstrated that tweezer **68** (Figure I-55) had the desired property of binding carboxylate-terminating peptides in an essentially aqueous enviroment, it was of interest to determine if the process could be reversed, i.e. whether a solid phase library of tweezers could be screened to identify a selective receptor for a specific peptide sequence.

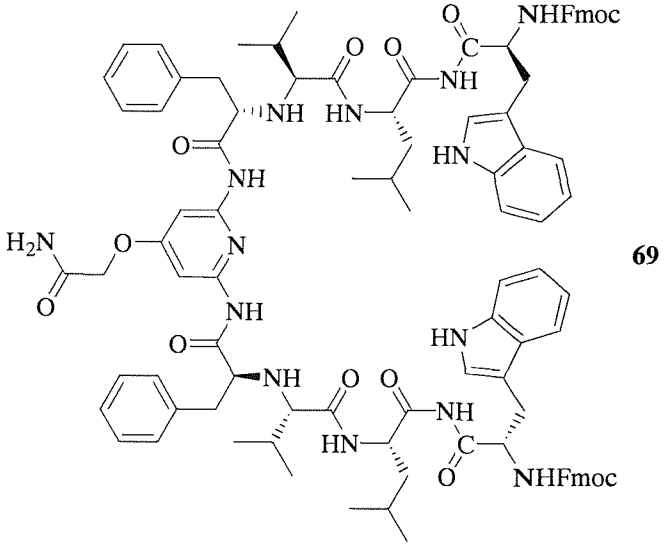


Figure I-56 Kilburn's 2,6 diamidopyridine based tweezer receptor 69

Kilburn [114] described a novel tweezer structure that incorporated a diamidopryridine unit as the head group to provide a binding site for the carboxylic acid terminus of peptide guests (Figure I-56).

A small (2197 members) resin-bound library **69** of tweezer receptors was prepared using the 'split and mix' strategy, and was used to demonstrate the potential of such libraries to identify selective receptors for tripeptide guests with a carboxylic acid terminus. The screening experiments were carried out in chloroform using the tripeptide DNS-L-Glu(O^{t}Bu)-L-Ser(O^{t}Bu)-L-Val-OH as a guest. High fluorescent beads were selected and sequenced by Edman degradation. The results of the sequencing experiments gave as a consensus sequence: Val-Leu-Trp. In order to study its binding properties and establish that the observed binding of DNS-labeled peptide, with the resin bound tweezer, was also operating in free solution, a single tweezer receptor (Figure I-56) with side arm sequence Val-Leu-Trp, was synthesised on solid phase. UV binding studies gave an estimated binding constant of 2.6×10^5 M^{-1}.

Although the experiments with DNS-L-Glu(O^{t}Bu)-L-Ser(O^{t}Bu)-L-Val-OH gave pleasing results with good selectivity, it was of interest to investigate the role of the dye in the overall recognition Further work in the group have described the synthesis and screening of libraries of tweezer receptors featuring a 2,6-diamidopyridine as a carboxylic acid binding site. [115]

Following the success of tweezer receptors library featuring a 2,6 diamindopyridine as a carboxylic binding site it was of challenging interest to extend the concept of screening selective-sequence receptor libraries to libraries capable of peptide recognition in aqueous media. In pursuit of this idea a library of tweezer receptors with an unprotected guanidinium head group was synthesised and screened to identify a receptor for a chosen peptidic guest in aqueous media (Figure I-57). [116]

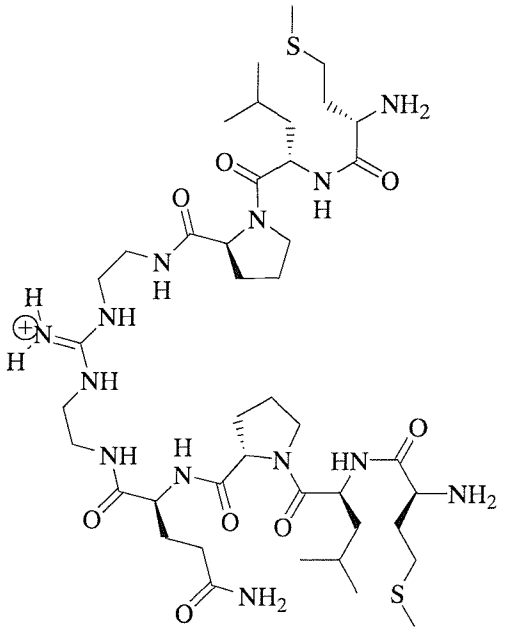
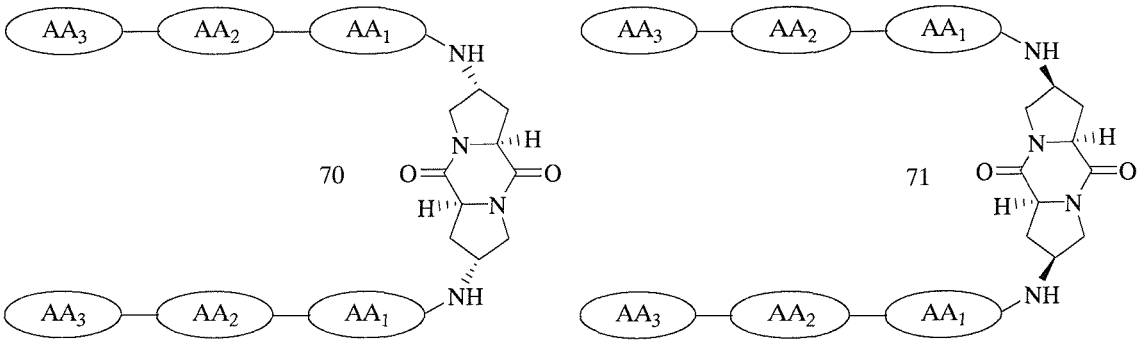


Figure I-57 Kilburn's guanidinum based receptors library

Screening experiments were carried out using an aqueous buffer solution (pH 9.2 borate). At this pH the guanidinuim moiety is still protonated and the peptidic guests exist as deprotonated carboxylates. The library was screened against a range of dye-labeled peptides: Red dye-spacer-L-Glu(O^{t}Bu)-L-Ser(O^{t}Bu)-L-Val-OH, Red dye-spacer-L-Glu-L-Ser-L-Val-OH, Red dye-spacer-D-Glu(O^{t}Bu)-D-Ser(O^{t}Bu)-D-Val-OH and Red dye-spacer- D-Ala-D-Ala-OH (given its well-known biological relevance). [117]

The selectivity of the receptor library (Figure I-57) was good, showing < 1% highly coloured beads. Five of the most intensively stained beads were selected and sequenced by Edman degradation. From the sequencing data a selective tweezer receptor was identified and was resynthesised on solid phase with the peptide arms incorporating the following amino acids: L-Pro-L-Leu-L-Met. Binding studies were carried out with UV tritation (considering the the UV absorption maximum (at 500 nm) of red dye moiety of the peptide guest). The data from this experiment showed a good fit for the presumed 1:1 binding and allowed an estimate of the binding constant, $K = 8.2 \times 10^4\ M^{-1}$.

Wennemers has recently described a family of two-armed receptors **70-76** (Figure I-58). These receptors consist of a central diketopiperazine derived from 4-aminoproline and two peptidic side chains as receptor arms. (Figure I-58). [118,79,119-123]



72 L-Phe-L-Asn(Tr)-L-Tyr(dye)
73 L-Phe-L-Asn(Tr)-D-Tyr(dye)
74 L-Asn(Tr)-L-Phe-D-Tyr(dye)
75 L-Phe-L-Gln(Tr)-L-Tyr(dye)
76 L-Gln(Tr)-L-Phe-D-Tyr(dye)

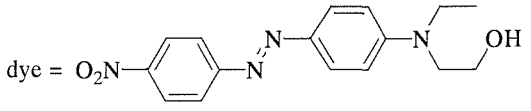


Figure I-58 Wennemer's diketopiperazine receptors 70 and 71

In order to examine the influence of small structural changes in the template on the binding properties of two-armed diketopiperazine receptors, two-armed diketopiperazine molecules based on the diastereomeric di(cis-4-aminoproline)diketopiperazine were synthesised. The binding properties of the two-armed molecules **72-76** (Figure I-58) were examined against a resin-bound tripeptide library with the general structure Ac-AA_3-AA_2-AA_1-$NH(CH_2)_5CONH$-PS (PS = polystyrene resin). The library was prepared following the standard procedure for encoded split-and-mix synthesis, 29 different D- and L- amino acids were employed to generate a library of 29^3 = 29389 components. It was found that the diastereomeric molecules based on the trans,trans-diketopiperazine were highly selective receptors with distinct binding preferences. Conformational analysis revealed significant differences between the two templates. The trans,trans-diketopiperazines showed to be highly preorganised molecules with a defined conformation while the cis,cis- diketopiperazines were found to be rather flexible without defined conformation.

I.11 Dynamic combinatorial chemistry

Dynamic combinatorial chemistry is a recently introduced supramolecular approach that uses self-assembly processes to generate libraries of chemical compounds [124-128]. It allows for the generation of libraries based on the continuous interconversion between the library constituents. The self-assembly of the building blocks occurs through reversible chemical reactions and addition of the target ligand or receptor creates a driving force that favours the formation of the best binding constituents (Figure I-59). [124]

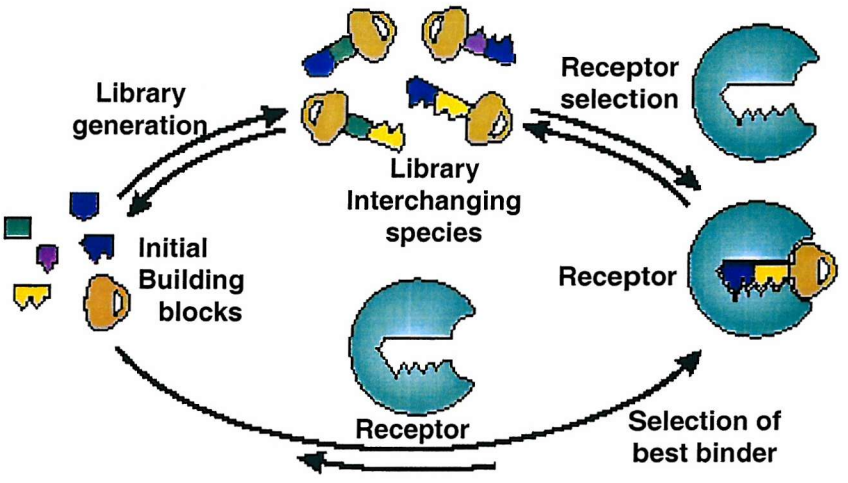


Figure I-59 A schematic representation of the concepts behind dynamic combinatorial chemistry [124]

The advantages of this novel strategy are the number of possible receptor structures that can be assayed, and the relative ease with which a successful receptor structure can be identified.

An alternative strategy for the identification of synthetic receptors, which has been pursued by Sanders and by others, involves incubation of a template (guest) for a receptor in a mixture of components that can reversibly form covalent bonds. Reversible covalent bond formation between components (e.g. alkene metathesis, imine formation, acylhydrazone formation, disulfide formation or transesterification) allows, in principle, all possible combinations of the components to be accessed. The presence of the template should stabilise and subtract from the equilibrium those combinations of

components that provide good receptors for the template. To date all strategies based on thermodynamic sorting have attempted to quench the process to prevent further equilibration. The generation of dynamic libraries can in essence, be accomplished using any type of reversible physical or chemical mechanism. They can make use of several reversible connections of either covalent or non-covalent character. The building blocks must have functional groups that can undergo reversible exchange (Figure I-60).

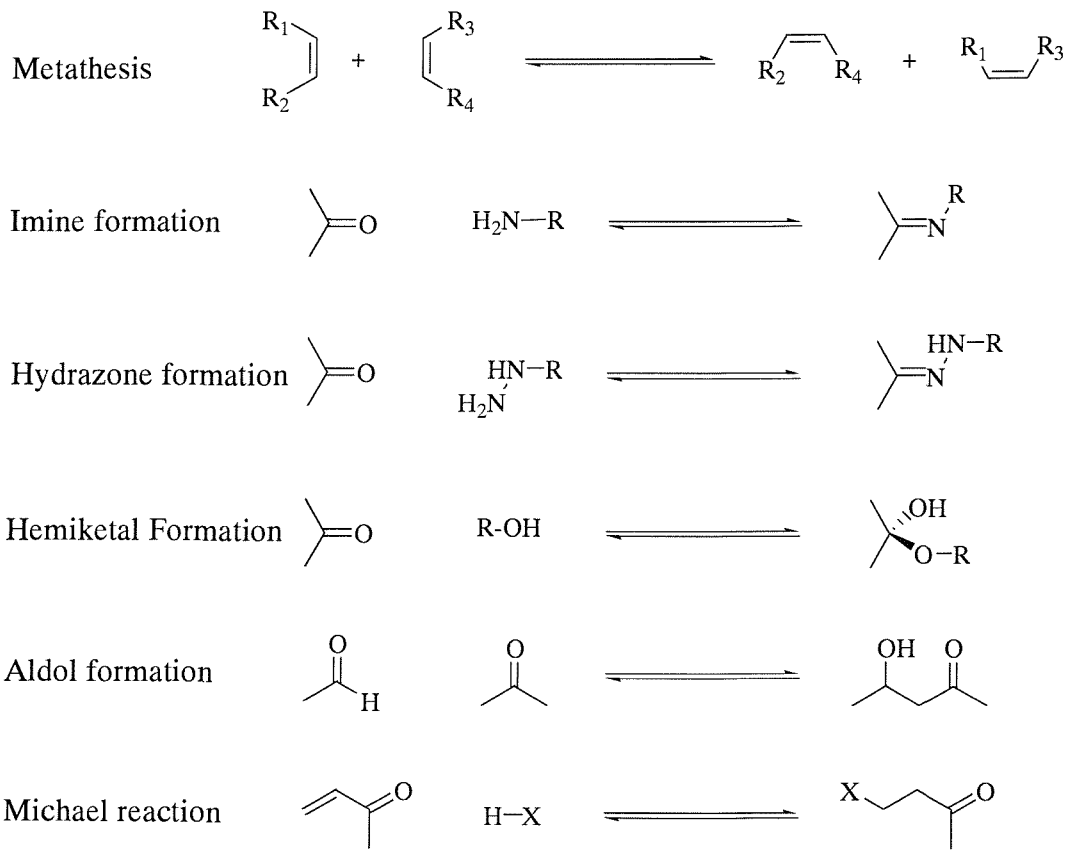


Figure I-60 Dynamic processes for potential use in DCC systems

Addition-elimination reactions at carbonyl groups are by far the most important class of reaction, especially imine exchange. For example primary amines undergo rapid imine formation and exchange with common aldehydes but the equilibrium is towards the starting materials in aqueous medium. With hydroxylamines and acyl hydrazides, the situation is the opposite: the stability of the imines is high whereas the kinetics of the reaction is slower. In the case of alkene metathesis, recent advances in catalyst development have enabled the use of this reaction in dynamic systems. The generation

of DCLs (Dynamic Combinatorial Libraries) can also involve more than one type of connection chemistry; two or more reactions or interactions can be used to vastly extend the diversity of the library.

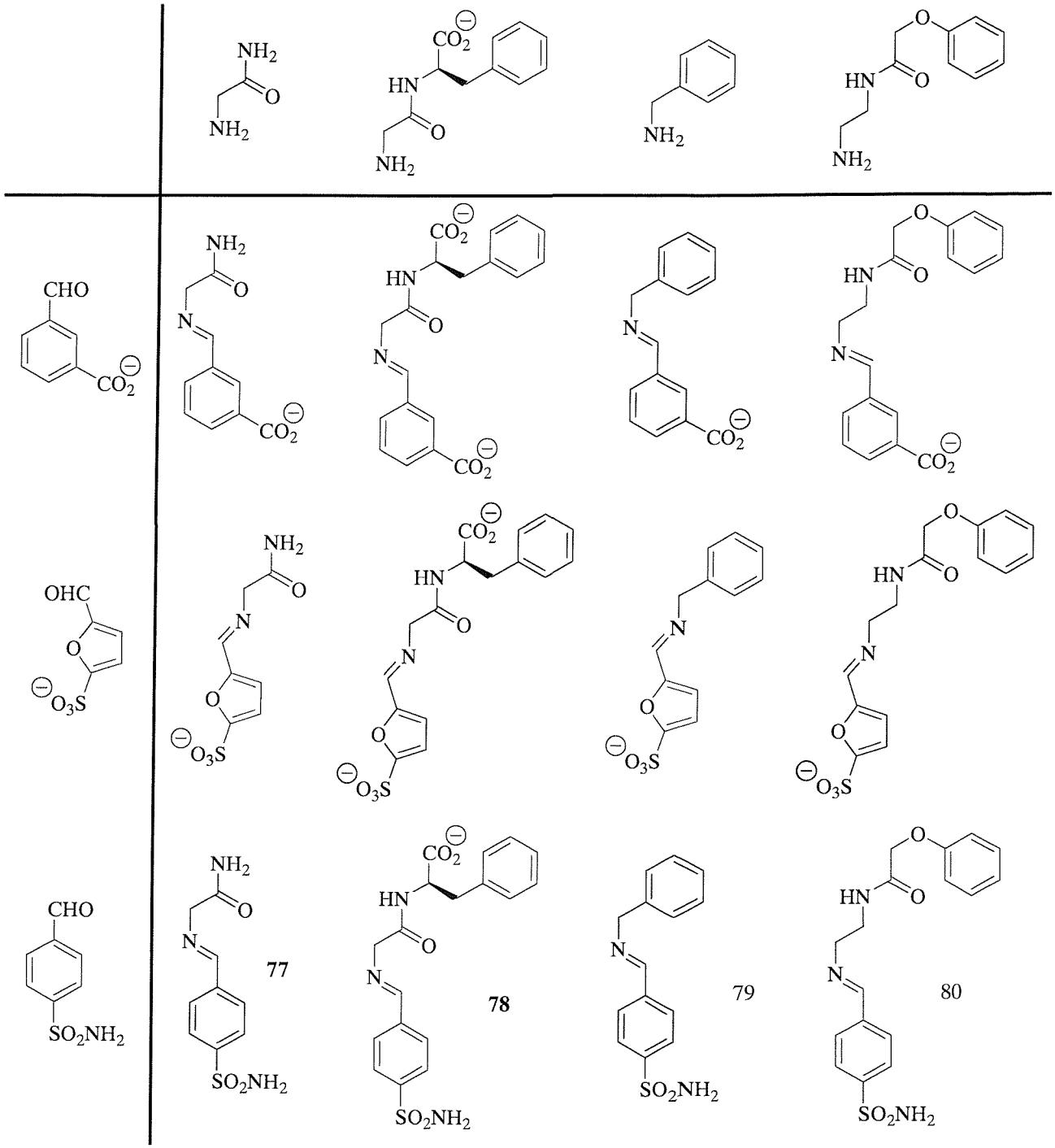


Table I-3 Dynamic combinatorial library of imines generated by reversible combination of a set of three aldehydes and four amines

Huc and Lehn [129] reported the synthesis of a dynamic library of imines generated by three aldehydes and four amines (Table I-3). This concept was applied to identify inhibitors of carbonic anhydrase (CA). [130] A library of twelve imines was generated reversibly which could be trapped by reduction to afford a mixture presenting structural features close to those of efficient inhibitors such as sulfonamides. Reactions were performed with and without CA to investigate if the presence of the enzyme would influence the abundance of the best ligands. The two chromatograms of the experiments conducted with and without CA were very similar. However a closer comparison revealed several novel features. It was found that the presence of CA caused a 80 % decrease in the abundance of **77** and **80** while it increased of two fold the abundance of **79** and to a lesser extent **78**. These findings proved that CA has induced some selectivity, which is consistent with the steric requirement of the enzyme.

Sanders [126,131,132] described a hydrazone based dynamic combinatorial library of macrocycles. The dynamic system was prepared from building blocks derived from L-proline (Figure I-61). Acid-catalyzed cyclization generated a library of 15 interchanging macrocycles. At the addition of acetylcholine to the reaction mixture the best binder was subtracted from equilibrium and this forced the library to rearrange so as to produce more of this member. Thus the equilibrium was shifted to produce a 50-fold amplification of the cyclic trimer relative to cyclic dimer.

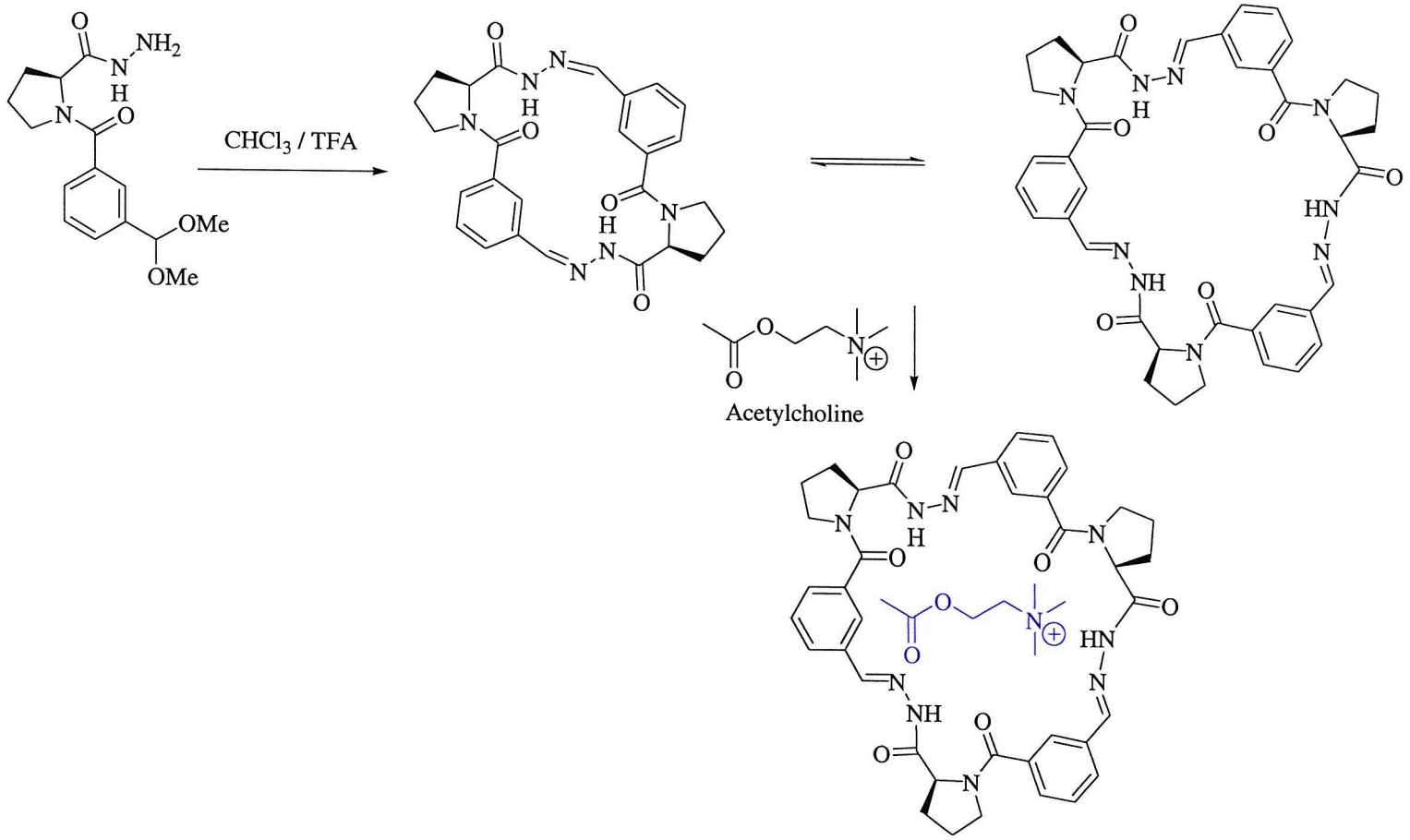


Figure I-61 Templating of a hydrazone-based library by acetylcholine

Amongst all the reversible process employed in dynamic systems, disulfide exchange is perhaps one of the most promising reaction. The advantage is that in solution dithiol readily oxides to disulfides upon exposure to air and the exchange can be modulated under slightly acidic conditions.

Sanders and co-workers [133] prepared a dynamic combinatorial library of structural diverse macrocycles in H_2O by mixing building blocks **81-86** (Figure I-62) including carbohydrate and amino acid derivative.

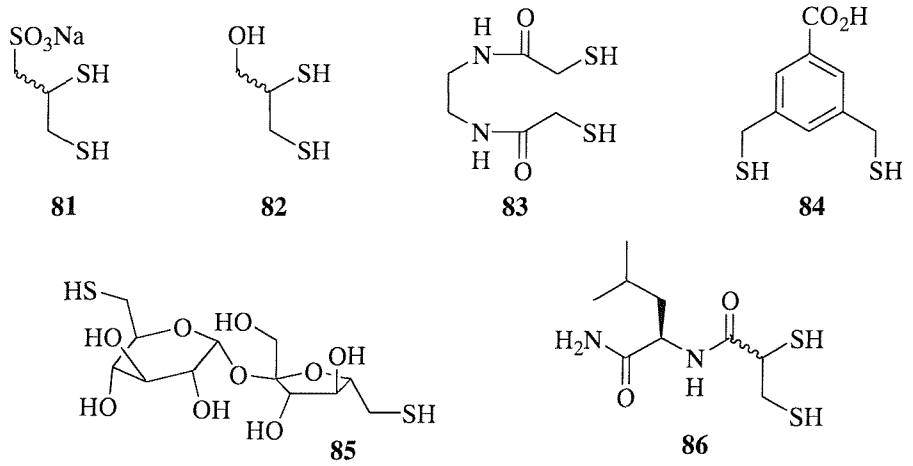


Figure I-62 Dithiol building blocks

The composition of the libraries (Figure I-63) was analysed using triple quadrupole electrospray ionization mass spectroscopy (QQQ ESI-MS) and Fourier transform ion cyclotron resonance (FTICR) ESI-MS and more than 100 members were identified. It was found that considerable structural diversity can be generated under extremely mild conditions.

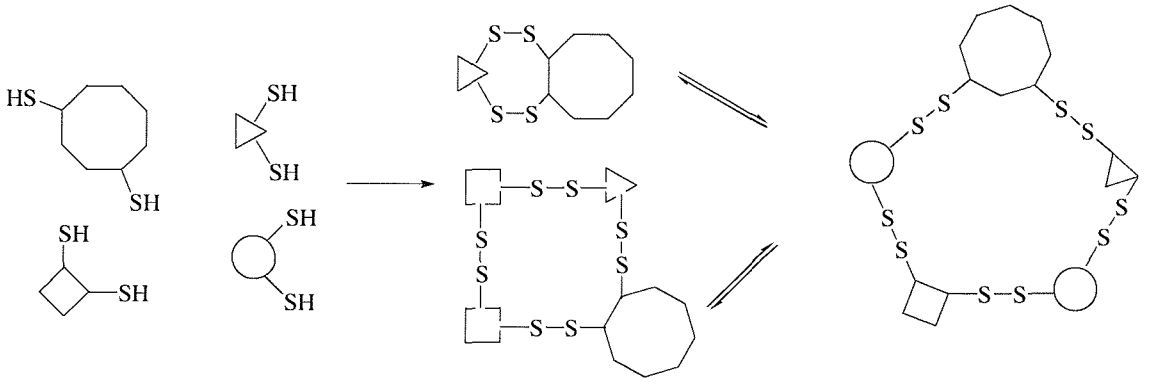


Figure I-63 Sander's dynamic combinatorial library of macrodisulfides from dithiol building blocks.

Chapter II

Synthesis of Combinatorial Libraries of Diamidopyridine-derived 'Tweezer' Receptors and Sequence Selective Binding of Peptides.

II.1 Background and Objectives

The design of enantioselective receptors is one of the central challenges of supramolecular chemistry. The increasing need for therapeutics and sensors drives the development of artificial receptors that recognise small peptides selectively. However, the many degrees of freedom of a simple di- or tripeptide make the rational design of a specific receptor for a given peptide an extremely difficult task. Combinatorial chemistry allows for the simultaneous synthesis and rapid testing for a desired property of large numbers of chemically related compounds. Our aim here is to employ combinatorial chemistry as a tool for the development of a novel class of two-armed receptors.

Our interest has focused on "tweezer receptors" which, despite their inherent flexibility, have proved to be highly selective for certain peptide sequences in both non-polar and aqueous solvent systems. Whereas in many of these systems the head group plays only a limited role in the binding of the guest, we became interested in developing tweezer receptors with two peptidic arms that incorporate a head group and that are capable of binding specifically to carboxylic acid functionalities (in analogy to our earlier macrocyclic peptide receptors. [68-71]

Such tweezers would ideally be receptors for peptides with a carboxylic acid terminus and it is anticipated that in their binding the peptidic arms could possibly form (parallel) β-sheet-like interactions with the backbone of the peptidic guest (Figure II-1).

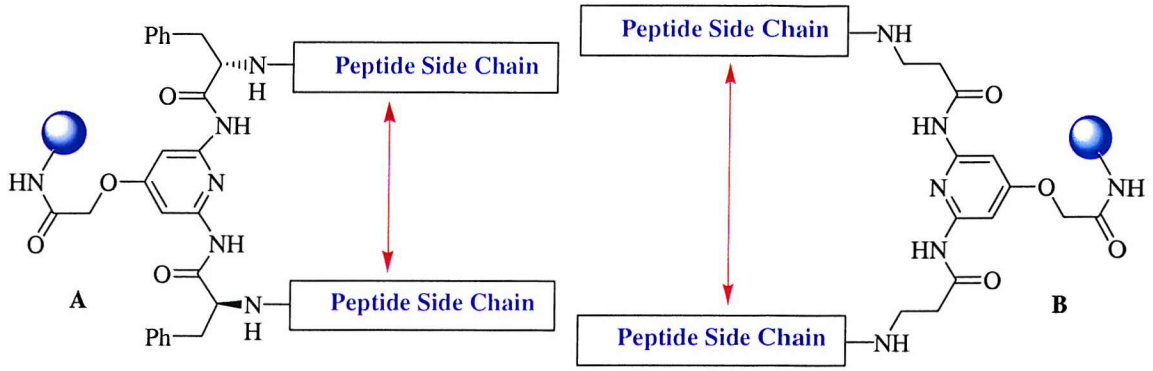


Figure II-1 Tweezer receptors

In pursuit of this idea, previous work [134,114,135,115] in the group has described the synthesis and screening of libraries of tweezer receptors such as **A** (Figure II-1), featuring a diamidopyridine functionality as a carboxylic acid binding site (CBS). [134,114,115,135]

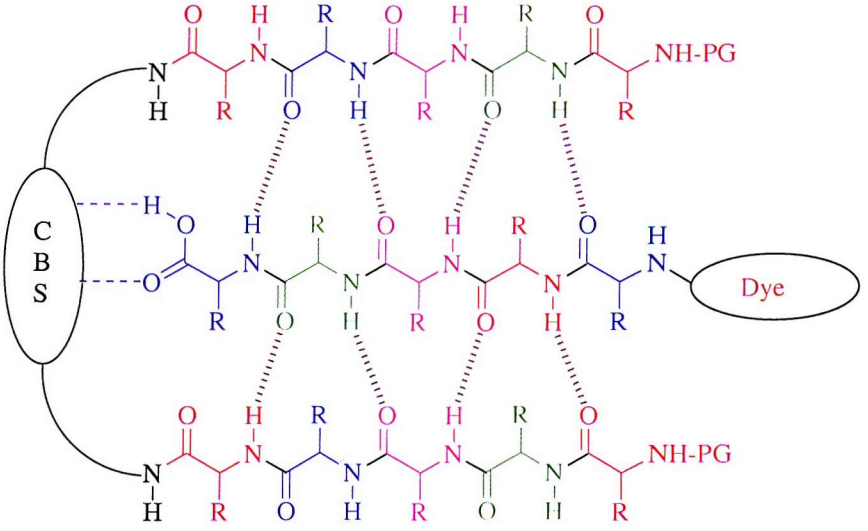


Figure II-2 Peptide receptors library.

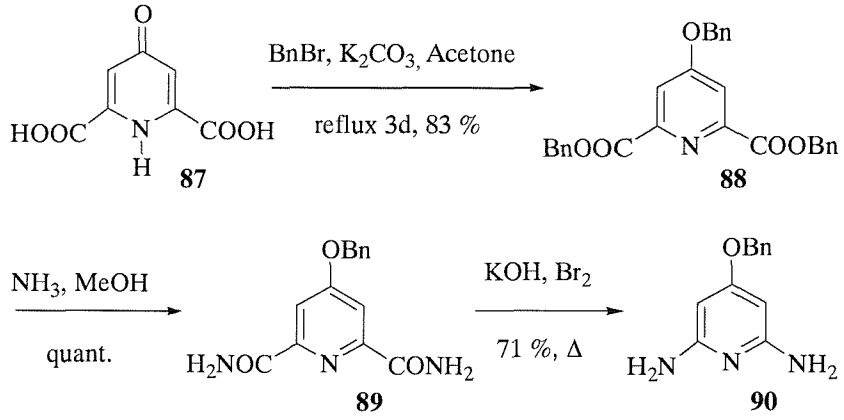
Libraries (Figure II-2) were screened with various dye-labelled tripeptides to identify suitable tweezer receptors. Individual tweezers identified from the screening process were resynthesised and subsequent binding studies revealed that the tweezers bind corresponding peptides with binding constants between 10^4 - 10^5 M^{-1} in $CHCl_3$. In order to investigate these systems in more detail and to probe the effect on binding properties of small structural changes in the library, we created similar libraries using a

diamidopyridine group with two β-alanine units instead of phenylalanine units as the starting point for the generation of the variable peptidic arms. As a consequence of this approach, the peptidic arms of the tweezer structure would be located one methylene unit further removed from the CBS than that provided by the diamidopyridine group (Figure II-1). [136]

II.2 Synthesis of the Carboxylic Binding Site (CBS).

The synthesis of a 2,6-diaminopyridine moiety with functionalised oxygen at the 4-position was carried out in order to allow attachment of the structure to the solid support. Carboxylic acid binding site **97** (Scheme II-8) was prepared from commercially available chelidamic acid **87** (Scheme II-1).

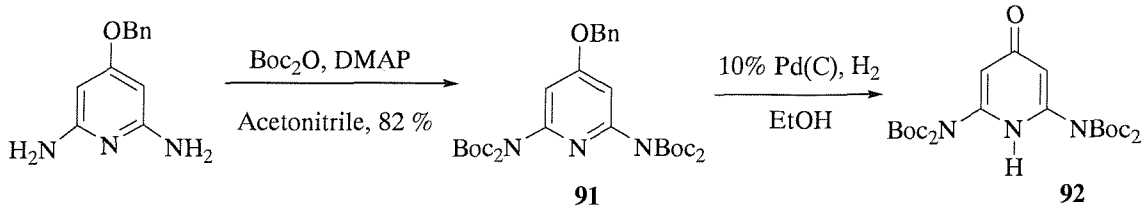
The key step in the synthesis of the CBS is a Hofmann rearrangement [137], which allows the transformation of an amide into an amine. The first step was the transformation of commercial chelidamic acid to the corresponding benzyl ester **88**. Treatment with a saturated solution of NH_3 in MeOH gave diamide **89**, which was transformed with excellent yield via Hofmann degradation into the corresponding diamine **90** (Scheme II-1) using an aqueous solution of KOH and bromine refluxed at 90 °C for 5 h.



Scheme II-1 Synthesis of diamine 90 via Hofmann degradation

The diamine **90** was protected with Boc-anhydride (di-tertbutyl dicarbonate) and DMAP in acetonitrile, then hydrogenolysis of benzyl ether **91** was carried out using

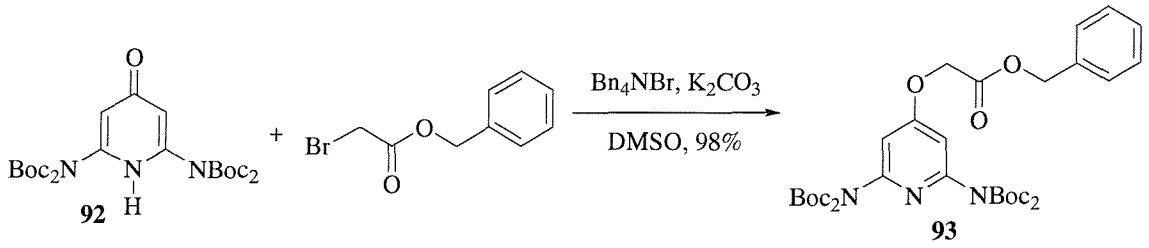
standard methods with 10 % Pd (C), H_2 in ethanol to give **92** in quantitative yield (Scheme II-2).



91 **92**

Scheme II-2 Hydrogenolysis of benzyl ether 91

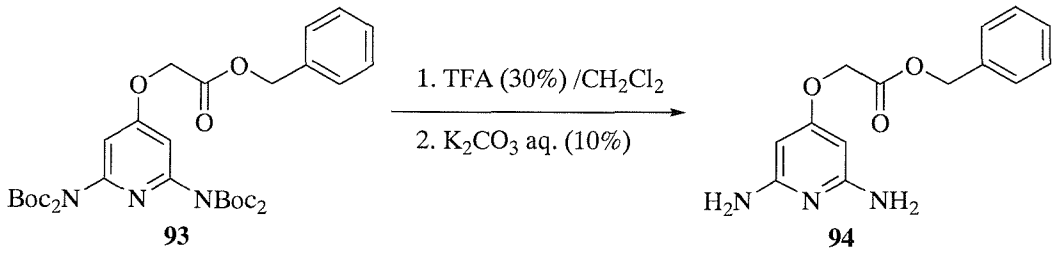
The following step was the alkylation of the oxygen group of pyridone **92** using DMSO as a solvent in the presence of K_2CO_3 and benzyl bromoacetate to give the desired compound **93** in 98 % yield, a convenient intermediate for the preparation of a range of diamidopyridine derivatives (Scheme II-3).



92 **93**

Scheme II-3 Alkylation of pyridone 92

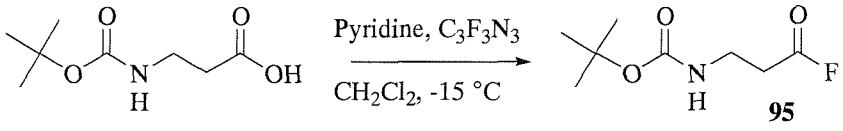
Finally, removal of the Boc protecting groups with 30 % TFA solution in DCM and extraction of the trifluoroacetate salt with 10 % aqueous solution of K_2CO_3 afforded the resulting diaminopyridine **94** ready for use in coupling experiments (Scheme II-4).



93 **94**

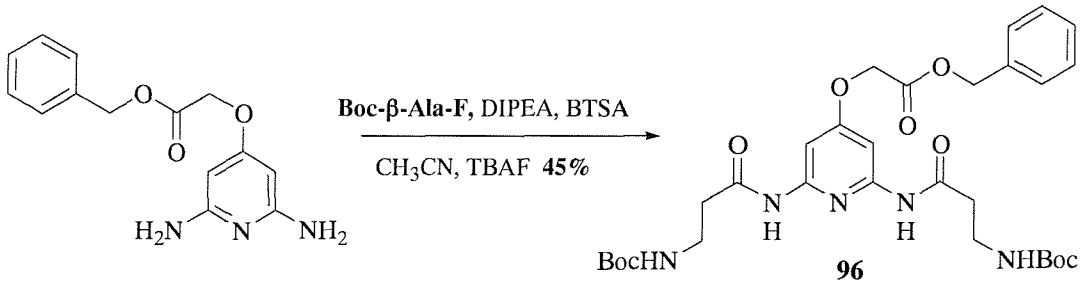
Scheme II-4 Synthesis of diamidopyridine 94

The coupling of *N*-Boc-β-alanine with diamine **94** was achieved using amino acid fluorides as peptide coupling reagents. For this purpose *N*-Boc-β-alanine was first converted to *N*-Boc-β-alanine acid fluoride **95** using cyanuric fluoride $(CFN)_3$ in quantitative yield (Scheme II-5). [138,139]



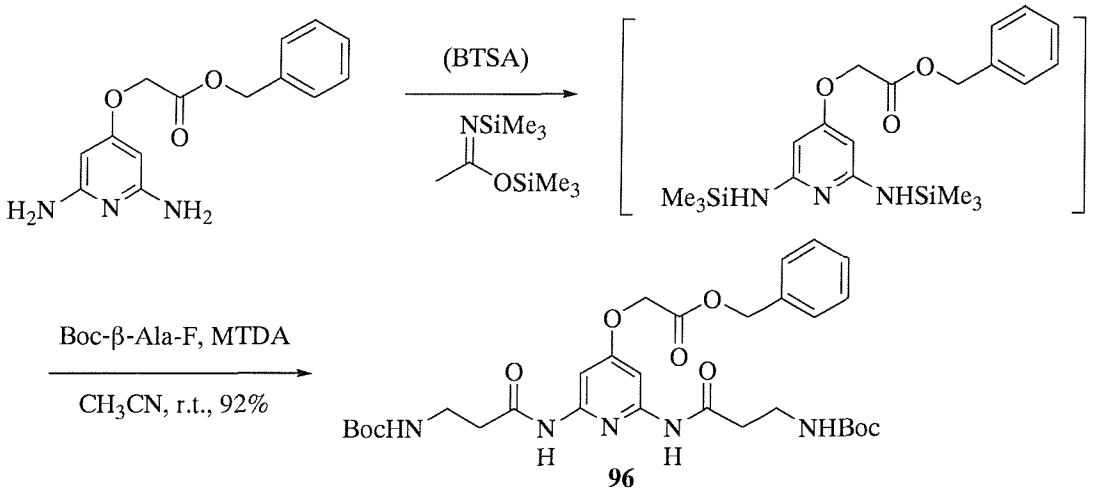
Scheme II-5 Synthesis of N-Boc-β-Ala-F 95

Diamine **94** (Scheme II-4) was preactivated with bis(trimethylsilyl)acetamide (BTSA) and then coupled with *N*-Boc-β-alanine acid fluoride **95** (Scheme II-5) in the presence of catalytic amount of TBAF to afford **96** in 45% yield (Scheme II-6).



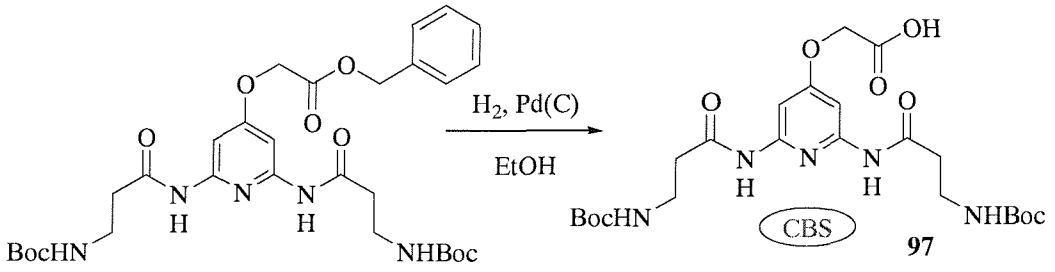
Scheme -II-6 Synthesis of diamidopyridine derivative 96

Coupling of *N*-Boc-β-alanine acid fluoride with diamine **94** (Scheme II-7) using BTSA (bis(trimethylsilyl)acetamide) and MTDA (methyl trimethylsilyl dimethylketene acetal) as a scavenger improved the yield of diamidopyridine derivative **96** (92%).



Scheme II-7 Coupling with N-Boc-Alanine-F using MTDA

The final step in the synthesis of the CBS was the hydrogenolysis of the benzyl ester to give the corresponding carboxylic acid **97** in 99% yield, suitable for attachment to the solid phase. (Scheme II-8)



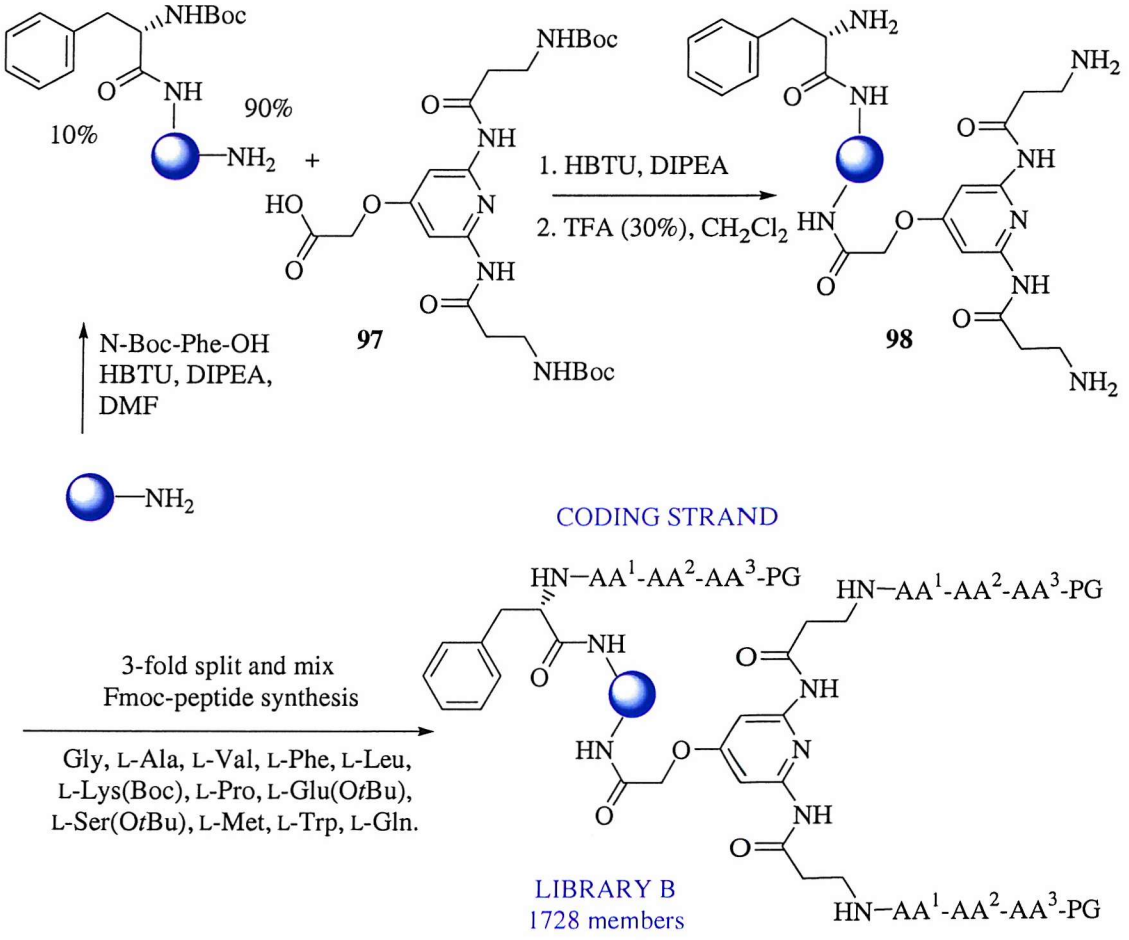
Scheme II-8 Final step of the synthesis of symmetrical protected Boc-CBS

II.3 Synthesis of a Peptide Receptor Library

The synthesis of a library of receptors was carried out using the 'split and mix' combinatorial strategy (See paragraph I.9.1). [90,91]

Boc protected CBS **97** was attached to Tentagel resin (Rapp Polymer, 130 μm) prepared with a 10 % loading of *N*-Boc-L-Phe-OH to serve as the coding strand. Removal of the Boc protecting groups yielded resin **98** ready for library generation.

A 1728 membered library of Fmoc protected tweezers **B** was prepared by three-fold coupling of 12 Fmoc protected amino acids (Gly, L-Ala, L-Val, L-Phe, L-Leu, L-Lys(Boc), L-Pro, L-Glu(O^{t}Bu), L-Ser(^{t}Bu), L-Met, L-trp, L-Gln) to the free amine groups of **98** using the 'split and mix' strategy (Scheme II-9).



Scheme II-9 Synthesis of tweezer receptors library B

II.4 Screening Experiments and Binding Studies

Screening experiments were carried out with tweezer receptors library **B** using the dye-labelled peptide guests Red dye-spacer-L-Glu(O^{t}Bu)-L-Ser(^{t}Bu)-L-Val-OH **99** and Red dye-spacer-L-Glu-L-Ser-L-Val-OH **100** (Figure II-3). These peptides were chosen as they had been used in previous studies with other libraries in the group. [113,114]

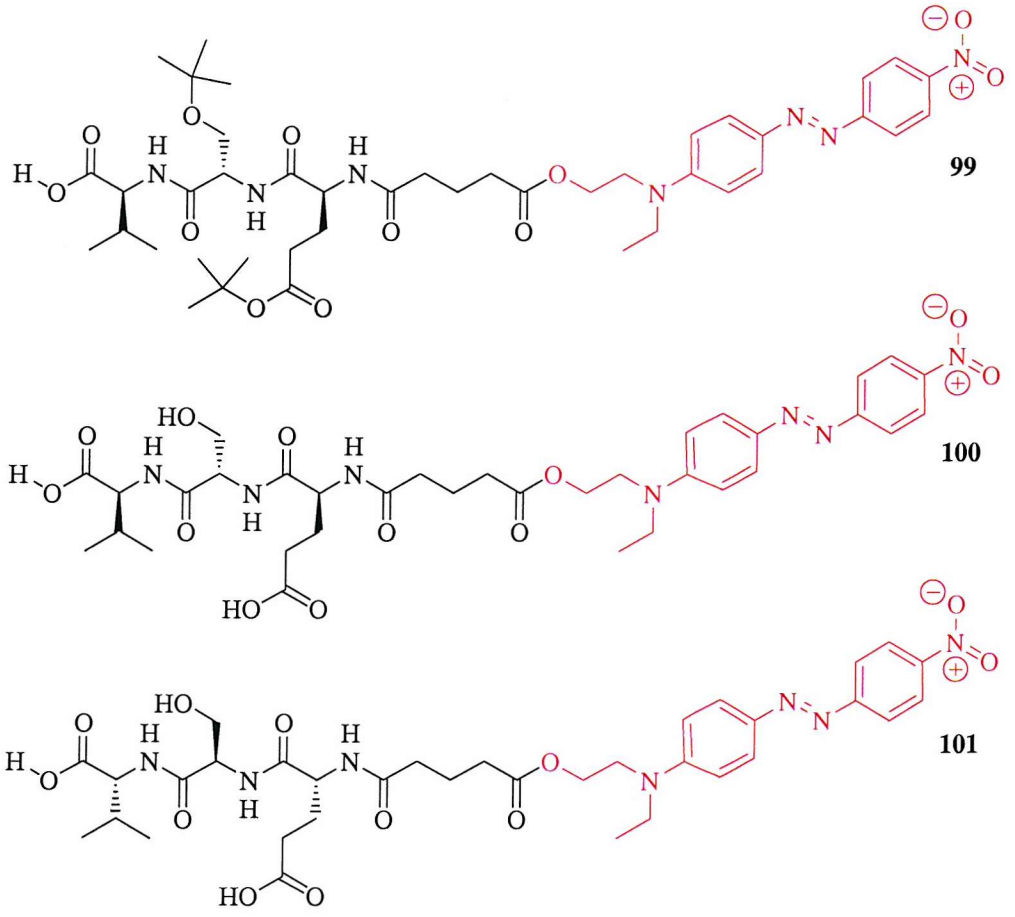


Figure II-3 Peptides used in the screening experiments with library B

In a typical screening experiment a sample of ~10 mg (~9000 beads) of the library material was equilibrated in a chosen solvent system for 24 h. Statistically, this represented an amount corresponding to at least five copies of the library. Dye-labelled tripeptide as a solution in the same solvent system was added and incubated for a further 24 h. Beads were analysed in flat-bottomed glass pots under a Leica inverted DML microscope (magnification, 40×). The concentration of dye-labelled peptide guests

could be increased to provide optimal selectivity as adjudged by the number of highly stained beads against a background of non- or lightly stained beads. Control experiments were carried out as in previous screening studies.

