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UNIVERSITY OF SOUTHAMPTON

SOLID PHASE AND COMBINATORIAL SYNTHESIS OF RECEPTORS FOR SMALL PEPTIDES

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ABSTRACT

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In the first chapter of this thesis recent developments in host-guest chemistry are reviewed, in particular receptors for carboxylic acids and small peptides. The applications of solid phase and combinatorial chemistry in the synthesis of receptors are also described. Chapter 2 describes the synthesis of a CBS (carboxylic acid binding site) functionalised to allow attachment to the solid support, and synthesis of a library of tweezer receptors. The library was screened with different labelled peptide guests and receptor structures were identified. One example of such a receptor library was resynthesised and binding studies in free solution were attempted. Chapter 3 describes the attempt to synthesise an "unsymmetrical" tweezer receptor library with different sequences of amino acids in the two peptidic arms. Chapter 3 describes also the synthesis of β-sulfonamido derivatives used in the synthesis of a further example of a tweezer receptor, and binding studies on this class of receptors showed selectivity for the peptide L-Ala-L-Ala. Chapter 3 includes also the synthesis of a further receptor library incorporating a bicyclic guanidinium as a CBS and screening experiments with dye labeled D-Ala-D-Ala. Chapter 4 describes the synthesis of the various peptides used as guests in the various screening experiments.

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Mariangela

Abbreviations

AA	Amino acid
Ac	Acetyl
Ar	Aryl
Aloc	Allyloxycarbonyl
Bn	Benzyl
Boc	*tert*-Butoxycarbonyl
BSA	*N*, O-bis(Trimethylsilyl)acetamide
Bu	Butyl
CBS	Carboxylic Binding Side
Cbz	Benzyloxycarbonyl
δ	Chemical shift (ppm)
d	Doublet
DBU	1, 8- Diazabicyclo[5.4.0]undec-7-ene
DCC	*N*, *N*'-Dicyclohexylcarbodiimide
Dde	2-(1-Hydroxy-2-phenyl-ethylidene)-5,5-dimethyl-cyclohexane-1,3-dione
Ddiv	2-(1-Hydroxy-3-methyl-butylidene)-5,5-dimethyl-cyclohexane-1,3-dione
DIC	*N*, *N*-Diisopropylcarbodiimide
DIPEA	Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	*N*, *N*-Dimethylformamide
DMSO	Dimethylsulfoxide
DNS	5-Dimethylaminonaphthalene-1-sulfonyl
EDC	1-3-(Dimethylaminopropyl)-3-ethylcarbodiimide
ES^+	Positive electrospray
EtOAc	Ethyl acetate
EtOH	Ethanol
FAB	Fast atom bombardment
Fmoc	Fluoren-9-ylmethoxycarbonyl
h	Hours
HBTU	*O*-(benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate

HMPB	4-Hydroxymethyl-3-methoxyphenoxybutyric acid
HOBt	*N*-Hydroxybenzotriazole hydrate
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
IR	Infrared
ITC	Isothermal Titration Calorimetry
J	Coupling constant (Hz)
Lit.	Literature
m	multiplet
MS	Mass Spectroscopy
MeOH	Methanol
Mins	minutes
M. p.	Melting point
NMM	*N*-Methylmorpholine
NMR	Nuclear Magnetic Resonance
PG	Protecting group
Ph	Phenyl
ppm	parts per million
Py	Pyridine
PyBOP^{TM}	Benzotriazole-1-yl-oxy-tri-pyrrolidinophosphonium hexafluorophosphate
RT	Room Temperature
s	Singlet
SKA	Methyl trimethylsilyl dimethylketene acetal
SPPS	Solid Phase Peptide Synthesis
t	Triplet
TBA	Tetrabutylammonium
TBAF	Tetrabutylammonium fluoride
TBTU	2-(1-H-Benzotriazole-1-yl)1,1,3,3-tetramethyluronium tetrafluoroborate
TEA	Triethylamine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography

UV	Ultraviolet

Amino Acids

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
Ser	Serine
Thr	Threonine
Trp	Tryptophan
Tyr	Tyrosine
Val	Valine

Chapter 1

Introduction to Supramolecular

1.1 Supramolecular Chemistry

"Supramolecular chemistry is the chemistry of the intermolecular bond, covering the structure and functions of the entities formed by association of two or more chemical species".[1] In contrast to the classic, molecular chemistry which is based on covalent bonds between single atoms, supramolecular chemistry relies on non-covalent bonds between molecules.

Molecular associations have been recognized and studied for a long time, indeed, the term "Übermoleküle", or supermolecules, was introduced in the mid-1930s to describe entities of higher organization resulting from the association of coordinatively saturated species.[2] Only in 1987 the concept and the term of supramolecular chemistry was introduced by Jean-Marie Lehn, who was awarded the Nobel prize for his work in this field. The partners of a supramolecular entity have been named molecular receptor or "host" and substrate or "guest",[1] the substrate being usually the smaller component which is involved in the binding. This terminology conveys the relation to biological receptors and substrate for which Paul Ehrlich stated[3] that molecules do not act if they are not bound ("*corpora non agunt nisi fixata*").

Molecular interactions form the basis of the highly specific recognition, reaction, transport and regulation processes that occur in biology, such as substrate binding to a receptor protein, enzymatic reactions, assembling of protein-protein complexes, immunological antigen-antibody association, intermolecular reading, translation and transcription of the genetic code, signal induction by neurotransmitters, and cellular recognition.
The objective of the host-guest chemist is to reproduce the binding observed in natural systems, using artificial receptors, to obtain selectivity and strength of binding similar to

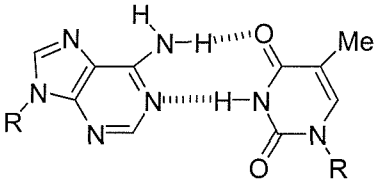
those found in biological events. The interest in artificial hosts is not only due to their direct relevance to the corresponding biological system, but also because of their potential to lead to new therapeutics, biosensors, and catalysts.[4]

Over the past two decades a vast number of artificial receptors have been devised and synthesised in numerous research groups all over the world. This has led to non-natural receptors for nucleotide bases,[5,6] amino acids,[7,8] peptides,[9,10] and many other entities.[1,10,11]

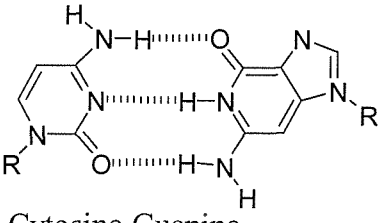
1.2 Molecular Recognition

Molecular recognition has been defined as a process involving *both binding* and *selection* of substrate(s) by a given receptor molecule.[12] In order to achieve a specific binding between receptor and substrate, complementarity in size, shape, and functional groups between host molecules and substrate are indispensable. However, since non-covalent interactions are generally much weaker than covalent bonds, host molecules rely on a multipoint binding motif to overcome the entropic cost of the organization of components into the final ordered state.[13] This amounts to a generalized double complementarity principle extending over energetic as well as geometric features, the celebrated "lock and key", steric fit concept enunciated by Emil Fischer for enzymes, in 1894.[14]

Probably the most important example for supramolecular chemistry in nature is the construction of the entire genetic code in DNA with only four nucleic acids, which always occur in the same distinct base-pairs.[15] Almost without exception adenine pairs with thymine and guanine binds cytosine (figure 1)



Adenine-Thymine



Cytosine-Guanine

Figure 1: Nucleic acids

1.3 Binding Energy

Host-guest complexation can be expressed as the equilibrium:

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

The left side of the equation describes the concentration of host [H] and guest [G] in the free, unassociated form. The right side describes the concentration of formed host-guest complex [HG] once the system is in equilibrium. The success of a synthetic receptor to bind a specific guest is measured as strength of binding or binding efficiency which is the equilibrium constant, K_a:

$$K_a = \frac{[\text{Host-Guest}]}{[\text{Host}]\,[\text{Guest}]}$$

The higher the value of the equilibrium constant the stronger the tendency for the complex to form and, therefore, the stronger the binding between the host and guest. The value of the equilibrium constant essentially reflects the change in the Gibbs free energy of the system on complexation. This is related by the following equation:

$$K_a = e^{(-\Delta G/RT)}$$

Where R is the gas constant, T is the temperature (in degrees Kelvin) and ΔG is the free energy change. If complexation is to occur the system must experience a decrease in Gibbs free energy which is governed by an enthalpic and an entropic term:

$$\Delta G = \Delta H - T\Delta S$$

ΔH is the change in enthalpy of the system and ΔS is the entropy term. The factors contributing to the enthalpic term include: the binding interactions between receptor and substate molecules and desolvation, which must take place before complexation can occur.

The entropic term is composed of the effects from conformational reorganization during association and an alteration in translational and rotational degrees of freedom of the receptor and substrate molecules. Desolvation can also contribute to this term. For successful complexation, the enthalpic term must be higher than the entropic term: that is the contribution from binding interactions must pay the energy penalty incurred to organize the host and guest during complexation. When designing a viable synthetic receptor it is therefore expedient to maximise the functional group complementarity between the host and guest and aim to reduce the conformational flexibility of the host, thereby reducing the reorganization required upon association.

1.4 Interactions Affecting Association of Host-Guest Complexes

The common interactions involved in host-guest complexes are essentially identical to those responsible for the reversible binding of a substrate to its receptor in a natural system. They are non-covalent and generally weak which necessitates use of many sites of interaction found in natural host-guest complexes and which have been used in some of the more sophisticated synthetic receptors that have recently been produced.

The common interactions utilised in synthetic host-guest complexes are as follows: 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 a large part in the association of hosts and guests and will also be considered in this section.

1.4.1 Electrostatic Interactions

All forces between atoms and molecules are electrostatic in origin. However, the term is normally reserved for molecular or ionic attractions involving static charges or dipolar moments. Coulomb's law gives the force (F) of such an interaction:

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

Where q_1 and q_2 are the charges of the two groups, r is the distance between them and D is the dielectric constant of the solvent; the optimal distance between the groups is approximately 2.8Å.[16]

Ion-ion interactions are non-directional, instead for ion-dipole interaction the dipole must be suitably aligned for optimal binding efficiency (figure 2).

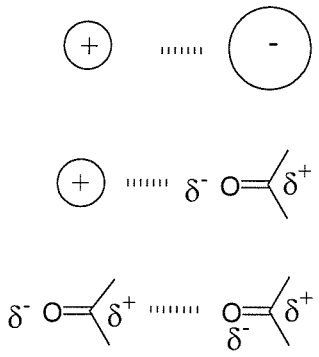


Figure 2: Electrostatic interactions (ion-ion, ion-dipole, dipole-dipole)

There are many receptors for cations and anions which employ electrostatic interactions to hold the guest in place. A relevant example is Pedersen's dibenzo[18]crown-6, **1**, which binds a range of alkali cations through electrostatic ion-dipole interactions between the alkali metal cation and the six oxygen donor atoms in the polyether rings with a general selectivity for K^+ in H_2O (figure 3).[17]

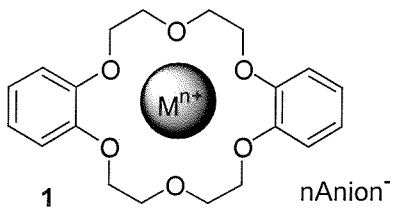


Figure 3:Pedersen's Cyclic Polyether

1.4.2 Hydrogen Bonds

Hydrogen bonds can be formed between charged and uncharged molecules; they are normally formed between an electronegative heteroatom with a lone pair of electrons, the hydrogen acceptor, and an electronegative heteroatom with an attached hydrogen atom, a hydrogen donor. The length of a hydrogen bond is dependent on the charges involved, and the electronegativity of the heteroatoms. The higher the charge on the interacting atoms the shorter and stronger the hydrogen bond. Typically, hydrogen bonded NH···O=C distances are 2.0-1.7 Å in length, though interactions in excess of 3.0 Å may also be significant.[18, 19] These value represent the distance between the donor and acceptor atoms, it should be noted that values of bond length and the strength of hydrogen bonds is extremely dependent on solvent. Hydrogen bond strength is maximal when the atoms are colinear,[18] however, that mean distribution of NH···O bond angles is 161.2°, and angles of 180° are rare. The thermodynamic strength of an hydrogen bond is extremely variable. For neutral molecules, it normally lies in the range of 10-65 KJ mol^{-1}, however, when one component of the hydrogen bond is ionic, the range of bond strengths rises to 40-190 KJ mol^{-1}.[20] Hydrogen bonds may be simple (involving only one donor and one acceptor), bifurcated (three-centre), or trifurcated (four-centre) (figure 4).[20]

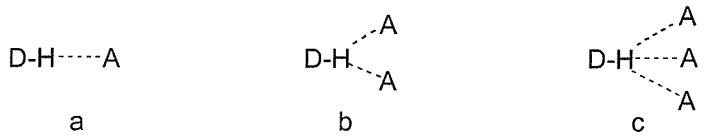


Figure 4: a) Simple; b) Bifuricated; c) Trifuricated

Hydrogen bond strengths are quite small, and so, strong complexation can be achieved only when several receptor sites act cooperatively.[20] A classic example is Hamilton's barbiturate receptor **2** (figure 5).[21] The incorporation of six hydrogen bonds into a synthetic receptor for barbiturates gave a binding constant of 10^{-5} M^{-1} in $CHCl_3$ for this important class of drug. Hamilton demonstrated the importance of hydrogen bonding in this complex by investigating the value of binding constant with a series of guests which were strategically modified to give a different number of potential hydrogen bonding sites. As

the number of hydrogen bonds formed between host and guest decreased a corresponding drop in the value of the binding constant was observed.

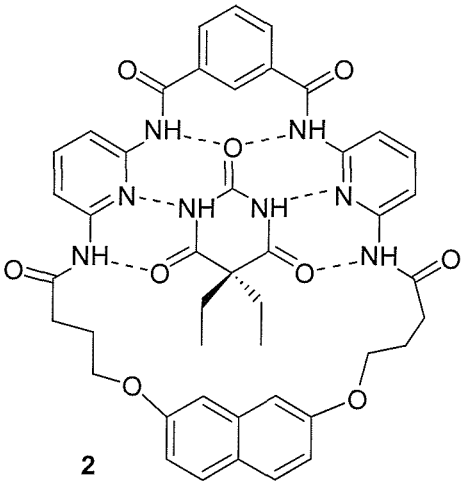


Figure 5: Hamilton's Barbiturate receptor

Hydrogen bonding is fundamental to many biological macromolecules in particular the secondary structure of global proteins and is also a feature of their interactions with natural ligands. For this reason it is important to synthetic hosts and bonding between them and their potential guests.

1.4.3 van der Waals Forces

These are particulary short range forces which are not effective at distances which significantly exceed molecular diameters. They are essentially a transient asymmetrical distribution of electron density that forms a dipole, this may induce an opposite temporary dipole in the neighbouring atom. For two atoms, the van der Waals force can be expressed by the Lennard-Jones potential (figure 6, Equation 1):

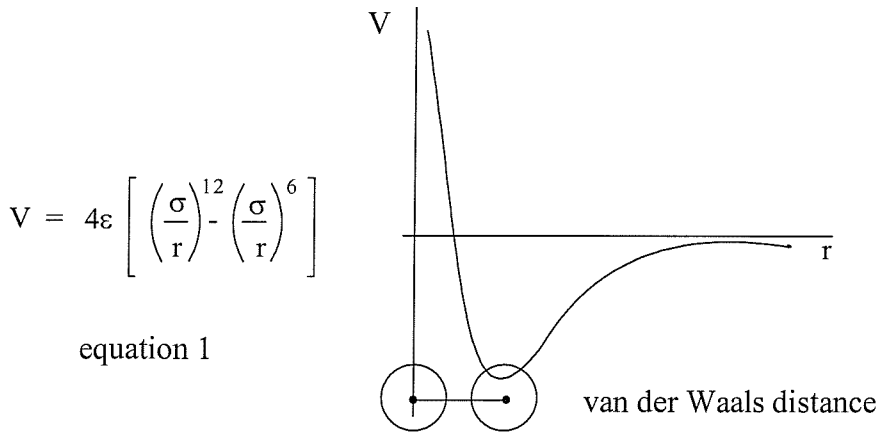


Figure 6: The Lennard Jones Potential

Where ε is the depth of the well and σ the separation at which V = 0. The first term becomes very large when both atoms try to penetrate within each others van der Waals radii, which results in a strong repulsion due to the negatively charged electron clouds. The second term represents an attractive potential due to London dispersion forces, as a temporarily unsymmetrical distribution of electrons in one atom induces an opposite polarity in the other. Hence the optimum distance between both particles is when the repulsive and the attractive forces are in equilibrium. This distance is called the van der Waals distance.[22] Although weak, these interaction are additive and across large molecules represent significant attractive forces. van der Waals forces depend on steric complementarity; a single van der Waals interaction provides virtually no specificity, however, specificity arises when there is an opportunity to make a large number of van der Waals contacts simultaneously.

1.4.4 π-π-Stacking Interactions

π-π-Stacking interactions are non-covalent interactions between delocalized π-systems including interactions between aromatic molecules.[23] Interactions between aromatics represent an important class of intermolecular forces in biochemical systems. They are, for example, involved in the base-stacking interactions which determine the sequence-dependent structure and properties of DNA as well as recognition of DNA by drugs and

regulatory proteins. They have implications in molecular recognition of drugs by biological receptors.[24]

The charge distribution on a benzene molecule has been represented by Hunter and Sanders as a positive σ-framer work sandwiched between two regions of negatively charged π-electron density; this is shown below in figure 7. Essentially, this means that the hydrogen atoms attached to the benzene molecule have a partial positive charge and will therefore, be attracted to the regions of high electron density above the plane of the benzene ring. This leads to the possibility of interaction between two benzene rings if they are oriented in either an "edge-to-face" or a "face-to-face" geometry that are favoured. For benzene rings, this "edge-to-face" geometry has been calculated to be a global energy minimum; this is more stable than the "face-to-face" geometry by approximately 6.3 kJ mol^{-1}.[24]

'face-to-face' stacking 'edge-to-face' stacking

Figure 7: Hunter's and Sanders's Model for the Stacking of Aromatic Rings

The use of π-π interactions in artificial receptors is showed by Kelly's tweezer receptor **3** (figure 8).[25] The peptide host contains a dibenzofuran moiety designed to bind to the *C*-terminal benzylamide unit of the guest *via* aromatic π-π stacking interactions in addition to the binding of the host to tetrapeptide guest to form an antiparallel β-sheet structure.

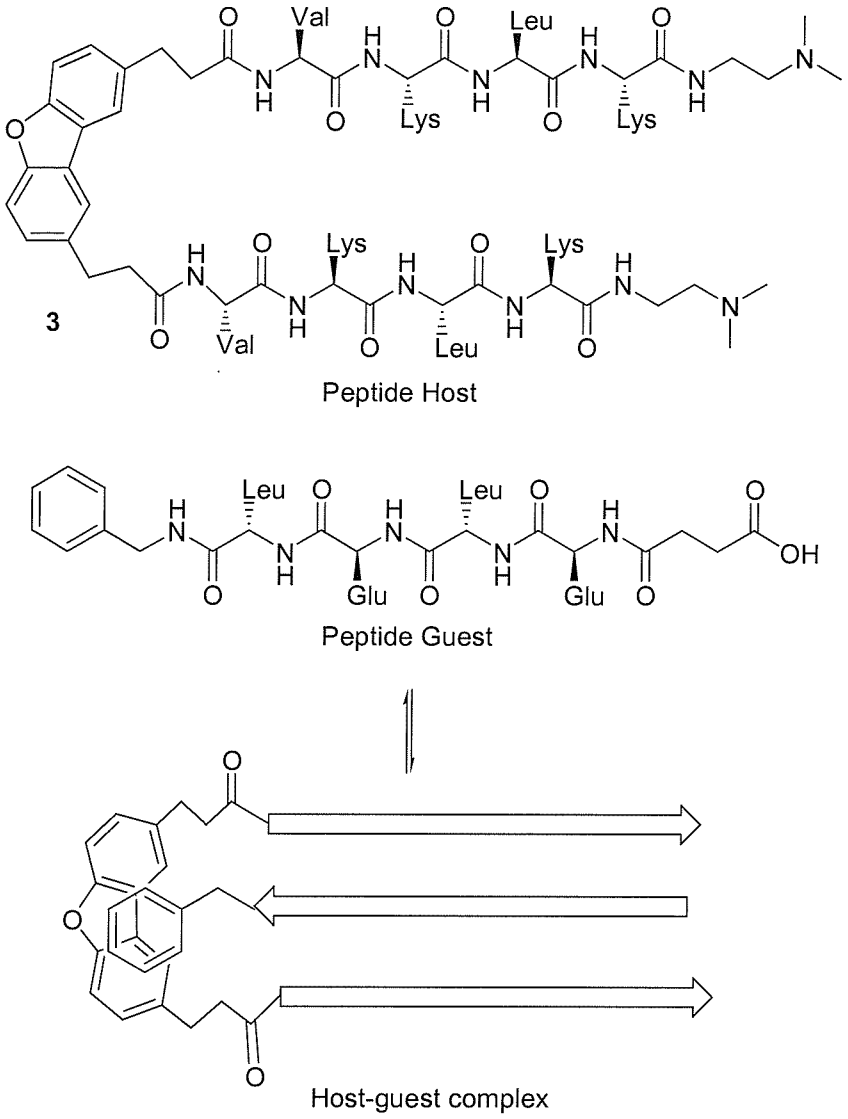


Figure 8: The Tweezer Receptor reported by Kelly and co-workers.[25]

1.4.5 Solvent Effects

Solvent is extremely important in the stabilization of the host-guest complex. The strength of electrostatic interactions is dependent on the dielectric constant of the solvent which measures its bulk polarity and reflects the dipole moment of an individual molecule of solvent. For organic molecules dissolved in organic solvents, solvation energies are usually negative, and the decreases in solvation which often accompany binding inhibit molecular complex formation.[26] Association is favoured by solvents which either weakly solvate the

uncomplexed components of the system or strongly solvate the molecular complex. For example, in a polar solvent such as water where the dieletric constant is high, formation of hydrogen bonds between solvent and solute molecules occurs, masking the charges on the host and the guest molecules. The attractive force between host and guest molecules are, therefore, significant only when a guest and host are in close proximity. In less polar solvents, for example chloroform, there is little hydrogen bonding between the solute molecules and the solvent so the forces that attract the host and guest will be stronger at greater distance.

1.4.6 Hydrophobic Effect

The drive for lipophilic molecules to associate is an important interaction in host-guest systems that operate under aqueous conditions.[27] Hydrophobic effects are of crucial importance in the binding of organic guests by hosts in water and may be divided into two energetic components: enthalpic and entropic. In the case that it is energetically favourable for a solvent to solvate the non polar surfaces of the host and guest, then the formation of the complex is not promoted, however, in case of weak solvation, complex formation is favoured. If the solvent molecules have strong cohesive interactions (as in the case of water) the solvation of non polar molecular surface and cavities is an enthalphically disfavorable process, because the solvent molecules on the surface of the solute can form fewer hydrogen bonds than those in the bulk solution and consequently are higher in energy. In the case of an association of two non polar surfaces, formation of the complex, the surface solvent molecules are released into the bulk and their energy content is lower, and this promotes complex formation with an enthalpic contribution. At the same time there is also an entropic contribution: the ordered solvent cage enveloping the host and guest molecules is disrupted, and the molecules of solvent released are transformed into a less-ordered state.[28]

1.5. Host-Guest Chemistry

It is impossible to give a complete overview summarising the entire field of host-guest chemistry in this thesis. The following section will therefore concentrate on receptors that

bind peptides and related guests. A more complete overview can be obtained from literature sources,[1,10,11,29-32]

1.5.1 Synthetic Receptors Designed to Bind Carboxylic Acids and Carboxylates

Several examples of binding sites for carboxylate and carboxylic acid functionalities are known and include the use of ammonium, metals, guanidinium, ureas, diamidopyridine and some of these are discussed below.

1.5.1.1 Receptors for Carboxylates

Carboxylates can be bound using bidentate hydrogen bonding array e.g. guanidinium **4**, ureas **5**, thioureas **6**, and amidines **7** (figure 9).

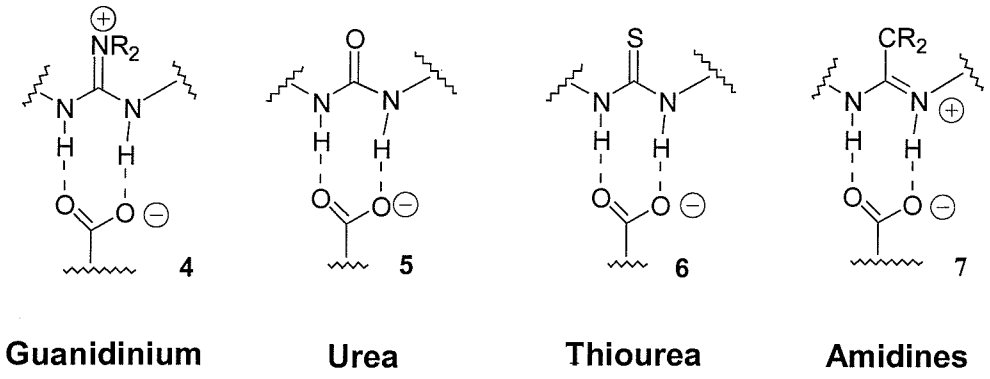
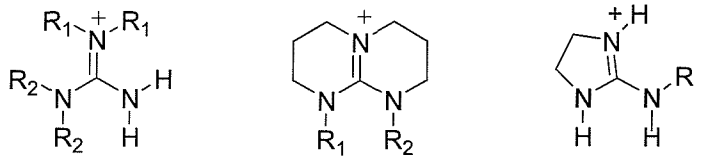


Figure 9: Different kind of carboxylate acid binding site

The guanidinium group presents several interesting features: it binds carboxylate salts with a bidentate hydrogen pattern and complementary electrostatic interaction, it has a very high pK_a (13.5) and is therefore able to bind carboxylates over a considerable pH range.
Lehn was among the first to investigate the use of the guanidinium group in the complexation of carboxylates by synthesising a series of structurally different guanidiniums and measuring their association constant with carboxylate salts in aqueous system *via* pH-metric titration experiments.[33]

A more recent study by Hamilton used isothermal titration calorimetry to study the association between a series of guanidinium derivatives **8-15**, as borate salts, with tetrabutylammonium (TBA) acetate in DMSO.[34] Sequential removal of hydrogen bonding sites results in a significant fall in the binding constants in the acyclic system **8-10** (K_a = 7.9 x 10^3 M^{-1}, K_a = 3.4 x 10^3 M^{-1}, and K_a = 110 M^{-1}, respectively). Similar bycyclic guanidinium **11** bound TBA-acetate strongly, (K_a = 5.6 x 10^3 M^{-1}) but no binding was observed for the corresponding methylated compounds **12** and **13**. Monocyclic guanidiniums **14** and **15** also display high affinities for acetate, particulary receptor **15**, as its iodide salt (K_a = 7.2 x 10^3 M^{-1}) in DMSO, as determined by isothermal titration calorimetry.[35]



8 $R_1 = R_2 = H$
9 R_1 = Me, $R_2 = H$
10 $R_1 = R_2$ = Me

11 $R_1 = R_2 = H$
12 R_1 = Me, $R_2 = H$
13 $R_1 = R_2$ = Me

14 R = H
15 R = Bn

Figure 10: Series of guanidinium derivatives

Schmidtchen first reported the use of a bicyclic guanidinium in the formation of host-guest complexes with simple carboxylates.[36] The use of bicyclic guanidinium presents all the advantages of a guanidinium and the advantage that forming a bicyclododecane framework becomes an almost perfect match for carboxylate anions with the two guanidinium protons aligned in the same direction. The most rigid conformation could increase the selectivity of the receptor to the substrate. An example of a receptor, which contain bicyclic guanidinium, that binds to a carboxylate was synthesised by de Mendoza (Figure 11).[37] p-Nitrobenzoate, in chloroform, was bound inside the (*S*,*S*)-receptor **16** by hydrogen bonding to the carboxylate and π-π-stacking between the aromatic rings (K_a = 1.6 x 10^3 M^{-1}).

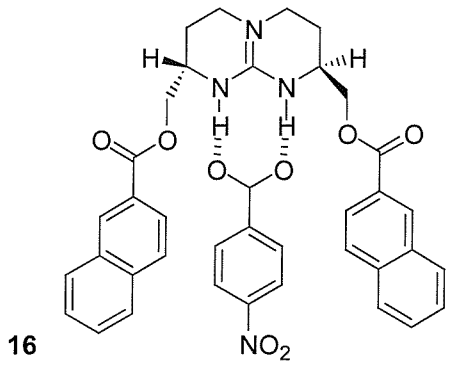


*Figure 11: de Mendoza's bicyclic guanidinium receptor **16***

By modifying the initial receptor, de Mendoza synthesised another receptor featuring three different recognition groups allowing enantioselective recognition of amino acids: hydrogen bonding between the carboxylate and guanidinium moiety, π-π-stacking between the aromatic rings, and electrostatic interaction between the ammonium ion and the aza-crown ether. Figure 12 shows the proposed structure for a 1:1 complex between receptor **17** and L-Trp.[38] 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. The extraction efficiencies (i.e. fraction of receptor molecules occupied by substrate) in the organic phase, determined by NMR integration, were *ca.* 40%. When the same experiment was carried out with the (*R*,*R*) enantiomer of de Mendoza's receptor **17** achieved the extraction of D-Phe and D-Trp but not their corresponding L-enantiomers.

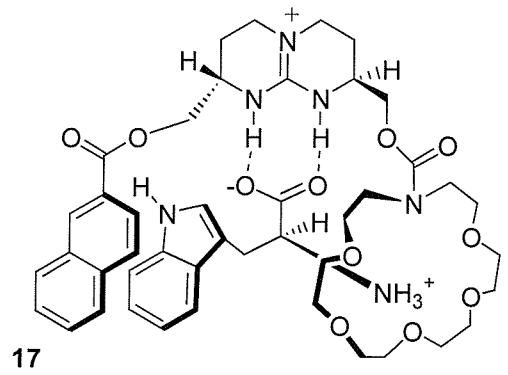
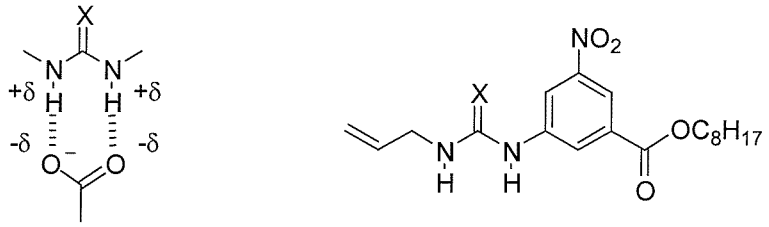


*Figure 12: de Mendoza's Receptor **17** binding L-tryptophan*

A similar binding pattern operates for carboxylate are ureas **5** and thioureas **6** but lacks the electrostatic complementarity. They have been shown to provide a strong binding site for carboxylate, using, as in the guanidinium case, a bidentate bonding motif. Wilcox was the first to utilise ureas and thioureas such as **18** and **19** in carboxylate binding.[39] Urea **20** was found to bind benzoate with an association constant of 2.7 x 10^4 M^{-1} in chloroform. Large shifts of the N-H protons were also observed, indicating strong hydrogen-bonding between the urea hydrogens and carboxylate oxygens. Further gains in the binding energy were achieved by increasing the acidity of the hydrogen-bonding donor sites by replacing the urea for a thiourea. Thiourea (pK_a = 21.0) is more acidic than urea (pK_a = 26.9), and therefore 1,3-dimethylthiourea complex **21** gave nearly a ten fold increase in stability over **20** with an association constant of 340 M^{-1}.



18 X = O, **19** X = S

20 X = O, **21** X = S

*Figure 13: Wilcox's urea/thiourea carboxylate receptors **20** and **21***

Kilburn has described recently the enantioselective binding of *N*-protected amino acids by a pyridyl thiourea receptor.[40] Receptor **22** was titrated with a range of amino acid carboxylates (TBA salts) and exhibited some selective particularly for amino acids with electron rich aromatic side chain e.g., *N*-Ac-D-Trp (K_a = 1.5 x 10^4 M^{-1}) in chloroform.

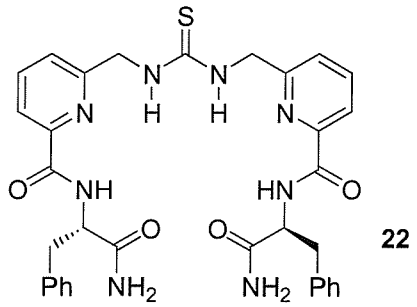
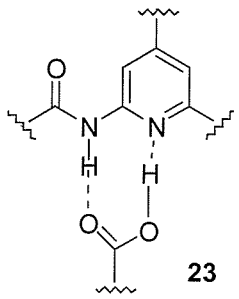


Figure 14: Kilburn's receptor contain a thiourea

Another unit which bounds carboxylates using bindentate hydrogen bonding is amidine **7**. The amidinium moiety provides a similar binding motif for carboxylate to that of the guanidinium with a pair of hydrogen bonds and a complementary electrostatic interaction. The reduced pK_a of the amidines compared to the guanidinium leads to a less potent binding site but this is compensated for by the relative ease of synthesis of the amidinium systems.

1.5.1.2 Receptors for Carboxylic Acids

A different binding pattern for carboxylic acid is 2,6-diamino pyridine units **23** (figure 15). Amidopyridines provide an excellent structural motif for binding carboxylic acids with the ability to form two complementary hydrogen-bonds from the carboxylic acid hydrogen to the pyridine nitrogen and the carboxylic acid carbonyl to the amide hydrogen.



2,6-diamidopyridine

Figure 15: The diamidopyridine unit

Hamilton was the first to use the motif to bind biscarboxylic acid derivatives when the amidopyridine units has been incorporated in the macrocycle **24** (figure 16).[41,42] Macrocycle **24** bound ethylmalonic acid (K_a = 7.3 x 10^3 M^{-1}) and diethylmalonic acid (K_a = 1.1 x 10^3 M^{-1}) in chloroform. An important aspect of this early work was the observation, by X-ray crystallographic analysis, that complexation does not involve proton transfer from the carboxylic acid to the pyridyl nitrogen.[41]

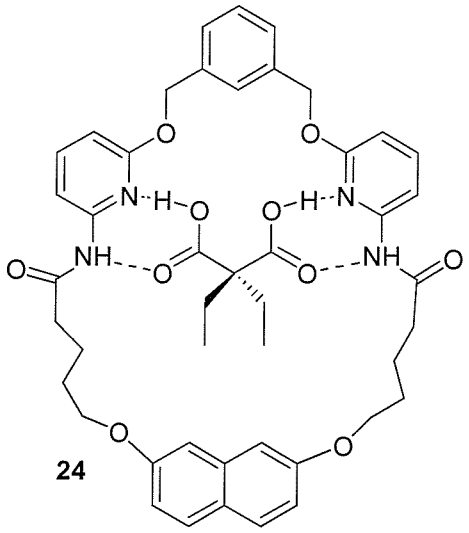


Figure 16: Hamilton's Macrocycle with amidopyridine units

Hamilton incorporated two amidopyridine units also in a structurally simpler series of acyclic receptors **25** and **26**. Receptor **25** has been shown to stabilise the s-cis rotamer of proline diacid **27** forming the complex shows in figure 17. The receptor **26** has been shown to stabilise the s-trans rotamer of prolin diacid **28** forming the complex illustrated in figure 17.[43]

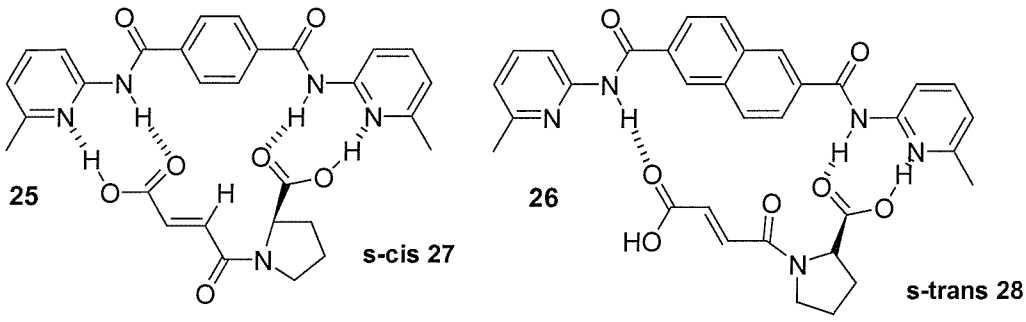


*Figure 17: Complexes between receptor **25** and **26** and substrate **27** and **28***

Helmchen has extended the two fold parallel hydrogen-bonds by incorporating an additional hydrogen-bond to the carbonyl oxygen *syn* lone pair in molecular clefts of type **29** (figure 18).[44] A series of sterically similar, but electronically different hosts were prepared and the interaction of R^1 and R^2 examined. The hosts were tested for their ability to induce chemical shifts of enantiotopic protons with various carboxylic acids as their guests by adding one equivalent of the reagent to a solution of carboxylic acid in chloroform. When R^1 = Ph or 1-naphthyl, carboxylic acid guests containing aromatic ring systems such as naproxen, phenylacetic acid and hydratropic acid **30** were found to cause the chemical shift of H^1 to alter in the range $\Delta\delta$ = 0.279-0.283 ppm. The fact that host **29** did not induce measurable changes of H^1 in any of the substrates when R^1 = cyclohexyl indicated π-π stacking interactions were occurring. Receptor **29** was also found to bind the *S*-enantiomer of hydratropic acid **30** (K_a = 1100 M^{-1}) with a stronger association constant than that observed for the *R*-enantiomer (K_a = 700 M^{-1}).

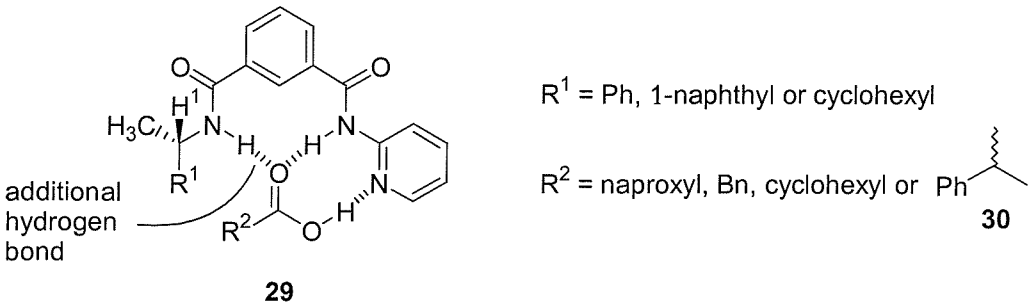
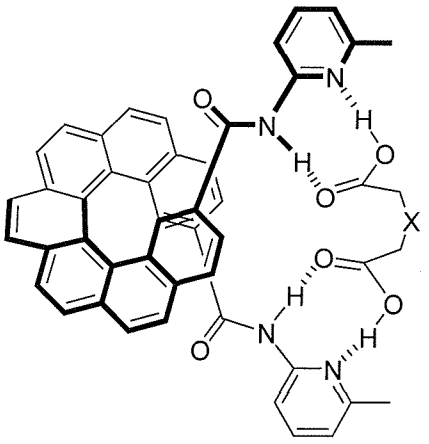


Figure 18: Helmchen's receptors for carboxylic acids

Diederich synthesised helicopodand **31** (figure 19), incorporating two pyridinecarboxamides as the dicarboxylic acid binding site.[45] In the productive 'in-in' conformation, **31** forms stable 1:1 complexes with α,ω–dicarboxylic acids in chloroform and a diasteroselectivity of complexation of $\Delta(\Delta G^{o}) = 5.8$ kJ mol^{-1} was measured for **32** and **33**, which differ only in the configuration at their double bond. Molecular modelling suggested only the *E* derivative **32** possessed the correct geometry to give four-fold hydrogen-bonding interaction between its two carboxylic acid residues and the two CONH(py) groups in **31**.



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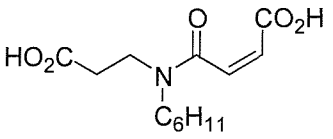

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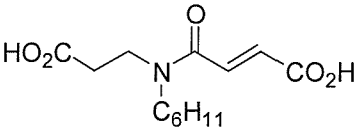
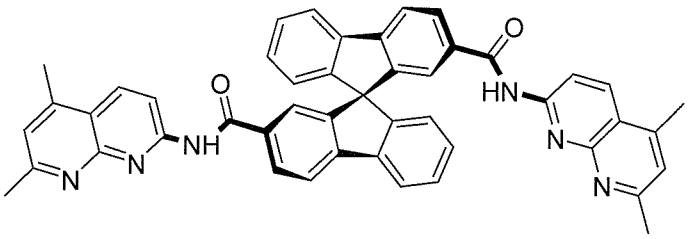


33

*Figure 19: Diederich's helicopodand receptor **31** for diacids*

Diederich also prepared optically active molecular cleft **34** and **35**, which incorporated a spirobi[fluorene] spacer and two carboxamide moieties as hydrogen-bonding sites (figure 20).[46] Solution binding studies in chloroform showed **34** and **35** were able to form stable 1 : 1 host-guest complex with *N*-protected Glu and Asp. The major intermolecular interaction in these associations is the hydrogen-bonding between the two COOH residues of the substrate and the two heterocyclic carboxamides of the receptors. The enantiomeric receptors selectively recognized *N*-Cbz-L-Asp and *N*-Cbz-*L*-Glu. NMR spectroscopic binding titrations showed the differences in stability between the two diastereoisomeric complexes, with $\Delta(\Delta G^{o}) = 4.0$kJ mol^{-1}. In particular, *N*-Cbz-L-Glu binds preferentially to the (S)-configured receptors while *N*-Cbz-L-Asp prefers association to the (R)-configured molecular clefts. Changing the hydrogen-bonding sites from naphthyridinecarboxamide in

34 to pyridinecarboxamide in **35** did not significantly change the free energy and enantioselectivity of complexation. This initially surprising observation was explained in consideration of two opposite contribution effects in the selective recognition. First of all naphthyridine *N*-atoms are weaker hydrogen-bond acceptors than pyridine *N*-atoms as a consequence of the fact that the pK_a value is 1.84 lower for naphthyridine. In contrast, binding to **34** should be strengthened as a result of a more favourable DAA/AD (*i.e.* donor-acceptor-acceptor/acceptor-donor) hydrogen-bonding pattern. This differs to the DA/AD hydrogen-bonding pattern in **35,** and should thus enable the formation of a bifurcated hydrogen-bond between the naphthyridine donor site and the carboxylic acid protons.



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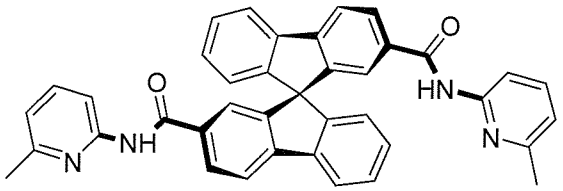
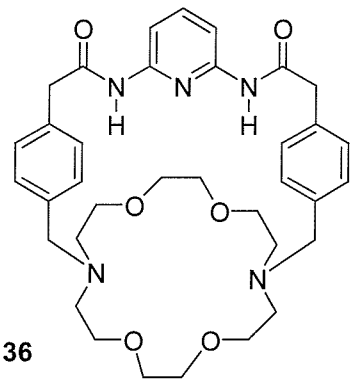


35

*Figure 20: Diederich's spirobi [fluorene] based receptors **34** and **35***

Kilburn has synthesised a number of complex macrocyclic architectures incorporating the 2,6-diamidopyridine unit. For example a simple receptor **36** (figure 21), has been prepared thus features a bis(amidopyridine) unit to serve as a binding site for carboxylic acid functionality and a diazacrown ether. This receptor was found to bind the monopotassium salts of various dicarboxylic acids in chloroform solution, using a combination of hydrogen bonding interactions and an electrostatic association between a carboxylate anion and a crown ether bound potassium cation.[47]



36

*Figure 21: Kilburn's receptor **36** containing a diamidopyridine unit*

1.5.2 Binding of Peptides

Perhaps the most intriguing challenge in molecular recognition is the creation of synthetic molecules that have properties similar to those of such remarkable biological substance as antibodies or enzymes. One very important class of substrates is the oligomers of α-amino acids. The efficient preparation of peptides with a completely or partially "non natural" configuration is a particularly attractive research area in medicinal chemistry because the enormous diversity of biological activity of many oligopeptides. While biological receptors manage to distinguish them by differences in their amino acid sequences, analogous selectivity is difficult to obtain with synthetic receptors.[48] One reason is that peptides are physically large molecules and the receptors which would bind them would bind them would need binding sites of comparable dimensions.[49]

1.5.2.1 A natural Peptide Receptor

The most classical example of a natural peptide receptor is vancomycin. Vancomycin is a clinically important antibiotic, against *Staphilococcus aureas* which are resistant not only penicillin, but also a number of other classical antibiotics.[50] Vancomycin disrupts bacterial cell wall biosynthesis by binding to the terminal L-Lys-D-Ala-D-Ala sequence of one of the peptidoglycan precursors. The active complex involves six hydrogen bonds between vancomycin and the dipeptide substrate. In addition, non-bonded interactions between the alanine methyl groups and hydrophobic regions of the antibiotic may account for its strong

substrate- and stereospecificity. Five of the six hydrogen bonds are found near the right-hand ring of the antibiotic, and they form a binding pocket for the carboxylate of the terminal D-alanine.[51]

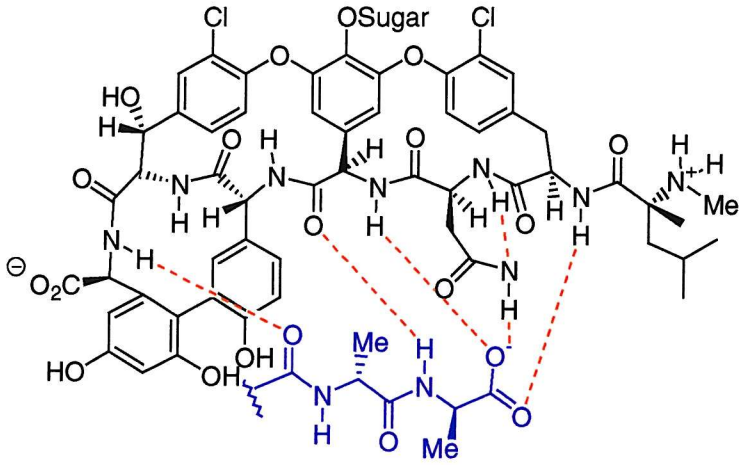


Figure 22: Vancomycin complex with D-Ala-D-Ala

The development of synthetic receptors for L-Lys-D-Ala-D-Ala, as a bacterial cell wall precursor, represents a desirable goal because a selective receptor for this peptide sequence might lead to mimics for the vancomycin family of antibiotics.

