Two-Armed Molecular Receptors – Peptide Recognition and Vesicle Formation Driven by Selective Non-Covalent Interactions

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- J. Grun, H. Wennemers, "Macrocyclic Diketopiperazine Receptors – Effect of Macrocyclization on the Binding Properties of Two-Armed Receptors", *in preparation*.

- J. Grun, K. Kita-Tokarczyk, M. Conza, W. Meier, C. Vebert, H. Wennemers, "Vesicle Formation in Aqueous Solution Driven by Selective Non-Covalent Interactions", *in preparation*.

- J. Grun, J. D. Revell, M. Conza, H. Wennemers, "Peptide-Polyethylene Glycol Conjugates: Synthesis and Properties of Peptides bearing a C-terminal Polyethylene Glycol Chain", *Bioorg. Med. Chem.* **2006**, *14*, 6197.

Excerpts from this work have been presented at the following Conferences:

- Poster: "Two-Armed Diketopiperazine and Carbazole Receptors: which is the best small peptide binder?",
  > 26th Regio-Symposium, International Symposium on Organic and Bioorganic Chemistry, 20–22 September 2006, Rheinfelden, Germany.

- Poster: "Diketopiperazine Receptor – Peptide Complexe: self-assembly in aqueous solution driven by selective non-covalent interactions",
  > Fall Meeting of the Swiss Chemical Society, 13th October 2006, Zurich, Switzerland;
  > 26th Regio-Symposium, International Symposium on Organic and Bioorganic Chemistry, 20–22 September 2006, Rheinfelden, Germany;
  > Polymers in Life Science, 4–5 April 2006, Murten, Switzerland;
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- Poster: "Diketopiperazine Receptor – Pegylated Ligand: driven self-assembly in aqueous solution",
  - > Abc Technologies 2006, 26-27 January 2006, Basel, Switzerland;
  - > Fall Meeting of the Swiss Chemical Society, 13th October 2005, Lausanne, Switzerland;
  - > 25th Regio-Symposium, International Symposium on Organic and Bioorganic Chemistry, 28-30 September 2005, Sornetan, Switzerland.

- Poster: "Receptor-ligand driven self-assembly: a key-lock mechanism with diketopiperazine receptor",
  - > Polymers in Life Science II, 22–23 March 2005, Pharmazentrum, Basel, Switzerland.

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  - > Fall Meeting of the Swiss Chemical Society, 7th October 2004, Zürich, Switzerland;
  - > 24th Regio-Symposium, Symposium on Organic and Bioorganic Chemistry, 29Th September – 1st October 2004, Lucelle, France.

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A mes parents

et

Jean-Philippe

"Si ce n'est aujourd'hui, ce sera demain:
rappelons-nous que la patience est le pilier de la sagesse."

--- Frédéric Mistral

"La grandeur de l'homme ne reside pas dans le fait que l'on ne doit pas tomber,
mais dans le fait que l'on doit savoir se relever. "

--- Confucius

ABSTRACT

Two-Armed Molecular Receptors –
Peptide Recognition and Vesicle Formation
Driven by Selective Non-Covalent Interactions

Jessica Grun

This thesis presents examples for applying encoded combinatorial chemistry to trace molecular interactions between two-armed receptors and peptidic substrates that could have not been predicted by conventional means. Starting from these selective non-covalent interactions, applications, like supramolecular self-assembly, were investigated in organic and aqueous media.

In the first part the synthesis of macrocyclic diketopiperazine receptors and their binding properties towards peptides is described. Combinatorial on-bead studies showed that both macrocyclization of the receptor and choice of the linker-type lead to significant changes in the binding properties compared to their flexible open-chain parent diketopiperazine receptors. Macrocyclization rigidifies the receptor and should induce a higher preorganisation. Thus, the conformations of the macrocyclic receptors were expected to differ from the open-chain diketopiperazine receptor prototype. Binding studies revealed that macrocyclization led not only to lower binding selectivities but also lower affinities toward peptidic guest compared to the open-chain parent receptors. Thus, the flexibility of the open-chain receptor allows the arms to better adjust to a peptidic guest and can be beneficial for selective and higher binding.

The second part describes the development of a new class of two-armed receptors consisting of a rigid carbazole backbone and peptidic side-chains which allow for structural as well as functional variations. Compared to the diketopiperazine template, a third functionality is present and allows for attachment of a dye, polymer chain or resin, at the opposite site of the recognition modules. Combinatorial binding studies and solid phase binding assays showed that these carbazole receptors interact with certain tripeptides, in organic solvents, with sequence selectivities and binding affinities that are comparable to

those of diketopiperazine receptors. These two-armed receptors have been the basis for the design of receptor libraries to identify selective receptors for interesting peptidic and non-peptidic substrates.

In the third part, selective non-covalent interactions between a diketopiperazine receptor and peptide-PEG conjugates were used to induce the assembly of vesicles in aqueous solution. The vesicles were analysed by a combination of light scattering, electron transmission and atomic force microscopy as well as surface pressure measurements. Vesicle formation was found to be independent of the ratio of receptor to ligand and relies upon selective receptor-peptide interactions. Other peptide-PEG conjugates did not assemble into vesicular structures when mixed with the receptor.

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A. General Section

1 INTRODUCTION

1.1 From Synthetic Receptors to Tweezer-like Receptors

Over the past decade a lot of effort has been made in order to design synthetic receptors with specific binding properties towards small peptides.[1-5] Many research groups have focused on the study of small synthetic hosts in order to gain insight into the principles governing non-covalent molecular interactions such as hydrogen bonds and ionic and hydrophobic interactions. Understanding the basis of these interactions by synthesizing molecules able to mimic and block the action of natural compounds with a high degree of specificity, is of enormous importance in the discovery of new therapeutic agents for the treatment of many diseases. Studying the selective recognition of peptides by synthetic receptors is not only of importance as a model for the study of biological protein-protein complex interactions but also for the development of novel specific chemosensors.[1,6] Furthermore, synthetic receptors can be immobilized on a solid phase and used as chiral stationary phases for the separation of peptides.[7] In this respect, the discrimination between enantiomeric as well as diastereomeric peptides is of particular interest.

The interaction between two molecules is a very sensitive process and slight structural modifications can lead to tremendous changes in the binding properties. The example of the antibiotic vancomycin demonstrates this subtlety of intermolecular interactions between small molecules.[8] Vancomycin is a macrotricyclic glycoheptapeptide which exerts its main bactericidal effect through inhibition of cell wall formation of gram-positive bacteria. Although rather small in size it binds strongly and selectively to the tripeptide *N*-acyl-Lys-D-Ala-D-Ala, a crucial component in the cell wall crosslinking of gram positive bacteria.[9] In the last few years this defence strategy against pathogenic bacteria appears to be endangered by the ability of bacteria to evolve rapidly into drug-resistant strains. Resistance is effected by the biosynthesis of an altered cell wall precursor ending in *N*-acyl-Lys-D-Ala-D-Lac.[10] Replacement of the terminal D-alanine with D-lactate introduces a repulsive electrostatic interaction of the oxygen with vancomycin instead of a hydrogen bond resulting in an approximately 1000-fold reduction in binding affinity (*Figure 1*).[11] This is one clear example showing that slight structural modifications (the substitution of a single atom) can induce significant changes in the binding properties. The identification of synthetic receptors that bind with high affinity to *N*-acyl-Lys-D-Ala-D-Lac could provide a powerful strategy for overcoming vancomycin resistance.[12]

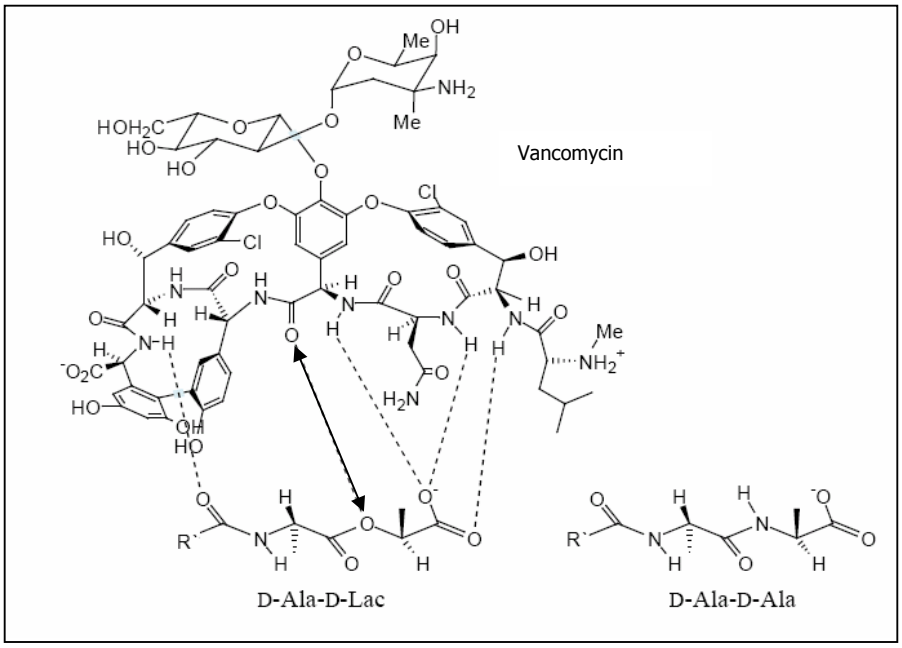


Figure 1: *Repulsive electrostatic interaction in Vancomycin binding N-acyl-Lys-D-Ala-D-Lac*

In recent years, much work in the area of peptide receptors has focused on 'tweezer-like receptors'.[13] This simple design for receptors consists of a template with two peptidic or sulfonopeptidic recognition elements (*Figure 2*).[14-19] Within the receptor structure, the receptor arms are considered as the selectivity determining modules, while the template serves as a rigid, structure-directing module. In spite of their structural flexibility many of such two-armed receptors bind peptidic guests with moderate to excellent selectivities and affinities. The lack of apparent preorganization within the receptor structure, combined with the many degrees of freedom of even simple di- and tripeptides, renders the rational design of such receptors for specific peptide sequences an extremely challenging task. During the last decade, combinatorial chemistry has become very promising in the search of suitable substrates for a given receptor and *vice versa*.[20,21]

Yet, most of the examined receptors exhibit drawbacks such as either poor binding selectivities, rather laborious syntheses or limited possibilities for tuning the receptor structures.