Thus, we incubated the peptide guests with a simple peptide library directly attached to Tentagel resin (analogous to the coding strand). No evidence for any selective binding was observed in these experiments. Thus we conclude that any observed binding selectivity (of the dye-labelled guest peptide for the resin-bound library members) is not a consequence of interaction of the tripeptide guest simply with the peptide side arm of the tweezer receptor, or with the coding strand on the library beads. Furthermore, the simple acetylated Red-dye **111** (Figure II-7) was incubated with the tweezer library, but again no selective binding to any of the resin-bound library members was observed, confirming that the selectivity observed with the dye-labelled peptide guests was not a consequence of binding to the dye moiety alone.

When library **B** was incubated with the deprotected peptide Red dye-spacer-L-Glu-L-Ser-L-Val-OH **100** in 1 % DMSO: 99 % chloroform, excellent binding selectivity was observed. Thus, at a peptide concentration of 18 μM, ~ 0.2 % of beads were highly coloured after 24 h of incubation (Figure II-4).

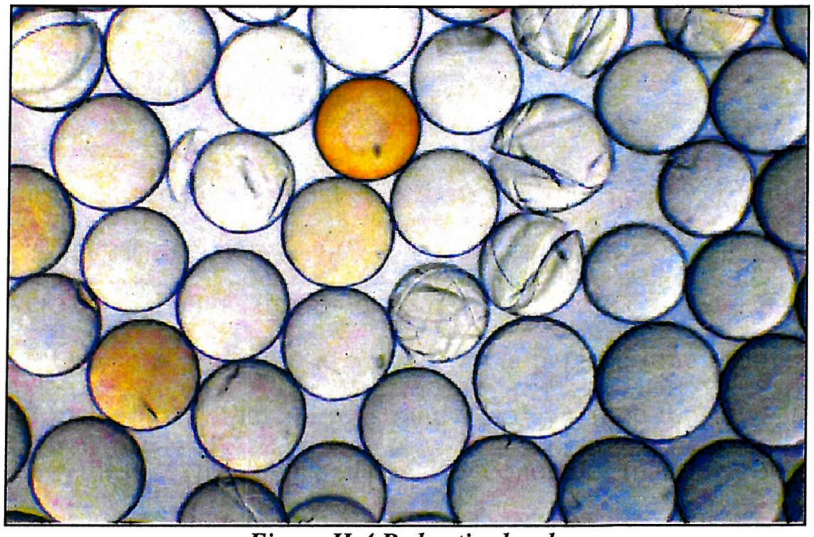


Figure II-4 Red active beads

It was essential that the receptor library/peptide guest was left longer than 12 h to reach equilibrium, indicating that binding with the resin-bound receptors has relatively slow

kinetics. The observed binding selectivity was also highly solvent dependent as no beads remained intensely stained after addition of 10 % DMSO.

BEAD	AA^0	AA^1	AA^2	AA^3
1	Phe	Gln	Val	Gln
2	Phe	Gln	Val	Gln
3	Phe	Gln	Val	Gln
4	Phe	Gln	Lys(Boc)	Gln
5	Phe	Gln	Lys(Boc)	Gln
6	Phe	Val	Ala	Glu(O^{t}Bu)
7	Phe	Val	Gln	Leu

Table II-1 Sequencing data for seven dyed beads selected from screening experiment of Red dye-L-Glu-L-Ser-L-Val-OH 100 with library B in $CHCl_3$.

Seven highly coloured beads were taken from the screening experiment and sequenced by Edman degradation to identify the structures of the tweezer receptors on each bead.[104] The sequencing results gave a high degree of consensus with glutamine at the first position for five beads and at the third position for four beads, with the Gln-AA^2-Gln sequence clearly favoured, and Val as the most common residue at the second position (Table II-1).

In comparison, previous screening experiments with peptide **100** and library **A** (Figure II-5) had shown moderate binding selectivity (~1 % of beads were highly stained in the screening experiment), but not as high as that observed for peptide **100** with library **B**. The sequences identified from the former screening experiment (Table II-2) have some similarities with the sequences identified from the latter (Table II-1).

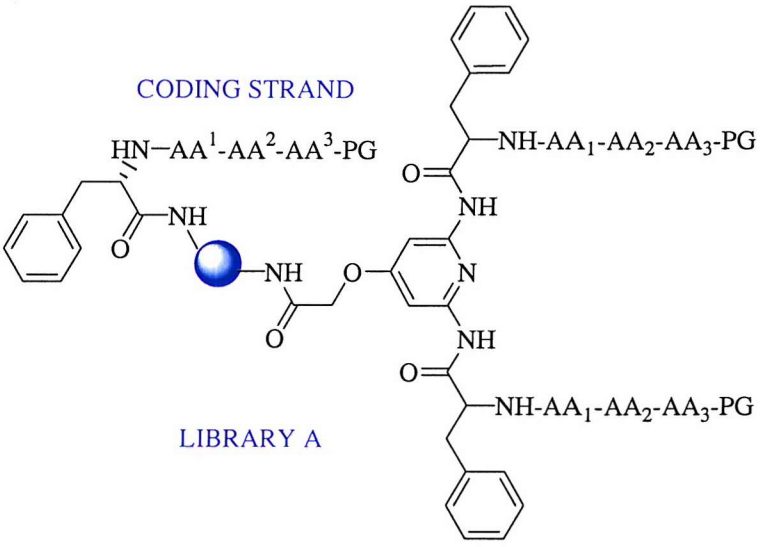


Figure II-5 Library A

Thus for peptide **100** and library **A**, Gln was found at the first position for two out of the five beads sequenced, and Val or the structurally similar Leu was found consistently at the second position. At the third position however, Ala was found for each bead.

BEAD	AA^0	AA^1	AA^2	AA^3
1	Phe	Ala	Leu	Ala
2	Phe	Ala	Leu	Ala
3	Phe	Gln	Leu	Ala
4	Phe	Gln	Val	Ala
5	Phe	Met	Val	Ala

Table II-2 Sequencing data for five dyed beads selected from screening experiment of Red dye-spacer-L-Glu-L-Ser-L-Val-OH 100 with library A in $CHCl_3$.

The similarity in sequences at the first and second position suggests that the binding of peptide **100**, by tweezers from libraries **A** (Figure II-5) and **B** (Scheme II-9), involves similar interactions with the tweezers side-arms.

In contrast, no binding selectivity was observed when library **B** was incubated with he protected peptide Red dye-spacer-L-Glu(O^{t}Bu)-L-Ser(^{t}Bu)-L-Val-OH **99** at a peptide concentration of 18 μM, and even at a peptide concentration of 100 μM only slight selectivity could be observed by inspection of the beads under the microscope.

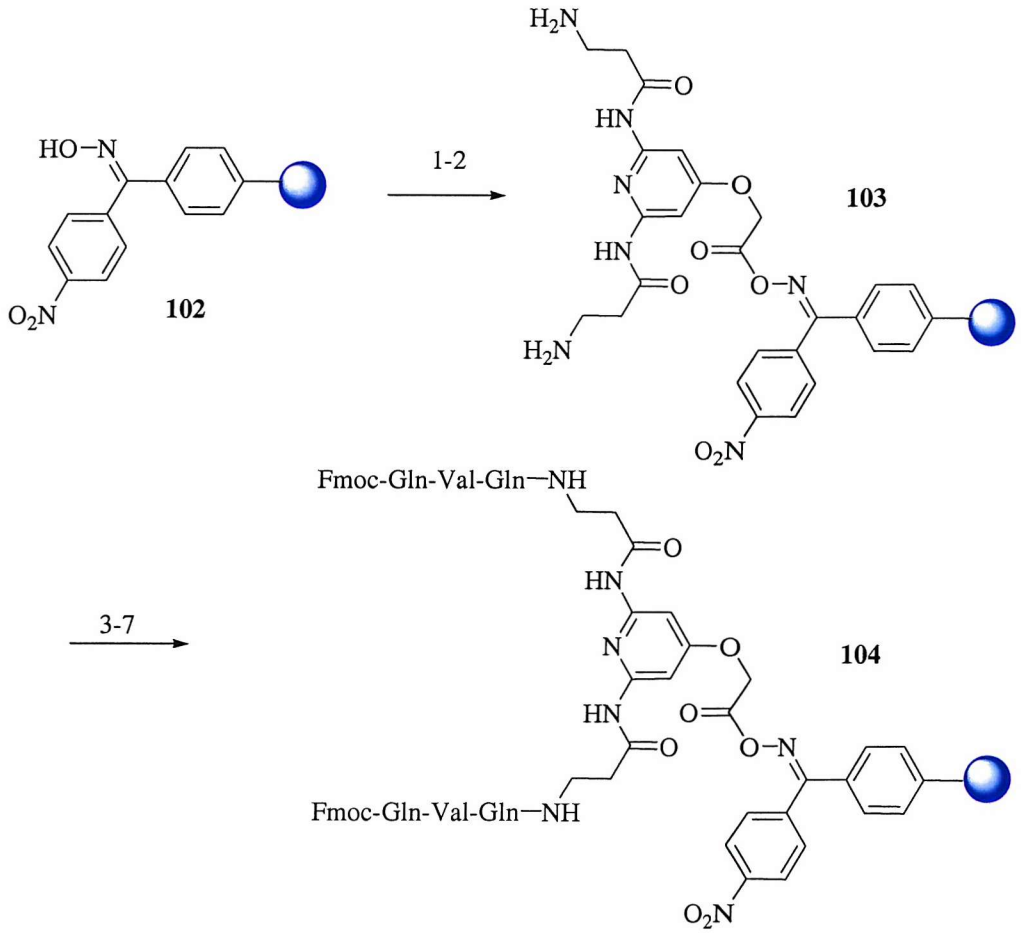
These latter results are in marked contrast to the screening results obtained previously using the same peptide guest **99** with receptor library **A**. In that case excellent selectivity was observed and four beads were sequenced. Three beads gave identical results for the tweezer side-arm structure: Val—Ala—Pro while the fourth bead yielded the closely related sequence Val—Met—Pro (Table II-3).

BEAD	AA^0	AA^1	AA^2	AA^3
1	Phe	Val	Ala	Pro
2	Phe	Val	Ala	Pro
3	Phe	Val	Ala	Pro
4	Phe	Val	Met	Pro

Table II-3 Sequencing data for four dyed beads selected from scrrening experiment of Red dye-spacer-L-Glu(O^{t}Bu)-L-Ser(^{t}Bu)-L-Val-OH 99 with library A in $CHCl_3$

II.5 Synthesis of the Single Tweezer Receptor.

As in previous studies, we sought to establish that the observed guest binding with the resin bound tweezer receptor was also operating in free solution. This was done with the synthesis of the single tweezer receptor which was first variably constructed on solid phase. The protected CBS **97** was linked to Oxime resin **102** (Scheme II-10) using HBTU and DIPEA. [140-143] Further treatment with 25 % TFA in CH_2Cl_2 afforded the deprotected resin bound CBS **103** ready for the generation of tripeptide side-arms Gln-Val-Gln, by standard Boc peptide synthesis.



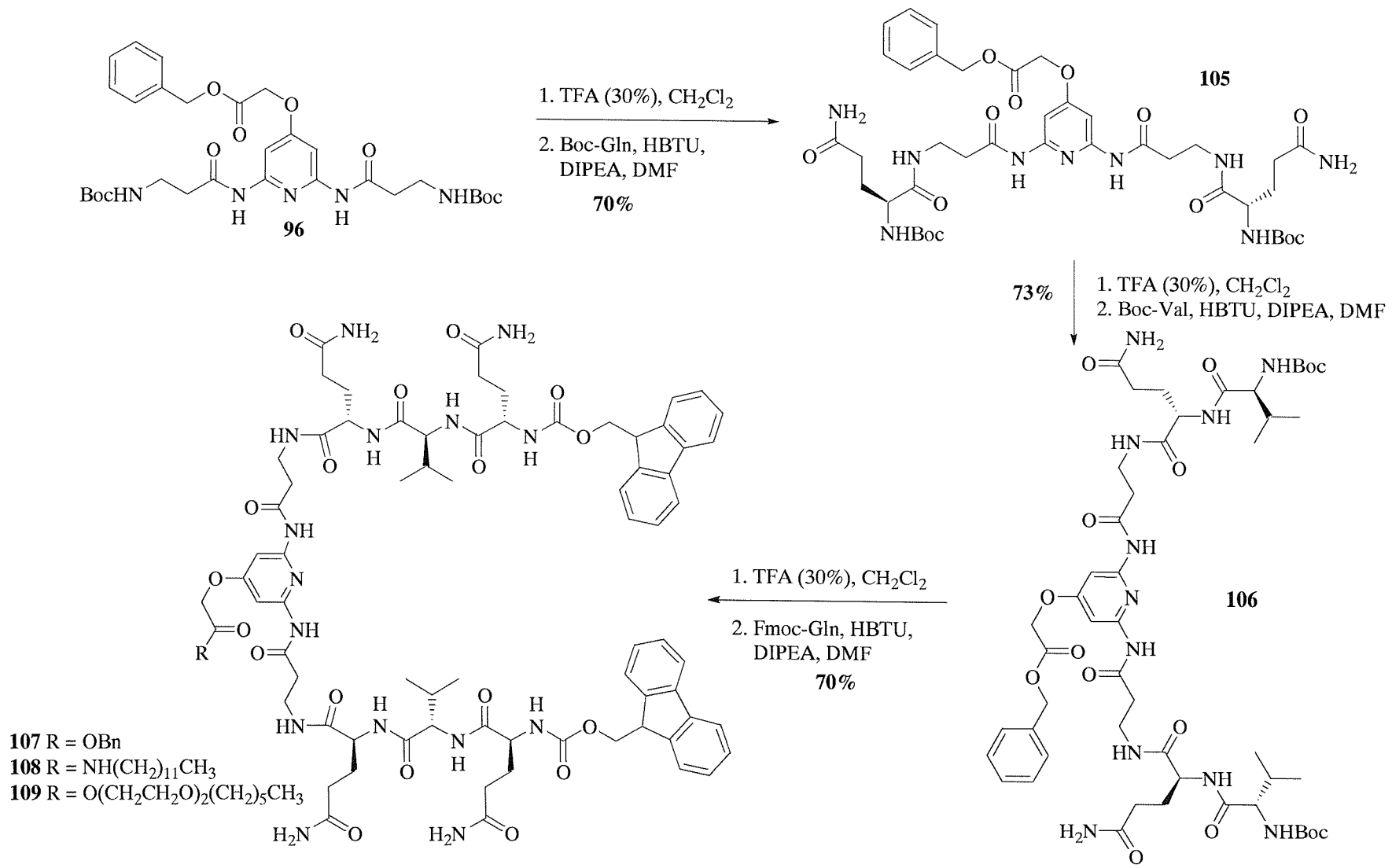
1. HBTU, DIPEA, CBS, DMF; **2.** 25%TFA in CH_2Cl_2; **3.** Boc-L-Gln-OH, HBTU, DIPEA, DMF; 4. 20% piperidine, DMF; **5.** Boc-L-Val-OH, HBTU, DIPEA, DMF; **6.** 20% piperidine, DMF; **7.** Fmoc-L-Gln-OH, HBTU, DIPEA, DMF.

Scheme II-10 Synthesis of single tweezer receptor on solid phase.

The cleavage of the tweezer of known structure from resin **104** (Scheme II-10) was performed using a solution of dodecylamine in CH_2Cl_2. [144,145] The dodecyl alkyl chain should have increased the lypophilicity of the tweezer and therefore the solubility in organic solvents such as $CHCl_3$. Unfortunately no product was recovered from the cleavage. This was possibly due to the insolubility of the product.

Consequently, tweezer **107** was prepared by solution-phase synthesis (Scheme II-11) as a single compound with tetrapeptide side-arms β-Ala-L-Gln-L-Val-L-Gln corresponding to the most commonly found sequence in the screening experiments.

The synthesis of **107** (Scheme II-11) using sequential coupling of Boc-amino acids and TFA deprotection was straightforward since each of the intermediates could be isolated cleanly by precipitation, without use of chromatography. However, unsurprisingly the final compound **107** proved to be completely insoluble in $CDCl_3$, which precluded any binding studies in this solvent. Tweezer **107** could be dissolved in DMSO, but there was no evidence for any binding in this solvent, which is consistent with the screening experiments in which the addition of as little as 10 % DMSO to the chloroform solution resulted in a complete loss of binding. Derivatives **108** and **109** of the tweezer receptor were also prepared, incorporating a long (dodecyl)alkyl chain or a diethyeneglycol hexyl ether, however these derivatives were also insoluble in $CHCl_3$.



Scheme II-11 Synthesis of the identified tweezer receptor 107

As an alternative to binding studies with the tweezer receptor in free solution, [146] we established that the resin bound receptor **110** (Scheme II-12) with the same tetrapeptide side arms, β-Ala-L-Gln-L-Val-L-Gln as **107**, is genuinely selective. Portions of this resin-bound tweezer were added to solutions of the peptides **99** and **100** and of the enantiomer Red dye-spacer-D-Glu-D-Ser-D-Val-OH **101** and the extent to which the peptides were adsorbed onto the resin beads was determined (Figure II-6).

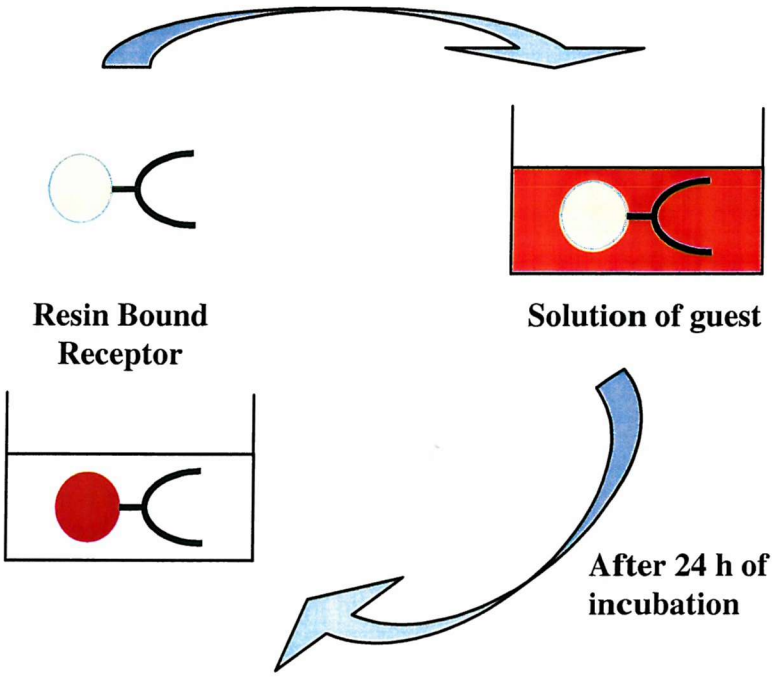
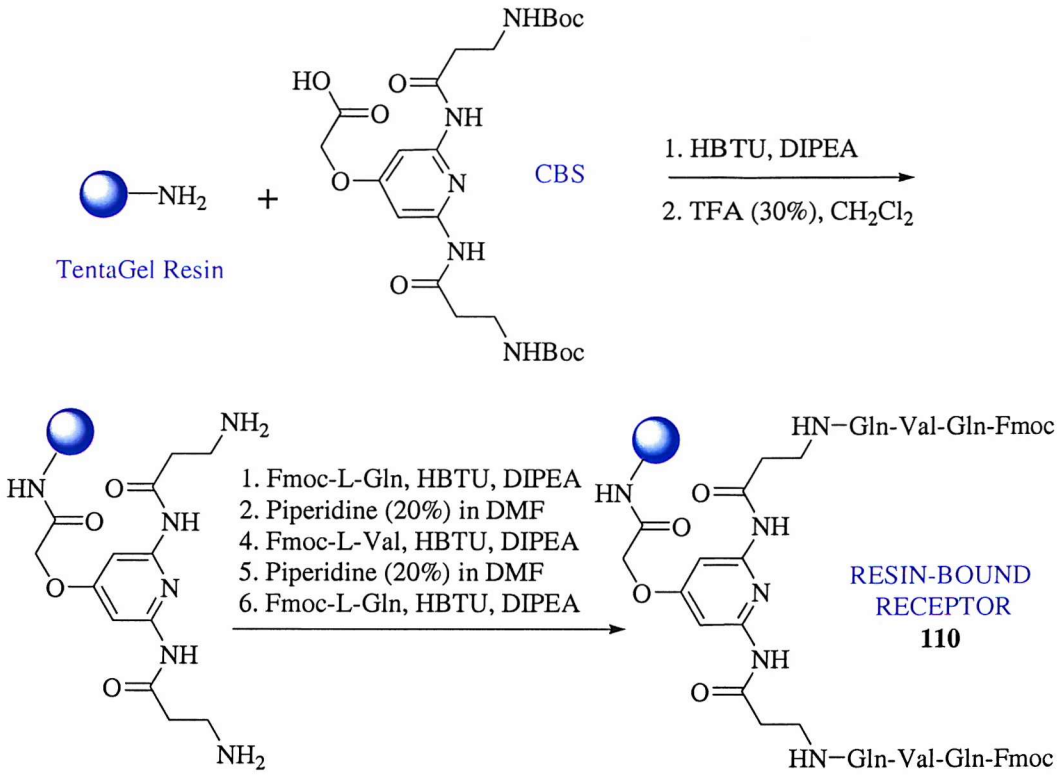


Figure II-6 Resin bound receptor extract guest from the solution

That is, the receptor was resynthesised on solid phase to give **110** with an estimated loading of 0.05 mmol of receptor/g resin (Scheme II-12).



Scheme II-12 Synthesis of identified resin-bound receptor.

Addition of 3.0 mg of resin-bound tweezer receptor **110** (~ 0.15 μmol of receptor) to a solution of Red dye-spacer-L-Glu-L-Ser-L-Val-OH **100** (0.5 mL, 0.3 mM, ≅0.15 μmol of peptide) in $CHCl_3$ led to adsorption of 86 % (± 13 %) of the peptide (Table II-4 and 5) as determined by HPLC (Figure II-8) (using the acetylated Red dye **111** and Red Dye **112** as an internal standard) after 24 h of incubation. (After only 2 h the amount of peptide adsorbed by the resin was ~ 40 %, Table II-5, again indicating the relatively slow rate at which binding equilibrium is reached with the resin-bound receptors).

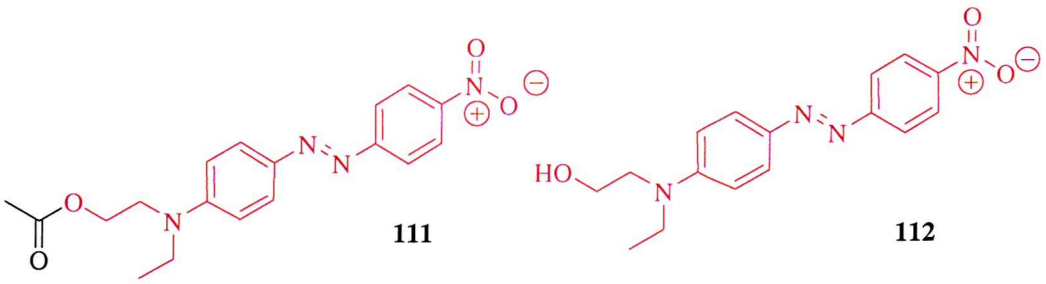


Figure II-7 Acetylated Red Dye and Red Dye: internal standards 111 and 112

Since the addition of acetyl-capped tentagel resin beads led to no significant absorption of the peptides or red dye **112** and **111**, this provides an estimate of the binding constant of $K \sim 1.5 \times 10^5\ M^{-1}$. With a starting concentration of peptide of 0.3 mM, and an equivalent amount of resin-bound receptor present (0.15 μmol in 0.5 mL solution), if y is the fraction of peptide absorbed, then concentration of free guest [G] = 0.3(1-y) mM; and $K = [HG]/[H][G] = y/0.3(1-y)^2\ mM^{-1}$.

Red Dye-spacer-L-Glu-L-Ser-L-Val-OH			
	STD	**2 h**	**24 h**
A % GUEST	73.1952	6.3801	3.2653
A%REF	26.8048	93.6199	96.7347
A% GUEST / A% REF	2.7307	0.0681	0.0338
% Guest extract		**97.50**	**98.76**
	STD		**24 h**
A % GUEST	35.9898		14.1555
A%REF	64.0102		85.8445
A% GUEST / A% REF	0.5623		0.1649
% Guest extract			**70.67**

Table II-4 HPLC experiment: Red dye-spacer-L-Glu-L-Ser-L-Val-OH 100 and Red dye 112 as internal standard

Red Dye-spacer-L-Glu-L-Ser-L-Val-OH				
	STD	**2h**	**24 h**	**24 h**
A % GUEST	35.5958	26.1372	5.1567	8.4846
A%REF	64.404	73.8628	94.8433	91.5154
A% GUEST / A% REF	0.5527	0.3539	0.0544	0.0927
% Guest extract		**35.98**	**90.16**	**83.23**

Table II-5 HPLC experiment: Red dye-spacer-L-Glu-L-Ser-L-Val-OH and acetylated Red dye 111 as internal standard

Addition of the same amount of resin-bound tweezer receptor (~ 0.15 μmol of receptor) to an equivalent solution of the side-chain protected peptide **99** (Red dye-spacer-L-Glu(O^{t}Bu)-L-Ser(^{t}Bu)-L-Val-OH, 0.5 mL, 0.3 mM, ≅0.15 μmol of peptide) led to a absorption of ~9% (±1%) (Table II-6) of the peptide, giving an estimated K ~ 3.6×10^2 M^{-1}. For peptide **99** was not possible to repeat the HPLC experiment using acetylated red dye **111** (Figure II-7) as internal standard because the Red dye-spacer-L-Glu(O^{t}Bu)-L-Ser(^{t}Bu)-L-Val-OH had the same retention time (Figure II-8-10). Thus it can be concluded that the resin-bound tweezer receptor **110** binds the peptide **100** > 400 times stronger than side-chain protected analogue **99**, which is consistent with the screening experiments.

Red Dye-spacer-L-Glu-(O^{t}Bu)-L-Ser-(O^{t}Bu)-L-Val-OH			
	STD	**2 h**	**24 h**
A % GUEST	32.8694	32.4598	30.814
A%REF	67.1306	67.5402	69.186
A% GUEST / A% REF	0.4896	0.4806	0.4454
% Guest extract		**1.85**	**9.04**
	STD	**2 h**	**24 h**
A % GUEST	32.9223	32.4683	31.011
A%REF	67.0777	67.5317	68.989
A% GUEST / A% REF	0.4908	0.4808	0.4495
% Guest extract		**2.04**	**8.41**

Table II-6 HPLC experiments: Red dye-spacer-L-Glu(O^{t}Bu)-L-Ser(^{t}Bu)-L-Val-OH 99 and Red dye 112 as internal standard

Identical experiments with the enantiomeric peptide Red dye-spacer-D-Glu-D-Ser-D-Val-OH **101** (Figure II-3, II-9), led to absorption of 54% (±15%) (Table II-7 and 8) of the peptide, giving an estimated K ~8.5×10^3 M^{-1}, suggesting that resin-bound tweezer receptor **110** binds the L-configured peptide **100** > 17 times stronger than its enantiomer.

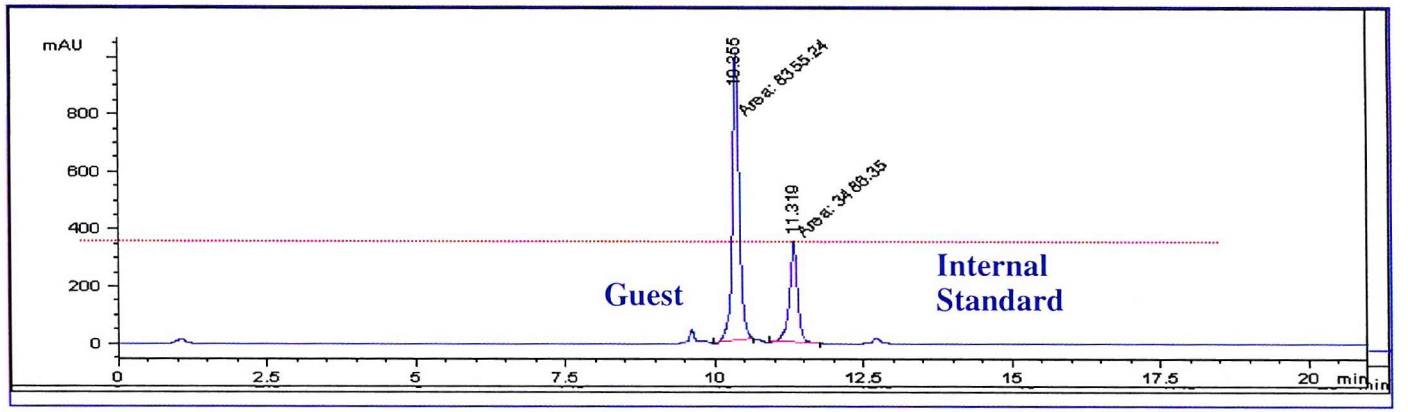
Red Dye-spacer-D-Glu-D-Ser-D-Val-OH			
	STD	**2 h**	**24 h**
A % GUEST	39.1976	26.0664	16.5877
A% REF	60.8024	73.9336	83.4123
A% GUEST / A% REF	0.6447	0.3526	0.1989
% Guest extract		**45.31**	**69.15**
	STD	**2 h**	**24 h**
A % GUEST	45.6614		20.3445
A% REF	54.3386		79.6555
A% GUEST / A% REF	0.8403		0.2554
% Guest extract			**69.61**

Table II-7 HPLC experiments: Red dye-spacer-D-Glu-D-Ser-D-Val-OH 101 and acetylated Red dye 111 as internal standard

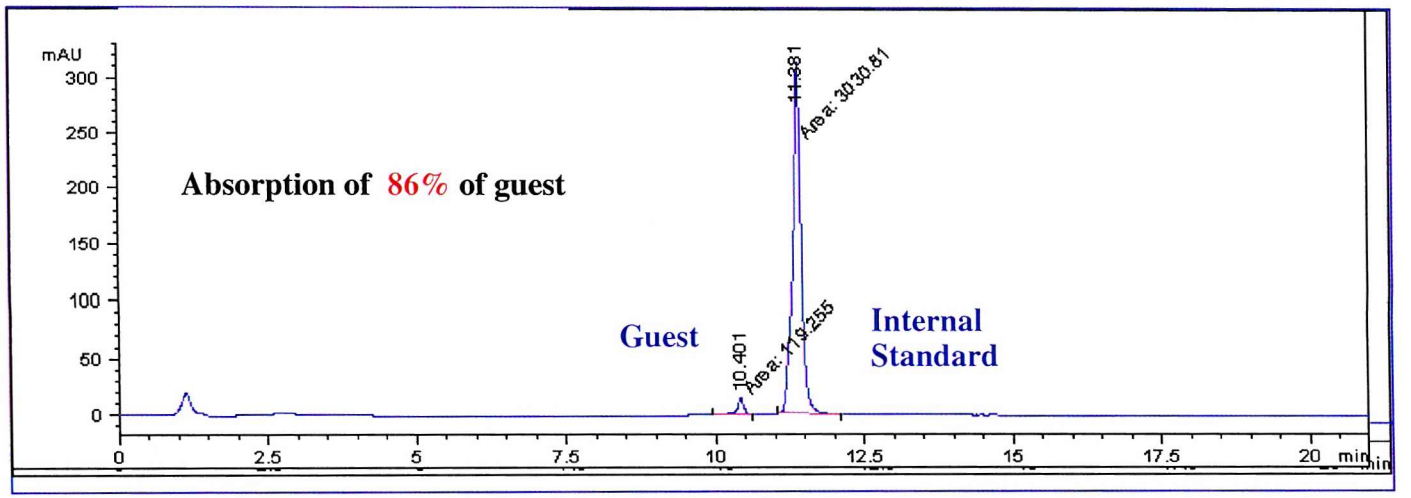
Red Dye-spacer-D-Glu-D-Ser-D-Val-OH			
	STD	**2 h**	**24 h**
A % GUEST	20.3494		13.5995
A% REF	79.6506		86.4005
A% GUEST / A% REF	0.2555		0.1574
% Guest extract			**38.39**
	STD	**2 h**	**24 h**
A % GUEST	18.311	15.1399	12.294
A% REF	81.689	84.8601	87.706
A% GUEST / A% REF	0.2242	0.1784	0.1402
% Guest extract		**20.41**	**37.47**

Table II-8 HPLC experiments: Red dye-spacer-D-Glu-D-Ser-D-Val-OH 101 and Red dye 112 as internal standard

These derived binding constants should only be considered as estimates given the experimental errors in determining the loading of the receptor on the resin, and in determining the amount of peptide absorbed.

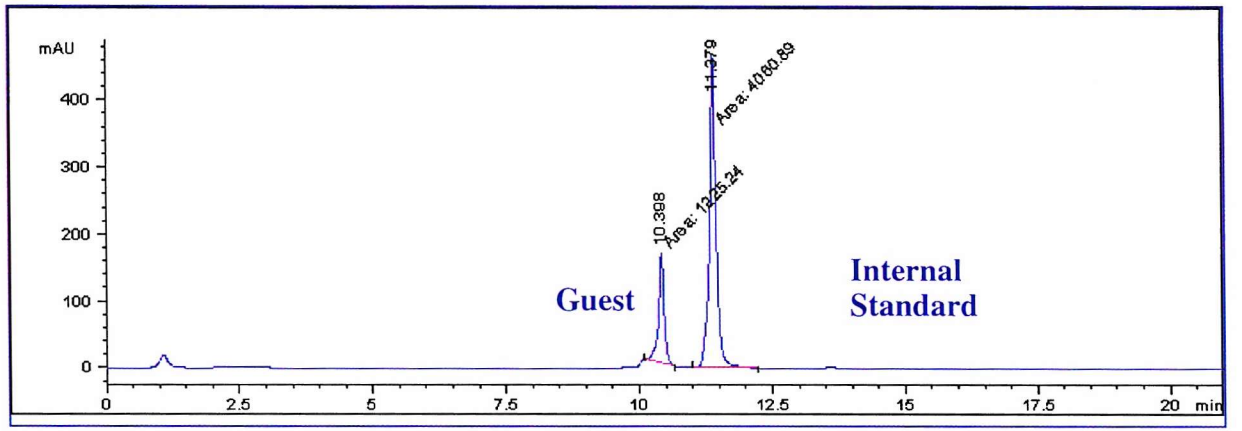


Standard solution of Red dye-L-Glu-L-Ser-L-Val-OH (guest) 100 and Red dye 112 (internal standard)

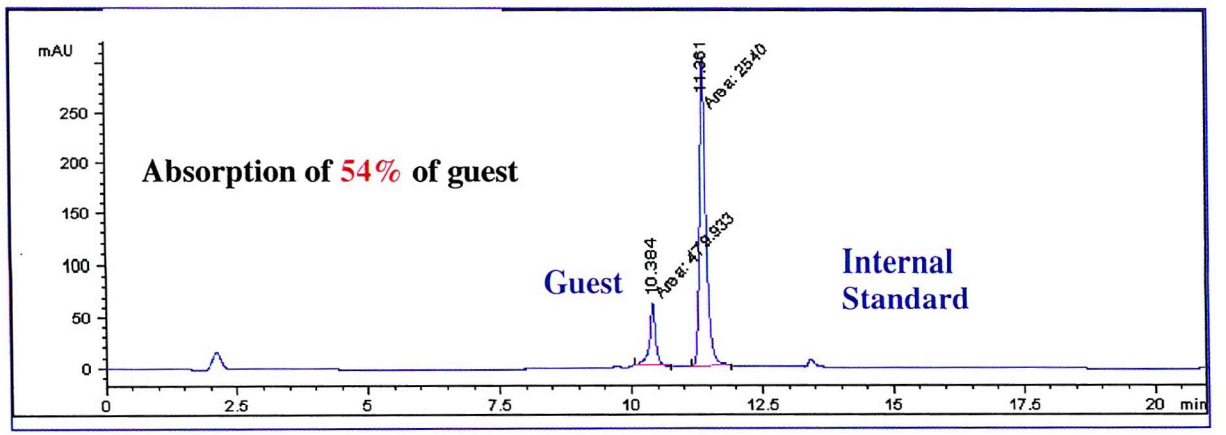


Solution of Red dye-L-Glu-L-Ser-L-Val-OH (guest) 100 and Red dye 112 (internal standard) after 24 hours of incubation

Figure II-8 Binding experiment with Red dye-spacer-L-Glu-L-Ser-L-Val-OH 100

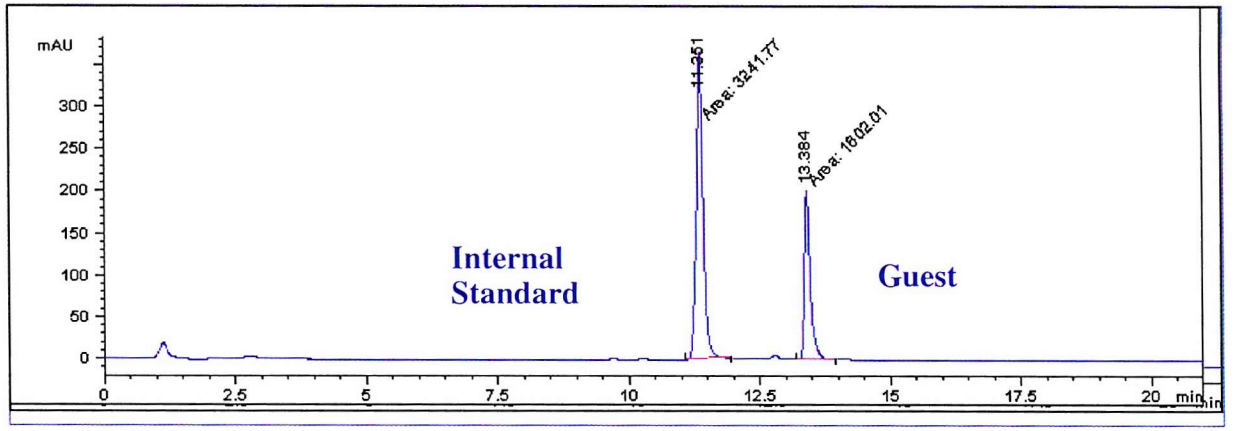


Standard solution of Red dye-D-Glu-D-Ser-D-Val-OH (guest) 101 and Red dye 112 (internal standard)

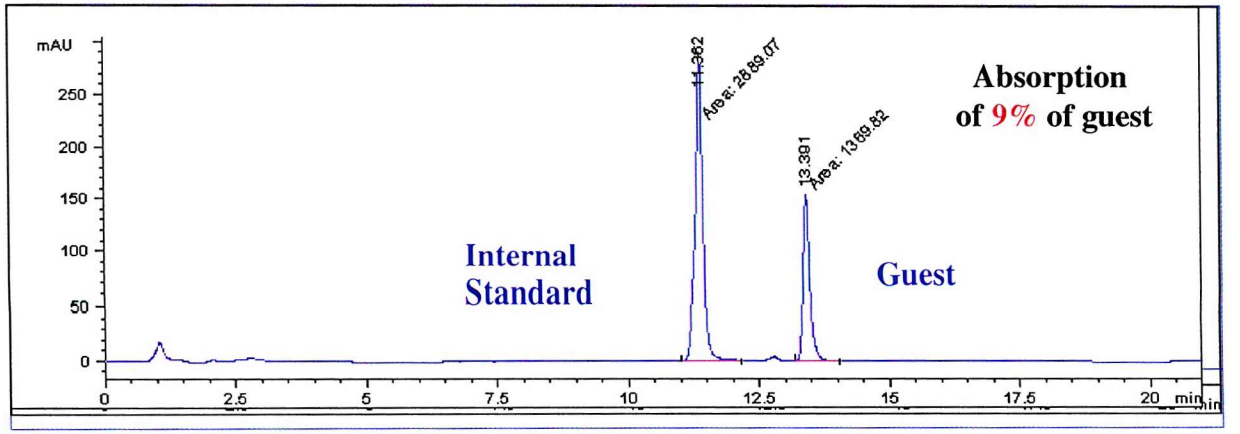


Solution of Red dye-D-Glu-D-Ser-D-Val-OH (guest) 101 and Red dye 112 after 24 h of incubation

Figure II-9 Binding experiment Red dye-spacer-D-Glu-D-Ser-D-Val-OH 101



Standard solution of Red dye-L-Glu(O*tBu*)-*L*-Ser(*t*Bu)-L-Val-OH (guest) 99 and Red dye (internal standard)



Solution of Red dye-L-Glu(O*tBu*)-L-Ser(*t*Bu)-L-Val-OH (guest) 99 and Red dye 112 after 24 of incubation

Figure II-10 Binding experiment withRed dye-spacer-L-Glu(OtBu)-L-Ser(tBu)-L-Val-OH 99

II.6 Discussion

The screening of the combinatorial library **B** of diamidopyridine derived tweezer receptors led to the identification of a relatively limited number of tweezer structures from a sizeable library that appear to bind the chosen peptide guest **100**, while attached to the solid-phase. The selective binding properties of one of these receptors **110** (Scheme II-12), while attached to the solid phase is demonstrated by the ability to discriminate between peptide **100** (Figure II-3) and the side chain protected analogue **99** by a factor of ~400 and by the ability to discriminate between peptide **100** and its enantiomer **101** by a factor of ~ 17. The screening results using the same peptide guest **100** with the two receptor libraries **A** (Figure II-5) and **B** (Scheme II-9) gave sequences with clear structural similarities at the first and second positions, which suggested that there are similar interactions between the backbone of the peptide guest and the tweezer receptor side-arms.

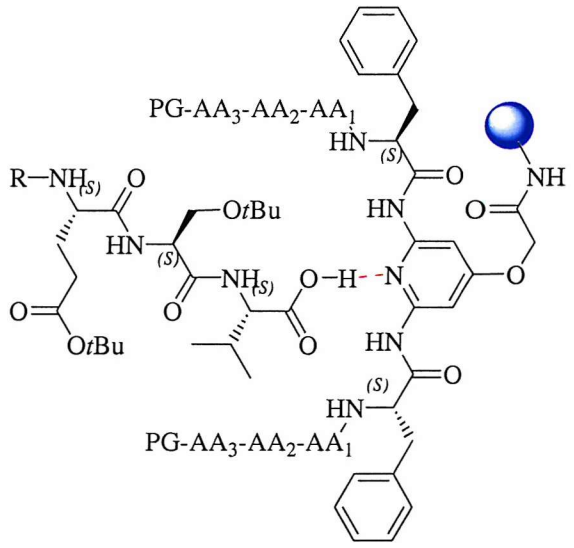


Figure II-11 Binding of the side-chain deprotected peptide with the glutamic acid carboxylic acid.

The side-chain protected peptide **99** however, interacts very differently with the two libraries **A** and **B**, with excellent selectivity observed in the screening experiments with library **A** and no apparent selectivity in the screening experiments with library **B**.

A possible explanation for these observations is that for the side-chain protected peptide **99** with receptors derived from library **A**, binding involves a strong amidopyridine-carboxylic acid interaction that places the backbone of the peptide guest in a suitable position to bind with the tweezer side-arms of the receptor, possibly using parallel β-sheet like interactions (Figure II-11).

In comparison, introduction of an additional methylene group to the side-arm of the receptor (i.e. using library **B** derived from amidopyridine derivative **97**), disrupted the alignment of the backbone of the peptide guest in relation to the side-arms of the receptor and no selective binding was observed.

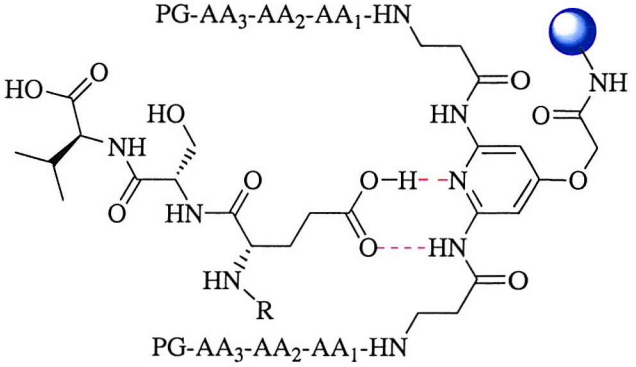


Figure II-12 Binding of the side-chain protected peptide with the C-terminal carboxylic acid.

Binding of the deprotected peptide **100** on the other hand, is complicated by the fact that either the terminal carboxylic acid moiety of the peptide (Figure II-12) or the side-chain carboxylic acid moiety from the glutamic acid unit may bind to the amidopyridine. If the glutamic acid side-chain carboxylic acid is used, the link from the carboxylic acid unit to the rest of the peptide backbone is both longer and more flexible such that the increased separation of the amidopyridine unit and the receptor side-arms in library **B** become more appropriate for binding the peptide guest. However, the increased flexibility of the side-chain carboxylic acid also allows binding with tweezers from library **A** (Figure II-5). Unfortunately, although we have been able to prepare individual tweezer receptors identified from the screening experiments, the poor solubility of the receptors off-resin and in the non-polar solvents suitable for binding (that is, chloroform) has precluded any detailed studies (eg. by NMR) on the structure of the receptor-substrate complex, so that the proposed differences in binding cannot be

confirmed experimentally. Indeed, in the present case the complete insolubility of tweezer receptor **107** in chloroform means that no binding in free solution can be demonstrated. This is clearly a limitation of this type of receptor and begs the question as to whether the binding properties of these systems, while resin-bound, is influenced by the environment created by the solid support.

II.7 Conclusion and Outlook

The studies described here demonstrate the power of the combinatorial approach for identifying receptors for a chosen substrate, but also explain some of the limitations of the approach when using diamidopyridine derived tweezers. It is clear from this and earlier work, that such diamidopyridine derived tweezers, despite their inherent flexibility, can act as receptors for specific peptide sequences particularly when attached to the solid-phase. These receptors may therefore have applications, particularly in the development of chromatographic supports for the separation or purification of peptides. However, the diamidopyridine-carboxylic acid interaction is probably too weak to promote peptide binding by the tweezer receptors other than in relatively non-polar solvents in which the receptors may have poor solubility—thus limiting their usefulness. There is also very little information regarding the actual mode of binding either in free solution or while the receptor is still attached to the solid-phase. Further studies are aimed at answering the unresolved questions regarding the mode of binding of this class of tweezer receptor and are also evaluating the binding properties of monolayers of such receptors while attached to a surface. However, it is clearly desirable to extend the concept of screening receptor libraries to libraries suitable for peptide recognition in water. Successful realisation of this objective and a clear understanding of the structure of resulting tweezer-substrate complexes formed in water has considerable potential for providing model systems of peptide-peptide interactions and application to a range of therapeutic problems.

Chapter III

III.1 Collaboration within EU Network

In this chapter work conducted as part of a collaborative program within a European Network consisting of Prof. Gennari group (Univ. of Milan), Prof. Anthony Davis (Univ. of Bristol), Prof. Javier De Mendoza (Univ. of Madrid), Prof. Jeremy D. Kilburn and DSM (Netherlands) will be described.

The overall aim of the project was the development of effective enantioselective receptors suitable for use in medium to large-scale separations of racemic mixtures of carboxylic acid derivatives. The receptor structures were developed by rational design and the combinatorial approach.

The groups in Madrid and Bristol were involved in developing enantioselective receptors suitable for extracting carboxylic acid derivatives from aqueous to organic solution and testing these in a prototype 'aqueous-organic-aqueous' membrane system (DSM) with appropriate racemic mixtures (aromatic carboxylic acids, naproxen etc).

Finally, the group in Southampton and Milan were interested in developing receptors for enantioselective extraction of carboxylic acids from organic solution into aqueous solution based upon guanidinium and diamidopyridine systems. Furthermore, there was great interest in synthesising combinatorial libraries of potential enantioselective receptors and in the development of screening procedures to identify particularly selective receptors that would subsequently be tested in the DSM system.

III.2 Collaboration with the Milan Group

III.2.1 Synthesis, Conformational Studies and Binding Properties of Acyclic Receptors.

Following the research conducted at the University of Southampton on developing a novel class of peptide receptors called tweezer receptors [112-115,136], the group in Milan focused on the synthesis of receptors based on peptidosulfonamide side arms. The conformational properties of unnatural biopolymer scaffolds containing β-sulfonamidopeptides [147] and vinylogous sulfonamidopeptides [148] were studied.

The sulfonamidopeptide backbone structure contains both hydrogen-bond donors (strong: CONH; very strong: SO_2NH) and hydrogen-bond acceptors (strong: CO; weak: SO_2N), a framework that is essential for the formation of folded structures by intramolecular hydrogen bonding. Following the successful synthesis of tweezer receptor structures that make use of a diamidopyridine moiety as the head group (Chapter II), the group in Milan focused on the synthesis of tweezer receptors featuring sulfonamidopeptide units as side arms. Initial studies were conducted on rationally designed symmetrical structures. The synthesis was accomplished on solid phase from symmetrically protected 2,6-diamidopyridine (Figure III-1).

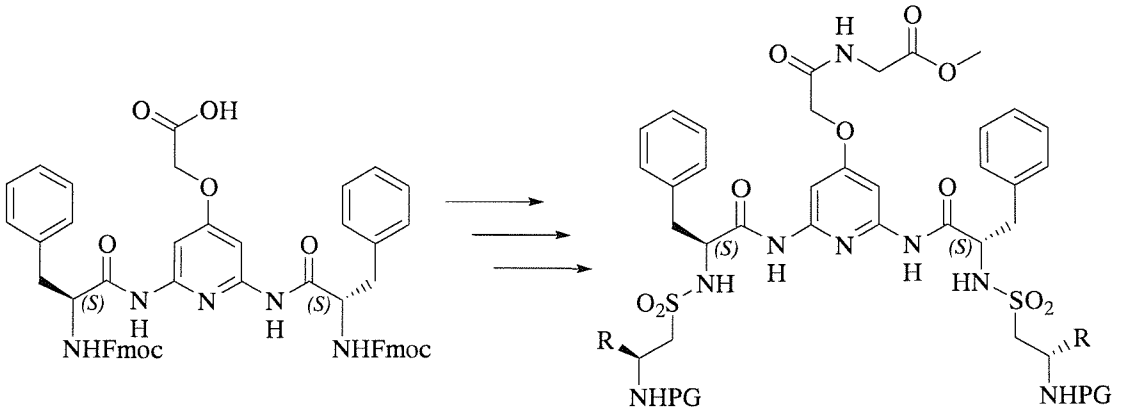


Figure III-1 Synthesis of tweezer receptors

Conformational studies of sulfonamidopeptide tweezer structures **113-115** (Figure III-2) were conducted by NMR spectroscopy. Similar results were obtained with structures **113** and **114**. In both cases the tweezer collapses into intramolecularly folded structures stabilised by a network of intramolecular hydrogen bonds in $CDCl_3$ solution.