1.5.2.2 Synthetic Peptide Receptors

Still has describes one example of a large synthetic receptor which has only minimal structural complexity and yet has binding selectivities approaching those of biological receptors. This receptor **37** is an A_4B_6 cyclooligomer of trimesic acid (**A**) and (*R*,*R*)-diaminocyclohexane (**B**) (figure 23).[52]

The design of the receptor aimed for minimal receptor flexibility by using fragments having few opportunities for conformational isomerism and by joining them with planar amide bonds. Among the ways in which **A** and **B** can be combined, the structure is appealing because of its well-defined binding cavity and appropriately positioned hydrogen-bonding groups. ^{1}H NMR spectroscopic titrations in chloroform showed that Still's receptor binds amino acid residues in peptide chain with very high selectivity. In

particular, it was found to bind L-amino acids enantioselectively (70-90% ee) and to select for amino acid sidechains having a particular size (*e.g.* phenyl>>benzyl, ethyl>>methyl). It was also observed a very strong binding to a certain tripeptide (*N*-Boc-Gly-(L)Val-Gly-NHBn) which presumably involved additional hydrogen bonding to the outlying amides which are part of the **B** fragments of the receptor.

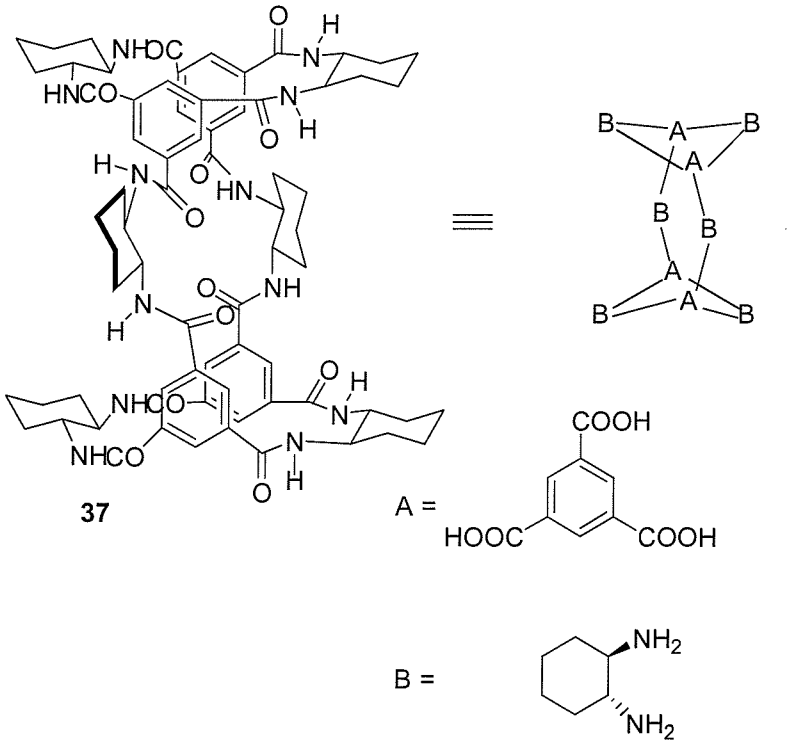


Figure 23: An example of Still's A_4B_6 Receptors

Still reported also a series of chiral C_3-symmetric receptors having only limited conformational flexibility and deep basket-like binding sites (figure 24).[53] The receptors bind Boc-protected *N*-methylamides of α-amino acids with high selectivity displaying a preference for the L-isomer. In particular serine and threonine were found to be preferentially bound instead of amino acids such as alanine, valine and leucine. The key features of those receptors are a deep binding cavity with appropriately-positioned hydrogen bond donor/acceptor functionalities and conformational stability. NMR spectroscopic and molecular modelling studies suggested such a binding mode for the Boc,

L-amino acid *N*-methylamides which were tightly bound by the receptor *via* three hydrogen bonds such that the methylamide terminus projected into the cavity.[54]

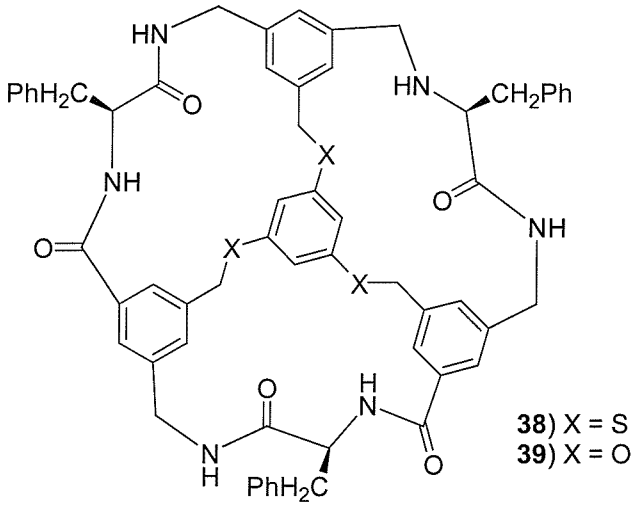
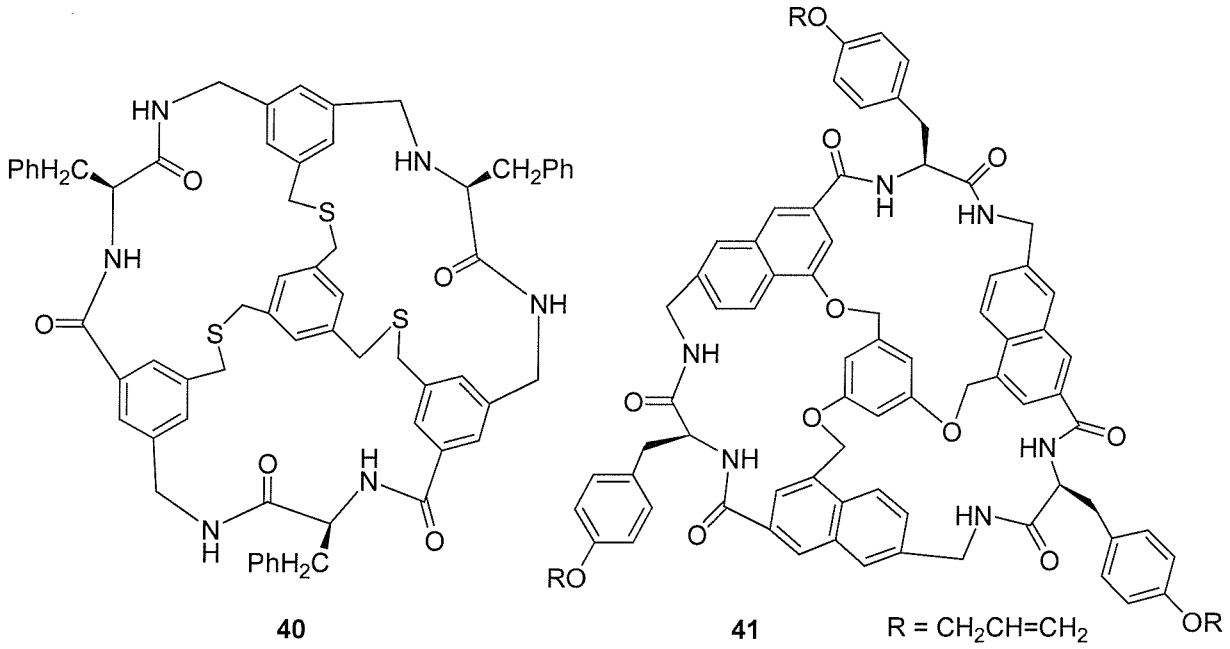


Figure 24: Still's C_3-Symmetric receptors

Further modification to the structure **38** involved the incorporation of additional methylene groups, between the sulfur atoms and the benzene ring, built into the apolar 'bottom' of its binding cavity. These modifications should increase the flexibility of the receptor and allow its binding cavity to assume a variety of forms having differing depths and shapes. The new receptor **40** bound substrates with significantly less enantioselectivity than the original host **38**, supporting the proposal that binding selectivity can be highly sensitive to the conformational mobility of the receptor. Thus, while flexible receptors may adapt their structures to fit a guest, there is a price in binding energy as well as in selectivity which must be paid.[55]

Later modifications to the original structure involved replacing the benzene rings with naphthalene rings and employing oxygen atoms instead of sulfur atoms (figure 25).[56] The receptor **41** shows an analogous capability to distinguish closely related peptidic substrates in organic solvents based on stereochemistry and, in some cases, on subtle differences in residue size. It was also shown that **41** not only exhibits high selectivity for binding tripeptides containing an internal L-proline (>99% diastereomeric excess (de) for L-Pro *vs* D-Pro) but also bind L-Pro more tightly than cyclic analogs which are either smaller or

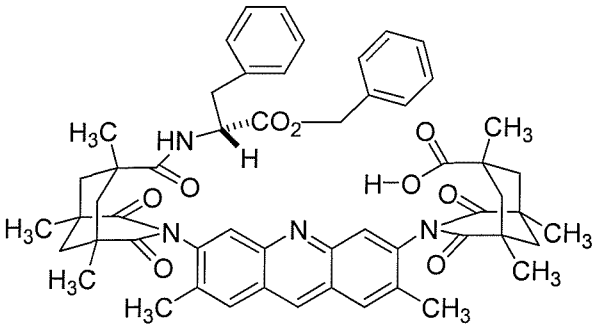
larger than Pro itself. Furthermore, **41** stereoselectively binds substrates having L-amino acids adjacent to L-Pro (90-99% de for L-Ala) and with binding constants of $K_a = 2.5 \times 10^5\ M^{-1}$ for *i*PrCO-L-Ala-L-Pro-L-Ala.



40 **41** R = $CH_2CH{=}CH_2$

*Figure 25: Still's C_3-Symmetric receptor **40**. Still's Naphthalene based C_3-Symmetric Receptor **41***

Rebek has also described a series of receptors for amino acid and peptides.[57] The unique structure of the molecules arise from the use of Kemp's triacid, where the three carboxyl groups are constrained to a triaxial conformation giving a U-shaped relationship between any two of them and so creating chiral cavities (figure 26). Receptor **42** features three different domains which are represented by the carboxylic acid, the acridine π-π-stacking surface and the bulky phenylalanine derivatives and those converge to create a chiral cavity.[58] The receptor recognised derivatives of phenylalanine, although no enantiomeric discrimination occurred with phenylalanine methyl ester as the guest. Modification of receptor **42** to include a 2,6-disubstituted naphthalene as the spacer unit provided receptor **43**. NMR spectroscopic titrations showed that receptor **43** was able to complex *cyclo*-L-Leu-Gly and *cyclo*-L-Leu-L-Leu with binding constants of $73 \times 10^3\ M^{-1}$ and $82 \times 10^3\ M^{-1}$, respectively.[59]



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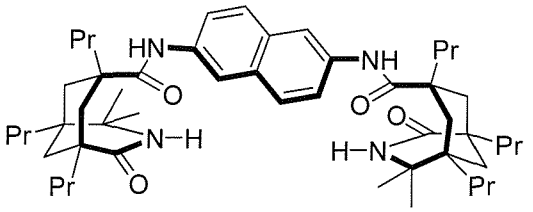
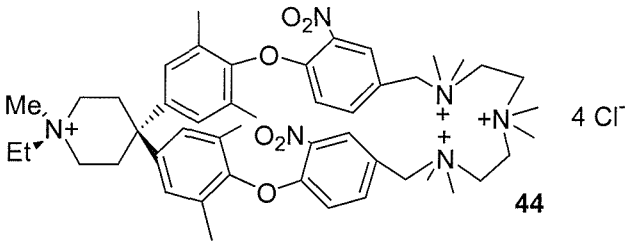


43

*Figure 26: Rebek's Acridine Receptor **42**. Rebek's Naphthalene Receptor **43***

Diederich described the synthesis and properties of the polyammonium cyclophane **44** (figure 27) which was designed for the complexation of aromatic and aliphatic carboxylates as well as *N*-protected α-amino acids and small peptides in water. The potential carboxylate-binding site in the receptor is formed by three adjacent quaternary ammonium ions. In analogy with vancomycin, the carboxylate-binding sites in the receptor are preorganized through their incorporation into a cyclophane structure. However, titration binding experiments indicated that it did not provide a cavity for inclusion of the guests and instead binding occurs exclusively on the external surface.[60]



44

Figure 27: Diederich's receptor

Breslow has used cyclodextrin dimers to bind peptide side chains in water with sequence selectivity.[61] A series of different receptors as dimers of β-cyclodextrin have been made and their binding with different peptides and with dipeptides Phe-Phe and Trp-Trp was examined. The receptors produced showed cooperative chelate binding to several peptides. All the binding studies were performed by titration microcalorimetry and showed the

largest association constant between the two guests shown in figure 28, giving values of 2590 M^{-1} and 1100 M^{-1} for the cyclic peptide **45** and linear peptide **46**, respectively. Those receptors are the first example in which the double binding of hydrophobic side chain is used to chelate a receptor to a peptide.

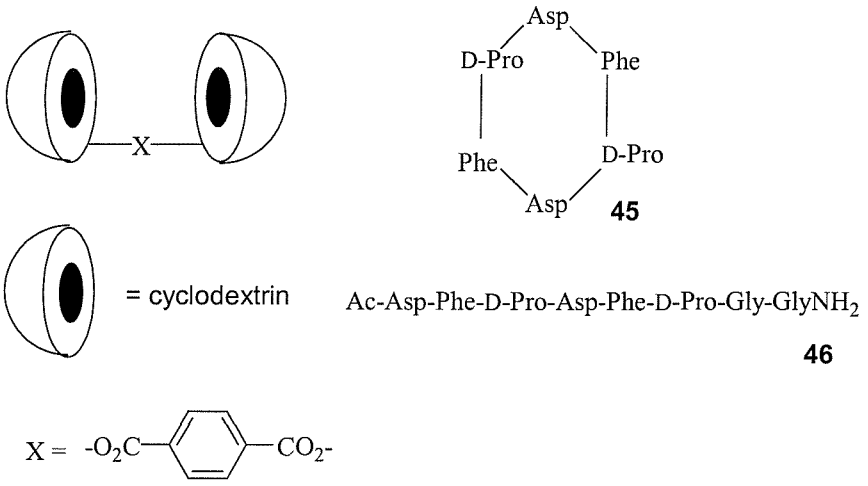


Figure 28: Breslow's Cyclodextrin Receptor and Guests

Hossain and Schneider described the incorporation of multi functionality in the synthesis of receptors **47** and **48** for peptides in water.[62] The receptor was designed to give a double complementarity between the 18-crown-6-unit with the N-terminus of a tripeptide and at the same time between a peralkylammonium group with the C-terminus of a tripeptide. In addition, secondary interactions along the peptide chain would provide sequence selectivity (figure 29). NMR spectroscopic titrations in water ($K_a \sim 200\ M^{-1}$) or methanol ($K_a \sim 10^4\ M^{-1}$) demonstrated binding of this system.

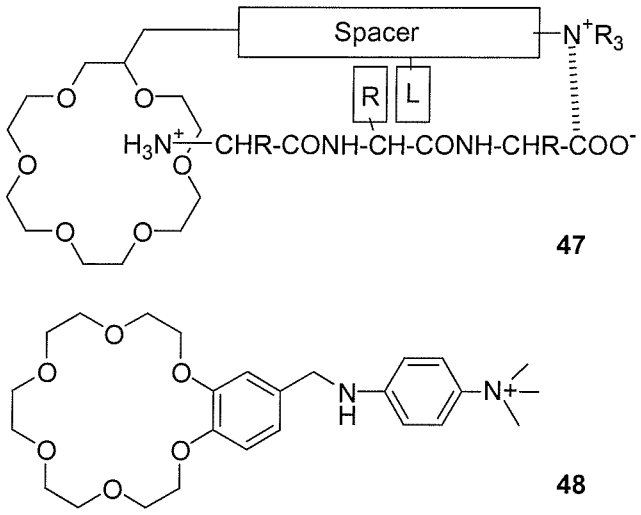


*Figure 29: The design concept for Hossain and Schneider's receptor **47**, Hossain and schneider's receptor **48***

Schneider described a porphyrin-crown ether derivatives **49** which exhibit affinities to unprotected peptides in aqueous solution. The incorporation of a crown ether unit into the porphyrin framework provided a binding site for primary ammonium moieties, thus facilitating the complexation of unprotected di-and tri-peptides (figure 30).[63] UV-visible spectroscopic titration showed that the binding constant increases as a function of the peptide length and with the number of aromatic units in the amino acid site chains, as expected from π-π-stacking contributions.

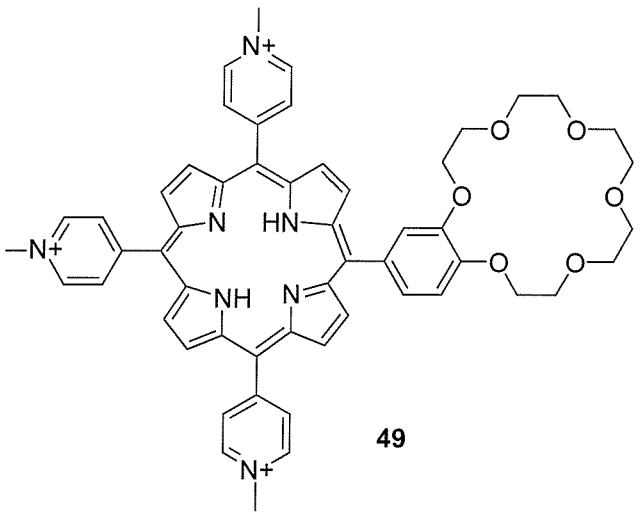
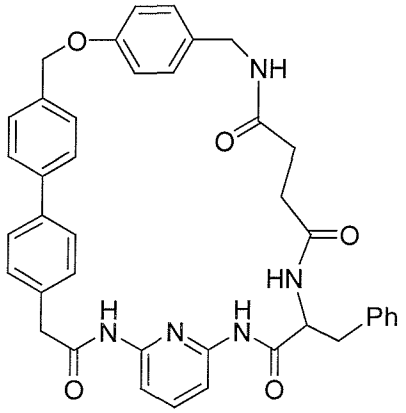


Figure 30: Schneider's Porphyrin Receptor

A series of macrocyclic receptors **50, 51** incorporating an amidopyridine moiety have also been synthesised by Kilburn for the purpose of binding small peptides and amino acids derivatives (figure 31).[64, 65] The hosts feature a binding site for a carboxylate or carboxylic acid terminus of peptide, amide functionality to provide potential hydrogen bonding sites and a rigid spacer unit to maintain the open conformation of the macrocycle. Binding studies, with the racemic mixture of receptors, indicate that **50** was able to bind simple peptides with some selectivity. NMR spectroscopic titration experiments in chloroform showed that the receptor complexes several *N*-benzyloxycarbonyl amino acids with values of K_a ranging from 308 to 588 M^{-1}.[64] *N*-benzyloxycarbonyl β-alanyl-L-alanine was the best substrate with an estimated binding constant of K_a = 2515 $M^{-1}$and K_a = 691 M^{-1}, respectively for the two diastereomeric complex formed, representing a binding enantioselectivity of ~ 80 :20.

Macrocycle **51** has been synthesised by linking together a diamidopyridine with suitable amino acids, followed by a double intermolecular cyclisation of a suitable activated precursor. Macrobicycle **51** features a diamidopyridine unit at the base of an open, bowl-shaped cavity. Incorporation of additional amide functionality around the rim of the bowl-shaped structure provides further hydrogen bonding sites to interact with peptidic guests. The binding properties of **51** have been investigated by NMR spectroscopic titration experiments, which revealed that **51** is a strong and selective receptor for peptides with a carboxylic acid terminus in chloroform; the strongest interaction observed was with Cbz-β-Ala-D-Ala which was bound with a binding constant of $K_a = 12.2 \times 10^3$ M^{-1}.[66]



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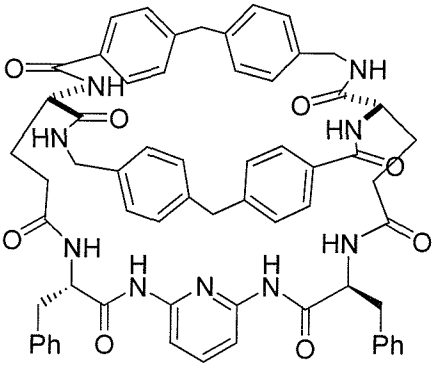
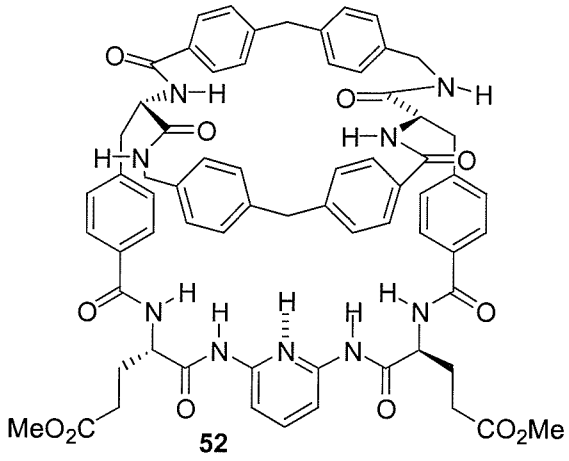


51

*Figure 31: Kilburn's receptor **50** and **51** containing a diamidopyridine unit*

A further modification was made to the diamidopyridine receptor to reduce its flexibility and produce a more preorganized host **52** (figure 32).[67] The new bowl-shaped macrobicyclic receptor was found to be a particularly strong and selective receptor for the dipeptide Cbz-L-Ala-L-AlaOH with a very high association constant of 33 x 10^3 M^{-1}. A result such as this is of particular interest due to the relevance to binding L-Lys-D-Ala-D-AlaOH, the bacterial cell wall precursor peptide.



52

Figure 32: Kilburn's Macrobicyclic receptor ***52***

1.5.2.3. Tweezer Receptors

Tweezer receptors are an important class of receptors for small peptides and simple amino acids. The basic design of tweezer receptors for peptides incorporates a "head" group or "hinge" bearing two side arms that incorporate appropriate functionality for binding with the backbone of suitable peptide substrate (figure 33).[68,69] Incorporation of a head group with a specific recognition site or "anchorpoint", for the C-terminus of the peptide guest, in addition to binding interactions from the tweezer side arms should increase the binding affinity of such receptors with suitable substrates and should ultimately lead to receptors for the C-terminal sequence of larger peptides. These kinds of receptor, despite their inherent flexibility, have proved to be highly selective for certain peptide sequences in both non-polar [70] and aqueous solvent systems.[71] The important point in the synthesis of tweezer receptors for peptides is that they allowed the use of combinatorial chemistry to

afford a large number of compounds in a short time. These will be discussed in details in section **1.7** after a brief introduction to combinatorial chemistry.

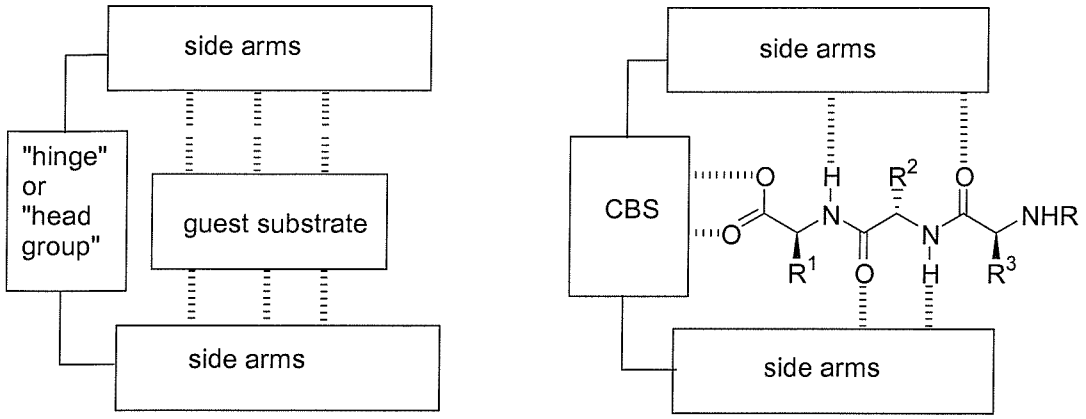


Figure 33: Design of tweezer receptors

1.6 Combinatorial Chemistry

Combinatorial Chemistry allows the synthesis of a large numbers of products at the same time by combining a set or sets of chemical building blocks (or monomers) in a few synthetic steps. The term has been used to describe the synthesis of many compounds by running reactions in parallel, as well as preparation of compound mixtures in the same reaction vessel. The new approach is in contrast to the traditional activity of a synthetic chemist, where the reactions are performed to obtain a single well-defined products. The ability of combinatorial synthesis to produce large collections (or libraries) of molecules gives an efficient source of molecular diversity, which in theory should speed up the process of discovery of potential leads.[72]

1.6.1. Synthesis of Peptide Libraries the "Split-and-Mix" Approach

Furka and coworkers were the first to describe the "portion-mixing" methodology in peptide synthesis.[73,74] The utility of this strategy has been further investigated by Houghten[75] and Lam[76] in their respective "divide-couple-recombine" and "split and mix" process. This simple procedure involves dividing the resin into equal fractions, coupling a

single monomer to each aliquot in a separate reaction and then thoroughly mixing all the resin particles together. Repeating this protocol for a total of x cycles with n monomers for each cycle allows a library of up to n^x adducts to be formed. Figure 34 provides an excellent explanation of this methodology. If we consider a three-step split synthesis using three building blocks (A, B, C) in each step, then it would be possible to generate 27 trimer combinations. Hence this combinatorial process is highly efficient as large libraries of compounds can be formed from relatively few reactive monomers units.[77]

The most important characteristic of a "split and mix" synthesis is that although the resin has the potential to contain many different compounds, only one product can ever be attached to a single bead. This is known as the "one bead one compound" principle.[78] This principle also provides a convinient way of manipulating the individual components within the process as there is only ever one reactive site avaliable when the monomer units are introduced, due to the fact that the products are linked to the solid support.

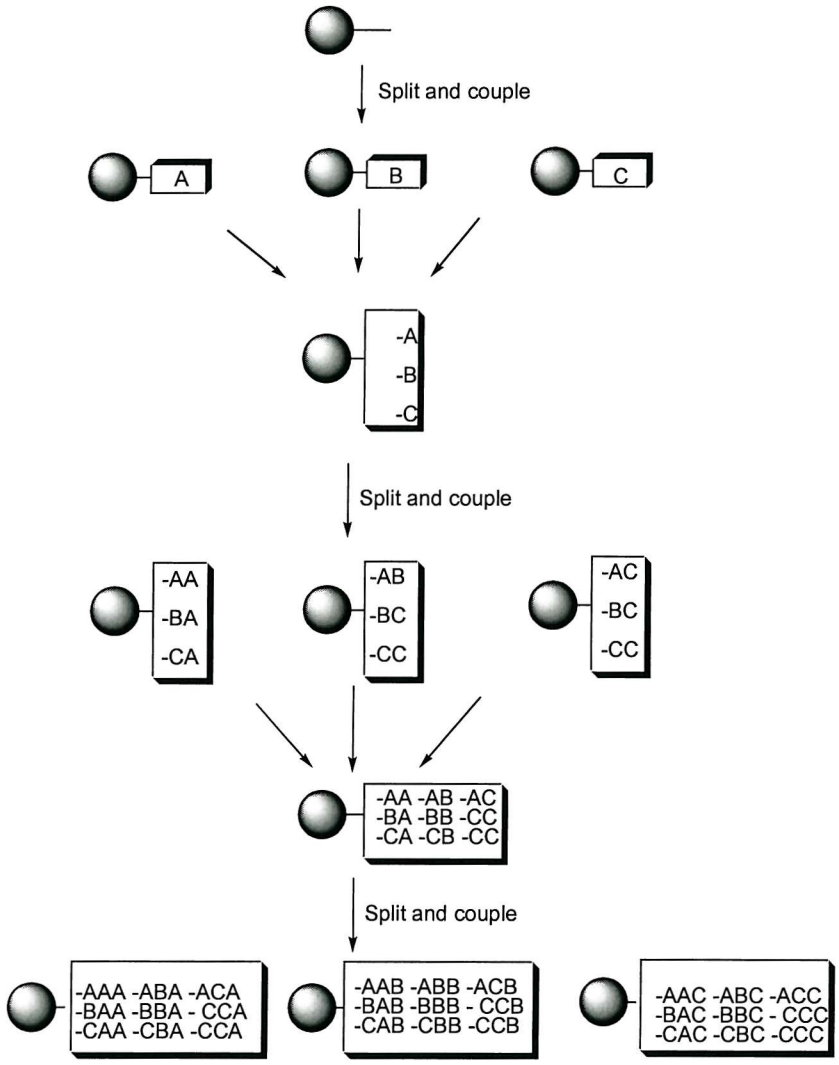


Figure 34: Preparation of a combinatorial library by "split and mix" method

1.6.2. Screening

As previously explained, individual beads from a "split and mix" synthesis bear single compounds, and it requires the use of sensitive analytical methods to directly determine the chemical structures of molecules isolated from just one resin bead. This was first demonstrated for peptide libraries prepared from naturally occurring L-amino acids,[79] where Edman degradation provides the sequence information from less than 10 pmols of material. These libraries have been screened using soluble receptor molecules "on bead", and in the mean time the ligand present in solution interacts with the receptor attached to

the resin, and the selective recognition is detected via a colorimetric or fluorogenic assay.[80, 81] In this way individual positive beads are selected and subjected to Edman peptide sequencing to give the structure of the active compound.

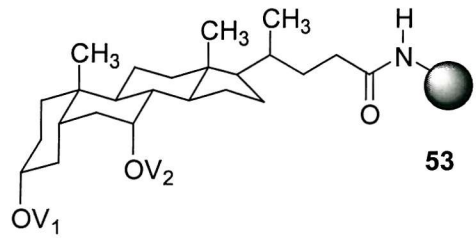
Another general approach to chemical structure determination for libraries exploits a variant of the split synthesis termed encoded combinatorial synthesis. In this strategy, the chemical history of any resin particle is recorded (i.e. encoded) through addition of a series of surrogate analytes, or identifier tags, which can detected with either greater ease or sensitivity than the chemical building blocks or reagents. This tagging method, devised by Still,[78] has already been used successfully by the Gennari group.[82]

1.7 Combinatorial Approach in the Synthesis of Libraries of Receptors

Solid phase and combinatorial chemistry are widely used for the generation of libraries of compounds which can be subjected to high-throughput screening for lead-finding and lead-optimisation. However, most of the libraries of receptors have been screened against combinatorial libraries of resin-bound peptides and have allowed a rapid evaluation of the binding properties of the receptor.[71,80,81]

In 1994, Still reported the first library of receptors that was synthesised using combinatorial synthesis.[83] The receptors are simple peptidosteroid which consist of A,B-*cis*-steroidal cores and peptidic appendages which were synthesised with Glu fixed in the first position of the sequence. The two other positions were randomised with 10 different amino acids to give 10^4 receptors **53** (figure 35). The library's only preorganization comes from the rigid nucleus cholic acid, which serves as a scaffold that carries functionality for appending diversity and connecting to the solid support. To test the receptor library for receptor substrate binding and sequence selectivity, four closely related opioid peptides (enkephalins) **54-57** as substrates were chosen, and screened against library **53** in chloroform. Analysis of active beads from screening experiments against guest **54** to **57** showed significant preferences for certain amino acids at each of the receptor's four AAn

sites. Moreover, for peptide red-**56** and red-**57** proline was by far the dominant amino acids in position AA_2 and AA_3 with 60% and 90%, occupancy respectively. To verify the selectivity of the receptor they developed a related screening experiment with different dye-labels (*"two-colour assay"*). The idea is to attach differently coloured dyes to substrates to be distinguished and then to screen for library beads that pick up only one colour. The receptor library was treated with a mixture of red-**55** and blue-**56** and they found a variety of purplish to reddish-purple beads and as well as a few very blue beads. Upon decoding these blue beads, they found mainly appendage sequences for **53** which were closely related to the consensus sequences found with the single colour red-**56** including AA_1-AA_4 = PheProProLeu (**1FPPL**) and AspProProVal (**1DPPV**). To prove that receptors from the blue beads were actually selective for **56**, both receptors were resynthesised and using HPLC, the relative binding of **55** and **56** were measured. Was found that both receptors bind red-**56** more tightly than red-**55** and the selectivity ($\Delta G_{4\text{-}3}$) was –4.2 and –6.7 kJ mol^{-1} with **1FPPL** and **1DPPV** respectively.



53

54 dye-CO$(CH_2)_3$COGly-Gly-(L)Phe-(L)Leu

55 dye-CO$(CH_2)_3$CO-(L)Tyr-(D)Ala-Gly-(L)Phe-(L)Leu

56 dye-CO$(CH_2)_3$CO-(L)Tyr-Gly-Gly-(L)Phe-(L)Leu

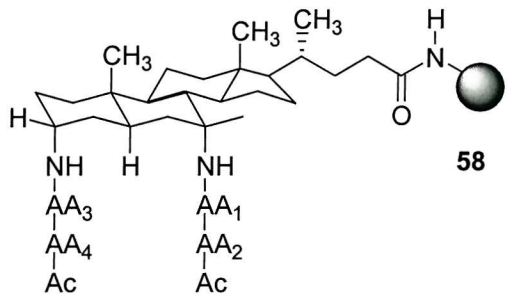
57 dye-CO$(CH_2)_3$CO-(L)Tyr-Gly-Gly-(L)Phe-(L)Met

Figure 35: Library of receptors based on A,B-cis-steroidal cores

In 1996, Still's group reported a second example of a library of receptors.[84] They described a related receptor library **58** with 10^4 components synthesised around an A,B-*trans*-steroidal core that is axially 3,7-disubstituted with conformationally less flexible dipeptidic arms compared to the cholic acid scaffold described in the previous example (figure 36). In previous screening experiments the library of the *cis*-steroid **53** (figure 36) showed that 1% of all the different receptors bound pentapetide **56**. In this case screening experiments showed that a much smaller fraction (0.1%) of this library binds N-acylated Leu enkephalin methyl ester **59** and every one of these has the same AA1-AA3: (L)Asn, (D)Asn, and (D)Phe, respectively. By resynthesising one of these enkephalin-binding receptor, they found that it binds Leu enkephalin derivatives with binding energies that diminish by 4.2-12.6 kJ mol^{-1} when many single-residue modifications to the Leu enkaphalin substrate are made.

Conformational analysis showed that the structure was stabilized by two strong interchain hydrogen bonds that create a compact, highly ordered core. The remaining hydrogen bond donor and acceptors were apparently unassociated and available for hydrogen-bonding to peptide guests in organic solvents. The examples demonstrate the power of this approach which relies more on generation of diversity and screening than rational and deterministic design of receptors. These receptors are clearly able to make some remarkable distinctions

between closely related oligopeptides which differ in only one amino acid. Furthermore library **58** based on more rigid *trans*-steroid showed higher selectivity than library **53** based on more flexible *cis*-steroid.



58

59

dye-CO(CH_2)$_3$CO-(L)Tyr-Gly-Gly-(L)Phe-(L)Leu-OMe

Figure 36: Library of receptors based on A,B-trans-steroidal cores

Liskamp's synthesised peptidosulfonamide "tweezer" receptor **60** and **61**. Those "tweezerlike" synthetic receptors consist of three different parts: a hinge to which the tweezer arms are attached; a dye or a solid bead attached to this hinge, and two arms, which consist of peptidosulfonamide peptidomimetics. Those receptors feature a different hinges into the structure to vary the flexibility and interchain distance, between the amino nitrogen atoms (figure 37).[81] Those tweezer receptors were screened for binding against a 24389 (= 29^3) membered encoded sidechain-deprotected tripeptide library on TentaGel (AA_3-AA_2-AA_1-N(H)-TentaGel). Both receptors showed high selectivity for the peptide sequence D-Ala-L-Asp-D-Ser. However, UV spectroscopic titration studies showed that receptor **61** bound the peptide sequence more than ten times stronger than receptor **60** with K_a of 4.1 x 10^3 M^{-1} and 320 M^{-1}, respectively.

The example demonstrated clearly that by varying the "hinge" part of tweezer-like receptor molecules it was possible to increase the binding affinity while maintaining the selectivity.

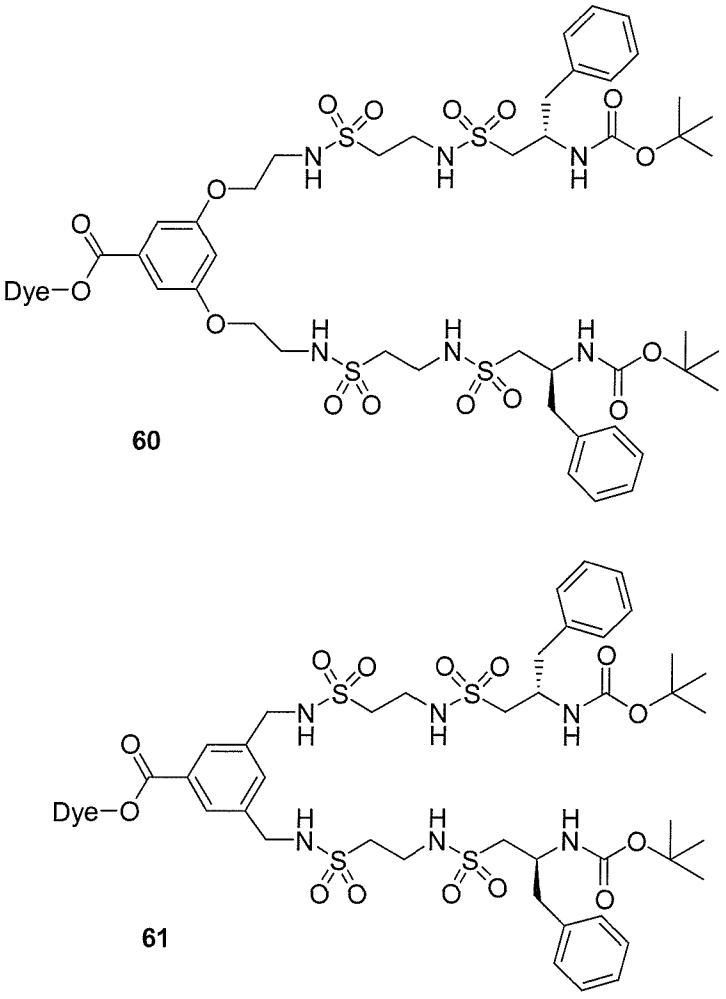


Figure 37: Liskamp's peptidosulfonamide tweezer receptor

Kilburn has synthesised a tweezer receptor **62** for peptides with a guanidinium carboxylate terminus, using guanidinium for the peptide carboxylate, as a specific binding site (figure 38).[85]

A guanidinium based CBS can bind to the deprotonated carboxylic terminus of peptides *via* hydrogen bounds as shown in structure **63**. The synthesis of this receptor was carried out using an appropriately functionalised guanidine which was attached by conventional solid phase synthesis. The resin functionalised with a guanidine unit allowed subsequent coupling of amino acids to give the peptidic arms.

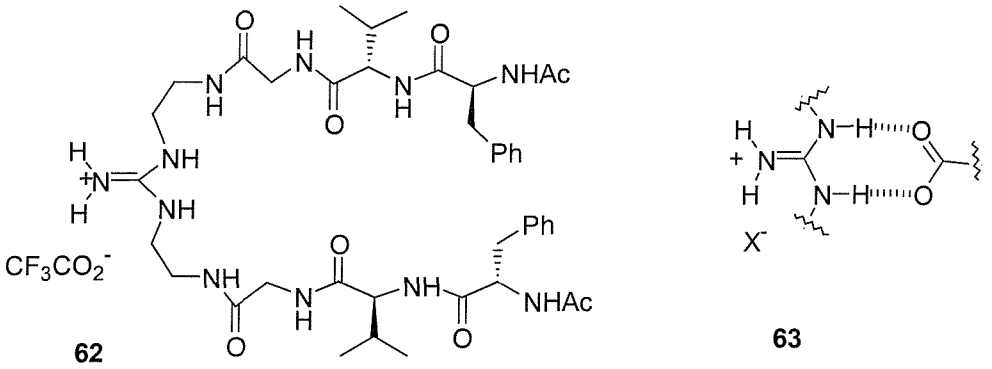


Figure 38: Guanidinium based tweezer receptor

Tweezer receptor **62** was modified with dansyl chloride (DNSCl) to give receptor **64** (figure 39).[71] Dansylated receptor **64** was used in screening experiments against a peptide library with free carboxylic terminus in an aqueous solvent system. Tweezer receptor **64** was found to bind to approximately 3% of the library members and following sequencing of 20 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 binding constant (K_a = 4 x 10^5 M^{-1}) for one of the peptides selected from the screening experiments (Cbz-Glu(O^{t}Bu)-Ser(^{t}Bu)-Val-OH), with receptor **64** was measured using titration calorimetry.

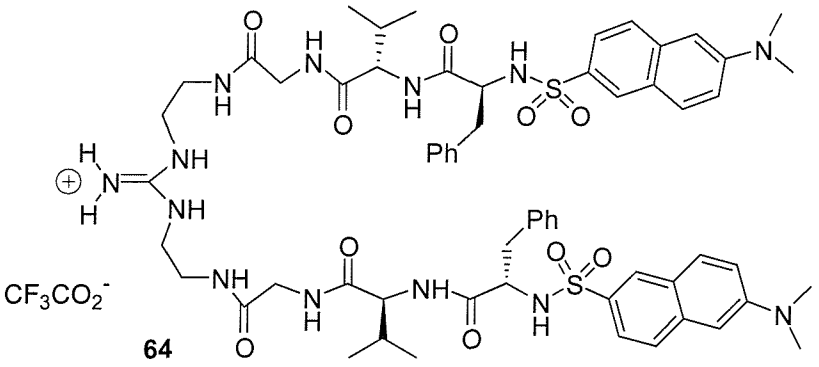
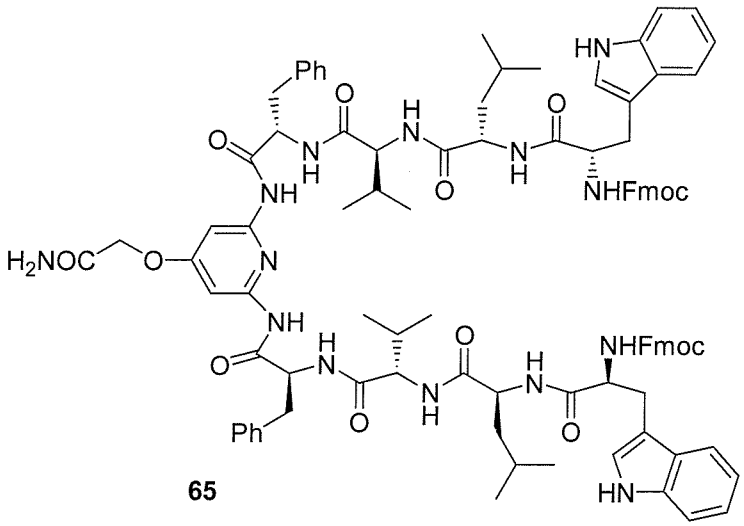


Figure 39: Guanidinium based tweezer receptor

Kilburn has described a novel tweezer structure **65** that incorporates a diamidopyridine unit as the head group to provide a binding site for the carboxylic acid terminus of peptide guests. In addition the arms of the tweezer are themselves simple peptides which can

potentially provide selective interactions with the backbone of the peptide guests. A small (2197 membered) resin-bound library of such tweezer receptors has been prepared using the "split and mix" strategy, and has been used to demonstrate the potential of such libraries to identify selective receptors for selected tripeptide guests with a carboxylic acid terminus. The screening experiments were carried out in chloroform using the tripeptide DSN-L-Glu(O^{t}Bu)-L-Ser(^{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 DSN-labelled peptide, with the resin bound tweezer, was also operating in free solution a single tweezer receptor **65** with side arm sequence Val-Leu-Trp, was synthesised on solid phase. UV spectroscopic binding studies gave an estimated binding constant of 2.6 x 10^5 M^{-1}.[70]

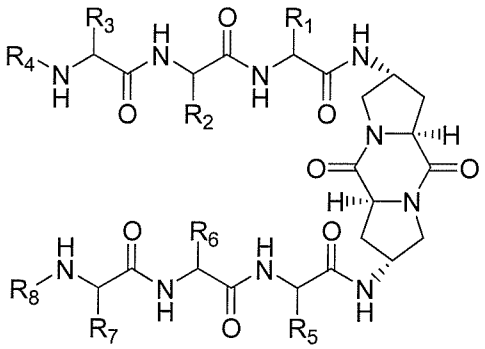


65

Figure 40: 2,6 diamidopyridine based tweezer

Wennemers has recently described a series of tweezer receptors featured a rigid diketopiperazine moiety. The diketopiperazine derived from 4-hydroxyproline provides the rigid backbone and anchor for the peptidic side chains, that is "the arms". Natural L-, as well as D-amino acids, were employed as building blocks for the receptor arms as they offer structural and functional variety (figure 41).[86] The dye-marked receptor prototypes **66** were tested for their peptide binding affinities by screening them against an encoded resin-

bound tripeptide library with the general structure Ac-AA_3-AA_2-AA_1-$NH(CH_2)_6$-CONH-PS. The library had been synthesised on solid phase by encoded split synthesis employing 29 L- and D-amino acids at each position. Thus, the library contained maximally 29^3 = 24389 different acylated tripeptides. Those receptors demonstrated not only the high binding specificity of the diketopiperazine towards peptides but also revealed that small structural changes induce significant changes in their binding properties.