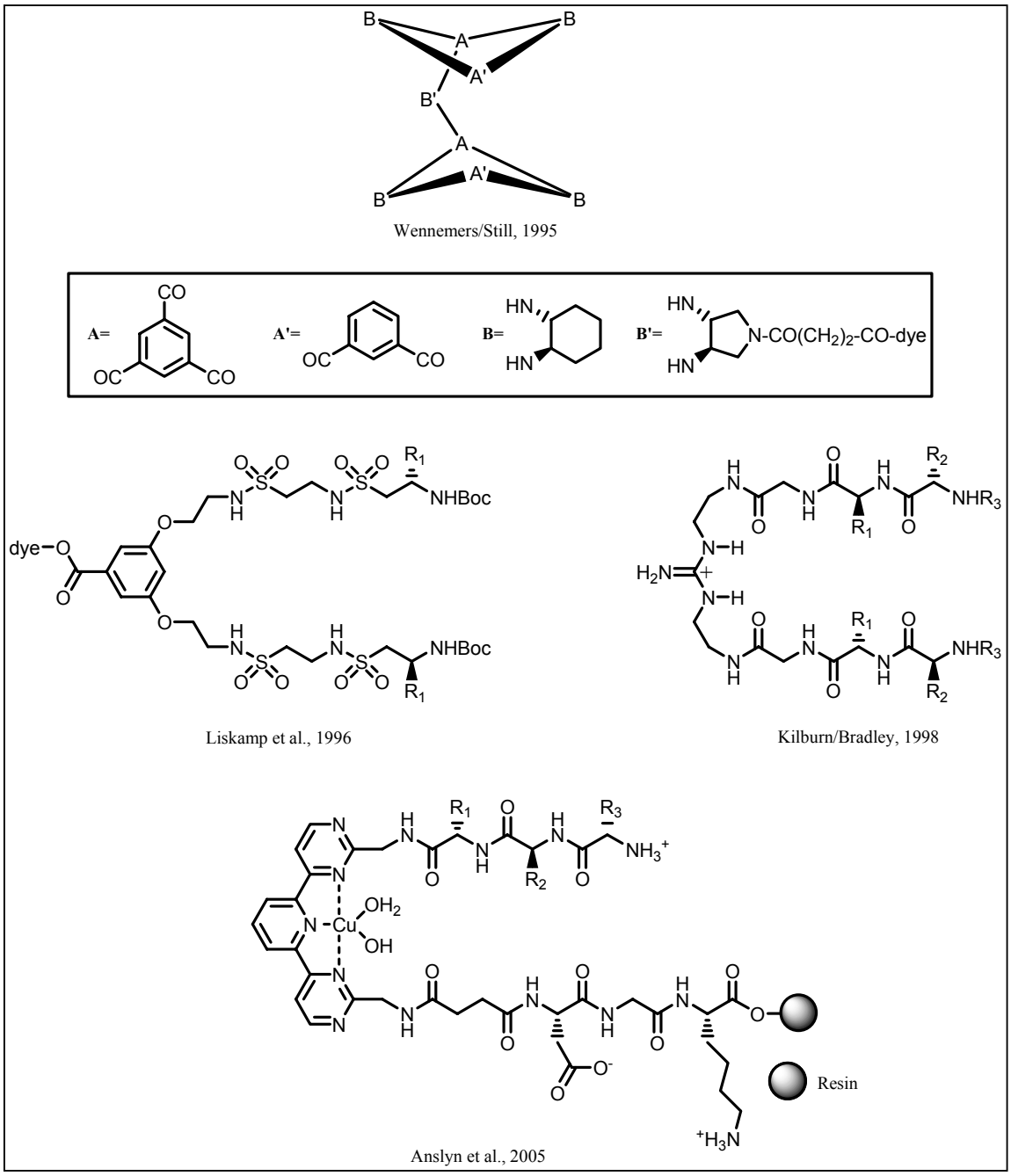


Figure 2: *Examples of two-armed receptors that bind short peptides (dye = disperse red 1)*

In order to overcome these problems, we aimed to develop a new class of two-armed receptors based on diketopiperazine and carbazole scaffolds that offer facile synthesis, high variability and structural rigidity. Furthermore, the use of amino acids as building blocks offers the possibility to employ these receptors as models to understand peptide-peptide interactions.

1.2 Combinatorial Chemistry

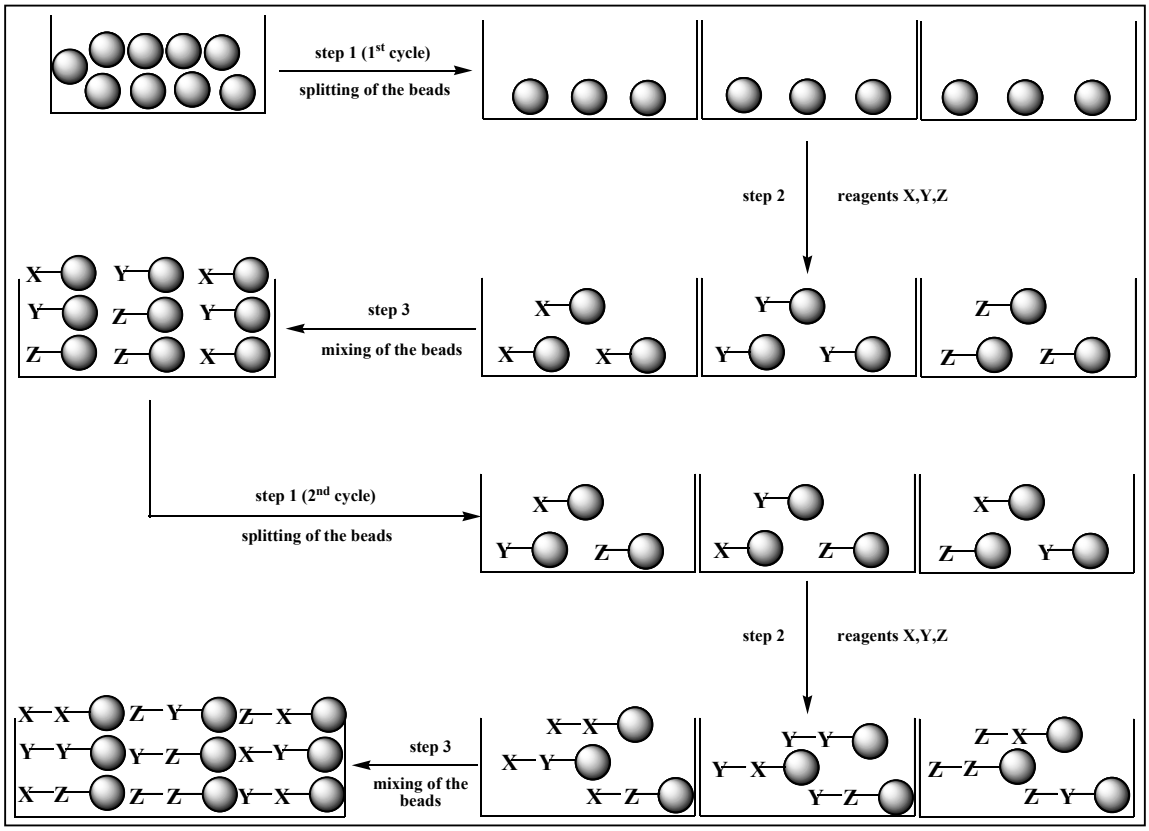
The advent of combinatorial chemistry in the early 1990s has revolutionized the discovery process of new therapeutics.[20,21] While it was first applied mainly in bioorganic and medicinal chemistry, it has since spread to other fields like material science[22] and catalyst development.[23-24]

The principle of combinatorial chemistry is to synthesize a large number of different molecules at the same time - instead of synthesizing compounds in a conventional one-at-a-time manner - and then to identify the most promising compound by high-throughput screening, for further development. The characteristics of combinatorial synthesis are that different compounds are generated simultaneously under identical reaction conditions in a systematic manner, so that in the ideal case the products of all possible combinations of a given set of starting materials (building blocks) will be obtained at once. The collection of these synthesized compounds is referred to as a *combinatorial library*. A very simple method to create molecular diversity is the "split-and-mix synthesis" that was presented by Furka and Lam in 1991.[25-28] Although many other methods have been introduced,[29-31] split and mix synthesis is still one of the most elegant approaches to create highly diversified pools of small molecules.

1.2.1 Split-and-mix synthesis

The concept of split-and-mix synthesis is based on the generation of a large number of related compounds simultaneously on solid support. The method works as follows (*Scheme 1*): a sample of resin is divided into a number of x equal portions (step 1), and each of these is reacted with a different set of reagents (step 2). After completion of the reactions, and subsequent washing to remove excess reagents, the portions are recombined and mixed (step 3). After splitting the resin again into equal portions, the modified solid support is ready for a next synthesis cycle. Reaction with a further set of activated reagents gives the complete set of possible dimeric units as a mixture, and then the whole process may be repeated if necessary (for a total of n times). The number of compounds obtained arises from the exponential increase of products (x^n). In the example illustrated in *Scheme 1*, two cycles (n = 2) with three different reagents each (x = 3) are performed, yielding $3^2 = 9$ combinations of dimeric products. Since each bead is subjected to only one reaction during any cycle, each bead carries only one compound ('one-bead-one-compound'). X, Y and Z

could be amino acids, in which case the final products would be dipeptides, but more generally they could be any type of monomeric unit.