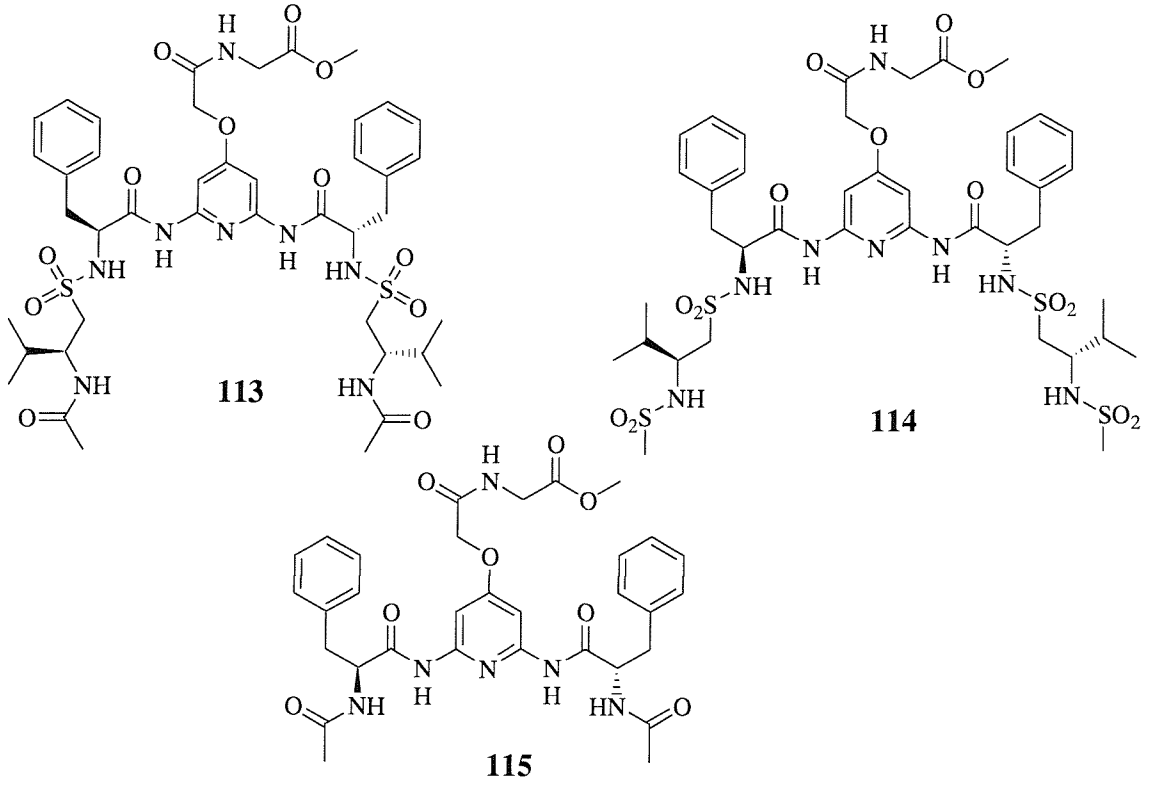
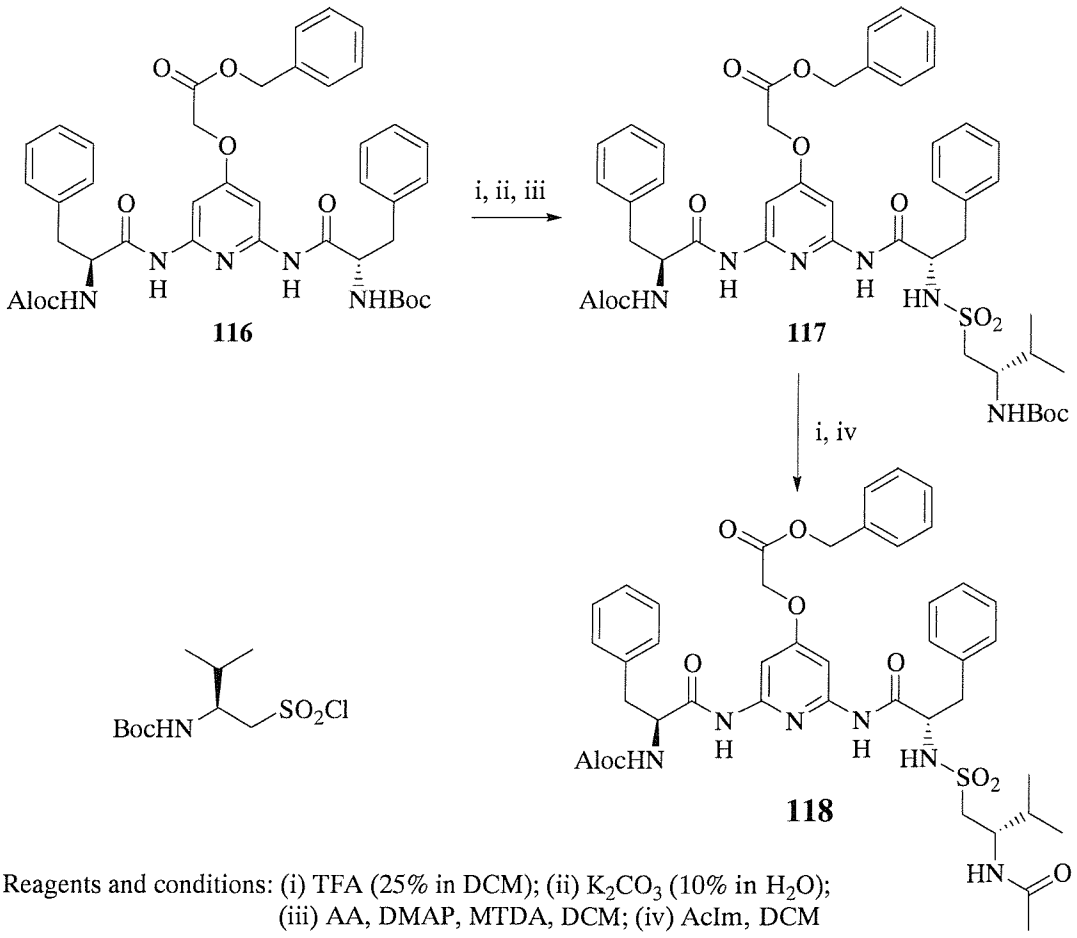


Figure III-2 Two-arm receptors 113-115

In structure **114** (Figure III-2) the presence of a poor hydrogen-bond acceptor such as the methanesulfonamide group in the terminal position was compensated for by the very strong hydrogen bond donating ability of the methanesulfonamide NH group.

Such conclusions were supported by NOESY and ROESY experiments, which revealed a large number of NOE contacts consistent with a folded structure. In contrast, control experiments conducted on structure **115** (Figure III-2) suggested that no aggregates were formed in that case within the concentration range under consideration.

In order to obtain a simplified view of the system and to study the properties of receptors featuring only one leg, a differently substituted scaffold was synthesised starting from the orthogonally protected CBS **116** (Scheme III-1).



Reagents and conditions: (i) TFA (25% in DCM); (ii) K_2CO_3 (10% in H_2O); (iii) AA, DMAP, MTDA, DCM; (iv) AcIm, DCM

Scheme III-1 Synthesis of the single leg receptor118

Conformational studies on compound **118** revealed that the hydrogen-bonding network in **113** and **115** was strengthened by the cooperative effect of both legs.

Furthermore, to investigate the effect of the length of the legs and possibly to increase the affinity of the tweezer receptors, structures **113** and **119** featuring sulfonamidopeptide legs of different length were synthesised (Figure III-3).

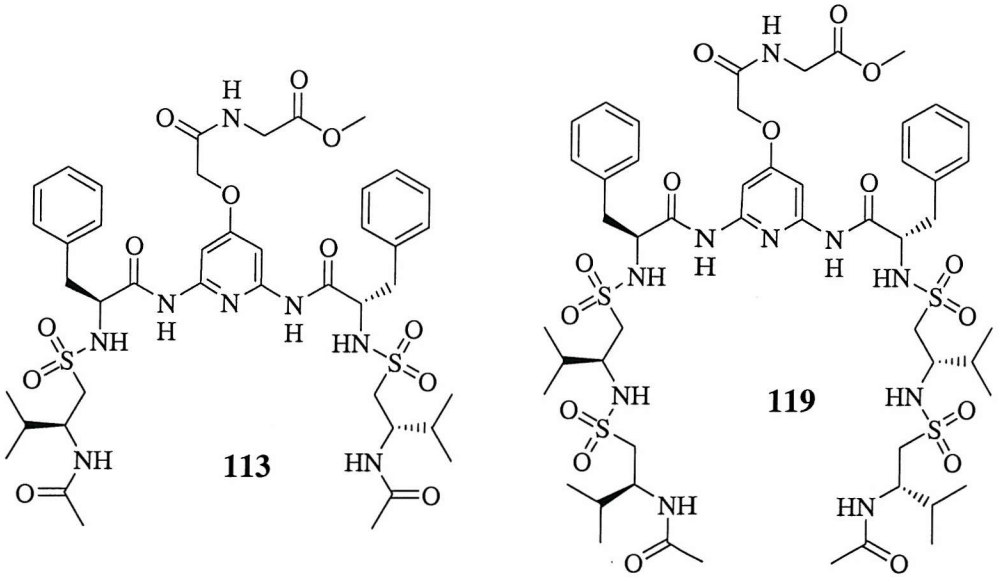


Figure III-3 Acyclic receptors for N-protected amino acids and dipeptides 113 and 119

Binding studies conducted on hosts **113** and **119** (Figure III-3) confirmed that binding involves a degree of unfolding of the receptor, and breaking of intramolecular hydrogen bonds (with an associated energetic cost) to allow interaction with the guest, resulting in rather low binding constants (Table III-1). Interesting enantioselectivity was shown by receptor **119** in the recognition of *N*-Cbz-D-Ala- D-Ala-OH. Binding of this guest was sufficiently strong to overcome the penalty of unfolding the receptor. Such high enantioselectivity (>20:1) has rarely been observed in synthetic receptors.

	K_{ass}/M^{-1} Host 113	**K_{ass}/M^{-1} Host 119**
N-Cbz-L-Ala-OH	207	32
N-Boc-L-Ala-OH	119	
N-Cbz-L-Ala-OH	270	
N-Cbz-L-Ala-L-Ala-OH	245	107
N-Boc-L-Ala-L-Ala-OH	361	
N-Cbz-D-Ala-D-Ala-OH	242	2404

Table III-1 Binding Constants K_{ass} (M^{-1}) for the 1:1 complex formed between receptors 113 and 119 and various amino acids and dipeptide derivatives calculated from the chemical shifts of various signals of 113 and 119, in $CDCl_3$ at 25 °C.

Once again it was confirmed that tweezer receptors despite their inherent flexibility are capable of selective binding as confirmed by the result obtained with similar structures described in Chapter II.

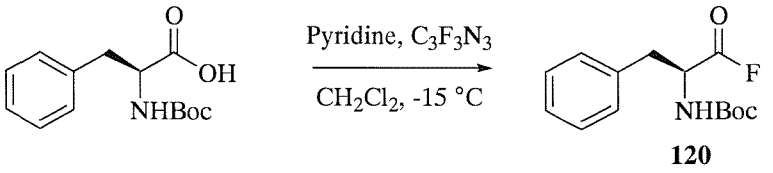
Following the successful synthesis of tweezer receptors employing the combinatorial approach, it was decided to pursue a combinatorial approach to the binding of dipeptides by screening a library of receptors against a selected template or by the screening of a library of dipeptides against a single receptor. Additional degrees of library diversification could be achieved by using the orthogonally protected CBS (carboxylic acid binding site) as a starting point for the generation of tweezer arms.

III.2.2 Synthesis of the Orthogonally Protected 2,6 Diamidopyridine CBS

The focus of our efforts in this project was to synthesise the orthogonally protected 2,6-diamidopyridine **116** featuring Phe as first amino acid for the generation of the sulfonamidopeptide arms.

The synthesis of this CBS was not straightforward in comparison with the synthesis of the symmetrical CBS developed in Southampton (Chapter II).

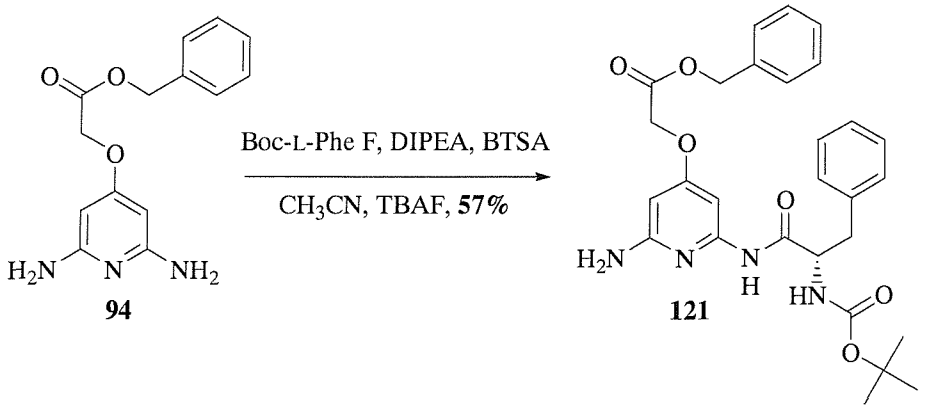
The synthesis of 2,6-diaminopyridine **94** (Scheme III-3) was performed following the methodology already described in Chapter II. For this purpose *N*-Boc-L-Phe-OH was first converted to *N*-Boc-L-Phe-F **120** using cyanuric fluoride $(CFN)_3$ in quantitative yield (Scheme III-2). [138,139]



120

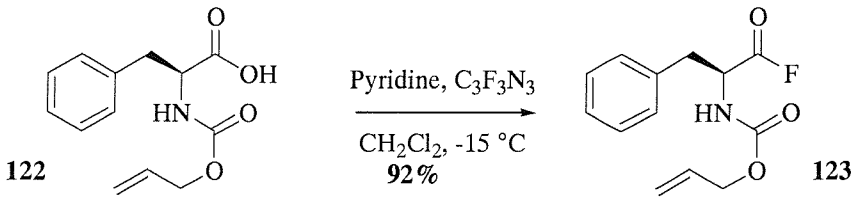
Scheme III-2 Synthesis of N-Boc-L-Phe-F 120

The coupling of *N*-Boc-L-Phe-F **120** with the free diamine **94** was carried out using BTSA (bis(trimethylsilyl)acetamide) and TBAF in dry acetonitrile to give the intermediate **121** in 57 % yield (Scheme III-3).



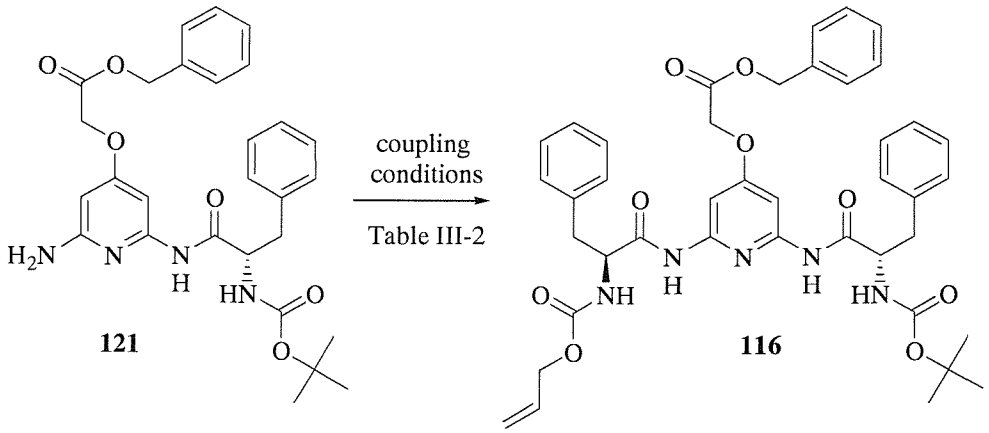
Scheme III-3 Synthesis of intermediate 121

The moderate yield was due to the complex reactivity of the system. Using an excess of the *N*-Boc-L-Phe-F (2 eq.) the bis coupling occurs in 10 % yield.



Scheme III-4 Synthesis of N-Alloc-L-Phe-F

Several methodologies were investigated to synthesise product **116** (Scheme III-5) as reported in Table III-2. Coupling of *N*-Alloc-L-Phe-F **123** (Scheme III-4) with **121** using BTSA (Bis(trimethylsilyl)acetamide) and MTDA (Methyl trimethylsilyl dimethylketene acetal) gave the desired product **116** (Scheme III-5) in 92 % yield (Table III-2).



Scheme III-5 Synthesis of orthogonally protected 2,6-diamidopyridine CBS 116

Amino Acid	Coupling conditions	Temperature	Yield
N-Alloc-L-Phe-OH	HOBt, DCC, $HCON(CH_3)_2$	rt.	No Product
N-Alloc-L-Phe-OH	HOBt, DCC, $HCON(CH_3)_2$	50 °C	No Product
N-Alloc-L-Phe-OH	HOBt, DCC, $HCON(CH_3)_2$, CH_2Cl_2	70 °C	24%
N-Alloc-L-Phe-OH	PyBOP, DIPEA, $HCON(CH_3)_2$	rt.	No Product
N-Alloc-L-Phe-OH	PyBOP, DIPEA, $HCON(CH_3)_2$	70 °C	No Product
N-Alloc-L-Phe-OH	PyBrOP, DIPEA, $HCON(CH_3)_2$	70 °C	12%
N-Alloc-L-Phe-F	BTSA, TBAF, DIPEA, dry CH_3CN	rt	No Product
N-Alloc-L-Phe-F	BTSA, TBAF, DIPEA, dry CH_3CN	70 °C	No Product
N-Alloc-L-Phe-F	BTSA, MTDA, DIPEA, dry CH_3CN	70 °C.	92%

Table III-2 Coupling conditions to synthesise the 2,6-diamidopyridine CBS 116

Thus orthogonally protected 2,6-diamidopyridine CBS **116** (Scheme III-5) was successfully synthesised.

Product **116** (Scheme III-5) was used by the Milan team to synthesise a differently substituted receptor in order to investigate binding properties of acyclic receptors. In particular it was used to synthesise a receptor featuring only one leg **118** (Scheme III-1)

III.3 Collaboration with the Bristol group

Professor Davis` group (University of Bristol) focused their research on designing enantioselective receptors for amino acid derivatives based on cholic acid. They were interested in receptors based on cholic acid in order to use the chirality of the steroidal framework to achieve enantioselective recognition. They developed steroidal guanidinium cations (Figure III-4) capable of extracting chiral carboxylates from the aqueous phase with encouraging enantioselectivities. [149-151]

In collaboration with the group of Prof. Davis a combinatorial library of cholic acid derivative receptors on solid phase was investigated.

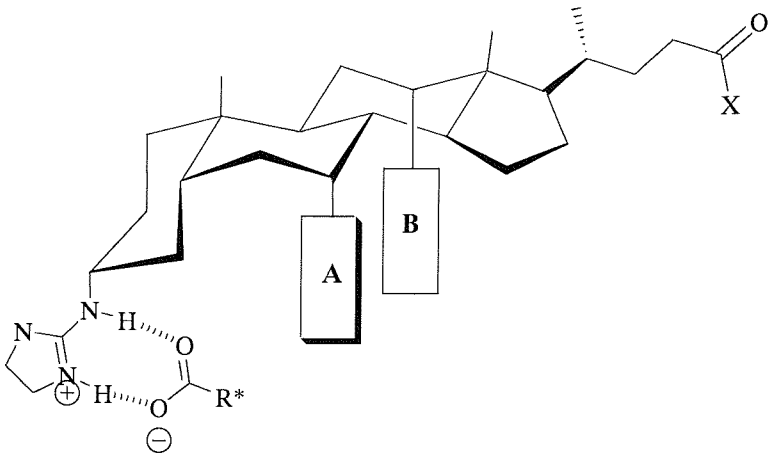


Figure III-4 General structure for steroidal guanidinium cations.

The synthesis of a library of receptors was carried out using the 'split and mix' combinatorial strategy (Chapter II).

Screening experiments as previously described (Chapter II) were carried out with the receptor library using the dye-labelled peptide guests Red dye-spacer-L-Ala-L-Ala-OH **125** and Red dye-spacer-D-Ala-D-Ala-OH **126** (Figure III-6).

The synthesis of the structure identified by screening experiments was attempted in solution but the synthesis route was shown to be unrealistically long. For this reason it was resynthesised on solid phase as a resin bound receptor **124** (Figure III-5).

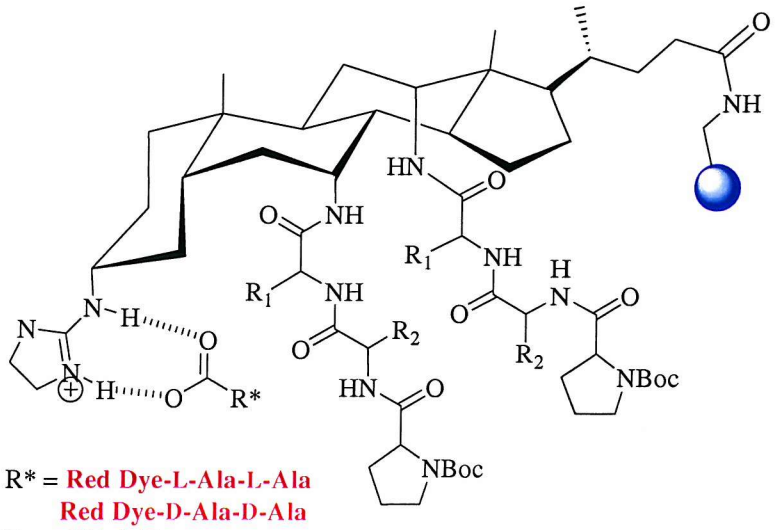


Figure III-5 Resin-bound receptor 124

Our previous studies have shown that as an alternative to conducting binding studies with the tweezer receptor free in solution, the resin bound tweezer receptor was capable of showing genuine selectivity by adding portions of the resin-bound tweezer to solutions of the guest which were subsequently adsorbed onto the resin beads. [146,136] A similar experiment was carried out with compound **124** (Figure III-5). The resin bound receptor **124** was synthesised (Prof. Davis' group) on solid phase with an estimated receptor loading of 0.28 mmol/g resin.

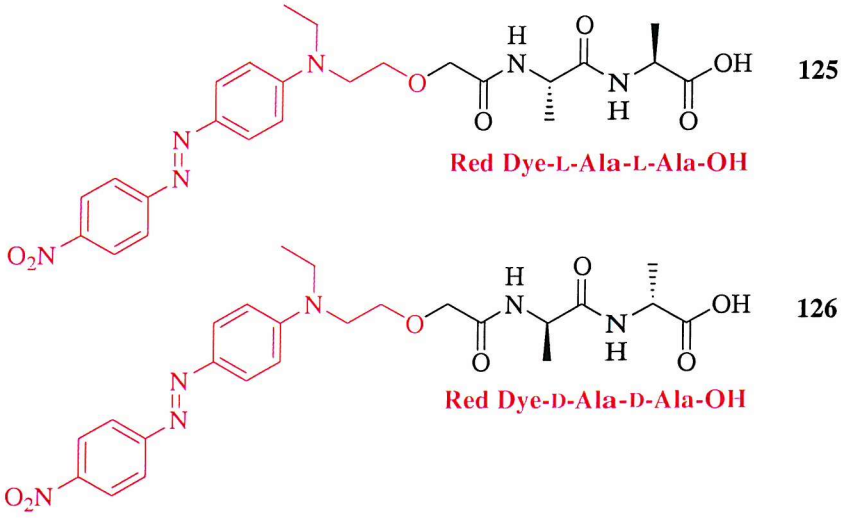
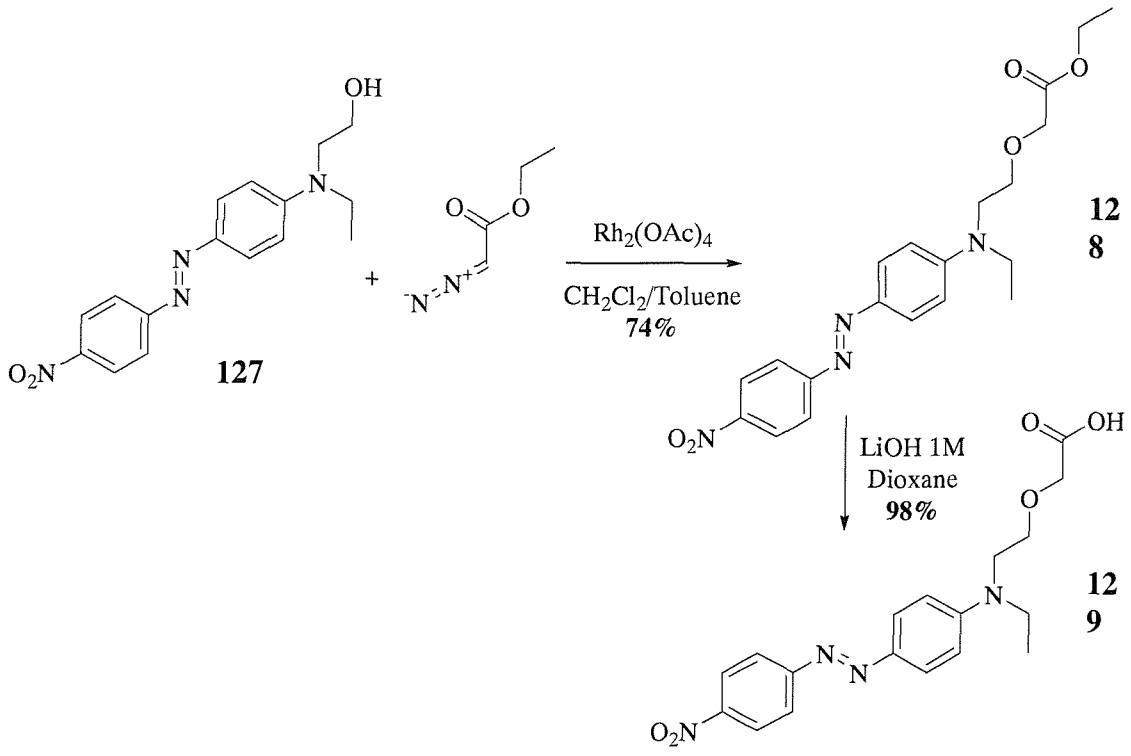


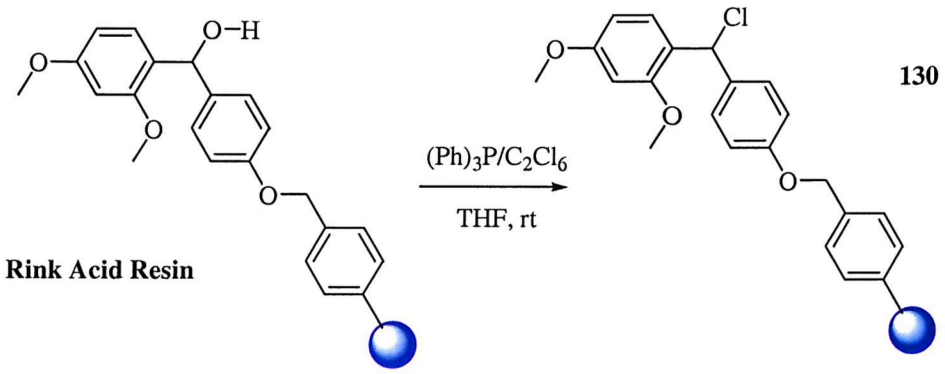
Figure III-6 Dye labelled peptide guests 125 and 126

The synthesis of the guests, Red Dye-spacer-L-Ala-L-Ala-OH **125** (Scheme III-8) and Red Dye-spacer-D-Ala-D-Ala-OH **126** (Scheme III-9) was carried out on solid phase. Disperse Red Dye **127** was previously functionalised with a carboxylic acid moiety (Scheme III-6). [152,153]



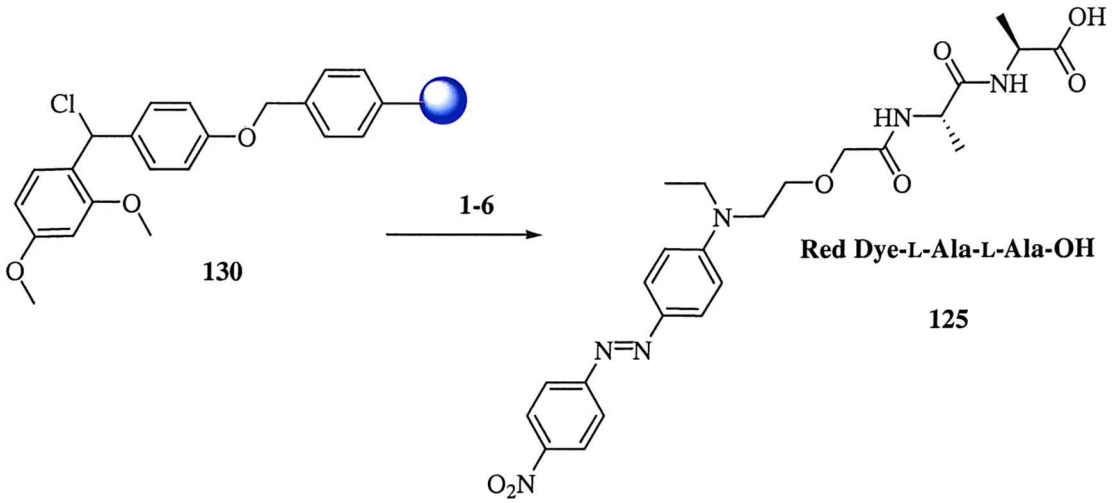
Scheme III-6 Synthesis of the Red Dye derivative 129

Disperse Red Dye **127** was reacted with ethyl diazoacetate in the presence of a catalytic amount of rhodium tetra-acetate to afford the ester derivative **128** (Scheme III-6). Hydrolysis of ester **128** gave the functionalised Red Dye **129** ready to be coupled on solid phase. The resin of choice was the Rink resin. The greater acid sensitivity of Rink linkers is due to the presence of electron-donating methoxy groups.



Scheme III-7 Rink chloride resin 130

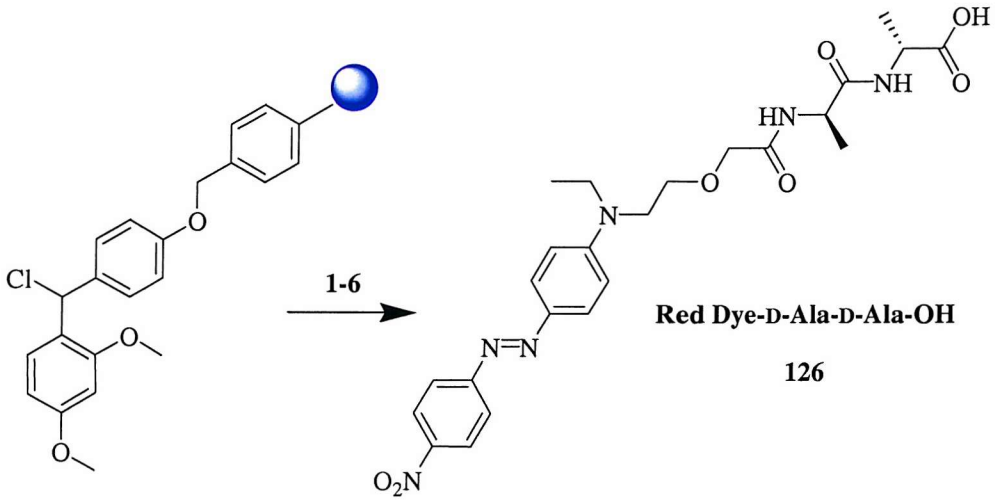
These groups provide considerable stabilisation of the intermediate carbonium ion generated under acidic cleavage conditions. Rink resin was first transformed in Rink-chloride **130** [154] by treatment with hexafluoroethane and triphenylphosphine (Scheme III-7). The dipeptide -L-Ala-L-Ala was synthesised on solid phase by standard Fmoc synthesis, while final coupling of Red Dye unit functionalised with a terminal carboxylic acid and cleavage with 10 % AcOH in dichloromethane afforded Dye labelled peptide Red Dye-spacer-L-Ala-L-Ala-OH **125** (Scheme III-8).



1. Fmoc-L-Ala-OH, DIPEA, $C_2H_4Cl_2$; **2.** 20% Piperidine in DMF; **3.** Fmoc-L-Ala-OH, DIC, HOBt, DIPEA, DMF; **4.** 20% Piperidine in DMF; **5. 129**, DIC, HOBt, DIPEA, DMF; **6.** 10% AcOH in CH_2Cl_2

Scheme III-8 Synthesis of Red Dye-spacer-L-Ala-L-Ala-OH 125

The synthesis of the enantiomer Red Dye-spacer-D-Ala-D-Ala-OH **126** (Scheme III-9) was performed following the general procedure described above.



1. Fmoc-D-Ala-OH, DIPEA, $C_2H_4Cl_2$, **2.** 20% Piperidine in DMF, **3.** Fmoc-D-Ala-OH, DIC, HOBt, DIPEA, DMF; **4.** 20% Piperidine in DMF; **5. 129**, DIC, HOBt, DIPEA, DMF; **6.** 10% AcOH in CH_2Cl_2

Scheme III-9 Synthesis of Red Dye-spacer-D-Ala-D-Ala-OH 126

Addition of 1.0 mg of resin-bound receptor (~ 0.28 μmol of receptor) to a solution of Red dye-spacer-L-Ala-L-Ala-OH **125** (0.3 mM) and Red dye-spacer-D-Ala-D-Ala-OH **126** (0.3 mM) in buffer solution (pH 8.5) led to adsorption of the peptide as determined by HPLC (using Red Dye as an external standard) after several hours of incubation (Table III-3-5). Since the addition of acetyl-capped tentagel resin beads led to significant adsorption of the peptides, the same experiment was also repeated on acetyl-capped tentagel to estimate the adsorption of the resin.

The experiment was carried out by adding a known quantity of resin (1 mg) to solutions of guest that contained different quantities of the peptide (1/2 eq, 1 eq., 1.5 eq.).

Red-Dye-D-Ala-D-Ala-OH 0.3 mM (Borax 0.025 M)				
1/2 equivalent of guest	**STD**	**3 h**	**24 h**	**48 h**
A % GUEST	39.2350	27.6228	19.1985	11.1960
A%REF	60.7650	72.3772	80.8015	80.8040
A% GUEST / A% REF	0.6457	0.3817	0.2376	0.1386
% Guest extract		**40.89**	**63.20**	**78.54**
Red-Dye-L-Ala-L-Ala-OH 0.3mM (Borax 0.025 M)				
1/2 equivalent of guest	**STD**	**4 h**	**24 h**	**48 h**
A % GUEST	42.7013	26.7468	26.6357	26.1199
A%REF	57.2987	73.2532	73.3643	73.8801
A% GUEST / A% REF	0.7452	0.3651	0.3631	0.3535
% Guest extract		**51.01**	**51.28**	**52.56**
Tentagel-Ac/Red-Dye-D-Ala-D-Ala-OH 0.3mM (Borax 0.025 M)				
1/2 equivalent of guest	**STD**	**4 h**	**24 h**	**48 h**
A % GUEST	39.2350	26.6421	25.2996	23.4067
A%REF	60.7650	73.3579	74.7004	76.5903
A% GUEST / A% REF	0.6457	0.3632	0.3387	0.3056
% Guest extract		**43.75**	**47.55**	**52.67**

Table III-3 HPLC experiment 1/2 equivalent of guest (Borax 0.025 M).

Red-Dye-D-Ala-D-Ala-OH 0.3mM (Borax 0.025 M)				
1 equivalent of guest	**STD**	**3 h**	**24 h**	**48 h**
A % GUEST	38.5295	24.5838	24.9413	21.4806
A% REF	61.4705	75.4162	79.0587	78.5194
A% GUEST / A% REF	0.6268	0.3260	0.3155	0.2736
% Guest extract		**47.99**	**49.67**	**56.35**
Red-Dye-L-Ala-L-Ala-OH 0.3mM (Borax 0.025 M)				
1 equivalent of guest	**STD**	**3 h**	**24 h**	**48 h**
A % GUEST	42.7013	26.7468	26.6357	26.1199
A% REF	57.2987	73.2532	73.3643	73.8801
A% GUEST / A% REF	0.7452	0.3651	0.3631	0.3535
% Guest extract		**51.01**	**51.28**	**52.56**
Tentagel-Ac/Red-Dye-D-Ala-D-Ala-OH 0.3mM (Borax 0.025 M)				
1 equivalent of guest	**STD**	**3 h**	**24 h**	**48 h**
A % GUEST	38.5295	26.6421	25.2996	23.4097
A% REF	61.4705	73.3579	74.7004	76.5903
A% GUEST / A% REF	0.6268	0.3632	0.3387	0.3056
% Guest extract		**42.06**	**45.97**	**51.24**

Table IH-4 HPLC experiment 1 equivalent of guest (Borax 0.025 M).

Red-Dye-D-Ala-D-Ala-OH 0.3 mM (Borax 0.025 M)			
1.5 equivalent of guest	**STD**	**24 h**	**48 h**
A % GUEST	38.5295	26.7495	27.0569
A%REF	61.4705	73.2505	72.9431
A% GUEST / A% REF	0.6268	0.3652	0.3709
% Guest extract		**41.74**	**40.82**
Red-Dye-L-Ala-L-Ala-OH 0.3 mM (Borax 0.025 M)			
1.5 equivalent of guest	**STD**	**24 h**	**48 h**
A % GUEST	42.7013	34.0935	34.4328
A%REF	57.2987	65.9065	65.5672
A% GUEST / A% REF	0.7452	0.5173	0.52515282
% Guest extract		**30.59**	**29.53**
Tentagel-Ac/Red-Dye-D-Ala-D-Ala-OH 0.3 mM (Borax 0.025 M)			
1.5 equivalent of guest	**STD**	**24 h**	**48 h**
A % GUEST	38.5295	30.4912	29.2107
A%REF	61.4705	69.5088	70.7893
A% GUEST / A% REF	0.6268	0.4387	0.412642871
% Guest extract		**30.01**	**34.17**

Table III-5 HPLC experiments 1.5 equivalent of guest (Borax 0.025 M).

The results obtained were not sufficient to allow a good estimation. However further investigations, conducted by the bristol team, on the structure on solid phase **124** proved the the latter was not correct. Consequently, the experiments were abandoned.

III.4 Collaboration with the University of Madrid.

Naproxen (Figure III-7) was introduced to the market by Syntex in 1976 as a non-steroidal anti-inflammatory drug (NSAID). Studies on biological activity revealed the importance of the α-methyl group and its (*S*) configuration. [155]

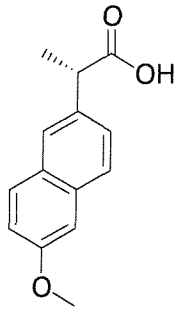


Figure III-7 Naproxen: NSAID Non steroidal anti-inflammatory drugs

Naproxen and all the NSAIDs (including Aspirin) have therapeutic effects as a result of the inhibition of the enzyme COX (cyclo-oxygenase), also known as PGHS (prostaglandin H_2 synthase).

Naproxen acts as a competitive inhibitor of arachidonic acid interacting with Arg 120 in the enzyme. Naproxen blocks the hydrophobic channel such that arachidonic acid cannot be bound and so be converted to prostaglandin.

Several methods for the synthesis of chiral (*S*)-Naproxen have been developed. An economic study of all the industrial syntheses showed that all of the routes are more expensive than the racemic synthesis developed by Syntex. [156]

Chiral chromatography and bio-transformations are other methods used in the industrial resolution of racemates. Methods like BLM (bulk liquid membrane) transport are very interesting but cannot be scaled up for industrial purposes.

Prof. De Mendoza`s group (the University of Madrid) was interested in a multi-stage continuous current extraction (from the aqueous layer to the organic). To be of economic interest extraction selectivity needs to be in the order of three to one or higher.

Several receptors (Figure III-8) have been designed for selective binding of (*S*)-Naproxen. Although in some cases studies reported observation of two different complexes, none of these reported any enantioselective recognition of Naproxen. [157-163]

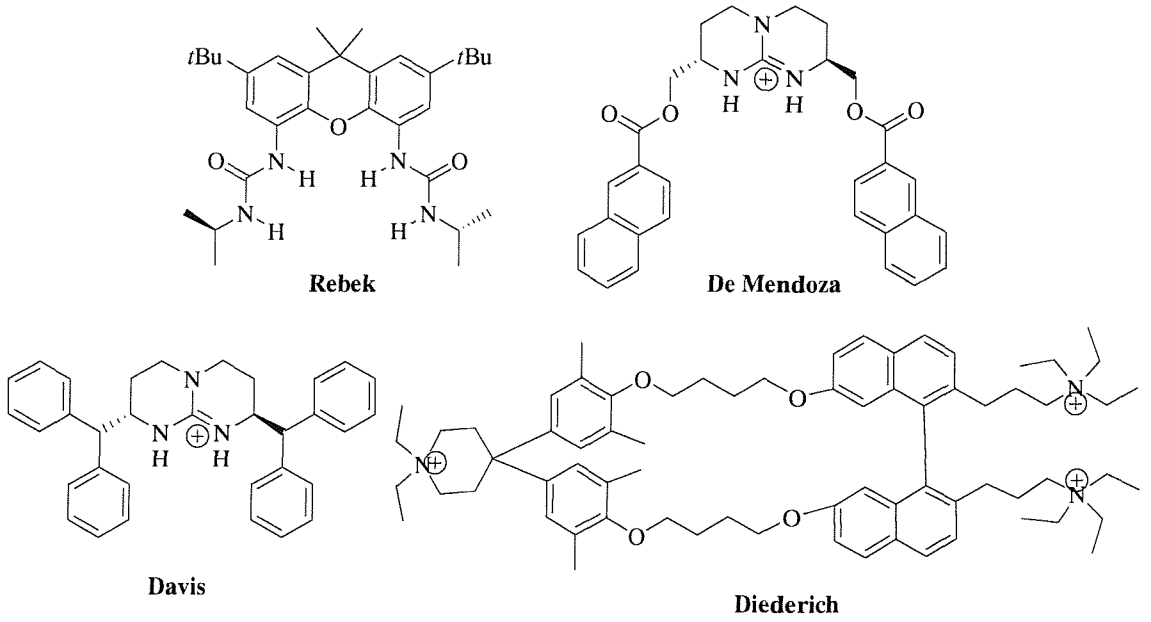


Figure III-8 Receptors for selective binding of (S)-Naproxen

Nature uses guanidinium moieties to coordinate different anionic groups. In order to improve its binding strength, the guanidinium group can be incorporated into a bicyclic framework. As a result, the hydration of the cation by accumulation of hydrophobic hydrocarbon residues is reduced and conformational freedom is restricted. Forming part of a bicyclodecane, the guanidinium cation becomes a perfect complement for carboxylate anions, with both protons being docking sites for the two syn lone pairs of the carboxylate. [48,49]

Initial studies for chiral recognition of Naproxen were based on the bicyclic guanidinium moiety. Further studies proved that an additional bond helps the fixture of the guest (Figure III-9). Calculations showed that for an effective hydrogen bond there should be five atoms between the guanidinium nitrogen and the donor.

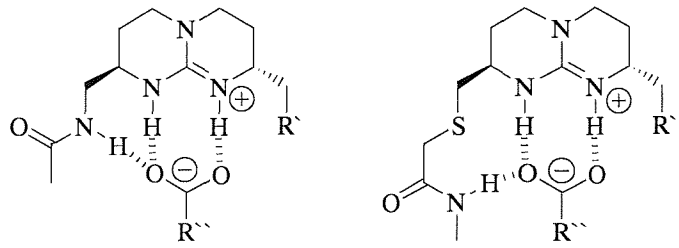


Figure III-9 Improvement in the efficiency of the additional hydrogen bond.

Studies by rational design identified three different features essential for chiral recognition: the carboxylate, the aromatic rings and the chiral methyl group. The carboxylate interacts strongly with the guanidinium moiety, while the additional hydrogen bond with the amide group helps in fixing the carboxylate and in placing the aromatic surfaces for interaction (π–π interactions) (Figure III-10).

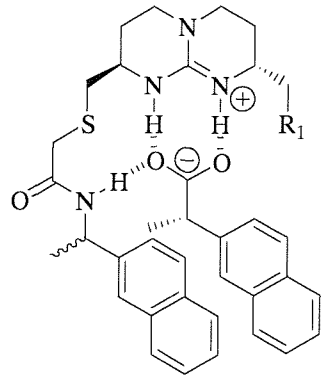
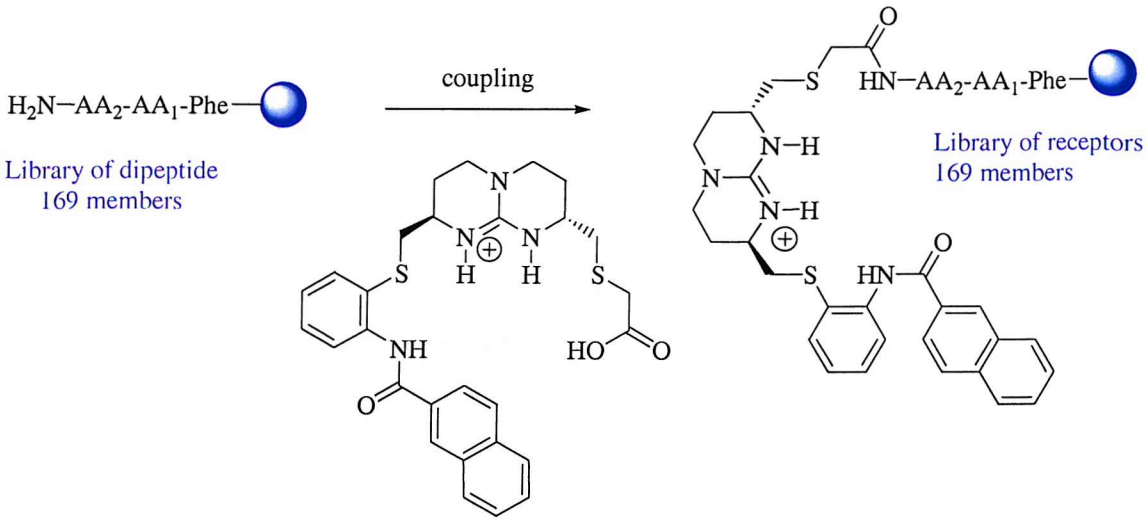
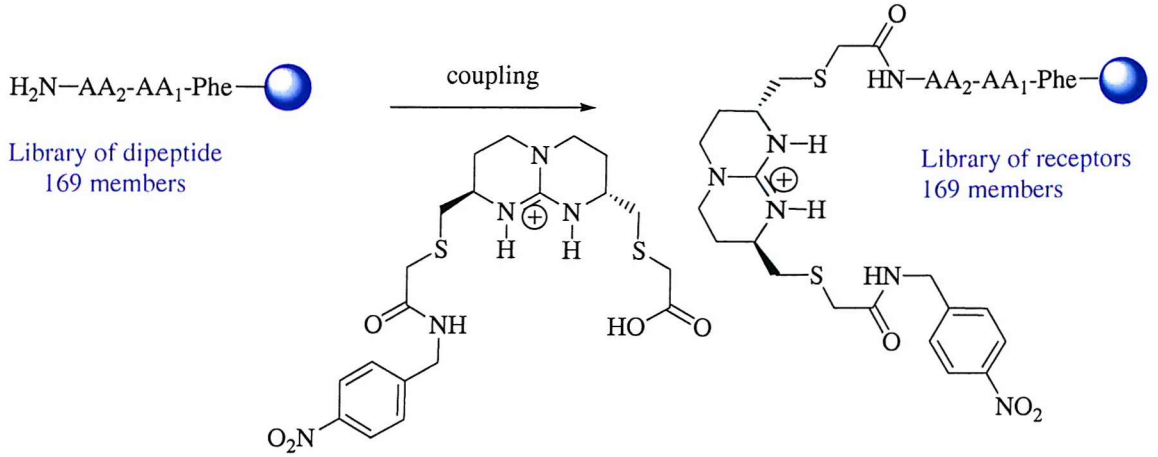


Figure III-10 Guanidinium-carboxylate interaction

Finally, the second arm, responsible for the chiral discrimination could be introduced by the combinatorial approach. A library of dipeptides could increase the number of chiral centres in the receptor and the presence of peptide bonds could increase the number of hydrogen bonds between host and guest. Libraries of receptors (13^2 =169 members) were synthesised by the split and mix strategy.



Scheme III-10 Synthesis of library MVG 102



Scheme III-11 Synthesis of library MVG 138

Therefore, it was decided to screen the libraries MVG 102 (Scheme III-10) and MVG 138 (Scheme III-11) against the enantiomers of the dye-labelled Naproxen (Figure III-11). In the first instance, the screening was conducted against the (*S*)-Naproxen in $CHCl_3$.

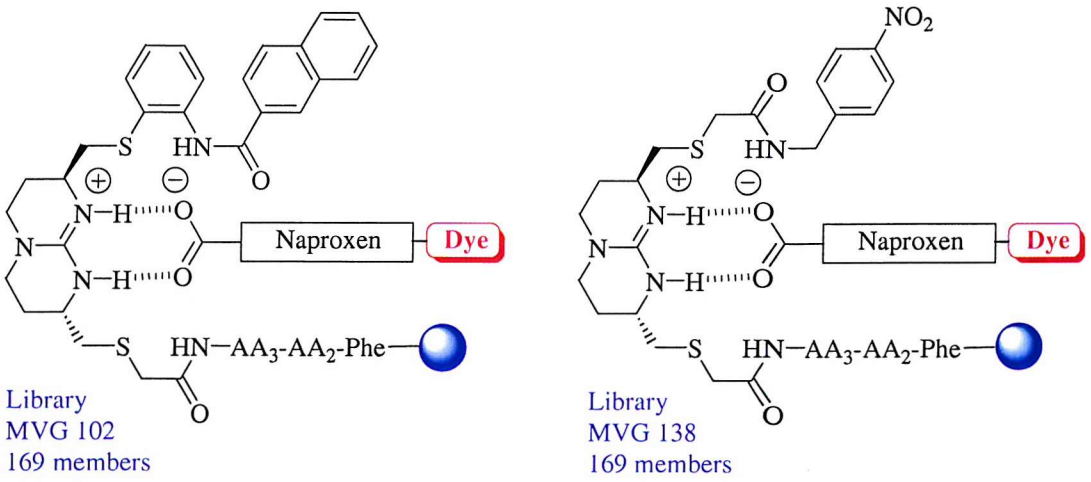


Figure III-11 Libraries MVG 102 and MVG 138

Excellent selectivity was observed. Of 2000 beads analysed less then 1% showed strong coloration (Figure III-12).