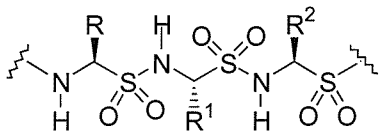


66

Figure 41: Two-armed diketopiperazine receptor ***66****. (Stereochemistry not shown–a mixture of D and L amino acid bilding blocks was used).*

Peptides are attractive targets for drug discovery because of their affinities and specificities toward biological receptors and the simplicity with which large peptide libraries can be synthesized in a combinatorial format. However, the poor stability and bioavailability of peptides *in vivo* have generally limited their therapeutic application. One approach to overcome this obstacle has been the development of non-natural biopolymer scaffolds (carbamates, peptoids, ureas, sulfonamides, etc.) with improved pharmacological properties compared to peptides.[87]

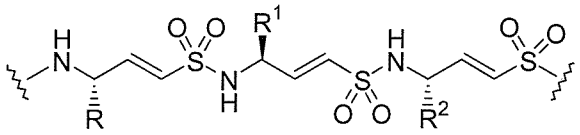
The first example is Liskamp's peptidosulfonamide "tweezer" receptor where α-sulfonopeptides **67** instead of regular peptides are used.[88] The peptidosulfonamide peptidomimetics are interesting because the sulfonamide moiety might act as a transition-state isostere of the hydrolysis of the amide bond. It is more resistant to degradation by proteases, more flexible than the amide bond, and more acidic hydrogen may give rise to stronger hydrogen bonds.[89]



67

Figure 42: α-sulfonopeptides

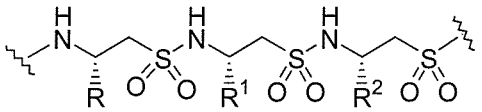
Gennari described the synthesis of vinylogous sulfonopeptides **68** (*vs*-peptides) *via* an interative process, both in solution,[90] and in the solid phase.[91] In collaboration with Still and Nestler, they also described the binding of tweezer-like molecular receptors based on *vs*-peptides to an encoded combinatorial tripeptide library, showing not only that *vs*-peptide based receptors bind oligopeptides, but also that the binding selectivity was just as high as that of receptors containing α-amino acids.[77]



68

Figure 43: vinylogous sulfonopeptides

β-Sulfonopeptides **69** were synthesized by Gennari's group *via* 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) mediated sulfonyl chloride coupling,[87] and by Liskamp's group *via* N-methylmorpholine (NMM) mediated sulfinylchloride coupling followed by oxidation of the resulting sulfinamides to sulfonamides.[89]



69

Figure 44: β-sulfonopeptides

Chapter 2

2.1. Introduction

The ability to prepare synthetic receptors displaying ligand specificity is a highly desirable goal. In particular, selective receptors for specific peptide sequences would have great potential for separation of peptide mixtures, in the field of biosensors and as novel therapeutics. Additionally these receptors would provide model systems for biological protein-peptide complexes. Much recent work in the area of peptide receptors has focused on "tweezer" molecules which despite their inherent flexibility, have proved to be highly selective for certain peptide sequences in both non-polar[70] and aqueous solvent systems.[71] The basic design of tweezer receptors incorporates a "head group" or "hinge" bearing two side arms which include appropriate functionality for binding with the backbone of a suitable substrate. Incorporation of such a "head group" with a specific recognition site for the terminal functional group of the peptide guests, in addition to binding interactions from the tweezer side arms should increase the binding affinity of these tweezer receptors. This process should ultimately lead to receptors for the *C*-terminal sequence of larger peptides.

In the introduction Kilburn's solid phase synthesis of tweezer receptor **64** based on a guanidinium CBS was described.[71] In addition to the interactions with the tweezer side arms the guanidinium species can bind to the deprotonated carboxylic terminus of peptides *via* hydrogen bonds and strong electrostatic interactions.
Following the success of guanidinium derived tweezers, Kilburn described preliminary studies with a combinatorial library of tweezer receptors, incorporating a 2,6-diamidopyridine unit,[70] as the binding site for the carboxylic acid terminus of peptide guests. Both arms of the tweezer were, again simple peptides, which can potentially provide selective interactions with the backbone of the guest. This library was screened successfully to identify a sequence selective receptor for a dye-labelled peptide guest with a carboxylic acid terminus.

The initial aim of this project was to repeat the synthesis of the Fessman library[92] using a combinatorial approach and to screen the library with a range of different guests. The synthesis of the tweezer receptors library was carried out using 2,6-diaminopyridine unit as a specific binding side (CBS), suitably functionalised with a carboxylic acid moiety to allow attachment to the solid support.

The first objective was to screen the tweezer receptor library with a set of peptide guests containing different dye-labels attached to the same amino acid sequence. This should allow a study of the role of the dye label in the selective recognition events in the screening experiments.

The second objective was to study the influence of the position of the dye label on the peptide. To increase the distance between the peptide and the dye label, the introduction of a spacer obtained from previous coupling of the dye with glutaric anhydride, was carried out.

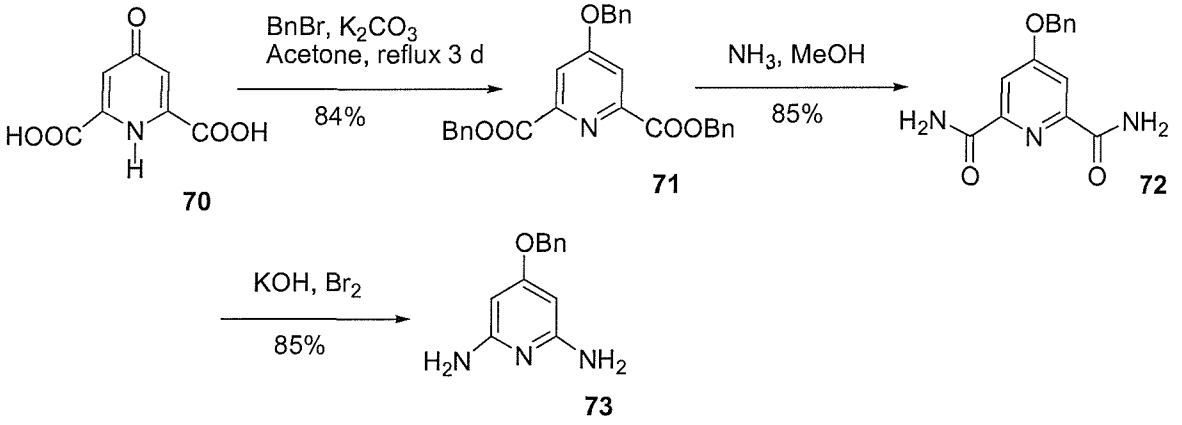
The synthesis of the peptide guests required the use of amino acids with the side-chain functional groups protected. The possibility of screening the library with several peptide guests, with protected as well as unprotected side chains introduces a further way of proving the binding selectivity of the library. An increased number of free functional groups (alcohol, amide and carboxylic acid) in the peptide side-chain could increase the number of possible interactions between the side arms of the tweezer and the peptide.

Another way to introduce diversity in the study of selective recognition between the tweezer library and peptide guests was to create different sub-libraries from the same parent library. The amino acids used to synthesise the tweezer's arms had their side-chain functional groups protected. Furthermore, the amino acid in the last position in the tweezer arm was present with the terminal amino group protected. All the possible combinations with protected and unprotected side-chain and terminal amino function gives a set of four different sub-libraries.

In this chapter these studies are described in full and full details of the synthesis of the tweezer library are provided.

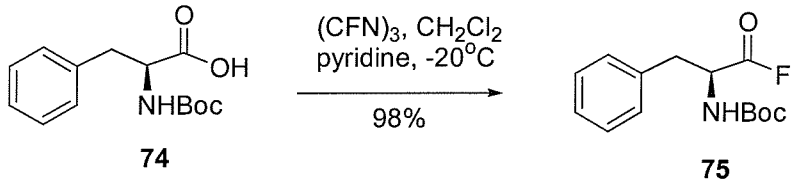
2.2 Synthesis of the CBS

The first aim of the project was the synthesis of a 2,6-diaminopyridine moiety with a functionalised oxygen at the 4-position in order to allow attachment to the solid support. At the beginning the route devised by Fessman[92] was used. The first step was the transformation of commercial chelidamic acid **70** to the corresponding benzyl ester **71**. Initially this step afforded a maximum 54% yield, but the use of excess benzylbromide allowed an increase of the yield to 84%. The following step was the treatment of benzyl ester **71** with a saturated solution of NH_3 in methanol which gave diamide **72** in 85% yield. The key step of the synthesis, which allowed the transformation of the diamide **72** into a diamine **73** was carried out *via* Hofmann rearrangement,[93] using an aqueous solution of KOH and bromine refluxed at 90°C for 5 h which yielded the desired compound in an excellent 85% yield.



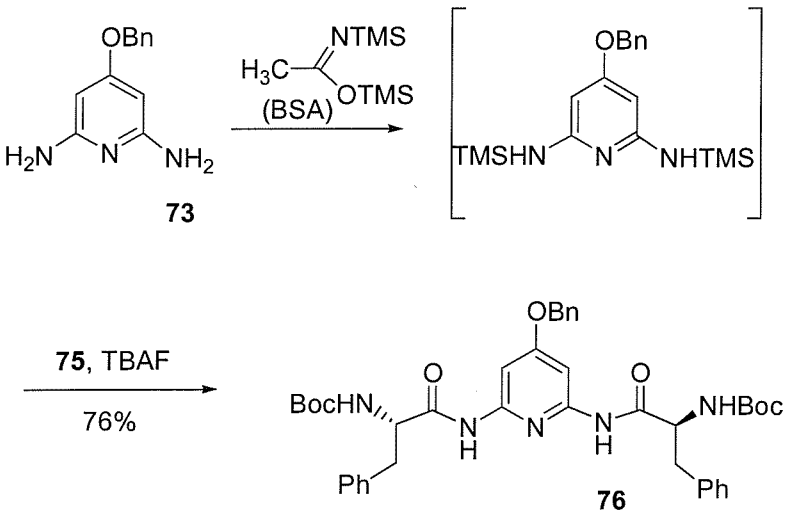
Scheme 1: Synthesis of diamine 73 via Hofmann rearrangement

At this point two different routes were available. In the initial work the next step was the coupling of Boc-Phe-OH **74** with diamine **73**. Boc-Phe-OH was first converted to Boc-Phe-F **75** using cyanuric fluoride in quantitative yield.[94]



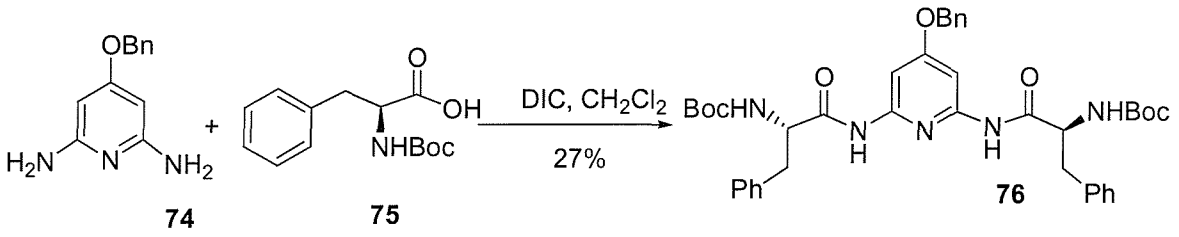
Scheme 2: Synthesis of Boc-Phe-F 75

Treatment of **73** with *N,O*-bis(trimethylsilyl)acetamide, followed by addition of Boc-Phe-F **75** in the presence of TBAF in acetonitrile gave **76** in 76% yield (scheme 3).



Scheme 3: Coupling with Boc-Phe-F

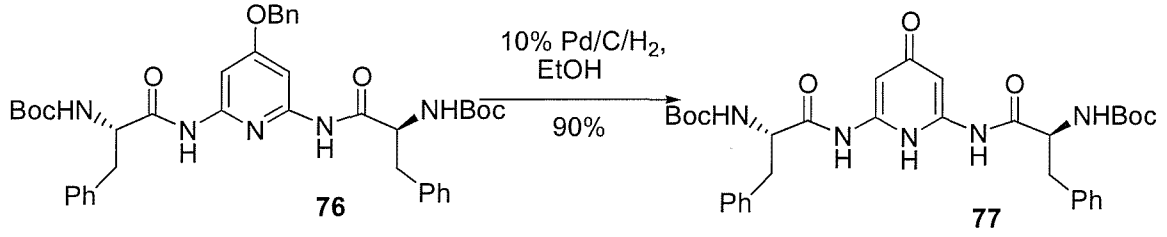
The coupling with DIC was also tried as an alternative but after purification by flash column chromatography, the yield of **76** was only 27% (Scheme 4).



Scheme 4: Coupling with DIC

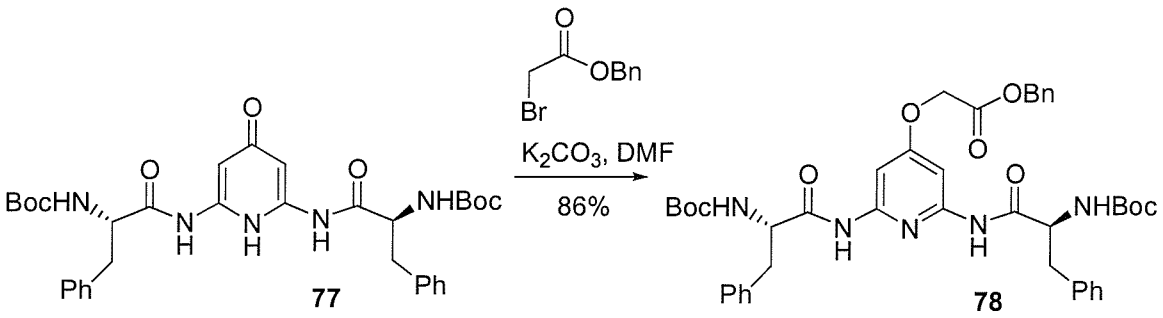
After consideration of the poor coupling yield with DIC it was decided to turn back to the more reliable Boc-Phe-F chemistry.

Hydrogenolysis of the benzyl ester **76** was carried out using standard methods (10% Pd/C in ethanol) to give **77** in 90% yield (scheme 5).



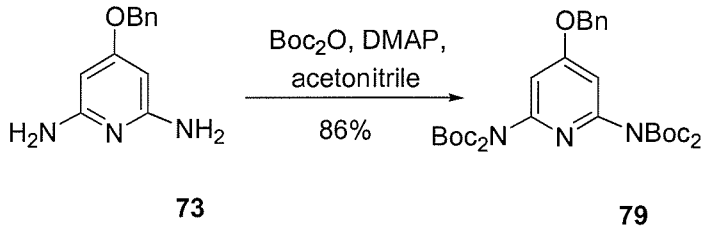
Scheme 5: Hydrogenolysis of benzyl ester 76

The following step was the alkylation of the oxygen of pyridone **77** using DMF as a solvent in presence of K_2CO_3 and benzyl bromoacetate to give the desired compound **78** in 86% yield (scheme 6).



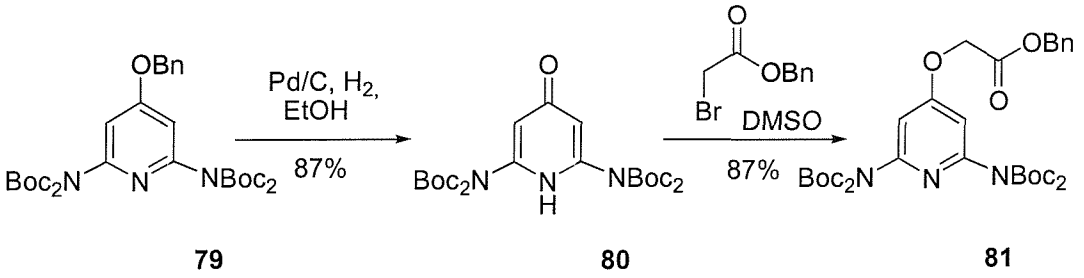
Scheme 6: Alkylation of pyridone 77

An alternative, new route to **78** was achieved by treatment of the diamidopyridine **73** with Boc-anhydride (di-*tert*-butyldicarbonate) and DMAP in acetonitrile. The purification on column chromatography gave **79** in 86% yield (scheme 7).



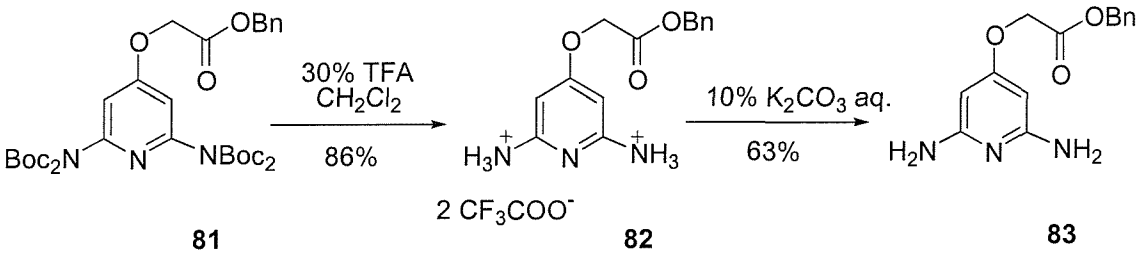
Scheme 7: Boc-protection of diamidopyridine 73

The next step was hydrogenolysis of benzyl ester **79** using the standard procedure with Pd/C in ethanol to give **80** in 87% yield. Alkylation of **80** with benzyl bromoacetate, as before, give **81** in 99% yield, which was a convenient intermediate for the preparation of a range of diamidopyridine derivatives (scheme 8).



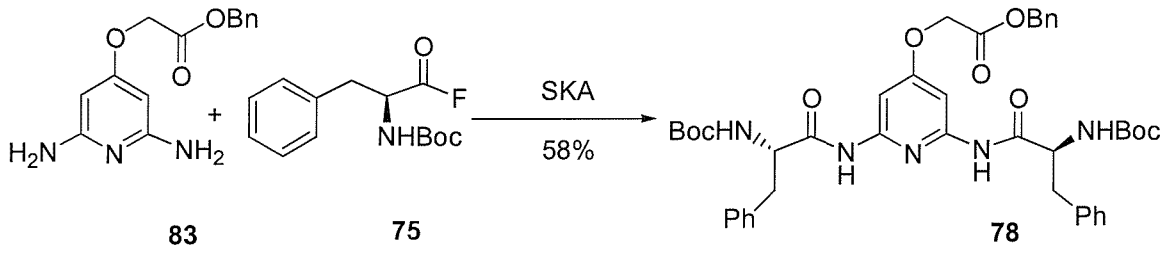
*Scheme 8: Synthesis of benzyl ester **81***

To allow the coupling step with Boc-Phe-F as before it was necessary to remove the protecting groups from the amino functions. The benzyl ester **81** was stirred for 2 h with a 30% TFA solution in CH_2Cl_2 to give the TFA salt **82**. The yield of the subsequent coupling could be improved by extracting the salt with 10% aqueous solution of K_2CO_3 to afford the diamidopyridine **83** ready for coupling (scheme 9).



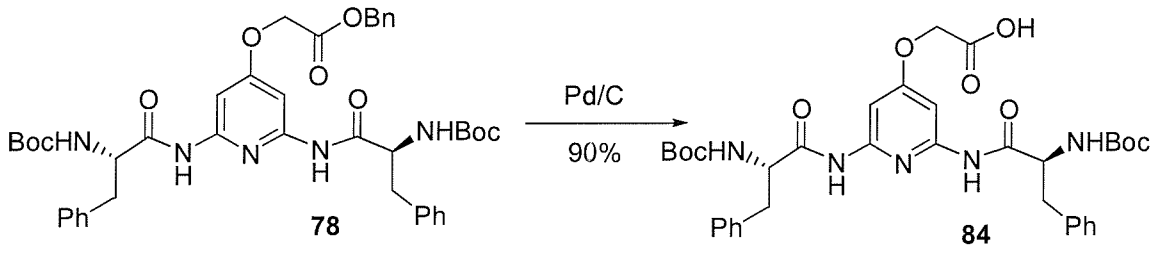
*Scheme 9: Synthesis of diamidopyridine **83***

The coupling with Boc-Phe-F **75** was achieved by activating the diamidopyridine **83** with BSA in CH_2Cl_2 followed by addition of Boc-Phe-F and methyl trimethylsilyl dimethylketene acetal (SKA) to scavenge the flouride to yield the compound **78** in 58% yield (scheme 10).



Scheme 10: Coupling with Boc-Phe-F in presence of SKA

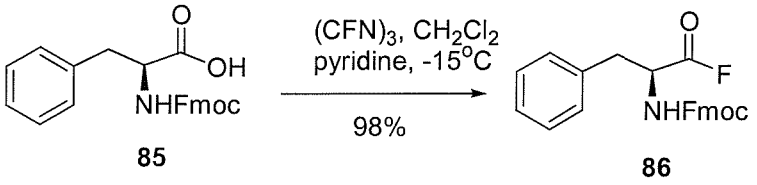
The final step for both routes was the hydrogenolysis of the benzyl ester **78** to give a free carboxylic group for attachment to the solid supported amine. This was achieved using Pd/C, under an atmosphere of hydrogen to give the desired pyridine **84** as Boc-CBS in 90% yield.[92]



*Scheme 11: Final step to give the Boc-CBS **84***

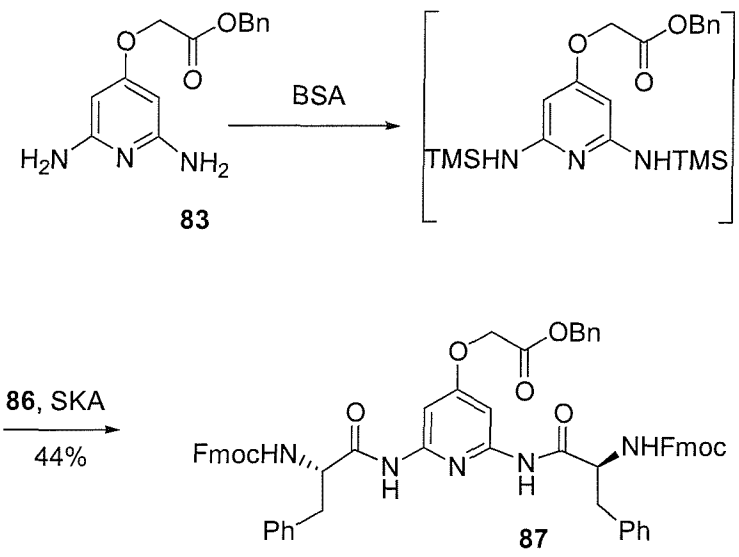
Applying the same procedures the analogous CBS with Fmoc protecting groups was synthesised for use with different solid phase linkers.

Fmoc-Phe-OH **85** was transformed to Fmoc-Phe-F **86** using $(CFN)_3$ in CH_2Cl_2 at $-15^{\circ}C$ (scheme 12).[94]



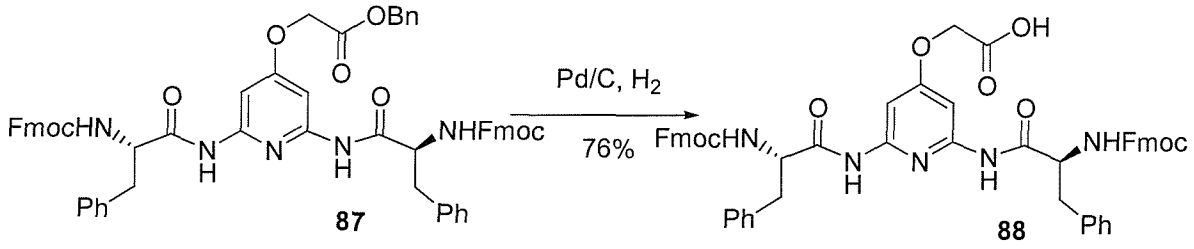
*Scheme 12: Synthesis of Fmoc-Phe-F **86***

The diamidopyridine **83** was treated with BSA and following addition of Fmoc-Phe-F **86** and SKA in CH_2Cl_2 yielded the final Fmoc-CBS **87** in 44% yield (scheme 13).



Scheme 13: Coupling with Fmoc-Phe-F in presence of SKA

The final step was the hydrogenolysis of **87** using Pd/ C to give the Fmoc-CBS **88** in 78% yield (scheme 14).



*Scheme 14: Final step to give Fmoc-CBS **88***

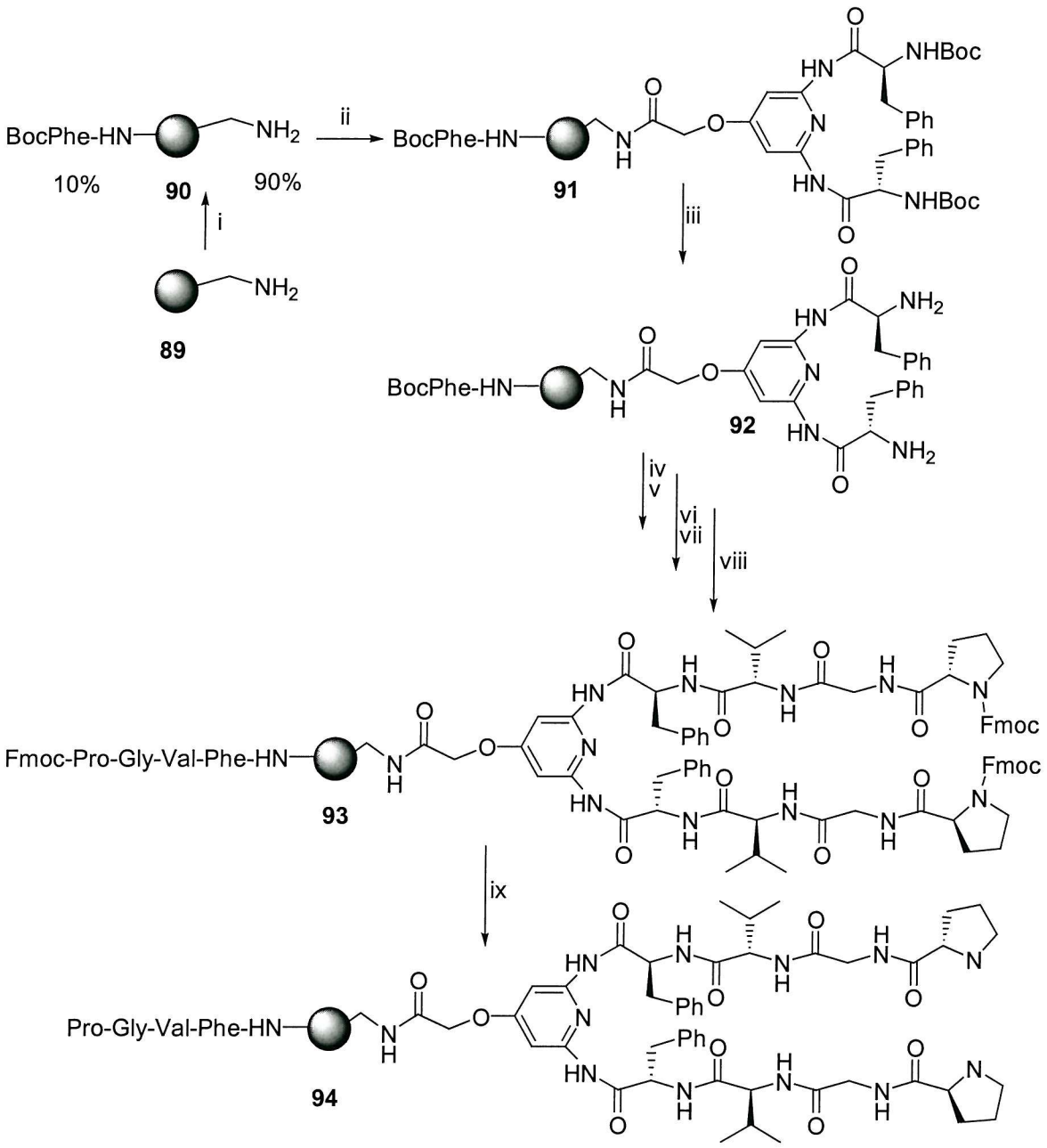
2.3. Synthesis of a Single Tweezer Receptor

In previous work it had also been found that attempted sequencing of individual beads carrying diamido pyridine tweezer, but no coding strand, using Edman degradation was only partially successful (typically only the first two amino acids could be unambiguously identified). It was concluded that under the conditions of Edman degradation the amide bonds to the diamidopyridine core were being cleaved, leading to loss of the peptide side-arm. As a consequence 10% of the free amino groups from TentaGel resin had been used to incorporate a coding strand. In Fessmann's work the tweezer library had been prepared using a linker between the resin and the CBS allowing cleavage of the tweezer prior to Edman sequencing of the coding strand.[70]

It was subsequently realised that cleavage of the tweezer might not be necessary to allow successful sequencing of the coding strand. It was therefore decided to construct a library without the use of a linker but including a coding strand. To check that the approach was appropriate a single tweezer was synthesised initially on the solid phase.

The first step was the coupling of 10% of the binding sites of the TentaGel resin **89** with Boc-Phe-OH to obtain the first amino acid of the peptide chain to use as coding strand for Edman degradation. The remaining amine sites of TentaGel NH_2 resin **90** were coupled with Boc-CBS **84** using *N*-hydroxybenzotriazole hydrate (HOBt), 2-(1H-benzotriazole-1-yl)1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) and diisopropylethylamine (DIPEA) to give resin **91**. The Boc protecting groups were removed using a 20% solution of TFA in CH_2Cl_2 to afford resin **92**. Sequential coupling with three amino acids was carried out using HOBt, TBTU and DIPEA to generate resin **93**. The Fmoc protecting group was removed using a 20% solution of piperidine in DMF to give the final compound **94** (scheme 15). Every step was confirmed using the ninhydrin test.[95] Edman degradation carried out with final compound **94** successfully returned the correct sequence.

Thus, it was confirmed that it was possible to determine the amino acid sequence in the tweezer receptor without using a linker.



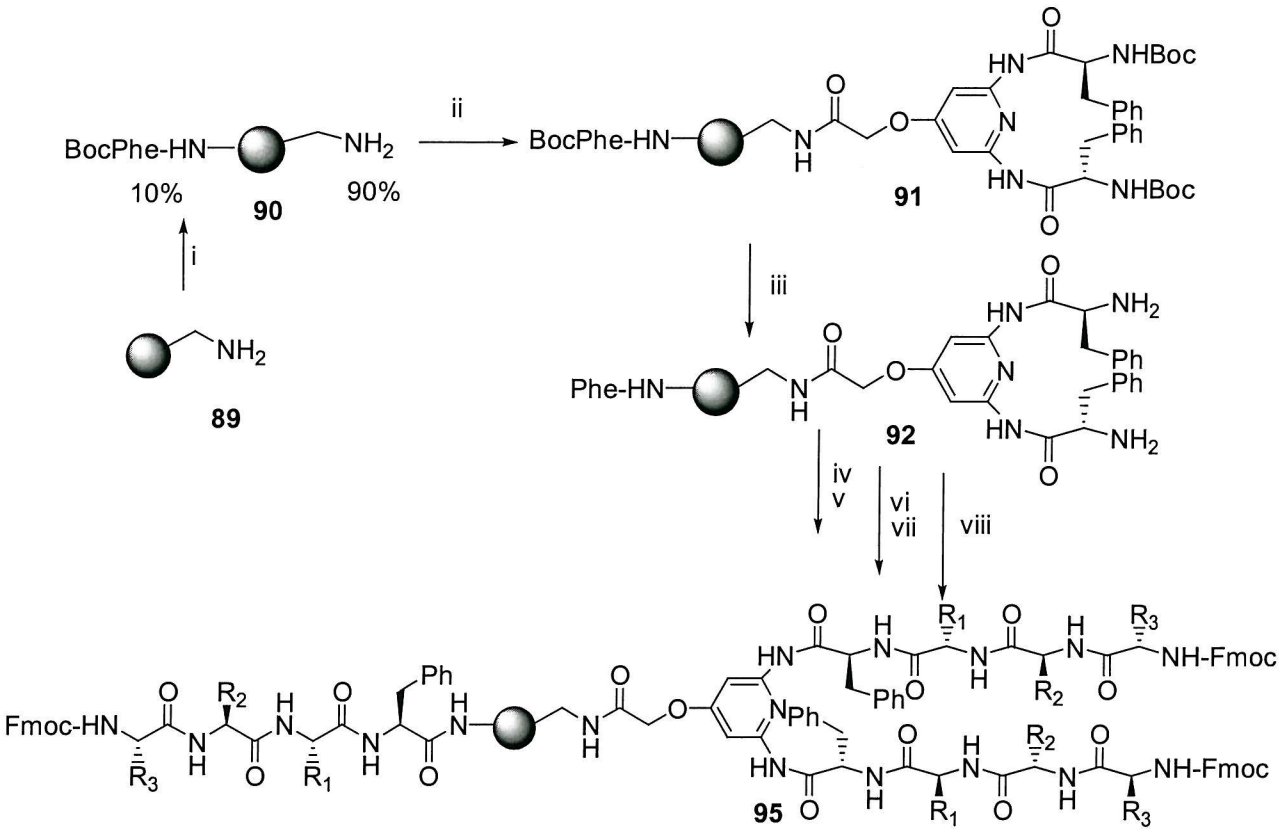
i) Boc-Phe-OH, TBTU, HOBt, DIPEA; ii) Boc-CBS, TBTU, HOBt, DIPEA iii) 20% TFA in CH_2Cl_2 iv) Fmoc-Val-OH, TBTU, HOBt, DIPEA; v) 20% piperidine in DMF vi) Fmoc-Gly-OH, TBTU, HOBt, DIPEA; vii) 20% piperidine in DMF; viii) Fmoc-Pro-OH, TBTU, HOBt, DIPEA; ix) 20% piperidine in DMF.

Scheme 15: Synthesis of a single tweezer ***94***

2.4. Synthesis of a Library of Tweezer Receptors.

The synthesis of a library of receptors was carried out using the "split and mix" combinatorial strategy.[76,78]

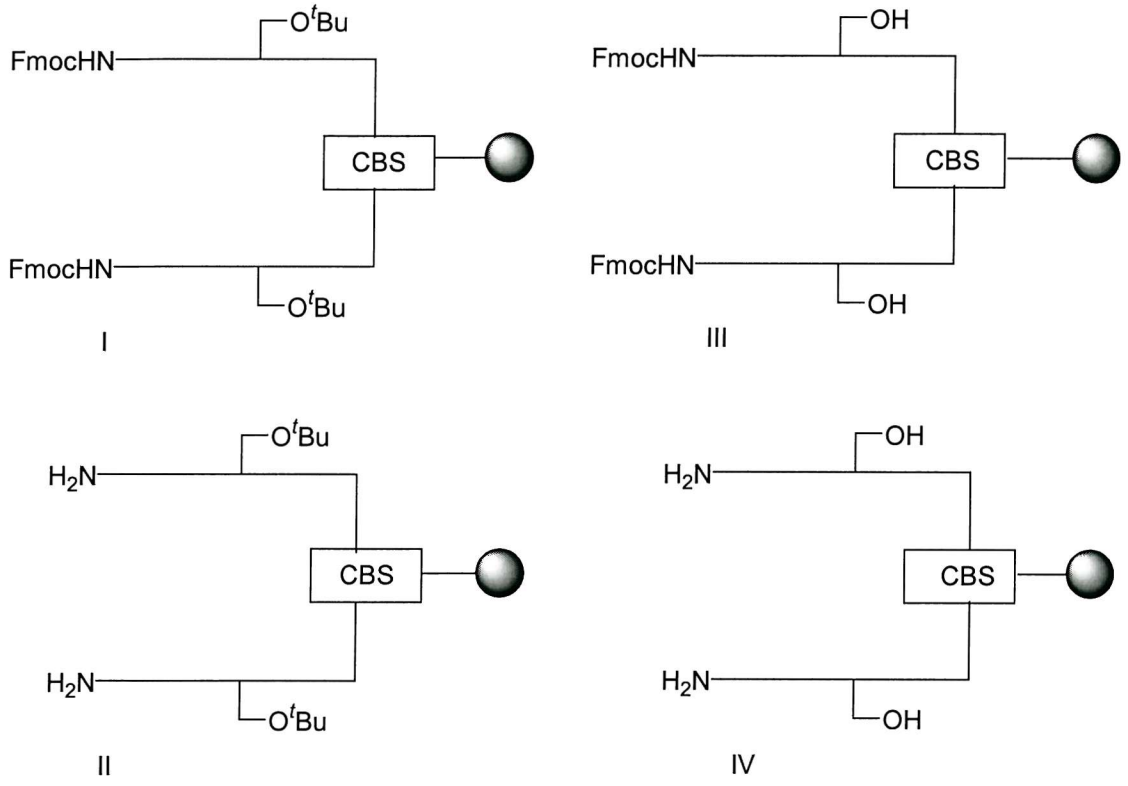
10% of the free amino sites of the Tentagel resin **89** were coupled with *N*-Boc phenylalanine to serve as a coding strand. The remaining amino functions were coupled with Boc-CBS **84**, using HOBt, TBTU, and DIPEA to give **91**. Removal of the Boc protecting groups yielded a resin **92** ready for library generation. A 2197 membered library of Fmoc protected tweezer **95** was prepared by a three-fold coupling of thirteen Fmoc protected amino acids (Gly, L-Val, L-Ala, L-Phe, L-Leu, L-Lys (Boc), L-Pro, L-Glu(O^{t}Bu), L-Ser(^{t}Bu), L-Met, L-Trp, L-Asn, L-Gln) using the "split and mix" technology.



i) Boc-Phe-OH, TBTU, HOBT, DIPEA; ii) Boc-CBS, TBTU, HOBT, DIPEA, DMF; iii) TFA 20% in CH_2Cl_2; iv) Fmoc-NHCHR$_1$COOH, TBTU, HOBT, DIPEA, DMF; v) 20% piperidine in DMF; vi) Fmoc-NHCHR$_2$COOH, TBTU, HOBT, DIPEA; vii) 20% piperidine in DMF; viii) Fmoc-NHCHR$_3$COOH, TBTU, HOBT, DIPEA, DMF.

Scheme 16: Synthesis of tweezer receptors library ***95***

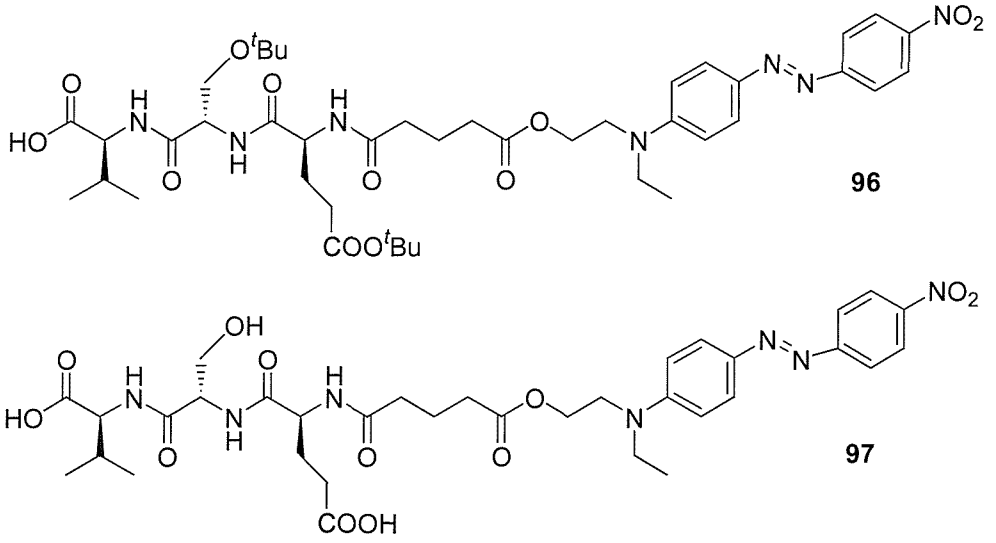
Library **95** was divided to give four different sub-libraries (scheme 17). Sub-library I, was *N*-terminus (Fmoc) and side chain protected. Sub-library II, with free terminal amino group but still protected side chains, was prepared by treatment of a portion of library **95** with 20% solution of piperidine in DMF. Sub-library III, with a *N*-terminus protected and side chain deprotected, was prepared by treatment of a portion of library **95** with a 50% solution of TFA in CH_2Cl_2. Sub-library IV was fully deprotected and was prepared by treatment of a portion of library **95** with a 20% solution of piperidine in DMF followed by a 50% solution of TFA in CH_2Cl_2.



Scheme 17: Four different sub-libraries

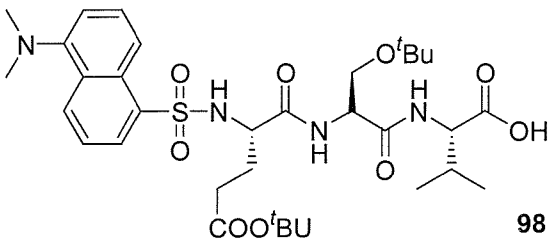
2.5 Screening Experiments

Screening experiments were initially carried out with the four different sub-libraries of tweezers using two different dye-labelled peptide guests: Red dye-spacer-L-Glu(O^{t}Bu)-L-Ser(^{t}Bu)-L-Val-OH **96**, and Red dye-spacer-L-Glu-L-Ser-L-Val-OH **97** (scheme 18, synthesis described in section 6.3).



Scheme 18: Dye-labelled peptide guests

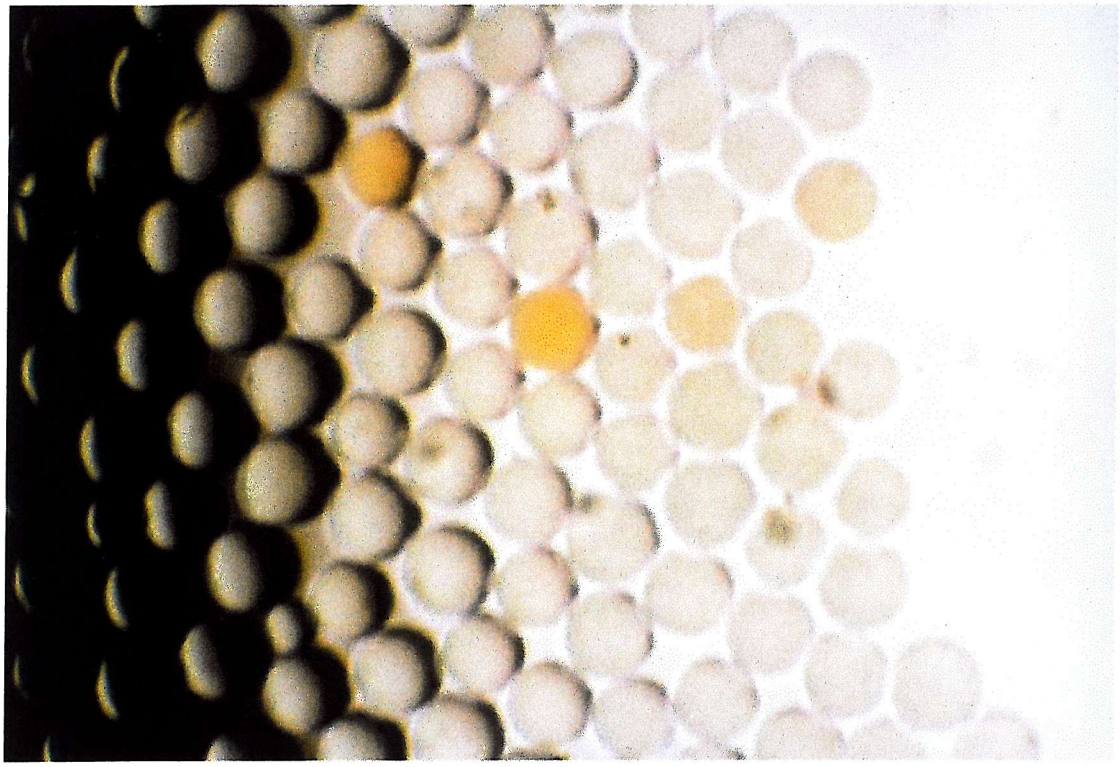
These peptide were chosen as they had been used in previous studies with other libraries in the group.[71] Fessman had already screened library I against dansyl labelled peptide **98** (scheme 19)



Scheme 19: Dansyl labelled peptide

All four sub-libraries were screened with peptide guests 96 and 97 in aqueous solvent at pH = 6.0 and pH = 9.2 and also in chloroform.

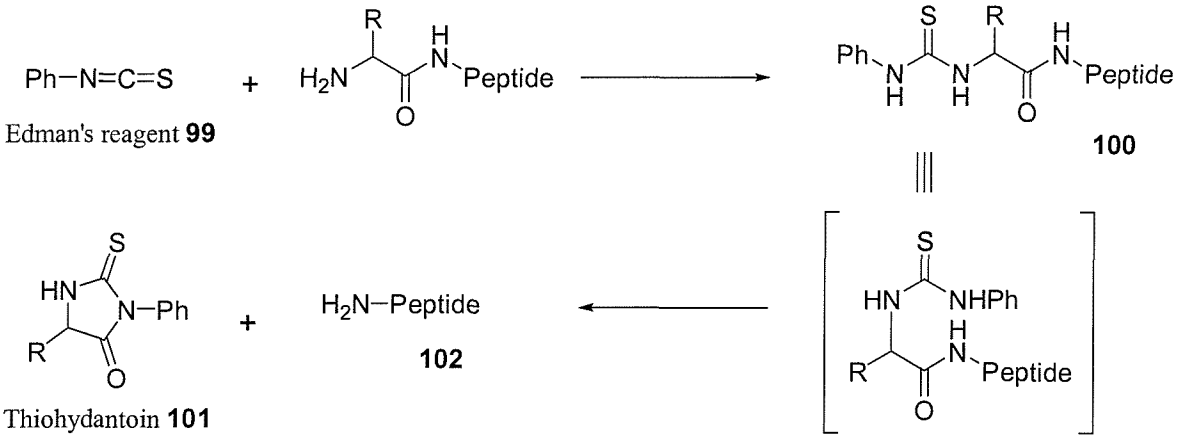
For a typical screening experiment a sample of 10 mg (~ 9000 beads) of each library of tweezer receptors bound to the TentaGel resin was used. Statistically, this represented an amount corresponding to at least five copies of each library member. The resin was equilibrated in the chosen solvent system for 24 hours, followed by addition of aliquots (3 x 30 μL) of dye-labelled tripeptide guest (20 μM), as a solution in the same solvent system to give a guest concentration of 1.8 μM and equilibration was continued for further 24 hours. Beads were analysed in flat-bottomed glass pots under a Leica inverted DML microscope (magnification x 40). The highly red beads were removed manually from the pot and sent to be analysed (example of active beads are shows in Picture 1).