Scheme 1: *Application of the split-and-mix library procedure exemplified for the solid phase synthesis of a 9 component dimer library*

After the generation of a library, the further steps are the screening for the identification of active compounds and subsequently their structural determination.

1.2.2 Encoded Split-and-Mix Synthesis

The power of combinatorial libraries is only evident if structural information on active components may easily be obtained. The amount of compound on each bead depends on the type of resin used and is typically in the order of 100 pmol per bead. With this amount linear peptides or oligonucleotides can be analyzed *via* Edman-sequencing[32] and PCR-(polymerase chain reaction) techniques, respectively. However, the analysis of non-sequenceable molecules is not as straightforward. Analytical methods like mass spectrometry[33-36] or ^{13}C-NMR and ^{1}H-NMR spectroscopy[37-39] have also been applied for the analysis of compounds on single bead. However, these detection methods have some limitations like the impossibility to distinguish isomers and enantiomers (for example L- and D- amino acids). Moreover, incomplete reactions during the synthesis result in compound mixtures which are difficult to analyse. In order to avoid these problems a number of new methods have been developed in the last years, in which the information concerning the active compound is carried on the bead in the form of a 'tag'. The idea of encoded combinatorial synthesis was to attach molecular tags to the solid support, in each reaction vessel of each reaction cycle, which can later be cleaved off the resin and analyzed easily and unequivocally.[40] Thus the tags can report the synthetic history of each individual bead.

The tag molecules should have the following properties: They should be inert under various reaction conditions, detectable on a very small scale and they should be easily attached and detached from the solid support.

Still *et al.* reported the first method using such chemically stable moieties.[41] The tags consist of polyhalogenated aromatic alcohols which can be detected at subpicomolar concentrations using electron capture gas chromatography (EC-GC), which is a very sensitive detection method of halogenated compounds. 26 tags can be separated in the EC-GC just by varying the length of the hydrocarbon chain and the substitution pattern of the halogenated aromatic ring. Two different linkers, one photolabile and one oxidatively labile, were developed in order to allow the tag alcohols to be attached, and then detached from the beads. Both linkers incorporate a carboxylic acid for attachment to the synthesis beads (*Figure 3*). For the photolabile linker, a photochemically labile *ortho*-nitrobenzylcarbonate was chosen for subsequent detachment of the tags[41a] *via* irradiation with UV-light, while vanilic acid[41b] was chosen as an oxidative-labile linker segment which allows the release of the tag alcohols *via* oxidation with ceric ammonium nitrate (CAN). The tag alcohols are then analyzed by EC-GC after silylation of the alcohol function. Amide chemistry was implemented

to attach the tags to the beads. These polyhalogenated aromatic reagents acylate the same synthesis sites used for the ligand synthesis, but due to the sensitivity of the tag detection this competition could be minimised. Only 1-2 % of the active sites on the solid support are acetylated with the tag during each reaction step. The tags attached to the oxidatively labile linker can be incorporated directly on the solid support *via* carbine insertion. This has the advantage that there is no dependence on the functional group of the solid support and so they can be used for any compound library.[41b]

Since this kind of tagging is the most versatile, we used polyhalogenated tags to encode our libraries.

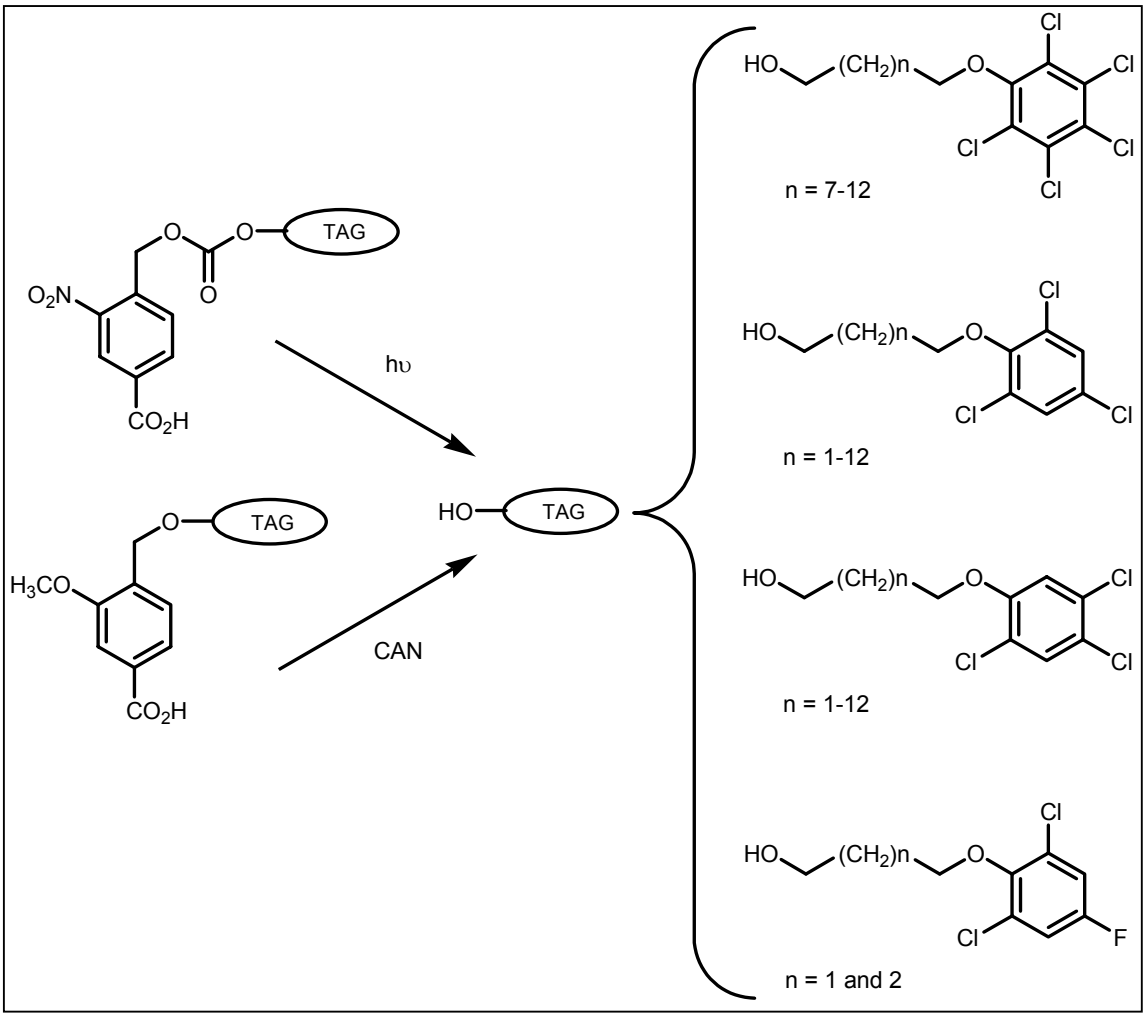
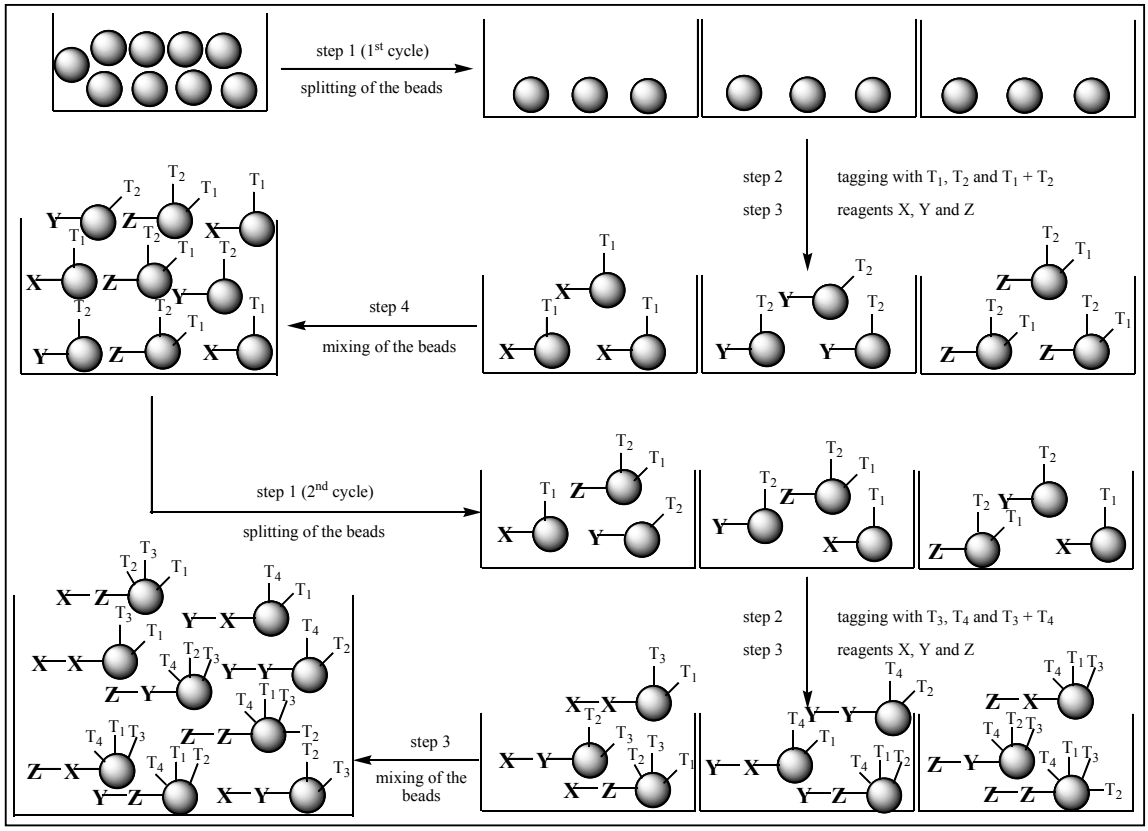