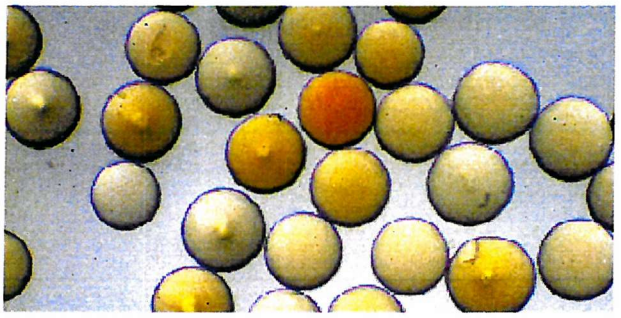
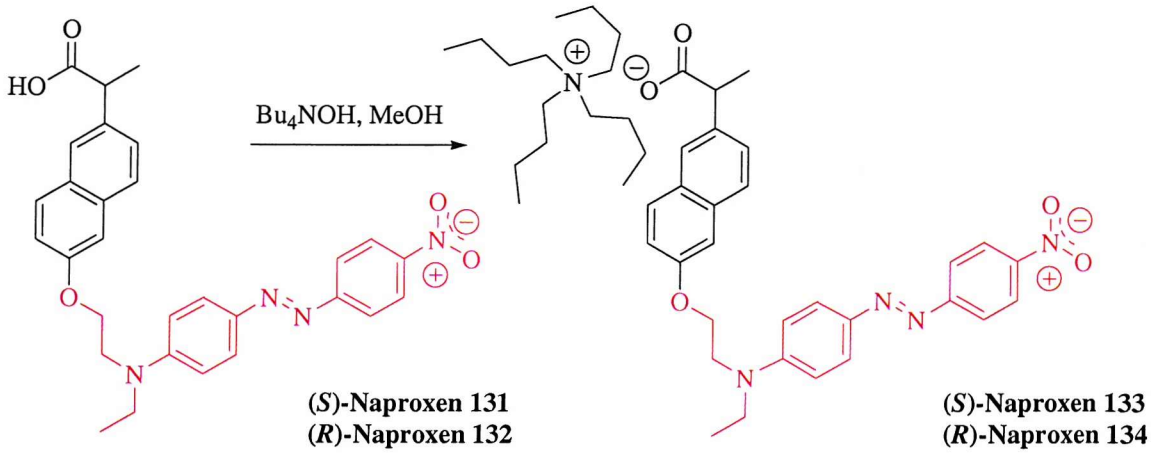


Figure III-12 Screening of library MVG 138 and (S)-Naproxen in $CHCl_3$.

The stained beads were collected, washed to remove the bound (*S*)-Naproxen and then screened against (*R*)-Naproxen. However in this case those beads that did not form a complex with the (*R*) enantiomer were isolated. The motivation behind this double screening was to unambiguously isolate tweezers capable of enantioselective binding to (*S*)-Naproxen. In order to generate the carboxylate anion of the substrate, (*S*) or (*R*) Dye-labelled Naproxen **131**, **132** (provided by Dr. Michiel Van Gool) was first converted to its tetrabutylammonium salt **133**, **134** (Scheme III-12).[164]



(*S*)-Naproxen 131 **(*S*)-Naproxen 133**
(*R*)-Naproxen 132 **(*R*)-Naproxen 134**

Scheme III-12 Synthesis of Bu_4N^+ salt of Naproxen 133 and 134

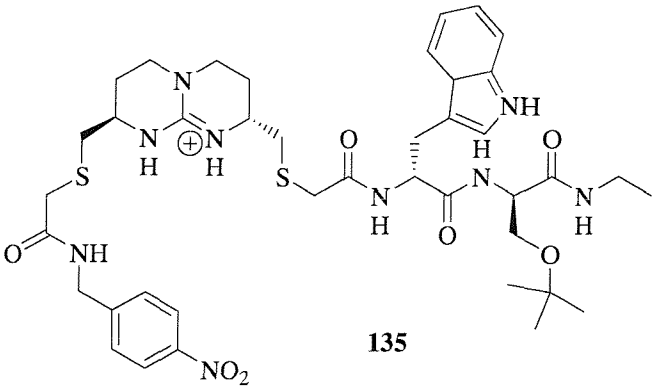
Five highly coloured beads were taken from the screening experiment with (*R*)-Naproxen in $CHCl_3$ and library MVG 138, four beads from MVG 102 and sequenced by Edman degradation to identify the structures of the tweezer receptors on each bead. From sequencing results it became apparent that library MVG 102 did not show selectivity. Instead, library MVG 138 showed discrete selectivity with the most favoured sequences being Trp, Ser, Val and Gln. (Table III-6)

Beads	**AA_0**	**AA_2**	**AA_1**
MVG 102	Phe	Ala	Met
MVG 102	Phe	Asn	Trp
MVG 102	Phe	Leu	Trp
MVG 102	Phe	Trp	Ser
MVG 138	Phe	Trp	Ser
MVG 138	Phe	Val	Gln
MVG 138	Phe	Trp	Ser
MVG 138	Phe	Lys	Gln
MVG 138	Phe	Val	Ala

Table III-6 Sequencing data for four beads (MVG 102) and five beads (MVG 138) selected from screening experiments of Red dye Naproxen with library MVG 102 and MVG 138 in $CHCl_3$.

Phenylalanine was used as an encoding strategy to verify that the Edman sequencing data were reliable. The synthesis of the single receptors was carried out for further binding studies.

Between all the possible structures it appeared that the most promising receptor as elucidated by the screening result was the compound **135** (Figure III-13) with an aromatic substituent for aromatic stacking and a bulky group for steric hindrance.



135

Figure III-13 Compound 135

In order to study the selective recognition of compound **135**, the group in Madrid performed titration, extraction and transport experiments.

A solution receptor **135** in chloroform was shaken with a solution of the sodium salt of racemic Naproxen for 10 min. The organic layer was analysed by ^{1}H-NMR. The chiral methyl group of (*R*) and (*S*)-Naproxen appeared at different shifts when the diastereomeric complex was formed. The result indicated that no selectivity was observed in the extraction and also that two equivalents were being extracted. The interpretation of this result was not an easy task since a 2:1 complex was not possible because the guanidinium moiety can bind only one Naproxen. A 1:1:1 complex (one Naproxen could bind at the guanidinium binding pocket and the second at a different point in the receptor) would however be possible. This hypothesis supposes that the second binding site is a neutral binding pocket. The extracted crude material was analysed also by chiral HPLC which confirmed the lack of selectivity.

To confirm the 1:1:1 complex hypothesis, titration experiments were performed in chloroform. The experiments were performed by addition of increasing amounts of

tetraethylammonium Naproxen salt to a solution of constant receptor concentration. The calculated binding constants were too high to be measured or for comparison with each other. During the addition of the first equivalent of Naproxen, the guanidinium NH showed a large downfield shift as expected. After the addition of the first equivalent this signal remained stationary however others began to shift. This fact clearly confirms that receptor **135** is binding a second molecule of Naproxen.

Transport through BLM (Bulk Liquid Membrane) was performed in an U-tube, the source phase being a buffered (TEAA buffer, pH = 7) aqueous solution of Naproxen and the organic phase a solution of compound **135** in chloroform. The receiving phase was an aqueous solution of $NaPF_6$. The analysis by HPLC with an achiral column of the receiving phase showed that receptor **135** transports Naproxen, however no enantioselectivity was observed.

III.5 Conclusions

In this chapter work conducted as part of a collaborative program within a European Network consisting of Prof. Gennari group (Univ. of Milan), Prof. Anthony Davis (Univ. of Bristol), Prof. Javier De Mendoza (Univ. of Madrid), Prof. Jeremy D. Kilburn and DSM (Netherlands) have been described.

The collaboration with the group in Milan focused on the synthesis of tweezer receptors featuring sulfonamidopeptide units as side arms. Conformational studies of sulfonamidopeptide tweezer structures **113-115** (Figure III-2) were conducted by NMR spectroscopy. Orthogonally protected 2,6-diamidopyridine **116** featuring Phe as first amino acid for the generation of the sulfonamidopeptide arms was successfully synthesised. Product **116** (Scheme III-5) was used by Milan in order to investigate binding properties of acyclic receptors in particular to synthesise a receptor featuring only one leg **118** (Scheme III-1).

The work conducted in collaboration with Professor Davis` group (University of Bristol) focused on designing enantioselective receptors for amino acid derivatives based on cholic acid. In order to perform binding experiments Red Dye-spacer-L-Ala-L-Ala-OH **125** (Scheme III-8) and Red Dye-spacer-D-Ala-D-Ala-OH **126** (Scheme III-9)

were synthesised. Unfortunately during this work it became evident that the structure on solid phase (provided by Davis' group) **124** was not correct. Consequently, the experiments were abandoned.

Finally the collaboration with the group in Madrid involved in developing enantioselective receptors suitable for extracting carboxylic acid derivatives from aqueous to organic solution and testing these in a prototype 'aqueous-organic-aqueous' membrane system (DSM) with appropriate racemic mixtures (aromatic carboxylic acids, naproxen etc). Screening experiments on receptor libraries **MVG 102** and **MVG 138** were conducted with Dye-labelled (*S,R*)-Naproxen **133-134** (Scheme III-12). The synthesis of the single receptor structure **135** identified was carried out for further binding studies.

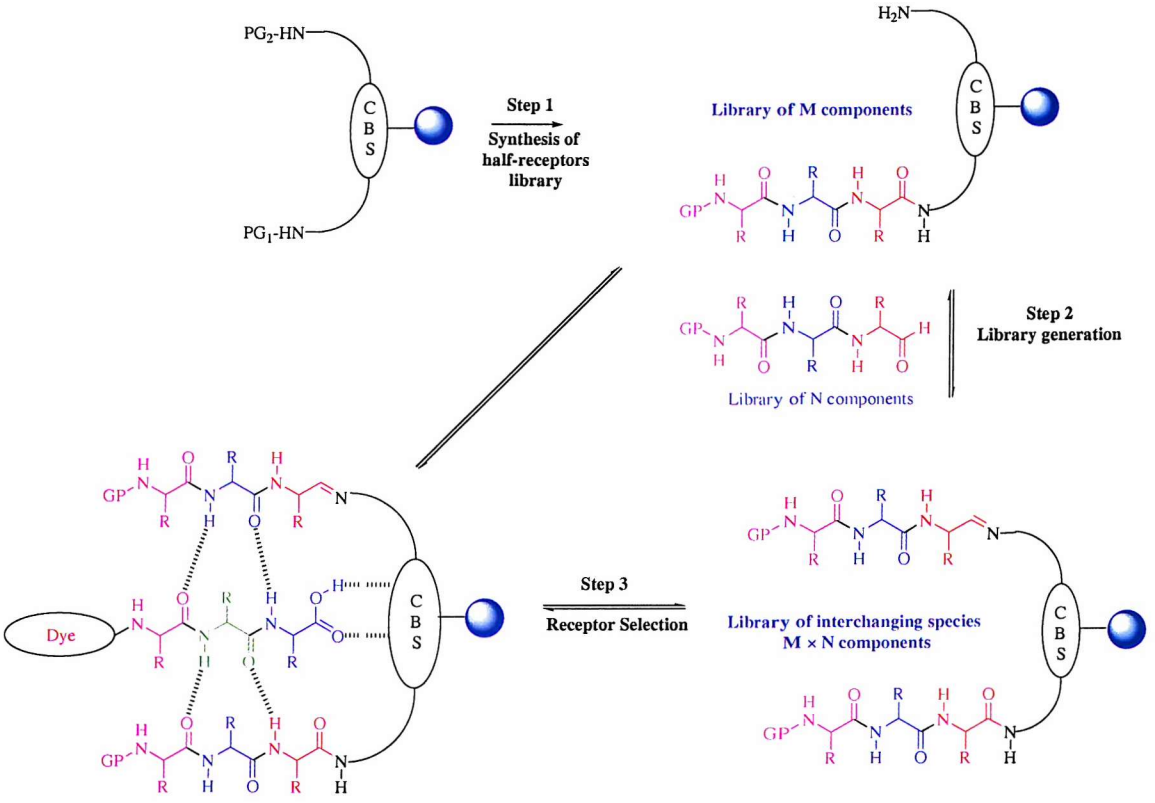
Transport through BLM (Bulk Liquid Membrane) was performed in an U-tube. The experiment showed that receptor **135** transports Naproxen, however no enantioselectivity was observed.

Chapter IV

IV.1 Background and Objectives

The combinatorial approach using a 'split and mix' strategy as described in Chapter II, generated a successful library of 'tweezer receptors' allowing us to identify selective receptors for a chosen peptide substrate.

The overall objective of the work described in this chapter was to develop a new strategy for identifying a synthetic receptor for any given substrate using a combination of combinatorial chemistry and template self-assembly, obviating any difficulty in identifying a receptor from the 'dynamic combinatorial libraries' produced. In order to achieve this it was necessary develop a strategy. The approach was to synthesise a library of 'half- tweezer' receptors in which the single arm was synthesised using the split and mix strategy (Step 1, Scheme IV-1). Using, for example, 12 different amino acids a library of 12^3= 1728 different components (library of M different components) would be generated.



Scheme IV-1 Generation of dynamic library of tweezer receptors

If the resulting resin bound 'half- tweezer' receptor library with a residual functional group (amine, alkene, etc.) could be equilibrated with a solution library of peptides (library of N components) with a complementary functional group (aldehyde, alkene, etc.) then a reversible process could generate a library of (M × N components) tweezer receptors based on continuous interconversion between the library constituents.

The addition of a chosen substrate (dye-labelled) creates a driving force that favours the assembly of the best binding constituents (template self-assembly). The best binder will be subtracted from the equilibrium forcing the library members to rearrange so as to produce more of this member. The steps are summarised as follows:

1) preparation of resin bound split-and-mix libraries (containing M library members) of half-receptor structure with a residual functional group (amine, alkene, thiol, hydrazone etc).
2) preparation of solution libraries (containing N library members) with a complementary residual functional group (alkene, aldehyde, thiol, etc).
3) selection and identification of receptors from the screening experiments.

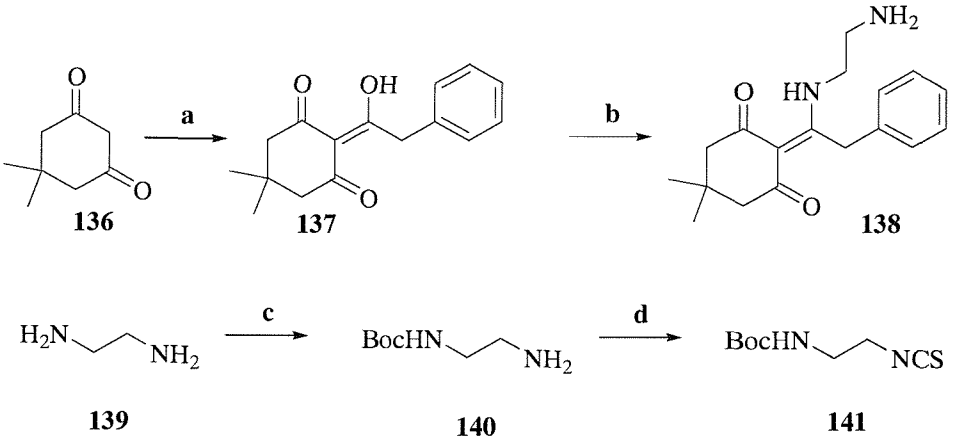
Selected beads (dyed) could then be picked out and sequenced using Edman degradation of the peptide coding strand (see Chapter II) to identify the structure of the half tweezer receptor responsible for selective recognition. Re-synthesis of the half tweezer receptor with the identified structure, followed by split and mix synthesis of the second arm, would generate a full receptor structure based on covalently linked peptide arms. Further equilibration of the resin bound library with the chosen substrate (dye-labelled guest) would allow the identification of the second arm responsible for selective recognition. Thus, a library of M × N different structures could be screened. It is important to observe that the structure identified from the second equilibration will necessarily differ from the structure selected by the first template self-assembly process. A reversibly formed bond could be substituted by a covalent irreversible process and this might interfere in substrate recognition with subsequent loss of selectivity.

The objectives of this work focused on identification of suitable building blocks to be used in the dynamic combinatorial approach described above. The interest focused on dynamic combinatorial systems based on catalysed alkene metathesis and imine formation for selective binding of C-terminal peptides.

IV.2 Towards Dynamic Combinatorial Libraries of Guanidinium and Diamidopyridine Derived Receptors.

IV.2.1 Synthesis of Orthogonally Protected CBS Derivatives

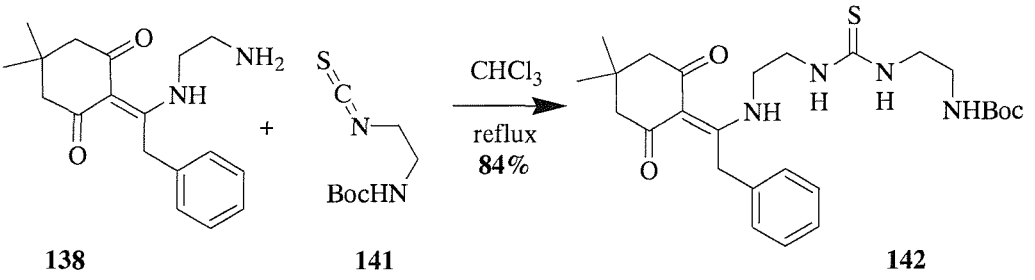
In order to prepare resin bound split-and-mix libraries (containing N library members) of half-receptor structures with a residual functional group suitable for imine formation (amine) guanidinine and amidopyridine derivative CBS were synthesised. The interest focused first on orthogonally protected guanidine derivative (CBS) suitably functionalised with a carboxylic acid moiety to allow attachment to resin-beads. (Figure IV-1). Amongst all of the described preparations of guanidines[165], the guanidinylation of a thiourea is used most widely. The guanidine derivative **145** (Scheme IV-4) was prepared starting from diamine **139** (Scheme IV-2). The first step was the synthesis of the Ddpe protecting group precursor **137**. The synthesis was readily achieved by acylation of dimedone **136** with a DCC-DMAP activated phenyl acetic acid[166] to afford the product **137** in 60% yield. Diamine **139** was first converted into the mono-Boc protected amine **138** and **140**; the latter was treated with thiophosgene to give isothiocyanate **141** (Scheme IV-2).



Legend: **a)** $PhCH_2COOH$, DCC, DMAP, DMF, **60% b)** $NH_2CH_2CH_2NH_2$, CF_3COOH, DCM, **100% c)** $(Boc)_2O$, CH_2Cl_2, **98% d)** $CSCl_2$, $NaHCO_3$ (aq.), $CHCl_3$,**96 %**

Scheme IV-2 Synthesis of mono-protected amine 138 and isothiocyanate 141

Subsequent reaction of **141** with amine **138** gave orthogonally protected thiourea **142** in 84% yield (Scheme IV-3).



138 **141** **142**

Scheme IV-3 Synthesis of thiourea 142

Research in our group focused on the synthesis of the guanidinium "head" group as a peptide receptor. Previous work [116] described the synthesis of guanidinium based receptors, using solid-phase and solution methodology. The choice of protecting group for the guanidine moiety was the tosyl group. Cleavage of this protecting group required very harsh conditions, such as HF treatment. Investigations were therefore performed to find alternative protecting groups, which are orthogonal to protecting groups used in peptide synthesis. Our attention has focused on the trifluoroacetyl group [167], which is a well-known protecting group for amine functionality and is easily cleaved [168] but, to date, has not been used as a protecting group for guanidines. The scope and limitation of the trifluoroacetyl group as a protecting group for guanidines has therefore been investigated; it was easily cleaved under mild basic conditions and was complementary to the Boc, Cbz and Ddpe protecting groups (Figure IV-1).

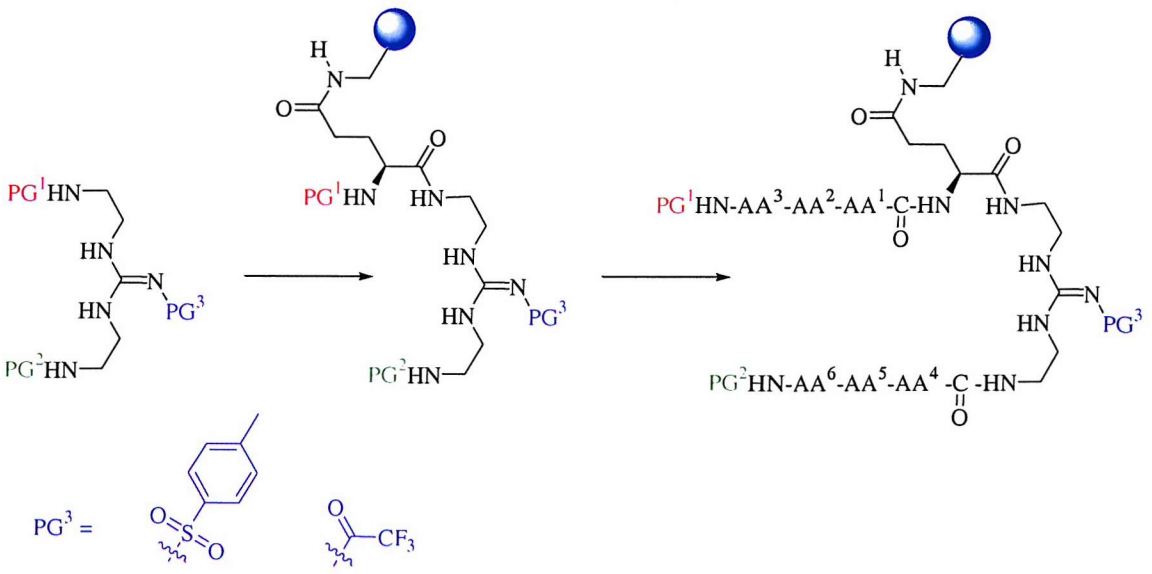
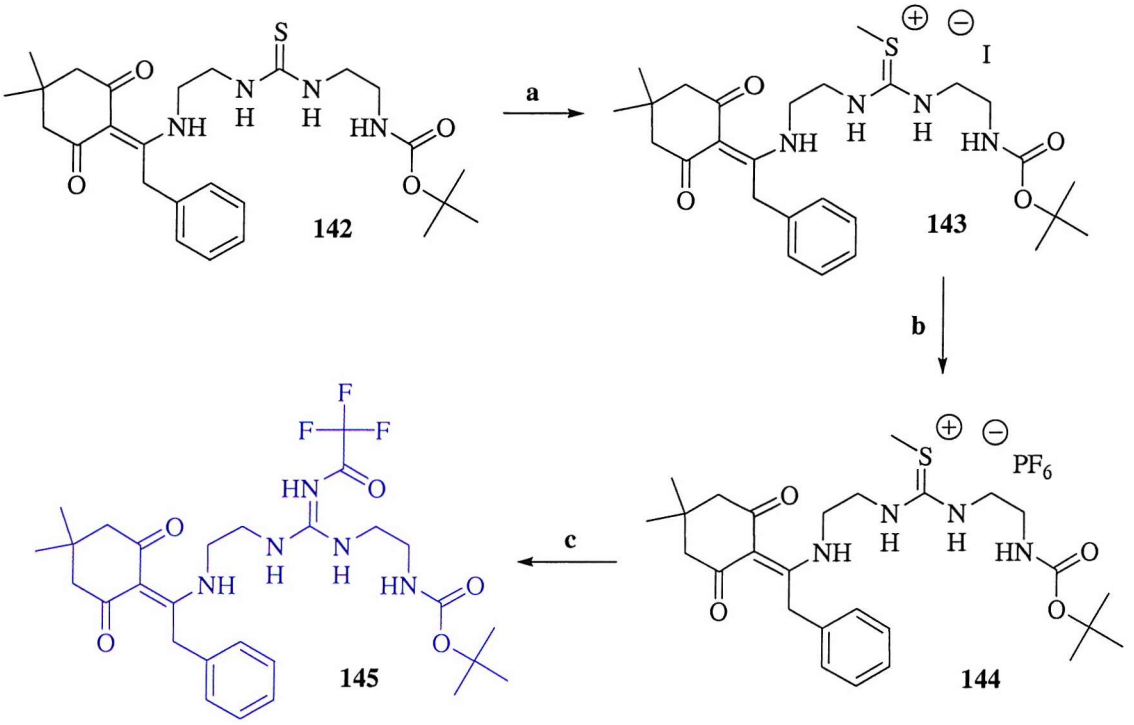


Figure IV-1 Guanidine orthogonal protecting groups

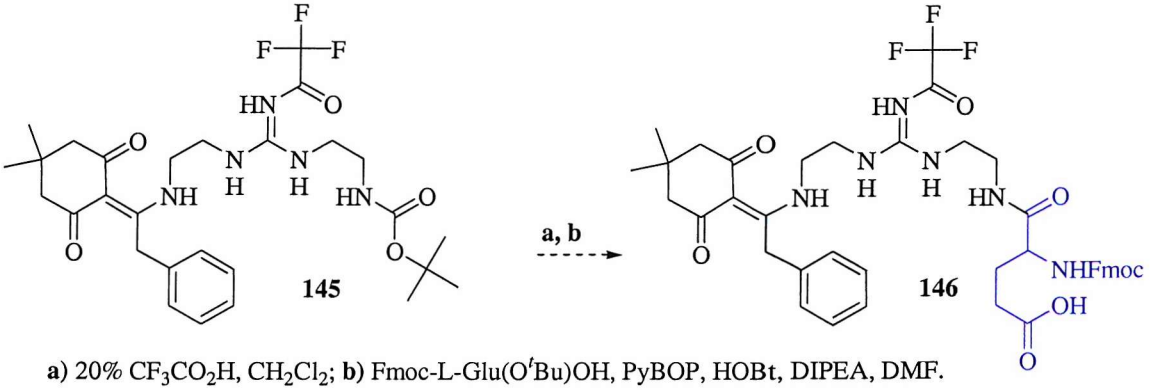
Alkylation of the thiourea **142** with methyl iodide and counter-ion exchange gave the sulfonium hexafluorophosphorate **144**, which on treatment with trifluoroacetamide in the presence of DBU led to the orthogonally protected guanidine **145** (Scheme IV-4).



a) CH_3I, acetone, **100%** **b)** NH_4PF_6, CH_2Cl_2, CH_3OH, **96%** **c)** CF_3CONH_2, DBU,toluene, $CHCl_3$, reflux, **37%**

Scheme IV-4 Synthesis of ortogonally protected CBS 145

Boc deprotection and coupling with *N*-α-Fmoc-L-glutamic acid γ-tert-butyl ester using PyBOP/HOBt would lead to the product which could be hydrolysed, using TFA, to give acid **146**.



a) 20% CF_3CO_2H, CH_2Cl_2; **b)** Fmoc-L-Glu(O^{t}Bu)OH, PyBOP, HOBt, DIPEA, DMF.

Scheme IV-5 Synthesis of orthogonally protected guanidine derivative 146

The orthogonal protecting groups PG_1-PG_3 (Figure IV-1) could allow for the synthesis of libraries of 'unsymmetrical' tweezer receptors, that is, tweezer structures in which the two-peptide arms can be randomised independently (Figure IV-2).

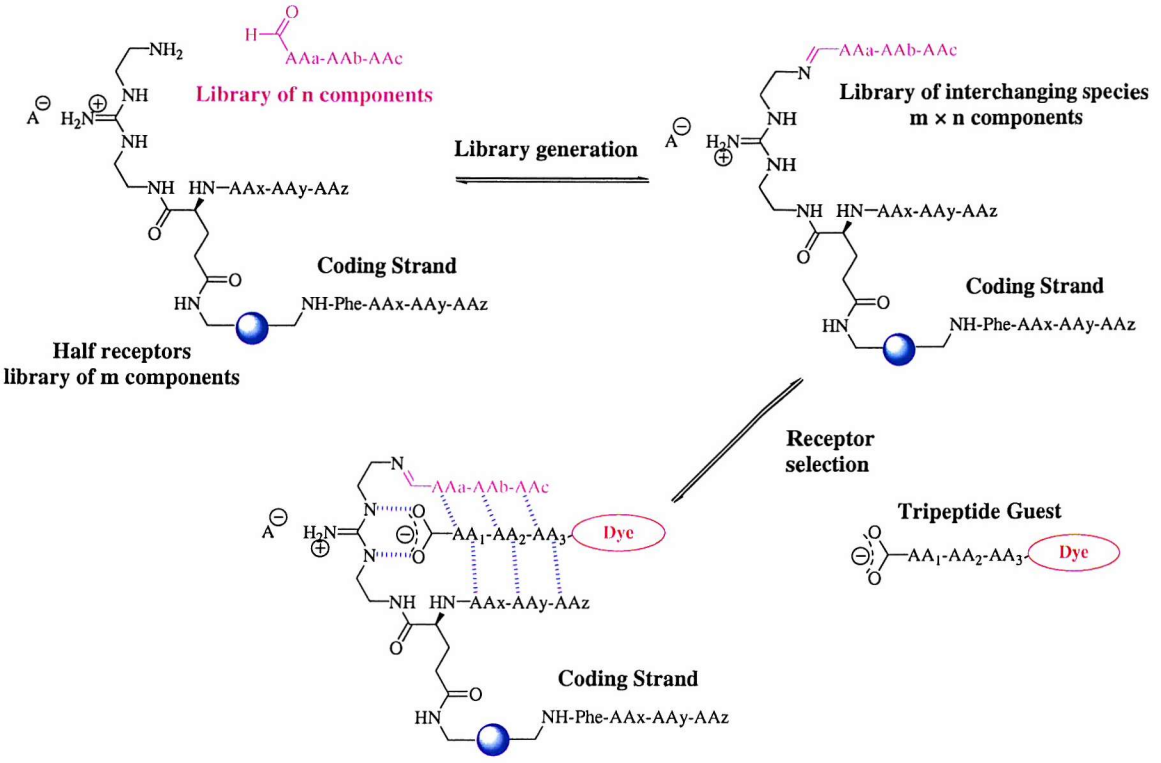
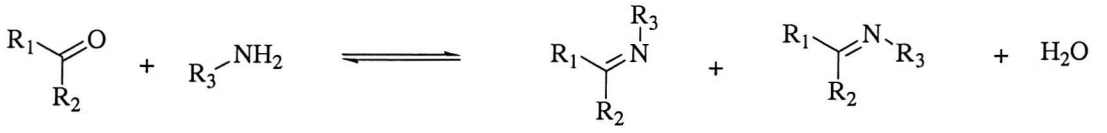


Figure IV-2 Representation of dynamic combinatorial library of receptors for carboxylate recognition.

Thus orthogonally protected guanidinium CBS as suitable building block for the generation of dynamic libraries for recognition of carboxylates derivatives was synthesised.

The reaction of aldehydes and ketones with ammonia or primary amines gives imine derivatives (Scheme IV-6). As for acetal formation, water is eliminated in this reaction, which is also acid-catalysed and reversible.



Scheme IV-6 Imine formation

Imines are difficult to isolate and purify due to their sensitivity to hydrolysis. The rate at which these imine-like compounds are formed is generally greatest near a pH of 5, dropping at higher and lower pHs. This agrees with general acid catalysis in which the conjugate acid of the carbonyl reactant combines with a free amino group. At high pH there will be an imperceptibly low concentration of the carbonyl conjugate acid and at low pH most of the amine reactant will be in the ammonium conjugate acid form, however it was reported that there exists the possibility to form imines in basic media. [169] At low pH (pH = 5) the C-terminal peptide will be in its neutral carboxylic acid form. The guanidinium group does not present the right bidentate hydrogen pattern for binding carboxylic acids. However amidopyridines provide an excellent structural motif for binding carboxylic acids (Figure IV-3).

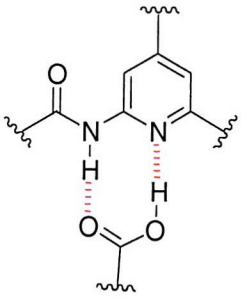


Figure IV-3 Diamidopyridine unit

Therefore interest also focused on the synthesis of an orthogonally protected amidopyridine derivative (CBS) suitable for carboxylic acid recognition. A schematic representation of a dynamic library of receptors for recognition of neutral carboxylic acid in organic solvents is given below (Figure IV-4). A library of interchanging receptors could be formed through reversible imine formation from a limited number of initial building blocks. The latter in this case are represented by half resin bound receptors featuring a free amine group as residual functional group and a solution library of tripeptide aldehydes as the complementary functional group.

On addition of a molecular 'lock' (for example, a dye-labelled tripeptide guest) the best binder is subtracted from the equilibrium and this could force the library to rearrange so as to produce more of this member.

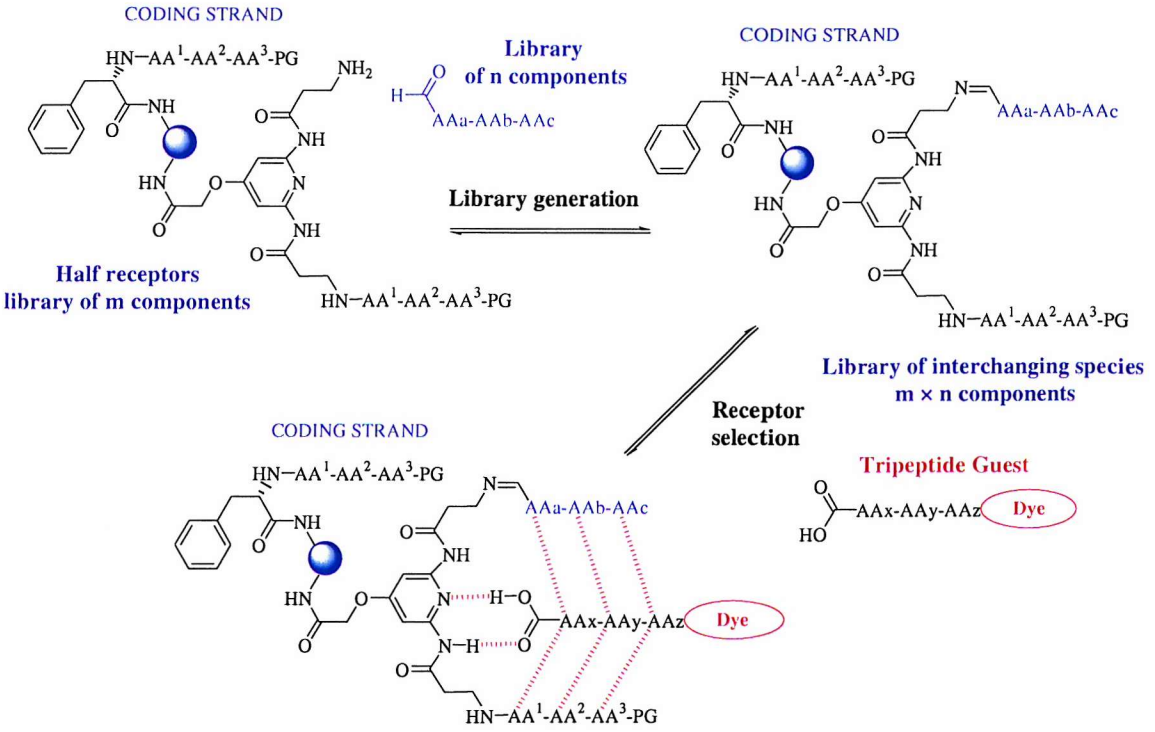
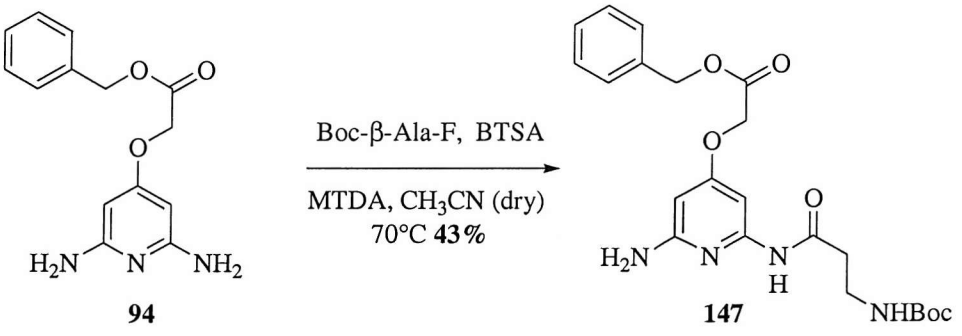


Figure IV-4 Representation of dynamic combinatorial library of receptors for carboxylic acid recognition.

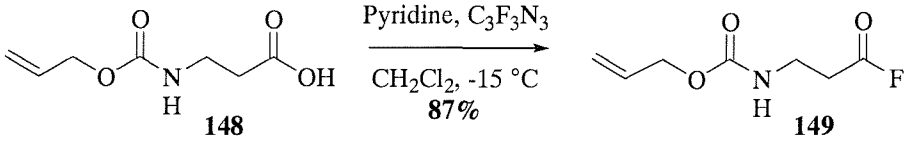
The synthesis of an orthogonally protected diamidopyridine CBS (carboxylic acid binding site) was performed following the methodology already described in Chapter II and Chapter III. A 2,6-diaminopyridine moiety bearing Alloc-β-Ala and Boc-β-Ala at the first position in each side chain respectively (Scheme IV-9) was synthesised.

The coupling of Boc-β-Ala-F **95** with the free diamine **94** was carried out using BTSA (bis(trimethylsilyl)acetamide) and MTDA (Methyl trimethylsilyl dimethylketene acetal) to give the intermediate **147** in 43 % yield (Scheme IV-7).



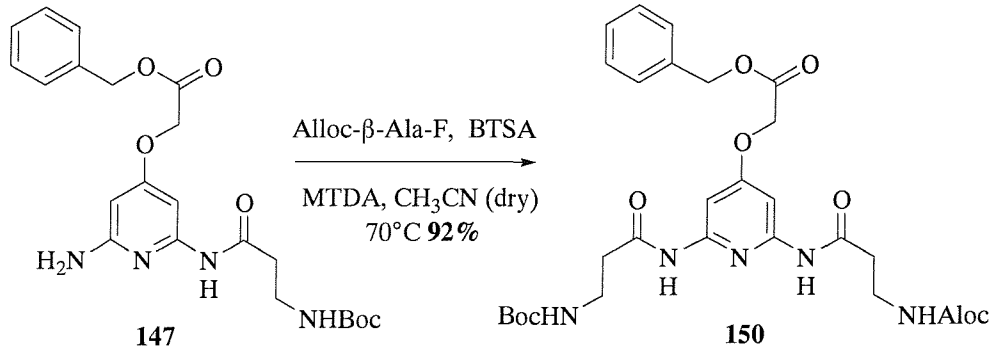
Scheme IV-7 Synthesis of intermediate 147

Subsequent coupling of *N*-Alloc-β-Ala-F **149** (Scheme IV-8) with **147** gives the desired product **150** in 92 % yield (Scheme IV-9).



Scheme IV-8 Synthesis of N-Alloc-β-Ala-F

For this purpose *N*-Alloc-β-Ala-OH **148** was first converted to N-Alloc-β-Ala-F **149** using cyanuric fluoride $(CFN)_3$ in 87% yield (Scheme IV-8). [138,139]



Scheme IV-9 Synthesis of an orthogonally protected diamidopyridine CBS 150

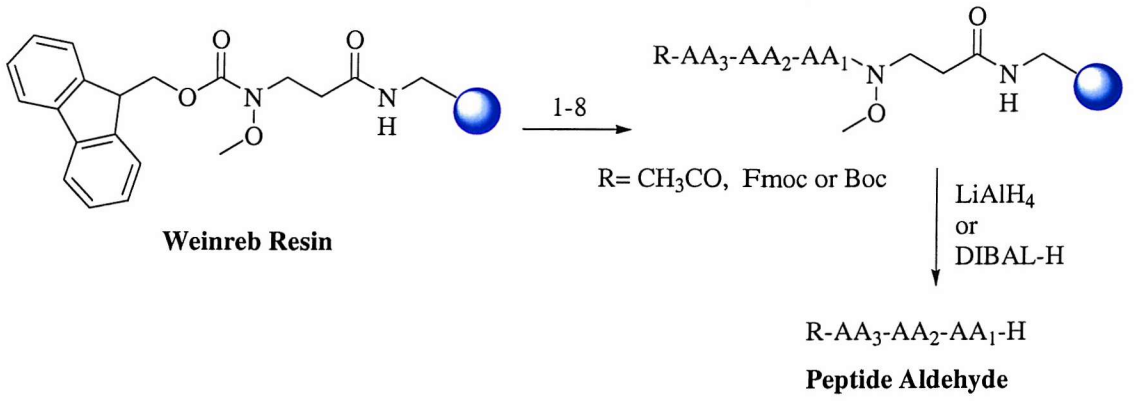
Thus orthogonally protected amidopyridine CBS **150** as suitable building block for the generation of dynamic library by imine formation for caxboxylic acid recognition was successful synthesised.

IV.3 Synthesis of C-terminal Peptide Aldehydes

Several strategies have been attempted for the preparation of solution libraries (containing M library members) of peptide aldehydes with a complementary residual functional group (aldehyde).

Various methods for the synthesis of peptide C-terminal aldehydes in solution have been described in the literature. [170-174] Recently, publications reporting the solid-phase preparation of peptide aldehydes and promotion of interest in such peptides are more

frequent. Amongst the described preparations of N-protected amino aldehydes and peptide aldehydes, the reduction of Weinreb amides is used most widely. In the first instance we have focused our attention on the solid phase synthesis *via* the Weinreb amide linker. (Scheme IV-9)



1. 20% Piperidine in DMF; **2.**Fomc-AA_1, HBTU, DIPEA, DMF; **3.**20%Piperidine in DMF **4.** Fmoc-AA_2, DIC, HOBt, DIPEA, DMF; **5.** 20% Piperidine in DMF; **6.** Fmoc-AA_3, DIC, HOBt, DIPEA, DMF; **7.** 20% Piperidine in DMF; **8.** Ac_2O, DMAP, CH_2Cl_2

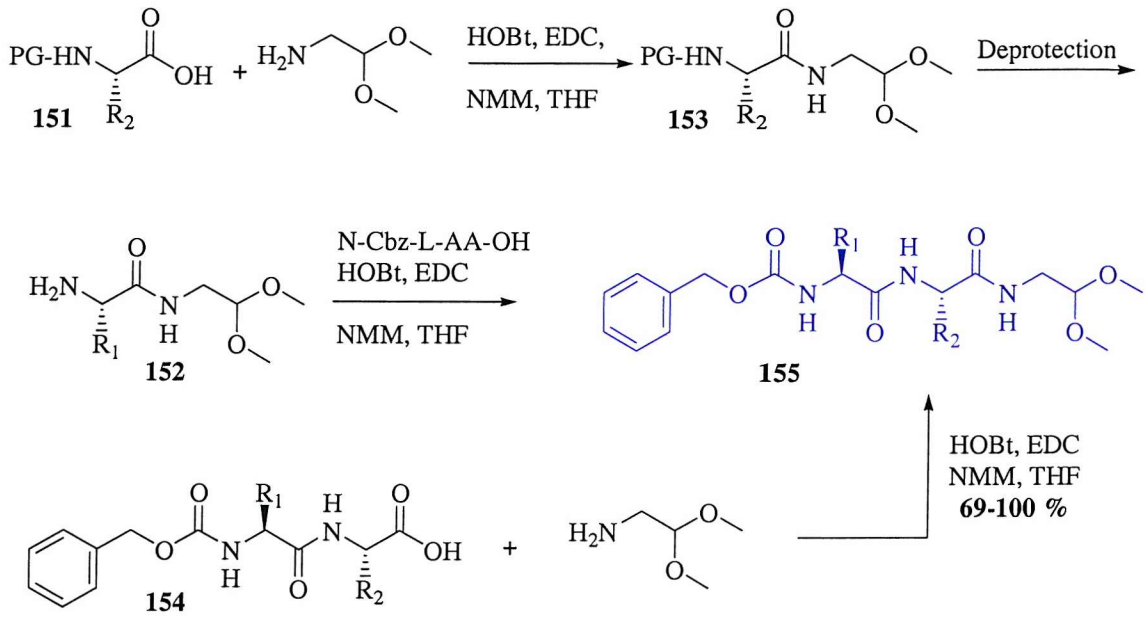
Scheme IV-10 Solid phase synthesis of C-terminal Peptide Aldehydes

The results are reported in Table IV-1. The products of the synthesis *via* the Weinreb amide linker were studied by reverse-phase high performance liquid chromatography (RP-HPLC) and by ^1H NMR in $CDCl_3$. Examination of the latter revealed the presence of the aldehydic proton signal but also the presence of impurities that did not allow the characterisation of the compounds.

Peptide aldehyde	Cleavage conditions	Yield
Fmoc-Phe-Gly-Val-H	$LiAlH_4$ 3 eq.	40% Product not pure
Ac-Val-Ala-Gly-H	$LiAlH_4$ 3 eq.	No product
Ac-Phe-Gly-H	$LiAlH_4$ 3 eq.	No product
Boc-Gly-H	$LiAlH_4$ 0.25 eq.	40% Product not pure
Ac-Gly-H	DIBAH 1 eq.	40% Product not pure

Table IV-1 Synthesis of peptide aldehydes via Weinreb amide linker

For this reason a new strategy in solution was attempted. [172] The synthesis of tripeptide dimethyl acetal was performed either starting from a simple *N*-protected amino acid **151** (Scheme IV-11) or an *N*-protected dipeptide **154**.



Scheme IV-11 Solution phase synthesis of C-terminal Peptide Aldehydes 155

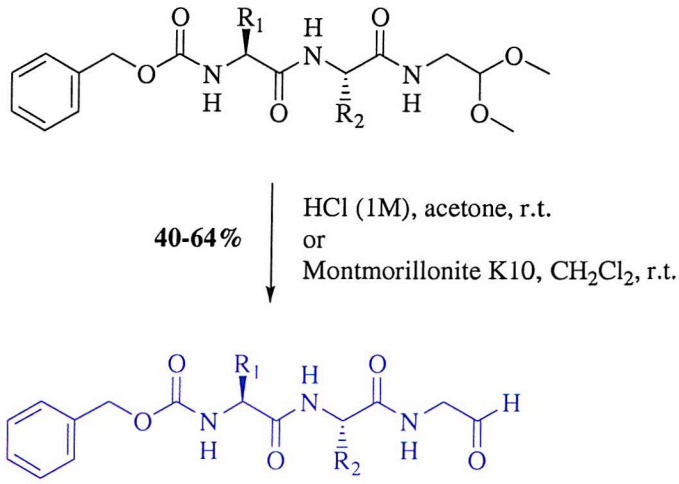
Peptide aldehydes synthesised with the methodology [175] described in Scheme IV-11-12 are reported in Table IV-2

	Z-AA$_1$-AA$_2$COCH(OMe)$_2$	Yield %	Solubility	Peptide Aldehydes
156	Z-βAlaGlyCOCH(OMe)$_2$	71	Insoluble in acetone	Decomposed
157	Z-βAlaValCOCH(OMe)$_2$	85	Insoluble in acetone	Decomposed
158	Z-AlaAlaCOCH(OMe)$_2$	69	Insoluble in acetone	**162** 64% yield
159	Z-GlyGlyCOCH(OMe)$_2$	100	Soluble in Acetone	Decomposed
160	Z-LeuGlyCOCH(OMe)$_2$	86	Soluble in Acetone	41 % yield Not pure
161	Z-GlyLeuCOCH(OMe)$_2$	100	Soluble in Acetone	**163** 40% yield

Table IV-2 Peptide aldehydes synthesised

Problems of insolubility were observed for some of the tripeptides (**156-158**). It was therefore necessary develop two different methodologies to deprotect the aldehydes.

Where the peptide was soluble in acetone (**159-161**), a classical hydrolysis was carried out with 1M HCl. Very mild conditions were used for peptides soluble in dichloromethane (**156-158**). Hydrolysis was performed with an acid clay mineral Montomorillonite K10. (Scheme IV-12)

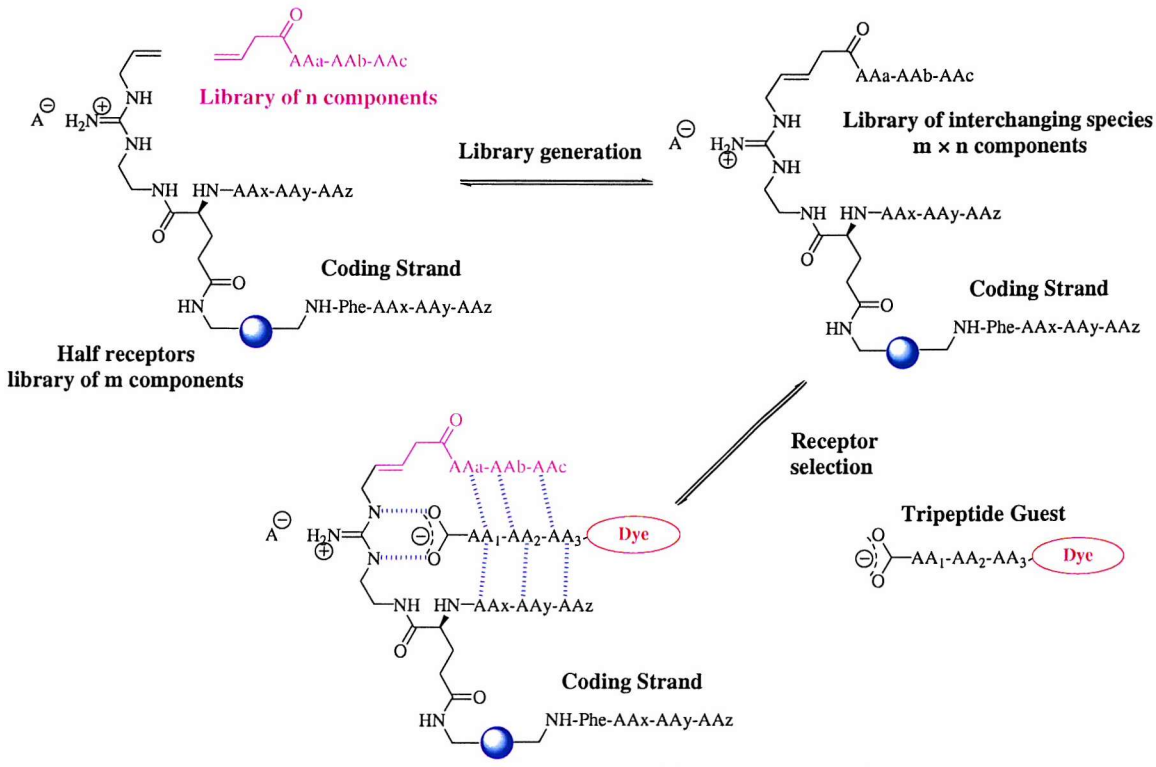


Scheme IV-12 Solution phase synthesis of C-terminal Peptide Aldehydes

Not surprisingly, the peptide aldehydes showed low stability and we were unable to obtain the desired aldehydes in sufficient yield and purity. This suggested that for dynamic combinatorial purposes it was necessary to use peptide aldehydes freshly synthesised. However in the lights of the results obtained in addition to the recognition that imines are difficult to isolate due to their sensitivity to hydrolysis, the investigations for the generation on dynamic library based on imines formation was not pursued further. Different reversible processes were considered, the interest moved to the metathesis described in the following section.