Picture 1: Red Active beads

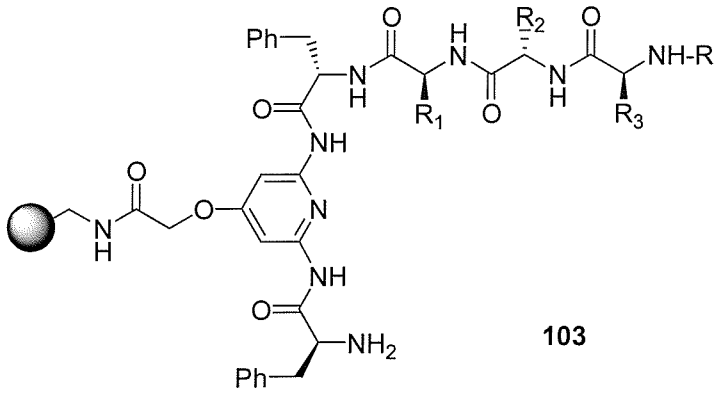
To find out which particular library member is present on the selected bead Edman degradation was used.[96,97] With this method only the first amino acid of the sequence is analysed leaving the rest of the sequence. The Edman degradation involves the reaction of

the N-terminal functionality of the first amino acid with phenylisothiocyanate (Edman's reagent) **99** to give thiourea derivative **100** (scheme 20). The resulting thiourea rearranges in the presence of strong acid to form a thiohydantoin **101** which is identified by HPLC analysis. Repetition of the Edman degradation cycle allows the stepwise identification of all amino acids residues of the peptide. The entire procedure is completely automated as a consequence of the fact that the sequence of reaction is repeated exactly the same for each residue. The only limitations of this procedure can be that the single bead does not provide enough peptide but we found that this procedure is very sensitive and accurate even with the small amount of compound provided by the coding strand on one bead (0.27 pmol/bead). A second limitation of the Edman method (although not relevant to our work) can be an increase of impurities as the cycle is repeated and in the case of peptides with more than 20 residues may have to be cut down first into shorter sequences.



Scheme 20: Edman degradation

Several control tests were first carried out in order to show that any observed selectivity was 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. A simple 2197 membered peptide library directly attached to TentaGel resin (analogous to the coding strand) was prepared. Incubation of this library with the dye-labelled peptide guest showed no selectivity. Similarly, incubation of the guest with a library **103** (scheme 21) of diamidopyridines with a single tweezer side arm showed no selectivity.



Scheme 21: Library of single tweezer side arm

In order to probe the role of the dye in the recognition process the simple acetylated red dye substrate was used as a guest, but incubation of that compound with the tweezer libraries again showed no selective binding properties. Taken together these results confirm that any observed selectivity in the screening of the tweezer libraries with the dye-labelled peptide guests are a consequence of the desired selective binding between the tweezer and the dye-labelled peptide guest.

2.6 Screening Experiment Results

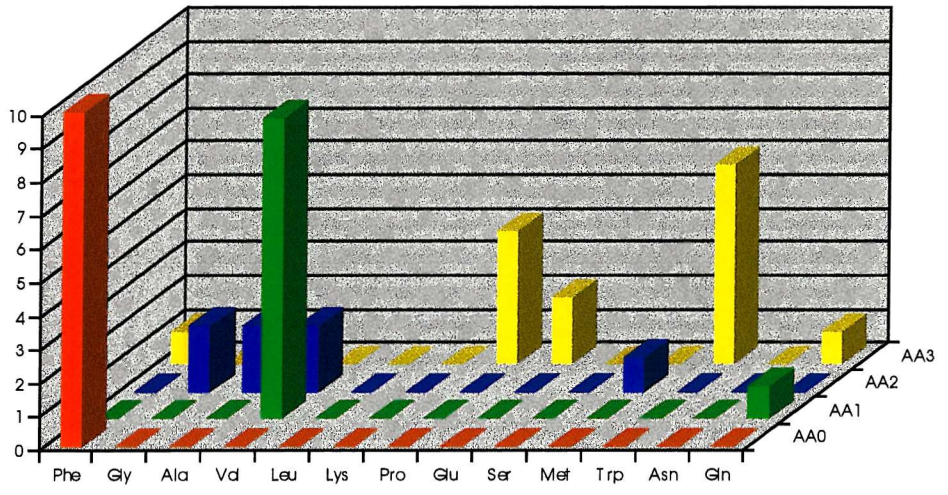
Sequencing of individual beads was carried out by Edman degradation as described above. The results are presented in tables, with the identity of the amino acids from the Edman sequencing AA_0-AA_3 where AA_3 is the *N*-terminal amino acid of the tweezer arm (and therefore the first amino acid sequenced) and AA_0 is the last amino acid sequenced which should be phenyalanine in all cases.

*a) Screening between sub-library I and peptide guest **96**, **98** and **104** in chloroform*

i) The first screening experiment by Fessman using the sub-library I and guest DNS-L-Glu(O^{t}Bu)-L-Ser(^{t}Bu)-L-Val-OH **98** in chloroform gave the sequence Phe-Val-xxx-Trp found for six of the ten beads analysed, and more specifically, Phe-Val-Leu-Trp for three of the beads (table 1 and graphic 1).[70]

Bead	AA_0	AA_1	AA_2	AA_3
1	Phe	Val	Leu	Trp
2	Phe	Val	Leu	Trp
3	Phe	Val	Leu	Trp
4	Phe	Val	Ala	Trp
5	Phe	Val	Val	Trp
6	Phe	Val	Met	Trp
7	Phe	Val	Gly	Phe
8	Phe	Val	Ala	Val
9	Phe	Val	Gly	Val
10	Phe	Gln	Val	Gln

*Table 1: Sub-library I with DNS-peptide **98** in chloroform*



*Graphic 1: Sub-library I with DNS-peptide **98** in chloroform*

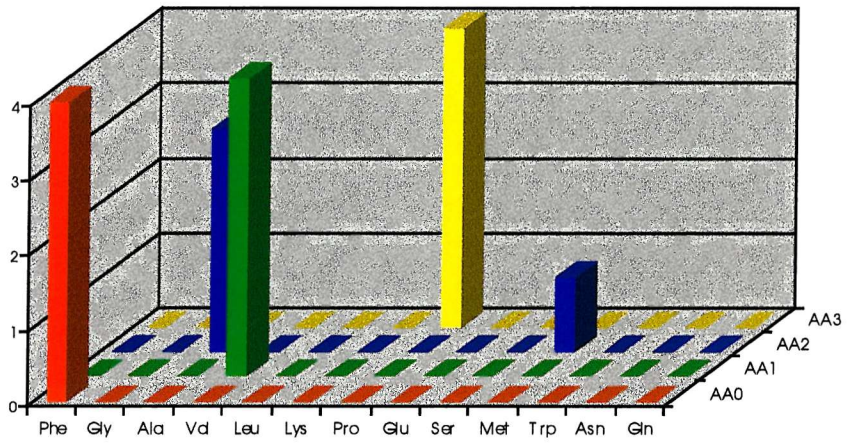
The results clearly identify that Valine is almost exclusively preferred at the first position (AA_1) of the sequence, but determination of the position of the second and third amino acids in the tweezer arm is less precise, which is not surprising since it is at the end of a non-rigid peptide strand.

ii) In order to probe the effect of changing the dye on the preferred receptor structure screened sub-library I with the Red-dye-spacer-L-Glu(O^{t}Bu)-L-Ser(^{t}Bu)-L-Val-OH **96** which has the same sequence of amino acids but a different dye and spacer unit, was screened. The screening experiment used about 9000 beads of which 5 beads were very strongly coloured and about 0.1% moderately coloured.

From the screening experiment the five most highly coloured beads were picked and four were successfully analysed by Edman degradation to identify the structure of the tweezer receptors on each bead. The sequencing results showed the presence of phenylalanine as the fourth residue in the sequencing for all beads, which was expected as it was introduced by the synthesis, and provided a useful check that the coding strand was operating correctly. Identical results for three beads showed the tweezer side arm structure: Phe-Val-Ala-Pro; and the fourth bead yielded the closely related sequence Phe-Val-Met-Pro (table 2 and graphic 2).

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

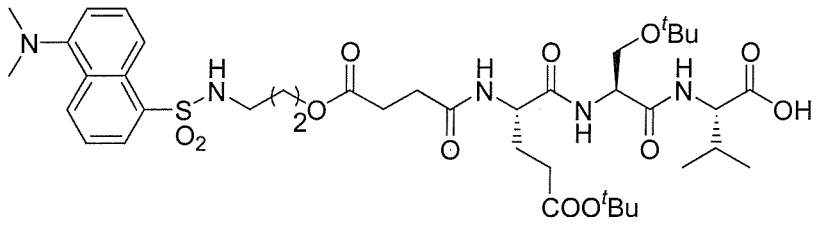
*Table 2: Sub-library I with red dye-labelled **96** in chloroform*



*Graphic 2: Sub-library I with red dye-labelled **96** in chloroform*

A comparison between those two screening experiments shows the influence of the position of the dye in the selective recognition event. With peptide **96** Pro is exclusively found at position 3, whereas with peptide **98** Trp is favoured and Pro was not found in any of the selected beads. The fact that the first amino acid is the same (Val) for both experiments is consistent with the idea that this part of the receptor is binding the C-terminal end of the peptide guest, which is the same in both **96** and **98**.

iii) Further experiments were carried out with DNS dye-spacer-L-Glu(O^{t}Bu)-L-Ser(^{t}Bu)-L-Val-OH **104**, where the dye was attached to the peptide through a spacer, obtained from previous coupling of the DNS with glutaric anhydride. Sequencing of seven beads from this screening experiment are given in table 3.

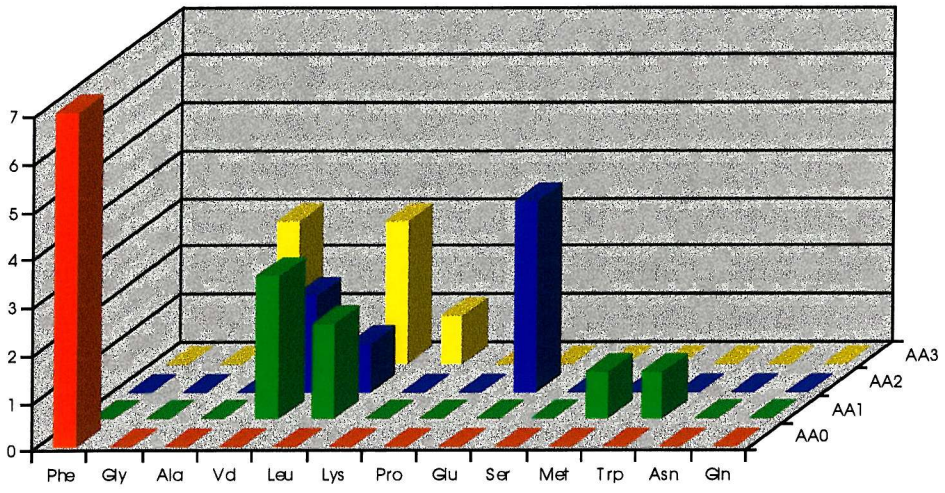


104

Scheme 22: Dansyl labelled peptide

Bead	AA_0	AA_1	AA_2	AA_3
1	Phe	Val	Glu	Leu
2	Phe	Val	Glu	Leu
3	Phe	Val	Glu	Leu
4	Phe	Leu	Glu	Ala
5	Phe	Leu	Val	Ala
6	Phe	Trp	Val	Ala
7	Phe	Met	Leu	Lys

*Table 3: Sub-library I with DNS-Spacer-peptide **104** in chloroform*



*Graphic 3: Sub-library I with DNS-Spacer-peptide **104** in chloroform*

From these results it is clear that a larger number of receptor structures can bind guest **104**. The first position AA_1 is still Valine most frequently, but not exclusively as with **96** and **98**. A preference for the sequence Glu-Leu for AA_2 and AA_3 can also be seen with **104** which differs from the results with guest **98** (Leu-Trp) and **96** (Ala-Pro). Related studies described by Still *et al.*[98], using different libraries, found that the dye label did not influence the observed binding selectivities found in their screening experiments. However, the results from the three screening experiments using peptides **96**, **98**, **104** show that the dye-label can play a significant role in the binding with tweezer receptors, even when separated from the peptide by a spacer unit.

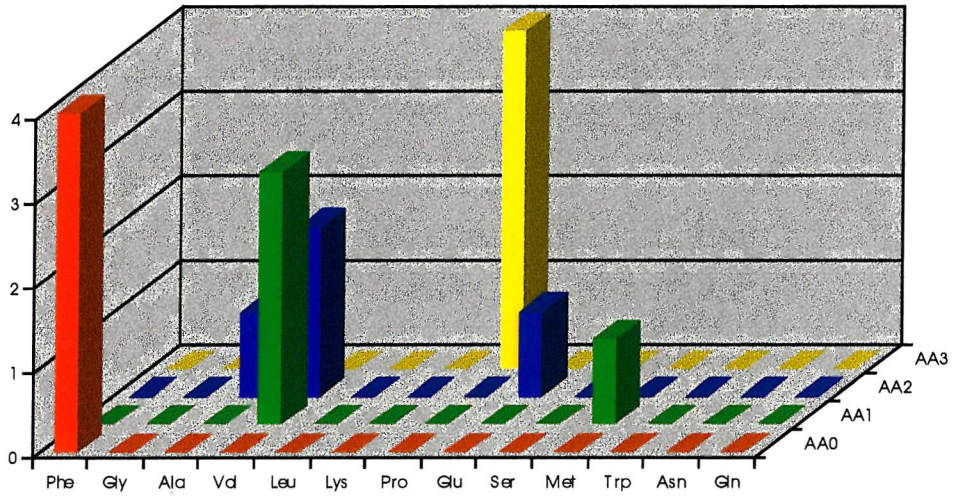
*b) Screening between sub-library II with peptide **96**, **98** in Chloroform*

For sub-libray II, the screening experiments with peptide guest **96**, gave AA_0 = Phe for all 4 beads; AA_1 was again Val for three beads and Met for one; AA_2 was split between Val (for two beads), Ala and Glu. The last position, AA_3 was once again Pro for all four beads

(table 4). Screening the same library with Fessman's peptide DNS-L-Glu(O^{t}Bu)-L-Ser(^{t}Bu)-L-Val-OH **98** (table 5) gave sequence Phe-Val-Leu-xxx, found for four of the ten beads analysed (table 6), and Met or Phe for the last position for 7 out of the 10 beads analysed.

Bead	AA_0	AA_1	AA_2	AA_3
1	Phe	Val	Ala	Pro
2	Phe	Val	Glu	Pro
3	Phe	Val	Val	Pro
4	Phe	Met	Val	Pro

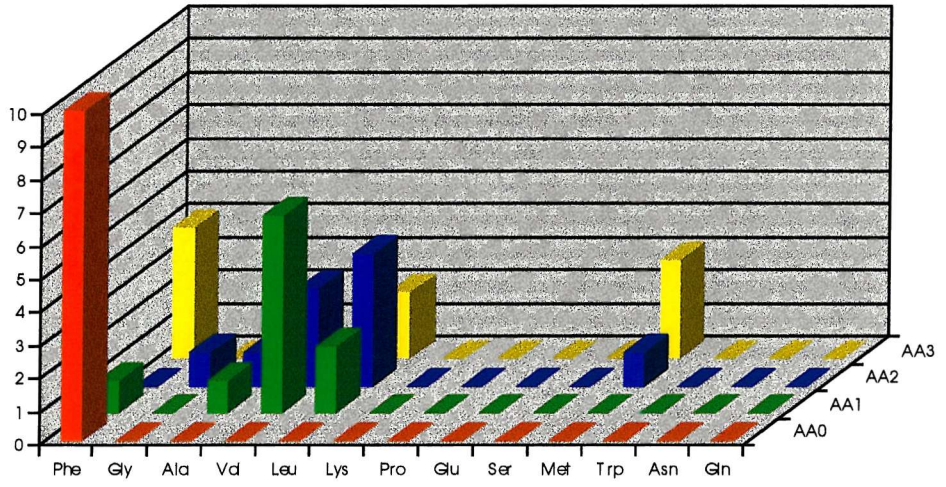
Table 4: Sub-library II with red dye labelled peptide ***96***



Graphic 4: Sub-library II with red dye labelled peptide ***96***

Bead	AA_0	AA_1	AA_2	AA_3
1	Phe	Val	Leu	Met
2	Phe	Val	Leu	Met
3	Phe	Val	Leu	Leu
4	Phe	Val	Leu	Leu
5	Phe	Val	Gly	Ala
6	Phe	Val	Met	Met
7	Phe	Leu	Val	Phe
8	Phe	Leu	Val	Phe
9	Phe	Phe	Val	Phe
10	Phe	Ala	Ala	Phe

*Table 5: Sub-library II with DNS peptide **98***



*Graphic 5: Sub-library II with DNS peptide **98***

Once again the difference in the last amino acid of the sequence can be noticed when the dye changes but the preferred sequence of binding the C-terminus portion of the guest remains constant.

*c) Screening between sub-library III and IV with peptide **96** in chloroform*

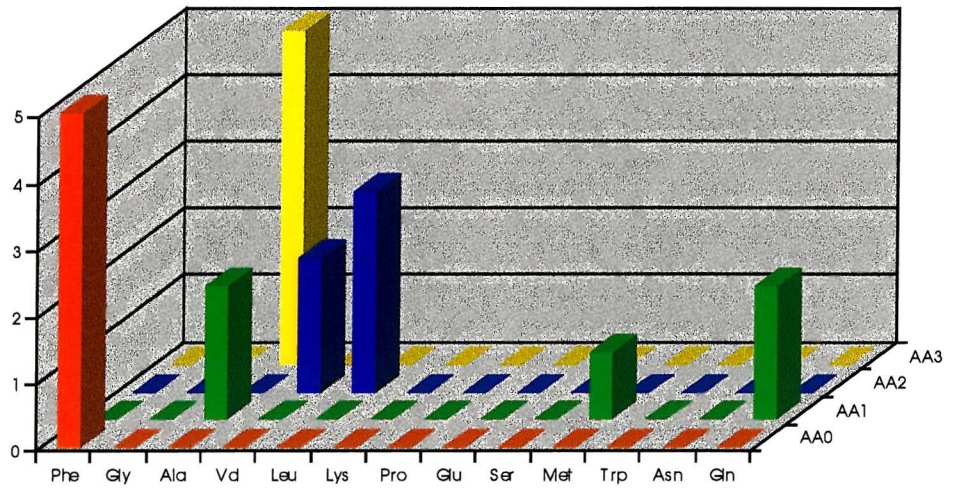
Screening experiments were set up as previously, but increasing the concentration of the guest unfortunately did not give any selectivity with peptide **96**. A possible explanation could be that removing the protecting groups from the side chain of the tweezer receptors increases the number of free OH and COOH groups in the structure. The increased number of possible interactions between the tweezer and the peptide guest decreases the selectivity and a larger number of members of the library were able to bind a given peptide guest.

*d) Screening between sub-library I, II, III and IV with peptide **97** in chloroform*

All four sub-libraries were screened in chloroform with red dye-labelled, side chain deprotected peptide **97**. High selectivity was observed with sublibrary I, and the five most highly coloured beads (0.05%) were sent for analysis by Edman degradation, but significantly different sequences for the tweezer side arms were identified in comparison with the screening results with the structurally related red dye-labelled protected peptide **96**. Ala was found at the AA_3 position in each of the five beads sequenced, and Val (or closely related Leu) at the AA_2 position in each case (table 6). For this peptide, however, Val was not found at the AA_1 position with any of the beads as it had been for some or all selected beads, in each of the previous screening experiments and with **96** Gln was found for two beads. Of course, with the deprotected peptide **97**, the free acid of the glutamic acid side chain may now be bound to the diamidopyridine in preference to the *C*-terminus carboxylic acid group, leading to the different sequence observed in this experiment. None-the-less, it is clear that selective binding of the side chain deprotected peptide **97** is possible with this set of tweezer receptors.

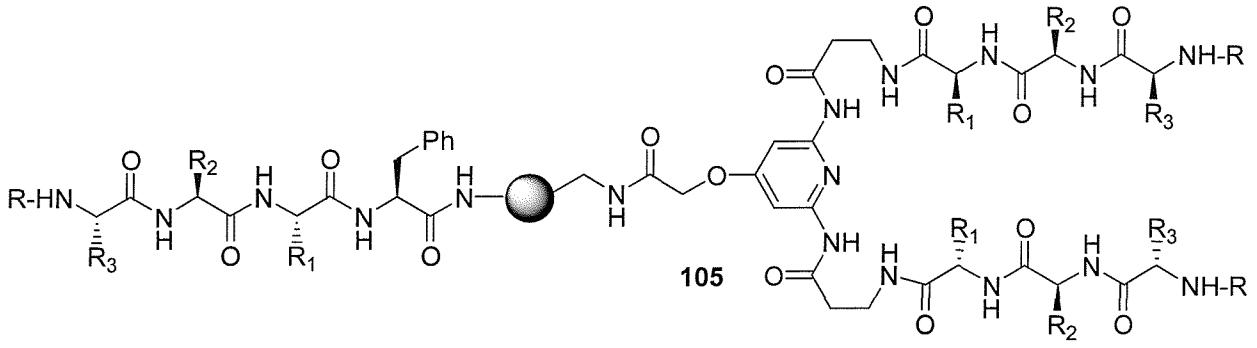
Beads	AA_0	AA_1	AA_2	AA_3
1	Phe	Ala	Leu	Ala
2	Phe	Ala	Leu	Ala
3	Phe	Gln	Leu	Ala
4	Phe	Gln	Val	Ala
5	Phe	Met	Val	Ala

Table 6: Sub-library I with red dye–labelled deprotected peptide ***97***



Graphic 6: Sub-library I with red dye–labelled deprotected peptide ***97***

At the same time that these studies were being carried out, other work in the group by Arienzo had produced related tweezer receptor libraries, but using a β-alanine in place of phenylalanine as the first amino acid on the tweezer side arms.[99] Screening experiments were carried out by Arienzo with library **105** to see how a small structural change to the receptor affected binding selectivity (scheme 23).

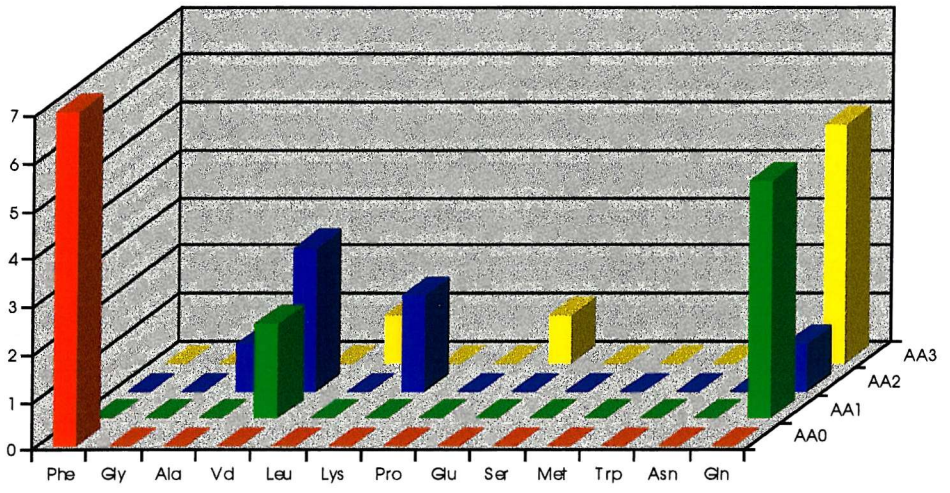


Scheme 23: Tweezer receptors library incorporating β-alanine

Side chain protected peptide **96** gave no apparent selectivity in a screening experiment with library **105**. However, screening of library **105** (with *N*-terminus and side chain protected analogous to sub-library I) in chloroform with red dye-labelled deprotected peptide **97** gave good selectivity. Seven highly coloured beads were taken from the screening experiment and sequenced by Edman degradation. The sequencing results gave a high level of consensus with glutamine at the AA_1 position for five beads and at the AA_3 position for four beads, with the sequence Gln-xxx-Gln clearly favoured and Val as the most common residue at the AA_2 position (table 7).

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	Gln
5	Phe	Gln	Lys	Gln
6	Phe	Val	Ala	Glu
7	Phe	Val	Gln	Leu

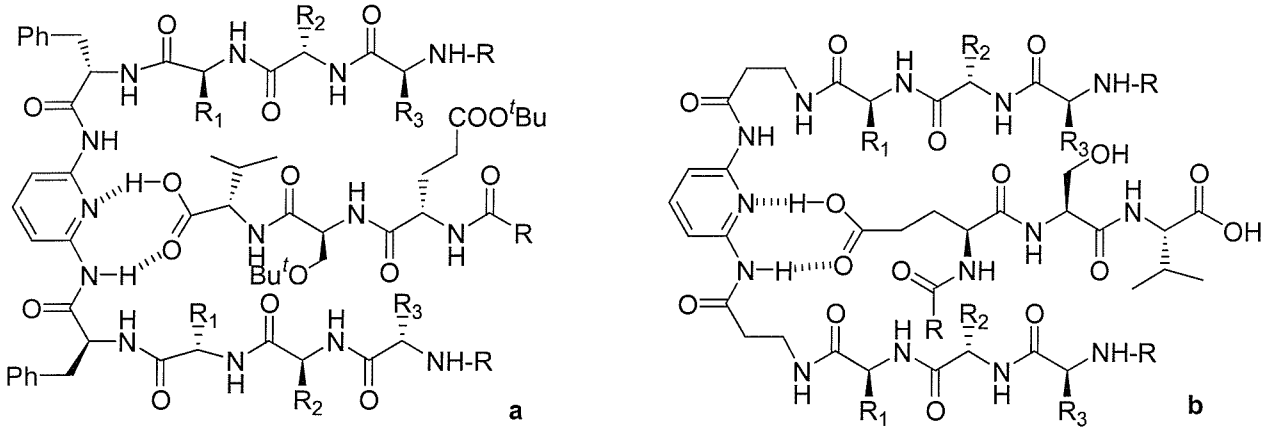
*Table 7: Screening results for library **105** with red dye-labelled deprotected peptide **97**, obtained by Arienzo.*[99]



*Graphic 7: Library **105** with red dye-labelled deprotected peptide **97***

With the two receptor libraries **95** and **105**, the screening results using the same peptide guest **97**, gave sequences with clear similarities at their first and second position, which suggest that there are similar interactions between the backbone of the peptide guest and the tweezer receptor side-arms. The side-chain protected peptide **96**, however, behaved very differently with the two libraries **95** and **105**: excellent selectivity was observed with library **95** and no apparent selectivity with library **105**. A possible explanation for these observations is that, for the side-chain protected peptide **96** with receptor derived from amidopyridine derivative **84** (library **95**), binding involves a strong amidopyridine-carboxylic acid interaction, which places the backbone of the peptide guest in a suitable position to bind with the tweezer side-arms of the receptor, possibly using a β-sheet-like interaction (scheme 24). The substitution of phenylalanine with β-alanine in the first position in the tweezer receptor with a consequence of an increase of the distance between the two arms destroyed the alignment of the backbone of the peptide guest in relation to the tweezer side-arms of the receptor, and no selective binding is observed. Binding to the deprotected peptide **97**, on the other hand, is complicated by the presence of two terminal

carboxylic acid moieties from the peptide, and from the side-chain of the glutamic acid and both may bind to the amidopyridine. If the glutamic acid side-chain is used, the distance between the carboxylic acid unit and the rest of the peptide backbone is both longer and more flexibile, and also in consideration of the increased separation of the amidopyridine unit and the receptor side-chain in the library **105** may now be more appropriate for binding the peptide gust, but the increased flexibility of the side-chain carboxylic acid also allows binding with tweezers from library **95**.



*Scheme 24: a. Binding the sidechain protected peptide **96** with the C-terminal carboxylic acid. b. Binding the sidechain deprotected peptide **97** with the glutaric acid sidechain carboxylic acid*

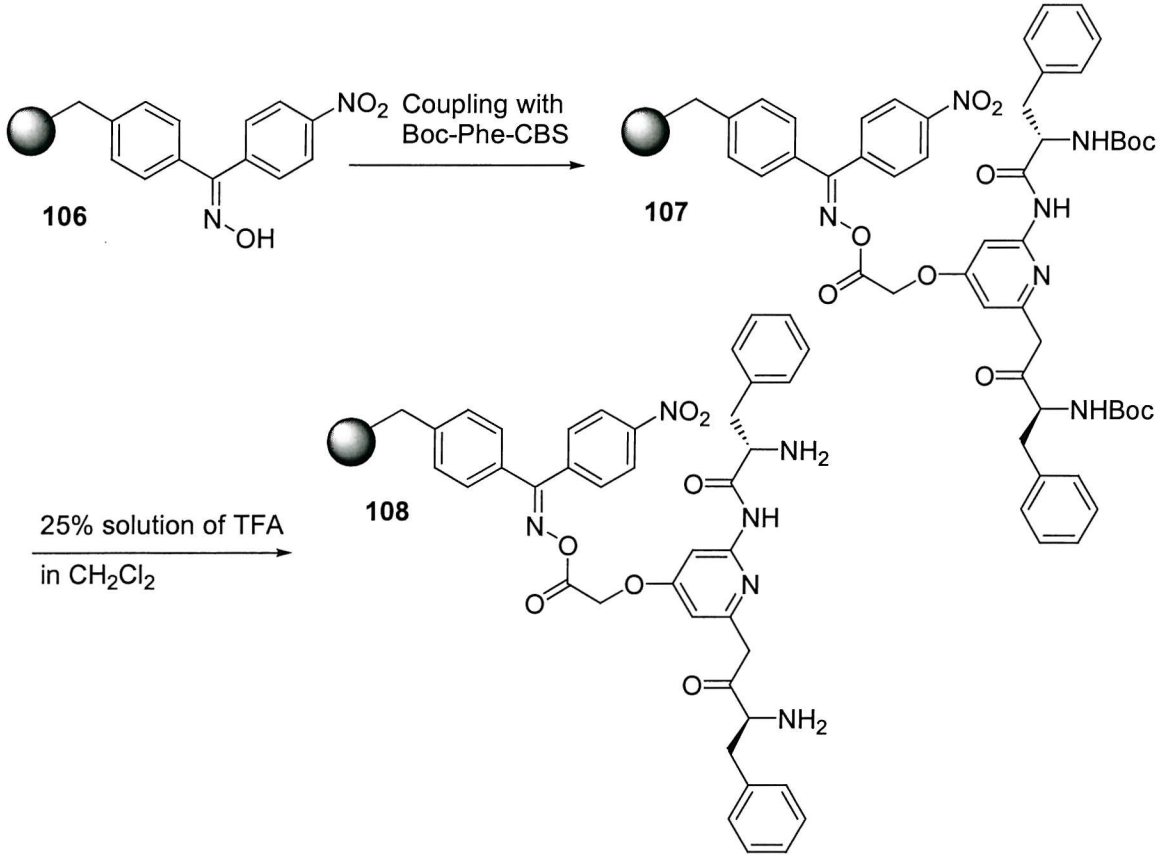
*e) Screening between sub-library I, II, III and IV with peptide **96** and **97** in aqueous solution*

As in the previous work from Fessman, no selective binding was observed with either of the guest-library combinations in a range of buffered aqueous solvents. All four sub-libraries were screened with guest **96** and **97** in buffered aqueous solution at different pH (6.0 and 9.2). It was concluded is that the diaminopyridine unit is not a good carboxylic binding site in water, which is not surprising since in polar solvents there is a significant energetic penalty in desolvating hydrogen bonding functionalities.

2.7 Synthesis of Single Tweezer Receptor

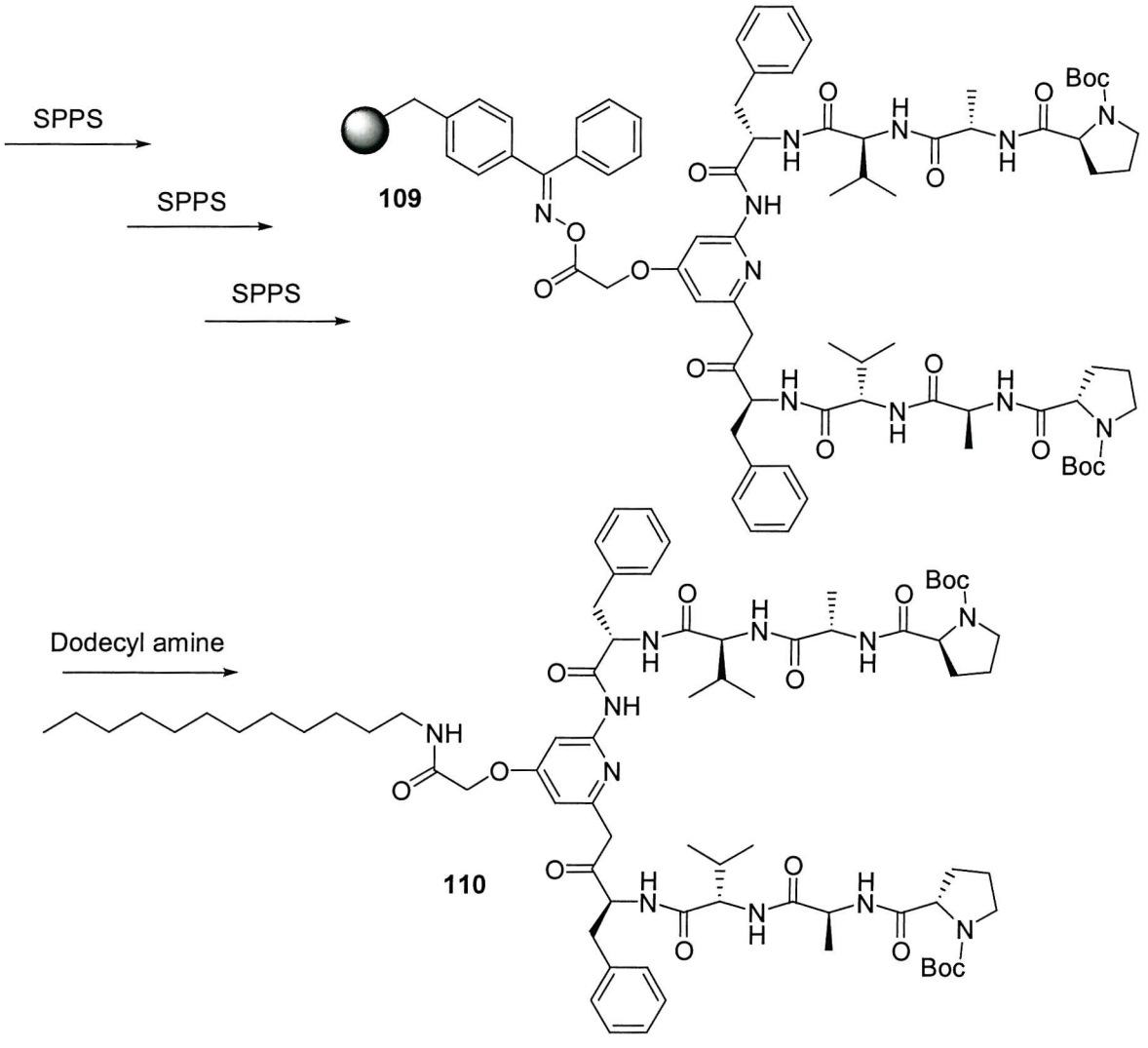
In order to establish that the observed binding of a red dye-labelled peptide, with resin bound tweezers, was also operating in free solution, a single tweezer with side arm sequence Phe-Val-Ala-Pro which was identified as the consensus sequence for binding peptide **96** was synthesised

The synthesis of the single tweezer receptor was carried out on solid phase. The Boc-CBS (carboxylic binding site) **84** was attached to Oxime resin **106**,[100,101] using PyBOP and DIPEA in CH_2Cl_2, to afford the solid supported CBS **107**. The Boc protecting groups were removed using a 25% solution of TFA to afford the free amine **108**.



*Scheme 25: Synthesis of solid supported Boc-CBS **108***

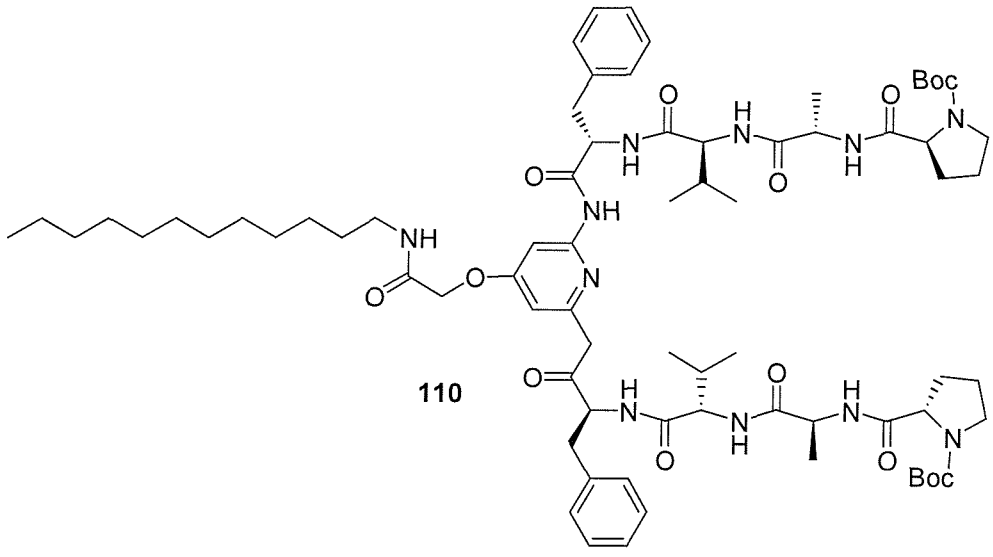
After three couplings with the familiar amino acids: *N*-Boc-L-Valine, *N*-Boc-L-Alanine and *N*-Boc-L-Proline, using TBTU, HOBt and DIPEA, and a 25% solution of TFA for Boc deprotection after each coupling, compound **109,** attached to the solid support, was prepared. The cleavage of the compound from the resin was carried out using a solution of dodecyl amine.[102,103] The presence of dodecyl amine bound to the tweezer, as a lipophilic residue, helped the solubility of the tweezer in chloroform. The final compounds so cleaved from the resin underwent further purification by semi-preparative HPLC analysis to afford the single tweezer receptor **110** ready for binding studies.



*Scheme 26: Synthesis of tweezer receptor **110***

2.8 Solution Binding Studies

Binding studies with tweezer receptor **110** and peptide guest **96** were attempted using UV spectroscopy and using NMR spectroscopic titration. [104, 105]



*Scheme 27: Host tweezer receptor **110***

For binding studies with UV spectroscopy two solutions were prepared. A solution of host tweezer receptor **110** was prepared by dissolving in chloroform giving a solution 2.6×10^{-5} M. A solution of peptide guest **96** was prepared in chloroform (concentration of **96** = 2.08×10^{-3} M). Titration studies using these solutions, however, did not give reproducible data. Similarly with NMR spectroscopic titration no evidence of binding was obtained, as none of the signals for the receptor shifted on addition of the guest. It is possible that the receptor concentration required for the NMR spectroscopic experiment leads to significant aggregation of the receptor, and consequently no binding. The failure to measure a binding constant in solution was disappointing, but does suggest that the nature of the solid support plays a role in promoting binding of receptors when attached to the solid phase. The tweezer receptor in free solution can give a considerable number of intramolecular and intermolecular interactions between the peptidic arms. On the other hand when the tweezer receptor is attached to the solid support such interactions may be suppressed. Despite the failure to measure a binding constant in solution, the combination

of rational and combinatorial approaches for the synthesis of a large number of receptors and screening of the libraries with a given peptide guest has proved to be a powerful and efficient method for discovering novel resin-bound receptors for peptides.

Chapter 3

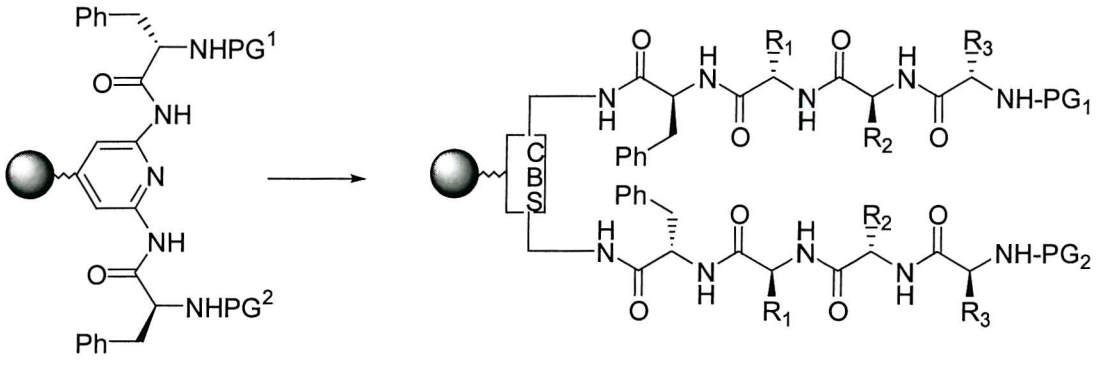
3.1 Introduction

The main aim of this thesis is the synthesis of tweezer receptors library using combinatorial chemistry to allow the synthesis of a large number of compound. The basic structure of a tweezer receptor incorporates a specific moiety as binding site (CBS), for carboxylic acid and carboxylate, and as well as a starting point of the synthesis of two peptidic arms. The synthesis of these systems requests a specific design. First of all the choice of the CBS, which is a rational approach in the synthesis followed by the combinatorial approach to give in a short time a large number of compounds. The union of these two different approaches allows the chemist to study the specific affinity of this system to the given peptide guest where one of these components is changed. In the first part, the possibility to introduce a sequence of amino acids in the two peptidic arms is a non-symmetrical way is describe, which afford a larger number of receptor increasing the possibility to find the specific receptor for the given peptide guest. In the second part the use of peptidomimetic residues in the two peptidic arms instead of amino acid is described. This change can increase the affinity of the tweezer receptor to the peptide guest. The last part describes the synthesis of a library of receptor incorporating a bicyclic guanidinium as a specific binding site for carboxylates. All these modifications and variations have proven the power of this double approach in the synthesis of a library of receptors.

3.2 Non-symmetrical tweezer library

Chapter 2 shows the synthesis of a tweezer receptor library featuring a diaminopyridine as a carboxylic acid binding site (CBS). A diaminopyridine unit, suitable for attachment to the solid support, was prepared and incorporated two phenylalanine units as the starting point for the generation of the variable peptidic arms. The libraries were successfully screened with various dye-labelled tripeptides to identify tweezer receptors. Following the success of these system in the selective recognition of peptide guests and in order to investigate them in more details, the next aim was the synthesis of a tweezer receptor

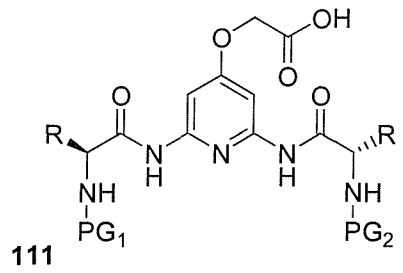
library with the two peptidic arms carrying a different sequence of amino acids. The design of the new library provides receptors with non-symmetrical structures, compared with the previous "symmetrical" receptor library (scheme 28).



Scheme 28: non-symmetrical library

The design of the library involved the use of the diaminopyridine unit as a specific binding site (CBS). Once again, the diaminopyridine unit incorporated two phenylalanine units as the starting point for the synthesis of the peptidic arms. The introduction of two different sequences of amino acids in the synthesis of the tweezer receptor library significantly increases the number of different receptors which can be accessed in the library, which in turn increases the possibility of finding the optimal match in the selective recognition of the peptide guest. The aim of this project was to find a selective receptor for the peptide red dye-labelled D-Ala-D-Ala. The bacterial cell precursor peptide L-Lys-D-Ala-D-Ala-OH represents a particularly interesting target because a selective receptor for this peptide sequence might lead to mimics for the vancomycin family of antibiotics.[50]

The introduction of two orthogonal protecting groups in the synthesis of the CBS is the key step in the synthesis of an asymmetric library. The first target was therefore a synthesis of CBS **111** with two different orthogonal protecting groups.