Figure 3: *Photolabile and oxidative-labile linkers and tag alcohols*

1.2.3 Synthesis of an Encoded Split-and-Mix Library

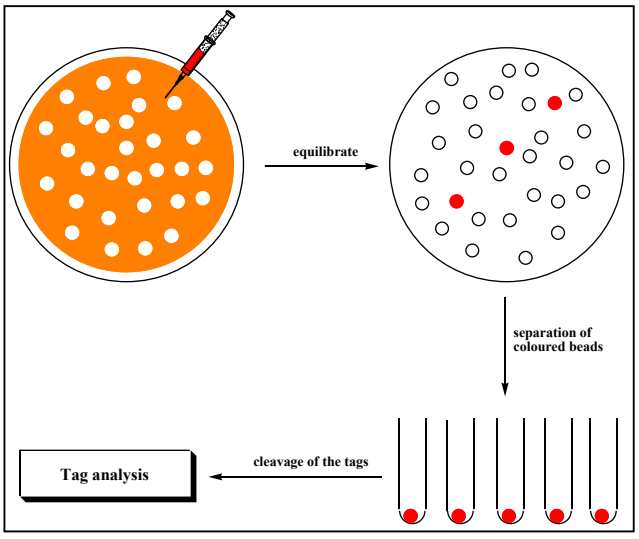
As illustrated in *Scheme 2*, during each synthesis cycle, a different array of tags is attached to the beads in each reaction vessel prior to the actual reaction step. The use of binary codes of tags instead of only a single tag allows for encoding of a large number of compounds.[41a] In the following example, two tags (T_1 and T_2) encode three reactions performed in the first cycle, the two tags T_3 and T_4 encode the three reactions performed in the second cycle. If the analysis of the tags on one bead of the library shows tags T_1, T_3 and T_4 the bead was subjected to reagent X in the first step and to reagent Z in the second step of the synthesis. Thus, using this binary encoding scheme N different tags can encode 2^N-1 reagents.[41a]



Scheme 2: *Application of the encoded split-and-mix library procedure exemplified for the solid phase synthesis of a 9 component dimer library*

1.2.4 Screening of Encoded Combinatorial Libraries

A highly efficient way for screening all members of a combinatorial library simultaneously for their binding properties is 'on-bead screening'. This selection system allows for the detection of activity by simply looking at the beads that carry the compounds of interest. The compound that is screened against the library is in solution while the library members are immobilized on the solid support. Intermolecular interactions with members of the library can be detected directly if the binding partner in solution carries a marker that allows for visual detection. Such markers can be dyes or fluorescent molecules[42] for the direct visual detection, enzymes or antibodies that allow for their detection by a color reaction[41b,43] or radioactive isotopes that can be detected by photoimaging techniques or microradiography.[44-47] In our group, dyes or fluorescent molecules have been employed as markers for the screening in aqueous and organic solvents because of their high sensitivity in detection. For example, a library is mixed with the solution of a receptor marked with a dye. Beads of the library members that interact with the receptor will appear colored and can be detected visually through a low power light microscope. After isolation of the colored beads, the tags are cleaved and analyzed by EC-GC to reveal the structure of the active compound (*Scheme 3*).



Scheme 3: *'On-Bead Screenings' of an encoded combinatorial library against a colored receptor*

Screenings are performed using an amount of beads that corresponds to several theoretical copies of the tested library. This is mandatory to assure a representative screening result because the distribution of individual sequences obeys Poissons statistics, thus a sample of 50000 beads of a 50000 membered library does not contain each member.[48,49]

1.3 Research Project

The work described here is based on three main aspects concerning two-armed receptors and their binding properties, which are explained below.

1) Our group has recently developed two-armed diketopiperazine receptors that bind peptides with high sequence selectivities and binding affinities. These receptors consist of a rigid diketopiperazine backbone bearing two peptidic side-chains, the "receptor arms".[50-54] The conformation of the template is well understood. In contrast, the conformation of the receptor arms and their orientation towards each other is difficult to elucidate due to their high structural flexibility.

In the first part of this thesis, we have addressed this question by comparing the binding selectivities of flexible two-armed receptors with those of macrocyclic receptors in which the termini of the receptor arms are connected (*Figure 4*). If flexible two-armed receptors bind their substrates with a somewhat parallel arrangement of the receptor arms, connecting the arms should not alter the binding properties significantly. In contrast, the binding selectivities should be altered by macrocyclization in the case of a rather "unordered" non-parallel arrangement of the receptor arms upon binding of their substrates. The binding properties have been investigated by combinatorial screenings and conventional binding studies.

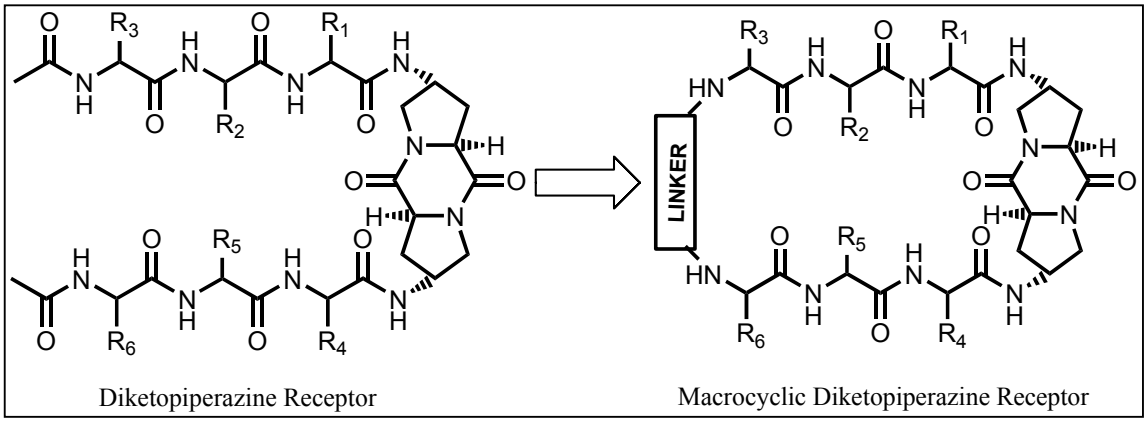


Figure 4: *General concept of macrocyclic receptor prototypes*

The following points are discussed:

- Design and binding properties of four macrocyclic diketopiperazine receptor prototypes.
- Comparison with the binding properties of the corresponding open-chain diketopiperazine receptor prototypes.
- Binding energies of macrocyclic and open-chain diketopiperazine receptor prototypes with tripeptides in chloroform.

2) Previous studies revealed that an exchange of the diketopiperazine template (e.g. simpler diamino scaffold) leads to significantly lower or the entire loss of binding selectivity.[52]

In the second part of this thesis, we investigated an alternative template that would offer an additional site for the attachment of receptors to a solid support or a soluble polymer. For this purpose, we chose the carbazole template which offers a third functionality in addition to those required for tethering of the two peptide arms (*Figure 5*).[55] The design and the binding properties of this new class of two-armed receptors based on a carbazole backbone are presented.

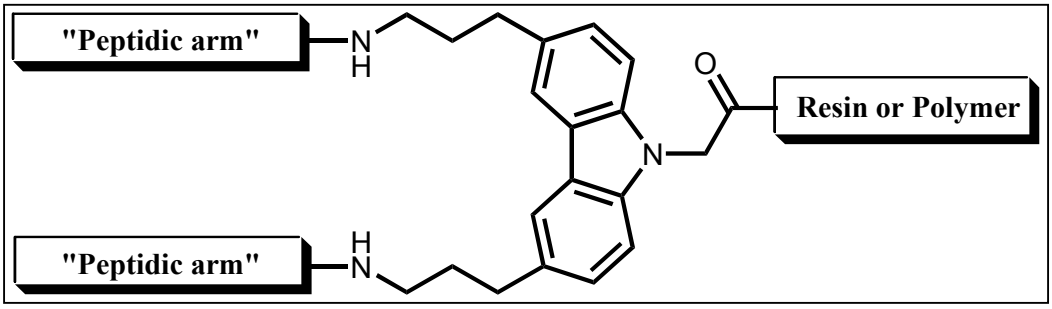


Figure 5: *Two-armed carbazole receptors.*

The following points are discussed:

- Design and binding properties of five carbazole receptor prototypes and comparison with the binding properties of the corresponding diketopiperazine receptor prototypes.
- Binding energies of the two-armed carbazole receptor prototypes and tripepides in chloroform.
- Synthesis of a receptor library on solid support and its screening towards water soluble peptidic target molecules.

3) We were also interested in the study of the self-assembly process of a diketopiperazine receptor with pegylated tripeptides. Combinatorial binding studies had revealed that, for example, diketopiperazine receptor **1** binds to the tripeptide Ac-D-Val-D-Val-D-His-linker-resin (resin = polystyrene) in chloroform solution with high selectivity and a binding affinity of ΔG = -4.7 kcal mol^{-1}.[50,54] Due to the low solubility of the non-resin bound peptide and in order to understand this highly selective host-guest interaction, we prepared the pegylated tripeptide **2**[56] to perform NMR studies in chloroform. Upon mixing the receptor **1** with the pegylated peptide **2** in chloroform, the formation of a gel was observed, demonstrating a supramolecular assembly process (*Figure 6*). Since a 3D network structure occurring *via* non covalent interactions may explain the gel formation in chloroform, we also investigated a possible self-assembly in aqueous solution.