IV.4 Dynamic Library by Metathesis

Recent advances in catalyst development [176] have enabled the use of alkene metathesis in dynamic systems. [125,127] A schematic representation of the approach based on the catalysed alkene metathesis process is shown below (Scheme IV-13)



Scheme IV-13 Dynamic combinatorial library via metathesis.

A dynamic library of interchanging receptors could be formed through a reversible alkene metathesis process from a limited number of initial building blocks. The latter are represented by half resin bound receptors featuring an allyl group as residual functional group and a solution library of tripeptides featuring an allyl group as the complementary functional group. On addition of a molecular 'lock' (for example, a dye-labelled tripeptide guest) the best binder is subtracted from the equilibrium and this forces the library to rearrange so as to produce more of this member.

IV.5 Synthesis of Building Blocks

In order to prepare resin bound split-and-mix libraries (containing N library members) of half-receptor structures with a residual functional group (allyl), the synthesis of an orthogonally substituted guanidine derivative (CBS), [116] suitably functionalised with a carboxylic acid moiety to allow attachment to resin-beads, was designed (Figure IV-5)

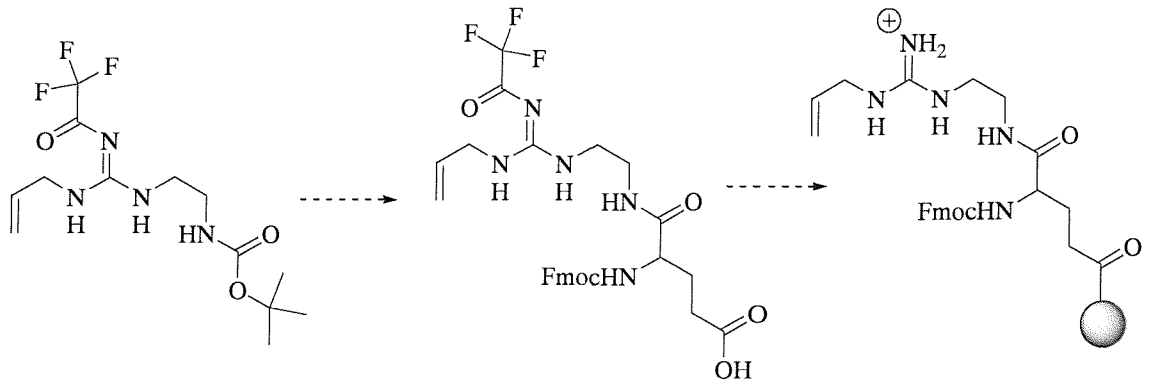
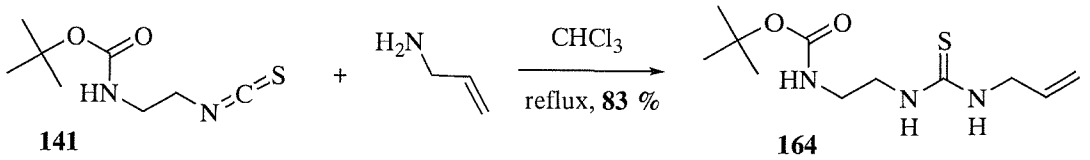


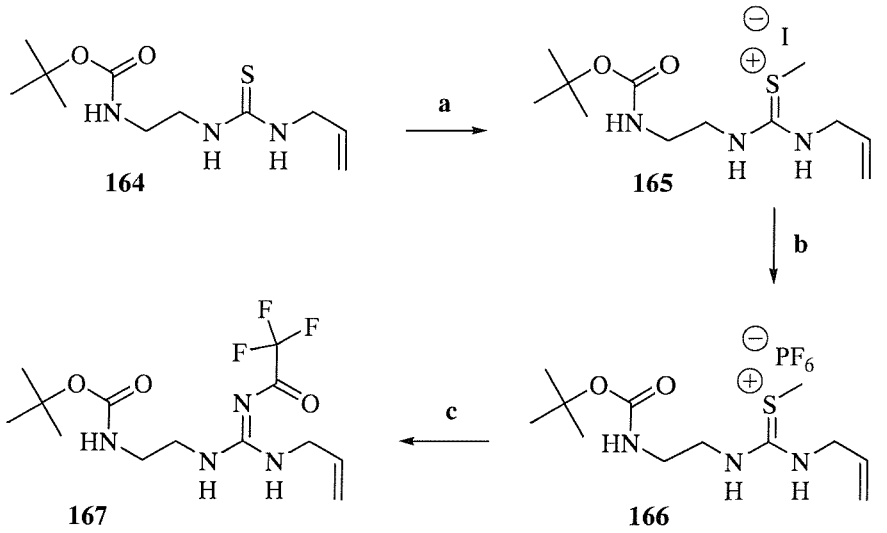
Figure IV-5 Orthogonally substituted guanidine derivative (CBS) functionalised with a carboxylic acid moiety for attachment to solid phase

The unsymmetrical substituted guanidine building block was synthesised following the standard procedure already described in paragraph IV-2.1. The ethylene diamine was first converted to the Boc monoprotected amine **140** (Scheme IV-2), which was treated with thiophosgene to give the isothiocyanate derivative **141** (Scheme IV-2).



Scheme IV-14 Synthesis of thiourea 164

Subsequent reaction of the isothiocyanate derivative with allyl amine gave an unsymmetrical substituted thiourea **164** (Scheme IV-14).

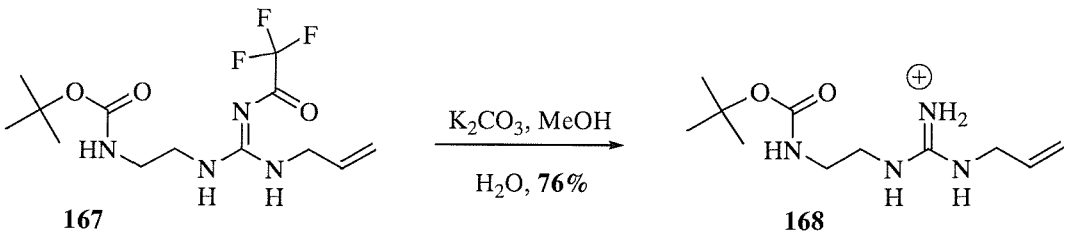


a) CH_3I, acetone, **100%** **b)** NH_4PF_6, CH_2Cl_2, CH_3OH, **100%** **c)** CF_3CONH_2, DBU, toluene, $CHCl_3$, reflux, **88%**

Scheme IV-15 Synthesis of orthogonally protected guanidinum 167

Alkylation of thiourea **164** with methyl iodide and counter-ion exchange gave the thiouronium hexafluorophosporate **166**, which after treatment with DBU and trifluoroacetamide gave the protected guanidine **167** (Scheme IV-15).

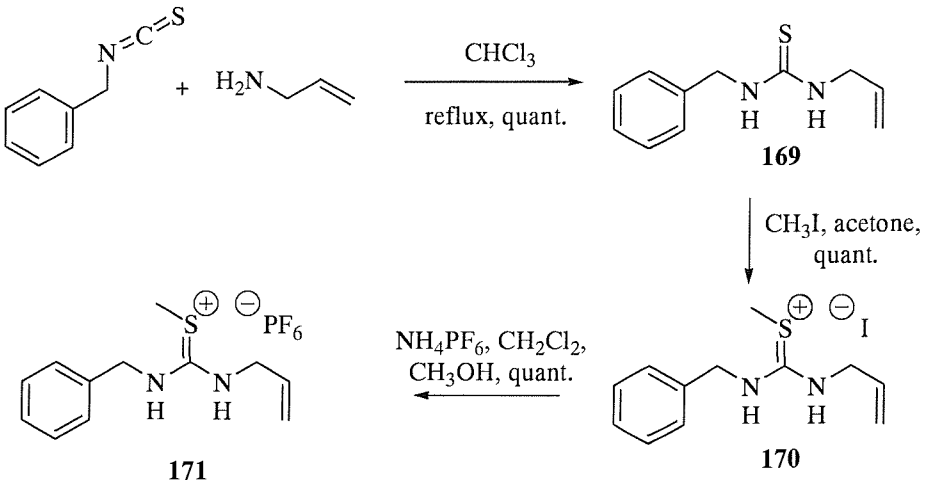
In order to verify the feasibility of alkene methathesis on solid phase applied to our particular system, preliminary investigations were carried out in solution on trifluoroacetyl-protected guanidine and on deprotected guanidinium building blocks.



Scheme IV-16 Synthesis of deprotected guanidinium 168

As anticipated, the trifluoroacetyl protecting group was easily cleaved with potassium carbonate in a mixture of methanol and water to afford the guanidinium derivative **168** (Scheme IV-16).

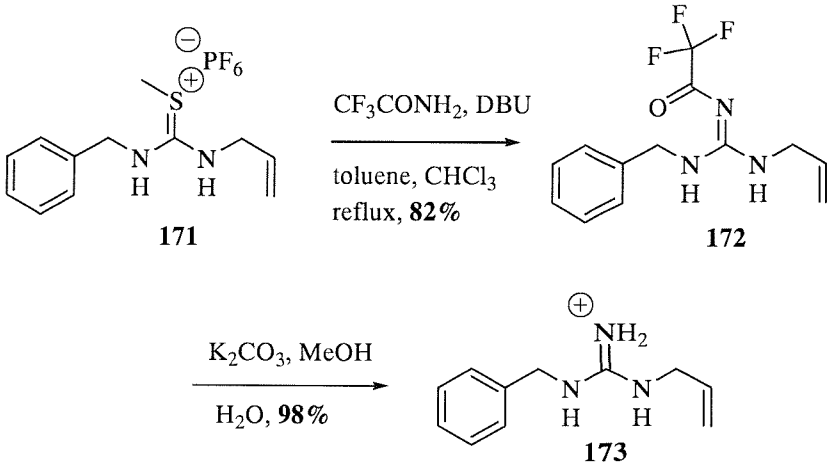
A second orthogonally substituted guanidine derivative was synthesised to generalize the approach in solution (Scheme IV-18).



Scheme IV-17 Synthesis of intermediate 171

The unsymmetrical substituted thiourea **169** (Scheme IV-17) was synthesised according to the general procedure described above. Benzyl isocyanate was reacted with allyl amine to afford thiourea **169** in quantitative yield (Scheme IV-17).

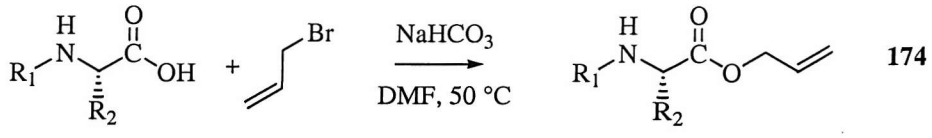
Further guanidinylation gave the protected guanidinium moiety **172** (Scheme IV-18) that after deprotection with potassium carbonate afforded the building block **173** ready for metathesis investigations.



Scheme IV-18 Synthesis of deprotected guanidinium 173

IV.6 Synthesis of Protected Amino Acid for Metathesis Investigations

A small library of protected amino acids featuring an allyl group as complementary functional group for metathesis investigations was synthesised.



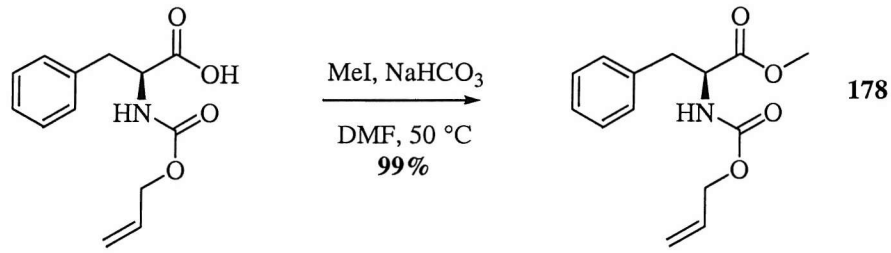
Scheme IV-19 General procedure for the synthesis of allyl ester amino acids

N-protected amino acids were reacted with allyl bromide and sodium hydrogen carbonate in *N,N*-dimethyl-formamide to afford allyl esters **174** in excellent yield. [177]

	R_1	R_2	Yield %
175	Boc	Ph	92
176	Benzoyl	Me	100
177	Benzoyl	Ph	95

Table IV-3 Allyl esters amino acids 174

In order to obtain an *N*-terminal amino acid featuring an allyl group, *N*-Alloc-L-Phe-OH was treated with methyl iodide and sodium hydrogen carbonate to afford methyl ester **178** (Scheme IV-20) in 99 % yield. [178]



Scheme IV-20 Synthesis of N-Alloc-L-Phe-OMe

IV.7 Metathesis Investigations

Second generation Grubbs' catalyst is a more active analogue of the first generation catalyst for ring-closing metathesis, cross-metathesis [176] and ROMP. It presents excellent functional group tolerance and selectivity. [179,180]

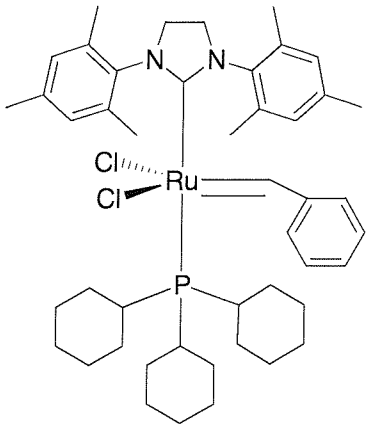
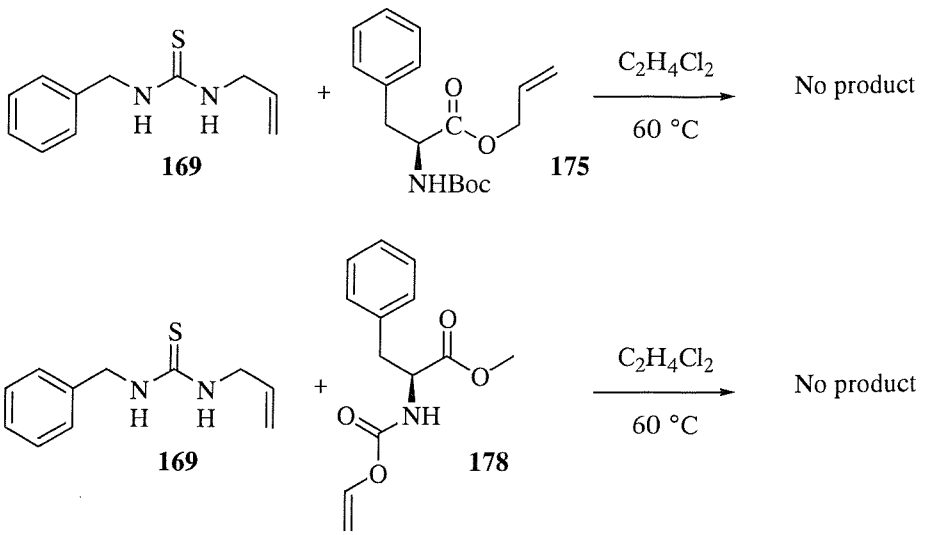


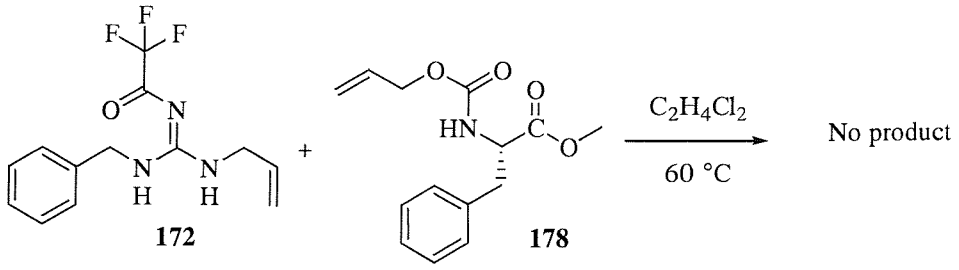
Figure IV-6 Second generation Grubbs' catalyst

In order to test the feasibility of alkene metathesis on guanidinium-based receptors, initial investigations were carried out on small building blocks in solution. Several reaction conditions were examined. Although recent literature [179] reported that second generation catalyst should be compatible with a large range of functional groups, no formation of product was observed with thiourea moieties. It was possible that the catalyst was not compatible with the thiourea functional group (Scheme IV-21).



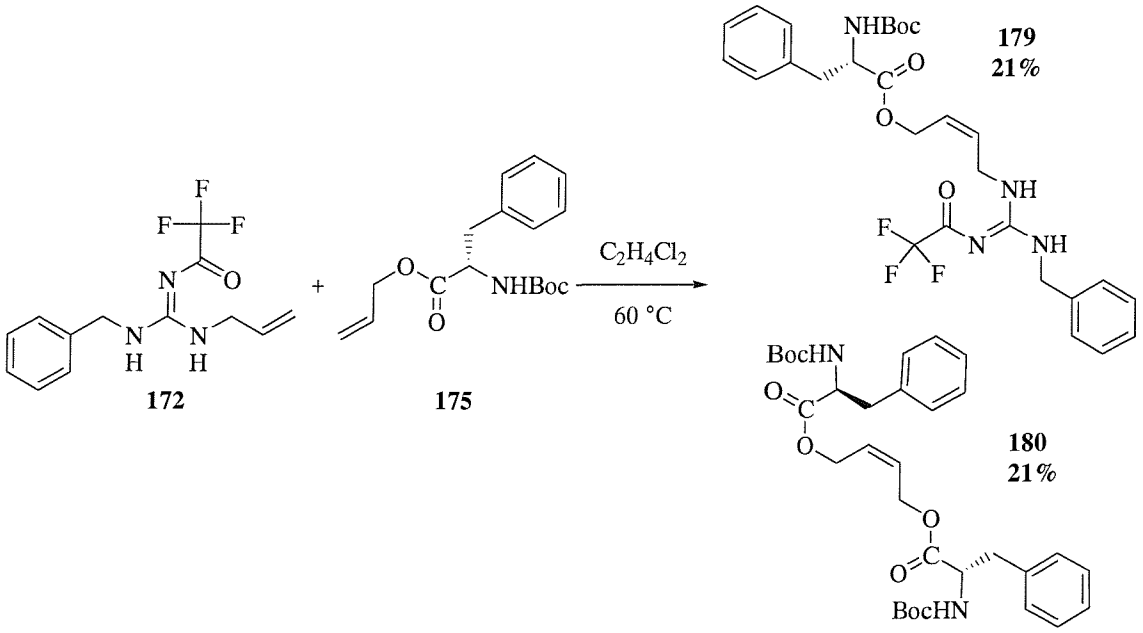
Scheme IV-21 Metathesis investigations on thiourea 169

For this reason further experiments were carried out first on the protected guanidinium moiety.



Scheme IV-22 Metathesis investigations on protected guanidinium 172

The trifluoacetyl guanidine **172** (Scheme IV-22) was reacted first with *N*-Aloc-L-Phe-OMe **178** however no formation of products was observed. Instead, when **172** (Scheme IV-23) was reacted with *N*-Boc-L-Phe allyl ester **175** using the same reaction conditions products of dimerisation on the amino acid **180** and products of cross-coupling **179** in 21 % yield were formed, however mostly unreacted starting material was recovered (Scheme IV-23).



Scheme IV-23 Metathesis investigations on protected guanidinium 172 and N-Boc-L-Phe allyl ester

Compound **179** (Scheme IV-23) was characterised by NMR and LRMS, but, the trifluoroacetyl group was cleaved (Figure IV-7) during HRMS analysis.

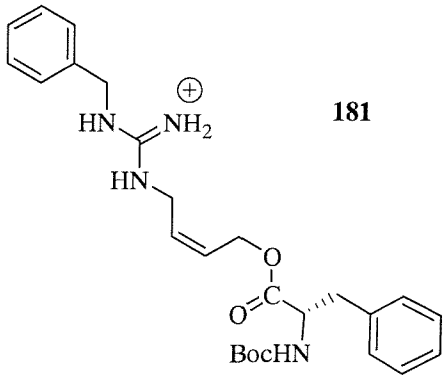
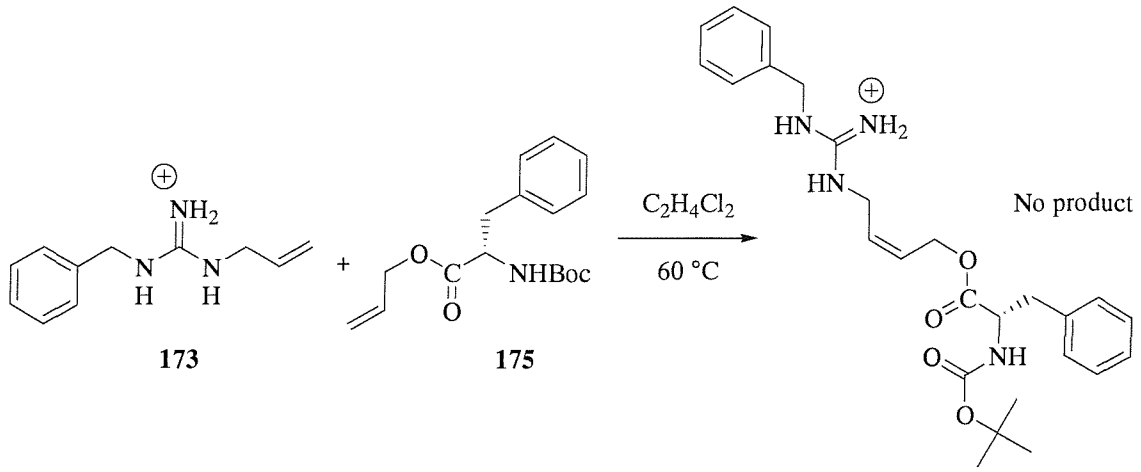


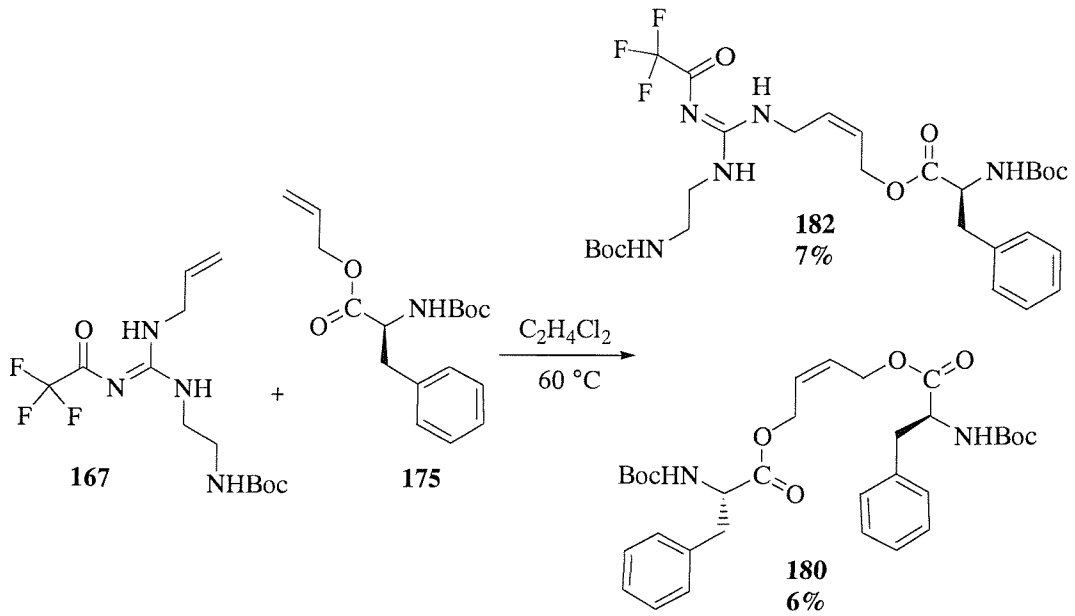
Figure IV-7 Deprotected guanidinium metathesis product 181

In order to prove that catalysed alkene metathesis was compatible with the deprotected guanidinium in screening experiments, the guanidinium **173** (Scheme IV-24) was reacted with *N*-Boc-L-Phe allyl ester **175** , but no formation of product was observed. These preliminary investigations were carried out in dichloroethane, however further investigations in different solvents were suggested.



Scheme IV-24 Metathesis investigations on deprotected guanidinium 173

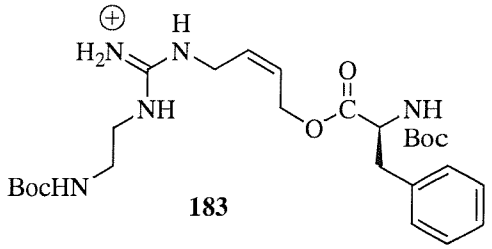
The same experiment was performed on trifluoroacetyl guanidine moiety **167** (Scheme IV-25). In this case the same pattern of products was obtained: the product of cross coupling in 7 % yield and dimerisation of N-Boc-L-Phe allyl ester in 6 % yield.



Scheme IV-25 Metathesis investigations on protected guanidinium 167

In the case of compound **182** (Scheme IV-25) this was deprotected by standing at room temperature to obtain **183** (Figure IV-8) as a pure product. These results suggest that the trifluoroacetyl group was unstable to catalysed alkene metathesis. However this is not

relevant for the scope of this investigation because template self-assembly processes should be carried out on a free guanidinium functionalised moiety.

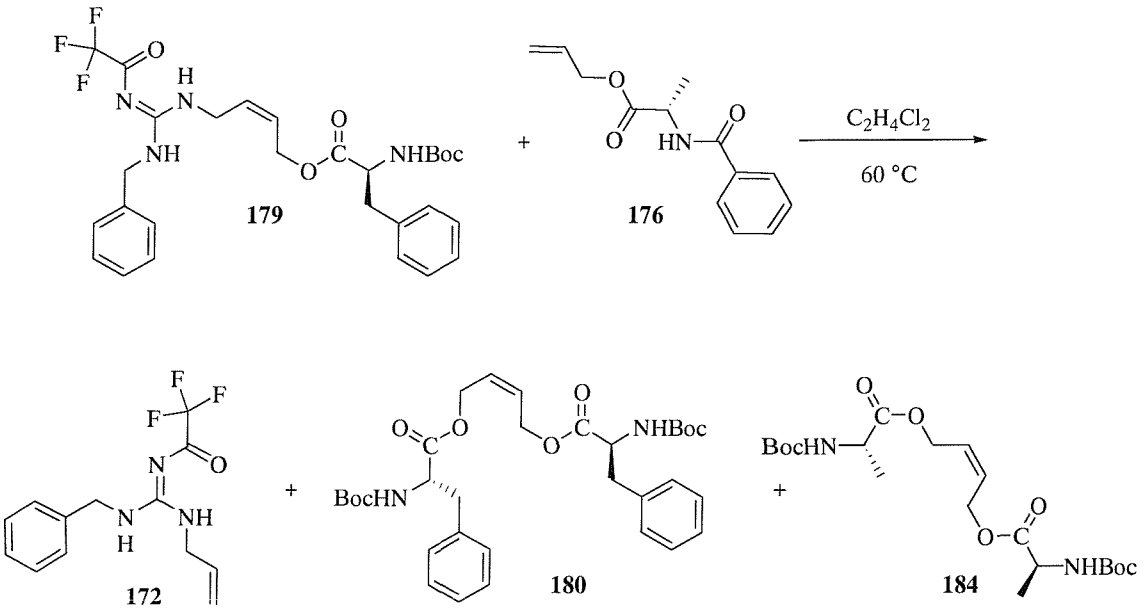


183

Figure IV-8 Deprotected Guanidinium 183

Although the yields were very poor which suggested that it would not be possible to transfer the methodology onto solid phase, an experiment to prove that alkene metathesis is indeed a potential reversible process was carried out. An interesting result was obtained when the product of cross coupling **179** (Scheme IV-23) was reacted with *N*–Benzoyl-Ala allyl ester **176** in the presence of catalytic amounts of Grubb`s catalyst (Scheme IV-26).

A mixture of products was obtained. The presence of products **172, 180** and **183** (Scheme IV-26) suggested that equilibrium was established between the reactants. Products obtained from this experiment were not characterised because it was not possible to isolate pure products. This experiment confirms that it was possible to generate a library of interchanging species using a reversible process such as alkene metathesis.



Scheme IV-26 Metathesis investigations

In light of results obtained from these preliminary investigations it can be asserted that alkene metathesis has the potential to be used in dynamic library generation, but further optimisation of the reaction conditions is required.

IV.8 Summary and Future Work

Synthesis of libraries of building blocks was performed. Several CBS orthogonally functionalised were synthesised: guanidinium and amidopyridine based derivatives. A library of peptide aldehydes was synthesised for imine formation purposes. Unfortunately peptides aldehydes were not sufficiently stable for use in screening experiments. Preliminary investigation on alkene methathesis were conducted on protected guanidine derivative however further investigations in different solvent systems should be performed on free guanidinium derivatives.

Amongst the potential reversible processes described in the literature, disulfide formation would appear to be the most suitable process.

Chapter V

V.1 General Experimental

Reactions, which required a dry atmosphere, were conducted in flame-dried glassware under an atmosphere of nitrogen. Reactions were carried out in solvents of commercial grade and where necessary were distilled prior to use. [181] Tetrahydrofuran was distilled under argon from benzophenone and sodium and dichloromethane was distilled from calcium hydride, as was petroleum ether where the fraction boiling between 40 and 60 °C was used.

Solvents for peptide synthesis were purchased from Rathburn Chemicals, HPLC grade solvents from Riedel-de-Haën. Tentagel S NH_2 resin was used as solid support in screening experiments and in peptide synthesis unless otherwise indicated and purchased from Rapp Polymere, Germany. Amino acids and coupling reagents were purchased from NovaBiochem. All other chemicals were purchased from Aldrich or Fluka. Peptide and library syntheses on solid phase were performed in glass vessels with sinter frits or polypropylene filtration tubes with polyethylene frits on a Visiprep SPE Vacuum Manifold from Supelco. The reaction containers were agitated either on a shaker (Stuart Scientific Flask Shaker SF1) or on a blood tube rotator (Stuart Scientific Blood Tune Rotator SB1). Thin layer chromatography (TLC) was performed on Aluminium-backed plates, Merck silica gel 60 F254. Sorbisil C60, 40-60-mesh silica was used for column chromatography.

V.2 Instrumentation

All melting points were determined in open capillary tubes using a Gallenkamp Electrothermal Melting Point Apparatus. Infrared Spectra were recorded on a Thunderdome Golden Gate attenuated Total Reflectance FT-IR spectrometer. ^{1}H-NMR spectra were obtained at 300 MHz on Bruker AC 300 and at 400 MHz on a Bruker DPX 400. Carbon NMR spectra were recorded at 75 MHz on a Bruker AC300 and at 100 MHz on a Bruker DPX 400.

All Electrospray (ES) spectra were recorded on a Micromass Platform quadrupole mass analyser (Fisons VG platform through a Hewelett Packard 1050 HPLC system) with an

electrospay ion source using acetonitrile as solvent. UV absorbance from ninhydrin tests were measured on a Hewlett-Packard 84532 K Diode Array Spectrometer using two-way quartz cells.

Semipreparative HPLC methods

Semipreparative HPLC experiments were accomplished using a Hewlett Packard Chem Station HP 1100, details of the gradient and solvents are given in Table V-1
Column: Phenomenex Prodigy ODS (3) (C-18, 250 × 10mm) semipreparative column (Flow 2.5 mL/min).

40STD254 Time (min)	**20STD254 Time (min)**	**20STD220 Time (min)**	**0.1% TFA/H_2O**	**0.1% TFA/CH_3CN**
0	0	0	100	0
40	20	20	0	100
50	25	25	0	100
55	30	30	100	0
60			100	0

Table V-1 Semipreparative HPLC methods

Analytical HPLC methods

Analytic HPLC was accomplished on a Hewlett Packard Chem Station HP1100, details of the gradient and solvents are given in Table V-2.
Column: Phenomenex Discovery (C-18, 150 × 4.6 mm, 3u) for the following methods S5OD, S15OD, L15OD; Prodigy ODS (3) for S15PROD

S5OD Time (min)	S15OD Time (min)	S15PROD Time (min)	L15OD Time (min)	H_2O/0.1% TFA	CH_3CN/ 0.1% TFA
0	0	0	0	90	10
3	10	10	20	10	90
4	15	15	25	10	90
5	20	20	30	90	10
6	21	21	31	90	10

Table V-2 Analytic HPLC methods.

V.3 Quantitative Ninhydrin Test [182]

Dry resin (2 to 5 mg) was weighed into a test tube. Reagent A (3 drops) and reagent B (1 drop) was added, mixed and heated to 100 °C for 10 min. As a control, the reagents were added in the same proportions as above without any resin. The tubes were then cooled, and 60% ethanol in water (2 mL) was added and the solutions mixed thoroughly. The solutions were filtered through a Pasteur pipette containing glass wool, washed twice with 0.5 M Et_4NCl (0.5 mL) and diluted to 5 mL with 60 % ethanol. The solution was measured against a blank at 570 nm. The loading was measured using the following equation

$$\text{mmol/g} = A_{570} \times V \times 10^3 / E \times m$$

Where V = Volume (mL), E = Extinction coefficient (1.5 × 104 Mcm^{-1}), m = Mass of resin (mg).

Reagent A

Solution 1 — Reagent grade phenol (40 g, 0.43 mol) was mixed with absolute ethanol (10 mL). The mixture was warmed until dissolved and IWT TMD-8 ion exchange resin (4 g) was added. The mixture was stirred for 45 min and then filtered.

Solution 2 — KCN (65 mg, 1mmol) was dissolved in water (100 mL). 2 mL were then diluted to 100 mL with pyridine (freshly distilled). IWT TMD-8 ion exchange resin (4 g) was added and stirred for 45 min and then filtered. Solution 1 and 2 were mixed.

Reagent B

Ninhydrin (2.5 g, 14 mmol) was dissolved in absolute ethanol (50 mL). The solution was stoppered and stored in the dark under nitrogen.

V.4 Quantitative Fmoc Test [183]

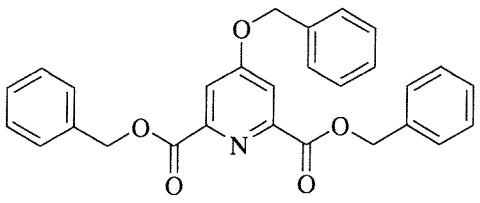
A known mass of dried resin was weighed into an eppendorf tube, 20% piperidine in DMF (1.5 mL) was added and the tube shaken for 10 min to remove the Fmoc group from the peptide. The resin was then filtered through a glass wool plug in a pipette and the filtrate made up to 10 mL with 20% piperidine in DMF. The solution was measured against a blank at 302 nm. The loading was measured using the following equation:

$$\text{Substitution (mmol/g)} = (A_{302} \times V \times 103)/E \times m$$

Where V = Volume (mL), E = Extinction coefficient (7800 Mcm^{-1}), m = Mass of resin (mg)

V.5 Experimental for Chapter II

Dibenzyl 4-(benzyloxy)-2,6-pyridinedicarboxylate (88)

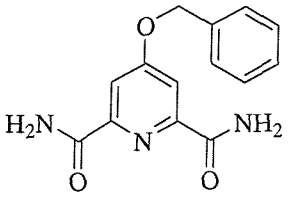


$C_{28}H_{23}NO_5$
Exact Mass: 453.1576

Chelidamic acid (5 g, 24.87 mmol), dry potassium carbonate (17.19 g, 0.12 mol) and benzyl bromide (24 mL, 0.2 mol) were refluxed in dry acetone for 3 days. After addition of hydrochloric acid (2N, 100 mL) the aqueous layer was extracted with ethyl acetate (3 × 150 mL) and the combined organic layers were dried over magnesium sulphate. The solvent was removed under reduced pressure to give a pale yellow oil. Trituration of the resulting oil with petrol ether gave a pale yellow solid (9.35 g, 20.62 mmol, 83% yield). Analytical data: mp 65 °C (lit. [114] 65 °C); ν_{max} = 3036 (w), 1750 (w), 1713 (s), 1592 (m), 1336 (s), 1103 (m), 1026 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ = 7.86 (s, 2H, C_5H_2N), 7.49-7.32 (m, 15H, ArH), 5.45 (s, 4 H, CO_2CH_2), 5.20 (s, 2H, $ArOCH_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 166.4 (0), 164.3 (0), 149.9 (0), 135.3 (0), 134.6 (0), 128.8 (1), 128.7 (1), 128.5 (1), 128.4 (1), 128.3 (1), 127.7 (1), 114.7 (1), 70.7 (2), 67.7 (2). LRMS (ES^+) *m/z* (%) = 454.2 (40) $[M+H]^+$, 476.1 (20) $[M + Na]^+$, 907.5 (20) $[2M + H]^+$, 929.5 (90) $[2M + Na]^+$.

Spectroscopic data were consistent to those reported in literature [114,115]

4- (Benzyloxy)-2,6-pyridinedicarboxamide (89)

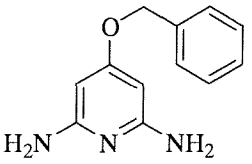


$C_{14}H_{13}N_3O_3$
Exact Mass: 271.0957

To a saturated solution of ammonia (g) in methanol (60 mL) dibenzyl ester **88** (7.64 g, 16.8 mmol) was added and the resulting reaction mixture stirred for 3 hours at room temperature. The solvent was removed under reduced pressure to give the corresponding diamide **89** as a white powder (4.24g, 15.62 mmol, 93% yield). Analytical data: mp > 240 °C (lit.[134] >220 °C); ν_{max} = 3427 (w), 1694 (s), 1662 (s), 1594 (s), 1559 (m), 1431 (S), 1379 (s), 1111 (s), 1018 (s), 877 (m) cm^{-1}; ^1H NMR (300 MHz, [D_6] DMSO) δ = 8.87 (s, 2H, NH_2), 7.76 (s, 2H, C_4H_2N + NH_2), 7.68 (s, 2H, NH_2), 7.51-7.36 (m, 5H, ArH), 5.37 (s, 2H, OCH_2Ar); ^{13}C NMR (75 MHz, [D_6] DMSO) δ = 166.9 (0), 165.2 (0), 151.3 (0), 135.9 (0), 128.7 (1), 128.3 (1), 127.8 (1), 110.5 (1), 69.9 (2); LRMS (ES^+) *m/z* (%) = 272.2 (50) $[M+H]^+$.

Spectroscopic data were consistent to those reported in literature [114,115]

4-(Benzyloxy)-2,6-diaminopyridine (90)

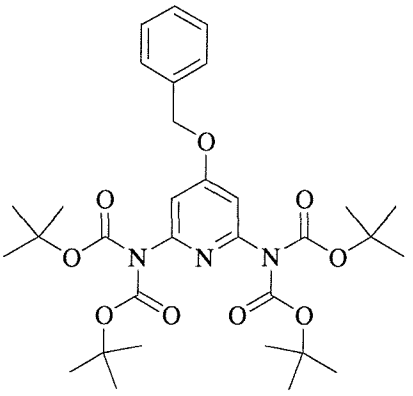


$C_{12}H_{13}N_3O$
Exact Mass: 215.1059

The diamide **89** (1.36 g, 5 mmol) was added to a solution of potassium hydroxide (5 M, 15 mL) and bromine (0.64 mL, 12.4 mmol). The resulting reaction mixture was stirred for 5 hours at 90 °C. After extraction with dichlomethane (5 × 50 mL), the combined organic layers were dried over magnesium sulfate. The solvent was removed under reduced pressure to give the corresponding diamine **90** as a light brown solid, (0.76 g, 3.5 mmol, 71%). Analytical data: mp 164-166 °C (lit. [134] 164-166 °C); ν_{max} = 3433 (w), 1634 (s), 1446 (m), 1184 (s) cm^{-1}; ^1H NMR (300 MHz, [D_6] DMSO) δ = 8.86 (s, 2H, C_5H_2N), 7.75 (s, 4H, NH_2), 7.50-7.36 (m, 5H, ArH), 5.36 (s, 2H,OCH_2Ar); ^{13}C NMR (75 MHz, [D_6] DMSO) δ = 166.8 (0), 165.1 (0), 151.2 (0), 135.8 (0), 128.6 (1), 128.2 (1), 127.7 (1), 110.5 (1), 69.8 (2); LRMS (ES^+) *m/z* (%) = 216.2 (100) $[M+H]^+$.

Spectroscopic data were consistent to those reported in literature [135]

4-Benzyloxy-2,6-bis[bis(tert-butyloxycarbonyl)amino]pyridine (91)



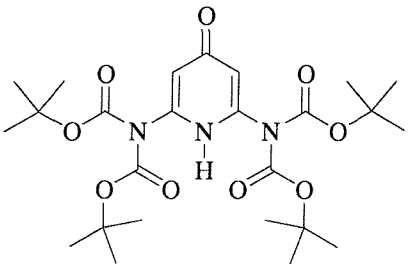
$C_{32}H_{45}N_3O_9$
Exact Mass: 615.3156

4-Dimethylamino-pyridine (0.118 g, 0.97 mmol) and a solution of di-tert-butyl dicarbonate (2.66 g, 12.1 mmol) in acetonitrile (10mL) was added to a solution of 4-(benzyloxy)-2,6-diaminopyridine **90** (0.52 g, 2.43 mmol) in a mixture of acetonitrile (25 mL) and dichloromethane (25 mL). The resulting reaction mixture was stirred for 24 hours at room temperature. The solvent was removed under reduced pressure and the residue purified by column chromatography on silica gel (dichloromethane with 1% methanol) to give **91** as a colourless solid (1.34 g, 90% yield). Analytical data: mp 140 °C (lit.[135] 146-148 °C); ν_{max} = 2981 (w), 1783 (m), 1769 (s), 1754 (s), 1600 (m), 1572 (m), 1151 (s), 1097 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 7.41-7.36 (m, 5H, ArH), 6.80 (s, 2H, C_5H_2N), 5.12 (s, 2H, OCH_2Ar), 1.43 (s, 36H, $(CH_3)_3C$); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 167.3 (0), 152.1 (0), 150.8 (0), 135.3 (0), 128.8 (1), 128.5 (1), 127.5 (1), 106.5 (1), 83.1 (0), 70.3 (2), 27.8 (3); LRMS (ES^+) *m/z* (%) = 616.3 (80) $[M+H]^+$, 638.2 (90) $[M+Na]^+$, 1253.8 (30) $[2M+Na]^+$; HRMS calcd for $C_{32}H_{46}N_3O_9$ $[M+H]^+$: 616.3234, found 616.3229.

Spectroscopic data were consistent to those reported in literature. [135,115]

2,6-Bis[bis(tert-butyloxycarbonyl)amino pyridone (92)

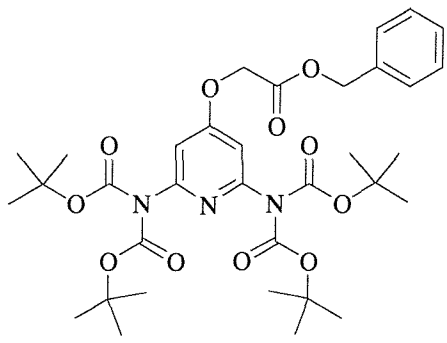
***tert*-Butyl ((*tert*-butoxycarbonyl)6-[di(*tert*-butoxycarbonyl)amino]-4-oxo-1,4-dihydro-2-pyridinylamino)methanoate (92)**



$C_{25}H_{39}N_3O_9$
Exact Mass: 525.2686

To a solution of **91** (1.34 g, 2.18 mmol) in ethanol (20 mL) 10% palladium on charcoal (0.116 g, 0.109 mmol) was added. The resulting reaction mixture was stirred vigorously for 18 hours at room temperature under an atmosphere of hydrogen. The catalyst was separated by filtration through a plug of celite. Evaporation of the solvent and drying of the residue at high vacuum yielded the corresponding pyridone **92** as a colourless solid (1.13 g, 99%). Analytical data: mp > 230 °C (lit.[115] > 230 °C); ν_{max} = 2979 (w), 1773 (m), 1729 (s), 1612 (m), 1277 (s), 1252 (s), 1153 (s), 1106 (s) cm^{-1}; ^1H NMR (400 MHz, [D_6] DMSO) δ = 11.06 (s, 1H, NH), 6.67 (s, 2H, C_4H_2N), 1.41 (s, 36H, $(CH_3)_3C$); ^{13}C NMR (100 MHz, [D_6] DMSO) δ = 166.4 (0), 151.1 (0), 150.4 (0), 106.8 (1), 82.2 (0), 27.1 (3); LRMS (ES^+) *m/z* (%) = 526.2 (100) $[M+H]^+$, 548.7 (40) $[M + Na]^+$, 1051.2 (10) $[2M + H]^+$ 1073.2 (10) $[2M + Na]^+$.

Benzyl 2-{(2,6-bis[bis(tert-butyloxycarbonyl)amino]-4-pyridyloxy}acetate (93)

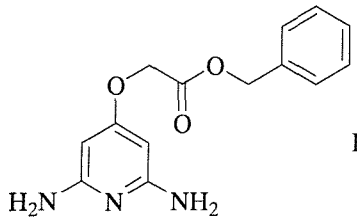


$C_{34}H_{47}N_3O_{11}$
Exact Mass: 673.3211

Benzyl bromoacetate (0.600 g, 2.62 mmol) was added to a suspension of pyridone **92** (1.21 g, 2.3 mmol), potassium carbonate (1.32 g, 9.576 mmol) and tetrabutylammonium bromide (0.74 g, 2.3 mmol) in DMSO (16 mL). The resulting reaction mixture was stirred for 18 hours at room temperature and then partitioned between diethyl ether (200mL) and water (200 mL). After separation of the organic layer, the aqueous layer was extracted with diethyl ether (200 mL). The combined organic layers were washed with water (2 × 200 mL) and dried over magnesium sulphate. Evaporation of the solvent and drying of the residue at high vacuum yielded **93** (1.53 g, 2.27 mmol, 99% yield) as a colourless solid. Analytical data: mp 114-117 °C (lit.[135] 115-117 °C); ν_{max} = 2982 (w), 1782 (m), 1754 (m), 1741 (s), 1601 (m), 1284 (s), 1252 (s), 1100 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 7.37 (m, 5H, ArH), 6.77 (s 2H, C_4H_2N), 5.24 (s 2H, OCH_2Ar), 4.61 (s, 2H, OCH_2CO), 1.44 (s, 36H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 167.2 (0), 166.3 (0), 152.0 (0), 150.7 (0), 134.7 (0), 128.7 (1), 128.5 (1), 128.4 (1), 105.8 (1), 83.2 (0), 67.3 (2), 65.0 (2), 27.8 (3); LRMS (ES^+) *m/z* (%) =674.5 (100) $[M+H]^+$, 696.5 (85) $[M+Na]^+$, 1369.6 (20) $[2M+Na]^+$; HRMS (ES^+) calcd for $C_{34}H_{48}N_3O_{11}$ $[M+H]^+$: 674.3289, found 674.3286.

Spectroscopically identical to material previously described [115]

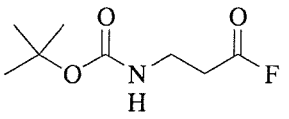
Benzyl 2-[(2,6-diamino-4-pyridyl)oxy]acetate (94)



$C_{14}H_{15}N_3O_3$
Exact Mass: 273.1113

TFA (6.4 mL) was added dropwise to a solution of **93** (1.5 g, 2.2 mmol) in dichloromethane (32 mL) and the resulting mixture was stirred for 2 hours. The solution was concentrated and trituration of the resulting oil with diethyl ether gave the bis TFA salt as a white solid. The salt was suspended in an aqueous solution of potassium carbonate (10%) and extracted with dichloromethane to give the free 2,6 diaminopyridine. The solvent was evaporated and the resulting residue was purified by column chromatography (dichloromethane and 5% methanol) to give the free 2,6-diamino pyridine **94** (0.4 g, 86%) as a white foam. Analytical data: HPLC = 9.6 min (S15OD). ν_{max} = 3445 (w), 3355 (w), 3177 (w), 1768 (m), 1734 (m), 1588 (s), 1453 (m), 1169 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 7.36-7.33 (m, 5H, ArH), 5.41 (s, 2H, C_4H_2N), 5.23 (s, 2H, OCH_2CO), 4.57 (s, 2H, OCH_2Ar) 4.11 (br s, 4H, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 168.2 (0), 167.4 (0), 158.9 (0), 135.1 (0), 128.6 (1), 128.5 (1), 128.4 (1), 84.5 (1), 67.1 (2), 64.6 (2); LRMS (ES^+): *m/z* (%) =274.1 (100) $[M+H]^+$, 547.1 (10) $[2M+H]^+$; HRMS (ES^+) calcd for $C_{14}H_{16}N_3O_3$ $[M+H]^+$: 274.1192, found 274.1189.

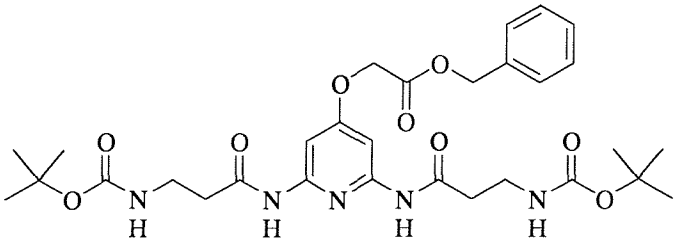
N-tert-Butoxycarbonyl-β-alanyl fluoride (95)



$C_8H_{14}FNO_3$
Exact Mass: 191.0958

A mixture of *N*-Boc-protected β-alanine (1.134 g, 6 mmol) and pyridine (0.484 mL, 6 mmol) in dry dichloromethane was cooled down to – 15 ^{0}C. After addition of cyanuric fluoride (1.1 mL, 13 mmol) the resulting mixture was stirred for 3 hrs at – 15 ^{0}C under a nitrogen atmosphere. The solution was poured on ice (10-20 mL) and more dichloromethane (50 mL) was added. The organic layer was separated and the aqueous layer extracted with dichloromethane (2 × 200 mL). The combined organic layers were dried over magnesium sulphate. Evaporation of the solvent at room temperature gives the product as colourless oil (1.06 g, 93% yield). The compound was unstable on standing. Analytical data: ν_{max} = 3349 (w), 2979 (w), 1839 (s), 1691 (s), 1512 (s), 1367 (s), 1167 (s) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 4.95 (1H, br s, NH), 3.43 (q, *J* = 5.5 Hz, 2H, NHC*H*$_2$), 2.75 (t, *J* = 5.5 Hz, 2H, CH_2CH_2), 1.44 (s, 9H, $(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 164.4-160.8 (d, J $_{C-F}$ = 360 Hz, 0), 155.6 (0), 79.9 (0), 35.4 (2), 33.3-32.8 (d, J $_{C-F}$ = 49.3 Hz, 2), 28.3 (3); LRMS (ES$^+$) *m/z* (%) = 383.4 (100) [2M+H]$^+$.