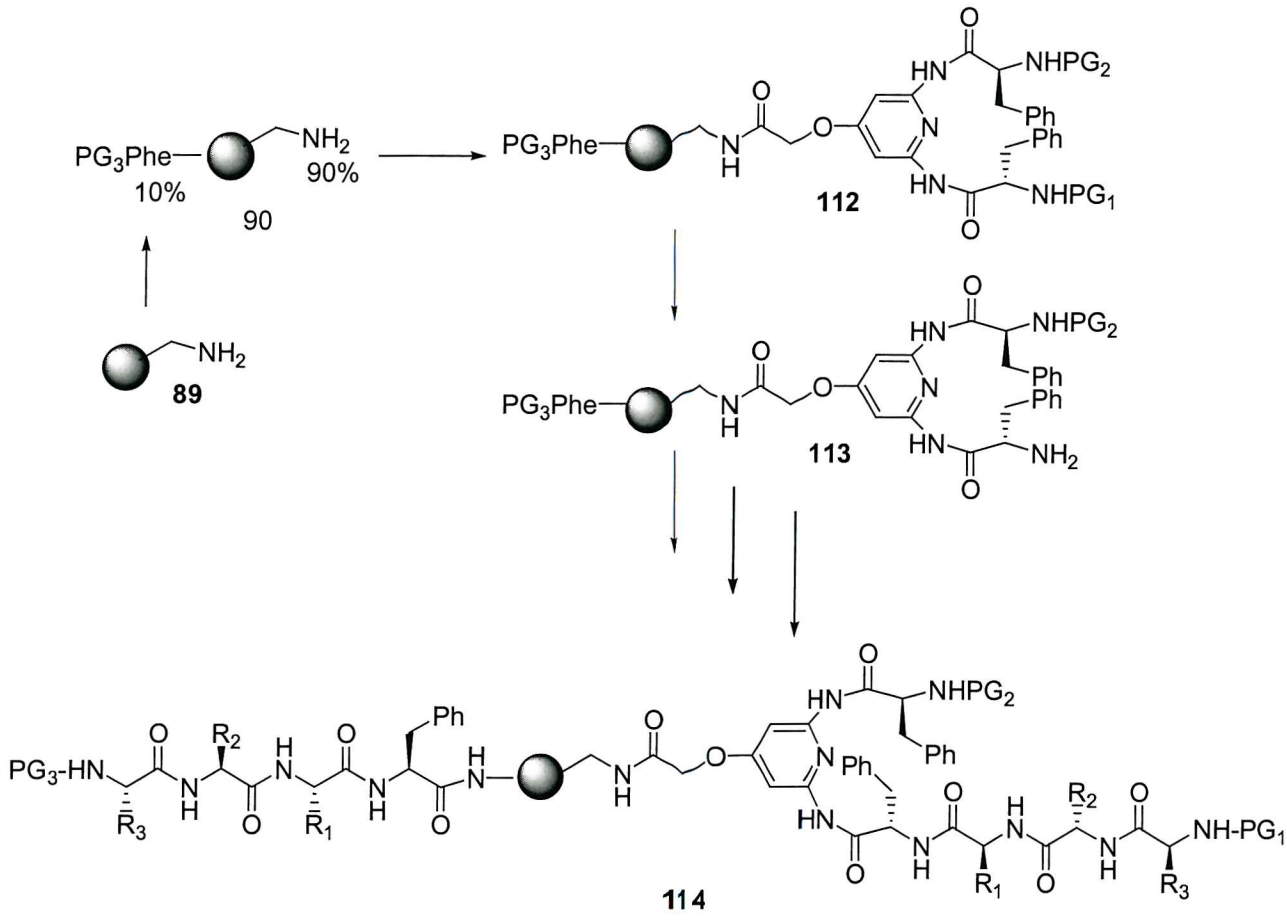


111

Scheme 29: Asymmetric CBS 111

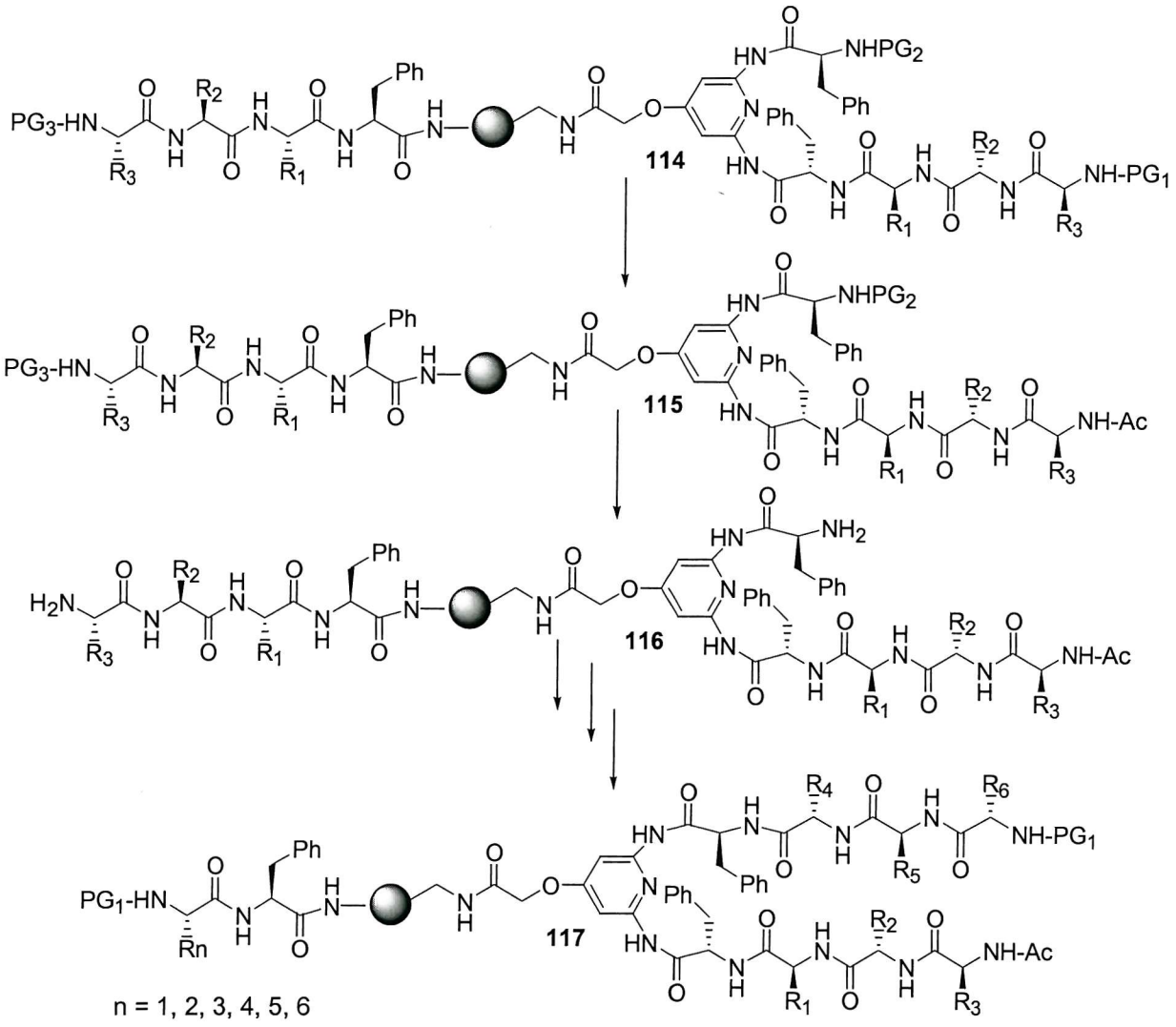
The strategy was to start the synthesis of the library using TentaGel resin **89**. A coding strand would be introduced by coupling 10% of the free amino groups with a protected phenylalanine. The remained 90% of the free amino groups in the resin would be coupled with the asymmetric CBS **111**, containing two orthogonal protecting groups, to afford the solid supported CBS **112**. Removal of the PG_1 protecting group would afford resin **113** with a free amino functionality ready for three consecutive coupling steps with different amino acids using "split and mix" strategy.[76]

Resin **113** would be divided in a number of portions and different amino acids $PG_1NHCH(R)COOH$, coupled to each portion. The following step was to remove the protecting group from the coding strand PG_3 and to couple the $PG_3NHCH(R)COOH$ to the same portion of resin. This procedure guarantees the same amino acids sequence in the arm of the tweezer and in the coding strand affording exactly the same compound in every single beads, in total respect of the principle "one bead one compound".[78] At that stage the resin would be mixed again and the same procedure repeated for other two cycles to give a library of "half" tweezer **114** (scheme 30).



Scheme 30: Synthesis of tweezer receptors library

Remove of PG_1 protecting group and acetylation with acetic anhydride would afford the solid supported library **115**. At that point the PG_3 and the PG_2 could be removed to give resin **116** (scheme 31). The resin could now be divided in "n" different portions and coupled with different amino acid, using again "split and mix" procedure for other three cycles to afford the final asymmetric tweezer receptors library **117**, with a heptapeptide coding strand ready for screening experiments.



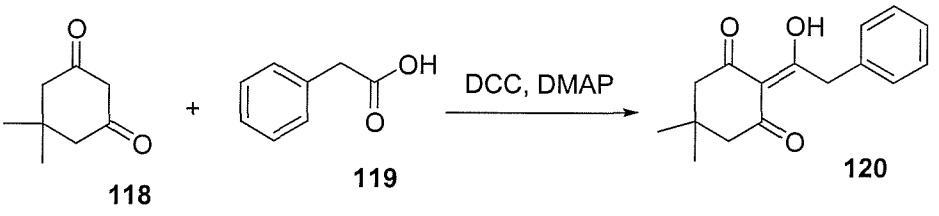
Scheme 31: Synthesis of tweezer receptors library

3.3 Synthesis of the CBS

The first part of the project required the synthesis of the CBS **111**. The key aspect of the synthesis was to find three orthogonal protecting groups: one for the coding strand and one for each arm of the tweezer.[106] We decided to use Fmoc in one of the arms of the tweezer receptor and Boc in the coding strand. The choice of Fmoc, base sensitive protecting group for amine functionality, was based on the versatility of this group in solid phase chemistry and the compatibility with a large number of other amino function protecting group. The choice of Boc protecting group allowed the use of Boc strategy in the synthesis of the

coding strand in presence of the Fmoc group. The main force was also the large access of all different amino acids commercial available as protected Boc and Fmoc. However, the choice of the third orthogonal protecting group was not straightforward. One possibility was the use of the primary amine protecting group N-1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde) and the analogous, Ddiv. Due to the ease of removal (2% hydrazine hydrate in DMF within 10 mins) and its relative stability to TFA and piperidine-DMF the use of those protecting group was attempted.[107] The selective primary amine protecting properties of Dde have been exploited successfully for the solid phase synthesis of polyamines such as spider toxins[108] and trypanothione.[109] However, the protection of α-amino acids has not been reported.

The first step was the synthesis of the Dde protecting group precursor **120**. The synthesis was readily achieved by acylation of dimedone **118** with a DCC-DMAP activated phenyl acetic acid **119**,[110] the reaction mixture was stirred at room temperature for 60 hours, to afford after crystallization from methanol the pure compound **120** in 58% yield (scheme 32).



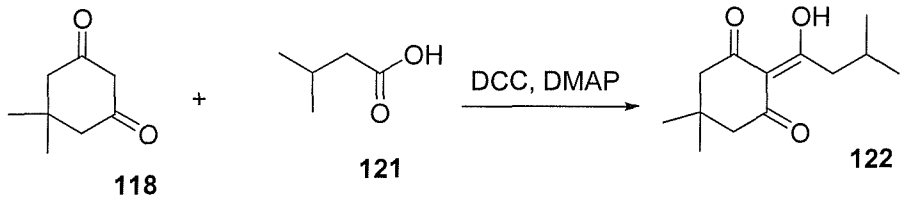
118 **119** **120**

*Scheme 32: Synthesis of Dde derivative **120***

The following step was the protection of amino acids. Several attempts were carried out. The first attempt was to try to protect phenylalanine using a catalytic amount of TFA in CH_2Cl_2 at room temperature for 12 hours. The reaction was monitored by TLC analysis and showed only the presence of starting material. The same conditions were used refluxing the reaction's mixture in CH_2Cl_2 for three days. In this case TLC analysis showed the presence of a new spots but still the major spot was the starting material. Prolonging the reaction time to three days did not afford the desired compound. Indeed under standard conditions (catalytic TFA, EtOH or CH_2Cl_2, at room temperature or

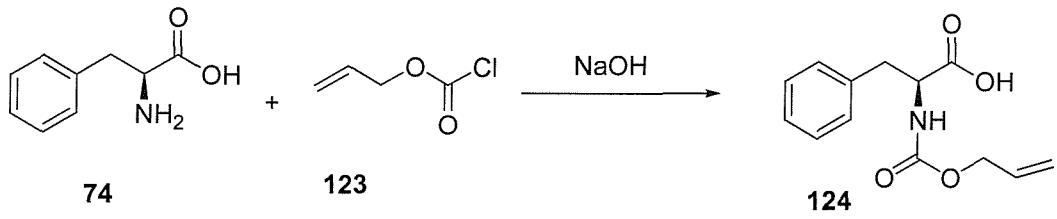
refluxing) it was not possible to protect any of phenyalanine, glycine or β-alanine, with Dde.

The use of Ddiv was also tried. The reaction to synthesise the Ddiv protecting group was performed in the same way: acylation of dimedone **118** with the DCC-DMAP activated valeric carboxylic acids **121**,[110] the reaction mixture was stirred at room temperature for 60 hours, to afford after crystallization from ether the pure compound **122** in 14% yield (scheme 33).



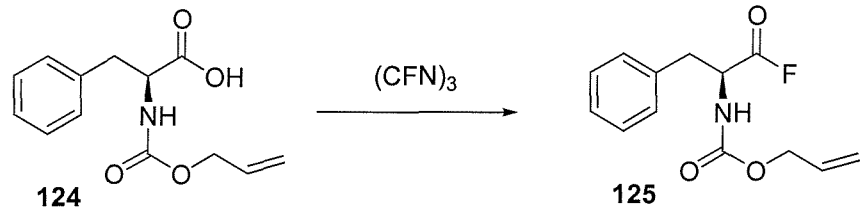
*Scheme 33: Synthesis of Dde derivative **122***

However, again, the use of Ddiv protecting group to protect phenylanine and β-alanine was not successful. With the failure of Dde and Ddiv it was decided to use the Aloc protecting group. The Aloc group is robust enough to withstand both the basic condition of the removal of Fmoc groups and the acidic condition required to remove the Boc group. Normally the Aloc deprotection can be achieved on solid phase using tetrakis(triphenylphosphine)palladium(0) in the presence of methylmorpholine.[111] The decision for this protecting group was not straightforward because it was observed in previous studies, in the group that in a model system this method gave a dark colour to the resin making visualisation of the active beads during the screening experiment difficult. Later improvements in the use of Aloc group on solid phase chemistry showed the possibility to loading the Aloc protected amino acid on solid support without leaving coloured residues. The first aim was the synthesis of Aloc-phenylalanine. The reaction was achieved using a standard procedure, allyl chloroformate **123** and L-phenylalanine **74** were mixed in aqueous NaOH at 0°C and stirred for 15 hours at room temperature to afford the desired compound **124** as a colourless oil (scheme 34).[112]



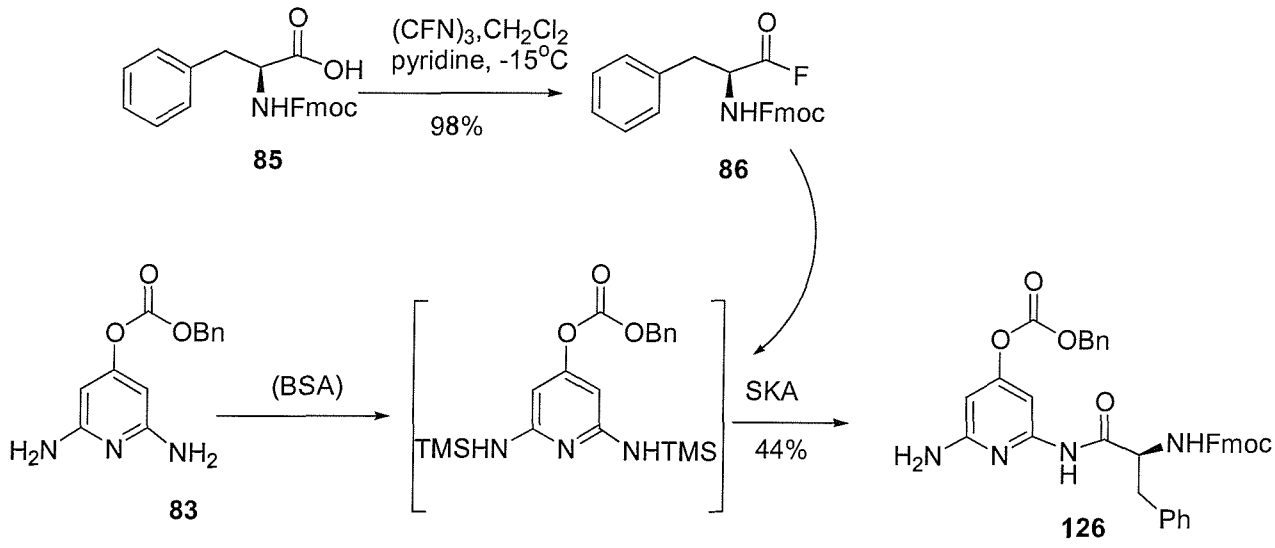
*Scheme 34: Synthesis of Aloc-phenylalanine **124***

Aloc-phenylalanine **124** was then transformed in Aloc-Phe-F **125** using cyanuric fluoride in dry CH_2Cl_2 to afford the final compound as colourless oil in >99% yield (scheme 35).[94]



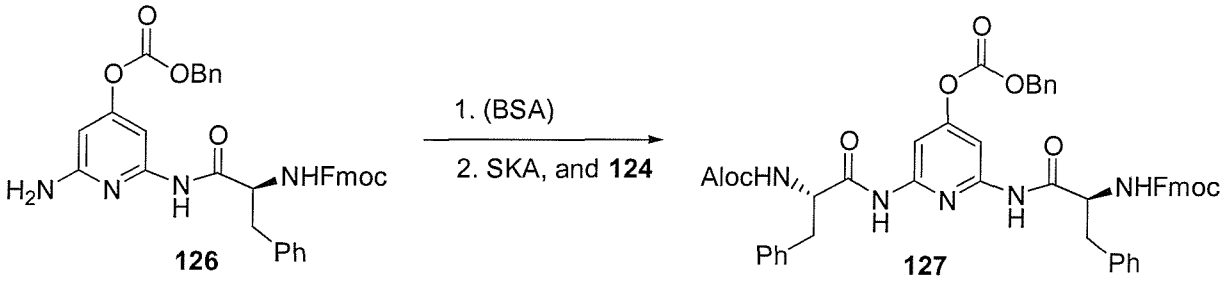
*Scheme 35: Synthesis of Aloc-phenylalanine fluoride **125***

The coupling of Fmoc-Phe-F was achieved using precursor **83** under standard procedure. The precursor **83** was stirred with BSA for two hours in dry CH_2Cl_2 and Fmoc-Phe-F **86** was added followed by SKA and stirred at room temperature for 12 hours to afford the desired compound **126**, with only one amino group coupled with a Fmoc protected phenylalanine.



Scheme 36: Coupling with Fmoc-Phe-F in presence of SKA

The last step in the synthesis of the asymmetric CBS was the coupling of Aloc-phenylalanine fluoride to compound **126**, to afford the final CBS **127**. The first attempt used the same conditions as before (CH_2Cl_2, room temperature, overnight) but did not yield the desired compound.



Scheme 37: Coupling with Aloc-Phe-F in presence of SKA

Many more coupling reagents were used such as EDC and TBTU but none yielded the desired product and it was only later that Arienzo[113] was able to optimise the conditions of this coupling to get the desired compound by refluxing the reaction over night in acetonitrile.

3.4 β-Sulfonamidopeptides in tweezer structures

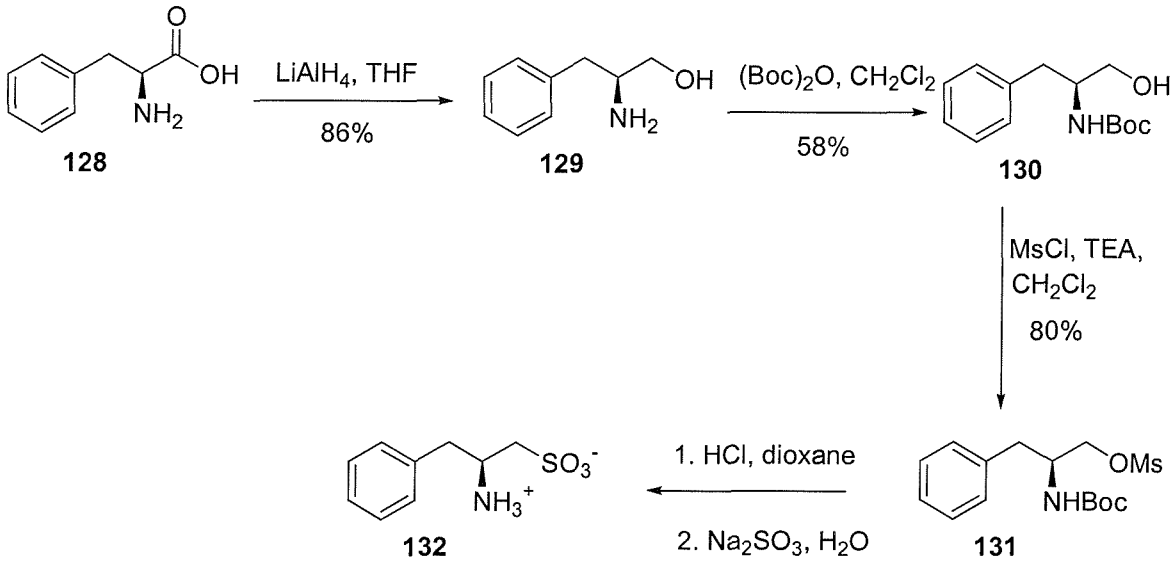
Peptides are attractive targets for drug discovery because of their affinities and specificities towards biological receptors. However, the poor bioavailability and the rapid enzymatic degradation of peptides *in vivo* have generally limited their therapeutic application. One approach to overcome this obstacle has been the development of non-natural biopolymer scaffolds which may show improved pharmacological properties relative to peptides.[88,89,114] The ability to assemble large synthetic oligomers efficiently also provides an opportunity to generate unnatural polymers with defined secondary and tertiary structures. Such structures should provide increased insight into the relationships between monomer structure and polymer conformation and may provide new classes of folded polymers with novel properties.

Gennari's group has developed an efficient synthesis to replace the labile peptide bond with mimetic groups, particularly pseudopeptides characterized by the presence of a sulfonamide bond. This modification creates a peptide bond surrogate with significant changes in polarity, hydrogen-bonding capabilities, and acid-base character. In contrast to the CONH moiety of peptides, which provides both a strong hydrogen bond donor and acceptor, the SO_2NH moiety of sulfonamidopeptides provides a very strong donor NH, but the SO_2 group is only a weak acceptor.[115] Furthermore, the sulfonamide bond should show enhanced metabolic stability and structural similarity to the tetrahedral transition state involved in the amide bond enzymatic hydrolysis.[116] This makes sulfonamidopeptides promising candidates in the development of protease inhibitors and new drugs. The oligomers and the polymers should also be interesting probes of the molecular scaffolds, with specific pseudopeptide backbone conformations based on the hydrogen bonding network. The introduction of peptide surrogate, that increase polarity and hydrogen bond donation capability, in a tweezer structure should increase the affinity of the tweezer for a peptide substrate. The introduction has already described the use of β-sulfonamidopeptides in tweezer structures. This chapter describes the synthesis of diamidopyridine derived tweezer with β-sulfonamidopeptides side arms. The use of β-sulfonamidopeptides for the arms of the tweezer receptors should increase the interaction with the peptidic backbone of a given peptide guest. A screening experiment of two dye-labelled tweezer receptors

against an inverted peptide library and the binding studies of a tweezer receptor with a peptide guest are also described.

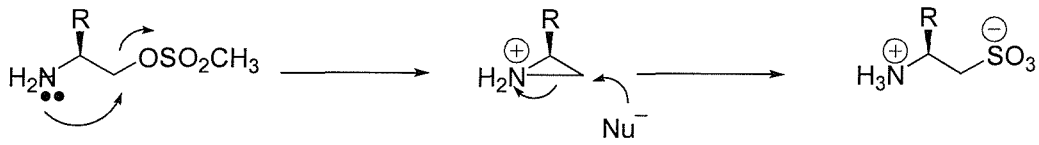
3.5 Synthesis of β-sulfonamidopeptides

β-Sulfonamide chloride **134** derived from the commercial single enantiomer L-phenylalanine was prepared. The amino acid **128** was reduced to the corresponding alcohol derivative **129** using $LiAlH_4$ in dry THF under reflux at 90°C for 4 hours, to afford the pure crystalline compound in 86% yield.[117] The free amino group from alcohol derivative **129** was protected with Boc using di-*tert*-butyl dicarbonate in CH_2Cl_2 to gave the desired compound **130** in 58% yield.[118] The next step was the transformation of Boc-alcohol derivative **130** to the mesylate **131** using triethylamine and methanesulfonyl chloride in CH_2Cl_2. The pure compound was obtained in 80% yield, after purification by flash chromatography, as a white crystalline product.[119] The Boc protecting group was removed from mesylate **131** using a solution of HCl in dioxane. The reaction was completed in 1 hour, the salt was not isolated but treated with a solution of Na_2SO_3 in water to displace the mesylate group and afford the sulfonic acid **132** (scheme 38).



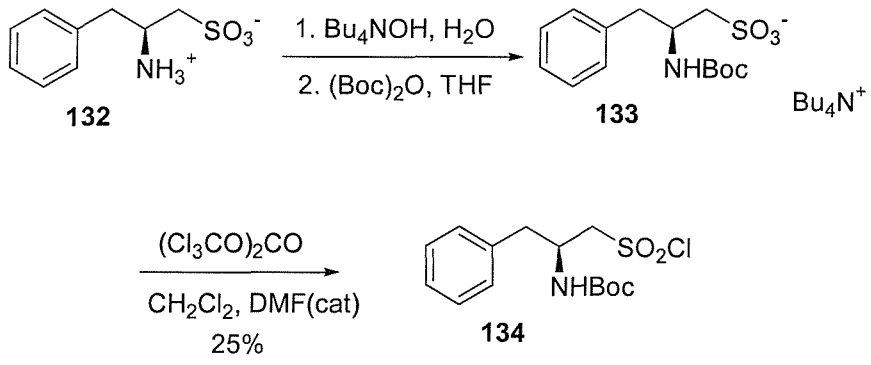
*Scheme 38: Synthesis of sulfonic acid **132***

This reaction can be seen as S_N2 or S_N1 *via* the aziridine intermediate (scheme 39).



Scheme 39: Mechanism via aziridine intermediate ($Nu^- = SO_3^{2-}$)

The β-amino sulfonic acid **132** was transformed to the corresponding tetrabutyl ammonium salt using a solution of $Bu_4NOH.30H_2O$ in water and stirred for 10 mins. In the same reaction vessel a solution of di-*tert*-butyl dicarbonate was added in THF to afford the amine group protected as its Boc derivative in the final tetrabutylammonium salt **133**. The last step was the transformation of tetrabutyl ammonium salt **133** into the sulfonyl chloride **134** using *bis*-trichloromethyl carbonate in CH_2Cl_2. The reaction afforded the compound in 25% yield (scheme 40).[120]

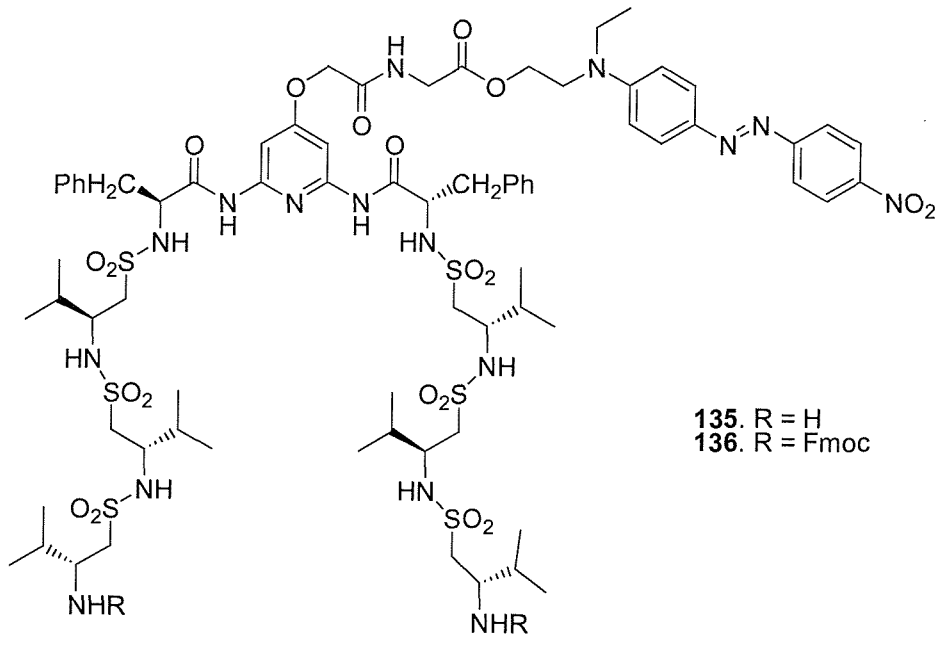


*Scheme 40: Synthesis of sulfonyl chloride **134***

3.6 Screening Experiments with Tweezer 135 and 136

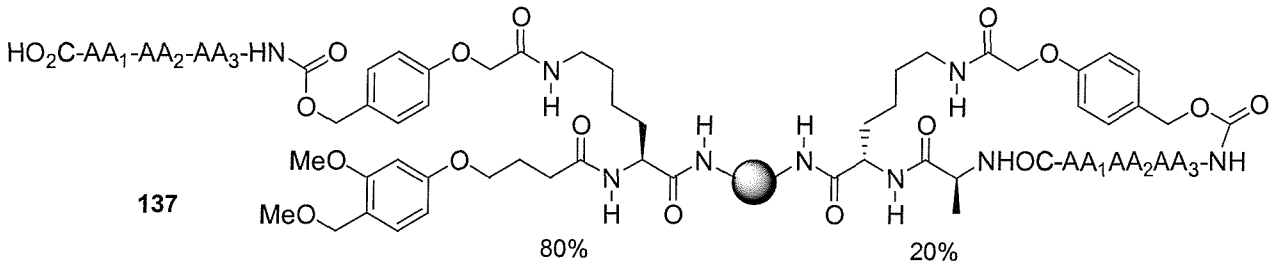
As part of a collaborative study, Gennari's group in Milan synthesised two different tweezer receptors using the 2,6-diamidopyridine derived CBS (prepared in Southampton), and following the synthetic strategy discussed in section 2.2. The tweezer receptors **135**

and **136** were dye-labelled to allow visualization during screening experiments (scheme 41).



*Scheme 41: Gennari's tweezer **135** and **136***

Screening experiments using tweezer **135** and **136** were carried out in Southampton. For the screening experiment a sample of inverted peptide library **137**,[92] (previously prepared in the group by Fessman) (scheme 42) was equilibrated in chloroform for 24 hours. Aliquots of red-dye labelled tweezer **135** or **136** in chloroform were added and equilibrated for a further 24 hours. Unfortunately, the screening experiment showed no selectivity and no red beads could be sent for Edman degradation.



Scheme 42: Inverted peptide library

The absence of any selectivity in this case suggested the presence of strong intramolecular hydrogen bonds between the receptor strands. Interaction with a guest would be possible only by breaking those bonds and unfolding the receptor with an energetic cost.

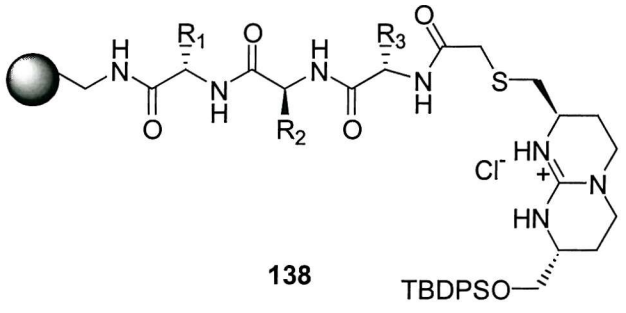
With the failure of the screening experiment (described above) the sulfonamide tweezer were investigated in more detail. NMR spectroscopic binding studies were carried out in Milan and was found that these receptors showed selectivity for *N*-Cbz-D-Ala-D-Ala peptide, but confirmed the presence of strong intermolecular interactions which significantly reduce the binding potential of the receptor.[121]

In conclusion, these studies showed the power of the combination of different components which in a rational approach can allow the synthesis of enantioselective receptors for peptide guests: the tweezer receptors, despite their inherent flexibility showed high selectivity for a given peptide guest; the use of a CBS as specific binding site for the terminal carboxylic group of the peptide guest gave a large contribution in the recognition process, and the sulfonamide peptides in the sidearms of the tweezer receptors are actively involved in the recognition event through selective interaction with the peptide backbone.

3.7 Use of a cyclic guanidinium as a CBS

The synthesis of specific receptors for peptide guests can be approached in a lot of different ways. The advantages of inserting different binding components into the receptor structure for interaction with a guest have been already described (*vide supra*). The most active receptor can be obtained from a combination of two different approaches: firstly a rational approach in the choice of a specific moiety for the terminal carboxylic group of the peptide guest; secondly a combinatorial approach, which gave a high number of different combinations of active functionality able to give additional interactions with the peptide guest. This chapter describes the synthesis and the studies of the binding properties of a library of receptors **138** (figure 43), which contained a cyclic guanidinium as a specific recognition site for the carboxylate group. The receptor contained arms composed from a sequence of amino acids attached to the CBS. The synthesis of the receptor arm was carried out using a combinatorial approach, which gave a large number of different combinations of peptide sequences. As in the tweezer the interaction between the receptor and the peptide guest will be a result of additional factors such as CBS-carboxylic group

and all possible interaction between the arm and the backbone of the peptide guest.



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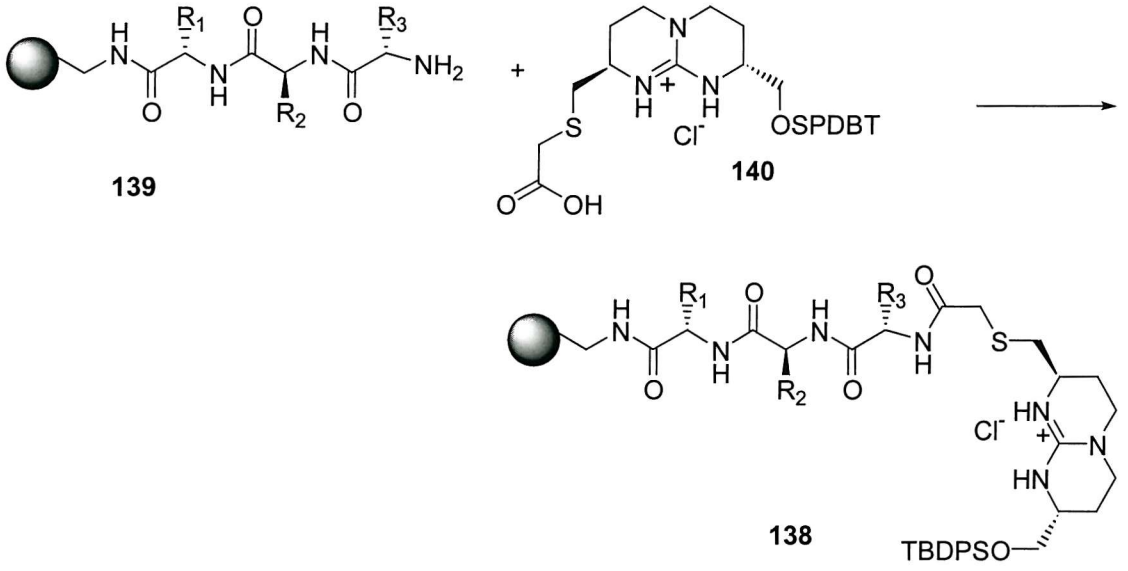
Scheme 43: Library of receptors contained a bicyclic guanidinium

The guanidinium unit presents the advantages that it remains protonated at a wide pH range and the binding event of carboxylate groups combines an electrostatic interaction with a bidentate hydrogen-bonding pattern. The bicyclic guanidinium offers these advantages in addition to the bicyclodecane framework forming an almost perfect match for carboxylate anions with the two guanidinium protons aligned in the same direction.

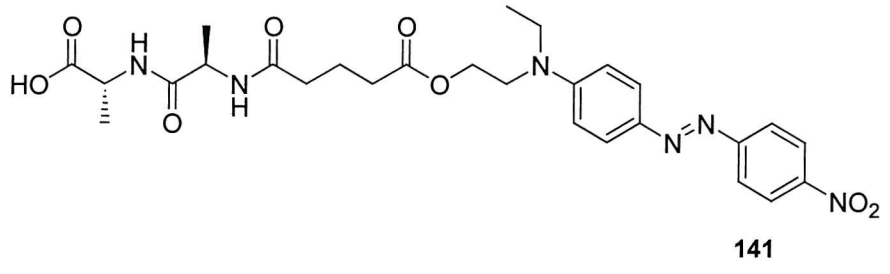
3.8 Synthesis and Screening of a Library of Receptors

This project was in collaboration with the research group of de Mendoza at the University of Madrid. The synthesis of guanidinium CBS **140** was carried out in Madrid using established procedures.[122] The synthesis of a library of receptors **138** was carried out in Southampton using a combinatorial "split and mix" approach on solid phase. Thirteen amino acids were used in a 2197 membered library of tri-peptides on TentaGel resin **139**. 10% of the library was reacted with Boc-Phe-OH to be use as a coding strand for Edman sequencing. The remaining 90% of the library were reacted with the guanidinium CBS, to give the final library of receptors **138**.



*Scheme 44: Synthesis of receptor' library **138***

The library was screened with red dye-labelled D-Ala-D-Ala **141** (scheme 45). The screening experiment was carried out in a buffered aqueous solution at pH=9.2. Beads were analysed in flat-bottomed glass pots under a Leica inverted DML microscope (magnification x 40) and they showed a 0.1% selectivity. Four active beads were manually removed from the pot and sent for analysis by Edman degradation.

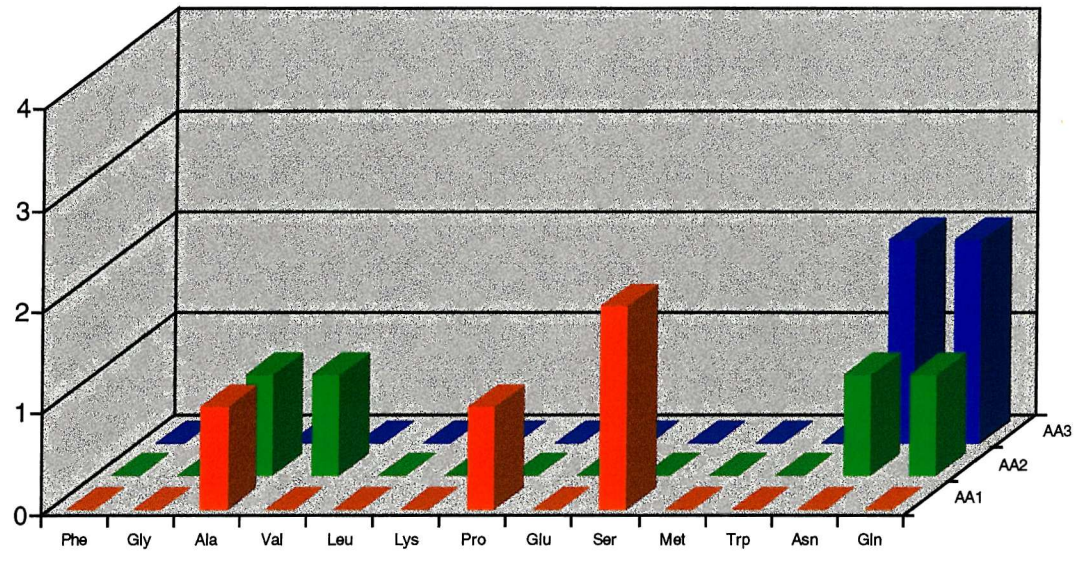


Scheme 45: Red dye-labelled D-Ala-D-Ala peptide guest

In the first position Ser(^{t}Bu) was found in two beads analysed and Ala and Pro were found in the third and fourth bead. The second position gave four different results: Ala, Asn, Val and Gln were found. In the third position Asn was found for two beads and Gln for the other two, results are summarised in table 8.

Bead	AA_1	AA_2	AA_3
1	Ser(^{t}Bu)	Asn	Gln
2	Ser(^{t}Bu)	Val	Gln
3	Ala	Ala	Asn
4	Pro	Gln	Asn

*Table 8: Screening between library **138** and peptide guest **141***

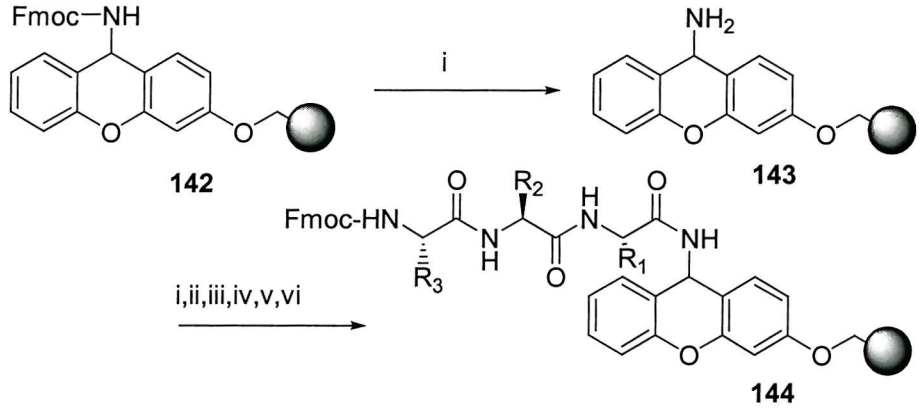


*Graphic 8: Screening between library **138** and peptide guest **141***

This result clearly shows the relevant importance of Asn and Gln in the first two positions closest to the CBS unit. Unfortunately, the small number of beads sent to be analysed could not give a well-defined consensus sequence of amino acids for a single receptor. Instead it was decided to synthesise all four receptors and study the binding properties in solution in order to determine if any of the compounds bound to the D-Ala-D-Ala sequence in free solution.

3.9 Synthesis of Single Receptors

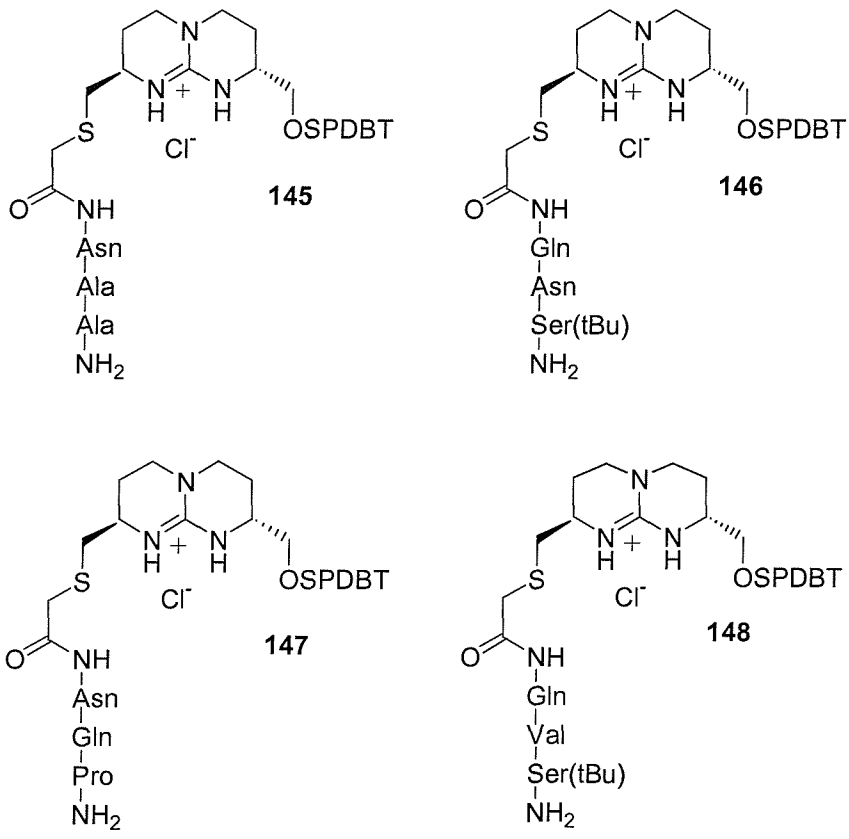
The starting point of the synthesis (carried out in Southampton) was the synthesis of the sequence of amino acids, using solid phase chemistry. The synthesis was carried out using Sieber amide resin **142**. The cleavage of the final compound from the Sieber amide resin affords a terminal amide group avoiding the presence of a carboxylic group at the end of the receptor arm, which may interact with the CBS, competing with the terminal carboxylic group from the peptide guest. The removal of the Fmoc group from Sieber resin **142** was carried out using a 20% solution of piperidine in DMF to afford resin **143** with a free amino group. The coupling of amino acids was carried out using the Fmoc strategy and DIC and HOBt as coupling reagents to afford the final peptides **144** attached to the solid support.



i) 20%piperidine in DMF; ii) Fmoc-NHCHR$_1$COOH, HOBt, DIC; iii) 20% piperidine in DMF; iv) Fmoc-NHCHR$_2$COOH, HOBt, DIC; v) 20% piperidine in DMF; vi) Fmoc-NHCHR$_3$COOH, HOBt, DIC.

Scheme 46: Synthesis of peptides

The cleavage of the final compounds from the resin required only 1% TFA solution in CH_2Cl_2 leaving the protecting group on the side chain of serine intact. The final pure compounds were obtained from ether precipitation and were sent to Madrid for the final coupling with the cyclic guanidinium CBS to afford the final receptors **145**, **146**, **147**, **158**.

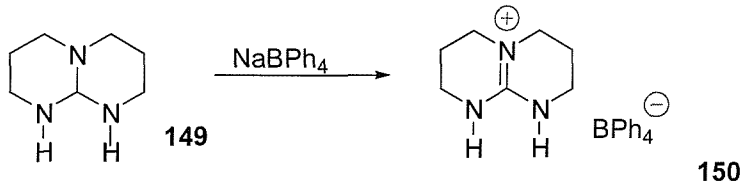


Scheme 47: Final receptors

3.10 Model Binding Studies

With the final receptors in hand, it was intended to determine the strength of the interaction using an isothermal titration calorimetry (ITC) experiment. ITC provides a measure of association strength, stoichiometry of binding, as well as thermodynamic parameters of association from a single experiment by directly measuring the heat produced from host-guest complexation. The heat evolved or absorbed during the addition of aliquots of guest into host solution is measured. This technique has been employed to investigate strongly associating species such as protein-DNA complexes,[123,124] enzyme substrate,[125] cyclodextrins,[126,127] and antibodies.[128] This method is superior to other techniques not only in the combination of association and thermodynamic information provided from just one experiment, but also in the ability to successfully evaluate multiple binding equilibria.