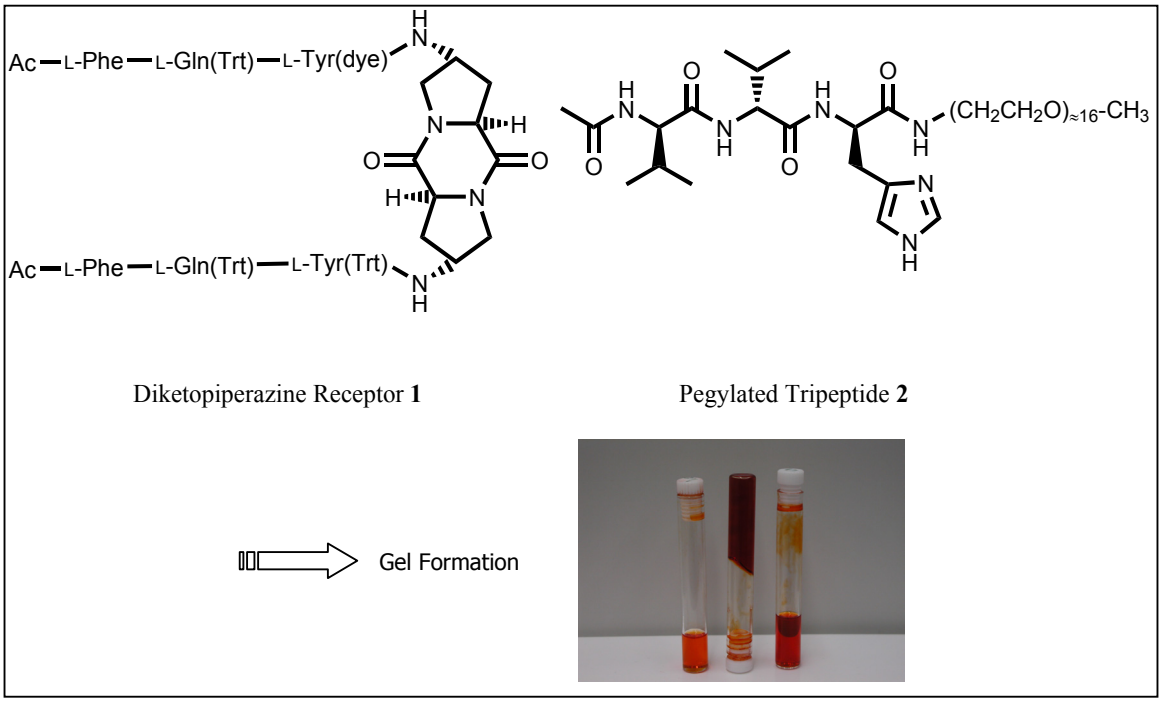


Figure 6: *Diketopiperazine receptor **1** mixed with the pegylated tripeptide **2***

To gain insight into the supramolecular assembly process, the following points are discussed:

- Synthesis of two selected pegylated tripeptides with different PEG lengths and two peptide-PEG conjugates which were not selected by the receptor in combinatorial on-bead assay.
- Dynamic Light Scattering studies of the self-assembled receptor-pegylated tripeptide system.
- Transmission Electron (TEM) and Atomic Force (AFM) Microscopy investigations.
- Surface pressure measurements.

2 DESIGN AND BINDING PROPERTIES OF MACROCYCLIC DIKETOPIPERAZINE RECEPTOR PROTOTYPES

2.1 Receptor Design

A receptor class containing members, which are capable of binding any desired peptide selectively, should consist of a rigid, structure-directing backbone as well as functional groups that allow for the formation of non-covalent interactions such as hydrogen bonds, ionic and hydrophobic interactions. Furthermore, the receptor structure should offer the possibility for combinatorial structural and functional variations and should be accessible by a simple synthesis strategy both in solution and on solid supports, to ultimately permit the generation of a combinatorial receptor library using a split-and-mix protocol.[26-27]

Two-armed diketopiperazine receptors fulfil all of the above-mentioned requirements for a versatile class of receptors. The design of the receptor is based on using *trans,trans*-diketopiperazine as a rigid scaffold which itself is derived from 4-hydroxyproline. The two peptidic receptor arms, which are attached to the scaffold *via* amide linkage, are built of L- as well as D- amino acids and offer structural and functional variety for binding peptide sequences. Standard peptide synthesis can be used to assemble these diketopiperazine receptors.

Moreover, conformational analysis demonstrated that the *trans,trans*-diketopiperazine adopts a well-defined turn-conformation that proved crucial for selective peptide binding (*Figure 7*).[52] Thus, exchange of the template leads to significantly lower or entire loss of binding selectivity. The analysis of the preferred conformation adopted by the receptors arms has not been straightforward due to their structural flexibility. To get an understanding of their relative orientation towards each other, we connected the termini of the peptidic receptor arms by different linkers and describe the synthesis and binding properties of these macrocyclic diketopiperazine receptors. To test for the effect of macrocyclization, the termini of the peptidic side-chains were connected by a) a metathesis reaction[57], b) amide bonds and c) a disulfide bond.[58]

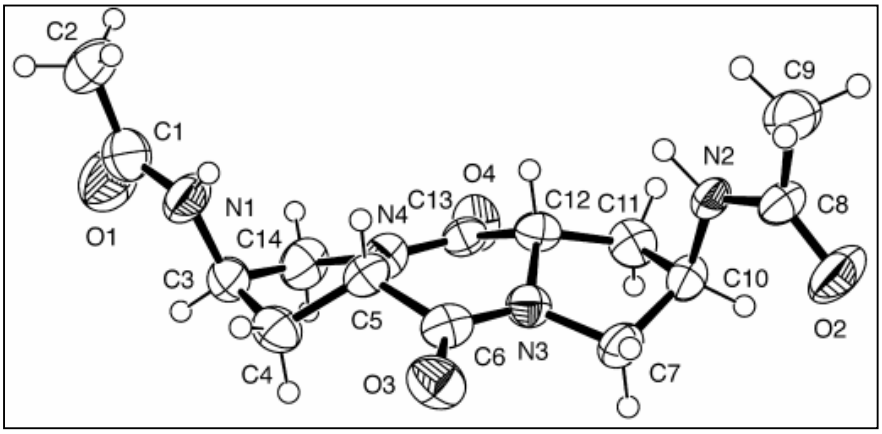


Figure 7: *Crystal structure of the trans,trans-diketopiperazine*

Macrocyclization was expected to result in improved binding selectivities and particularly binding affinities in the case of the open-chain diketopiperazine receptors bind with linearly aligned peptidic recognition modules. Conversely, reduced binding affinities were expected as a result of macrocyclization in the case of the recognition modules are not aligned parallel to each other in the binding conformation (*Figure 8*).