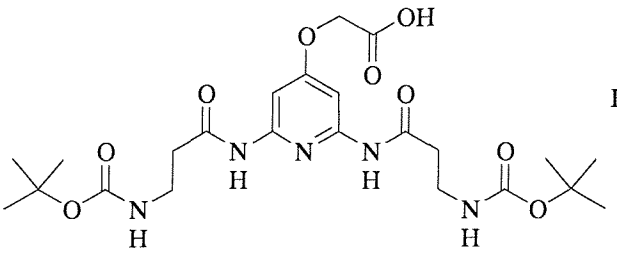
[2,6-Bis-(3-*tert*-butoxycarbonylamino-propionylamino)-pyridin-4-yloxy]-acetic acid benzyl ester (96)



$C_{30}H_{41}N_5O_9$
Exact Mass: 615.2904

Diamine **94** (0.74 g, 2.7 mmol) was dissolved in dry acetonitrile (63 mL). After addition of N,O-bis(trimethylsilyl)acetamide (0.84 ml, 2.7 mmol) the reaction mixture was stirred at rt for 2 h. Then, N-tert-Butoxycarbonyl-β-alanyl fluoride **95** (1.58 g, 13.5 mmol) and MTDA (1.68mL, 13.5 mmol) were added and the reaction mixture was stirred for additional 20 hours at room temperature. The solvent was removed under reduced pressure and the residue was redissolved in dichloromethane (300 mL). The organic layer was washed with an aqueous solution of sodium hydrogencarbonate (5%, 300 mL) and water (300 mL) then dried over magnesium sulphate. The solvent was evaporated and the resulting residue was purified by column chromatography (dichloromethane and 2% methanol) to give diamidopyridine derivative **96** as colourless foam (1.53 g, 92%). Analytical data: ν_{max} = 3289 (w), 2976 (w), 1756 (w), 1677 (s), 1616 (m), 1584 (m), 1500 (s), 1435 (s), 1158 (s) cm^{-1}; ^1H NMR (400 MHz, [D_6] DMSO) δ = 10.02 (s, 2H, NHCO), 7.42-7.35 (m, 7H, ArH, C_4H_2N), 6.81 (br s., 2H, NHBoc), 5.23 (s, 2H, OCH_2Ar), 4.91 (s, 2H, OCH_2CO), 3.22 (q, *J* = 6.5 Hz, 4H, CH_2NHBoc), 2.57 (t, *J* = 6.5 Hz, 4H, , $NHCOCH_2$), 1.39 (s, 18H, $(CH_3)_3C$); ^{13}C NMR (100 MHz, [D_6] DMSO) δ = 170.6 (0), 167.9 (0), 166.3 (0), 155.5 (0), 151.3 (0), 135.5 (0), 128.4 (1), 128.1 (1), 127.9 (1), 95.6 (1), 79.2 (0),77.6 (0), 66.2 (2), 64.5 (2), 36.6 (2), 36.2 (2), 28.2 (3). LRMS (ES^+) *m/z* (%) = 616.2 (100) $[M+H]^+$, 638.2 (10) [M + Na] $^+$; HRMS (EI^+) calcd for $C_{30}H_{42}N_5O_9$ $[M+H]^+$: 616.2982, found 616.3007.

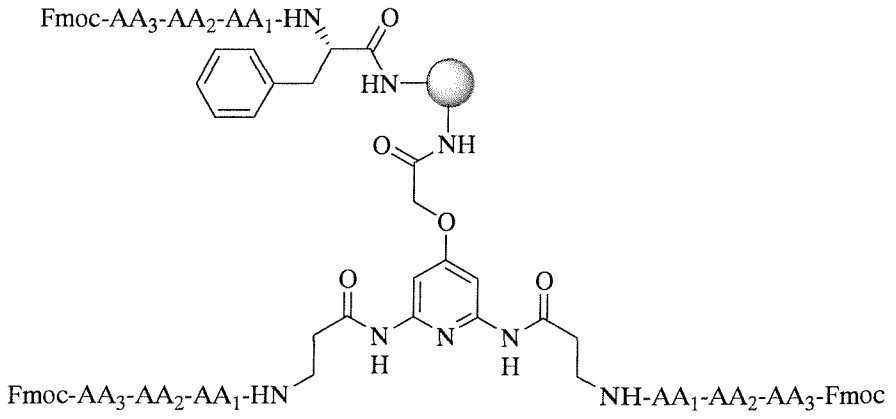
2-[2,6-Bis (*N-tert*-Butoxycarbonyl-β-alaninyl-4-pyridyloxy) acetic acid (97)



$C_{23}H_{35}N_5O_9$
Exact Mass: 525.2435

10% Palladium on charcoal (1.1 g) was added to a solution of benzyl ester **96** (1.22 g, 2 mmol) in ethanol (25mL). The reaction mixture was stirred vigorously for 18 hours at room temperature under an atmosphere of hydrogen. The catalyst was separated by filtration through a plug of celite. Evaporation of the solvent and drying of the residue under high vacuum yielded the corresponding carboxylic acid **97** as a colourless solid (1.05 g, 99%). Analytical data: HPLC: 10.125 min (S15OD2); mp 60 °C; ν_{max} = 3400 (b w), 2976 (m), 2934 (m), 1686 (s), 1654 (s), 1583 (m), 1510 (m), 1158 (s), 848 (m) cm^{-1}; ^1H NMR (400 MHz, [D_6] DMSO) δ = 10.14 (s, 2H, NHCO), 7.32 (s, 2H, C_4H_2N), 6.81 (br s., 2H, NHBoc), 4.71 (s, 2H, OCH_2CO), 3.21 (4H, t, *J* = 6.5 Hz, CH_2NHBoc), 2.55 (4H, t, *J* = 6.5 Hz, $NHCOCH_2$), 1.37 (18H, s, $(CH_3)_3C$); ^{13}C NMR (100 MHz, [D_6] DMSO) δ = 170.8 (0), 169.1 (0), 166.7 (0), 155.5 (0), 150.9 (0), 95.4 (1), 77.6 (0), 64.4 (2), 36.6 (2), 36.2 (2), 28.2 (3); LRMS (ES^+) *m/z* (%) =526.3 (100) $[M+H]^+$, 548.2 (20) $[M+Na]^+$, 1051.4 (10) $[2M+H]^+$; HRMS (ES^+) calcd for $C_{23}H_{36}N_5O_9$ $[M+H]^+$: 526.2506, found 526.2508.

Synthesis of the tweezer receptors library B



A solution of Boc-Phe (9.8 mg, 0.037 mmol), PyBOP (19.2 mg, 0.037 mmol), HOBt (6 mg, 0.037 mmol) in DMF (1 mL) was added to Tentagel-NH_2 resin (1.28 g, 0.37 mmol, NH_2), pre-swollen in DMF (2 mL) followed by DIPEA (29 μL, 0.166 mmol) and the suspension was shaken for 20 hours. A solution of acid **97** (220 mg, 0.417 mmol), PyBOP (217 mg, 0.417 mmol), HOBt (56.3 mg, 0.417 mmol) in DMF (2 mL) was added to the resin followed by DIPEA (303 μL, 1.74 mmol) and the suspension was shaken for 20 hours. Any remaining amine residues were capped by treating the resin with an excess of acetic anhydride The resin was filtered and washed with dichloromethane (3 × 5 mL), dimethylformamide (3 × 5 mL), dichloromethane (3×5 mL). A qualitative ninhydrin test was negative. Subsequent Boc deprotection was achieved with a solution of TFA (30%) in CH_2Cl_2 for 2 hours. Resin **98** was washed with CH_2Cl_2 (3×5 mL), dimethylformamide (3 × 5 mL), dichloromethane (3×5 mL) and washed with a solution of DIPEA (10%) in dichloromethane, MeOH (3×5 mL) and Et_2O (5 × 5 mL) and dried *in vacuo*. The tweezer library was then prepared using three cycles of split and mix synthesis. The resulting resin was divided in 12 equal portion. To each resin portion one of the following Fmoc amino acids was added: L-Ala, L-Gln, L-Glu(OtBu), Gly, L-Leu, L-Lys(Boc), L-Met, L-Phe, L-Pro, L-Ser(tBu), L-Trp, L-Val(0.08 mmol amino acid per resin portion), along with HBTU (30 mg, 0.08 mmol) and DIPEA (47 μL, 0.27 mmol) in DMF (2 mL). The reaction mixtures were shaken for 18 hours. Qualitative ninhydrin test were carried out to check all transformations were

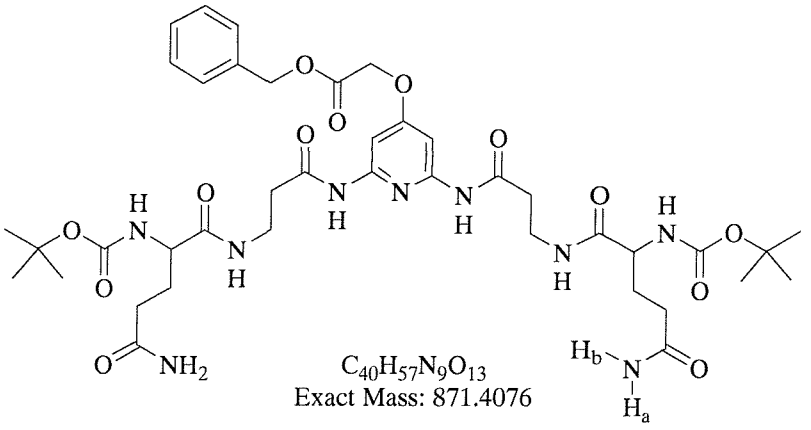
complete. The resin was mixed and the terminal Fmoc-groups removed with 20% piperidine in DMF. The resin was split again into 12 equal portions and the procedure repeated twice in order to build up the tweezer receptor library **B**

Screening

The resin-bound receptors library **B** (11 mg.) was equilibrated in chloroform for 24 hours. A solution of Red-Dye-spacer-L-Glu-L-Ser-L-Val-COOH in chloroform (150 μL, 20 μM,) was added to the resin sample and equilibration was continued for further 24 h. Beads were analysed in flat-bottomed glass pot under a Leica inverted DML microscope (magnification × 40). The selectivity was high, showing 15 highly red coloured beads on a total of about 9 thousands beads. Ten of the most intensively stained beads were selected. Fmoc deprotection was carried out using a solution (5 mL) of piperidine (20 %) in dimethylformamide. Boc deprotection was carried out using a solution (5 mL) of TFA (30 %) in dichloromethane. The active beads were selected from the screening experiment and submitted for Edman degradation. The results obtained are reported in the table II-1.

Synthesis of identified tweezer receptor

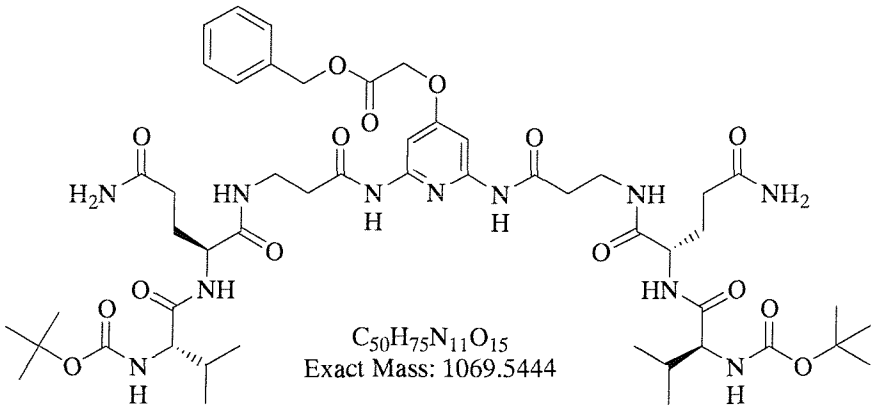
Benzyl 2-(3,5-di{[3-({5-amino-2-[*tert*-butoxycarbonyl)amino]-5-oxopentanoyl} amino)propanoyl]amino}phenoxy)acetate (105) [136]



TFA (2mL) was added dropwise to a solution of the diamidopyridine **96** (0.16 g, 0.26 mmol) in dichloromethane (4 mL) and the resulting mixture was stirred for 2 hours. The solution was concentrated and the trituration of the resulting oil with Et_2O gave the bis TFA salt as a white solid, which was dissolved in dimethylformamide (2 mL). N-Boc-L-Gln-OH (0.196 g, 0.76 mmol) HBTU (0.3 g, 0.76mmol) and DIPEA (0.23 mL, 1.32 mmol) were added and the resulting mixture stirred for 18 hours. The precipitate formed was isolated by centrifugation to give tweezer **105** as a white solid (0.16 g, 70% yield). Analytical data: mp > 240 °C; $[\alpha]_D$ = -5.31 (c 0.8, DMSO, 22 °C); ν_{max} = 3302 (br m), 2938 (w), 1746 (m), 1649 (s), 1519 (m), 1439 (m), 1162 (s), 1018 (s) cm^{-1}; ^1H NMR (400 MHz, [D_6] DMSO) δ = 10.05 (s, 2H, NHpyr), 7.82 (br s, 2H, N*H*CH_2CH_2), 7.41-7.31 (m, 7H, ArH+ pyrH), 7.22 (s, 2H, CON*H*$_a$H$_b$), 6.82 (d *J* = 7.5 Hz, 2H, NHBoc), 6.72 (s, 2H, NH_aH_b), 5.21 (s, 2H, CH_2Ar), 4.88 (s, 2H, OCH_2CO), 3.84 (m, 2H, C*H*NHBoc), 3.38 (m, 4H, CH_2CH_2NH), 2.57 (m, 4H, CH_2CH_2NH), 2.07 (s, 4H, CH_2CONH_2), 1.81 (m, 2H, $CH_aH_bCHNHBoc$), 1.66 (m, 2H, $CH_aH_bCHNHBoc$), 1.35 (s, 18H, $C(CH_3)_3$); ^{13}C NMR (100 MHz, [D_6] DMSO) δ = 173.2 (0), 171.2 (0), 170.1 (0), 167.4 (0), 165.7 (0), 154.6 (0), 150.8 (0), 134.9 (0), 127.8 (1), 127.6 (1),

127.4 (1), 95.1 (1), 77.5 (0), 65.7 (2), 63.9 (2), 54.3 (2), 53.5 (1), 35.6 (2), 34.2 (2), 31.0 (2), 27.6 (3); LRMS (ES^+) *m/z* (%) = 872.3 (80) $[M+H]^+$, 894.3 (10) $[M+Na]^+$.

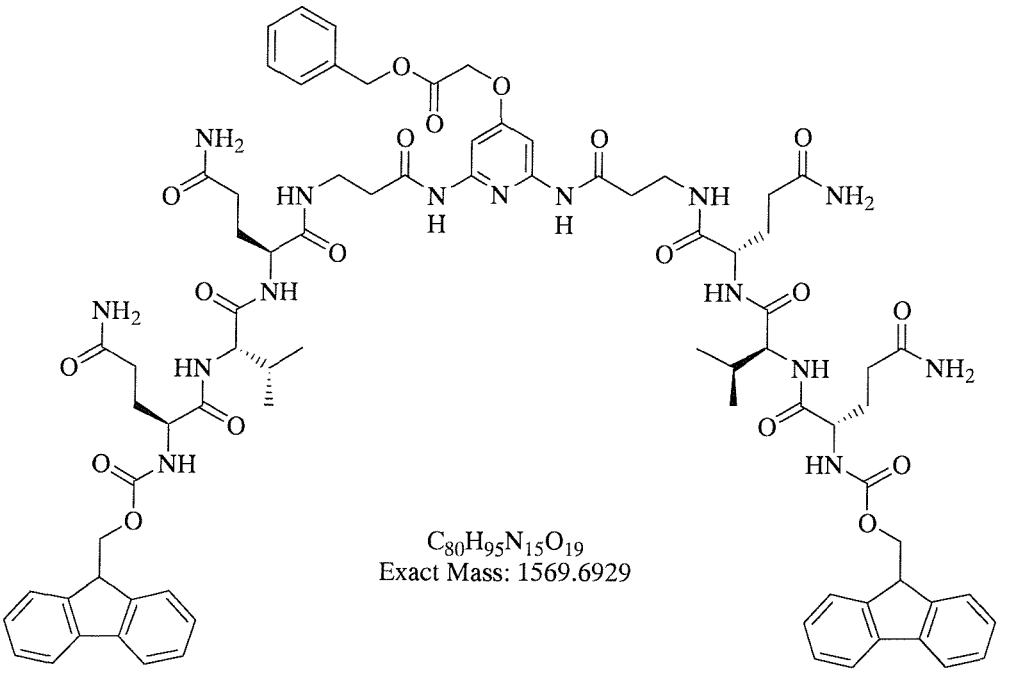
Benzyl 2-(3,5-di[(3-{[5-amino-2-({2-[(tert-butoxycarbonyl)amino]-3-methyl butanoyl} amino)-5-oxopentanoyl]amino}propanoyl)amino]phenoxy}acetate (106)
136



TFA (2 mL) was added dropwise to a solution of the diamidopyridine **105** (0.16 g, 0.18 mmol) in CH_2Cl_2 (4 mL) and the resulting mixture was stirred for 2 hours. The solution was concentrated and the trituration of the resulting oil with diethyl ether gave the bis-TFA salt as a white solid, which was dissolved in dimethylformamide (2 mL). N-Boc-L-Val-OH (0.12 g, 0.55 mmol), HBTU (0.21 g, 0.55 mmol) and DIPEA (0.16 mL, 0.93 mmol) were added and the resulting mixture stirred for 18 h. The precipitate formed was isolated by centrifugation to give tweezer **106** as a white solid (0.14 g, 73% yield). Analytical data: HPLC: 13.03 min (LONG220); 8.03 min (STD220); mp decompose >210 °C; $[\alpha]_D$ = -5.96 (c 1.0, DMSO, 22 °C); ν_{max} = 3285 (br m), 2959 (w), 1632 (s), 1519 (s), 1436 (m), 1163 (s) cm^{-1}; ^1H NMR (400 MHz, $[D_6]$ DMSO) δ = 10.00 (s, 2H, NHCO), 7.92 (br s, 2H, br CH_2CH_2NHCO), 7.78 (d, *J* = 8.0 Hz, 2H, CHN*H*CO), 7.37-7.29 (m, 7H, ArH and pyrH), 7.15 (s, 2H, $CONH_aH_b$), 6.71 (m, 4H, N*H*Boc, $CONH_aH_b$), 5.17 (s, 2H, CH_2Ar), 4.85 (s, 2H, OCH_2CO), 4.18 (q, *J* = 7.5 Hz, 2H, $CHCH(CH_3)_2$), 3.75 (br s, 2H, $CHCH_2CH_2CONH_2$), 3.33 (m, 4H, $NHCOCH_2CH_2$), 2.53 (m, 4H, $NHCOCH_2CH_2$), 2.01 (t, *J* = 7.5 Hz, 4H, CH_2CONH_2), 1.91 (m, 2H, $CH(CH_3)_2$) 1.78 (m, 2H, $CHaHbCH_2CONH_2$) 1.68 (m, 2H, $CH_aH_bCH_2CONH_2$), 1.34 (s,

18H, $C(CH_3)_3$), 0.79 (d, J = 6.5 Hz, 6H, $CH(CH_3)_2$), 0.75 (d, J = 6.5 Hz, 6H, $CH(CH_3)_2$); ^{13}C NMR (100 MHz, [D_6] DMSO) δ = 173.6 (0), 171.0 (0), 170.4 (0), 167.9 (0), 166.3 (0), 155.5 (0), 151.3 (0), 135.5 (0), 128.4 (1), 128.1 (1), 127.9 (1), 95.6 (1), 78.1 (0), 66.2 (2), 64.5 (2), 59.8 (1), 52.0 (1), 36.1 (2), 35.8 (2), 34.7 (2), 31.3 (2), 30.2 (1), 28.2 (3), 19.2 (3), 17.9 (3); LRMS (ES^+) m/z (%) = 1070.1 (100) $[M+H]^+$, 1091.9 (40) $[M+Na]^+$.

Benzyl 2-{3,5-di[(3-{[5-amino-2-({ 2-[(5-amino-2-{[(9H-9-fluorenylmethoxy) carbonyl]amino}-5-oxopentanoyl)amino]-3-methylbutanoyl}amino)-5-oxo pentanoyl]amino}propanoyl)amino]phenoxy}acetate (107) [136]

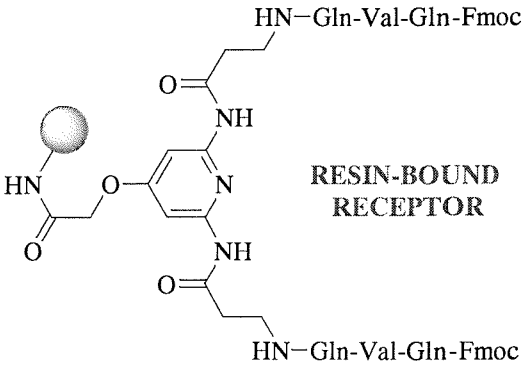


TFA (1 mL) was added dropwise to a solution of the diamidopyridine **106** (0.08 g, 0.075 mmol) in dichloromethane (3 mL) and the resulting mixture was stirred for 2 hours. The solution was concentrated and the trituration of the resulting oil with diethyl ether gave the bis-TFA salt as a white solid, which was dissolved in dimethylformamide (2 mL). N-Fmoc-L-Gln-OH (0.06 g, 0.16 mmol), HBTU (0.06 g, 0.16 mmol) and DIPEA (0.05 mL, 0.3 mmol) were added and the resulting mixture stirred for 18 hours. The precipitate formed was isolated by centrifugation to give tweezer **107** as a white

solid (0.08 g, 70% yield). Analitical data: HPLC 9.67 min (STD220, analytical), 20.7 min (LONG 254); mp decompose >240 °C; $[\alpha]_D$ = -6.38 (c 0.53, DMSO, 22 °C); ν_{max} = 3375 (br m), 1668 (s), 1629 (m) 1543 (m), 1210 (m), 1007 (s) cm^{-1}; ^1H NMR (400 MHz, [D_6] DMSO) δ = 9.94 (s, 2H, pyrNH), 7.96 (d, *J* = 7.5Hz, 2H, NH), 7.90 (d, *J* = 7.5 Hz 4H, fluorenylH), 7.85 (t, *J* = 6 Hz, 2H, CH_2CH_2NHCO), 7.75-7.70 (m, 6H, NH and fluorenylH), 7.53 (d, 2H, N*H*), 7.45-7.32 (m, 15H, ArH, fluorenylH,+pyrH), 7.24 (s, 2H, $CONH_aH_b$), 7.16 (s, 2H, $CONH_cH_d$), 6.72 (s, 2H, $CONH_aH_b$), 6.67 (s, 2H, $CONH_cH_d$), 5.1 (s, 2H, OCH_2Ph), 4.79 (s, 2H, OCH_2CO), 4.16-4.07 (m, 10H, $CHCH(CH_3)_2$, $CHCH_2CH_2CONH_2$, $CHCH_2$fluorenyl), 3.94 (m, 2H, $CHCH_2CH_2CONH_2$), 3.40 (m, 4H, CH_2CH_2NH), 2.20-1.70 (m, 14H, $COCH_2CH_2NH$ and $CH(CH_3)_2$ and $CH_2CH_2CONH_2$), 0.85 (d, 6H, $CH(CH_3)_2$), 0.83 (d, 6H, $CH(CH_3)_2$); ^{13}C NMR (100 MHz, [D_6] DMSO) δ = 173.9 (0), 173.7 (0), 171.1 (0), 170.7 (0), 170.5 (0), 167.9 (0), 166.3 (0), 157.3 (0), 155.9 (0), 151.3 (0), 143.8 (C), 140.7 (0), 135.5 (0), 128.4 (1), 128.1 (1), 128.0 (1), 127.6 (1), 127.1(1), 125.3 (1), 120.1 (1), 95.7 (1), 66.2 (2), 65.7 (2), 64.5 (2), 57.5 (1), 54.3(1), 52.3 (1), 46.6 (1), 36.1 (2), 34.7 (2), 31.6 (2), 31.4 (2), 30.5 (1), 27.9 (2), 27.7 (2), 19.2 (3), 17.8 (3); MS (TOF LD^+) *m/z* (%) = 1571.07 (100) $[M+H]^+$, 3301.65 (30) $[2M+H]^+$.

Synthesis of the resin bound tweezer receptor

Resin Bound Receptor (110)



A solution of CBS **97** (32.8 mg, 0.624 mmol), HBTU (23.35 mg, 0.0624mmol) in DMF (2 mL) was stirred for 5 min and then added to TentaGel resin (200 mg, 0.26 mmol/g) pre-swollen in dichloromethane (1 mL) followed by DIPEA (45 μL, 0.26 mmol), and the suspension was shaken for 20 hours. Any remaining amine residues were capped by treating the resin with an excess of acetic anhydride. The resin was filtered and washed with dichloromethane (3 × 5 mL), dimethylformamide (3 × 5 mL), dichloromethane (3 × 5 mL). A qualitative ninhydrin test was negative. Subsequent Boc deprotection was achieved with a solution of TFA (30%) in dichloromethane for 2 hours. The resin was filtered and washed with dichloromethane (3 × 5 mL), dimethylformamide (3 × 5 mL), dichloromethane (3 × 5 mL) and washed with a solution of DIPEA (10%) in dichloromethane, MeOH (3 × 5 mL), and Et_2O (5×5 mL) and dried *in vacuo*. The tweezer receptor **110** was then prepared, using 3 cycles of Fmoc peptide synthesis. A solution of Fmoc-L-Gln (57.5 mg, 0.156 mmol), HBTU (59.2 mg, 0.156 mmol) in dimethylformamide (2 mL) was stirred for 5 min and then added to TentaGel resin pre-swollen in dichloromethane (1 mL) followed by DIPEA (45 μL, 0.26 mmol), and the suspension was shaken for 3 hours. The resin was filtered and washed with dichloromethane (3 × 5 mL), dimethylformamide (3 × 5 mL), dichloromethane (3 × 5 mL). A qualitative ninhydrin test was negative. Fmoc deprotection and subsequent coupling of Fmoc-L-Val-OH, followed by Fmoc

deprotection and coupling of Fmoc-L-Gln-OH as described above provided the resin bound tweezer **110** with an estimated loading of 0.05 mmol of receptor/g resin.

General Procedure for HPLC experiment in $CHCl_3$

Standard solution A_x (30 μL) was taken, the solvent evaporated under reduced pressure and the residue dissolved in (230 μL) of acetonitrile; and 30 μL of this solution were injected into HPLC.
Solution X (500 μL) was added to 3.0 mg of resin and left to equilibrate. After equilibration the solution (30 μL) was evaporated under reduced pressure and the residue dissolved in 230 μL of acetonitrile. The resulting solution (30μL) was directly injected into HPLC. The procedure was repeated after equilibration time (refer to Table II-1, 2, 3)

Solution $\mathbf{A_1}$ of Red dye-spacer-L-Glu-L-Ser-L-Val-OH **100** (0.3 mM, 1.2 mg/5 mL) and Red Dye **112** (0.3 mM, 0.5 mg/5 mL) as internal standard was prepared in $CHCl_3$ (1%DMSO).

Solution $\mathbf{A_2}$ of Red dye-spacer-L-Glu-L-Ser-L-Val-OH **2** (0.3 mM, 1.2 mg/5 mL) and Acetylated Red Dye **111** (0.3 mM, 0.5 mg/5 mL) as internal standard was prepared in $CHCl_3$ (1%DMSO).

Solution $\mathbf{A_3}$ of Red dye-spacer-L-Glu(O^{t}Bu)-L-Ser(^{t}Bu)-L-Val-OH **99** (0.3 mM, 1.2 mg/5 mL) and Red Dye **112** (0.3 mM, 0.5 mg/5 mL) as internal standard was prepared in $CHCl_3$ (1%DMSO).

Solution $\mathbf{A_4}$ of Red dye-spacer-D-Glu-D-Ser-D-Val-OH **101** (0.3 mM, 1.2 mg/5 mL) and Red Dye **112** (0.3 mM, 0.5 mg/5 mL) as internal standard was prepared in $CHCl_3$ (1%DMSO).

Solution $\mathbf{A_5}$ of Red dye-spacer-D-Glu-D-Ser-D-Val-OH **101** (0.3 mM, 1.2 mg/5 mL) and Acetylated Red Dye **111** (0.3 mM, 0.5 mg/5 mL) as internal standard was prepared in $CHCl_3$ (1%DMSO).

Retention time:

Red dye-spacer-(L,D)-Glu-(L,D)-Ser-(L,D)-Val-OH **100** and **101**: 10.3 min.

Red dye-spacer-L-Glu(O^{t}Bu)-L-Ser(^{t}Bu)-L-Val-OH **99**: 13.5min.

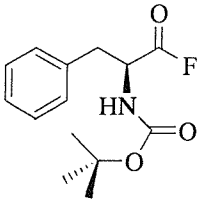
Red Dye **112**: 11.3 min.

Red Dye **111**: 13.5 min.

V.6 Experimental Part Chapter III

Synthesis of Unsymmetrical CBS Gennari's Group Collaboration

tert-Butyl *N*-(1-benzyl-2-fluoro-2-oxoethyl)carbamate (120) [138]

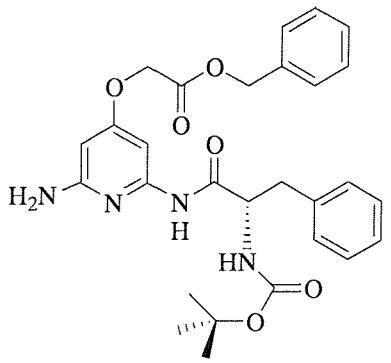


$C_{14}H_{18}FNO_3$
Exact Mass: 267.1271

A mixture of *N*-Boc-L-Phe-OH (0.318 g, 1.2 mmol) and pyridine (0.98 mL, 1.2 mmol) in dry dichloromethane was cooled down to −15 ^{0}C. After addition of cyanuric fluoride (0.222 mL, 2.6 mmol) the mixture was stirred for 3 hours at – 15 ^{0}C under an atmosphere of nitrogen. The solution was poured on ice (10-20 mL) and more dichloromethane (50 mL) was added. The organic layer was separated and the aqueous layer was extracted with dichloromethane (2 × 200 mL). The combined organic layers were dried over magnesium sulphate. Evaporation of the solvent at room temperature gave the product as pale yellow solid (0.33 g, 1.2 mmol, 100% yield). Analytical data: mp = 65-69 °C; $[\alpha]_D$ = 52.4 (c 1.08, $CHCl_3$, 24 °C); ν_{max} = 3369 (w), 2973 (w), 1832 (s), 1784 (s), 1679 (s), 1512 (s), 1156 (s) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.36-7.18 (m, 5H, ArH,), 4.80 (br dd, J = 5.9 Hz, 1H, 14 Hz, CHCOF), 3.17 (d, J = 5.9 Hz, 2H, CH_2Ar), 1.43 (s, 9H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.9 (d, J_{CF} = 371 Hz, CO), 157.5 (CO), 134.5 (C), 129.3(CH), 129.2(CH), 127.8 (CH), 80.8 (C) 66.0 (C), 53.42 (d, J_{CF} = 60Hz, C*H*NH), 36.9 (CH_2), 28.2 (CH_3); LRMS (ES+) *m/z* (%) = 306.3 (80) $[M+K]^+$.

Spectroscopic data were consistent with those reported in literature [138,184]

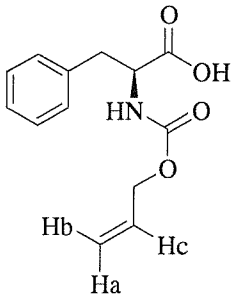
Benzyl2-{[2-amino-6-({(2*S*)-2-[(tert-butoxycarbonyl)amino]-3-phenylpropanoyl}amino)-4-pyridyl]oxy}acetate (121)



$C_{28}H_{32}N_4O_6$
Exact Mass: 520.2322

To a solution of **94** (0.740 g, 2.7 mmol) in dry acetonitrile (36 mL), DIPEA (0.940 mL, 5.4 mmol), BTSA (0.33 mL, 1.35 mmol) and N-Boc-L-Phe-F (0.710 g, 2.7 mmol) were added. After stirring at room temperature for 1.5 hours, a catalytic amount of a solution of TBAF (1M in THF) was added to the mixture. Stirring was continued for 20 hours at room temperature. After evaporation of the solvent the residue was dissolved in dichloromethane (350 mL), washed with an aqueous solution of sodium hydrogencarbonate (5%, 350 mL) and water (300 mL). The organic layer was dried over magnesium sulphate and the solvent was evaporated to give a yellow oil. The residue was purified by flash chromatography on silica gel (eluant, 98:2 CH_2Cl_2/MeOH) to obtain the product as pale yellow waxy solid (0.796 g, 1.53 mmol, 57%) and staring material **94** (0.147 g, 0.54 mmol, 20%). Analytical data: ν_{max} = 2965 (w), 2866 (w), 1690 (m), 1617 (s), 1460 (m), 1159 (s), 1078 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ = 8.39 (s, 1H, NH), 7.37-7.18 (m, 11H, ArH and C_4H_2N), 5.76 (d, J = 1.5 Hz, 1H, C_4H_2N), 5.26 (2H, s, OCH_2Ar), 5.0 (br s, 1H, NH), 4.7 (2H, s, CH_2OCO), 4.49 (br d, 3H, $CHNH+NH_2$), 3.1 (br dd, J = 5.9 Hz, 2H, $CHCH_2Ph$), 1.4 (s, 9H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 169.7 (0), 167.8 (0), 167.0 (0), 158.1 (0), 155.1 (0), 150.6 (0), 136.0 (0), 134.8 (0), 129.0 (1), 128.5 (1), 128.4 (1), 128.3 (1), 128.3 (1), 126.9 (1), 126.8 (1), 90.8 (1), 90.2 (1), 80.3 (0), 66.9 (2), 64.6 (2), 56.2 (1), 38.0 (2), 28.0 (3); LRMS (ES^+) *m/z* (%) = 521.1 (100) $[M+H]^+$, 1041 (40) $[2M+H]^+$.

2-{[(allyloxy) carbonyl]amino}-3-phenylpropanoic acid (122) [185]

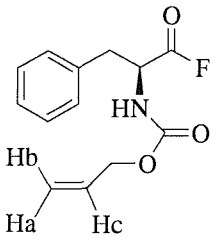


$C_{13}H_{15}NO_4$
Exact Mass: 249.1001

To a solution of phenylalanine (2.44 g, 1.476 mmol) in aqueous 4N NaOH (20 mL) allyl chloroformate (1.96 g 16.2 mmol) was added dropwise at 0 °C (ice/water bath). The reaction mixture is allowed to warm up to room temperature and stirred overnight. After 18 hours reaction time the solution was acidified by adding a concentrated solution of potassium hydrogen sulphate until pH 2. During this process an equal amount of dichloromethane was added. The aqueous layer was extracted with dichloromethane (3 × 150 mL) and the combined organic layers were dried over magnesium sulphate. Evaporation of the solvent and drying of the residue at high vacuum afforded the product in 96% (3.518 g, 14.12 mmol). Analytical data: HPLC = 10.23 min (S15OD2); ν_{max} = 3316 (br w), 2933 (w), 1719 (m), 1659 (s), 1256 (s), 740 (m) cm^{-1}; ^1H NMR (400 MHz, CDCl3) δ = 7.16-7.09 (m, 5H, ArH); 6.42 (br s, 1H, NH), 5.86-5.76 (ddt, J_{trans} = 17 Hz, J_{cis} = 11 Hz, J_{vic} = 5.5 Hz, 1H, CH_2=C*H*CH_2O), 5.20 (d, J_{trans} = 17 Hz, 1H, CH_aH_b=$CHCH_2OCO$), 5.13 (d, J_{cis} = 11 Hz, 1H, CHH_a=$CHCH_2OCO$), 4.63-4.58 (m, 1H, NHC*H*CH_2), 4.48 (d, J_{vic} = 5.5 Hz, 2H, CH_2CH=CH_2), 3.15-3.11 (dd, J = 5.5 Hz, 14 Hz, 2H, CH_2Ph), 3.06-3.01 (dd, J = 6 Hz, 14 Hz, 2H, CH_2Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 176.0 (0). 155.9 (0), 135.7 (1), 132.6 (0), 129.5 (1), 128.8 (1), 127.4 (1), 118.1 (2) 66.2 (2), 54.7 (1), 37.9 (2); LRMS (ES^+): *m/z* (%) =250 $[M+H]^+$, 499 $[2M+H]^+$, 521 $[2M+Na]^+$.

Spectroscopic data were consistent with those reported in literature. [185]

Allyl *N*- (1-benzyl-2-fluoro-2-oxoethyl) carbamate (123)

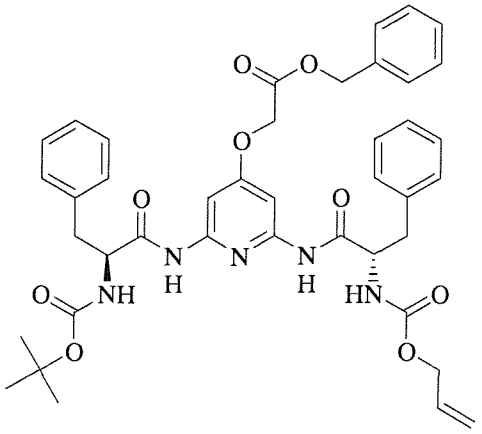


$C_{13}H_{14}FNO_3$
Exact Mass: 251.0958

A mixture of N-Aloc-protected phenylalanine **122** (0.736 g, 2.95 mmol) and pyridine (0.238 mL, 2.95 mmol) in dry dichloromethane was cooled down to – 15 ^{0}C. After addition of cyanuric fluoride (0.548 mL, 6.49 mmol) the mixture was stirred for 3 hours at – 15 ^{0}C under a nitrogen atmosphere. The solution was poured on ice (10-20 mL) and more dichloromethane (50 mL) was added. The organic layer was separated and the aqueous layer is extracted with dichloromethane (2 × 200 mL). The combined organic layers were dried over $MgSO_4$. Evaporation of the solvent at room temperature gave the product as a colourless oil (0.683 g, 2.72 mmol, 92% yield). Analytical data: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.29-7.16 (m, 5H, ArH), 5.89 (ddt, J_{trans} = 17Hz, J_{cis} = 11 Hz, J_{vic} = 7 Hz, 1H, CH_2=C*H*CH_2O), 5.21 (m, 1 H, CH_{2a}=C*H*CH_2O), 5.15 (dd, J_{cis} = 11 Hz, J_{gem} = 1 Hz, 1H, CH_{2b}=C*H*CH_2O), 5.03 (br s, 1H, NH), 4.82 (d, J = 7 Hz, 1H, NHC*H*CH_2)), 4.58 (d, J = 7 Hz, 2H, COCH_2CH), 3.20 (d, J = 7 Hz, 2H, CH_2Ar); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 163.6-159.9 (0) (d, $^1J_{CF}$ = 370 Hz), 155.3 (0), 134.2 (0), 132.2 (1), 129.2 (1), 129.1 (1), 127.8 (1), 118.3 (2), 66.3 (2), 53.9-53.4 (1) (d, $^2J_{CF}$ = 60.Hz), 36.9 (2); LRMS (ES^+): m/z (%) = 290.3 $[M+K]^+$.

Spectroscopic data were consistent with those previously reported. [135]

Benzyl 2-{[2-[((2*S*)-2-{[(allyloxy)carbonyl]amino}-3-phenylpropanoyl)amino]-6-({(2*S*)-[*tert*-butoxycarbonyl)amino]-3-phenylpropanoyl}amino)-4-pyridyl]oxy}acetate (116)



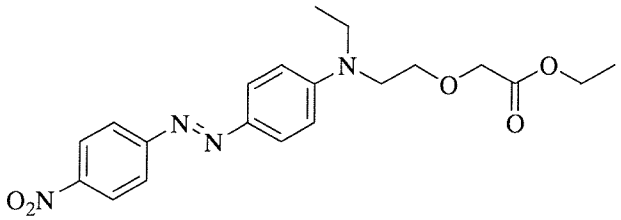
$C_{41}H_{45}N_5O_9$
Exact Mass: 751.3217

To a solution of **121** (0.30 g, 0.57 mmol) in dry acetonitrile (5 mL), BTSA (0.140 mL, 0.57 mmol) was added. After stirring at room temperature for 2 hours N-Alloc-L-Phe-F (0.42 g, 1.7 mmol) and MTDA (0.34 mL, 1.7 mmol) were added to the mixture. Stirring was continued for 20 hours at 70 °C. The solvent was removed under reduced pressure, the residue was dissolved in dichloromethane (350 mL) and washed with an aqueous solution of sodium hydrogencarbonate (5%, 150 mL) and water (100 mL). The organic layer was dried over magnesium sulphate. The residue was purified by flash chromatography on silica gel (eluant, 98:2 CH_2Cl_2/MeOH) to obtain the product as pale yellow oil (0.39 g, 0.52 mmol, 92 %). Analytical data: HPLC: 38.4 min (40STD254); 4.5 min (S5OD), 14.4 min (S15OD2); $[\alpha]_D$ = -4.95 (c 1.04, $CHCl_3$, 22 °C); ν_{max}: 3282 (m), 3025 (w), 2977 (w), 2361 (w), 1758 (m), 1684 (s), 1584 (m), 1511 (s), 1437 (s), 1214 (s), 1159 (s) cm^{-1}; ^1H NMR (400 MHz, CD_3CN) δ = 9.59 (br s, 2H, NH), 7.38-7.23 (m, 17H, ArH), 6.23 (br s, 1H, NH), 5.87-5.80 (m, 1H, CH_2=C*H*CH_2O), 5.22-5.12 (m, 2H, C*H*$_2$CHCH$_2$OCO), 5.22 (s, 2H, OC*H*$_2$Ar), 4.82 (s, 2H, OCH_2OCO), 4.58-4.44 (m, 4H, C*H*NHCH$_2$ and COOC*H*$_2$CH=CH$_2$), 3.27-3.16 (m, 2H, NHCHC*H*$_2$Ph), 2.99-2.89 (m, 2H, NHCHC*H*$_2$Ph), 1.33 (9H, s, $(CH_3)_3C$); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 172.2 (0), 170.4 (0), 166.8 (0), 156.3 (0), 155.9 (0), 147.7 (0), 135.7 (0), 134.8 (0), 132.4 (1), 129.4 (1), 129.0 (1), 128.9 (1), 128.8 (1), 128.7 (1),

127.4 (1), 127.3 (1), 118.2 (2), 96.4 (1), 96.2 (1), 80.9 (0), 67.8 (2), 66.4 (2), 65.7 (2), 57.4 (1), 57.2 (1), 37.8 (2), 37.9 (2), 28.3 (3); LRMS (ES^+): *m/z* (%) = 752 $[M+H]^+$, 774, $[M+Na]^+$, 1504 $[2M+H]^+$, 1526 $[2M+Na]^+$; HRMS (ES^+) calcd for $C_{41}H_{45}N_5NaO_9^+$ $[M+Na]^+$: 774.3130, found 774.3109.

Synthesis of guests Davis' Collaboration

1-(2-{ethyl-4-[(*E*)-2-(4-nitrophenyl)-1-diazenyl]anilino}ehoxy)-2-butanone (128)



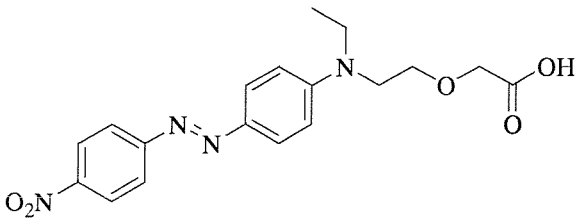
$C_{20}H_{24}N_4O_5$
Exact Mass: 400.1747

To a solution of Disperse Red Dye **127** (0.78 mg, 2.5 mmol) in a 1:1 mixture of dichloromethane (25 mL) and toluene (25 mL), $Rh_2(OAc)_4$ (20 mg, 0.05 mmol) was added and the resulting reaction mixture was stirred at 40 °C for 15 min. A solution of ethyl diazoacetate (1 mL, 10mmol) in toluene (5 mL) was added drop wise over a period of 1 hour. The evolution of N_2 was observed during the addition. The resulting reaction mixture was stirred for 18 hours at room temperature. The solvent was removed under reduced pressure and the resulting residue was purified by column chromatography on silica gel (Petrol Ether/EtOAc, 80:20) to give the product as a red solid in 74% yield (0.74 mg, 1.86 mmol). Analytical data: HPLC (see general remarks) retention time: 13.5 min, 15.38 min (S15OD2). (lit: [152] 80 °C); ν_{max} = 2973 (m), 1758 (s), 1599 (s), 1511 (s), 1388 (m), 1335 (m), 1131 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 8.29 (d, 2H, *J* = 9 Hz, ArH), 7.89 (t, 4H, *J* = 9 Hz, ArH), 6.76 (d, 2H, *J* = 9 Hz), 4.21 (q, 2H, *J* = 7 Hz, OCH_2CH_3), 4.1 (s, 2H, OCH_2CO), 3.77 (t, 2H, *J* = 6 Hz, NCH_2CH_2O), 3.68 (t, 2H, *J* = 6 Hz, NCH_2CH_2O), 3.55 (q, 2H, *J* = 7 Hz, NCH_2CH_3), 1.27 (t, 3H, *J* = 7 Hz, OCH_2CH_3), 1.24 (t, 3H, *J* = 7 Hz, NCH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 170.3 (0), 156.9 (0), 151.5 (0), 147.5 (0), 143.8 (0), 126.4 (1), 124.8 (1), 122.7 (1), 111.5 (1), 69.2 (2), 68.8 (2), 61.1 (2), 50.3 (2), 46.1 (2), 14.3 (3), 12.3

(3); LRMS (ES^+) *m/z* (%) = 401.3 (100) $[M+H]^+$; HRMS (ES^+) calcd for $C_{20}H_{25}N_4O_5$ $[M+H]^+$: 401.1819, found 401.1819.

Spectroscopic data were consistent with those reported in literature. [152]

1-(2-{ethyl-4-[(*E*)-2-(4-nitrophenyl)-1-diazenyl]anilino}ethoxy)acetic acid (129)



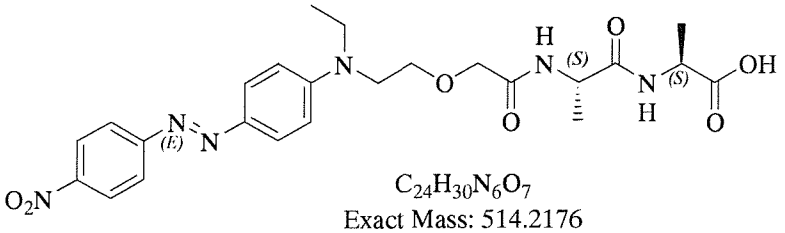
$C_{18}H_{20}N_4O_5$
Exact Mass: 372.1434

To a solution of **128** (0.30 mg, 0.75 mmol) in dioxane (30 mL) was added an aqueous solution of LiOH (1M, 30 mL). The resulting reaction mixture was stirred for 24 hours at room temperature. The solvent was removed under reduced pressure and the aqueous phase was acidified to pH ~ 3-4 with a solution of $KHSO_4$ 1M. The aqueous layer was extracted with dichloromethane (3 × 200 mL). The organic layer was dried over magnesium sulphate and the solvent removed under pressure to give the product as a red solid in 98% yield (0.27 mg, 0.73 mmol). Analytical data: HPLC retention time: 11.44 min (S15OD2); mp: 160 °C (lit: [152] 161 °C); ν_{max} = 2902 (br, m), 1723 (s), 1599 (s), 1584 (s), 1510 (s), 1379 (m), 1333 (s), 1127 (s) cm^{-1}; ^1H NMR (400 MHz, $[D_6]$ DMSO) δ = 8.35 (d, 2H, *J* = 9Hz, ArH), 7.93 (d, 2 H, *J* = 9Hz, ArH), 7.83 (d, 2H, *J* = 9 Hz, ArH), 6.89 (d, 2H, *J* = 9 Hz, ArH), 4.02 (s, 2H, OCH_2CO), 3.67 (m, 4H, NCH_2CH_2O), 3.55 (q, 2H, *J* = 7 Hz, NCH_2CH_3), 1.16 (t, 3H, *J* = 7 Hz., NCH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 171.5 (0), 156.2 (0), 151.7 (0), 146.8 (0), 142.6 (0), 126.1 (1), 124.9 (1), 122.4 (1), 111.6 (1), 68.2 (2), 67.9 (2), 49.5 (2), 45.2 (2), 11.9 (3); LRMS (ES^+) *m/z* (%) = 373.2 (100) $[M+H]^+$; HRMS (ES^+) calcd for $C_{18}H_{21}N_4O_5$ $[M+H]^+$ 373.1491, found 373.1506.

Spectroscopic data were consistent with those reported in literature. [152]

Red-Dye-L-Ala-L-Ala-OH

(2*S*)-2-[((2*S*)-2-{[2-(2-{ethyl-4-[(*E*)-2-(4-nitrophenyl)-1-diazenyl]anilino}ethoxy) acetyl]amino}propanoyl)amino]propanoic acid (125)



$C_{24}H_{30}N_6O_7$
Exact Mass: 514.2176

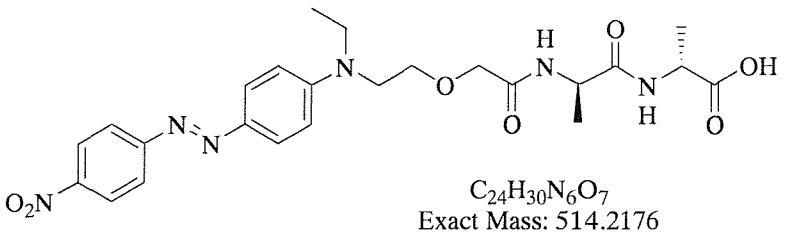
To a suspension of Rink acid resin (500 mg, 0.215 mmol) in dry tetrahydrofuran was added triphenylphosphine (310 mg, 1.18 mmol) and hexachloroethane (280 mg, 1.18 mmol) and the resulting mixture was shaken for 6 hours at room temperature under argon. After washing with tetrahydrofuran (3 × 10 mL) the resin was pre-swollen in 1,2-dichloroethane. Fmoc-L-Ala-OH (201 mg, 0.65 mmol) and DIPEA (225 μL, 0.65 mmol) were added to the resulting Rink chloride resin **130** and the mixture was shaken for 20 hours. Subsequent washing with dichloromethane (3 × 10 mL), methanol (3 × 10 mL) and dichloromethane (3 × 10 mL) yielded a resin which gave a negative ninhydrin test. A quantitative Fmoc test was applied to a small sample at this stage of the synthesis before removing Fmoc protecting groups from the remaining resin. Subsequent washing with dichloromethane (3 × 10 mL), N,N-dimethylformamide (3 × 10 mL) and dichloromethane (3 × 10 mL) yielded a resin which gave a positive ninhydrin test. A solution of Fmoc-L-Ala-OH (201 mg, 0.65 mmol), DIC (101 μL, 0.65 mmol) and HOBt (98 mg, 0.64 mmol) in DMF was stirred for 10 min and added to the resin. Following addition of DIPEA (168 μL, 0.96 mmol), the resulting mixture was shaken at room temperature for 20 hours. The resin was washed as before and a qualitative ninhydrin test indicated complete coupling.