In preparation for the binding studies with the receptors **145-148** some ITC studies were carried out using a simple guanidinium salt and acetate. Thus commercial guanidine **149** was converted into the tetraphenylborate salt **150** following Hamilton's procedure.[34]



*Scheme 49: Preparation of guanidinium tetraphenylborate salt **150***

The receptor **150** was dissolved in neat DMSO to afford a 5mM solution. Tetrabutylammonium acetate was dissolved in neat DMSO to afford a 100mM solution of guest. For the titration experiment the solution of receptor was added to the calorimetry cell and the guest solution was introduced in fifty separate 5 μL injections at 25°C. The solution was continuously stirred to ensure rapid mixing, and maintained at 25°C. Dilution effects were determined by a second experiment in which the same acetate solution was added to pure DMSO. Any heat changes were subtracted from the raw titration data to produce the final binding curve. The final binding isotherm displayed in Figure 45 is characteristic of moderately strong, exothermic 1:1 complex. The value for association constant (K_a = 4573 M^{-1}) was determined by a non-linear least squares fit for a one site binding model (figure 45).[129] The experiment was repeated 2 times to give the same K_a indicating a reproducible result. The same experiment has been reported in literature from Hamilton to give an association constant K_a = 5600 M^{-1}. The strength and exothermic nature of this association suggests a complex held together by strong hydrogen bonding such as the expected bidentate interaction between the cyclic guanidinium and carboxylate group from acetate.

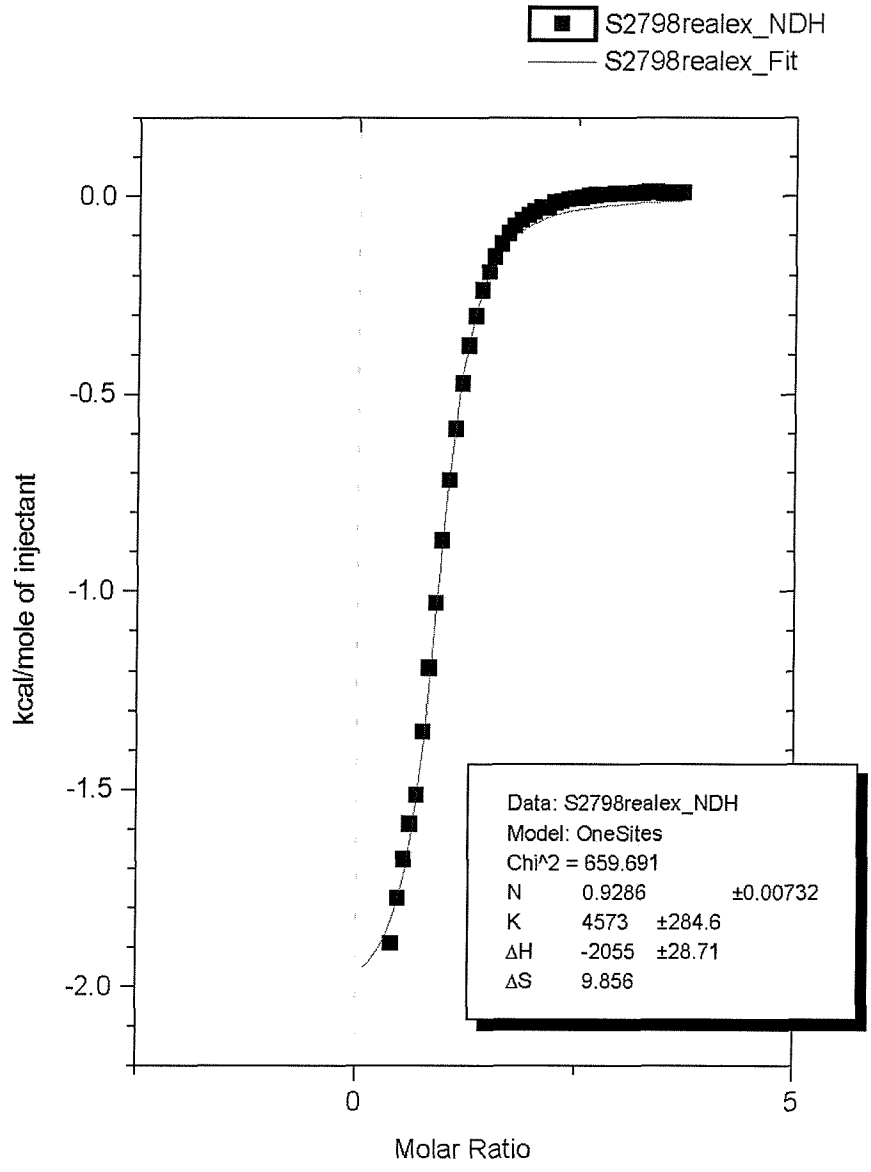


Figure 45: The binding isotherm in DMSO

Screening experiments with library **138** had been carried out in buffered aqueous solution. It was trerefore desirable to measure binding constants in acqueous conditions. The solubility of **150** in water was very low, however, and a DMSO/H_2O (70 : 30) mixture was required to solubilise **150**.

When the experiment was carried out using 70% DMSO in water, both the association strength and the thermodynamic nature of the complex are changed, due to increased solvation of both host and guest binding sites in water. Complex formation must be

preceded by energetically unfavourable desolvation of these sites, resulting in a lowered binding affinity ($K_a = 3500\ M^{-1}$) (figure 46).

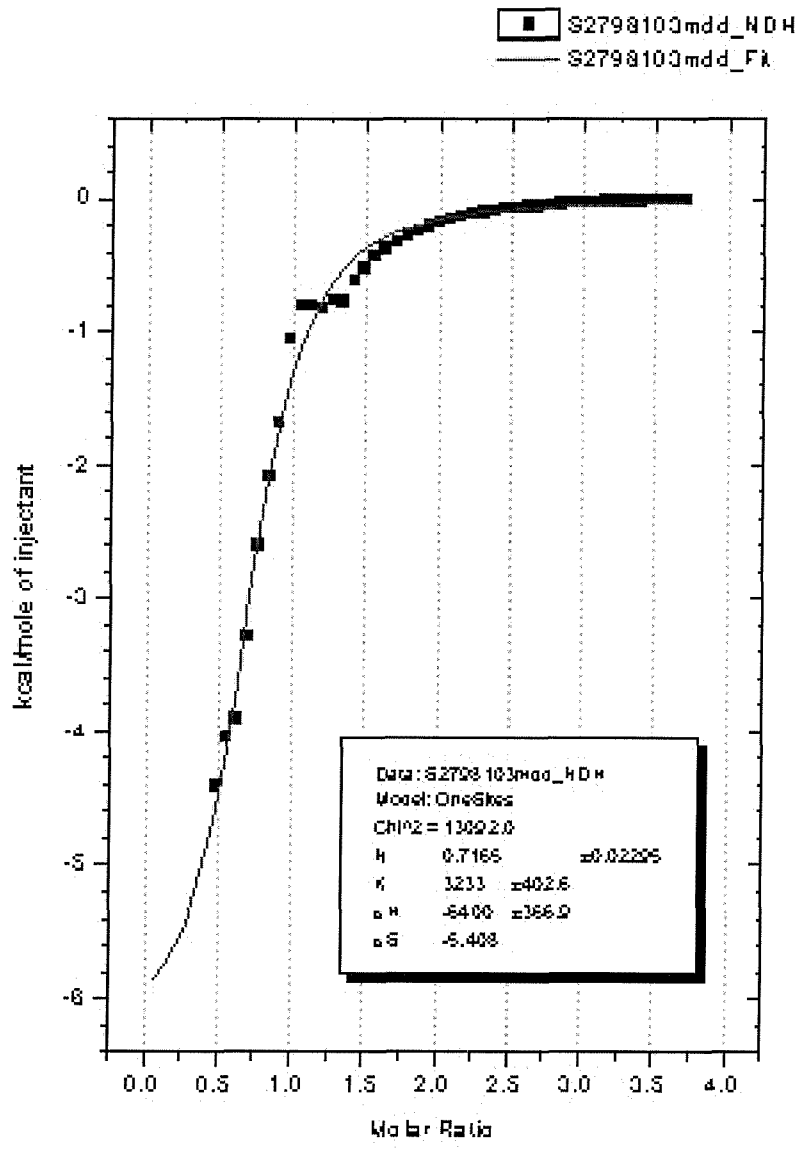


Figure 46: The binding isotherm in DMSO/H_2O

Unfortunately, to date, the synthesis of the final receptors (in Madrid) has not been completed. However, we have described the synthesis of a library of receptors, which contained a combination of rational design in the use of a specific CBS and a

combinatorial approach to produce all the different combination of amino acids in the arms. The screening experiment identified a specific sequence of amino acids in the arm of the receptor, to give a structure of a potential receptor for a biological relevant peptide guest D-Ala-D-Ala.

3.11 Conclusions

In this chapter a number of approaches taken for the further development of the combinatorial tweezer libraries have been described. The first approach, to create 'unsymmetrical' libraries, was unsuccessful becouse the required CBS could not be synthesised efficiently.

The second approach concerned the use of β-sulfonamides instead of amino acid building blocks in the tweezer side-arms, as part of a collaboration with Milan. Screening of a library of such tweezers was not successful in identifing novel receptors, but further studies with an individual receptor (carried out in Milan) revealed interesting binding properties, and such receptors are likely to form strong intramolecular interactions which reduce their binding potential.

In the third approach a simple peptide library was capped with a bicyclic guanidinium (as part of a collaboration with Madrid) and preliminary screening experiments have provided encouraging results suggesting that receptors for the dipeptide D-Ala-D-Ala may have been identified from this library, although final synthesis of these receptors has not jet been completed.

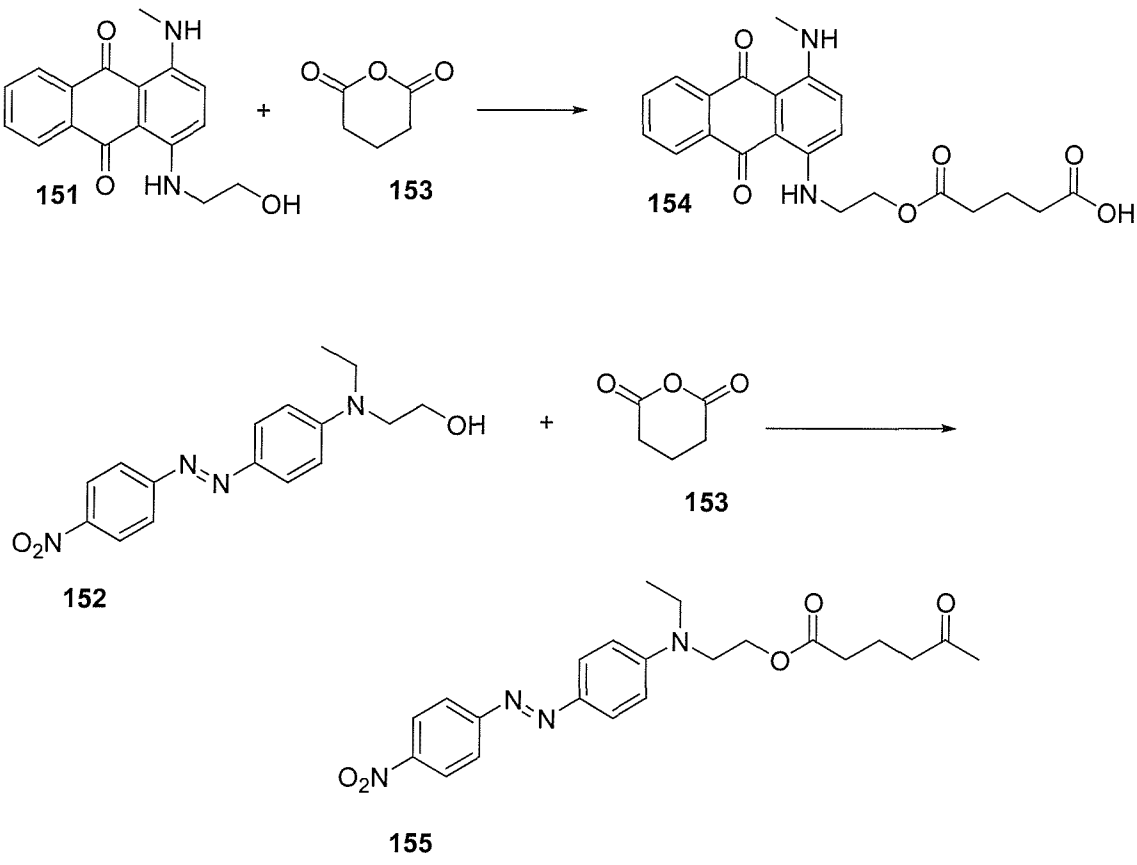
Chapter 4

4.1. Introduction

One of the most common methods for assessing substrate/receptor binding consists of labeling a substrate or receptor with a coloured or fluorescent dye. For example, the screening of library of receptors requires the synthesis of peptide-dye derivative as a guest [83,84] and the screening of library of peptide requires the synthesis of receptor-dye derivative.[71] The presence of a dye group attached to the peptide guest or host allows visualization during screening experiments. This chapter describes the synthesis of different dyed peptide guests. The synthesis of peptide guests was carried out using solid phase chemistry.

4.2 Choice of Dye

The first aim was to choose a reliable dye to use in the synthesis of peptides guest. We decided to use commercial disperse-blue dye **151** and disperse-red dye **152**. Both compounds needed purification to obtain a pure compound in only 30% yield. The disperse red and blue contained an alcohol as a terminal functional group which was reacted with glutaric anhydride to give acids **155** and **154** suitable for attachment to the amino terminus of a peptide.

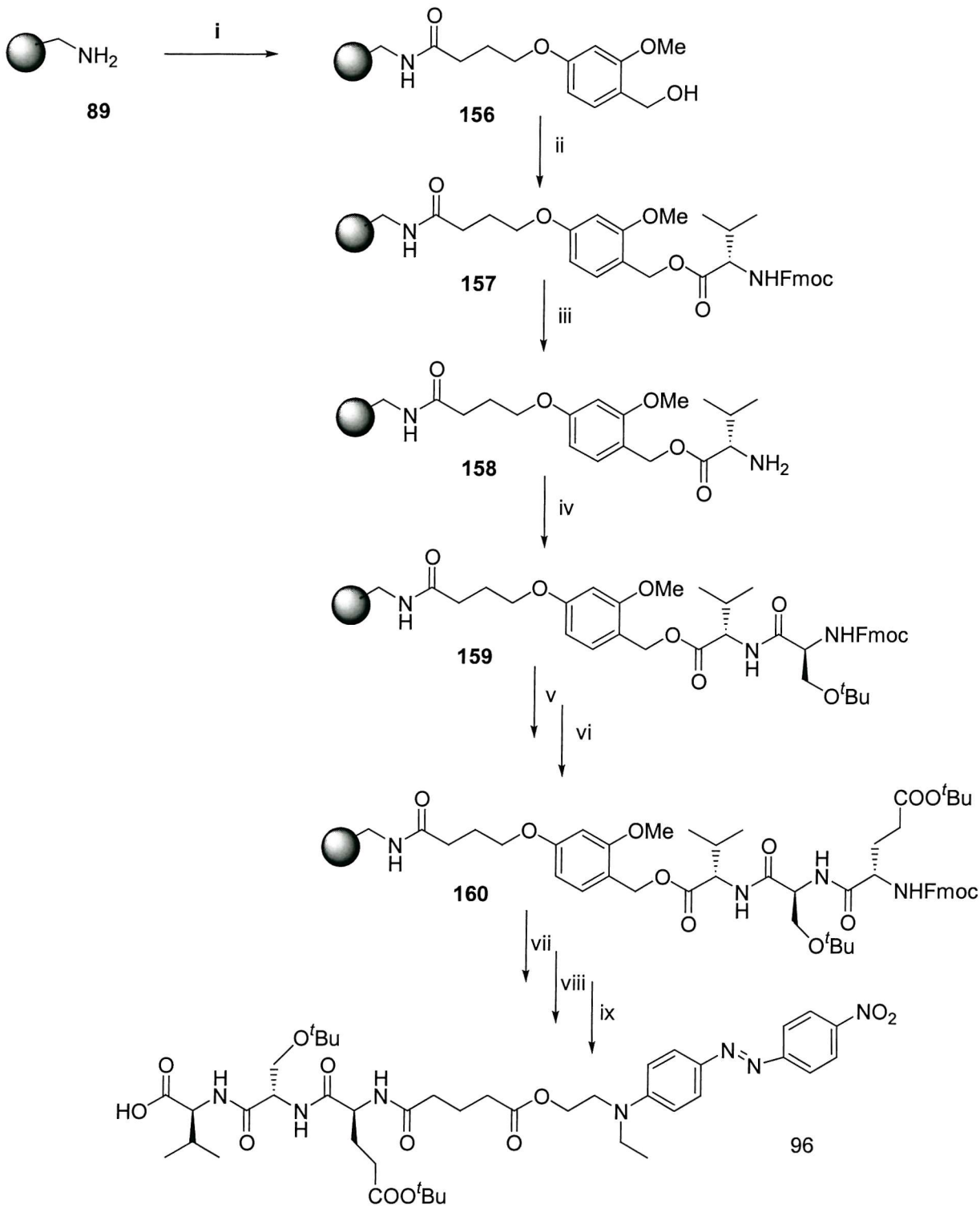


Scheme 49: Synthesis of dyes with carboxylic group

The coupling between disperse-blue and glutaric anhydride proved to be quite difficult and several attempts were made. The first attempt was to use 1 eq of dye and anhydride and DMAP in catalytic amount in CH_2Cl_2 but unfortunately the desired compound was not obtained. The second attempt using dioxane and $NaHCO_3$ returned only starting material. The last attempt was carried out using 1 eq of dye, anhydride and DMAP in CH_2Cl_2 to afford the final compound **154** in 30% yield. The coupling with red dye was more reliable and was carried out using 2 eq of anhydride, DMAP and TEA in CH_2Cl_2 to give the desired compound in excellent yield of 93% after purification on column chromatography.[130]

4.3 Synthesis of Peptide Guests

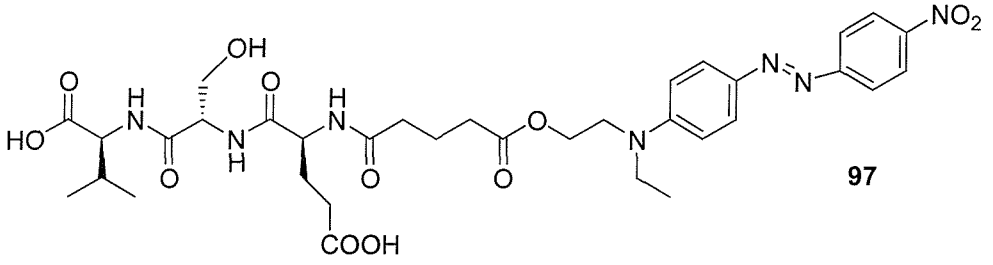
The synthesis of dye red-labelled L-Glu(O^{t}Bu)-L-Ser(^{t}Bu)-L-Val-OH was carried out using aminomethyl TentaGel resin **89** and HMPB (4-(4-hydroxymethyl-2-methoxyphenyl) butanoic acid) linker.[92] This linker was used because the peptide could be cleaved from the resin using only 1% of TFA in CH_2Cl_2 without causing any problem with acid labile side chain protecting groups. The first step of the synthesis was the coupling between aminomethyl TentaGel resin **89** with the HMPB linker using HOBt, TBTU and DIPEA as coupling reagents in DMF to afford resin **156**. The completion of the reaction was checked with a qualitative ninhydrin test. The coupling between the alcohol group on the linker bounded to the resin with the first amino acid, Fmoc-Val-OH was carried out using DIC as an activating agent in DMF and DMAP to give resin **157**. The following step was to remove the Fmoc terminal protecting group using a 20% solution of piperidine in DMF to afford the free amino group in resin **158** ready for the following coupling. The second amino acid, Fmoc-Ser(^{t}Bu)-OH, was coupled using HOBt, TBTU and DIPEA as coupling reagents to give **159**. The protecting Fmoc group was removed using 20% solution of piperidine in DMF. The final amino acid coupling was carried out using Fmoc-Glu(O^{t}Bu)-OH, HOBt, TBTU and DIPEA in DMF to give resin peptide bounded **160**. The final step on solid phase was the coupling of red dye-bounded glutaric anhydride **155** using HOBt, TBTU and DIPEA to afford the final product bound to the resin. The cleavage of the final compound from the resin was achieved treating the resin with 1% solution of TFA in CH_2Cl_2 to afford, after purification by column chromatography, the pure compound **96** in 20% yield (scheme 50).



i) HMPB, HOBt, TBTU, DIPEA; ii) Fmoc-Val-OH, DIC, DMAP; iii) 20% piperidine in DMF;
iv) Fmoc-Ser(O^{t}Bu)-OH, HOBt, TBTU, DIPEA; v) 20% piperidine in DMF; vi) Fmoc-Glu(O^{t}Bu)-OH, HOBt, TBTU, DIPEA; vii) 20% piperidine in DMF; viii) 157, HOBt, TBTU, DIPEA; ix) 1% TFA in CH_2Cl_2

Scheme 50: Synthesis of Dye-labelled L-Glu(O^{t}Bu)-L-Ser(^{t}Bu)-L-Val

The synthesis of dye red-labeled L-Glu-L-Ser-L-Val-OH was carried out using the same procedure as described above for the dye red-labeled L-Glu(O^{t}Bu)-L-Ser(^{t}Bu)-L-Val-OH but the cleavage from the resin was carried out using 50% solution of TFA in CH_2Cl_2 in order to remove the side chain protecting group of Ser and Glu to afford the final compound **97**.



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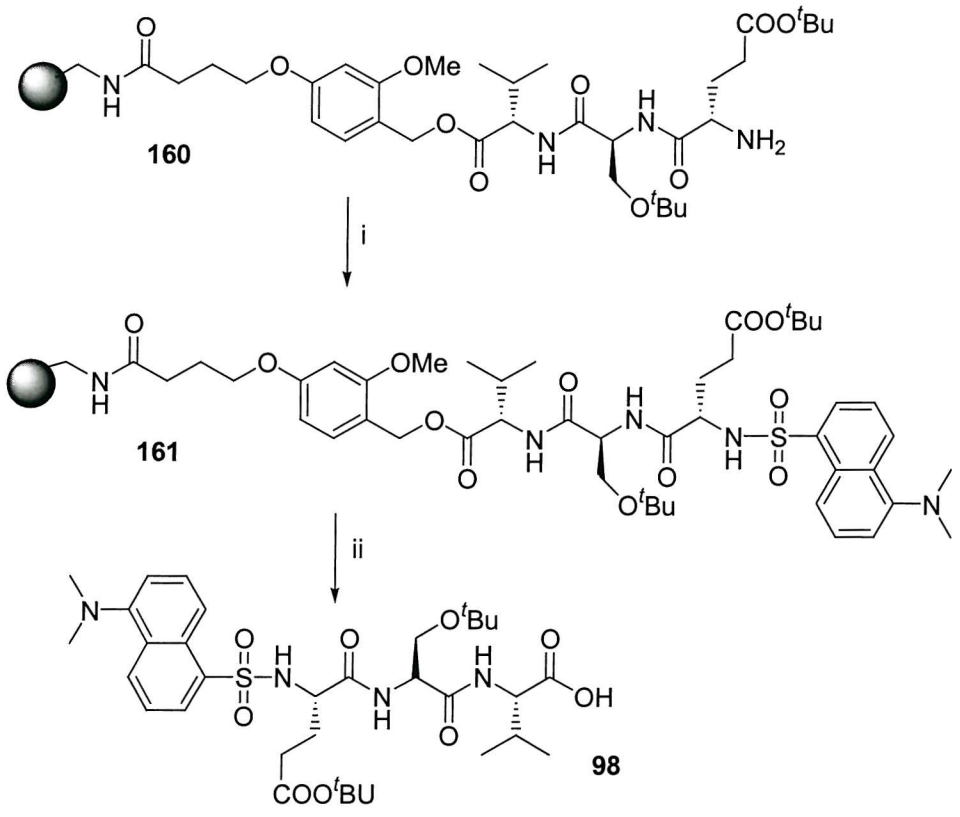
Scheme 51: Dye-labelled peptide L-Glu-L-Ser-L-Val-OH

The same synthetic strategy was used for the synthesis of DNS-L-Glu(O^{t}Bu)-L-Ser(^{t}Bu)-L-Val-OH **98**. A solution of DNSCl in CH_2Cl_2 was added to the resin **160** and stirred at room temperature for 6 hours to give the resin bound tripeptide **161**. Cleavage was carried out by treatment of the resin **161** with a solution of 1% TFA in CH_2Cl_2. The solvent collected from the cleavage step was evaporated and the crude was purified by column chromatography to afford the desired compound in 62% yield.



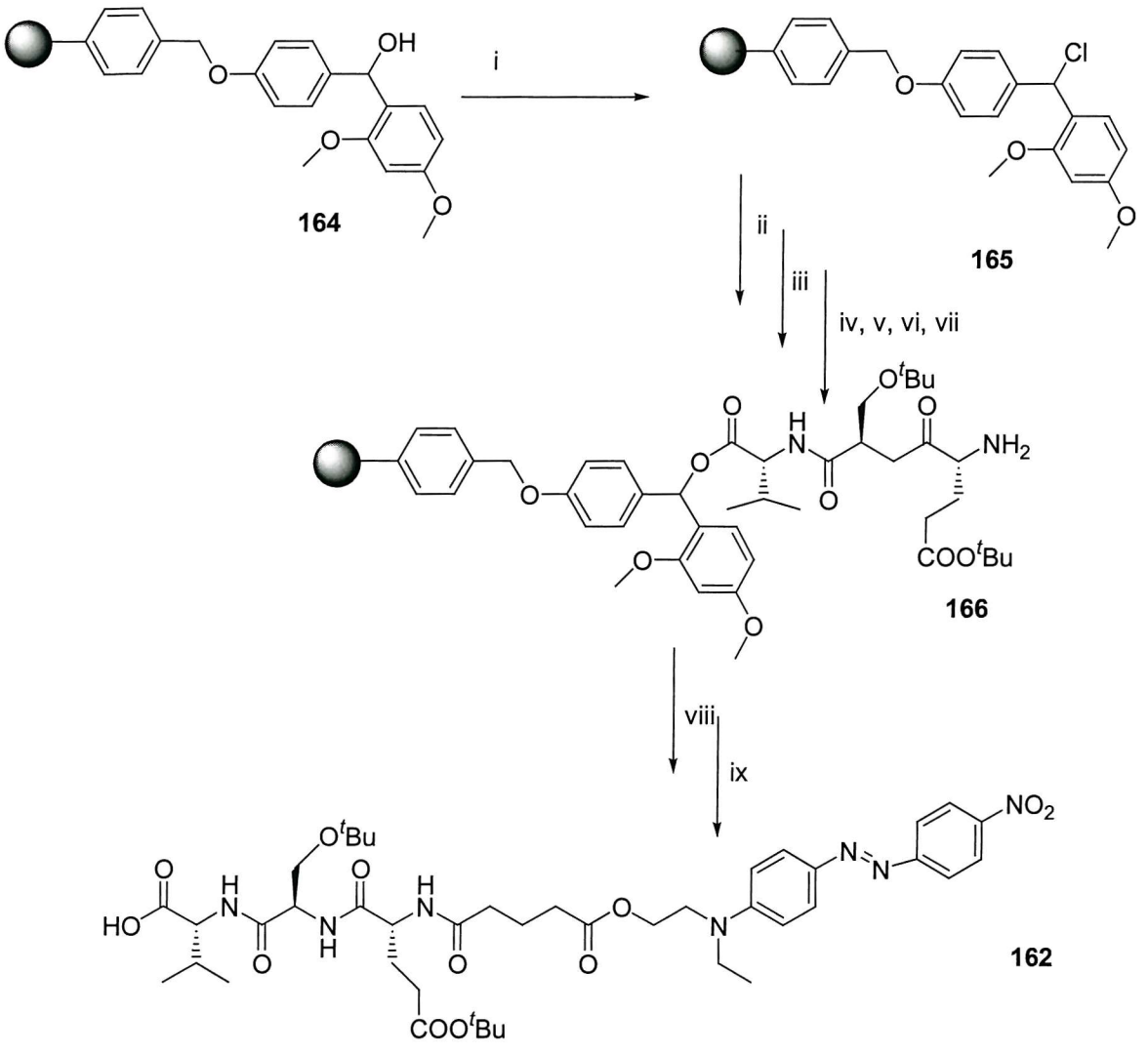
i) DNSCl in CH_2Cl_2; ii) 1% TFA in CH_2Cl_2

Scheme 52: Synthesis of DNS-labelled L-Glu(O^{t}Bu)-L-Ser(^{t}Bu)-L-Val-OH

To study the influence of a different chirality on the peptide guest during the selective recognition event the same peptides were synthesised using the opposite enantiomers of each single amino acid. The synthesis of Red dye-spacer-D-Glu(O^{t}Bu)-D-Ser(^{t}Bu)-D-Val-OH **162** and the analogous deprotected side chain Red dye-spacer-D-Glu-D-Ser-D-Val-OH **163** were synthesised using a different strategy and later used by Jensen in screening experiments with a different library.[131]

It was decided to use a different linker to improve the poor yield obtained with HMPB. The linker should be suitable for an easy cleavage of the reaction products under mild conditions. It was decided to use the Rink linker.[132] The Rink linker has been used in the synthesis of some chemical libraries because mild conditions (10% acetic acid) can be used for the release of the library components. Furthermore the use of Rink-chloride allows a very general and practical method for the attachment of amines, alcohol, carboxylic groups, and thiols to a solid support and also their release under mild condition.[133]

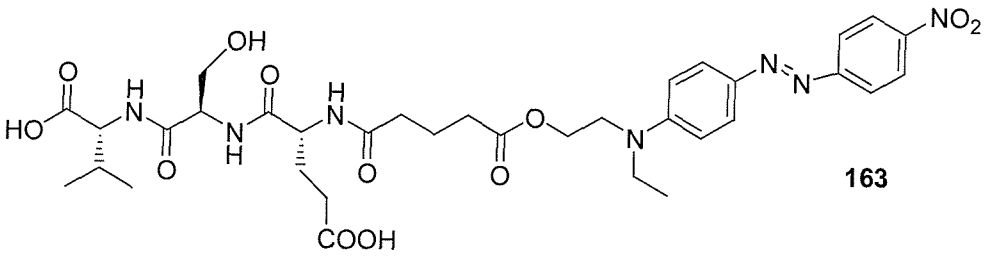
The Rink acid resin **164** was suspended in dry THF with triphenylphosphine and hexachloroethane for 6 hours at room temperature to afford the activated resin **165**. After washing with THF, the resin was pre-swollen in CH_2Cl_2. Fmoc-D-Val-OH together with DIPEA was added to the suspension and agitated for 20 hours to yield a resin, which gave a negative ninhydrin test. Fmoc deprotection was achieved using 20% solution of piperidine to give a free amine group ready for the coupling with the second amino acid. A solution of Fmoc-D-Ser(^{t}Bu)-OH, DIC and HOBt in DMF was stirred for 10 minutes and added to the resin followed by DIPEA and the resulting mixture was agitated at room temperature for 20 hours. Fmoc deprotection and subsequent coupling of Fmoc-D-Glu(O^{t}Bu)-OH followed by Fmoc deprotection as describe above provided the resin bound tripeptide **166**. A solution of red dye-linked glutaric acid, HOBt, HBTU in DMF was stirred for 10 minutes and added to the resin followed by DIPEA and agitated for 20 hours. Agitating the resin with 10% acetic acid in CH_2Cl_2 for 2 hours performed cleavage of the product, from the Rink acid resin. The solvent collected from the cleavage step was evaporated and the crude was purified by column chromatography to afford the desired compound **162** in 73% yield.



i) Ph_3P, C_2Cl_6, THF; ii) Fmoc-D-Val-OH, DIPEA; iii) 20% piperidine in DMF;
iv) Fmoc-D-Ser(^{t}Bu)-OH, DIC, HOBt, DIPEA; v) 20% piperidine in DMF;
vi) Fmoc-D-Glu(O^{t}Bu)-OH, DIC, HOBt, DIPEA; vii) 20% piperidine in DMF; viii) **155**, HOBt, HBTU, DIPEA; ix) 10% acetic acid in CH_2Cl_2

Scheme 53: Synthesis of Dye-labelled D-Glu(O^{t}Bu)-D-Ser(^{t}Bu)-D-Val-OH

The analogous side chain deprotected peptide red dye-spacer-D-Glu-D-Ser-D-Val-OH **163** has been obtained after treatment of peptide **162** with 50% solution of TFA in CH_2Cl_2.



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Scheme 54: Dye-labelled D-Glu-D-Ser-D-Val-OH

Chapter 5

Experimental

5.1 General Experimental

Reactions requiring a dry atmosphere were conducted in flame dried glassware under nitrogen. Where degassed solvents were used, a stream of nitrogen was passed through them immediately prior to use, unless otherwise stated. Solvents were of commercial grade and were used without further purification unless otherwise stated. THF was distilled under nitrogen over benzophenone and sodium, and CH_2Cl_2 was distilled over calcium hydride, as was petroleum ether where the fraction boiling between 40°C and 60°C was used.[134]
TLC analysis was carried out using aluminium-backed sheets coated with silica gel (0.25 mm) and containing the fluorescent indicator UV_{254}. UV (254 nm) was used to visualize compounds unless stated otherwise. Flash column chromatography was performed, according to the procedure outlined by Still,[135] on Sorbsil C60, 40-60 mesh silica. Unless otherwise stated, experiments were carried out at room temperature.

5.2 Instrumentation

Proton NMR spectra were obtained at 300 MHz on a Bruker AC 300 and at 400 MHz on a Bruker DPX 400 spectrometer. Carbon NMR spectra were recorded at 75 MHz on a Bruker AC 300 spectrometer and at 100 MHz on a Bruker DPX 400 spectrometer. Chemical shifts are reported in ppm on the δ scale. Coupling constants (J) are given in Hz. Signal multiplicities were determined using the distortionless enhancement by phase transfer (DEPT) spectral editing technique.

Infra-red spectra were recorded on a BIORAD Golden Gate FTS 135. All samples were run either as neat solids or as oils.

Melting points were determinated in open capillary tubes using a Gallenkamp Electrothermal melting point apparatus and are uncorrected.

Mass spectra were obtained on a VG analytical 70-250 SE normal geometry double focussing mass spectrometer. All electrospray (ES) spectra were recorded on a Micromass Platform quadrupole mass analyser with an electrospray ion source using acetonitrile as solvent. High resolution accurate mass measurements were carried out at 10,000 resolution using mixture of polyethylene glycols and/or polyethylene glycolmethyl ethers as mass calibrants for FAB.

Analytical HPLC spectra were obtained using a Hewlett Packard HP1100 Chemstation, using a Phenomenex C_{18} prodigy 5μm (150 mm x 3 mm) column. A gradient from water, 0.1% TFA to acetonitrile, 0.042% TFA was employed.

Semi-preparative reverse-phase HPLC were carried out using a Hewlett Packard HP1100 Chemstation with an automated fraction collector and a Phenomenex C18 prodigy 5 μm (250 mm x 10 mm) column. A gradient water, 0.1% TFA to acetonitrile, 0.042% TFA was employed.

UV titration experiments were recorded on a Shimadzu UV-1601 UV-visibile spectrophotometer. UV absorbance of ninhydrin and Fmoc quantitative tests were measured on a Hewlett-Packard 8452A Diode Array Spectrometer using two way quartz cells.

Amino acids were of L-configuration unless stated otherwise. Solid phase peptide and library syntheses were performed in glass vessels with sinter frits or polypropylene filtration tubes with polypropylene frits and a Visiprep SPE Vacuum Manifold (Supelco). The reaction containers were agitated either using a Stuart Scientific Flash Shaker SF1 or by a Stuart Scientific Blood Tube Rotator SB1.

Binding experiments were performed on an Isothermal Titration Calorimeter from Microcal Inc., Northampton, Massachussetts, USA.

5.3 Name of Compounds

Compound names were determined using the ACD/name program which followed the IUPAC Nomenclature. Version 2.51/ 30 Jan 1997, Toronto, Canada.

5.4 General Experimental Procedures

5.4.1 Quantitative ninhydrin test.[136]

Two reagents were prepared:

Reagent A:

Solution 1- Reagent grade phenol (40 g) was dissolved in absolute ethanol (10 mL) with warming and then stirred over Amberlite mixed-bed resin MB-3 (4 g) for 45 mins. The mixture was then filtered.

Solution 2- Potassium cyanide (65 mg) was dissolved in water (100 mL). A 2 mL aliquot of this solution was diluted with pyridine (freshly distilled from ninhydrin) and stirred over Amberlite mixed-bed resin MB-3 (4 g). The solution was filtered and mixed with solution 1 to form reagent A.

Reagent B:

Ninhydrin (2.5 g) was dissolved in absolute ethanol (50 mL).

A known mass of resin (< 5 mg) was treated in a small test tube with 7 drops of reagent A (described above) and 3 drops of reagent B (described above) and heated at 100°C for 10 mins. The tube was cooled and 60% aqueous ethanol solution (2 mL) was added to the tube. The contents of the tube were filtered through a pipette charged with a plug of glass wool and the blue filtrate collected in a 10 mL volumetric flask. The resin and the pipette

plug was washed using a solution of tetraethyl ammonium chloride (0.5 M in CH_2Cl_2, 2 x 0.5 mL) and the sample made up to 10 mL with 60% aqueous ethanol solution. The absorbance at 570 nm was then measured against a blank solution. The density of primary amine functionalities present on the resin was calculated using the equation:

Loading (mmol/ g) = (A x V) / (ε_{570} x W) x 1000

ε_{570} = Molar extinction co-efficient (15000 M^{-1} cm^{-1}).
V = diluted volume (10 mL).
W = mass of resin (mg).
A_{570} = absorbance measured at 570 nm.

5.4.2 Quantitative Fmoc test.[137]

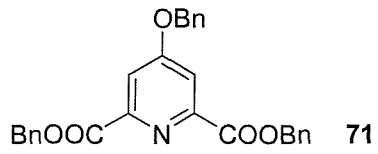
To a known mass (< 5 mg) of resin was added a solution of 20% piperidine in DMF (1 mL). The resin was allowed to stand for 15 mins and the solution filtered through a glass pipette with a glass wool plug. The filtrate was diluted to 10 mL with 20% piperidine in DMF. The absorbance at 302 nm was recorded, measured against a blank of 20% piperidine in DMF. The loading was calculated from the following equation:

Loading = (A_{302} x V) / (ε_{302} x W) x 1000

A_{302} = absorbance of the piperidyl-fulvene adduct.
V = diluted volume (10 mL).
W = mass of the resin sample (mg).
ε_{302} = molar extinction co-efficient of the adduct at 302 nm (7800 M^{-1} cm^{-1}).

5.5 Experimental for Chapter 2.

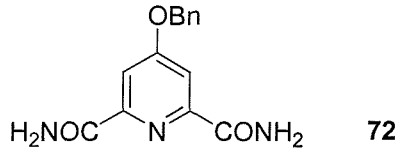
Dibenzyl 4-(benzyloxy) 2,6-pyridinedicarboxylate 71



71

Chelidamic acid (9.50 g, 47.2 mmol), dry potassium carbonate (29.3 g, 212 mmol) and benzyl bromide (34 mL, 283 mmol) were refluxed in dry acetone for 3 d. After addition of hydrochloric acid (2N, 250 mL) the aqueous layers were extracted with ethyl acetate (3 x 150 mL) and the combined organic layers were dried over magnesium sulfate. The solvent was removed under reduced pressure to give a yellow oil. After trituration of the resulting oil with petroleum ether, **71** was obtained as a light yellow solid (17.8 g, 84%). M.p. 63-65°C (lit.:[92] 65°C); IR: ν = 1716, 1594, 1342, 1154 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$): δ = 7.85 (s, 2H, PyrH), 7.47-7.30 (m, 15H, ArH), 5.43 (s, 4H, CO_2CH_2), 5.20 (s, 2H, OCH_2Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 166.7(0), 164.5(0), 150.1(0), 135.5(0), 134.8(0), 129.0(1), 128.9(1), 128.7(1), 128.6(1), 128.5(1), 127.9(1), 115.0(1), 70.9(2), 67.9(2); MS (ES^+): m/z (%): 454 (85) $[M + H]^+$, 929 (100) $[2M + Na]^+$. Spectroscopic data are consistent with those reported in literature.[92]

4-Benzyloxy-2,6-pyridinedicarboxamide 72

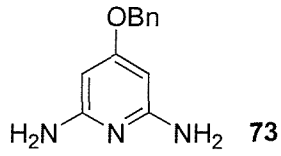


72

Compound **72** was prepared according to the literature procedure.[92] (3.55 g, 85%). M.p. > 220 °C (lit.:[92] >220°C); IR: ν = 3429, 1693, 1662, 1110 cm^{-1}; ^1H NMR (300MHz, DMSO-d_6): δ = 8.85 (s, 2H, PyrH), 7.73 (s, 4H, $CONH_2$), 7.49-7.30 (m, 5H, ArH), 5.35 (s, 2H, OCH_2Ar); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 166.8(0), 165.2(0), 151.3(0), 135.9(0),

128.6(1), 128.2(1), 127.8(1), 110.5(1), 69.9(2); MS (ES^+): m/z (%): 273 (33) $[M + H]^+$, 295 (87) $[M + Na]^+$, 565 (100) $[2M + Na]^+$. Spectroscopic data are consistent with those reported in literature.[92]

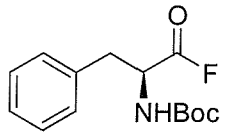
4-Benzyloxy-2,6-pyridinediamine 73



73

Compound **73** was prepared according to the literature procedure.[92] (1.38 mg, 85%): M.p. 163-165 °C (lit.:[92] 164-166°C); IR: ν = 3434, 1446, 1367, 1015 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$): δ = 7.52-7.40 (m, 5H, ArH), 5.42 (s, 2H, PyrH), 5.38 (s, 4H, NH_2), 5.06 (s, 2H, OCH_2Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 167.0(0), 160.0(0), 137.1(0), 128.4(1), 127.8(1), 127.5(1), 82.3(1), 68.2(2); MS (ES^+): m/z (%): 216 (100) $[M + H]^+$. Spectroscopic data are consistent with those reported in literature.[92]

***N-tert*-Butoxycarbonyl-L-phenylalanyl fluoride 75[94]**

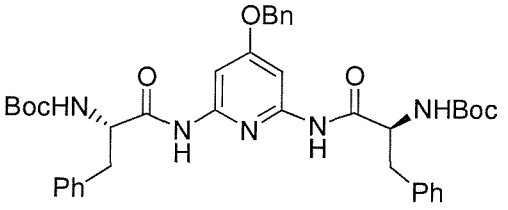


75

Cyanuric fluoride (1.40 mL, 16.6 mmol) was added to a mixture of *N*-Boc-L-phenylalanine (2.00 g, 7.55 mmol) and pyridine (608 µL, 7.55 mmol) in dry CH_2Cl_2 at –15°C and stirred for 3 h at –15°C. Crushed ice (50 g) and CH_2Cl_2 (100 mL) were added to the reaction mixture and the aqueous layer was extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were washed with ice-cold water (200 mL), dried over $MgSO_4$ and filtered. The solvent was removed under reduced pressure to give the acid fluoride as a white solid (2.01 g, 100%). M.p 66-68°C (lit.:[92] 66-68°C), IR: ν = 3358, 2974, 1831, 1686 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.38-7.18 (m, 5H, ArH), 4.86-4.76 (m, 1H, CHN*H*), 4.60

(dd, J=9 Hz, 1H, C*H*NH), 3.18-3.11 (m, 2H, CH_2Ar), 1.46 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 162.5(0) (d, $^1J_{CF}$ = 368 Hz), 155.2(0), 134.9(0), 129.6(1), 129.4(1), 128.0(1), 81.0(1), 53.8(1) (d, $^2J_{CF}$ = 53 Hz), 37.3(2), 28.6(3); MS (ES^+): m/z (%): 306 (90) $[M + K]^+$. Spectroscopic data are consistent with those reported in literature.[92]

4-Benzyloxy-2,6-bis(*N*-*tert*-butoxycarbonyl-L-phenylalaninylamino)pyridine 76



76

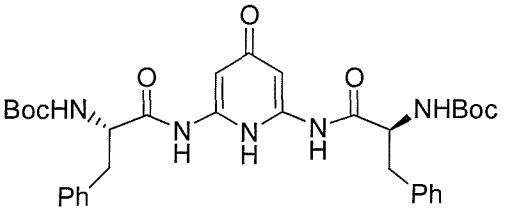
a) via coupling with Boc-Phe-F

Compound **76** was prepared according to the literature procedure.[92] (269 mg, 76%). M.p. 90-92°C (lit.:[92]88-90 °C); IR: ν = 3429, 1685, 1440, 1165 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 10.20 (s, 2H, NH), 7.62-7.25 (m, 19H, ArH, PyrH and NH), 5.29 (s, 2H, $ArOCH_2$), 4.57 (bs, 2H, C*H*NH), 3.12 (m, 2H, $CHCH_aH_bPh$), 2.92 (m, 2H, $CHCH_aH_bPh$), 1.42 (s, 18H, $C(CH_3)_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 170.4(0), 165.9(0), 154.1(0), 149.9(0), 136.5(0), 134.7(0), 127.9(1), 127.2(1), 126.8(1), 126.7(1), 126.5(1), 125.0(1), 94.7(1), 76.9(2), 68.2(0), 55.1(1), 35.6(2), 26.8(3); MS (ES^+): m/z (%): 710 (100) $[M + H]^+$; $[\alpha]_D$ = -19.14° ($CHCl_3$, c = 0.39, R.T). Spectroscopic data are consistent with those reported in literature.[92]

b) via coupling with Boc-Phe-OH in presence of DIC

Compound **76** was prepared according to the literature procedure.[92] (1.09 g, 27%). The spectroscopic data obtained was consistent with that reported above.