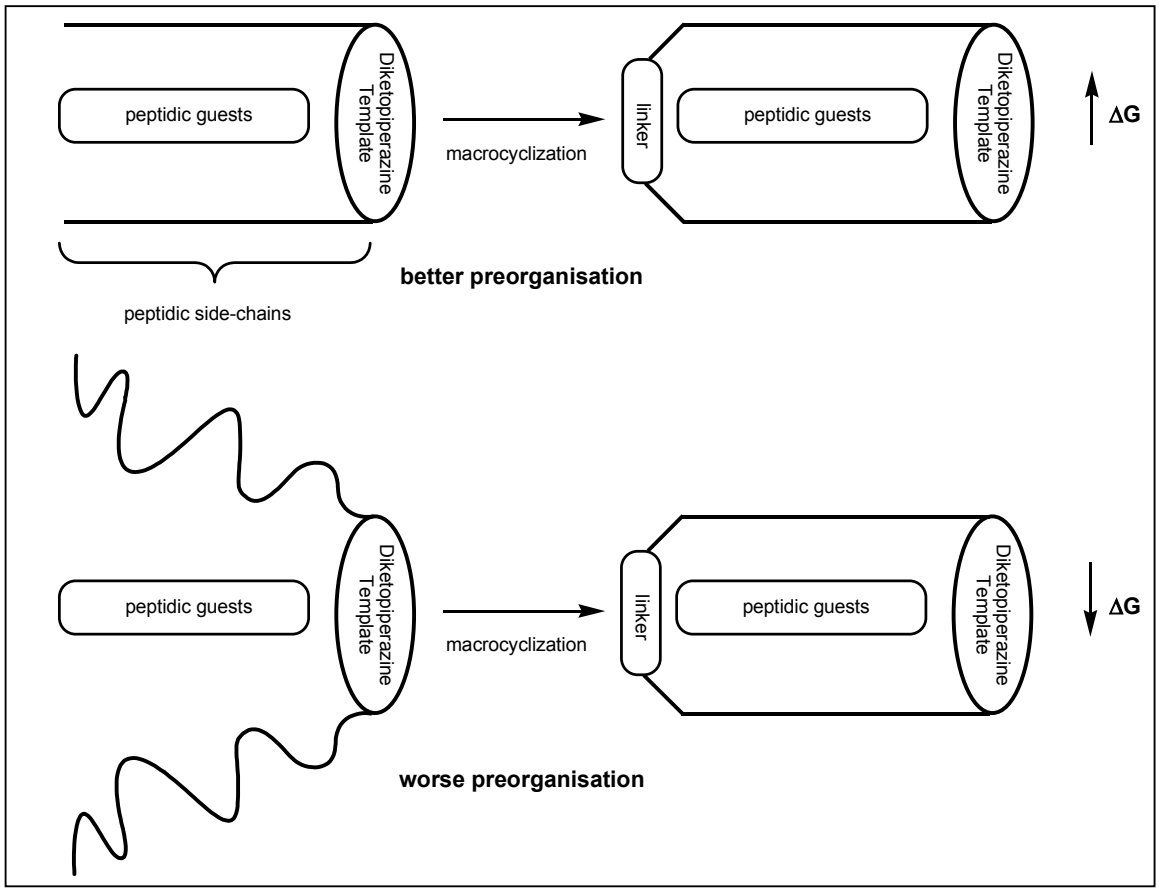


Figure 8: *Schematic representation of binding preorganisation*

To analyse the effect of macrocyclization on the binding properties of diketopiperazine receptors, we chose the open-chain receptor **1** as a receptor prototype. This receptor prototype consists of the *trans,trans*-diketopiperazine backbone and two symmetric tripeptide arms containing L-Tyr(dye), L-Gln(Trt) and L-Phe (*Figure 9*). Tyrosine was chosen as first amino acid because its phenolic hydroxyl group offers an attachment site for a dye, essential for the screening. As a dye, the red azo-dye Disperse Red 1 is used. We chose this specific receptor even though it is not the most selective or the one with the highest binding properties but because it is more soluble compared to the receptor containing Asn instead of Gln at the second position of the peptidic arms.[50]

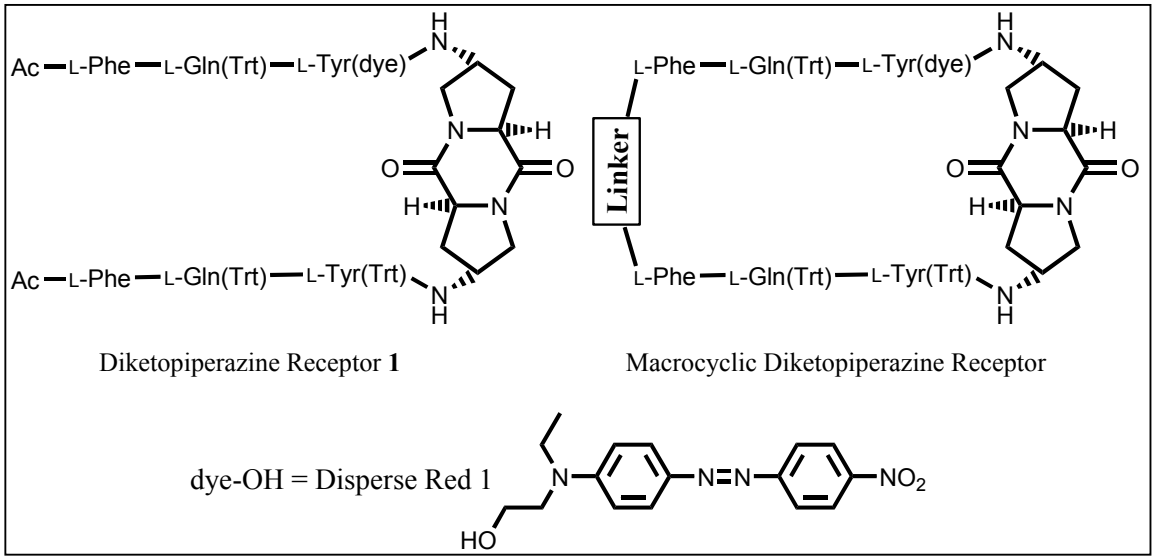
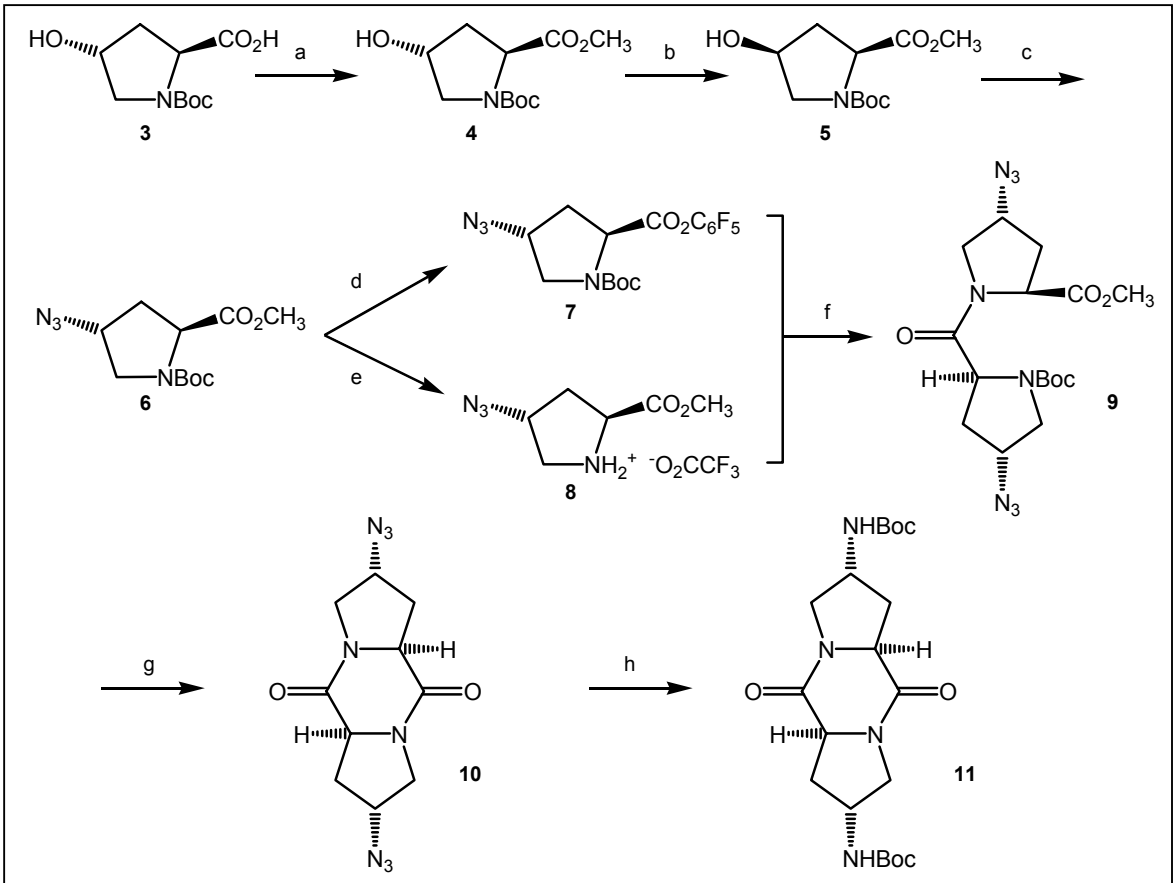


Figure 9: *Macrocyclic dye-marked diketopiperazine receptor prototype*

2.2 Synthesis of the Symmetric Diketopiperazine Template

The synthesis of the diketopiperazine template **10** (*Scheme 4*) started from *N*-Boc-γ-*trans*-hydroxy-L-proline-methylester **4** which was obtained from the reaction of the Cesium-salt of the commercially available *N*-Boc-γ-*trans*-hydroxy-L-proline **3** with MeI. Inversion of the configuration of the Cγ of **4**, under Mitsunobu conditions, yielded *N*-Boc-γ-*cis*-hydroxy-L-proline-methylester. Introduction of the azide-group was accomplished by a S_N2 substitution with NaN_3 of the corresponding mesylate of **5** in 96% yield.[59] Subsequently the *N*-Boc-γ-*trans*-azido-L-proline-methylester **6** was split into two portions. One portion was hydrolized and transformed into the pentafluorophenylester **7**. In the second portion of **6**, the Boc group was deprotected using a mixture of TFA/CH_2Cl_2 yielding the corresponding TFA salt **8**. Mixing of **7** and **8** in the presence of Hünig's base yielded the cyclization precursor **9**. *N*-Boc deprotection with TFA and addition of Hünig's base led to the diketopiperazine **10** in 81% yield. The reduction of the azide functionalities with Palladium on carbon in the presence of Boc_2O yielded the well storable *N*-Boc-protected diketopiperazine **11**.