A solution of dye acid **129** (1.5 eq, 0.32 mmol), HOBt (58 mg, 0.32 mmol), DIC (121 mg, 0.32 mmol) in DMF (10 mL) was stirred for 10 min and added to the resin followed by addition of DIPEA (186 μL, 1.07 mmol) and shaken for 20 hours. After this time the resin was washed with dichloromethane (3 × 10 mL), dimethylformamide (3 × 10 mL)

and dichloromethane (3 × 10 mL). Cleavage of the product from the Rink acid resin was performed by agitating the resin in 10% acetic acid in dichloromethane for 2 × 1 h. The filtrate from the cleavage step was evaporated and the crude product was either purified (if necessary) by column chromatography or by semi-preparative reverse-phase HPLC (Phenomenex Prodigy ODS(3) C-18, 250×10 mm) using a linear gradient from water + 0.1% TFA to acetonitrile + 0.042% TFA over 40 min, acetonitrile + 0.042% TFA for 10 min, a linear gradient from acetonitrile + 0.042% TFA to water + 0.1% TFA over 5 min, and water + 0.1% TFA for 5 min, with a flow rate of 2.5 mLmin–1, monitoring at 254 nm. Analytical data: HPLC (L15OD500 see general remarks) eluted after 10.48 min, (L15OD254) 15.7 min; mp: decomposition >170 °C; $[\alpha]_D$: sample too dark to achieve the measurement; ν_{max} = 3277 (br, s), 1650 (s), 1604 (s), 1510 (s), 1389 (m), 1338 (s), 1074 (br, s) cm^{-1}; ^1H NMR (400 MHz, [D_6] DMSO): δ = 8.40 (d, 2H, *J* = 9 Hz, ArH), 7.96 (d, 2 H, *J* = 9 Hz, ArH), 7.87 (d, 2 H, *J* = 9 Hz, ArH), 7.77 (d, 1H, *J* = 7 Hz, NH), 7.40 (m, 1H, NH), 6.95 (d, 2H, *J* = 9 Hz, ArH), 4.39 (m, 1H, NHC*H*CH_3), 4.05 (m, 1H, C*H*CH_3), 3.98 (s, 2H, OCH_2CO), 3.74 (br. s, 4H, NCH_2CH_2O), 3.61 (q, 2H, *J* = 7 Hz, N*C*H_2CH_3), 1.27-1.19 (m, 9H, N*C*H_2CH_3, 2CHCH_3); ^{13}C NMR (100 MHz, [D_6] DMSO) δ = 175.2 (0), 171.3 (0), 168.3 (0), 156.2 (0), 151.7 (0), 146.8 (0), 142.7 (0), 128.1 (1), 126.1 (1), 124.9 (1), 122.4 (1), 111.6 (1), 69.9 (2), 68.5 (2), 49.3 (2), 48.9 (1), 47.5 (1), 45.1 (2), 18.4 (3), 11.9 (3); LRMS (ES^+) *m/z* (%) = 515.4 (60) $[M+H]^+$; HRMS (ES^+) calcd for $C_{24}H_{31}N_6O_7$ $[M+H]^+$: 515.2251, found 515.2249.

Red-Dye-D-Ala-D-Ala-OH

(2*R*)-2-[((2*R*)-2-{[2-(2-{ethyl-4-[(*E*)-2-(4-nitrophenyl)-1-diazenyl]anilino}ethoxy) acetyl]amino}propanoyl)amino]propanoic acid (126)



$C_{24}H_{30}N_6O_7$
Exact Mass: 514.2176

To a suspension of Rink acid resin (500 mg, 0.215 mmol) in dry tetrahydrofuran was added triphenylphosphine (310 mg, 1.18 mmol) and hexachloroethane (280 mg, 1.18 mmol) and the resulting mixture was shaken for 6 hours at room temperature under argon [154]. After washing with tetrahydrofuran (3 × 10 mL) the resin was pre-swollen in 1,2-dichloroethane. N-Fmoc-D-Ala-OH (201 mg, 0.65 mmol) and DIPEA (225 μL, 0.65 mmol) were added to the resulting Rink chloride resin **130** and the mixture was shaken for 20 hours. Subsequent washing with dichloromethane (3 × 10 mL), methanol (3 × 10 mL) and dichloromethane (3 × 10 mL) yielded a resin which gave a negative ninhydrin test. A quantitative Fmoc test was applied to a small sample at this stage of the synthesis before Fmoc-deprotecting the remaining resin. Subsequent washing with dichloromethane (3 × 10 mL), dimethylformamide (3 × 10 mL) and dichloromethane (3 × 10 mL) yielded a resin, which gave a positive ninhydrin test. A solution of N-Fmoc-D-Ala-OH (201 mg, 0.65 mmol), DIC (101 μL, 0.65 mmol) and HOBt (98 mg, 0.64 mmol) in DMF was stirred for 10 min and added to the resin. Following addition of DIPEA (168 μL, 0.96 mmol), the resulting mixture was agitated at room temperature for 20 hours. The resin was washed as before and a qualitative ninhydrin test indicated complete coupling.

A solution of dye acid **129** (1.5 eq, 0.32 mmol), HOBt (58 mg, 0.32 mmol), DIC (121 mg, 0.32 mmol) in DMF (10 mL) was stirred for 10 min and added to the resin followed by addition of DIPEA (186 μL, 1.07 mmol) and shaken for 20 h. After this time the resin was washed with dichloromethane (3 × 10 mL), DMF (3 × 10 mL) and

dichloromethane (3 × 10 mL). Cleavage of the product from the Rink acid resin was performed by agitating the resin in 10% acetic acid in dichloromethane for 2 × 1 hour. The filtrate from the cleavage step was evaporated and (if necessary) the crude product was either purified by column chromatography or by semi-preparative reverse-phase HPLC (Phenomenex Prodigy ODS(3) C-18, 250×10 mm) using a linear gradient from water + 0.1% TFA to acetonitrile + 0.042% TFA over 40 min, acetonitrile + 0.042% TFA for 10 min, a linear gradient from acetonitrile + 0.042% TFA to water + 0.1% TFA over 5 min, and water + 0.1% TFA for 5 min, with a flow rate of 2.5 $mLmin^{-1}$, monitoring at 254 nm. Analytical data: HPLC: 10.4 min (S15OD, 500 nm), 15.6 min (L15OD, 254 nm); mp: decomposition > 170 °C; $[\alpha]_D$: sample too dark to achieve the measurement; ν_{max} = 3277 (br, m), 2952 (br m), 1656 (s), 1603 (s), 1510 (s) 1189 (s), 1129 (s), cm^{-1}; ^1H NMR (400 MHz, [D_6] DMSO) δ = 8.37 (d, 2H, *J* = 9 Hz, ArH), 8.12 (br. s,1H, NH), 7.93 (d, 2 H, *J* = 9 Hz, ArH), 7.83 (d, 2 H, *J* = 9 Hz, ArH), 7.64 (d, 1H *J* = 7 Hz, NH), 6.91 (d, 2H, *J* = 9 Hz, ArH), 4.36 (m, 1H, *J* = 7 Hz, $NHCHCH_3$), 4.14 (br. s, 1 H, $CHCH_3$), 3.94 (s, 2H, OCH_2CO), 3.69 (br s, 4H, NCH_2CH_2O), 3.57 (q, 2H, *J* = 7 Hz, NCH_2CH_3), 1.29-1.15 (m, 9H, NCH_2CH_3, $2CHCH_3$); ^{13}C NMR (100 MHz, [D_6] DMSO): δ = 175.1 (0), 171.3 (0), 168.3 (0), 156.2 (0), 151.7 (0), 146.8 (0), 142.7 (0), 126.1 (1), 124.9 (1), 122.4 (1), 111.6 (1), 69.9 (2), 68.5 (2), 49.3 (2), 47.3 (1), 45.1 (2), 43.9 (1), 18.6 (3), 12.0 (3); LRMS (ES^+) *m/z* (%) = 515.4 (60) $[M+H]^+$; HRMS (ES^+) calcd for $C_{24}H_{31}N_6O_7$ $[M+H]^+$: 515.2251, found 515.2249.

General Procedure for HPLC experiment in buffer solution

Standard solution **A** (50 μL) and standard solution $\mathbf{B_1}$ and $\mathbf{B_2}$ (50 μL) were added to 100 μL of acetonitrile and 30 μL of the resulting solution was directly injected into the HPLC.

466 μL of solution $\mathbf{B_1}$ or $\mathbf{B_2}$ were added to 1.0 mg of resin bound compound **124** (0.28 μmol) and left to equilibrate. After equilibration, 50 μL of each solution was added to 100 μL of acetonitrile and 30 μL of each resulting solution were injected into the HPLC. This procedure was repeated after equilibration time (refer to Table III-3, 4, 5).

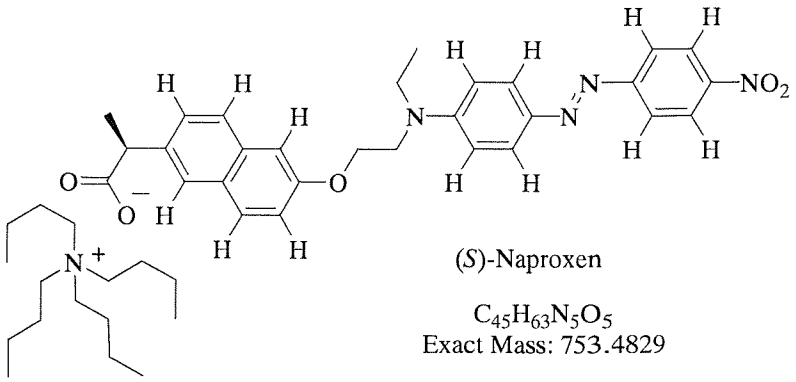
Solution **A** of Red Dye **112** (0.3mM, 0.9 mg/10 mL) in CH_3CN.

Preparation of buffer solution: Sodium borate (953.25 mg, 2.5 mmol) was dissolved in water (HPLC grade, 98.2 mL), NaOH 0.1 M was added to the solution (1.8 mL) to give a sodium borate concentration of 0.025M and pH 9.2.
Solution $\mathbf{B_1}$ of Red dye-spacer-D-Ala-D-Ala-OH **126** (0.3 mM, 1.5 mg/10 mL) in buffer solution pH 8.5.

Solution $\mathbf{B_2}$ of Red dye-spacer-L-Ala-L-Ala-OH **125** (0.3 mM, 1.2 mg/5 mL) in buffer solution pH 8.5.

Red dye-spacer-(D,L)-Ala-(D,L)-Ala-OH : retention time 10.4 min.
Red Dye **112** retention time: 11.3 min.

(*S*)-Naproxen Bu_4N^+ salt (133)
tetrabutylammonium (2*S*)-2-[6-(2-{ethyl-4-[(*E*)-2-(4-nitrophenyl)-1-diazenyl]anilino}ethoxy)-1,4-dihydro-2-naphthalenyl]propionate tetrabutylammoniun salt



(*S*)-Naproxen

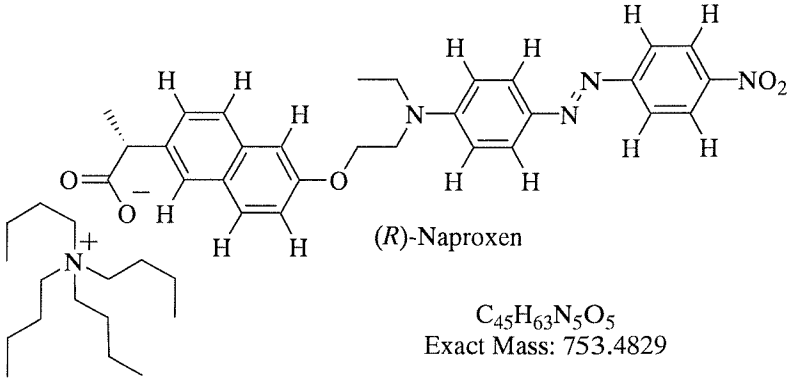
$C_{45}H_{63}N_5O_5$
Exact Mass: 753.4829

To a solution of (*S*)-Naproxen (3 mg, 5.8 μmol) in MeOH/DMSO (9:1, 2 mL), Bu_4NOH 1M in water (5.6 μL, 5.6μmol) was added. The resulting reaction mixture was stirred for 2 hours at room temperature. subsequently the solvent removed under reduced pressure. The (*S*)-Naproxen salt was lyophilised overnight. Analytical data: ^1H

NMR (400 MHz) δ = 8.32 (d, *J* = 9 Hz, 2H, ArH), 7.91 (m, 4H, ArH), 7.77 (s, 1H, Ar-napht), 7.67-7.64 (m, 2H, Ar-napht), 7.55 (d, *J* = 8.5 Hz, 1H, Ar-napht), 7.06 (m, 2H, Ar-napht), 6.84 (d, *J* = 9.0 Hz, 2 H, ArH), 4.28 (t, *J* = 5.5 Hz, 2H, CH_2O), 3.90 (t, *J* = 5.5 Hz, 2H, CH_2NAr), 3.74 (q, *J* = 7.0 Hz, 1H, $CHCH_3$), 3.64 (q, *J* = 7.0 Hz, 2H, CH_2CH_3), 3.15 (t, *J* = 8.5 Hz, 8H, $NCH_2CH_2CH_2CH_3$), 1.54-1.46 (m, 11H, $CH_2CH_2CH_2CH_3$, $CHCH_3$), 1.37-1.25 (m, 11H, $CH_2CH_2CH_2CH_3$, CH_2CH_3), 0.92 (t, *J* = 7.0 Hz, 12H, $CH_2CH_2CH_2CH_3$).

(*R*)-Naproxen Bu_4N^+ salt (134)

tetrabutylammonium (2*R*)-2-[6-(2-{ethyl-4-[(*E*)-2-(4-nitrophenyl)-1-diazenyl]anilino}ethoxy)-1,4-dihydro-2-naphthalenyl]propionate tetrabutylammonium salt.



(*R*)-Naproxen

$C_{45}H_{63}N_5O_5$
Exact Mass: 753.4829

To a solution of (*R*)-Naproxen (3 mg, 5.8 μmol) in MeOH/DMSO (9:1, 2 mL), Bu_4NOH 1M in water (5.6 μL, 5.6μmol) was added. The resulting reaction mixture was stirred for 2 hours at room temperature, subsequently the solvent was removed under reduced pressure. The (*R*)-Naproxen salt was lyophilised overnight. Analytical data: ^1H NMR (400 MHz): δ = 8.31 (d with fine splitting, *J* = 9.0 Hz, 2H, ArH), 7.91 (m, 4H, ArH), 7.77 (s, 1H, Ar-napht), 7.65 (m, 2H, Ar-napht), 7.55 (d, *J* = 8.5 Hz, 1H, Ar-napht), 7.05 (m, 2H, Ar-napht), 6.84 (d, *J* = 9.0 Hz, 2H ArH), 4.28 (t, *J* = 5.5 Hz, 2H, CH_2O), 3.90 (t, *J* = 5.5 Hz, 2H, CH_2NAr), 3.73 (q, *J* = 7.0 Hz, 1H, $CHCH_3$), 3.64 (q, *J* = 7.0 Hz, 2H, CH_2CH_3), 3.18 (t, *J* = 8.0 Hz, 8H, $NCH_2CH_2CH_2CH_3$), 1.52 (m, 11H,

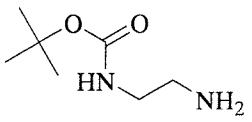
$CH_2CH_2CH_2CH_3$, $CHCH_3$), 1.38-1.24 (m, 11H, $CH_2CH_2CH_2CH_3$, CH_2CH_3), 0.93 (t, J = 7.0 Hz, 12H, $CH_2CH_2CH_2CH_3$).

Screening experiment: (*S*)-Naproxen 133 in $CHCl_3$

A sample of library **MVG 102** (2mg) and library **MVG 138** (2 mg) were equilibrated in $CHCl_3$ (200 μL) for 24 hours. A solution of (*S*)-Naproxen Bu4N$^+$ salt **133** (80 μM) in chloroform was added to the library samples to give ~ 26 μM concentration in guest, equilibration was continued for > 24h. Beads were analysed in flat-bottomed glass pots under a Leica inverted DML microscope (magnification × 40) and 40 highly red stained beads were selected. The 40 beads were washed with dichloromethane, dimethylformamide, dichloromethane, MeOH, HCl (1M), H_2O, MeOH, dichloromethane, dimethylformamide and chloroform to remove the (S)-Naproxen trapped and equilibrated again in chloroform (200 μL) A solution of (*R*)-Naproxen Bu4N$^+$ salt **134** (80 μM) in chloroform was added to the library samples to give ~ 26 μM concentration in guest, equilibration was continued for > 24 hours.

V.7 Experimental Chapter IV

1-(*tert*-butyloxycarbonyl)ethyldiamine (140) [186]

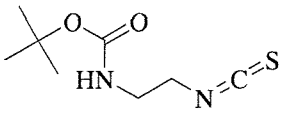


$C_7H_{16}N_2O_2$
Exact Mass: 160.1212

A solution of di-tert-butyl dicarbonate (6.1 g, 27.4 mmol) in dichloromethane (400 mL) was added dropwise to a solution of ethylenediamine **139** (9.2 mL, 137 mmol) in dichloromethane (50 mL) over 6 hours with vigorous stirring. Stirring was continued for a further 24 hours at room temperature. The solvent was removed under reduced pressure, the resulting residue was suspended in an aqueous solution of sodium carbonate (2M, 300 mL) and extracted with dichloromethane (2×300 mL). The organic layer was dried over magnesium sulphate and the solvent removed under reduced pressure to give the product **140** as colourless oil (4.30 g, 26.8 mmol, 98%). Analytical data: HPLC (STD 220) 1.7 min; ν_{max} = 3362 (w), 3323 (w), 2973 (w), 2928 (w), 1687 (s), 1520 (m), 1454 (m), 1247 (m), 1166 (s), cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 4.96 (br s, 1H, CON*H*CH_2), 3.17 (q, *J* = 6.0 Hz, 2H, CH_2CH_2NH), 2.80 (t, *J* = 6.0 Hz, 2H, CH_2CH2NH), 1.45 (s, 9H, $(CH_3)_3C$), 1.32 (br. s, 2H, NH_2).; ^{13}C NMR (100 MHz, $CDCl_3$) δ = 156.2 (0), 79.2 (0), 43.4 (2), 41.9 (2), 28.4 (3); LRMS (ES^+) m/z (%) = 161 (90) $[M+H]^+$.

All structural assignments were in agreement with the ^1H and ^{13}C reported in literature. [186]

tert-butyl _N_-(2-isothiocyanatoethyl)carbamate (141) [186]

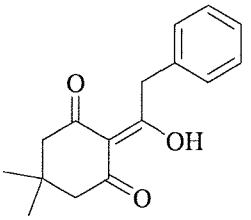


$C_8H_{14}N_2O_2S$
Exact Mass: 202.0776

Thiophosgene (2.2 mL, 29.2 mmol) was added to a solution of **140** (3.46 g, 21.6 mmol) in a mixture of chloroform (530 mL) and aqueous solution of sodium hydrogen carbonate 0.2 N (8.4 g, 99 mmol, in 500 mL water) at 0°C. The reaction mixture was stirred at room temperature for 18 hours. After separation of the organic layer, the aqueous layer was extracted with chloroform (200 mL). The combined organic layers were dried over magnesium sulphate and the solvent removed under reduced pressure to give the product as yellow oil with no notable impurities (4.2 g, 20.7 mmol, 96%). Analytical data: HPLC (S15OD) 15.4 min; ν_{max} = 3342 (m), 2975 (w), 2929 (w), 2194 (w), 2101 (m), 1689 (s), 1529 (s), 1433 (w), 1365 (m), 1282 (s), 1168 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 4.92 (br s, 1H, NH), 3.63 (t, J = 6.0 Hz, 2H, CH_2NCS), 3.37 (q, J = 6.0 Hz, 2H, CH_2NH), 1.44 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 155.6 (0), 132.3 (0), 80.1 (0), 45.4 (2), 40.6 (2), 28.3 (3); LRMS (ES^+) m/z (%) = 225.1 (100) $[M+Na]^+$.

Spectroscopically identical to material described in literature. [186]

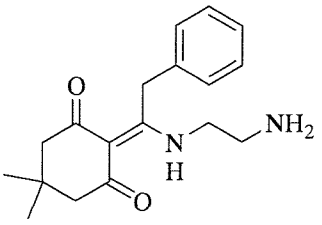
2-(1-Hydroxy-2-phenyl-ethylidene)-5,5-dimethyl-cyclohexane-1,3-dione (137)[187,188]



$C_{16}H_{18}O_3$
Exact Mass: 258.1256

Dimedone **136** (3.0g, 21.4 mmol), DCC (3.7 g, 18 mmol) and DMAP (2.2 g, 18 mmol) were added to a solution of phenylacetic acid (2.4 g, 18 mmol) in DMF (200 ml) and the resulting reaction mixture stirred at room temperature for 60 hours. Precipitated DCU was removed by filtration and the solvent removed under reduced pressure. The yellow residue was dissolved in dichloromethane (200 ml) and the organic solution washed with an aqueous solution of potassium hydrogen sulphate (1M, 200 ml) and a saturated aqueous solution of sodium hydrogen carbonate. The organic layer was dried over magnesium sulphate and the solvent removed under reduced pressure to give a yellow solid, which was crystallised from hot methanol to give the pure product as a white crystalline solid (2.6 g, 10 mmol, 60%). Analytical data: HPLC (S15OD) 18.6 min; mp = 102-104 °C; ν_{max} = 2961 (w), 2927 (w), 2858 (w), 2364(w), 2339 (w), 1647 (s), 1556 (s), 1496 (m), 1449 (m), 1404 (m), 1036 (m) cm^{-1}; ^1H NMR (400 MHz, CDCl3) δ = 7.32-7.22 (m, 5H, ArH), 4.38 (s, 2H, CH_2Ar), 2.53 (s, 2H, $COCH_2$), 2.36 (s, 2H, $COCH_2$), 1.06 (s, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 202.8 (0), 197.4 (0) 195.0 (0), 134.6 (0), 129.8 (1), 128.4 (1), 126.9 (1), 111.8 (0), 52.6 (2), 46.6 (2), 46.2 (2), 30.7 (C), 28.2 (3); LRMS (ES^-) *m/z* (%) = 257.1 $[M-H]^-$; HRMS (EI^+) calcd for $C_{16}H_{18}O_3$: 258.1256, found 258.1254.

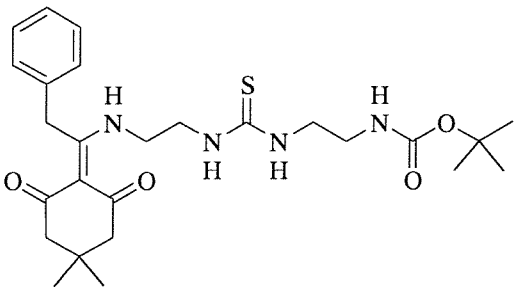
2-{1-[(2-aminorthyl)amino]-2phenylethylene}-5,5-dimethyl-1,3-cyclohexanedione (138)



$C_{18}H_{24}N_2O_2$
Exact Mass: 300.1838

A solution of **137** (2.5 g, 9.7 mmol) in dichloromethane (200 ml) was added dropwise to a solution of ethylene diamine (2.9g, 48.4 mmol) and TFA (76 ml, 0.99 mmol) in dichloromethane (100 ml), over a period of 10 hours, with vigorous stirring. The stirring was continued for further 36 hours at room temperature. The solution was washed with an aqueous solution of sodium carbonate (2M, 2 × 100 mL) and water (2 × 100 mL). The organic layer was dried over magnesium sulphate and the solvent removed under reduced pressure to give the product as a pale yellow oil in 100% yield (2.9 g, 100%). Analytical data: HPLC (S15OD) 7.18 min; ν_{max} = 2939 (w), 2867 (w), 1634 (s), 1573 (s), 1567 (s), 1557 (s), 1449 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 13.73 (br s, 1H, NH), 7.30-7.02 (m, 5H, ArH), 4.59 (s, 2H, $ArCH_2$), 3.37 (q, *J* = 6 Hz, 2H, CH_2NH), 2.88 (t, *J* = 6 Hz, 2H, CH_2NH_2), 2.40 (m, 4H, CH_2CO), 1.05 (s, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 199.7 (0), 196.3 (0), 173.5 (0), 135.8 (0), 128.7 (1), 127.9 (1), 126.5 (1), 108.1 (0), 53.6 (2), 52.5 (2), 46.1 (2), 40.8 (2), 35.3 (2), 30.0 (0), 28.3 (3); LRMS (ES^+) *m/z* (%) = 301.2 (100) $[M+H]^+$.

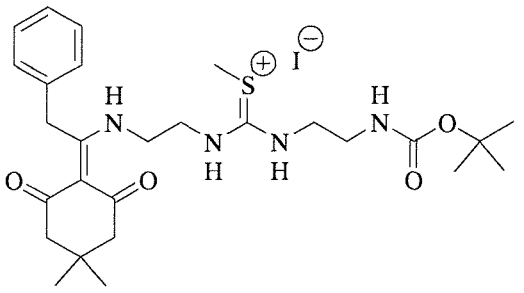
***tert*-butyl-*N*-[2-({[(2-{[1-(4,4-dimethyl-2,6-dioxocyclohexyliden)-2-phenylethyl]amino}-ethyl)amino]carbothioyl}amino)ethyl]carbamate (142)**



$C_{26}H_{38}N_4O_4S$
Exact Mass: 502.2614

Mono-protected diamine **138** (1.5 g, 5.1 mmol) was added to a solution isothiocyanate **141** (0.94 g, 4.7 mmol) in chloroform (60 mL) and the resulting reaction mixture was refluxed for 18 h. The solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel (Petrol Ether/EtOAc, 20:70) to afford the thiourea **142** as white foam (1.97 g, 3.94 mmol, 84%). HPLC (S15OD) 11.0 min. (99 % pure); ν_{max} = 3118 (m), 2909 (w), 2250 (w), 1659 (s), 1596 (s), 1475 (s), 1406 (s), 1304 (m), 1185 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 13.58 (br s, 1H, C=CNH), 7.22-7.06 (m, 5H, ArH), 6.73 (s, 2H, NHCSNH), 5.08 (s, 1H, NHCO) 4.52 (s, 2H, $ArCH_2$), 3.59 (br s, 4H, CH_2NH), 3.44 (br s, 2H, CH_2NH), 3.20 (q, 2H, *J* = 6 Hz, CH_2NH), 2.32 (s, 4H, CH_2CO), 1.35 (s, 9H, $(CH_3)_3C$), 0.97 (s, 6H, $(CH_3)_2C$); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 183.1 (0), 174.1 (0), 157.4 (0), 135.8 (0), 128.8 (1), 128.0 (1), 126.6 (1), 108.2 (0), 80.3 (0), 52.9 (2), 43.6 (2), 42.2 (2), 39.7 (2), 34.9 (2), 30.0 (0), 28.4 (3), 28.3 (3); LRMS (ES+) *m/z* (%) = 503.4 (100) $[M+H]^+$, 525.4 (10) $[M+Na]^+$, 1005.7 (10) $[2M+H]^+$; HRMS calcd for $C_{26}H_{39}N_4O_4S$ $[M+H]^+$ 503.2692, found 503.2685.

{1-({2-[(*tert*-butoxycarbonyl)amino]ethyl}amino)-1-[(2-{[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-2-phenyl-ethyl]amino}-ethyl)amino]-methylidene}(methyl)sulfonium iodide (143)

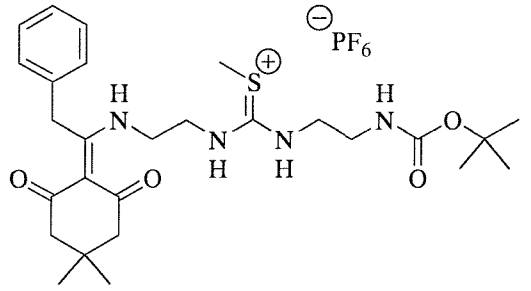


$C_{27}H_{41}IN_4O_4S$
Exact Mass: 644.1893

$C_{27}H_{41}N_4O_4S^+$
Exact Mass: 517.2849

Iodomethane (0.30 mL, 4.85 mmol) was added to a solution of **142** (1.2 g, 2.4 mmol) in acetone (70 mL) and the resulting reaction mixture was stirred for 18 hours at room temperature. The solvent and other volatile compounds were removed under reduced pressure to give the product as slightly yellow foam (1.55 g, 2.4 mmol, 100%). Analytical data: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 13.67 (br s, 1H, C=CNH), 9.41 (br. s, 1H, NHCS), 8.87 (br. s, 1H, NHCS), 7.23-7.10 (m, 5H, ArH), 5.54 (br s 1H, NHBoc), 4.56 (s, 2H, $ArCH_2$), 3.75 (m, 2H, CH_2NH), 3.50 (m, 4H, CH_2NH), 3.37 (m, 2H, CH_2NH), 2.63 (s, 3H, SCH_3), 2.33 (s, 4H, CH_2CO), 1.35 (s, 9H, $(CH_3)_3C$), 0.98 (s, 6H, $(CH_3)_2C$); ^{13}C NMR (100 MHz, $CDCl_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 174.0 (0), 168.2 (0), 159.2 (0), 135.6 (0), 128.8 (1), 128.1 (1), 126.7 (1), 108.7 (0), 81.4 (0), 53.5 (2), 52.5 (2), 48.5 (2), 44.1 (2), 41.8 (2), 38.6 (2), 34.9 (2), 29.9 (0), 28.3 (3), 28.2 (3), 14.8 (3); LRMS (ES^+) *m/z* (%) = 517.5 (100) $[M]^+$.

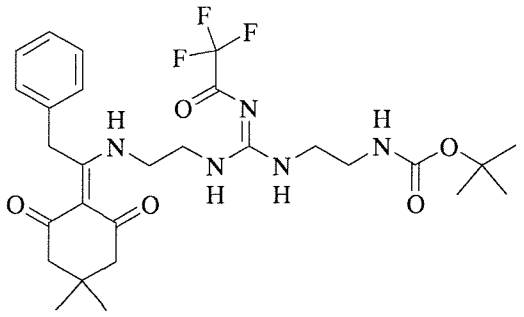
{1-({2-[(*tert*-butoxycarbonyl)amino]ethyl}amino)-1-[(2-{[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-2-phenyl-ethyl]amino}-ethyl)amino]-methylidene}(methyl)sulfonium hexafluorophosphate (144)



$C_{27}H_{41}F_6N_4O_4PS$
Exact Mass: 662.2490

To a solution of methyl-sulfonium iodide **143** in a 1:1 solvent mixture of dichloromethane (30 mL) and methanol (30 mL), ammonium hexafluorophosphate (0.79 g, 4.84 mmol) was added and the resulting solution stirred for 18 hours at room temperature. The solvents were evaporated under reduced pressure and the resulting oily residue redissolved in dichloromethane (100 mL) and washed with water (80 mL). The organic solution was dried over magnesium sulphate and the solvent was removed under reduced pressure to afford the product as white brittle foam (1.5 g, 2.4 mmol, 96%). Analytical data: ν_{max} = 2340 (w), 2250 (w), 1650 (s), 1590 (m), 1476 (m), 1386 (s), 1188 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 13.74 (br s, 1H, C=CNH), 8.39 (br s, 2H, NHCS), 7.29-7.13 (m, 5H, ArH), 5.56 (br. s. 1H, NHBoc), 4.56 (s, 2H, $ArCH_2$), 3.67 (m, 2H, CH_2NHC=C), 3.50 (br s, 4H, CH_2NH), 3.37 (m, 2H, CH_2NHBoc), 2.58 (s, 3H, SCH_3), 2.39 (s, 4H, CH_2CO), 1.41 (s, 9H, $(CH_3)_3C$), 1.04 (s, 6H, $(CH_3)_2C$); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 174.1 (0), 168.3 (0), 159.4 (0), 135.6 (0), 128.9 (1), 128.0 (1), 126.7 (1), 108.7 (0), 81.5 (0), 53.7 (2), 52.7 (2), 46.2 (2), 44.4 (2), 41.4 (2), 38.4 (2), 34.8 (2), 30.0 (0), 28.3 (3), 28.2 (3), 13.4 (3); LRMS (ES^+): m/z (%) = 517 (100) $[M-PF_6]^+$; LRMS (ES^-) *m/z* (%) = 144.9 (100) $[PF_6]^-$.

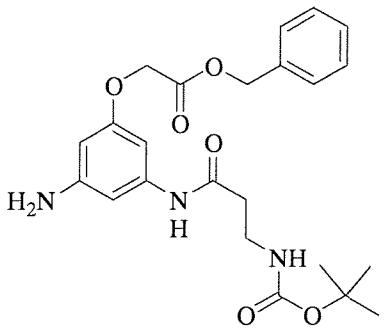
***tert*-butyl N-[2-({[(2-{[1-(4,4-dimethyl-2,6-dioxocyclohexyliden)-2-phenylethyl] amino}ethyl)amino][(2,2,2-trifluoroacetyl)imino]methyl}amino)ethyl]carbamate (145)**



$C_{28}H_{38}F_3N_5O_5$
Exact Mass: 581.2825

To a solution of sulfonium hexafluorophosphate salt **144** in a 4:1 mixture of toluene (4 mL), dichloromethane (1 mL) DBU (0.897 mL, 6 mmol), and trifluoroacetamide (1.35 g, 12 mmol) were added. The resulting reaction mixture was refluxed with vigorous stirring for 6 hours. The solvents were removed under reduced pressure. The residue was purified by column chromatography on silica gel (eluent $CHCl_3$) to afford the product as oil in 37% yield. Analytical data: HPLC 13.3 min (S15OD2); ν_{max} (film) = 3279 (w), 2926 (w), 2361 (w), 1706 (m), 1632 (s), 1568 (s), 1433 (m), 1273 (m), 1139 (s) cm^{-1}; ^1H NMR (400 MHz, [D_6] DMSO)) δ = 13.44 (br s, 1H, C=CNH), 9.17 (br s, 1H, NH), 7.73 (br , 1H, NH), 7.28 -7.08 (m, 5H, ArH), 6.94 (br s, 0.5 H, NH), 6.85 (br s, 0.5H, NH), 4.58 (s, 1H, $ArCH_2$), 4.53 (s, 1H, $ArCH_2$), 3.53 (br s, 2H, CH_2NH), 3.41 (br s, 2H, CH_2NH), 3.26 (m, 2H, CH_2NH), 3.11 (m, 2H, CH_2NH), 2.31 (s, 4H, CH_2CO), 1.36 (9H, $(CH_3)_3C$), 0.95 (s, 6H, $(CH_3)_2C$; LRMS (ES^+) *m/z* (%) = 582.2 (100) $[M+H]^+$, 604.3 (60) $[M+Na]^+$, 1185.6 (20) $[2M+Na]^+$; HRMS calcd for $C_{28}H_{39}N_5O_5F_3$ $[M+H]^+$ 582.2887, found 582.2898.

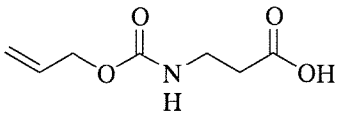
Benzyl 2-{[2-amino-6-({3-[(*tert*-butoxycarbonyl)amino]propanoyl}amino-4-pyridyl]oxy}acetate (147)



$C_{22}H_{28}N_4O_6$
Exact Mass: 444.2009

BSA (34 μL, 0.14 mmol) was added to a solution of 2,6-diamino pyridine **94** (39 mg, 0.14 mmol) in dry acetonitrile (2 mL). The resulting reaction mixture was stirred for 2 hours at room temperature. Subsequently N-Boc-β-Ala-F **95** (27 mg, 0.14 mmol) and MTDA (28 μL, 0.14 mmol) were added to the mixture. Stirring was continued for 20 h at 70 °C. The solvent was removed under reduced pressure, the residue was dissolved in dichloromethane (350 mL), washed with an aqueous solution of sodium hydrogencarbonate (5%, 350 mL) and water (300 mL). The organic layer was dried over $MgSO_4$ and the solvent was evaporated to give pale yellow oil. The residue was purified by flash chromatography on silica gel (eluant dichloromethane/MeOH 98:2) to give the product as a white foam (29 mg, 0.065 mmol, 47 %) and starting material (12 mg 0.044 mmol 31%). Analytical data: HPLC (S5OD) 3.17 min; ν_{max} = 3499 (w), 3383 (w), 3354 (w), 3333 (w), 2973 (w), 1758 (m), 1676 (m), 1535 (s), 1364 (m), 1156 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 8.1 (s, 1H, NH), 7.35 (br s, 6H, ArH, C_5H_2N), 7.22 (s, 1H, NH), 5.75 (d, *J* = 2Hz, 1H, C_5H_2N), 5.24 (s, 2H, OCH_2Ar) 4.66 (s, 2H OCH_2CO), 4.36 (s, 2H, NH_2) 3.45 (q, *J* = 6 Hz, 2H, CH_2NHBoc), 2.5 (t, *J* = 6Hz, 2H, $NHCOCH_2$) 1.4 (s, 9H, $(CH_3)_3C$); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 170.4 (0), 168.2 (0), 167.4 (0), 158.4 (0), 156.1 (0), 151.2 (0), 135.2 (0), 128.8 (1), 128.7 (1), 128.6 (1), 90.9 (1), 90.4 (1), 79.5 (0), 67.3 (2), 64.9 (2), 37.4 (2), 36.4 (2), 28.5 (3); LRMS (ES^+) *m/z* (%) = 445.2 (100) $[M+H]^+$; HRMS (ES^+) calcd for $C_{22}H_{29}N_4O_6$ $[M+H]^+$: 445.2086, found 445.2082.

3-{[(allyloxy)carbonyl]amino}-propionoic acid] (148)

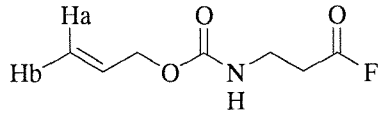


$C_7H_{11}NO_4$
Exact Mass: 173.0688

To a solution of β-Alanine (1.99 g, 16.5 mmol,) in aqueous 4N NaOH (20 mL) allyl chloroformate (1.34 g 15 mmol,) was added dropwise at -20 °C (ice/water bath). The reaction mixture was allowed to warm up to room temperature and stirring was continued for 18 hours. The solution was acidified by adding a concentrated solution of $KHSO_4$ until pH 2. During this process, the equal amount of dichloromethane was added. The aqueous layer was extracted with dichloromethane (3 × 150 mL) and the combined organic layers was dried over magnesium sulphate. The solvent was evaporated under reduced pressure to afford the product as a colourless oil (1.18 g, 6.8 mmol, 46 %). Analytical data: ν_{max} (film) = 3318 (br w), 2940 (w), 1693 (s), 1523 (m), 1407 (m), 1242 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 8.57 (br s,1H) 6.30 (br s, 1H, NH), 5.95-5.86 (ddt, J_{trans} = 16 Hz, J_{cis} = 11 Hz, J_{vic} = 5.5 Hz, 1H, CH_2=C*H*CH_2), 5.32 (d, J_{trans} = 16 Hz, 1H, CH_2CH=C*H*$_a$$H_b$), 5.20 (d, J_{cis} =11 Hz, 1H, CH2CH=CH_a*H*$_b$), 4.55 (d, J = 5.5 Hz, 2H, C*H*$_2$CH=CH_2), 3.40 (q, J = 6 Hz, 2H, NHC*H*$_2$$CH_2CO$), 2.60 (t, J = 6 Hz, 2H, $NHCH_2$C*H*$_2$CO); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 177.5 (0). 156.4 (0), 132.8 (1), 118.0 (2), 65.9 (2), 36.4 (2), 34.3 (2); LRMS (ES^+) *m/z* (%) = 369 (30) $[2M+H]^+$.

Spectroscopic data were consistent with those reported in literature [189]

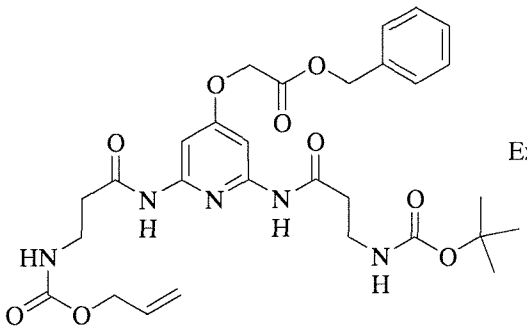
Allyl *N*-(3-fluoro-3-oxopropyl)carbamate (149)



$C_7H_{10}FNO_3$
Exact Mass: 175.0645

A solution of N-Aloc-β-Alanine **148** (0.113 g, 0.65 mmol) and pyridine (63 μL, 0.78 mmol) in dry dichloromethane was cooled down to – 15 0C. After addition of cyanuric fluoride (137 μL, 1.62 mmol) the mixture was stirred for 3 hours at – 15 °C under a nitrogen atmosphere. The solution was poured onto ice (10-20 mL) and more dichloromethane (50 mL) was added. The organic layer was separated and the aqueous layer was extracted with dichloromethane (2 × 200 mL). The combined organic layers were dried over magnesium sulphate. The solvent was removed under reduced pressure at rt. to give the product as colourless oil (0.099 g, 0.57 mmol, 87 % yield). Analytical data: ν_{max} = 3332 (w), 2951 (w), 1839 (s), 1696 (s), 1523 (m), 1248 (s), 1094 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 5.98-5.85 (ddt, J_{trans} = 17 Hz, J_{cis} = 11 Hz, J_{vic} = 5.5 Hz, 1H, CH_2=C*H*CH_2O), 5.32 (d, J_{trans} = 17Hz, 1H, C$H_a$$H_b$=$CHCH_2O$), 5.23 (d, J_{cis} = 11Hz, 1H, $CH_a$$H_b$=$CHCH_2O$), 4.57 (d, J = 5.5 Hz, 2H, CH_2CH=CH_2), 3.53-3.48 (q, J = 6Hz, 2H, NHC$H_2$$CH_2$COF), 2.79 (t, J = 6 Hz, 2H, CH_2CH_2COF), 1.72 (br s, 1H, NH) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ = 163.9 (d, J_{CF} = 358 Hz, 0), 155.2 (0), 132.8 (1), 118.1 (2), 65.9 (2), 35.9 (2) 33.2-32.7 (d, J_{CF} = 49 Hz, CH_2); LRMS: (ES^+) *m/z* (%) = 351.1 (50) $[2M+H]^+$.

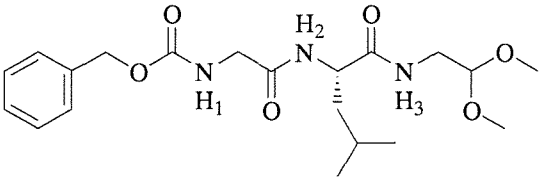
Benzyl 2-{[2-[(3-{[(allyloxy)carbonyl]amino}-propanoyl)amino]-6-({3-[(tert-butoxycarbonyl)amino]propanoyl}amino-4-pyridyl]oxy}acetate (150)



$C_{29}H_{37}N_5O_9$
Exact Mass: 599.2591

To a solution of **147** (29 mg, 0.065 mmol) in dry acetonitrile (2 mL), BSA (16 μL, 0.065 mmol) was added. After stirring at room temperature for 2 h N-Aloc-β-Ala-F **149** (68 mg, 0.39 mmol) and MTDA (68 mg, 0.39 mmol) were added to the mixture. Stirring was continued for 20 h at 70 °C. The solvent was removed under reduced pressure, the residue was dissolved in dichloromethane (350 mL) and washed with an aqueous solution of sodium hydrogencarbonate (5%, 150 mL)) and water (100 mL). The organic layer was dried over magnesium sulphate. The residue was purified by flash chromatography on silica gel (eluant, 98:2 DCM/MeOH) to obtain pale yellow oil (36 mg, 0.06 mmol, 92 %). Analytical data: HPLC (S15OD2) 12.06 min, (S5OD) 3.7 min; ν_{max} = 3299 (br m), 1981 (w), 2928 (w), 1755 (m), 1680 (s), 1584 (m), 1511 (s), 1434 (s), 1208 (s), 1158 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ = 8.54, (br s, 2H, NH), 7.44 (br s, 2H, C_5H_2N), 7.33 (br s, 5H, ArH), 5.86 (br s, 1H, CH_2=C*H*CH_2O), 5.72 (s, 1H, NH), 5.38 (s, 1H, NH), 5.27-5.14 (m, 2H, C*H*$_2$=CHCH_2OCO), 5.21 (s, 2H, OCH_2Ar), 4.68 (s, 2H, OCH_2CO), 4.52 (d, *J* = 5.5 Hz, 2H, $COCH_2CH$) 3.52 (d, *J* = 5.5 Hz, 2H, C*H*$_2CH_2CO$), 3.45 (d, *J* = 5.5 Hz, 2H, C*H*$_2CH_2CO$), 2.58 (br s, 4H, CH_2CH_2CO) 1.41 (s, 9H, $(CH_3)_3C$); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.8 (0), 167.9 (0), 167.4 (0), 156.7 (0), 156.3 (0), 150.7 (0), 135.1 (0), 132.9 (1), 128.7 (2×1), 128.6 (2×1), 128.6 (1), 117.8 (2) 96.5 (1), 79.6 (0), 67.4 (2), 65.8 (2), 65.1 (2), 37.6 (2), 37.3 (2) 37.0 (2), 36.6 (2) 28.5 (3); LRMS (ES^+) *m/z* (%) =600.3 $[M+H]^+$, 622.3 $[M + Na]^+$; HRMS (ES^+) calcd for $C_{29}H_{38}N_5O_9$ $[M+H]^+$: 600.2656 found 600.2664.

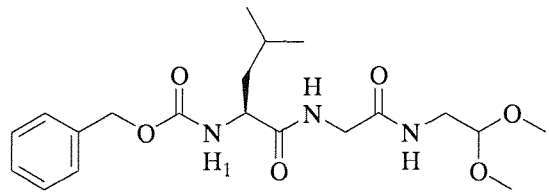
Benzyl *N*-{2-[((1*S*)-1-{[(2,2-dimethoxyethyl)amino]carbonyl}-3-methylbutyl) amino]-2-oxoethyl}carbamate (161)



$C_{20}H_{31}N_3O_6$
Exact Mass: 409.2213

To a solution of N-Cbz-L-Gly-L-Leu-OH (254.7 mg. .0.79 mmol) in a 1:1 mixture of tetrahydofuran (1mL) and acetonitrile (1mL), HOBt (110 mg, 0.72 mmol) was added and the resulting reaction mixture was stirred for 10 min. EDC (138 mg, 0.72 mmol) was added and the reaction mixture was stirred for further 10 min. Aminoacetaldehyde dimethyl acetal (72 μL, 0.66 mmol) and N-methyl morpholine were added and the reaction mixture stirred for 18 hours at room temperature. The solvent was removed under reduced pressure, the residue redissolved in dichloromethane (50 mL), and washed with an aqueous solution of sodium hydrogen carbonate (1M, 2 × 50 mL) and brine (1M, 2 × 50 mL). The organic layer was dried over magnesium sulphate and the solvent was removed under reduced pressure to afford the product as white foam (gel) (0.27 mg, 0.66 mmol, 100%). Analytical data: R_f = 0.39 (dichloromethane/MeOH 95:5); $[\alpha]_D$ = – 16.6 (c 1.01 g/100 mL, chloroform, 24 °C); ν_{max} (film) = 3285 (m), 3068 (w), 2954 (m), 2835 (w), 1711 (m), 1648 (s), 1513 (s), 1453 (m), 1382 (m), 1237 (m), 1128 (s), 1051 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 7.30 (m, 5H, ArH), 7.09 (d, J = 11 Hz, 1H, NH_2), 6.84 (br s, 1H, NH_3), 5.92 (br s, 1H, NH_1), 5.08 (s, 2H, $ArCH_2$), 4.50 (m, 2H, $CHCH_2$), 4.35 (t, *J* = 7 Hz, 1H, $CH(OCH_3)_2$), 3.87 (d, *J* = 7 Hz, 2H, $NHCH_2CO$), 3.4 (m, 2H, $NHCH_2CH(OCH_3)_2$), 3.32 (s, 6H, $CH(OCH_3)_2$), 1.6 (m, 3H, $CHCH_2CH(CH_3)_2$ $CHCH_2CH(CH_3)_2$, 0.88 (d, *J* = 7 Hz, 6H, $CH(CH_3)_2$; ^{13}C NMR (100 MHz, $CDCl_3$) δ = 172.4 (0), 169.2 (0), 156.7 (0), 136.2 (0), 128.5 (1), 128.2 (1), 128.0 (1), 102.3 (1), 67.1 (2), 54.2 (3), 54.1 (3), 51.8 (1), 44.4 (3), 41.3 (2), 39.9 (2), 24.7 (1), 22.7 (3), 22.2 (3); LRMS (ES^+) *m/z* (%) = 410.1 (40) $[M+H]^+$, 432.1 (60) $[M+Na]^+$, 819.0 (20) $[2M+H]^+$, 841.0 (50) $[2M+Na]^+$, 1249.9 $[3M+Na]^+$; HRMS (ES^+) calcd for$C_{20}H_{31}N_3O_6Na$ $[M+Na]^+$: 432.2112 found 432.2105.

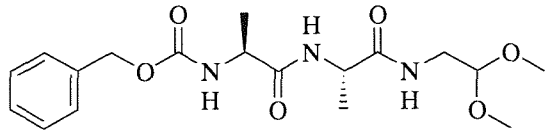
Benzyl *N*-{(1*S*)-1-[({2-[(2,2-dimethoxyethyl)amino]-2-oxoethyl}amino)carbonyl]-3-methylbutyl}carbamate (160)



$C_{20}H_{31}N_3O_6$
Exact Mass: 409.2213

Synthesised according to the general procedure on a 0.79 mmol scale: yield 86% (0.27 mg, 0.66 mmol). Analytical data: R_f = 0.39 (DCM/MeOH 95:5); $[\alpha]_D$ = – 17.27(c 1.04 g/100 mL,, chloroform, 22 °C); ν_{max} = 3289 (m), 2955 (m), 1650 (s), 1526 (s), 1454 (m), 1387 (m), 1239 (s), 1128 (s), 1044 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 7.31 (m, 5H, ArH), 6.81 (br s, 1H, NH), 5.70 (br s, 1H, NH), 5.12-5.03 (m, 2H, $ArCH_2$ several conformation), 4.37 (t, *J* = 5 Hz, 1H, $CH(OCH_3)_2$), 4.22 (m, 1H, $CHCH_2CH(CH_3)_2$), 3.99-3.94 (dd, *J* = 5 Hz, 16.5 Hz, 1H, $NHCH_2CH(OCH_3)_2$), 3.89-3.84 (dd, *J* = 5 Hz, 16.5 Hz, 1H, $NHCH_2CH(OCH_3)_2$), 3.36 (m, 2H, $NHCH_2CO$), 3.33 (s, 6H, $CH(OCH_3)_2$), 1.69-1.58, (m, 2H, $CHCH_2CH(CH_3)_2$), 1.53-1.49 (m, 1H, $CHCH_2CH(CH_3)_2$, 0.92 (d, *J* = 7 Hz, 6H, $CH(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 173.2 (0), 169.1 (0), 156.5 (0), 136.2 (0), 128.6 (1), 128.3 (1), 128.1 (1), 102.4(1), 67.2 (2), 54.3 (3), 53.8 (1), 43.2 (3) 41.5 (2), 41.0 (2), 24.8 (1), 23.0 (3), 21.9 (3); LRMS (ES^+) *m/z* (%) = 410.3 (40) $[M+H]^+$, 432.3 (90) $[M+Na]^+$, 819.0 (40) $[2M+H]^+$, 841.0 (70) $[2M+Na]^+$; HRMS (ES^+) calcd for $C_{20}H_{31}N_3O_6Na$ $[M+Na]^+$: 432.2112, found 432.2105.