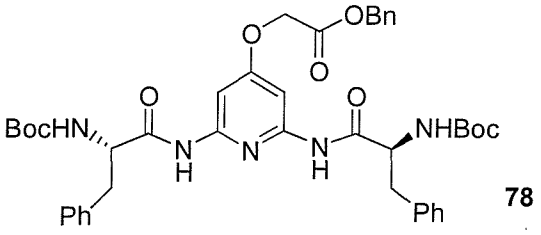
2,6-Di (*N-tert*-butoxycarbonyl-L-phenylalaninylamino)-4-oxo-1,4-dihydropyridine 77



77

Compound **77** was prepared according to the literature procedure.[92] (464 mg, 100%) which was recrystallised from ethanol. M.p. 115-118°C (lit.:[92] 115-118°C); IR: ν = 3400, 1684, 1440, 1165 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ = 10.65 (s, 1H, NH_{py}), 9.95 (s, 2H, NH), 7.40-7.15 (m, 12H, ArH), 4.45 (m, 2H, C*H*CH_2Ph), 3.05-2.77 (m, 4H, CHCH_2Ph), 1.29 (s, 18H, $C(CH_3)_3$); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 171.6(0), 167.0(0), 155.6(0), 151.1(0), 138.0(0), 129.4(1), 128.1(1), 126.4(1), 97.1(1), 78.3(0), 56.6 (1), 37.1(2), 28.2(3); MS (ES^+): m/z (%): 620 (100)$[M + H]^+$, 1240 (20) $[2M + H]^+$. Spectroscopic data are consistent with those reported in literature.[92]

Benzyl-2-{[2,6-bis(*N-tert*-butoxycarbonyl-L-phenylalanylamino)-4-pyridyl]oxy} acetate 78

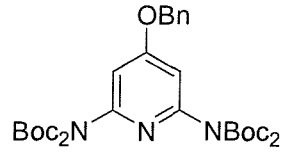


78

Diamine **83** (666 mg, 2.44 mmol), was dissolved in dry acetonitrile (30 mL). After addition of *N,O*-bis trimethylsilyl acetamide (1.20 mL, 4.88 mmol) the reaction mixture was stirred at room temperature for 2 h. Then *N*-Boc-phenylalanine fluoride (2.60 g, 9.76 mmol) and SKA (1.98 mL, 9.76 mmol) were added and the reaction mixture was stirred for additional 12 h. The solvent was removed under reduced pressure and the residue purified by column chromatography on silica gel (1% to 5% ethyl acetate in dichloromethane) to afford pure **78** as a white solid (1.098 g, 58%). M.p. 63-65°C (lit.:[92]

65-67 °C); IR: ν = 3441, 1691, 1586, 1513, 1437, 1161 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 10.03 (s, 2H, NH), 7.32-7.04 (m, 19H, ArH, PyrH and NH), 5.11 (s, 2H, OCH_2Ar), 4.84 (s, 2H, OCH_2CO), 4.37 (s, 2H, C*H*NH), 2.87-2.89 (m, 2H, CH_aCH_bPh), 2.73-2.67 (m, 2H, CH_aH_bPh), 1.20 (s, 18H, $C(CH_3)_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 169.9(0), 166.1(0), 164.7(0), 153.6(0), 149.4(0), 135.9(0), 133.6(0), 127.4(1), 126.6(1), 126.2(1), 126.2(1), 126.1(1), 124.4(1), 93.9(1), 76.4(2), 64.4(2), 62.8(0), 54.5(1), 35.1(2), 26.3(3); MS (ES^+): m/z (%): 768 (100)$[M + H]^+$; $[\alpha]_D$ = -15.65° ($CHCl_3$, c = 0.39, R.T.). Spectroscopic data are consistent with those reported in literature.[92]

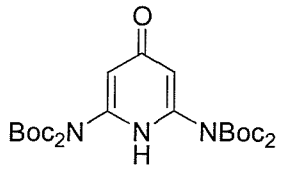
4-Benzyloxy–2,6-[bis(*N-tert*-butyloxycarbonyl)amino] pyridine 79



79

4-(Benzyloxy)-2,6-pyridinediamine **73** (3.18 g; 16.6 mmol), DMAP (2.03 g; 16.6 mmol) and di-*tert*-butyl dicarbonate (21.8 g; 100 mmol) was dissolved in acetonitrile (350 mL). The resulting reaction mixture was stirred for 24 h at RT. The solvent was removed under reduced pressure and the residue purified by column chromatography on silica gel (1% to 5% methanol in dichloromethane) to give the desired compound **79** as a colourless solid (8.78 g; 86%) which was recrystallized from diethyl ether. M.p. 146-148°C; IR: ν = 2980, 1769, 1734 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 7.41-7.27 (m, 5H, Ph), 6.81 (s, 2H, PyrH), 5.13 (s, 2H, CH_2), 1.43 (s, 36H, $C(CH_3)_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 167.5(0), 152.2(0), 151.0(0), 135.7(0), 128.9(1), 128.6(1), 127.6(1), 106.7(1), 83.2(0), 70.5(2), 27.9(3); MS (ES+): m/z (%): 616.6 (100) $[M + H]^+$, 638.6 (85) $[M + Na]^+$, 1254.1 (60) $[2M + Na]^+$; HRMS calcd for $C_{32}H_{45}N_3O_9$ $[M + H]^+$ 615.3156, found 615.3177; elemental analysis calcd (%) for $C_{32}H_{45}N_3O_9$ C 62.42, H 7.37, N 6.82; found C 62.37, H 7.32, N 6.75.

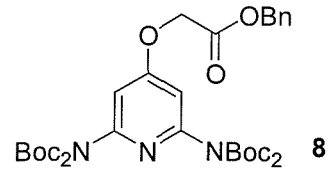
2,6-Bis[tetra (*N-tert*-butoxycarbonyl)amino]-4-oxo-1,4-dihydropyridine 80



80

10% Palladium on charcoal (1.51 g, 1.43 mmol Pd) was added to a solution of **79** (4.40 g, 7.16 mmol) in ethanol (100 mL). The reaction mixture was stirred vigorously for 18 h at RT under a hydrogen atmosphere. 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 **80** as a colourless solid (3.26 g, 87%) which was recrystallized from diethyl ether. M.p. >230°C; IR: ν = 2978, 1731, 1612 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 7.27 (s, 1H, NH), 6.65 (s, 2H, CH), 1.45 (s, 36H, $C(CH_3)_3$); ^{13}C (75 MHz, $CDCl_3$): δ = 166.5(0), 151.8(0), 151.3(0), 108.7(0), 83.4(1), 27.8(3); MS (ES^+): m/z (%): 526 (100) $[M + H]^+$, 564 (10) $[M + K]^+$, 1051 (20) $[2M + H]^+$; HRMS calcd for $C_{25}H_{39}N_3O_9$ $[M + H]^+$ 525.2686, found 525.2667.

Benzyl-2-[(2,6-tetrabis [(*N-tert*-butoxycarbonyl)-4-pyridyl) oxy]acetate 81

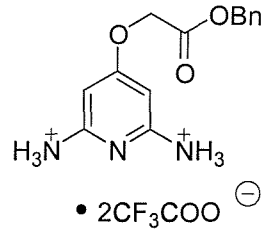


81

Benzyl bromoacetate (1.70 g, 7.46 mmol) was added to a suspension of pyridone **80** (3.26 g, 6.22 mmol), K_2CO_3 (3.61 g, 26.1 mmol) in DMSO (60 mL). The resulting reaction mixture was stirred for 18 h at RT and then partitioned between diethyl ether (70 mL) and water (70 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 x 200 mL), dried over magnesium sulfate and filtered. Evaporation of the solvent and drying of the residue under high vacuum yielded **81** (4.14 g, 99%) as a colourless solid: M.p. 115-117°C; IR: ν = 2982, 1782, 1740, 1600 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 7.36 (m,

5H, Ph), 6.76 (s, 2H, PyrH), 5.21 (s, 2H, OCH_2CO_2), 4.73 (s, 2H, OCH_2Ph), 1.43 (s, 36H, $C(CH_3)_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 167.3(0), 166.4(0), 152.2(0), 150.9(0), 134.9(0), 128.8(1), 128.7(1), 128.5(1), 106.0(1), 83.3(0), 67.5(2), 65.2(2), 27.9(3); MS (ES^+): m/z (%): 674 (100) $[M + H]^+$, 696 (85) $[M + Na]^+$, 1369 (20) $[2M + Na]^+$; HRMS calcd for $C_{34}H_{47}N_3O_{11}$ $[M + H]^+$ 673.3210, found 673.3205.

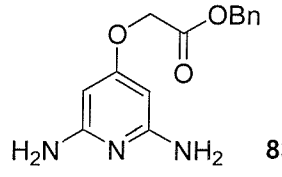
Benzyl-2-[2,6-diamino-4-pyridyloxy] acetate TFA salt 82



82

Benzyl-2-[(2,6-tetrabis (*N-tert*-butoxycarbonyl)-4-pyridyl)oxy]acetate **81** (4.54 g, 6.75 mmol) was stirred in 30% TFA in CH_2Cl_2 for 2 h at RT. Toluene (100 mL) was added and all solvents evaporated under reduced pressure to give a yellow oil. Following trituration with diethyl ether, **82** was obtained as a white solid (1.57 g, 86%). M.p. 146-148°C; IR: ν = 3393, 1752, 1654, 1617 cm^{-1}; ^1H NMR (300 MHz, CD_3OD): δ = 7.35 (s 5H, ArH), 5.54 (s, 2H, PyrH), 5.23 (s, 2H, CH_2CO), 4.90 (s, 6H, NH_3^+), 4.82 (s, 2H, CH_2Ar); ^{13}C NMR (75 MHz, CD_3OD): δ = 172.1(0), 169.0(0), 154.7(0), 136.6(0), 129.5(1), 129.4(1), 129.3(1), 83.3(1), 68.1(2), 66.0(2); MS (ES^+): m/z (%): 274 (100) $[M + H]^+$, 547 (10) $[2M + H]^+$; HRMS calcd for $C_{14}H_{16}N_3O_3$ $[M + H]^+$ 274.1192 found 274.1185.

Benzyl-2-[2,6-diamino-4-pyridyloxy] acetate 83

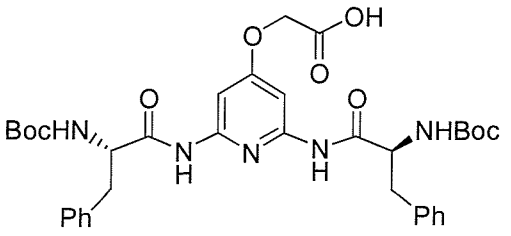


83

Diamine bis TFA salt **82** (591 mg, 2.15 mmol) was dissolved in CH_2Cl_2 (50 mL) and washed with aqueous K_2CO_3 solution. The aqueous washes were collected and extracted

with CH_2Cl_2 (3 x 50 mL). The combined organic phases were washed with brine (100 mL), dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to afford the pure diamine **83** (372 mg, 63%) as a solid. M.p. 98-100°C; IR: ν = 3438, 1767, 1733; ^{1}H NMR (300 MHz, CD_3OD): δ = 7.46 (s 5H, Ph), 5.42 (s, 4H, NH_2), 5.33 (s, 2H, PyrH), 5.28 (s, 2H, OCH_2CO) 4.28 (s, 2H, CH_2Ar); ^{13}C NMR (75 MHz, CD_3OD): δ = 174.0(0), 168.9(0), 167.9(0), 158.8(0), 128.8(1), 128.7(1), 128.6(1), 84.5(1), 67.2(2), 64.7(2); MS (ES^+): m/z (%): 274 (100) $[M + H]^+$, 547 (10) $[2M + H]^+$.

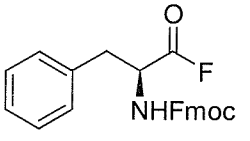
2-[2,6-Bis(N-*tert*-butoxycarbonyl-L-phenylalanilamino)-4-pyridyloxy]acetic acid 84



84

Compound **84** was prepared according to the literature procedure.[92] (230 mg, 92%). M.p. 100-102°C (lit.:[92] 100-102°C); IR: ν = 3440, 2360, 1684, 1436 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.92 (s, 1H, COOH), 10.19 (s, 2H, NH), 7.50-7.21 (m, 14H, ArH, PyrH and NH), 4.77 (s, 2H, OCH_2CO), 4.58 (m, 2H, C*H*NH), 3.15-3.05 (m, 2H, CH_aH_bPh), 2.95-2.89 (m, 2H, CH_aH_bAr), 1.42 (s, 18H, $C(CH_3)_3$); ^{13}C NMR (100MHz, DMSO-d_6): δ = 172.1(0), 170.0(0), 167.4(0), 155.9(0), 151.5(0), 138.3(0), 129.7(1), 128.4(1), 126.7(1), 96.2(1), 78.7(2), 65.3(0), 56.8(1), 37.4(2), 28.5(3); MS (ES^+): m/z (%): 678 (100), $[M + H]^+$. Spectroscopic data are consistent with those reported in literature.[92]

N-9-Fluorenylmethoxycarbonyl-L-phenylalanyl fluoride 86[94]

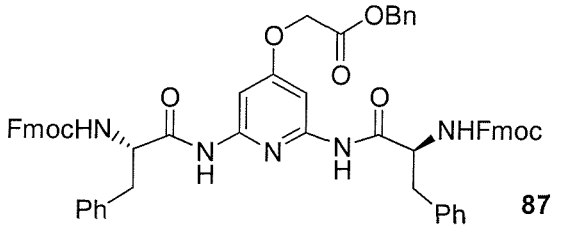


86

Cyanuric fluoride (958 μL, 11.3 mmol) was added to a mixture of *N*-Fmoc-L-phenylalanine (2.00 g, 75.1 mmol) and pyridine (416 μL, 5.16 mmol) in dry CH_2Cl_2 at

–15°C. After stirring for 3 h at –15°C crushed ice (50 g) and CH_2Cl_2 (100 mL) were added to the reaction mixture and the aqueous layer was extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were washed with ice-cold water (200 mL), dried over $MgSO_4$ and filtered. The solvent was removed under reduced pressure to give the acid fluoride as a white solid (2.00 g, 100%). M.p. 172-174°C; IR: ν = 3313, 1835, 1697, 1529 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.78 (d, J=9 Hz, NH), 7.56-7.14 (m, 13H, ArH), 5.12 (d, J=10 Hz, 1H, C*H*NH), 4.84 (d, J=7 Hz, 1H, C*H*CH_2), 4.51-4.39 (m, 2H, CH_2OCO), 3.20-3.13 (m, 2H, CH_2Ar); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 161.2(0) (d, $^1J_{CF}$=367 Hz), 154.8(0), 143.0(0), 142.9(0), 140.8(0), 133.7(1), 128.7(1), 128.591), 127.3(1), 126.5(1), 124.4(1), 119.5(1), 66.7(2), 53.2(1) (d, $^2J_{CF}$ = 60 Hz), 46.5(1), 36.4(2). MS (ES^+): m/z (%): 799 (15) $[2M + Na]^+$.

Benzyl 2-((2,6-di(fmoc-L-phenylalanylamino)-4-pyridyl)oxy)acetate 87

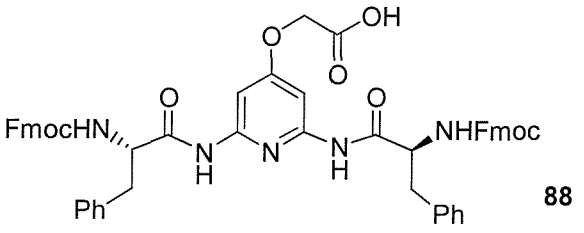


87

The free diamine **83** (400 mg, 1.46 mmol) was dissolved in dry CH_2Cl_2 (50 mL), BSA (721 µL, 2.92 mmol) was added under an argon atmosphere, and the solution was stirred at RT for 2 h. Fmoc-Phe-F (2.27 g, 5.84 mmol) was added in one portion followed by SKA (1.18 mL, 5.84 mmol). The resulting solution was stirred overnight at RT. The reaction mixture was directly poured into the top of a flash chromatography column and eluted with 5% to 10% ethyl acetate in CH_2Cl_2, to give the pure compound (649 mg, 44%). M.p. 93-95°C (lit.:[92] 94-96°C); IR: ν = 3424, 1695, 1585, 1437, 1215 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 7.84 (d, J=7 Hz, 2H, ArH), 7.79 (d, J=8 Hz, 2H, ArH), 7.44-7-18 (m, 33H, ArH and NH), 5.20 (s, 2H, OCH_2CO), 4.95 (s, 2H, OCH_2Ph), 4.57 (m, 2H, C*H*CH_2Ph), 4.20-3.98 (m, 6H, O*C*H_2*CH*), 3.06-3.03 (m, 2H, CHC$H_a$$CH_b$Ph), 2.88-2.82 (m, 2H, $CHCH_a$CH_bPh); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 170.4(0), 166.8(0), 154.8(0), 150.0(0), 142.5(0), 139.5(0), 136.6(0), 134.3(0), 128.1(1), 127.3(1), 126.9(1), 126.7(1),

126.4(1), 125.8(1), 125.2(1), 124.1(1), 124.0(1), 118.9(0), 94.7(1), 65.0(2), 64.5(2), 58.6(2), 55.5(1), 45.4(1), 35.8(2); MS (ES^+): m/z (%): 1012 (40) $[M + H]^+$. Spectroscopic data are consistent with those reported in literature.[92]

2-{2,6-Bis[*N*-9-fluorenylmethoxycarbonyl)-L-phenylalanilamino]-4-pyridyloxy} acetic acid 88



88

Compound **88** was prepared according to the literature procedure.[92] (151 mg, 78%). M.p. 143-145°C (lit.:[92] 143-145°C); IR: ν = 3424, 1695, 1585, 1437, 1215 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 10.19 (s, 1H, OH), 7.92 (d, J=7 Hz, 2H, ArH), 7.85 (d, J=8 Hz, 2H, ArH), 7.70-7.25 (m, 28H, ArH and NH), 4.61 (s, 2H, OCH_2CO), 4.38 (m, 2H, $CHCH_2Ph$), 4.27-4.20 (br m, 6H, OCH_2CH), 3.13-3.10 (m, 2H, CH_aCH_bPh), 2.95-2.88 (m, 2H, CH_aCH_bPh); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 171.7(0), 170.0(0), 156.4(0), 151.2(0), 144.1(0), 141.1(0), 138.2(0), 129.7(0), 128.5(1), 128.0(1), 127.5(1), 126.8(1), 125.7(1), 125.6(1), 120.5(0), 96.8(1), 66.1(2), 57.1(2), 46.9(1), 37.4(2); MS (ES+): m/z (%): 922 (50) $[M + H]^+$, 944 (20) $[M + Na]^+$; $[\alpha]_D$ = -8.77° ($CHCl_3$, c = 0.44, R.T.) Spectroscopic data are consistent with those reported in literature.[92]

Synthesis of Tweezer Receptor 94

A solution of *N*-Boc-L-phenylalanine (0.01 mmol, 3.58mg), HOBt (0.01 mmol, 2.00 mg), TBTU (0.01 mmol, 4.77 mg) and DIPEA (0.05 mmol, 9.40 μL) in CH_2Cl_2 and a few drops of DMF was stirred for 10 mins at RT and then added to TentaGel NH_2 resin **89** (500 mg, 0.13 mmol). The suspension was shaken with a nitrogen stream for 2 h. The resin was washed with CH_2Cl_2, DMF, and CH_2Cl_2 and dried to give resin **90**. A solution of Boc-CBS **84** (0.27 mmol, 114 mg), HOBt (0.27 mmol, 41.3 mg), TBTU (0.27 mmol, 86.6 mg) and

DIPEA (0.60 mmol, 105 μL) was stirred for 10 mins in CH_2Cl_2 with a few drops of DMF at RT and then added to the resin. After 3 h the coupling was repeated to give the resin **91**. A qualitative ninhydrin test was negative. The resin was agitated in a 20 % solution of TFA in CH_2Cl_2. The resin was washed with CH_2Cl_2, DMF, and CH_2Cl_2 and the deprotection step repeated. A qualitative ninhydrin test was positive. A solution of *N*-Fmoc-L-Valine (0.27 mmol, 92.0 mg), HOBt (0.27 mmol, 41.3 mg), TBTU (0.27 mmol, 86.6 mg) and DIPEA (0.60 mmol, 105 μL) in CH_2Cl_2 and a few drops of DMF was stirred for 10 mins and then added to the resin swollen in a minimum amount of CH_2Cl_2. The resin was agitated with a nitrogen stream for 2 h. The coupling step was repeated. A qualitative ninhydrin test was negative. The resin was agitated in 20% piperidine in DMF for 20 mins, washed with CH_2Cl_2, DMF, and CH_2Cl_2 and the deprotection step repeated. A qualitative ninhydrin test was positive. A solution of *N*-Fmoc-L-Glycine (0.27 mmol, 80.0 mg), HOBt (0.27 mmol, 41.3 mg), TBTU (0.27 mmol, 86.6 mg) and DIPEA (0.60 mmol, 105 μL) in CH_2Cl_2 and a few drops of DMF was stirred for 10 mins and then added to the resin and shaken 4 h at RT. A qualitative ninhydrin test was negative. The resin was shaken in 20% piperidine in DMF for 20 mins, washed with CH_2Cl_2, DMF, and CH_2Cl_2 and the deprotection step repeated. A qualitative ninhydrin test was positive. A solution of *N*-Fmoc-L-Proline (0.27 mmol, 91.0 mg), HOBt (0.27 mmol, 41.3 mg), TBTU (0.27 mmol, 86.6 mg) and DIPEA (0.60 mmol, 105 μL) in CH_2Cl_2 with a few drops of DMF was stirred for 10 mins and then added to the resin and agitated for 4 h. Qualitative ninhydrin test was negative. The resin was shaken with 20% piperidine in DMF for 20 mins, washed with CH_2Cl_2, DMF, and CH_2Cl_2 and the deproctetion step repeated to give the final receptor **94** attached to the resin.

Synthesis of Tweezer Receptor Library 95

A solution of *N*-Boc-L-phenylalanine (27.0 μmol; 7.16 mg), HOBt (29.7 μmol, 4.54 mg), TBTU (29.7 μmol, 9.54 mg) and DIPEA (108 mmol, 13.9 mg) in DMF (25 ml) was added to a TentaGel NH_2 resin (1.00 g, 0.27 mmol NH_2/g resin) and shaken for 2 h. A solution of Boc-CBS **84** (0.29 mmol, 201 mg), HOBt (0.29 mmol, 45.5 mg), TBTU (0.29 mmol, 95.5 mg) and DIPEA (1.19 mmol, 153 mg) in DMF was added to the resin and agitated

overnight. The resin was agitated for 1 h with a solution of TFA 50% in CH_2Cl_2 to remove the BOC protective groups. The resin was divided in 13 equal portions. To each resin portion one of the following *N*-Fmoc-protected amino acids was added: L-Ala, L-Asn, L-Glu(O^{t}Bu), L-Gln, L-Gly, L-Leu, L-Lys(Boc), L-Met, L-Phe, L-Pro, L-Ser(^{t}Bu), L-Trp, L-Val (0.08 mmol) with HOBt (0.08 mmol, 13.0 mg), TBTU (0.08 mmol, 27.0mg) and DIPEA (0.18 mmol, 33 μl) in DMF and agitated for 3 h. The resin was mixed and the terminal Fmoc-group removed with 20% piperidine in DMF for 20 mins for two cycles. The resin was split again into 13 equal portions and the procedure repeated twice in order to build up the tweezer-receptor library **95**.

Screening of Tweezer receptor Library 95

A sample of each sublibrary **1, 2, 3, 4** (7 mg) was equilibrated in chloroform (1 mL) for 24 h. Aliquots of guest **96** (20 μM), in chloroform, were added (3 x 30 μL) to the sample to give a guest concentration of 1.8 μM, equilibration was continued for 24 h. Beads were analysed in flat-bottomed glass pots under a Leica inverted DML microscope (x 40 magnification). Highly red stained beads were selected from each pot with sublibrary **1**, **2**, **3** and submitted for Edman sequencing.

Screening of Tweezer receptor Library 95

Preparation of the buffer solution: to an aqueous solution of borax (0.025M, 50 mL) was added aqueous sodium hydroxide (0.1M, 0.9 mL) and the pH of the resulting buffer solution was maintained at 9.2.

A sample of each sublibrary **1**, **2**, **3**, **4** (7 mg) was equilibrated in borax buffer solution (1 mL) for 24 h. Aliquots of guest **96** (15% DMSO in H_2O, 20 μM), were added (3 x 30 μL) to the sample to give a guest concentration of 1.8 μM, and equilibration was continued for 24 h. Beads were analysed in flat-bottomed glass pots under a Leica inverted DML microscope (x 40 magnification). Highly red stained beads were selected from each pot with sublibrary **3** and **4** and submitted for Edman sequencing.

Screening of Tweezer receptor Library 95

Preparation of the buffer solution: to an aqueous solution of potassium dihydrogen phosphate (0.1 M, 50 mL) was added aqueous sodium hydroxyde (0.1 M, 5.6 mL) and the pH of the resulting buffer solution was checked by a pH meter to be 6.0.
A sample of each sublibrary **1**, **2**, **3**, **4** (7 mg) was equilibrated in borax buffer solution (1 mL) for 24 h. Aliquots of guest **97** (15% DMSO in H_2O, 20 μM), were added (3 x 30 μL) to the sample to give a guest concentration of 1.8 μM, and equilibration was continued for 24 h. Beads were analysed in flat-bottomed glass pots under a Leica inverted DML microscope (x 40 magnification).

Screening of Tweezer receptor Library 95

A sample of each sublibrary **1, 2, 3, 4** (7 mg) was equilibrated in chloroform (1 mL) for 24 h. Aliquots of guest **97** (20 μM), in chloroform, was added (3 x 30 μL) to the sample to give a guest concentration of 1.8 μM, and equilibration was continued for 24 h. Beads were analysed in flat-bottomed glass pots under a Leica inverted DML microscope (x 40 magnification). Highly red stained beads were selected from pot with sublibrary **1**, and submitted for Edman sequencing.

Preparation of Tweezer receptor 110

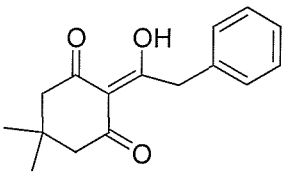
A solution of Boc-CBS **84** (80.0 mg, 0.12 mmol) and PyBOP (61.0 mg, 0.12 mmol) in dichloromethane (4 mL) was added to pre-swollen commercial available oxime resin (200 mg, 0.12 mmol NH_2) followed by DIPEA (0.24 mmol, 41.0 μL). The suspension was agitated on a tube rotator for 48 h, drained and then washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL The resin was agitated on a tube rotator for further 18 h after addition of acetic anhydride (2.36 mmol, 223 μL) in CH_2Cl_2 (5 mL). Washing with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL), and CH_2Cl_2 (3 x 5 mL) and subsequent drying yielded a resin which provided a negative ninhydrin test. Removal of Boc protection was achieved by adding and agitating the resin in 25% TFA in dichloromethane for 2 h. Subsequent washing with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL), methanol (3 x 5 mL), and

CH_2Cl_2 (3 x 5 mL) and drying yielded a resin which gave a positive ninhydrin test. A solution of *N*-Boc-L-Valine (51.0 mg, 0.24 mmol), TBTU (76.0 mg, 0.24 mmol), HOBt (36.0 mg, 0.24 mmol), and DIPEA (0.53 mmol, 93 μL) in DMF (5 mL) was added to the pre-swollen resin and the resulting suspension was agitated on a tube rotator for 18 h. After washing with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL), and CH_2Cl_2 (3 x 5 mL) the coupling cycle was repeated to yield a resin which give a negative ninhydrin test. Subsequent Boc removal (as described above) followed by cyclic coupling using *N*-Boc-L-Alanine and *N*-Boc-L-Pro-OH in alternative cycles with Boc deprotection, each coupling and deprotection step monitored by ninhydrin tests, yielded the resin bound tweezer receptor **109** with Boc-protected *N*-terminus.

Cleavage from solid support was achieved by agitating the pre-swollen resin with a solution of dodecylamine (0.5 M in chloroform) for 12 h. The mixture was drained, and washed with CH_2Cl_2 (3 x 5 mL) and methanol (3 x 5 mL). Concentration of the filtrate under reduced pressure yielded a yellowish oil (166 mg). Purification by column chromatography on silica (10% methanol in CH_2Cl_2) produced a colourless film (25 mg, 15%). Characterisation was achieved after purification by semi-preparative, reverse-phase HPLC analysis (Phenomenex Prodigy ODS(3) C-18, 250 x 10 mm using a linear gradient from water + 0.1% TFA to acetonitrile + 0.042% TFA over 40 mins, acetonitrile + 0.042% TFA for 10 mins, a linear gradient from acetonitrile + 0.042% TFA to water + 0.1% TFA over 5 mins, and water + 0.1% TFA for 5 mins, with a flow rate of 2.5 mL mins, monitoring at 220 nm. Under these conditions receptor **110** (15 mg, 10%) eluted after 45.93 mins. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.48-7.44 (m, 2H, PyrH), 7.25-7.12 (m, 19H, ArH and NH), 4.86-3.90 (m. 10H, COC*H*NH and OCH_2CO), 3.60-3.45 (m, 4H, CH_2Ar), 2.11-1.97 (m, 14H, CH_3CHCH_3 and CH_2 from Pro), 1.43 (s, 18H, $C(CH_3)_3$), 1.18-1.09 (m, 22H, $(CH_2)_{11}CH_3$), 0.82-0.78 (m, 9H, $CHCH_3$ and CH_2CH_3), 0.67-0.62 (m, 12H, CH_3CHCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 176.7(0), 174.6(0), 174.5(0), 172.9(0), 155.9(0), 146.3(0), 136.7(0), 129.2(1), 128.6(1), 127.1(1), 96.4(0), 81.6(0), 68.0(1), 61.8(1), 55.9(1), 52.3(1), 47.6(1), 40.0(2), 36.4(2), 32.0(2), 30.0(2), 29.7(2), 29.4(2), 29.3(2), 29.1(2), 28.3(1), 26.9(2), 24.7(2), 22.8(2), 18.6(3), 17.8(3), 17.1(3). 14.2(3); MS (ES^+): m/z (%): 1379 (100) $[M + H]^+$, 1401 (30) $[M + Na]^+$.

5.6 Experimental for Chapter 3.

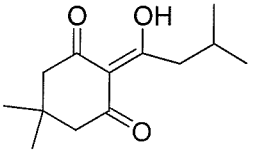
2-(1-Hydroxy-2-phenyl-ethylidene)-5,5-dimethyl-cyclohexane-1,3-dione 120[107]



120

Dimedone (3.00 g, 21.4 mmol), DCC (4.00 g, 19.6 mmol) and DMAP (2.40 g, 19.6 mmol) were added to phenylacetic acid (2.60 g, 19.4 mmol) in DMF (200 mL) and the mixture stirred at room temperature for 60 h. Precipitated DCU was removed and the solvent removed under reduce pressure. The yellow residue was redissolved in ethyl acetate (200 mL) and the organic solution washed first with aqueous potassium hydrogen sulfate (1M, 200 mL) and a saturated solution of sodium hydrogen carbonate. The organic layer was dried over $MgSO_4$ and filtered The solvent was removed under reduced pressure to give a crude yellow solid which was recrystallised from methanol to afford the pure product as a white crystalline solid (2.01 g, 40%). M.p. 102-104°C; IR: ν = 3056, 2929, 1648 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.35-7.23 (m, 5H, ArH), 4.40 (s, 2H, CH_2Ar), 2.54 (s, 2H, CH_2CO), 2.38 (s, 2H, CH_2CO), 1.08 (s, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 203.1(0), 197.8(0), 195.4(0), 135.0(0), 130.2(1), 128.8(1), 127.3(1), 112.2(0), 52.9(2), 47.0(2), 46.6(2), 31.0(0), 28.5(3); elemental analysis calcd (%) for $C_{16}H_{18}O_3$: C 74.39, H 7.02; found: C 74.34, H 7.01.

2-(1-Hydroxy-3-methyl-butylidene)-5,5-dimethyl-cyclohexane-1,3-dione 122[107]

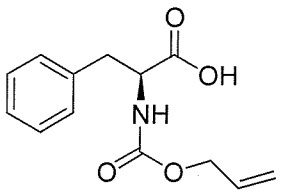


122

Dimedone (3.00 g, 21.4 mmol), DCC (4.00 g, 19.6 mmol) and DMAP (2.40 g, 19.6 mmol) were added to valeric acid (2.10 mL, 19.4 mmol) in DMF (200 mL) and the mixture stirred

at room temperature for 60 h. Precipitated DCU was removed and the solvent removed under reduce pressure. The yellow residue was redissolved in ethyl acetate (200 mL) and the organic solution washed first with aqueous potassium hydrogen sulfate (1M, 200 mL) and subsequently with a saturated sodium hydrogen carbonate solution. The organic layer was dried over $MgSO_4$, filtered and the solvent removed under reduce pressure to give a yellow solid which was recrystallised from ether to afford the pure product as a white crystalline solid (600 mg, 14%). M.p. 152-154°C; IR: ν = 3282, 2929, 1702, 1629 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 2.69 (d, J=9 Hz, 2H, CH_2CH), 2.25 (d, J=13 Hz, 4H, CH_2CO), 2.05-2.01 (m, 1H, $CH(CH_3)_2$), 1.03 (s, 6H, CH_3CCH_3), 0.89 (d, J=9 Hz, 6H, CH_3CHCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 200.6(0), 196.8(0), 196.6(0), 115.8.(0), 54.3(2), 53.1(2), 52.4(2), 48.9(1), 31.1(3), 29.3(3), 26.7(3), 26.6 (3), 24.1(0); elemental analysis calcd (%) for $C_{13}H_{20}O_3$: C 69.61, H 8.99; found: C 69.85, H 8.99.

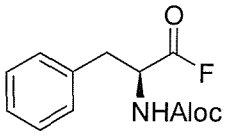
2{[(Allyloxy)carbonyl]amino}-3-phenylpropanoic acid 124[112]



124

Allyl chloroformate (1.40 mL, 13.1 mmol) was added dropwise to a solution of commercially available L-phenylalanine (2.00 g, 12.1 mmol) in aqueous NaOH (4 N, 30 mL) at 0°C. The reaction mixture was allowed to warm up to ambient temperature and stirred for 15 h. The reaction mixture was then acidified (HCl) to pH 2 and the aqueous layers were extracted with CH_2Cl_2 (3 x 50 mL). The combined organic layers were dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure to give allyl carbamate **124** (2.01 g, 67%) as a colourless oil. IR: ν = 3360, 1841, 1702 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.70 (bs, 1H, NH), 7.35-7.17 (m, 5H, ArH), 5.96-5.90 (m, 1H, $CH_2CH=CH_2$), 5.37-5.24 (m, 2H, $CH=CH_2$), 4.74-4.72 (m, 1H, CHNH), 4.60 (d, 2H, J=5 Hz, $CH_2CH=CH_2$), 3.28-3.13 (m, 2H, CH_2Ar); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.8(0), 154.8(0), 134.6(0), 131.4(1), 128.3(1), 127.7(1), 126.2(1), 116.9(2), 65.1(2), 53.5(1), 36.7(2).

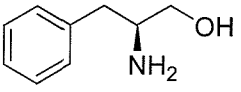
N-Allyloxycarbonyl-L-phenylalanyl fluoride 125[94]



125

Cyanuric fluoride (1.63 mL, 19.4 mmol) was added to a mixture of *N*-Aloc-Phenylalanine (2.20 g, 8.83 mmol) and pyridine (712 μL, 8.83 mmol) in dry CH_2Cl_2 at –15°C and stirred for 3 hours at –15°C. Crushed ice (50 g) and CH_2Cl_2 (100 mL) were added to the reaction mixture and the aqueous layer was extracted with CH_2Cl_2 (100 mL x 3). The combined organic layers were washed with ice-cold water (200 mL), dried over $MgSO_4$ and filtered. The solvent was removed under reduced pressure to give the acid fluoride as a yellow oil (2.00 g, 96%). IR: ν = 3360, 1841, 1702, 1523 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.09-6.91 (m, 5H, ArH), 5.84-5.73 (m, 1H, C*H*=CH_2), 5.23-5.06 (m, 2H, CH_2=CH), 4.73 (d, J=5 Hz, 1H, C*H*NH), 4.58-4.48 (m, 2H, CH_2OCO), 3.15-3.02 (m, 2H, CH_2Ar); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 162.2(0) (d, $^1J_{CF}$=368 Hz), 155.8(0), 134.6(0), 132.5(1), 129.5(1), 129.0(1), 128.2(1), 118.6(2), 66.4(2), 54.1(1) (d, $^2J_{CF}$=59 Hz), 37.2(2); MS (ES^+): m/z (%): 260 (10) $[M + Na]^+$.

2-amino-3-phenyl-1-propanol 129

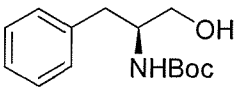


129

Under anhydrous conditions, a suspension of $LiAlH_4$ (1.52 g, 40.0 mmol) in THF (75 ml) was stirred at 0°C for 10 mins. L-Phenylalanine (2.00 g, 12.1 mmol) was added slowly and the suspension was stirred at 0°C for 20 mins. The reaction was placed under nitrogen and refluxed at 90°C for 4 h. Once the reaction had cooled to room temperature water (25 mL) was added at 0°C followed by a solution of 10% H_2SO_4. The mixture was stirred for a further 15 mins. The suspension changed from grey to yellow. The reaction mixture was filtered and the aqueous phase was neutralised by the addition of 50% NaOH (30 mL). The organic layer was extracted, dried over $MgSO_4$ and filtered. The solvent was removed

under reduced pressure to give a pure white crystalline product. (1.57 g, 86%). M.p. 78-80°C; IR: ν = 3357; 3298; 2874; 1574 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.25-7.11 (m, 5H, ArH); 3.57 (dd, 1H, J=3.76, 4.04 Hz, CH_aH_bOH); 3.32 (dd, 1H, J=7.28 Hz, CH_aH_bOH); 3.09-3.03 (m, 1H, CHN); 2.73 (dd, 1H, J=5.52, 5.24 Hz, CH_aH_bAr); 2.73 (dd, 1H, J=8.52 Hz, CH_aH_bAr); 1.81 (s, 2H, NH_2); ^{13}C NMR (100MHz, $CDCl_3$): δ = 138.9(0), 129.6(1), 129.0(1), 126.8(1), 66.6(1), 54.5(2), 41.2(2); MS (ES^+): m/z (%): 152 (100) $[M + H]^+$. Spectroscopic data are consistent with those reported in literature.[87]

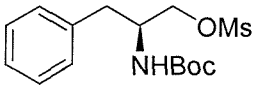
Synthesis of *tert*-butyl N-(1-benzyl-2-hybroxyethyl)carbamate 130



130

To a solution of the amino alcohol **129** (1.34 g, 8.84 mmol) in CH_2Cl_2 (25 mL) at 0°C a solution of di-*tert*-butyl dicarbonate (2.23 g, 8.84 mmoL) in CH_2Cl_2 (25 mL) was added dropwise. The solution was stirred for 3 h at RT. The solvent was removed under reduced pressure. The residue was redissolved in ethyl ether and washed with $KHSO_4$ (0.33 M), brine (50 mL), saturated $NaHCO_3$ (50 mL) and brine (50 mL). The organic solution was dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to give a white crystalline solid. (1.28 g, 58%). M.p. 88-90°C; IR: ν = 3349, 1685, 1526, 1166 cm^{-1}; ^1H NMR (400MHz, $CDCl_3$): δ = 7.24-7.13 (m, 5H, ArH), 4.67 (br s, 1H, NH), 3.79 (m, 1H, CHN), 3.59 (dd, 1H, J=3.76, 3.52 Hz, CH_aH_bOH), 3.48 (dd, 1H, J=5.28 Hz, CH_aH_bOH), 2.76 (d, 2H, J=7.28 Hz, CH_2Ar), 1.34 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (100MHz, $CDCl_3$): δ = 156.5(0), 138.2(0), 129.6(1), 128.9(1), 126.9(1), 80.1(0), 64.7(1), 54.1(2), 37.8(2), 28.7(3); MS (ES^+): m/z (%): 252 (75) $[M + H]^+$; 525 (85) $([2M + Na]^+$. Spectroscopic data are consistent with those reported in literature.[87]

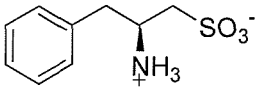
2-[(*tert*-Butoxycarbonyl)amino]-3-phenylpropyl methane sulfonate 131



131

Under a nitrogen atmosphere at 0°C a solution of methanesulfonyl chloride (729 μL, 6.30 mmol) in CH_2Cl_2 (25 mL) was added dropwise to a solution of the *N*-Boc-amino alcohol **130** (1.26 g, 4.90 mmoL), triethylamine (661 μL, 6.50 mmoL) in CH_2Cl_2 (25 mL) over a period of 30 mins. The reaction was stirred for two hours at room temperature. The solvent was removed under reduced pressure and the residue redissolved in ethyl acetate. The organic phase was washed with aqueous $NaHCO_3$ (30 mL) and Brine (30 mL), dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to give a crude solid. The crude compound was purified using flash chromatography (ethyl acetate:petroleum ether 40:60) to give a white crystalline product. (1.28 g, 80%). M.p. 110-112°C; IR: ν = 3353, 1691, 1526, 1160 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.28-7.13 (m, 5H, ArH), 4.66 (br s, 1H, NH), 4.17 (m, 1H, C*H*NH), 4.06-4.03 (m, 2H, CH_2OSO_2), 2.94 (s, 3H, SO_2CH_3), 2.84-2.65 (m, 2H, CH_2Ar), 1.34 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 154.9(0), 136.4(0), 128.8(1), 128.7(1), 128.5(1), 79.8(0), 69.6(1), 53.6(2), 41.3(3), 37.1(2), 28.7(3); MS (ES^+): m/z (%): 330 (20) $[M + H]^+$; 352 (28) $[M + Na]^+$; 281 (80) $[2M + Na]^+$. Spectroscopic data are consistent with those reported in literature.[87]

2-Amino-3-phenyl-1-propanesulfonate 132

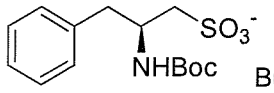


132

The mesylated amino alcohol **131** (1.70 g, 5.40 mmoL) was dissolved in a solution of anydrous hydrogen chloride (4M in dioxane, 11 mL) and stirred for 1 h. The solvent was removed under reduced pressure, and the residue was dissolved in water. Na_2SO_3 (0.66 g, 5.00 mmoL) was added to the solution. The reaction was stirred for 36 h. The solution was concentrated under reduced pressure and methanol was added to remove the excess

$NaSO_4$. The solution was filtered and the solvent removed under reduced pressure to afford the desired compound (1.10 g, 95%). IR: ν = 3008, 2920, 1511, 1352 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.74 (br s, 3H, NH_3), 7.49-7.35 (m, 5H, ArH), 4.45-4.42 (m, 1H, CH_aH_bO), 4.31-4.26 (m, 1H, CH_aH_bO), 3.85 (br s, 1H, $CHNH_3$), 3.20 (dd, J=5.52 Hz, 1H, CH_aH_bAr), 2.99 (dd, J=9.04, 9.28 Hz, 1H, CH_aH_bAr); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 135.9(0), 129.8(1), 129.1(1), 127.5(1), 68.1(1), 51.0(2), 37.3(2); M/z (ES^+): 230 (100) [M + NH_3]$^+$. Spectroscopic data are consistent with those reported in literature.[87]

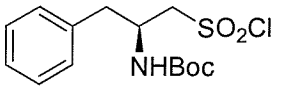
Synthesis of 2-[{*tert*-butoxycarbonyl}amino]-3-phenyl-1-propane sulfonate tetrabutyl ammonium salt 133



133

The betaine **132** (300 mg, 1.46 mmol) was dissolved in water (1.00 mL). A solution of tetrabutylammonium hydroxide (1.07 g, 1.50 mmoL) in water (1.90 mL) was added and the resulting solution stirred for 10 mins. A solution of di-*tert*-butyl dicarbonate (0.38 g, 1.50 mmoL) in THF (1.50 mL) was added to the reaction in a dropwise fashion. The resulting solution was stirred for 16 h. The reaction was concentrated under reduced pressure to half the original volume and the aqueous phase extracted with CH_2Cl_2 (5 x 10 mL). The organic layers were combined, dried over $MgSO_4$, and filtered. The solvent was removed under reduced pressure to give a light-yellow oil. (267 mg, 58%). IR: ν = 1766, 1527 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 7.32-7.24 (m, 5H, ArH), 5.30 (bs, 1H, NH), 3.40-3.34 (m, 9H, $N(CH_2)_4$ and CH_2SO_2), 3.09-2.87 (m, 1H, CHN), 2.76-2.61 (m, 2H, CH_2Ar), 1.69-1.25 (m, 25H, $C(CH_3)_3$ and $NCH_2CH_2CH_2$), 1.02 (t, 12H, $NCH_2CH_2CH_3$). Spectroscopic data are consistent with those reported in literature.[87]

***tert*-Butyl *N*-(1-benzyl-2-chlorosulfonlyethyl)carbamate 134**



134

The tetrabutyl ammonium salt **133** (1.52 g, 3.10 mmoL) and DMF (49.0 μL, 0.63 mmoL) were dissolved in CH_2Cl_2 (20 mL) at 0°C. A solution of bis-trichloromethyl carbonate (1.31 g, 4.40 mmol) in CH_2Cl_2 (10 mL) was added in a dropwise fashion. The reaction was stirred at 0°C for 30 mins and then at RT for 1 h. The solvent was reduced under reduced pressure to give the crude sulfonyl chloride (258 mg, 25%). IR: ν = 2259, 1753, 1702 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$): δ = 7.26-7.22 (5H, m, ArH); 4.28-4.22 (1H, m, NH); 3.88-3.38 (3H, m, CH_2SO_2Cl and CHN); 3.28-2.89 (2H, m, CH_2Ar); 1.53 (9H, s, $C(CH_3)_3$). Spectroscopic data are consistent with those reported in literature.[87]

Screening the Inverted Peptide Library 137 with Red-dye labelled tweezer 135 and 136

A sample of inverted peptide library **137** (10 mg) was equilibrated in $CHCl_3$ (1 mL) for 24 h. Aliquots of red-dye labelled tweezer **135** (20 μM), in $CHCl_3$, was added (2 x 1.5 mL) to the sample to give 15 μM concentration in host, equilibration was continued for 24 h. Beads were analysed in flat-bottomed glass pots under Leica inverted DML microscope (40 x magnification). The same screening experiment was carried out with red dye labelled twezeer **136.**

Screening of Receptor Library 138

Preparation of the buffer solution: to an aqueous solution of borax (0.02 M, 50 mL) was added aqueous sodium hydroxide (0.10 M, 0.90 mL) and the pH of the resulting buffer solution was maintained at 9.2.