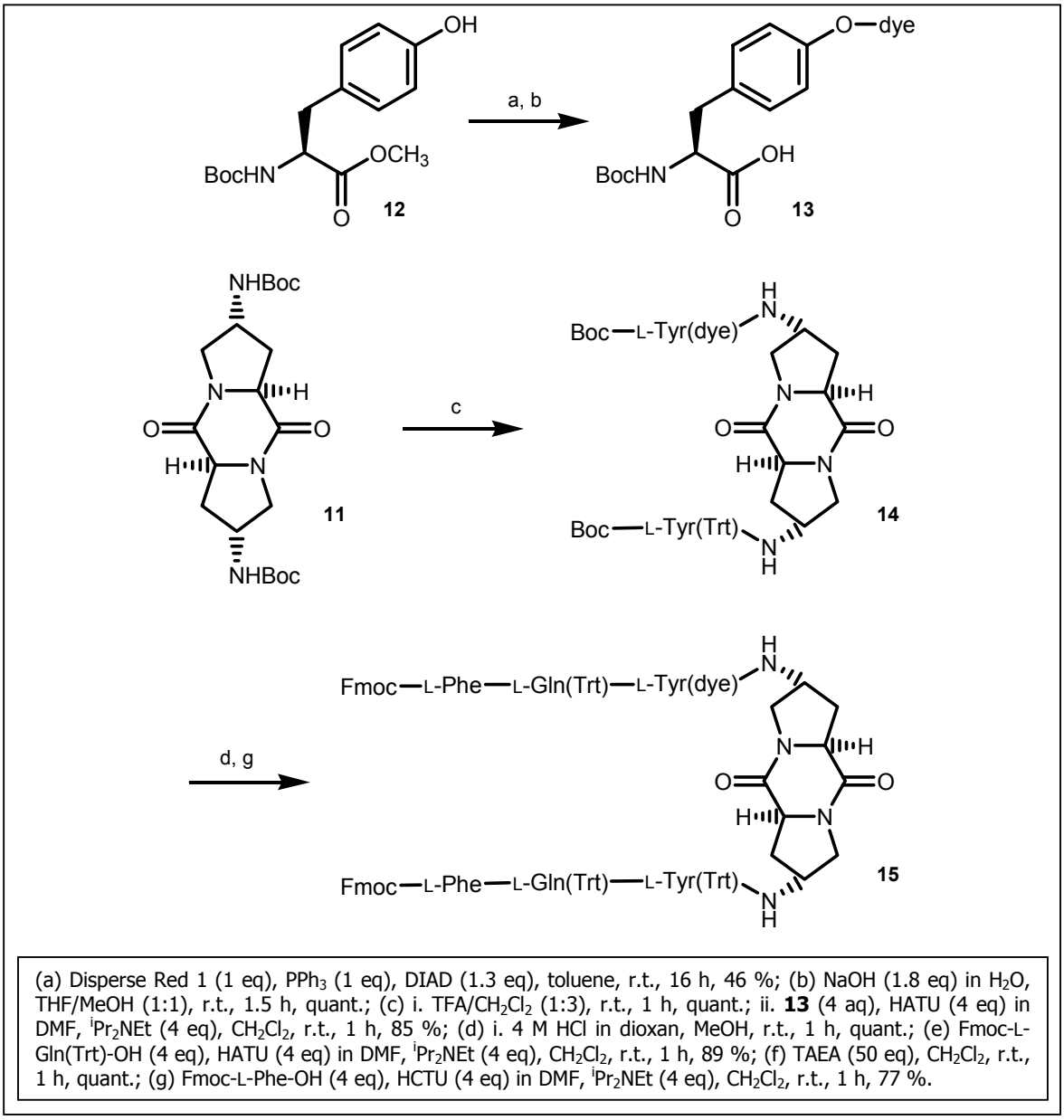


(a) i. Cs_2CO_3 (0.55 eq), MeOH, H_2O, r.t., 1 h; ii. MeI (2 eq), DMF, r.t., 30 min, 94 %; (b) i. $C_6H_5CO_2H$ (1.15 eq), PPh_3 (1.16 eq), DIAD (1.1 eq), THF, 0°C then r.t., 2.5 h,; ii. $NaOCH_3$ (0.95 eq), MeOH, r.t., 15 min, 66 %; (c) i. MsCl (1.2 eq), Et_3N (1.2 eq), CH_2Cl_2, 0°C then r.t., 30 min; ii. NaN_3 (5 eq), DMF, 80°C, 2 h, 96 %; (d) i. NaOH (1.8 eq) in H_2O, THF/MeOH (1:1), r.t., 1.5 h; ii. C_6F_5OH (1.1 eq), EDC (1.5 eq), CH_2Cl_2, r.t., 1 h, 80 %; (e) TFA/CH_2Cl_2 (1:3), r.t., 1.5 h, quant.; (f) $^iPr_2NEt$ (2 eq), CH_2Cl_2, r.t., 16 h, 69 %; (g) i. TFA/CH_2Cl_2 (1:3), r.t., 1.5 h; ii. $^iPr_2NEt$ (5 eq), THF, r.t., 16 h, 81 %; (h) Pd/C (10%), H_2, Boc_2O (4.1 eq), MeOH, r.t., 3 h, 13 %.

Scheme 4: *Synthesis of the symmetric diketopiperazine template*

2.3 Synthesis of the Two-Armed Diketopiperazine Receptor Precursor

The red azo-dye Disperse Red 1 was attached to the phenolic hydroxyl group of the *N*-Boc-L-Tyrosine-methylester **12** by a Mitsunobu reaction, followed by conversion of the methylester into the carboxylic acid to yield the dye-marked tyrosine derivative **13** (*Scheme 5*). For the assembly of the receptor, the Boc groups of **11** were removed and the resulting diamine was coupled with the dye-marked carboxylic acid of *N*-Boc-L-Tyrosine to yield **14**. After *N*-Boc deprotection with HCl, the remaining amino acids of the arms were assembled by standard couplings of *N*-α-Fmoc-protected amino acids using *O*-(7-azabenzotriazol-1-yl)*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HATU) or *O*-(1H-6-chlorobenzotriazole-1-yl)1,1,3,3-tetramethyluronium hexafluorphosphate (HCTU) as coupling reagents and tris(2-aminoethyl)amine (TAEA) for Fmoc-deprotection. Using this synthetic route the receptor precursor **15** with two identical arms consisting of a dye-marked L-Tyr as initial amino acid followed by L-Gln(*N*-trityl) and L-Phe was prepared.



(a) Disperse Red 1 (1 eq), PPh_3 (1 eq), DIAD (1.3 eq), toluene, r.t., 16 h, 46 %; (b) NaOH (1.8 eq) in H_2O, THF/MeOH (1:1), r.t., 1.5 h, quant.; (c) i. TFA/CH_2Cl_2 (1:3), r.t., 1 h, quant.; ii. **13** (4 aq), HATU (4 eq) in DMF, $^{i}Pr_2NEt$ (4 eq), CH_2Cl_2, r.t., 1 h, 85 %; (d) i. 4 M HCl in dioxan, MeOH, r.t., 1 h, quant.; (e) Fmoc-L-Gln(Trt)-OH (4 eq), HATU (4 eq) in DMF, $^{i}Pr_2NEt$ (4 eq), CH_2Cl_2, r.t., 1 h, 89 %; (f) TAEA (50 eq), CH_2Cl_2, r.t., 1 h, quant.; (g) Fmoc-L-Phe-OH (4 eq), HCTU (4 eq) in DMF, $^{i}Pr_2NEt$ (4 eq), CH_2Cl_2, r.t., 1 h, 77 %.

Scheme 5: *Synthesis of two-armed diketopiperazine receptor precursor*

2.4 Synthesis of Macrocyclic Diketopiperazine Receptor Prototypes

After the synthesis of the Fmoc-protected macrocyclic receptor precursor **15**, we envisaged different methods of macrocyclization. The termini of the peptidic side-chains were connected by a) ring closing metathesis;[57] b) amide bond formation and c) formation of a disulfide bridge.[58] All of the linkers were designed to reduce the conformational flexibility of this receptor to test the binding properties towards tripeptides of these rigidified macrocyclic receptors. Thus, after Fmoc-deprotection different linking moieties for macrocyclization were introduced by standard coupling methods using HATU or HCTU and Hünig's base as coupling reagents and TAEA for Fmoc-deprotection. Specifically, we prepared four different macrocyclic receptor prototypes (*Figure 10*).