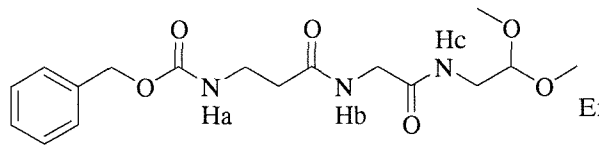
Benzyl *N*-[(1*S*)-2-({(1*S*)-2-[(2,2-dimethoxyethyl)amino]-1-methyl-2-oxoethyl]-carbamate (158)



$C_{18}H_{27}N_3O_6$
Exact Mass: 381.1900

Synthesised according to the general procedure on a 0.14 mmol scale: 69% yield Analytical data: $[\alpha]_D$ = -35.19 (c 0.94, chlorofom, 22 °C); ν_{max} = 3278 (m), 2975 (w), 2934 (w), 2414 (w), 1692 (m), 1637 (s), 1535 (m), 1445 (m), 1256 (m), 1051 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 7.37-7.31 (m, 5H, ArH), 6.71 (br s, 1H, NH), 6.40 (br s, 1H, N*H*$CH_2CH(OCH_3)_2$), 5.41 (br s, 1H, NH), 5.11 (s, 2H, CH_2Ar), 4.50-4.43 (m, 1H, C*H*CH_3), 4.37 (t, *J* = 5 Hz, 1H, C*H*$(OCH_3)_2$), 4.25 (br s, 1H, C*H*CH_3), 3.40 (m, 2H, NHC*H*$_2$CH$(OCH_3)_2$), 3.37 (s, 3H, $(OCH_3)_2$), 1.37 (t, *J* = 7 Hz, 6H, CHC*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 172.2 (0), 156.2 (0), 136.3 (0), 128.71 (1), 128.4 (1), 128.26 (1), 102.5 (1), 67.3 (2), 54.4 (1), 49.1 (1), 41.1 (2), 18.8 (3), 18.5 (3); LRMS (ES^+) *m/z* (%) = 382.2 (80) $[M+H]^+$, 404.3 (100) $[M+Na]^+$; HRMS (ES^+) calcd for $C_{18}H_{27}N_3O_6Na$ $[M+Na]^+$: 358.1373, found 358.1373.

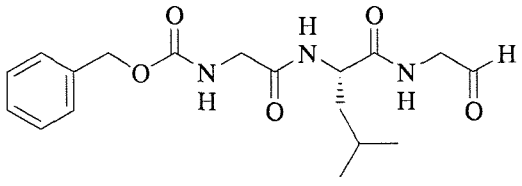
Benzyl N-[3-({2-[(2,2-dimethoxyethyl)amino]-2-oxoethyl}amino)-3-oxopropyl]carbamate (156)



$C_{17}H_{25}N_3O_6$
Exact Mass: 367.1743

Synthesised according to the general procedure on a 0.79 mmol scale: yield 100%. Analytical data : ν_{max} = 3303 (m), 3248 (m), 2922 (w), 1720 (m), 1639 (s), 1543 (s), 1230 (s), 1126 (s), 703 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 7.30 (br s, 5H, ArH), 7.00 (br s, 1H, NHb), 6.82 (br s, 1H, NHc), 5.86 (br s, 1H, NHa), 5.04 (s, 2H, CH_2Ar), 4.35 (t, J = 5 Hz, 1H, $CH(OCH_3)_2$), 3.87 (d, J = 5 Hz, 2H, $NHCH_2CO$), 3.48-3.42 (q, J = 5.5 Hz, 2H, $NHCH_2CH_2CO$), 3.37 (t, J = 5Hz, 2H, $CH_2CH(OCH_3)_2$), 3.32 (s, 6H, $(OCH_3)_2$), 2.45 (br s, 2H, 2H, $NHCH_2CH_2CO$); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 172.3 (0), 169.3 (0), 156.7 (0), 136.6 (0), 128.6 (1) 128.2 (1), 128.1 (1), 102.3 (1), 66.7 (2), 54.2 (3), 43.3 (2), 40.9 (2), 37.4 (2), 35.9 (2); LRMS (ES^+): m/z (%) = 368.1 (40) $[M+H]^+$, 390.1 (90)$[M+Na]^+$, 735.0 (20) $[2M+H]^+$, 756.9 (50) $[2M+Na]^+$; HRMS (ES^+) calcd for: $C_{17}H_{25}N_3O_6Na$ $[M+Na]^+$: 390.1634, found 390.1635.

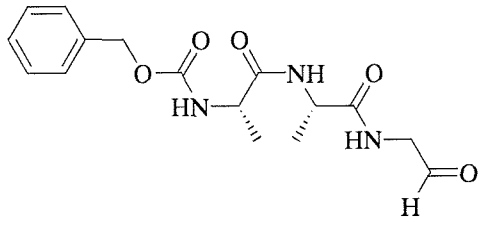
Benzyl *N*-{2-[((1*S*)-3-methyl-1-{[(2-oxoethyl)amino]carbonyl}butyl)amino]-2-oxoethyl}carbamate (163)



$C_{18}H_{25}N_3O_5$
Exact Mass: 363.1794

To a solution of **161** (270 mg, 0.66 mmol) in acetone (4 mL), 1N HCl (3.3 mL) was added. The resulting reaction mixture was stirred for 24 hours. The solution was neutralised by adding an aqueous solution of 1N $NaHCO_3$. The product was extracted in dichloromethane, the organic layer dried over magnesium sulphate and the solvent removed under reduced pressure. The residue was purified by column chromatography ($CHCl_3$) to afford the product as foam in 41% yield. Analytical data: R_f = 0.2 (DCM/MeOH 95:5); $[\alpha]_D$ = 13.2 (c 0.50, $CHCl_3$, 22 °C); ν_{max} = 3284 (m), 3062 (w), 2957 (m), 1708 (m), 1655 (s), 1525 (s), 1240 (s), 1152 (m), 1240 (s), 1019 (s), 697 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 9.54 (s, 1H, CHO), 7.31 (m, 5H, ArH), 7.00 (d, *J* = 7.5 Hz, 1H, NH), 5.71 (1H, NH), 5.10 (s, 2H, $ArCH_2$), 4.58 (m, 3H, NHC*H* + CH_2CHO), 3.89 (m, 2H, CH_2NH), 1.58, (m, 3H, $CHCH_2CH(CH_3)_2$ and $CHCH_2CH(CH_3)_2$, 0.90 (t, *J* = 6 Hz, 6H, $CH(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 196.5 (0), 173.0 (0), 170.2 (0), 157.1 (0), 136.1 (0), 128.7 (1), 128.5 (1), 128.2 (1), 127.8 (1), 67.6 (2), 51.9 (2), 50.2 (1), 44.7 (2), 10.9 (2), 24.9 (1), 22.9 (3), 22.0 (3); LRMS (ES^+) *m/z* (%) = 386.3 (100) $[M+Na]^+$, 418.4 (100) $[M+Na+CH_3OH]$, 749.5 (40) $[2M+Na]^+$; HRMS (ES^+) calcd for $C_{20}H_{31}N_3O_6Na$ $[M+Na]^+$: 432.2109, found 432.2105.

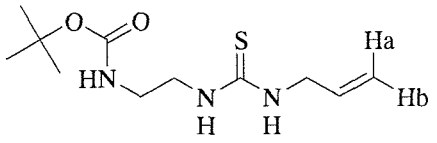
Benzyl *N*-[(1*S*)-1-methyl-2-({(1*S*)-1-methyl-2-oxo-2-(2oxoethyl)amino]ethyl}amino) 2-oxoethyl]carbamate (162)



$C_{16}H_{21}N_3O_5$
Exact Mass: 335.1481

To solution of **158** (38.2 mg, 0.1 mmol) in dichloromethane, montmorillonite K10 (300 mg) was added. The resulting reaction mixture was stirred for 24 hours. The reaction mixture was filtered under reduced pressure on celite. The solvent was removed under reduced pressure and the residue purified by column chromatography (eluant chloroform) to afford the aldehyde as an oil (21 mg, 0.064mmol, 64%). Analytical data: R_f = 0.26 (DCM/MeOH 95:5); $[\alpha]_D$ = -7.57(c 1.0, $CHCl_3$, 22 °C); ν_{max} (film) = 3277 (m), 2977 (w), 2935 (w), 1691 (m), 1639 (s), 1538 (m), 1261 (m), 1068 (m), 692 (m) cm^{-1}; ^1H NMR (400 MHz, CD_3CN) δ = 9.42 (s, 1H, CHO). 7.34 (m, 5H, Ph), 7.08 (br s, 1H, NH), 6.96 (br s, 1H, NH), 6.00 (br s, 1H, NH), 5.05 (s, 2H, $ArCH_2$), 4.36-4.29 (m, 1H, $CHCH_3$), 4.04 (m, 1H, $CHCH_3$), 3.88 (d, *J* = 5.5 Hz, 2H, CH_2CHO), 1.26 (d, *J* = 15 Hz, 6H, CH_3CH); ^{13}C NMR (100 MHz, CD_3CN) δ = 199.7 (0), 174.0 (0), 173.6 (0), 157.5 (0), 138.1 (0), 129.5 (1), 128.9 (1), 128.7 (1), 67.3 (2), 52.2 (1), 50.4 (2), 49.8 (1), 18.1 (3); LRMS (ES^+) *m/z* (%) = 336.2 (60) $[M+H]^+$, 358.2 (100) $[M+Na]^+$, 693.4 $[2M+Na]^+$. HRMS (ES^+) calcd for $C_{16}H_{21}N_3O_5Na$ $[M+Na]^+$: 358.1373, found 358.1373.

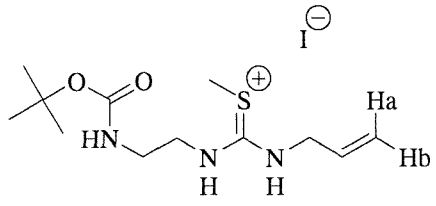
tert-butyl _N_ (2-{[allylamino)carbonothioyl]amino}ethyl)carbamate (164)



$C_{11}H_{21}N_3O_2S$
Exact Mass: 259.1354

Allylamine (0.412 mL, 5.5 mmol) was added to a solution of tert-butyl N-(2-isothiocyanatoethyl)carbamate **141** (1.01 g, 5 mmol) in chloroform (60 mL) and the resulting reaction mixture was refluxed with stirring for 18 hours. The solvent was removed under reduced pressure. The residue was purified by flash column chromatography on silica gel (eluting with chloroform) to afford the product as a white foam (1.08 g, 4.16 mmol, 83%). Analytical data: ν_{max} = 3304 (m), 3227 (m), 2966 (w), 2935 (w), 1686 (s), 1565 (s), 1500 (s), 1466(m), 1364 (m), 1309 (m), 1229 (s), 1157 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 6.90 (br s, 1H, NH), 6.38 (br s, 1H, NH), 5.84 (ddt, J_{trans} = 16.5 Hz, J_{cis} = 10.5 Hz, J_{vic} = 5.5 Hz, CH_2=C*H*CH_2), 5.27 (d, J_{trans} = 16.5 Hz, 1H, CH2CH=C*H*$_a$H$_b$), 5.18 (d, J_{cis} = 10.5 Hz, 1H, CH2CH=CH$_a$*H*$_b$), 5.07 (br s, 1H, NH), 3.99 (br s, 2H, C*H*$_2$CH=CH$_2$), 3.61 (br s, 2H, C*H*$_2$CH$_2$NHBoc), 3.32 (q, J = 5.5 Hz, 2H, C*H*$_2$NHBoc), 1.42 (s, 9H, C(CH$_3$)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 182.0 (0), 157.5 (0), 132.8 (1), 117.4 (2), 80.2 (0), 46.3 (2), 39.8 (2), 28.3 (3); LRMS (ES^+) *m/z* (%) = 260.1 (100) $[M+H]^+$, 541.2 (50) $[M+Na]^+$; HRMS (ES^+) calcd for $C_{11}H_{22}N_3O_2S$ $[M+H]^+$: 260.1426, found 260.1427.

[1-(allylamino)-1-({2-[(*tert*-butoxycarbonyl)amino]ethyl}amino)methylidene]-(methyl)-sulfonium iodide (165)

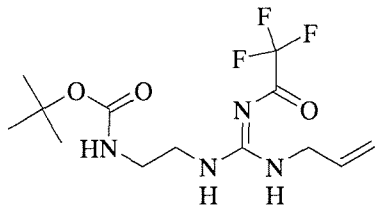


$C_{12}H_{24}IN_3O_2S$
Exact Mass: 401.0634

$C_{12}H_{24}N_3O_2S^+$
Exact Mass: 274.15892

Iodomethane (0.163 mL, 2.6 mmol) was added to a solution of thiourea **164** (0.34 g, 1.3 mmol) in acetone (30 mL) and the reaction mixture was stirred for 18 hours at room temperature. The solvent and other volatile components were removed under reduced pressure to give the product as pale yellow oil (0.520 g, 1.3 mmol, 100%). Analytical data: ν_{max} = 3363 (m), 3221 (m), 3111 (m), 3005 (m), 2355 (w), 1679 (s), 1607 (s), 1527 (s), 1277 (s), 1161 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 9.44 (s, 1H, NH), 8.75 (s, 2H, NH), 5.86 (ddt, J_{trans} = 16.0 Hz, J_{cis} = 10.5 Hz, J_{vic} = 5.5 Hz, CH_2=C*H*CH_2), 5.27 (m, 2H, C*H*$_2$=CHCH_2), 4.09 (s, 2H, CH_2=CHC*H*$_2$), 3.82 (br s, 2H, CH_2C*H*$_2$NHCS), 3.44 (d, J = 5.5 Hz, C*H*$_2$$CH_2$NHCS), 2.75 (s, 3H, CH_3S), 1.41 (s, 9H, $(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 167.9 (0), 158.7 (0), 130.8 (0), 119.3 (2), 81.0 (0), 46.9 (2), 46.8 (2), 38.7 (2), 28.3 (3), 15.0 (3); LRMS (ES^+) *m/z* (%) = 274.2 (100) $[M]^+$; HRMS (ES^+) for $C_{12}H_{24}N_3O_2S^+$ $[M]^+$ calcd: 274.1586 found 274.1584.

tert-butyl _N_-[2-({(allylamino)[(2,2,2-trifluoroacetyl)imino]methyl}amino)ethyl] carbamate (167)



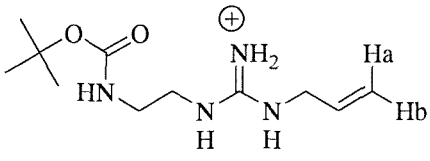
$C_{13}H_{21}F_3N_4O_3$
Exact Mass: 338.1566

To a solution of methyl-sulfonium iodide **165** (0.392 g, 0.97 mmol) in a 1:1 solvent mixture of dichloromethane (30 mL) and methanol (30 mL), ammonium hexafluorophosphate (0.318 g, 1.95 mmol) was added and the resulting solution stirred for 18 hours at room temperature. The solvents were evaporated under reduced pressure and the resulting oily residue redissolved in dichloromethane (100 mL) and washed with water (80 mL). The organic solution was dried over magnesium sulphate and the solvent was removed under reduced pressure to afford **166** as a white foam (0.406 g, 0.97 mmol, 100%). Analytical data: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.51 (s, 1H, NH), 8.18 (s, 2H, NH), 5.85 (ddt, J_{trans} = 16.0 Hz, J_{cis} = 10.5 Hz, J_{vic} = 5.5 Hz, CH_2=C*H*CH_2), 5.49-5.28 (m, 2H, C*H*$_2$=CHCH_2), 4.05 (s, 2H, CH_2=CHC*H*$_2$), 3.56 (br s, 2H, CH_2C*H*$_2$NHCS), 3.41 (br s, 2H, C*H*$_2$$CH_2$NHCS), 2.63 (s, 3H, CH_3S), 1.42 (s, 9H, $(CH_3)_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 168.6 (0), 159.1 (0), 130.5 (0), 119.6 (2), 81.3 (0), 47.3 (2), 45.7 (2), 38.6 (2), 28.2 (3), 13.2 (3). LRMS (ES^+): m/z (%) = 274.2 (100) $[M]^+$; LRMS (ES^-) *m/z* (%) = 144.9 (100) $[PF_6]^-$.

The foam was dissolved in a 4:1 mixture of toluene (4 mL) and dichloromethane (1 mL), DBU (0.373 mL, 2.5 mmol) and trifluoroacetamide (0.565g, 5 mmol) were added. The resulting reaction mixture was refluxed with vigorous stirring for 6 h. The solvents were removed under reduced pressure. The residue was purified by column chromatography on silica gel (eluant chloroform) to afford the product as a pale yello oil (0.288 g, 0.85 mmol, 88%). Analytical data: Rf = 0.5 (eluting with chloroform); ν_{max} = 3332 (w), 2983 (w), 1676 (m), 1626 (s), 1607 (s), 1438 (m), 1411 (m), 1256 (m), 1163 (s), 1134 (s) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.57 (s, 1H, NH), 6.43 (s, 1H, NH), 5.86 (s, 1H, NH), 5.31 (m, 3H, C*H*=C*H*$_2$), 3.85 (br s, 2H, C*H*$_2$CH=CH_2), 3.53 (br s,

2H, CH_2NHBoc), 3.31 (br s, 2H, CH_2CH_2NHBoc), 1.44 (s, 9H, $C(CH_3)_3$; ^{13}C NMR (100 MHz, $CDCl_3$) δ = 166.6 (0, q, J_{CF} = 35.0 Hz), 160.8 (0), 157.8 (0), 131.4 (1), 117.7 (2), 116.9 (0, q, J_{CF} =287 Hz), 80.0 (0), 43.7 (2), 43.5 (2), 40.3 (2), 28.2 (3); LRMS (ES^+) *m/z* (%) = 339.2 (100) $[M+H]^+$; HRMS (ES^+) calcd for $C_{13}H_{22}F_3N_4O_3$ $[M+H]^+$: 339.1634, found 339.1638.

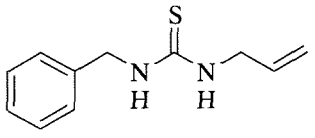
[(allylamino)({2-[(*tert*-butoxycarbonyl)amino]ethyl}amino)methylene]ammonium (168)



$C_{11}H_{23}N_4O_2^+$
Exact Mass: 243.1821

To a solution of (trifluoro-acetyl)-guanidine **167** (0.046 g, 0.134 mmol) in methanol (2 mL), potassium carbonate (0.093 g, 0.672 mmol) and water (1 mL) were added. The resulting reaction mixture was stirred for 3 hours at room temperature. The solvents were removed under reduced pressure. The crude product was dissolved in $CHCl_3$ and filtered. The solvent was evaporated and the residue was purified by semipreparative HPLC to afford the product as colourless oil (0.024 g, 0.10 mmol, 76%). Analytical data: R_f = 0.1 (eluting with chloroform); HPLC = 13.11 min (20STD220); ν_{max} = 3332 (br m), 3198 (m), 2984 (w), 1672 (s), 1630 (s), 1511 (m), 1368 (m), 1248 (s), 1200 (s), 1174 (s) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.26 (br s, 1H, NH), 7.02 (br s, 2H, NH), 5.82 (ddt, J_{trans} = 17.0 Hz, J_{cis} = 10.5 Hz, J_{vic} = 5.5 Hz, 1H, CH_2=C*H*CH_2), 5.40 (br s, 1H, NH), 5.32 (d, J_{trans} = 17 Hz, 1H, CH_aH_b=$CHCH_2$), 5.26 (d, J_{cis} = 10.5 Hz, 1H, CH_aH_b=$CHCH_2$), 3.82 (s, 2H, CH_2=$CHCH_2$), 3.30 (s, 2H, $NHCH_2CH_2NH$), 3.27 (s, 2H, $NHCH_2CH_2NH$), 1.42 (s, 9H, C $(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 156.8 (0), 132.0 (1), 118.0 (2), 81.7 (0), 43.8 (2), 39.6 (2), 28.2 (3); LRMS (ES^+) *m/z* (%) = 243.4 (100) $[M]^+$; HRMS (ES^+) calcd for $C_{11}H_{23}N_4O_3$ $[M]^+$: 243.1811, found 243.1815.

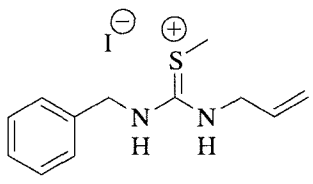
N-Allyl-N'-benzylthiourea (169)



$C_{11}H_{14}N_2S$
Exact Mass: 206.0878

Allylamine (0.620 mL, 8.25 mmol) was added to a solution of benzoisothiocyanate (1 mL, 7.5 mmol) in chloroform (60 mL) and the resulting reaction mixture was refluxed with stirring for 18 hours. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography on silica gel (eluting with chloroform) to afford the product as a waxy solid (1.54 g, 7.5 mmol, 100%). Analytical data: HPLC (S15OD) 8.37 min; ν_{max} = 3229 (m), 2936 (w), 1554 (s), 1518 (m), 1493 (m), 1222 (s), 1191 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 7.3-7.2 (m, 5H, ArH), 6.11 (br s, 1H, NH), 5.95 (br s, 1H, NH), 5.74 (ddt, J_{trans} = 16 Hz, J_{cis} = 11 Hz, J_{vic} = 6 Hz, CH_2=C*H*CH_2), 5.10 (m, 2H, CH_2=CHC*H*$_2$), 4.59 (d, J = 5 Hz, 2H, CH_2Ar), 3.96 (s, 2H, CH_2=CHC*H*$_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 182.0 (0), 136.9 (0), 133.0 (1), 128.8 (1), 127.8 (1), 127.5 (1), 117.2 (2), 48.5 (2), 46.7 (2); LRMS (ES^+) *m/z* (%) = 207.0 (100), $[M+H]^+$; HRMS (ES^+) for $C_{11}H_{15}N_2S$ $[M+H]^+$: calcd 207.0950, found 207.0950; Elemental analysis calcd (%) for $C_{11}H_{14}N_2S$ C 64.04, H 6.84, N 13.58, S 15.54, found C 64.15, H 6.78, N 13.69, S 15.48.

[1-(allylamino)-1-(benzylamino)-methylidene](methyl)sulfonium iodide (170)

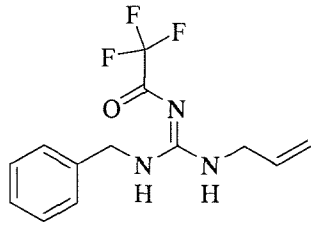


$C_{12}H_{17}IN_2S$
Exact Mass: 348.0157

$C_{12}H_{17}N_2S^+$
Exact Mass: 221.1112

Iodomethane (0.30 mL, 4.85 mmol) was added to a solution of thiourea **169** (0.5 g, 2.4 mmol) in acetone (70 mL) and the reaction mixture was stirred for 18 hours at room temperature. The solvent and other volatile compounds were removed under reduced pressure to give the product as an pale yellow oil (0.835 g, 2.4 mmol, 100%). Analytical data: HPLC (S15PROD) 6.04 min; (S15OD) 10.15min; ν_{max} = 3143 (m), 2997 (m), 1596 (s), 1512 (m), 1229 (m), 949 (w), 728 (m) cm^{-1}; ^1H NMR (400 MHz, CD_3CN) δ = 8.07 (br s, 2H, NH), 7.35-7.31 (m, 5H, ArH), 5.85 (br s, 1H, CH=CH_2), 5.23 (br s, 2H, CH=CH_2), 4.64 (br s, 2H, CH_2Ar), 4.1 (br s, 2H, CH_2CH=CH_2), 2.65 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.2 (0), 135.1 (1), 130.4 (0), 128.8 (1), 128.25 (1), 127.7 (1), 119.2 (2), 47.6 (2), 46.2 (2) 16.6 (3); LRMS (ES^+) *m/z* (%) = 221.1 (100) $[M]^+$; HRMS (ES^+) for $C_{12}H_{17}N_2S^+$ $[M]^+$: calcd 221.1105, found 221.1107.

***N*-Allyl-*N'*-benzyl-*N''*-(2,2,2-trifluoro-acetyl)-guanidine (172)**



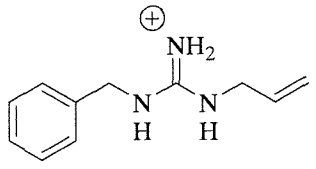
$C_{13}H_{14}F_3N_3O$
Exact Mass: 285.1089

To a solution of methyl-sulfonium iodide **170** (0.87 g, 2.5 mmol) in a 1:1 solvent mixture of dichloromethane (30 mL) and methanol (30 mL), ammonium hexafluorophosphate (2.4 g, 15 mmol) was added and the resulting solution stirred for 18 hours at room temperature. The solvents were evaporated under reduced pressure and the resulting oily residue redissolved in dichloromethane (100 mL) and washed

with water (80 mL). The organic solution was dried over magnesium sulphate and the solvent was removed under reduced pressure to afford the hexafluorophosphate salt **171** as white foam (0.915 g, 2.5 mmol, 100%).

The foam was dissolved in a 4:1 mixture of toluene (4 mL), and dichloromethane (1 mL) DBU (0.93 mL, 6.25 mmol) and trifluoroacetamide (1.41 g, 12.5 mmol) were added. The resulting reaction mixture was refluxed with vigorous stirring for 6 hours. The solvents were removed under reduced pressure and the residue was purified by flash column chromatography on silica gel (eluant chloroform) to afford the product **172** (0.585 g, 2.05 mmol, 82%). Analytical data: R_f = 0.45 (eluting with chloroform); HPLC (S15OD) 12.76 min, (S15PROD) 10.2 min; ν_{max} = 3318 (w), 3267 (w), 1575 (s), 1437 (m), 1236 (s), 1194 (s) 1160 (s) cm^{-1}; ^1H NMR (400 MHz, [D_6] DMSO) δ = 9.45 (s, 0.5 H, NH), 9.24 (s, 0.5 H, NH), 8.08 (s, 0.5 H, NH), 7.87 (s, 0.5 H, NH), 7.30 (br s, 5H, ArH), 5.86 (br s, 1H, CH_2=C*H*CH_2), 5.20 (m, 1H, C*H*$_a$H$_b$=CHCH$_2$), 5.06 (m, 1H, CH$_a$*H*$_b$=CHCH$_2$), 4.49 (d, *J* = 5 Hz, 2H, ArCH_2), 3.92 (s, 2H, CH$_a$*H*$_b$=CHCH$_2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 167.0 (0, J_{CF} = 35 Hz), 160.8 (0), 137.4 (1), 131.7 (1), 129.3 (1), 128.9 (1), 128.0 (1), 127.8 (1), 118.3 (2), 116.8 (0, J_{CF} = 286 Hz), 45.7 (2), 44.9 (2); LRMS (ES^+) *m/z* (%) = 286.2 (100) $[M+H]^+$; HRMS (ES^+) calcd for $C_{13}H_{15}F_3N_3O$ $[M+H]^+$: 286.1166, found 286.1162.

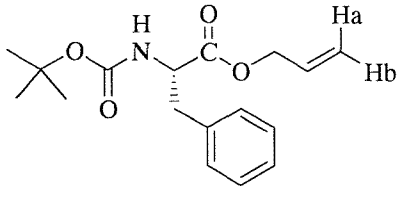
[(allylamino)(benzylamino)methylene]ammonium (173)



$C_{11}H_{16}N_3^+$
Exact Mass: 190.1344

Potassium carbonate (0.14 g, 1.02 mmol) and water (0.5 mL) were added to a solution of trifluoro-acetyl)-guanidine **172** (0.058 g, 0.20 mmol) in methanol (5mL). The resulting reaction mixture was stirred for 3 hours at room temperature. The solvents were removed under reduced pressure. The crude was redissolved in $CHCl_3$ and filtered to afford the product as colourless oil (0.038 g, 0.2 mmol, 98%). Analytical data: HPLC (SO15OD) 8.6 min; ν_{max} = 3174 (w), 1660 (s), 1623 (s), 1175 (s), 1126 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.18 (br s, 2H, NH_2), 7.44-7.16 (m, 5H, ArH), 6.96 (br s, 2H, NH), 5.65 (ddt, J_{trans} = 16 Hz, J_{cis} = 11 Hz, J_{vic} = 6 Hz, CH_2=C*H*CH_2), 5.10 (s, 1H, CH_aH_b=$CHCH_2$), 5.07 (s, 1H, CH_aH_b=$CHCH_2$), 4.28 (d, J = 6 Hz, 2H, ArCH_2). 3.68 (t, J = 6 Hz, 2H, CH_2=$CHCH_2$), 2.7 (br s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 156.8 (0), 135.5 (0), 132.1 (1), 129.24 (1), 128.3 (1), 126.9 (1), 117.8 (2), 45.2 (2), 43.8 (2); LRMS (ES^+): m/z (%) = 190.2 (100) $[M+H]^+$. HRMS (ES^+) calcd for $C_{11}H_{16}N_3$ $[M]^+$: 190.1336, found 190.1338.

Allyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-phenyl-propanoate (175) [190]



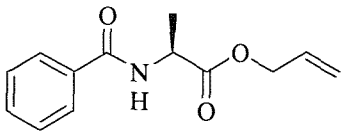
$C_{17}H_{23}NO_4$
Exact Mass: 305.1627

To a solution of *N*-Boc-L-Phe-OH (0.617 g, 2.32 mmol) in *N,N*-dimethyl formamide, sodium hydrogen carbonate (0.840 g, 10 mmol) and allyl bromide (0.254 mL, 3 mmol) were added. The resulting reaction mixture was stirred for 5 hours at 50 °C. The solvent was removed under reduced pressure. The residue was dissolved in

dichloromethane (100 mL) and washed with water. The organic layer was dried over magnesium sulphate and the solvent removed under reduced pressure. A further purification by column chromatography afforded the product as a pale yellow oil (0.653 g, 2.13 mmol, 92%). Analytical data: HPLC = (SO15OD) 14.05 min; $[\alpha]_D$ = 48.8 (c 1.0 g/100 mL, $CHCl_3$, 23 °C); $[\alpha]_D$ lit.[191,190] = - 10.2 (c 1.1 g/100 mL,, MeOH, 29 °C); ν_{max} = 3369 (w), 2978 (w), 1711 (s), 1495 (m), 1158 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 7.23-7.14 (m, 3H, ArH), 7.06 (d, J = 6.7 Hz, 2H, ArH), 5.78 (ddt, J_{trans} = 17 Hz, J_{cis} = 10.5 Hz, J_{vic} = 5.5 Hz, 1H, CH_2=C*H*CH_2), 5.22 (d, J = 17 Hz, 2H, CH_2CH=C*H*$_a$$H_b$), 5.17 (d, J = 10.5 Hz, 2H, CH_2CH=CH_aH_b), 4.89 (br s, 1H, NH), 4.52 (m, 3H, C*H*$_2$CH=$CH_2$and C*H*CH_2Ar), 3.03 (m, 2H, $ArCH_2$), 1.34 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.7 (0), 155.2 (0), 136.1 (0), 131.7 (1), 129.5 (1), 128.6 (1), 127.1 (1), 119.0 (2), 80.0 (0), 66.0 (2), 54.6 (1), 38.5 (2), 28.4 (3);^1H NMR (400 MHz, CD_3CN) δ = 7.24-7.20 (m, 5H, ArH), 5.90 (1H, ddt, J_{trans} = 17.0 Hz, J_{cis} = 10.5 Hz, J_{vic} = 5.5 Hz, CH_2=C*H*CH_2), 5.52 (br s, 1H, NH), 5.32 (d with fine splitting, *J* = 17.0 Hz, 2H, CH_2=CHC*Ha*Hb), 5.22 (d with fine splitting, *J* = 10.5 Hz, 2H, CH_2=CHCHa*Hb*), 4.58 (d, 2H, *J* = 5.5 Hz, CH_2=CHC*H*$_2$), 4.38 (br s, 1H, C*H*CH_2Ar), 3.12 (dd, *J* = 13.5 Hz, 5.5 Hz, 1H, CHC*H*$_c$$H_d$Ar), 2.93 (m, 1H, CHC$H_c$*H*$_d$Ar), 1.35 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (100 MHz, CD_3CN) δ = 172.7 (0), 156.4 (0), 138.2 (0), 133.3 (1), 130.3 (1), 129.4 (1), 127.8 (1), 118.3 (2), 80.1 (0), 66.3 (2), 56.1 (1), 38.2 (2), 28.5 (3); LRMS (ES^+) *m/z* (%) = 306.2 (40) $[M+H]^+$, 611.3 (10) $[2M+H]^+$, 633.4 (80) $[2M+Na]^+$.

Spectroscopic data were consistent with those reported in literature [190].

Allyl (2*S*)-2-(benzoylamino)propanoate (176) [192]

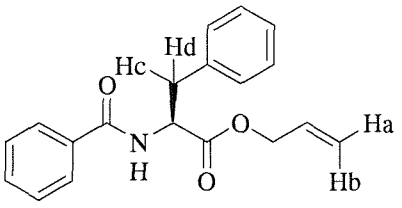


$C_{13}H_{15}NO_3$
Exact Mass: 233.1052

To a solution of *N*-Benzoyl-L-Ala-OH (1.03 g, 5.33mmol) in *N,N*-dimethyl formamide, sodium hydrogen carbonate (4.47 g, 53.3 mmol), and allyl bromide (0.600 mL, 6.93 mmol) were added. The resulting reaction mixture was stirred for 5 hours at 50 °C. The solved was removed under reduced pressure. The residue was redissolved in dichloromethane (200 mL) and washed with water. The organic layer was dried over magnesium sulphate and the solvent removed under reduced pressure. A further trituration affords the product as a pale yellow waxy solid (1.24 g, 5.33 mmol, 100%). Analytical data: $[\alpha]_D$ = 32.55 (c 1.0 g/100 mL, $CHCl_3$, 24 °C); $[\alpha]_D$ = -9.45 (c 1.0 g/100 mL,, MeOH, 23 °C); ν_{max} = 3291 (m), 3057 (w), 2998 (w), 2929 (w), 2882 (w), 1739 (s), 1634 (s), 1537 (s), 1452 (s), 1345 (s), 1307 (m), 1206 (s), 1168 (s), 1122 (s) cm^{-1}; ^1H NMR (400 MHz, $CHCl_3$) δ = 7.83 (d with fine splitting, *J* = 7 Hz, 2H, ArH), 7.51-7.40 (m, 3H, ArH), 6.81 (d, *J* = 5.5 Hz, 1H, NH), 5.92 (ddt, J_{trans} = 17.0 Hz, J_{cis} = 10.5 Hz, J_{vic} = 5.5 Hz, 1H, CH_2=C*H*CH_2), 5.34 (dd, *J* = 17. Hz, 1 Hz, 1H, CH_2CH=C*Ha*Hb), 5.26 (dd, *J* = 10.5 Hz, 1 Hz, 1H, CH_2CH=CHa*Hb*), 4.85-4.79 (q, *J* = 7Hz, 1H, C*H*CH_3), 4.78-4.62 (m, 2H, C*H*$_2CH$=CH_2), 1.52 (d, *J* = 7Hz, 3H, CHC*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 173.0 (0), 166.9 (0), 134.0 (0) 131.8 (1), 131.6 (1), 128.76 (1), 127.1 (1), 66.1 (2), 48.6 (1), 18.7 (3); LRMS (ES^+) m/z (%) = 234.1 (30), $[M+H]^+$, 489.2 (50) $[2M+Na]^+$.

Spectroscopic data were consistent with those reported in literature [192]

Allyl (2*S*)-2-(benzoylamino)-3-phenyl-propanoate (177) [193]

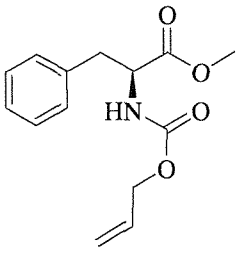


$C_{19}H_{19}NO_3$
Exact Mass: 309.1365

To a solution of N-Benzoyl-L-Phe-OH (1.03 g, 5.33 mmol) in N,N-dimethyl formamide, sodium hydrogen carbonate (4.47 g, 53.3 mmol), and allyl bromide (0.600 mL, 6.93 mmol) were added. The resulting reaction mixture was stirred for 5 hours at 50 °C. The solvent was removed under reduced pressure. The residue was redissolved in dichloromethane (200 mL) and washed with water. The organic layer was dried over magnesium sulphate and the solvent removed under reduced pressure. A further trituration affords the product as a white solid (1.565 g, 5.06 mmol, 95%). Analytical data: mp 90-92 °C; $[\alpha]_D$ = 110.9 (c 0.92 g/100 mL, $CHCl_3$, 24 °C); $[\alpha]_D$ = – 44.6 (c 0.99 g/100 mL,, MeOH, 23 °C); ν_{max} = 3301 (w), 1744 (s), 1610 (s), 1539 (s), 1341 (m), 1245 (s), 1210 (s), 1093 (m), 932 (m) cm^{-1}; ^1H NMR (400 MHz, $CHCl_3$): δ = 7.67 (d *J* = 7 Hz, 2H, ArHCO), 7.48-7.36 (m, 3H, ArHCO), 7.26-7.18 (m, 3H, ArH), 7.10 (d, *J* = 6 Hz, 2H, ArH), 6.52 (d, 1H, 7 Hz, NH), 5.85 (ddt, J_{trans} = 16.5 Hz, J_{cis} = 10.5 Hz, J_{vic} = 6 Hz, 1H, CH_2=C*H*CH_2), 5.29 (d with fine splitting, *J* = 16.5 Hz, 1H, CH_2CH=CH_aH_b), 5.23 (d with fine splitting, *J* = 10.5 Hz, 1H, CH_2CH=CH_aH_b), 5.07 (dt, *J* = 6 Hz, 7.5 Hz, 1H, NHC*H*CH_2), 4.60 (d, *J* = 6 Hz, 2H, CH_2=CHCH_2), 3.29-3.24 (dd, *J* = 6 Hz, 14 Hz, 1H, CHCH_cH_d), 3.22-3.18 (dd, *J* = 5.5 Hz, 14 Hz, CHCH_cH_d); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 171.5 (0), 166.9 (0), 135.9 (0), 134.1 (0), 131.9 (1), 131.5 (1), 129.6 (1), 128.7(1), 127.3 (1), 127.1 (1), 119.4 (2), 66.3 (2), 53.7 (1), 38.1 (2); LRMS (ES^+): *m/z* (%) = 310.1 (10) $[M+H]^+$, 641.3 (100) $[2M+Na]^+$.

Spectroscopically identical to material already described in literature [193].

Methyl (2*S*)-2-{[(allyloxy)carbonyl]amino}-3-phenyl-propanoate (178)

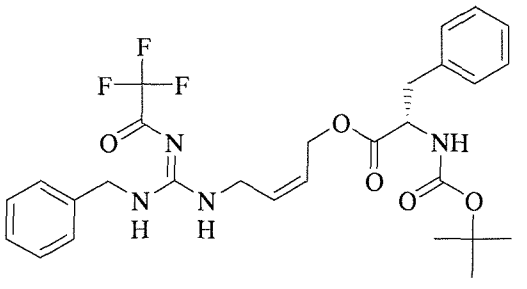


$C_{14}H_{17}NO_4$
Exact Mass: 263.1158

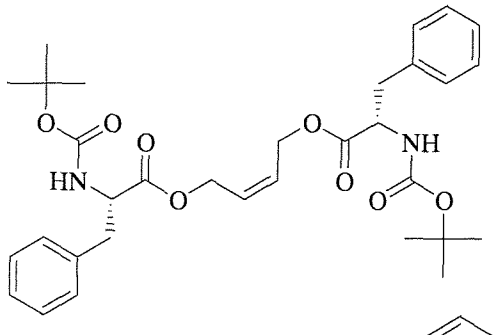
Sodium hydrogen carbonate (2.0 g, 24 mmol), and iodomethane (0.747 mL, 12 mmol) were added to a solution of *N*-Aloc-L-Phe-OH **122** (0.590 g, 2.4 mmol) in N,N-dimethyl formamide. The resulting reaction mixture was stirred for 5 hours at 40 °C. The solvent was removed under reduced pressure. The residue was redissolved in dichloromethane (100 mL) and washed with water. The organic layer was dried over magnesium sulphate and the solvent removed under reduced pressure to afford the product **178** (0.625 g, 0.237 mmol, 99%). Analytical data: HPLC = (S15OD) 11.73 min; $[\alpha]_D$ = 43.3 (c 0.8 g/100 mL, $CHCl_3$, 23 °C) lit.[178]; $[\alpha]_D$ = 39.5 (c 1.0 g/100 mL,, $CHCl_3$, 23 °C); $[\alpha]_D$ = – 4.48 (c 1.03 g/100 mL,, MeOH, 23 °C); ν_{max} (film) = 3317 (w), 2952 (w), 1719 (m), 1662 (s), 1527 (m), 1256 (m), 1213 (m), 1094 (m), 1052 (m), 702 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 7.95 (s, 1H, NH), 7.24-7.15 (m, 3H, ArH), 7.05 (d, *J* = 7 HZ, 2H, ArH), 5.86 (ddt, J_{trans} = 16.5 Hz, J_{cis} = 10.5 Hz, J_{vic} = 5.5 Hz, 1H, CH_2=C*H*CH_2O), 5.23-5.12 (m, 2H, CH_2=CHCH_2), 4.56 (m, 1H, NHC*H*CH_2), 4.47 (d, J_{vic} = 5.5 Hz, 2H, CH_2CH=CH_2), 3.64 (s, 3H, OCH_3), 3.09-2.98 (m, 2H, CH_2Ar); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 172.1 (0). 155.6 (0), 135.9 (0), 132.8 (1), 129.4 (1), 128.7 (1), 127.2 (1), 117.9 (2), 65.9 (2), 54.9 (1), 52.4 (3), 38.4 (2); LRMS (ES^+) *m/z* (%) = 264.2 (20) $[M+H]^+$, 549.3 (50) $[M+Na]^+$.

Spectroscopically identical to material already described in literature [178]

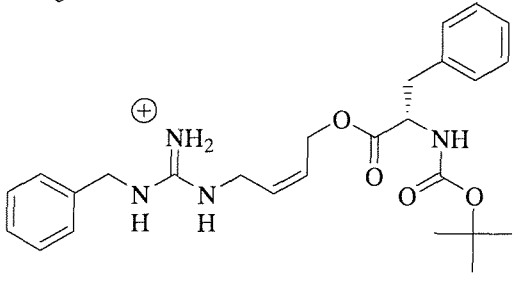
4-({(benzylamino)[(2,2,2-trifluoroacetyl)imino]methyl}amino)-2-butenyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-phenylpropanoate (179)



179
$C_{28}H_{33}F_3N_4O_5$
Exact Mass: 562.2403



180
$C_{32}H_{42}N_2O_8$
Exact Mass: 582.2941

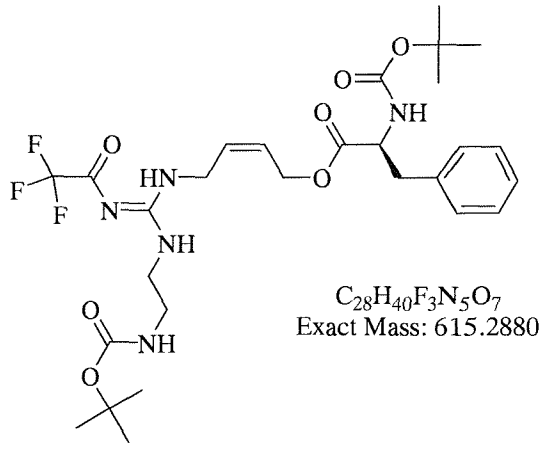


181
$C_{26}H_{35}N_4O_4^+$
Exact Mass: 467.2658

To a solution of allyl ester **175** (0.24 g, 0.787 mmol) and (trifluoro-acetyl)-guanidine **172** (0.173 g, 0.605 mmol) in degassed dichloroethane (5mL), the second generation Grubbs' catalyst (0.0146 g, 5 mol%) was added under an atmosphere of nitrogen. The resulting reaction mixture was stirred at 60 °C for 18 h under nitrogen atmosphere. The solvent was removed under reduced pressure and the crude product purified by column chromatography (eluent 7:3 PetEt/EtOAc) to obtain the product **179** in 21 % yield (0.071 g, 0.128 mmol), starting material (0.069 g, 0.241 mmol) in 40% yield, and the product of cross coupling of allyl ester **180** (0.0745 g, 0.126 mmol) in 21% yield. Analytical data: R_f = 0.26 (eluting with Petrol Ether/EtOAc 7:3), 0.18 (eluting with chloroform); HPLC (S15OD2) 14.3 min $[\alpha]_D$ = 8.18 (c 0.51, $CHCl_3$, 24 °C); ν_{max} = 3330 (w), 2975 (w), 1698 (m), 1622 (s), 1589 (m), 1496 (m), 1436 (m), 1240

(m), 1190 (s) cm^{-1}; ^1H NMR (400 MHz, CD_3CN) δ = 9.70 (br s, NH), 9.46 (br s, NH), 7.36-7.16 (m, 10H, ArH), 6.44 (br s, NH), 6.16 (br s, NH), 5.70 (d, *J* = 9 Hz, 2H, CH=CH), 5.52 (br s, NH), 4.54 (br s, 4H, CH=CHCH_2O and ArCH_2NH), 4.36 (d, *J* = 6 Hz, 1H, C*H*CH_2Ar), 4.01 (br s, NH), 3.84 (br s, 2H, CH=CHCH_2NH), 3.06 (dd, *J* = 6Hz, 14 Hz, 1H C*H*CH_2Ar), 2.89 (m, 1H, C*H*CH_2Ar), 1.33 (s, 9H, $(CH_3)_3C$); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 171.7 (0), 167.7-166.6 (q, J_{CF} = 34.8 Hz, $COCF_3$), 160.8 (0), 155.3 (0), 135.9 (0), 129.4 (1), 129.2 (1), 128.7 (1), 128.3 (1), 127.2 (1), 121.3-112.7 (q, J_{CF} = 285 Hz, CF_3), 80.2 (0), 64.5 (2), 54.7 (1), 45.5 (2), 42.6 (2), 38.4 (2), 28.4 (3); LRMS (ES^+) *m/z* (%) = 563.4 (100) $[M+H]^+$, 585.3 (50) $[M+Na]^+$, 1125.6 (10) $[2M+H]^+$, 1147.5 $[2M+Na]^+$; HRMS (ES^+) calcd for $C_{26}H_{35}N_4O_4$ $[M]^+$: 467.2658, found 467.2656. Was not possible to obtain the mass of the protected compound by HRMS

4-({({2-[(*tert*-butoxycarbonyl)amino]ethyl}amino)[(2,2,2-trifluoroacetyl)imino] methyl}amino)-2-butenyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-phenylpropanoate (182)



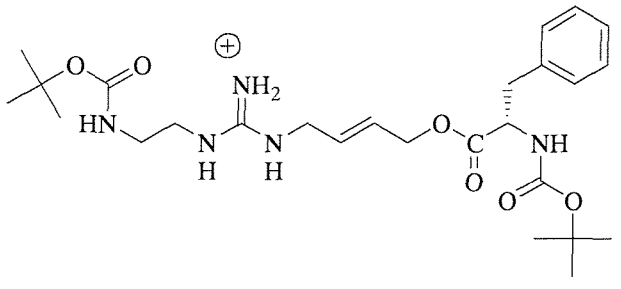
$C_{28}H_{40}F_3N_5O_7$
Exact Mass: 615.2880

To a solution of allyl aster **175** (0.063 g, 0.206 mmol) and trifluoro-acetyl-guanidine **167** (0.070 g, 0.206 mmol) in degassed dichloroethane (5mL), the second generation Grubbs' catalyst (2 × 0.008 g, 9 mol%) was added under nitrogen atmosphere in 4h. The resulting reaction mixture was stirred for 18 hours at 60 °C under nitrogen atmosphere. The solvent was removed under reduced pressure and the crude product purified by column chromatography (eluting with Petrol Ether/EtOAc 6:4) to obtain the

product **182** (0.009 g, 0.016 mmol, 8%), starting material (0.049 g, 0.147 mmol, 71%) and product of cross coupling of allyl ester (0.0076 g, 0.013 mmol, 6%). The NMR spectra shows that the N-(2,2,2-Trifluoro-acetyl)-guanidine exists in solution in more than one configuration. Analytical data: LRMS (ES^+): m/z (%) = 616.3 (100) $[M+H]^+$, 638.3 (10) $[M+Na]^+$

The product was deprotected by standing at room temperature to obtain a pure guanidinium **183**

(({2-[(*tert*-butoxycarbonyl)amino]ethyl}amino){[4-({(2S)-2-[(*tert*-butoxycarbonyl) amino]-3-phenylpropanoyl}oxy)-2-butenyl]amino}methylene)ammonium (183)



$C_{26}H_{42}N_5O_6^+$
Exact Mass: 520.3135

Analytical data: $[\alpha]_D$ = -41.44(c 0.27, $CHCl_3$, 22°C); ν_{max} = 3318 (w), 3198 (w), 2980 (w), 2360 (w), 1676 (s), 1509 (m), 1162 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 7.28-7.21 (m, 3H, ArH), 7.13 (d, *J* = 7 Hz, 2H, ArH), 5.81-5.68 (m, 2H, CH=CH), 5.38 (s, 1H, NH), 5.01 (d, *J* = 7.5 Hz, 1H, NH), 4.64-4.53 (m, 3H, C*H*NH and NHCH_2CH=CH), 3.81 (s, 2H, CH_2OCO), 3.28 (br s, 4H, NHCH_2 and CH_2NH), 3.12-3.03 (m, 2H, CHCH_2Ar), 2.16 (s, 2H, NH), 1.42 (s, 9H, $C(CH_3)_3$), 1.39 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 171.8 (0), 157.0 (0), 155.4 (0), 136.1(0), 129.43 (1), 129.4 (1), 128.9 (1), 128.7 (1), 127.2 (1), 127.1 (1), 80.9 (0), 80.3 (0), 64.6 (2), 54.8 (1), 42.8 (2), 39.8 (2), 38.4 (2), 28.4 (3); LRMS (ES^+) *m/z* (%) = 520.3 (100) $[M]^+$; HRMS (ES^+) calcd for $C_{26}H_{42}N_5O_6^+$ $[M]^+$: 520.3140, found 520.3130.

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