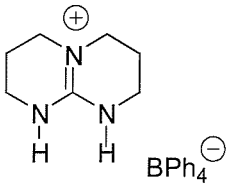
A sample of library **138** (12 mg) was equilibrated in borax buffer solution (1 mL) for 24 h. Aliquots of guest **141** (20 μM), in water-DMSO (85:15)%, were added (3 x 30 μL) to the

sample to give a guest concentration of 1.8 μM, and equilibration was continued for 24 h. Beads were analysed in flat-bottomed glass pots under Leica inverted DML microscope (40 x magnification). Highly red stained beads were selected, removed and submitted for Edman sequencing.

Tetrabutylammonium acetate salt 149

A solution of tetrabutylammonium hydroxide (5.00 mL, 5.00 mmol, 1 M solution in methanol) and acetic acid (0.42 mL, 7.50 mmol) was stirred for 15 mins. Toluene (10 mL) was added to the mixture and the solvents were removed under reduced pressure to give a white solid. The product was washed with petroleum ether and removed by filtration. The white solid obtained was dried under high vacuum to afford the pure compound **151** (1.50 g, 100%). M.p. 96-98°C (lit.: 95-98°C)[138]; IR: ν = 2958, 2874, 1671 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ = 3.27 (t, J=8 Hz, 8H, CH_2N), 1.83 (s, 3H, CH_3COO), 1.66-1.64 (m, 8H, CH_2), 1.40 (q, J=7.2 Hz, 8H, CH_2), 1.03 (t, J=7.2 Hz, 12H, CH_3); MS (ES^+): m/z (%): 242 (100) $[M + H]^+$.

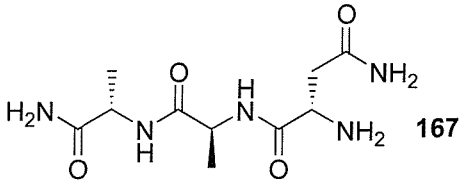
1,3,4,6,7,8-Hexahydro-2H-pyrimido[1,2-a]pyrimidine tetraphenylborate (1.BPh_4) 150[33]



150

Compound **150** was prepared according to the literature procedure.[34] (97 mg, 100%). M.p 178-180°C (lit.:[34] 180°C); IR: ν = 3379, 3055, 1617 cm^{-1}; ^1H NMR (400 MHz, CD_3CN): δ = 7.30 (br s, 8H, ArH), 7.02 (t, J=7.28 Hz, 8H, ArH), 6.87 (t, J=7.28 Hz, 4H, ArH), 6.05 (br s, 2H, NH), 3.28-3.19 (m, 8H), 1.97-1.90 (m, 4H); ^{13}C NMR (100 MHz, CD_3CN): δ = 135.4(0), 125.3(1), 125.2(1), 121.4(1), 117.0(0), 46.2(2), 37.6(2), 19.9(2). MS (ES^+): m/z (%): 139.9 (100) $[M + H]^+$. Spectroscopic data are consistent with those reported in literature.[34]

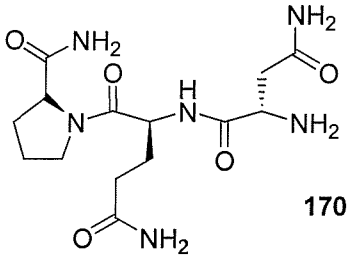
Synthesis of H_2N-L-Asn-L-Ala-L-Ala-$CONH_2$ 167



Sieber Amide Resin (500 mg, 0.52 mmol/g) was agitated in 20% piperidine in DMF for 40 mins. After this time the resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL). The qualitative ninhydrin test was positive. A solution of *N*-Fmoc-alanine (0.52 mmol, 162 mg), and HOBt (0.52 mmol, 79.56 mg) were dissolved in CH_2Cl_2 (20 mL) containing a few drops of DMF. DIC (0.57 mmol, 90.0 mL) was added and the mixture stirred for 20 mins before addition to pre-swollen resin. The resin mixture was agitated at RT for 3 h to ensure the coupling step was driven to completion. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL) and dried. The qualitative ninhydrin test was negative. The resin was agitated with 20 % piperidine in DMF for 20 mins. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL), and CH_2Cl_2 (3 x 5 mL) and the deprotection step repeated. Qualitative ninhydrin test was positive. A solution of *N*-Fmoc-Alanine (0.52 mmol, 162 mg), and HOBt (0.52 mmol, 79.5 mg) were dissolved in CH_2Cl_2 (20 mL) containing a few drops of DMF. DIC (0.57 mmol, 90.0 μL) was added and the mixture stirred for 20 mins before the addition to the resin swollen in a minimum amount of CH_2Cl_2. The resin was stirred overnight. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL) and dried. The qualitative ninhydrin test was negative. The resin was stirred with 20% piperidine in DMF for 20 mins. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL) and the deprotection step was repeated. Qualitative ninhydrin test was positive. A solution of *N*-Fmoc-Asparagine (0.52 mmol, 199 mg) and HOBt (0.52 mmol, 79.5 mg) were dissolved in CH_2Cl_2 (20 mL) containing a few drops of DMF. DIC (0.57 mmol, 90.0 μL) was added and the mixture stirred for 20 mins before addition to the resin swollen in a minimum amount of CH_2Cl_2. The resin was stirred at RT for 3 h. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL) and dried. The qualitative ninhydrin test was negative. The resin was stirred with 20% piperidine in

DMF for 20 mins. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL) and deprotection step was repeated. Qualitative ninhydrin test was positive. The resin was agitated in 1% TFA in CH_2Cl_2 for 2 h. Resin was drained and then washed with CH_2Cl_2 and methanol. Toluene (10 mL) was added to the combined filtrate and solvents removed under reduced pressure. The crude product was treated with ether to allow precipitation of the pure peptide as a white solid which was removed for filtration (68 mg, 96%). RP HPLC analysis (λ=254 nm) employing a mobile phase gradient from 90% to 10% water/TFA in acetonitrile/TFA: 6.262 mins; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.55 (d, J=7 Hz, 1H, NH from Ala), 8.01 (br, 2H, NH_2 from Asn), 7.92 (d, J=7 Hz, 1H, NH), 7.63 (s, 1H, CONH_aH$_b$), 7.20 (s, 1H, CONH$_a$$H_b$), 7.09 (s, 1H, CON$H_aH_b$), 6.91 (s, 1H, CONH$_a$$H_b$), 4.23-4.19 (m, 1H, C$HCH_3$), 4.08 (m, 1H, C$HCH_3$), 3.99 (br, 1H, C$HCH_2$CONH$_2$), 2.67-2.49 (m, 2H, C$H_2$CONH$_2$), 1.19-1.14 (m, 6H, CHC$H_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 172.3(0), 169.4(0), 169.06(0), 166.3(0), 47.4(1), 46.9(1), 46.4(1), 41.1(2), 16.4(3), 16.2(3); MS (ES$^+$): m/z (%): 274 (100) [M + H]$^+$, 547 (15) [2M + H]$^+$; HRMS calcd for $C_{10}H_{20}N_5O_4$ [M + H]$^+$ 274.1516 found 274.1511.

Synthesis of H_2N-L-Asn-L-Gln-L-Pro-$CONH_2$ 168



170

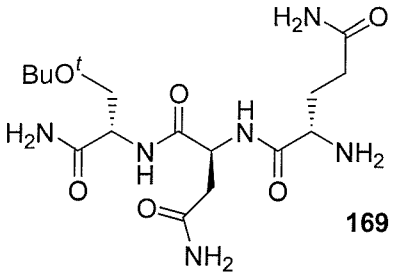
Sieber Amide Resin (500 mg, 0.52 mmol/g) was agitated with 20% piperidine in DMF for 40 mins. After this time the resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL). The qualitative ninhydrin test was positive. A solution of *N*-Fmoc-Proline (0.52 mmol, 175 mg), and HOBt (0.52 mmol, 79.5 mg) were dissolved in CH_2Cl_2 (20 mL) containing a few drops of DMF. DIC (0.57 mmol, 90.0 mL) was added and the mixture stirred for 20 mins before addition to pre-swollen resin. The resin mixture was agitated at RT for 3 h to ensure the coupling step was driven to completion. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL) and dried. The

qualitative ninhydrin test was negative. The resin was stirred with 20 % piperidine in DMF for 20 mins. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL) and the deprotection step repeated. Qualitative ninhydrin test was positive. A solution of *N*-Fmoc-Glutamine (0.52 mmol, 191 mg) and HOBt (0.52 mmol, 79.5 mg) were dissolved in CH_2Cl_2 (20 mL) containing a few drops of DMF. DIC (0.57 mmol, 90.0 μL) was added and the mixture stirred for 20 mins before the addition to the resin swollen in a minimum amount of CH_2Cl_2. The resin was stirred overnight. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL) and dried. The qualitative ninhydrin test was negative. The resin was stirred with 20% piperidine in DMF for 20 mins. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL) and the deprotection step was repeated. Qualitative ninhydrin test was positive. A solution of *N*-Fmoc-Asparagine (0.52 mmol, 199 mg) and HOBt (0.52 mmol, 79.5 mg) were dissolved in CH_2Cl_2 (20 mL) containing a few drops of DMF. DIC (0.57 mmol, 90.0 μL) was added and the mixture stirred for 20 mins before addition to the resin swollen in a minimum amount of CH_2Cl_2. The resin was stirred at RT for 3 h. The resin was washed with CH_2Cl_2 (3 times), DMF (3 times) and CH_2Cl_2 (3 times) and dried. The qualitative ninhydrin test was negative. The resin was stirred with 20% piperidine in DMF for 20 mins. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL) and the deprotection step repeated. Qualitative ninhydrin test was positive.

The resin was shaken with 1% TFA in CH_2Cl_2 for 2 h. Resin was drained and washed with CH_2Cl_2 and methanol. Toluene (10 mL) was added to the combined filtrate and solvents were evaporated under reduced pressure. The crude product was treated with ether to allow precipitation of the pure peptide as a white solid which was removed with filtration (96 mg, 100%). RP HPLC analysis (λ=254 nm) employing a mobile phase gradient from 90% to 10% water/TFA in acetonitrile/TFA: 6.702 mins; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.76 (d, J=7 Hz, 1H, N*H*CHCH$_2$CH$_2$), 8.21 (br, 2H, CHN*H*$_2$), 7.78 (s, 1H, CON*H*$_a$NH$_b$), 7.43 (s, 1H, CONH$_a$N*H*$_b$), 7.38 (s, 1H, CON*H*$_a$H$_b$), 7.34 (s, 1H, CONH$_a$N*H*$_b$), 7.00 (s, 1H, CON*H*$_a$NH$_b$), 6.90 (s, 1H, CONH$_a$N*H*$_b$), 4.67-4.60 (m, 1H, C*H*CH$_2$CH$_2$), 4.32 (m, 1H, C*H*CONH$_2$), 4.20 (br, 1H, C*H*NH$_2$), 3.75-3.67 (m, 2H, CH$_2$N), 2.80 (m, 1H, C*H*$_a$H$_b$CONH$_2$), 2.64 (m, 1H, CH$_a$*H*$_b$CONH$_2$), 2.29-2.25 (m, 2H, CH$_2$C*H*$_2$CONH$_2$), 2.19-1.82 (m, 4H, C*H*$_2$C*H*$_2$CH$_2$N), 180-1.74 (m, 2H, C*H*$_2$CH$_2$CONH$_2$); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 174.1(0), 173.4(0), 171.0(0), 169.7(0), 168.4(0), 59.8(1), 51.1(1), 50.6(1),

47.1(2), 46.7(2), 31.19(2), 29.7(2), 24.8(2), 22.6(2); MS (ES^+): m/z (%): 357 (100) $[M + H]^+$, 713 (15) $[2M + H]^+$; HRMS calcd for $C_{14}H_{25}N_6O_5$ $[M + H]^+$ 357.1887 found 357.1878.

Synthesis of H_2N-L-Gln-L-Asn-L-Ser(^{t}Bu)-$CONH_2$ 169

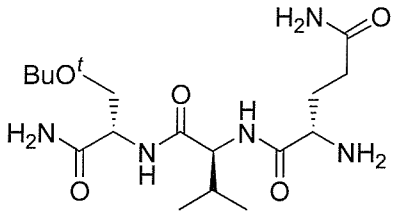


169

Sieber Amide Resin (500 mg, 0.52 mmol/g) was agitated with 20% piperidine in DMF for 40 mins. After this time the resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL). The qualitative ninhydrin test was positive. A solution of *N*-Fmoc-Serine(*t*Bu) (0.52 mmol, 199 mg), and HOBt (0.52 mmol, 79.5 mg) were dissolved in CH_2Cl_2 (20 mL) containing a few drops of DMF. DIC (0.57 mmol, 90.0 mL) was added and the mixture stirred for 20 mins before addition to the resin pre-swollen in a minimum amount of CH_2Cl_2. The resin was agitated at RT for 3 h to ensure the coupling step was driven to completion. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL) and dried. The qualitative ninhydrin test was negative. The resin was stirred with 20 % piperidine in DMF for 20 mins. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL), and CH_2Cl_2 (3 x 5 mL) and the deprotection step was repeated. The qualitative ninhydrin test was positive. A solution of *N*-Fmoc-Asparagine (0.52 mmol, 199 mg), and HOBt (0.52 mmol, 79.5 mg) were dissolved in CH_2Cl_2 (20 mL) containing a few drops of DMF. DIC (0.57 mmol, 90.0 μL) was added and the mixture stirred for 20 mins before the addition to the resin pre-swollen in a minimum amount of CH_2Cl_2. The resin was stirred overnight. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL) and dried. The qualitative ninhydrin test was negative. The resin was stirred with 20% piperidine in DMF for 20 mins. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL) and the deprotection step was

repeated. The qualitative ninhydrin test was positive. A solution of *N*-Fmoc-Glutamine (0.52 mmol, 176 mg), and HOBt (0.52 mmol, 79.5 mg) were dissolved in CH_2Cl_2 (20 mL) containing a few drops of DMF. DIC (0.57 mmol, 90.0 μL) was added and the mixture stirred for 20 mins before addition to the resin pre-swollen in a minimum amount of CH_2Cl_2. The resin was stirred at RT for 3 h. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL) and dried. The qualitative ninhydrin test was negative. The resin was stirred with 20% piperidine in DMF for 20 mins. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL) and the deprotetion step repeated. The qualitative ninhydrin test was positive. The resin was shaken with 1% TFA in CH_2Cl_2 for 2 h. Resin was drained and washed with CH_2Cl_2 and methanol. Toluene (10 mL) was added to the combined filtrate and solvents were evaporated under reduced pressure. The crude product was treated with ether to allow precipitation of the pure peptide as a white solid which was removed with filtration (34.0 mg, 40%). RP HPLC analysis (λ = 254 nm) employing a mobile gradient 0-100% acetonitrile/water (20 mins): 12.254; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.82 (d, J=8 Hz, 1H, N*H*CHCH$_2$), 8.27 (br, 2H, CHNH$_2$), 7.96 (d, J=8 Hz, 1H, N*H*CHCH$_2$O), 7.57 (s, 1H, CON*H*$_a$NH$_b$), 7.47 (s, 1H, CONH$_a$N*H*$_b$), 7.44 (s, 1H, CON*H*$_a$H$_b$), 7.24 (s, 1H, CONH$_a$N*H*$_b$), 7.11 (s, 1H, CON*H*$_a$NH$_b$), 7.07 (s, 1H, CONH$_a$N*H*$_b$), 4.79 (m, 1H, C*H*CH$_2$CONH$_2$), 4.30 (m, 1H, C*H*CH$_2$O), 3.93 (br, 1H, C*H*CH$_2$CH$_2$), 3.65 (m, 1H, C*H*$_a$H$_b$OC(CH$_3$)$_3$), 3.57 (m, 1H, CH$_a$*H*$_b$OC(CH$_3$)$_3$), 2.72 (m, 1H, C*H*$_a$H$_b$CONH$_2$), 2.53 (m, 1H, CH$_a$*H*$_b$CONH$_2$), 2.36-2.32 (m, 2H, CH$_2$C*H*$_2$CONH$_2$), 2.05-2.00(m, 2H, C*H*$_2$CH$_2$CONH$_2$), 1.22 (s, 9H, C(CH$_3$)$_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 172.0(0), 170.2(0), 169.9(0), 168.9(0), 166.8(0), 71.3(0), 60.2(2), 53.9(1), 52.1(1), 50.4(1), 35.6(2), 29.0(2), 27.7(2), 25.8(3); MS (ES$^+$): m/z (%): 403 (100) [M + H]$^+$, 425 (30) [M + Na]$^+$, 805 (15) [2M + H]$^+$.

Synthesis of H_2N-L-Gln-L-Val-L-Ser(^{t}Bu)-$CONH_2$ 170



170

Sieber Amide Resin (500 mg, 0.52 mmol/g) was agitated with 20% piperidine in DMF for 40 mins. After this time the resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL). The qualitative ninhydrin test was positive. A solution of *N*-Fmoc-Serine(^{t}Bu) (0.52 mmol, 199 mg), and HOBt (0.52 mmol, 79.5 mg) were dissolved in CH_2Cl_2 (20 mL) containing a few drops of DMF. DIC (0.57 mmol, 90.0 mL) was added and the mixture stirred for 20 mins before addition to the pre-swollen resin. The resin was shaken at RT for 3 h to ensure the coupling step was driven to completion. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL) and dried. The qualitative ninhydrin test was negative. The resin was stirred with 20 % piperidine in DMF for 20 min. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL), and CH_2Cl_2 (3 x 5 mL) and the deprotection step was repeated. The qualitative ninhydrin test was positive. A solution of *N*-Fmoc-Valine (0.52 mmol, 176 mg), and HOBt (0.52 mmol, 79.5 mg) were dissolved in CH_2Cl_2 (20 mL) containing a few drops of DMF. DIC (0.57 mmol, 90.0 μL) was added and the mixture stirred for 20 mins before the addition to the resin swollen in a minimum amount of CH_2Cl_2. The resin was stirred overnight. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL) and dried. The qualitative ninhydrin test was negative. The resin was stirred with 20% piperidine in DMF for 20 mins. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL) and the deprotetion step was repeated. The qualitative ninhydrin test was positive. A solution of *N*-Fmoc-Glutamine (0.52 mmol, 176 mg), and HOBt (0.52 mmol, 79.5 mg) were dissolved in CH_2Cl_2 (20 mL) containing a few drops of DMF. DIC (0.57 mmol, 90.0 μL) was added and the mixture stirred for 20 mins before addition to the resin swollen in a minimum amount of CH_2Cl_2. The resin was stirred at RT for 3 h. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL) and

dried. The qualitative ninhydrin test was negative. The resin was stirred with 20% piperidine in DMF for 20 mins. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL) and deprotetion step was repeated. The qualitative ninhydrin test was positive. The resin was shaken with 1% TFA in CH_2CL_2 for 2 h. Resin was drained and washed with CH_2Cl_2 and methanol. Toluene (10 mL) was added to the combined filtrate and solvents were evaporated under reduced pressure. The crude product was treated with ether to allow precipitation of the pure peptide as a white solid which was removed with filtration (76 mg, 100%). RP HPLC analysis employing mobile phase gradient from 90 to 10% water/TFA in acetonitrile/TFA: 0.767 mins; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.77 (d, J=8 Hz, 1H, N*H*CHCH), 8.43 (br, 2H, CHNH_2), 8.16 (d, J=8 Hz, 1H, N*H*CHCH$_2$O), 7.69 (s, 1H, CONH_aNH$_b$), 7.59 (s, 1H, CONH$_a$NH_b), 7.38 (s, 1H, CONH_aH$_b$), 7.26(s, 1H, CONH$_a$NH_b), 4.59-4.56 (m, 2H, C*H*CH(CH$_3$)$_2$ and C*H*CH$_2$O), 4.20 (br, 1H, C*H*CH$_2$CH$_2$), 3.78-3.72 (m, 2H, CH_2OC(CH$_3$)$_3$), 2.54-2.50 (m, 2H, CH$_2$CH_2CONH$_2$), 2.32 (m, 1H, CH$_3$C*H*CH$_3$), 2.22-2.16 (m, 2H, CH_2CH$_2$CONH$_2$), 1.39 (s, 9H, C(CH$_3$)$_3$), 1.19-1.14 (m, 6H, CH_3CHCH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 175.8(0), 173.7(0), 172.5(0), 170.7(0), 75.0(0), 64.1(2), 60.3(1), 55.5(1), 54.1(1), 32.8(2), 32.6(2), 29.5(3), 21.5(1), 20.2(3); MS (ES$^+$): m/z (%): 388 (100) [M + H]$^+$, 775 (15) [2M + H]$^+$; HRMS calcd for $C_{17}H_{34}N_5O_5$ [M + H]$^+$ 388.2561 found 388.2556.

ITC titration experiment

Preparation of solution A and B:

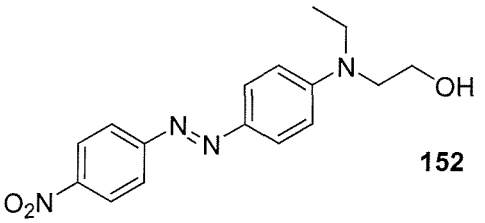
A) Guanidimium receptor **152** (22.9 mg, 50.0 μmol) was dissolved in dry DMSO (10 mL) to give a solution 5 mM.

B) **151** (301 mg, 1 mol) was dissolved in solution A (10 mL) to give a 100 mM guest solution.

Receptor solution (5 mM) was added to the calorimetry cell. Tetrabutylammonium acetate (250 μL, 100mM) **151** was introduced in fifty separate 5 μL injections at 25°C. The solution was stirred continuously to ensure rapid mixing, and maintained at 25°C. Dilution effects were determined by a second experiment in which the same acetate solution was added to pure DMSO. Any heat changes were subtracted from the raw titration data to produce the final binding curve.

5.9 Experimental for Chapter 4.

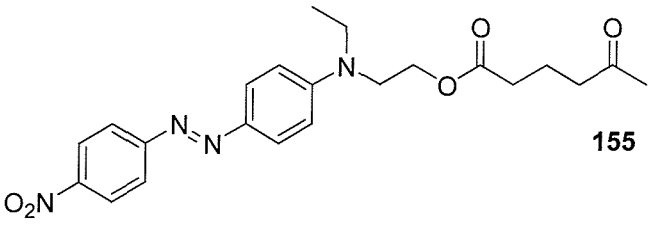
Disperse red 1 152



152

The commercial **disperse red 1** (3.00 g) was dissolved in a minimum amount of CH_2Cl_2 and put in a column which contains 300 g of silica which was eluted with a gradient from CH_2Cl_2 to CH_2Cl_2/ ethyl acetate (4.5:1). Collected fractions were evaporated under reduced pressure to afford the pure compound (1.20 g, 30%). (R_f : 0.41). M.p. 158-160 °C (lit.: 160-162); IR: ν = 3251, 1506 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.23 (d, J=9Hz, 2H, ArH), 7.84-7.79 (m, 4H, ArH), 6.73 (d, J=9Hz, 2H, ArH), 3.82 (t, J=6Hz, 2H, CH_2OH), 3.56-3.46 (m, 4H, NCH_2CH_2 and NCH_2CH_3), 1.18 (t, J=7Hz, 3H, CH_3); ^{13}C NMR (100MHz, $CDCl_3$): δ = 157.2(0), 152.1(0), 147.8(0), 144.2(0), 126.6(0), 125.1(0), 123.0(0), 112.0(0), 60.7(2), 52.8(2), 46.4(2), 12.5(3); MS (ES^+): m/z (%): 315 $[M + H]^+$ (20).

Synthesis of Red-Dye with glutaric anhydride 155

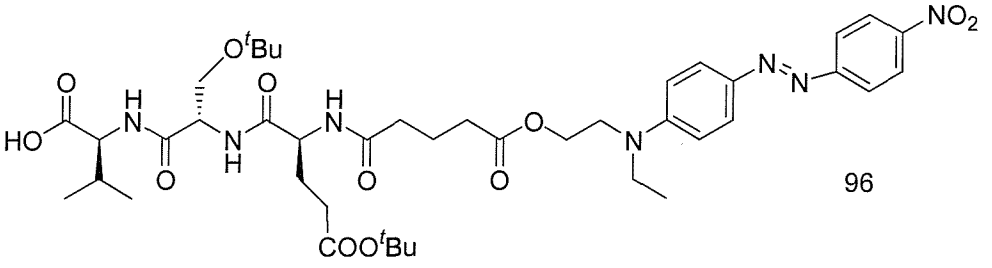


155

Disperse red 1 **152** (500 mg, 1.59 mmol), glutaric anhydride (322 mg, 2.70 mmol) and DMAP were dissolved in dry CH_2Cl_2 (50 mL) at RT. TEA (1.75 mmol, 244 µL) was then added and the reaction mixture was stirred at RT for 20 h. Solvent was removed under reduced pressure and the residue purified by flash chromatography (1% to 10% methanol in CH_2Cl_2) to give the pure compound (0.63 g, 93%). (R_f: 0.47) (10% methanol in

CH_2Cl_2); M.p. 117-119°C; IR: ν = 3481, 1731, 1697, 1597, 1510 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.37 (d, J= 9Hz, 2H, ArH), 7.98-7.93 (m, 4H, ArH), 6.85 (d, J= 9Hz, 2H, ArH), 4.36 (t, J=6Hz, 2H, CH_2O), 3.73 (t, J= 6Hz, 2H, NCH_2CH_2), 3.60-3.55 (m, 2H, NCH_2CH_2), 2.47-2.43 (m, 4H, $CH_2CH_2CH_2$), 2.03-1.95 (m, 2H, $CH_2CH_2CH_2$), 1.31 (t, J= 7Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.3(0), 173.1(0), 157.1(0), 151.6(0), 147.9(0), 144.3(0), 126.6(0), 125.1(0), 123.0(0), 111.9(0), 61.8(2), 49.1(2), 46.0(2), 33.4(2), 33.1(2), 20.1(2), 12.7(3); MS (ES^+): m/z (%): 429 $[M + H]^+$ (100); elemental analysis calcd (%) for $C_{21}H_{24}N_4O_6$: C 58.87, H 5.65, N 13.08; found: C 58.49, H 5.77, N 12.61.

Synthesis of Red Dye-L-Glu(O^{t}Bu)-L-Ser(^{t}Bu)-L-Val-OH 96

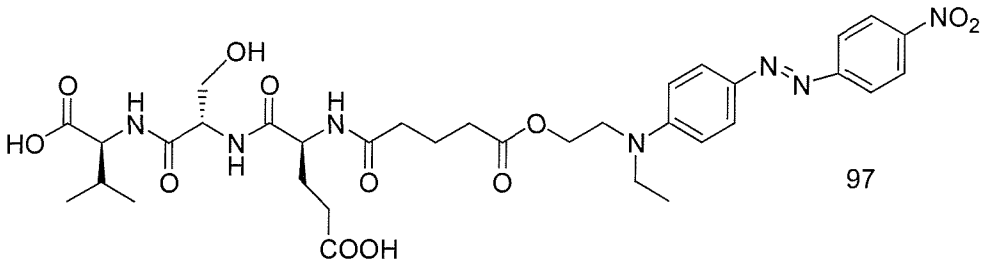


96

A solution of 4-(4-hydroxymethyl-2-methoxyphenyl) butanoic acid (HMPB-linker) (1.08 mmol, 347 mg), HOBt (1.08 mmol, 146mg), TBTU (1.08 mmol, 347 mg) and DIPEA (2.43 mmol, 425 μl) in DMF (25 ml) was added to TentaGel NH_2 resin (2.00 g, 0.54 mmol NH_2-sites) and agitated overnight. The resin was washed with CH_2Cl_2 (3 x 10 mL), DMF (3 x 10 mL), CH_2Cl_2 (3 x 10 mL) and dried. The qualitative ninhydrin test was negative. A solution of *N*-Fmoc-L-Valine (2.70 mmol, 916 mg) was stirred for 10 mins with DCI (2.70 mmol, 340 mg) in CH_2Cl_2. The symmetrical anhydride so produced was added, with DMAP to the HMPB functionalised resin and shaken overnight. The resin was washed with CH_2Cl_2 (3 x 10 mL), DMF (3 x 10 mL), CH_2Cl_2 (3 x 10 mL) and dried. The completion of the coupling step was monitored by a qualitative ninhydrin test. The Fmoc group was removed with 20% piperidine in DMF for 20 mins. The resin was washed with CH_2Cl_2 (3 x 10 mL), DMF (3 x 10 mL), CH_2Cl_2 (3 x 10 mL) and the deprotection step repeated. The qualitative ninhydrin test was positive. A solution of *N*-Fmoc-L-Serine(^{t}Bu) (1.08 mmol, 414 mg), HOBt (1.08 mmol, 165 mg), TBTU (1.08 mmol, 347 mg) and

DIPEA (2.43 mmol, 425 μl) in DMF (25 ml) was added to the resin and shaken 4 h. The resin was washed as before and a qualitative ninhydrin test indicated complete coupling. The resin was agitated with 20% piperidine in DMF for 20 mins. The resin was washed with CH_2Cl_2 (3 x 10 mL), DMF (3 x 10 mL) and CH_2Cl_2 (3 x 10 mL) and the deprotection step was repeated. A solution of *N*-Fmoc-L-Glutamic acid(O^tBu) (1.08 mmol, 460 mg), HOBt (1.08 mmol, 165 mg), TBTU (1.08 mmol, 347 mg) and DIPEA (2.43 mmol, 425 μl) was added to the resin and agitated overnight. The resin was washed as before and qualitative ninhydrin test was negative. The resin was agitated with 20% piperidine in DMF for 20 mins. The resin was washed with CH_2Cl_2 (3 x 10 mL), DMF (3 x 10 mL), CH_2Cl_2 (3 x 10 mL). A solution of red dye-linked glutaric acid **155** (1.08 mmol, 462 mg), HOBt (1.08 mmol, 165 mg), TBTU (1.08 mmol, 347 mg) and DIPEA (2.43 mmol, 425 μl) in DMF (25 ml) was added to the resin and shaken overnight. The resin was shaken again overnight with 1% TFA in DMF. The resin was drained and the combined filtrate evaporated under reduced pressure to yield the crude product. Purification by column chromatography using 10% methanol in CH_2Cl_2 afforded the pure compound **96** (23 mg, 20%). R_f=0.37 (10% methanol in CH_2Cl_2); M.p. 98-100°C; IR: ν = 3344, 2966, 2918, 1728, 1672, 1600 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.25 (d, J=9Hz, 2H, ArH), 7.86-7.81 (m, 4H, ArH), 7.26-7.24 (m, 2H, NHCO), 6.88 (br s, 1H, NHCO), 6.73 (d, J=9 Hz, 2H, ArH), 4.42-4.40 (m, 3H, C*H*$CH(CH_3)_2$ and CH_2O^tBu), 4.23 (t, J=6 Hz, 2H, OCH_2CH_2), 3.72 (m, 1H, C*H*CH_2CH_2), 3.61 (t, J=6 Hz, 2H, NCH_2CH_2), 3.49-3.43 (m, 2H, NCH_2CH_3), 3.36 (t, J=8 Hz, 1H, C*H*CH_2O), 2.33-2.29 (m, 4H, OCO$CH_2CH_2CH_2CO$ and $CH_2COOtBu$), 2.23-2.15 (m, 3H, CH_3CHCH_3 and CH_2CONH), 1.91-1.84 (m, 2H, $OCOCH_2CH_2CH_2CO$), 1.37 (s, 9H, $COOC(CH_3)_3$), 1.20-1.16 (m, 5H, NCH_2CH_3 and $CH_2CH_2COO^tBu$), 1.13 (s, 9H, $CH_2OC(CH_3)_3$), 0.90 (d, J=6.7 Hz, 3H, CH_3CHCH_3), 0.87 (d, J=6.7 Hz, 3H, CH_3CHCH_3); ^{13}C NMR (100MHz, $CDCl_3$): δ = 176.0(0), 175.1(0), 173.3(0), 172.0(0), 170.7(0), 163.2(0), 157.1(0), 151.6(0), 147.8(0), 144.2(0), 126.6(0), 125.0(0), 123.0(0), 111.8(0), 81.6(0), 74.7(0), 61.3(2), 61.6(2), 58.0(1), 53.7(1), 53.3(1), 49.1(2), 46.1(2), 34.3(2), 32.2(2), 29.8(3), 29.6(3), 28.4(2), 28.3(2), 27.7(2), 20.9(2), 20.9(2), 18.0(3), 12.6(3); MS (ES+): m/z (%): 856 (100%) $[M + H]^+$; HRMS calcd for $C_{42}H_{62}N_7O_{12}$ $[M + H]^+$ 856.4451 found 856.4437.

Synthesis of Red Dye-L-Glu-L-Ser-L-Val-OH 97

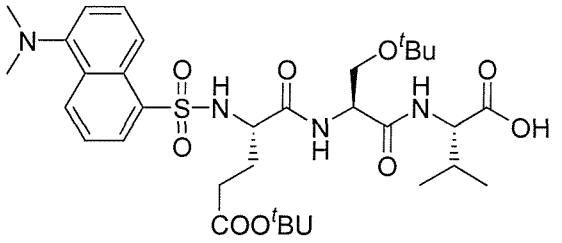


97

A solution of 4-(4-hydroxymethyl-2-methoxyphenyl) butanoic acid (HMPB-linker) (1.08 mmol, 347 mg), HOBt (1.08 mmol, 146mg), TBTU (1.08 mmol, 347 mg) and DIPEA (2.43 mmol, 425 µl) in DMF (25 ml) was added to TentaGel NH_2 resin (2.00 g, 0.54 mmol NH_2-sites) and agitated overnight. A solution of *N*-Fmoc-L-Val (2.70 mmol, 916 mg) was stirred for 10 mins with DCI (2.70 mmol, 340 mg) in CH_2Cl_2. The symmetrical anhydride so obtained was added to the HMPB functionalised resin followed by DMAP (10%) and agitated overnight. The resin was treated with 20% piperidine in DMF for 20 mins. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL), and CH_2Cl_2 (3 x 5 mL) and the deprotection step repeated. The qualitative ninhydrin test was positive. A solution of *N*-Fmoc-L-Serine(^{t}Bu) (1.08 mmol, 414 mg), HOBt (1.08 mmol, 165 mg), TBTU (1.08 mmol, 347 mg) and DIPEA (2.43 mmol, 425 µl) in DMF (25 ml) was added to the resin and agitated 4 h. The resin was shaken with 20% piperidine in DMF for 20 mins. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL), and CH_2Cl_2 (3 x 5 mL) and the deprotection step repeated. A solution of *N*-Fmoc-L-Glutaric acid(O^{t}Bu) (1.08 mmol, 460 mg), HOBt (1.08 mmol, 165 mg), TBTU (1.08 mmol, 347 mg) and DIPEA (2.43 mmol, 425 µl) was added to the resin and agitated overnight. The resin was agitated with 20% piperidine in DMF for 20 mins. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL), and CH_2Cl_2 (3 x 5 mL) and the deprotection step repeated. A solution of red dye-linked glutaric acid **155** (1.08 mmol, 462 mg), HOBt (1.08 mmol, 165 mg), TBTU (1.08 mmol, 347 mg) and DIPEA (2.43 mmol, 425 µl) in DMF (25 ml) was added to the resin and shaken overnight. The resin was agitated again overnight with 50% TFA in DMF. The solvent was removed by filtration and the solvent evaporated under reduced pressure to yield the crude. The crude material was purified by a flash chromatography to afford the pure compound **97** (80 mg, 20%). R_f= 0.9 (10% methanol in

CH_2Cl_2); IR: ν = 3283, 2920, 1666, 1600 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 8.22 (d, J=8.75Hz, 2H, ArH), 7.78 (d, J=7.76Hz, 1H, N*H*CHCH$_2$OH), 7.80 (d, J=8.76Hz, 2H, ArH), 7.71 (d, J=9.04Hz, 2H, ArH), 7.66 (d, J=8.52Hz, 1H, N*H*CHCH(CH$_3$)$_2$), 6.78 (d, J=9.04Hz, 2H, ArH), 4.71 (br s, 1H, N*H*CHCH$_2$CH$_2$), 4.21 (m, 1H, C*H*CH$_2$OH), 4.16 (m, 1H, C*H*CH$_2$CH$_2$), 4.10 (br t, 2H, CH$_2$C*H*$_2$OCOCH$_2$), 4.03-3.99 (m, 1H, C*H*CH(CH$_3$)$_2$), 3.58 (br t, 2H, NC*H*$_2$CH$_2$), 3.45-3.39 (m, 4H, NC*H*$_2$CH$_3$ and C*H*$_2$OH), 2.18-2.09 (m, 4H, OCOC*H*$_2$CH$_2$CH$_2$CO and C*H*$_2$COOH), 2.04-2.02 (m, 2H, CH$_2$CH$_2$C*H*$_2$CONH), 1.91-1.87 (m, 1H, CH$_3$C*H*CH$_3$), 1.76-1.74 (m, 2H, C*H*$_2$CH$_2$COOH), 1.61-1.57 (m, 2H, OCOCH$_2$C*H*$_2$CH$_2$), 1.02 (t, J=8Hz, 3H, NCH$_2$C*H*$_3$), 0.72 (d, J=7Hz, 6H, C*H*$_3$CC*H*$_3$); ^{13}C NMR (400 MHz, DMSO-d_6): δ = 172.8(0), 171.6(0), 171.5(0), 170.6(0), 170.2(0), 168.8(0), 156.6(0), 150.4(0), 145.8(0), 141.7(0), 124.9(0), 123.8(0), 121.4(0), 110.5(0), 60.3(2), 60.0(2), 55.9(1), 53.7(1), 50.7(1), 47.1(2), 44.0(2), 34.6(2), 32.9(2), 31.7(2), 29.0(2), 27.5(2), 20.9(2), 16.7(3), 12.8(3); MS (ES+): m/z (%): 744 (10) $[M + H]^+$, 766 (10) $[M + H]^+$ HRMS calcd. for $C_{34}H_{46}N_7O_{12}$ $[M + H]^+$ 744.3199 found 744.3198.

DNS-Glu(O^{t}Bu)-Ser(^{t}Bu)-Val-OH 98



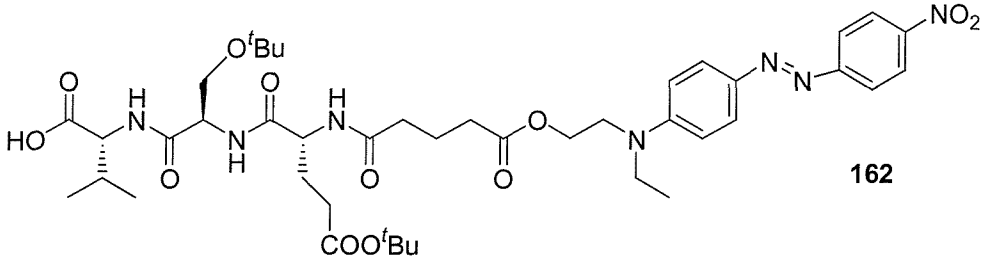
98

A solution of 4-(4 hydroxymethyl-2-methoxyphenyl) butanoic acid, HMPB-linker, (120 mg, 0.50mmol) and HOBt (337 mg, 2.50 mmol) in CH_2Cl_2 was added to TentaGel S NH_2 resin (500 mg, 0.50 mmol NH_2-sites), followed by DIC (391 μL, 2.50 mmol) and DMAP (10 mg) the suspension mixture was agitated with a nitrogen stream for 3 h. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL), and CH_2Cl_2 (3 x 5 mL). The qualitative ninhydrin test was negative. DCC (516 mg, 2.50 mmol) was added to a solution of *N*-Fmoc-L-Valine (1.7 g, 5.00 mmol) in CH_2Cl_2 and stirred for 10 mins. The symmetrical anhydride so obtained was added to the HMPB functionalised resin and the suspension mixture was shaken with nitrogen for 5 h. The coupling step was repeated three

times. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL), CH_2Cl_2 (3 x 5 mL). The resin was agitated with 20% piperidine in DMF for 20 mins. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL), and CH_2Cl_2 (3 x 5 mL) and the deprotection step repeated. A solution of *N*-Fmoc-L-Serine(^{t}Bu) (409 mg, 1.25 mmol) and HOBt (338 mg, 2.50 mmol) in CH_2Cl_2 was added to the resin followed by DIC (391 μL, 2.50 mmol) and DMAP (10 mg) and the suspension was shaken with a nitrogen stream for 3 h to yielded a resin which give a negative ninhydrin test. The Fmoc group was removed as described above (*vide supra*). A solution of *N*-Fmoc-L-Glutamic acid(O^{t}Bu) (532 mg, 1.25 mmol) and HOBt (338 mg, 2.50 mmol) was added to the resin followed by DCI (337 mg, 2.50 mmol) and DMAP (10 mg) and the suspension was shaken with a nitrogen stream for 3 h. The resin was washed with CH_2Cl_2 (3 times), DMF (3 times) and CH_2Cl_2 (3 times). The qualitative ninhydrin test was negative. The resin was shaken with 20% piperidine in DMF for 20 mins. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL), and CH_2Cl_2 (3 x 5 mL) and the deprotection step was repeated. Qualitative ninhydrin test was positive. The resin was shaken in CH_2Cl_2 in presence of Et_3N (348 mL, 2.5 mmol) for 10 mins. A solution of DNSCl (337 mg, 2.50 mmol) in CH_2Cl_2 was added and the suspension mixture was shaken for 6 h to give resin bound tripeptide **161**. Cleavage was carried out by treatment of the resin **161** with a solution of 1% TFA in CH_2Cl_2 (100 mL). The resin beads were removed by filtration and the solvent of the filtrate was removed under reduced pressure. The crude residue was further purified by column chromatography (0-10% methanol in CH_2Cl_2) to give tripeptide **98** (211 mg, 62%) as a green solid. M.p. >240°C (lit.:[92] >240°C); IR: ν = 3432, 2360, 1684, 1206, 1143; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.56 (d, 1H, J=7 Hz, NHDNS), 8.45 (d, 1H, J=8 Hz, DNSH), 8.31 (d, 1H, J=8 Hz, DNSH), 8.09 (d, 1H, J=8 Hz, DNSH), 8.08 (d, 1H, J=7 Hz, NH), 7.60 (dd, 1H, J=8 Hz, DNSH), 7.57 (dd, 1H, J=8 HZ, DNSH), 7.25 (bd, 2H, J=8 Hz, NH and DNSH), 4.31 (dt, 1H, J=7, 5 Hz, C*H*$CH_2(OC(CH_3)_2)$), 4.00 (dd, 1H, J=8, 5 Hz, C*H*$(CH(CH_3)_2)$), 3.45 (d, 2H, J=5 Hz, C*H*$_2(OC(CH_3)_2)$), 2.81 (s, 6H, $N(CH_3)_2$), 2.25 (m, 2H, C*H*$_2CO_2C(CH_3)_3$), 2.08 (dd, 1H, J=6,5 Hz, C*H*$(CH_3)_2$), 1.90-1.70 bm, 2H, C*H*$_2CH_2CO_2C(CH_3)_3$), 1.12 (s, 9H, C*H*$_2OC(CH_3)_3$), 0.90-0.82 (m, 15H, $CH(CH_3)_2$ and $CH_2OC(CH_3)_3$); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 171.4(0). 170.0(0), 169.0(0), 151.3(0), 136.5(0), 129.6(1), 129.2(1), 128.4(1), 127.8(1), 123.6(1), 119.7(1), 115.2(1), 80.5(0), 72.8(0), 61.7(2), 58.4(1), 56.1(1), 53.7(1), 45.3(3), 31.7(2), 31.1(1), 28.5(2),

27.3(3), 26.9(3), 19.7(3), 18.0(3); MS (ES^+): m/z (%): 679 (100) $[M + H]^+$, 701 (10) $[M + Na]^+$, 1357 (15) $[2M + H]^+$, 1379 (20) $[2M + Na]^+$. Spectroscopic data are consistent with those reported in literature.[92]

Synthesis of Red Dye-D-Glu(O^{t}Bu)-D-Ser(^{t}Bu)-D-Val-OH 162



162

To a suspension of Rink Acid Resin (500 mg, 0.21 mmol) in dry THF was added triphenylphosphine (310 mg, 1.18 mmol) and hexachloroethane (280 mg, 1.18 mmol). The resulting mixture was stirred for 6 h at room temperature. The resin was washed with THF (3 x 5 mL). The resin was swollen in CH_2Cl_2 and *N*-Fmoc-D-Valine (233 mg, 0.68 mmol) and DIPEA were added to the resin mixture and agitated 20 h. After this time the resin was washed with CH_2Cl_2 (3 x 5 mL), methanol (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL). The resin was suspended in a 20% piperidine in DMF for 20 mins. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL) and the deproctetion step repeated. A solution of *N*-Fmoc-D-Serine(^{t}Bu) (247 mg, 0.64 mmol), DIC (101 μL, 0.64 mmol) and HOBt (98.0 mg, 0.64 mmol) in DMF was stirred for 10 mins and added to the resin followed by DIPEA (168 μL, 0.96 mmol), and the resulting mixture stirred at RT for 20 h. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL). The resin was suspended in a 20% piperidine in DMF for 20 mins. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL) and the deprotection step repeated. A solution of *N*-Fmoc-D-Glutamic acid(O^{t}Bu) (275 mg, 0.64 mmol) DIC (101 μL, 0.64 mmol) and HOBt (98 mg, 0.64 mmol) in DMF was stirred for 10 mins and added to the resin followed by DIPEA (168 μL, 0.96 mmol) and stirred 20 h. After this time the resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL). The resin was suspended in a 20% piperidine in DMF for 20 mins. The resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL). A

solution of Red-Dye with glutaric anhydride (110 mg, 0.26 mmol), HOBt (47.0 mg, 0.26 mmol), HBTU (98.0 mg, 0.26 mmol) in DMF was stirred for 10 mins and added to the resin followed by DIPEA (150 μL, 0.86 mmol) and stirred for 20 h. After this time the resin was washed with CH_2Cl_2 (3 x 5 mL), DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL). The resin was agitated with 10% acetic acid in CH_2Cl_2 for 2 h to allow the cleavage from the resin. The solvent was collected though filtration and the solvent evaporated under reduced pressure to give crude. The crude was purified on column chromatography (10% methanol in CH_2Cl_2) to give the pure compound (133 mg, 73%). HRMS calcd for $C_{42}H_{62}N_7O_{12}$ $[M + H]^+$ 856.4451 found 856.4441.

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