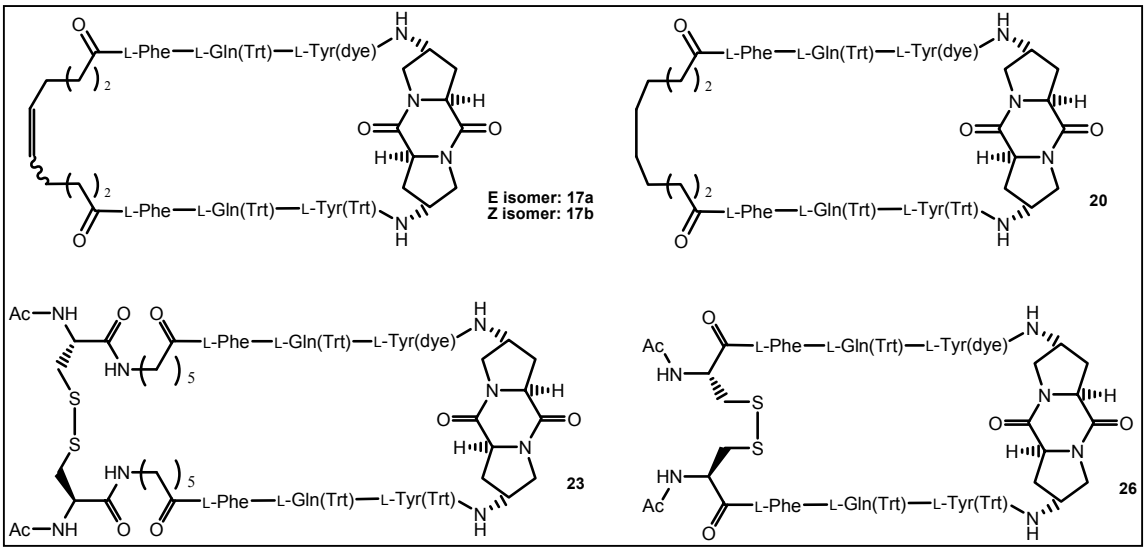


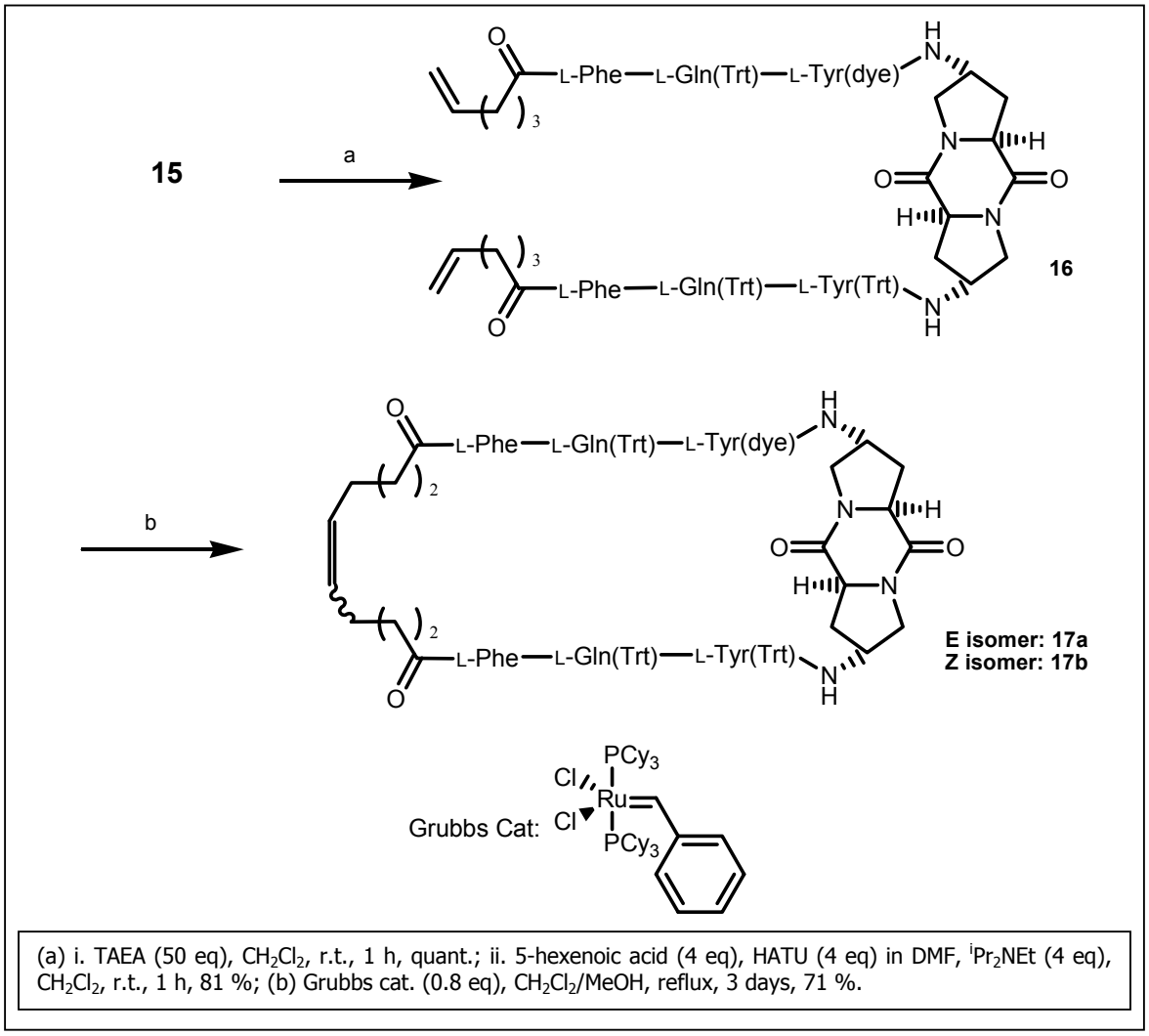
Figure 10: *Macrocyclic diketopiperazine receptor prototypes (dye = Disperse Red 1)*

2.4.1 Ring Closing Metathesis

Ring closing metathesis (RCM) has emerged as a powerful tool for the formation of C-C bonds in chemistry. Of particular significance, this type of metathesis utilizes no additional reagents beyond a catalytic amount of metal carbene and the only other product formed in the reaction is, in most cases, a volatile olefin, such as ethylene, and the cycloalkene. Furthermore, RCM is a very versatile method for the preparation of medium-sized and macrocyclic products.[60] It was discovered that neither a conformational predisposition of the starting material toward ring closure, nor the ring size formed are particularly relevant factors,[61] although this had been previously assumed.[62] The E/Z selectivity depends on the ring strain. Ruthenium alkylidene[63] and molybdenum alkylidene[64] are two of the most commonly used initiators for RCM, and reports of their use in organic synthesis have increased steadily.[65]

The Grubbs catalysts, based on ruthenium alkylidene, are some of the major catalysts employed in this reaction, as they exhibit high reactivity and have a remarkable tolerance towards many different organic functional groups. Catalytic activity is not reduced significantly by the presence of air, moisture or minor impurities in solvents.[66] However, the mere presence of a functional group (ester, ketone, ether, etc.) in the starting material was found to be of utmost importance, as well as the proper distance between this key substituent and the alkenes to be metathesized.[61a,d] To accomplish our macrocylization, we chose the first generation of Grubbs catalyst.

After Fmoc-deprotection, 5-hexenoic acid was coupled onto receptor precursor **15** using HATU as coupling reagent. Use of 0.8 eq of Grubbs catalyst at a concentration of 1 mM in a mixture of CH_2Cl_2 and MeOH, led to the macrocyclic receptor **17** in 71 % yield. Addition of the catalyst was accomplished over a period of three days to complete the reaction. The ring closing metathesis reaction resulted in a 1:2 mixture of the Z and E isomers that were separated by preparative HPLC (*Scheme 6*).



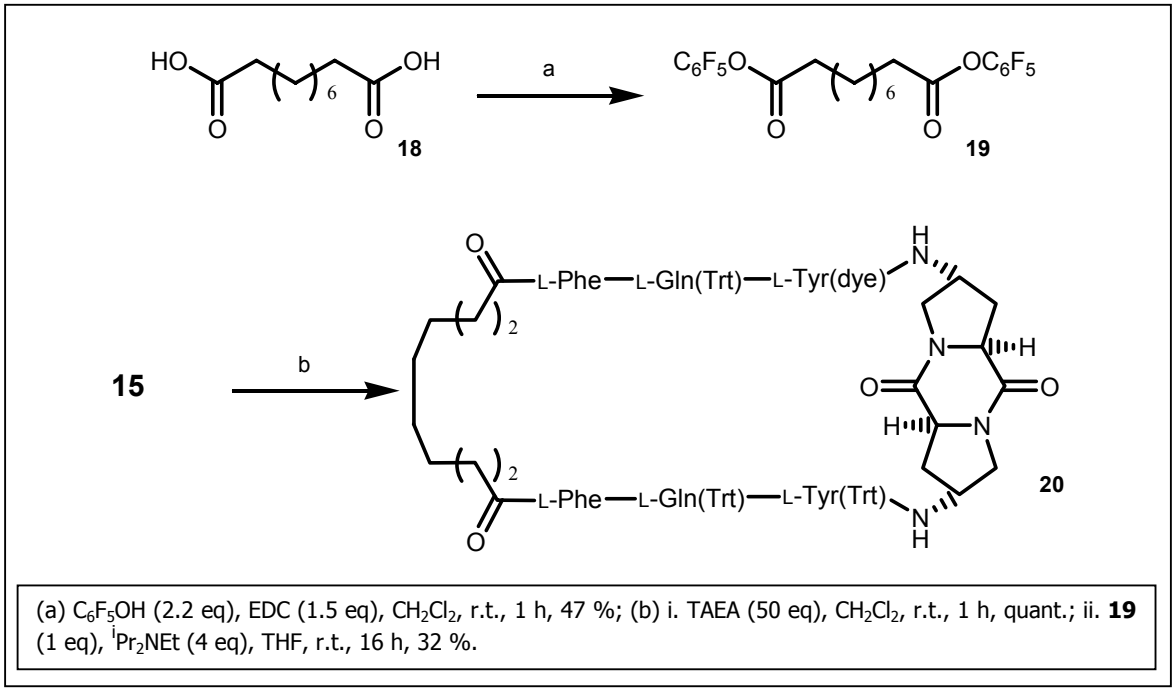
(a) i. TAEA (50 eq), CH_2Cl_2, r.t., 1 h, quant.; ii. 5-hexenoic acid (4 eq), HATU (4 eq) in DMF, $^{i}Pr_2NEt$ (4 eq), CH_2Cl_2, r.t., 1 h, 81 %; (b) Grubbs cat. (0.8 eq), CH_2Cl_2/MeOH, reflux, 3 days, 71 %.

Scheme 6: *Synthesis of the macrocyclic diketopiperazine receptors **17a** and **17b** via ring closing metathesis*

2.4.2 Amide Bond Formation

For the amide bond formation, the linkers between the termini of the peptidic side-chains were designed to have the same number of atoms (ten) as used in the RCM, thus allowing to analyze whether the linker would have an influence on the binding properties of macrocyclic diketopiperazine receptors with the same ring size.

After Fmoc deprotection, the free amines of the receptor precursor **15** were coupled with an activated dicarboxylic acid. Sebacic acid **18** was chosen and activated as pentafluorophenyl (Pfp) esters using Pfp-OH and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) as coupling reagent. The macrocyclic receptor **20** was obtained by simultaneous slow addition of the bis-pentafluorophenylester **19** and the free amines of receptor **15** to a solution of Hünig's base at a concentration of 1 mM in THF, in 32% yield (*Scheme 7*). This low concentration was used as a means to avoid intermolecular reactions and polymerization of the receptors through sebacic acid, instead of the desired macrocyclization.



(a) C_6F_5OH (2.2 eq), EDC (1.5 eq), CH_2Cl_2, r.t., 1 h, 47 %; (b) i. TAEA (50 eq), CH_2Cl_2, r.t., 1 h, quant.; ii. **19** (1 eq), $^iPr_2NEt$ (4 eq), THF, r.t., 16 h, 32 %.

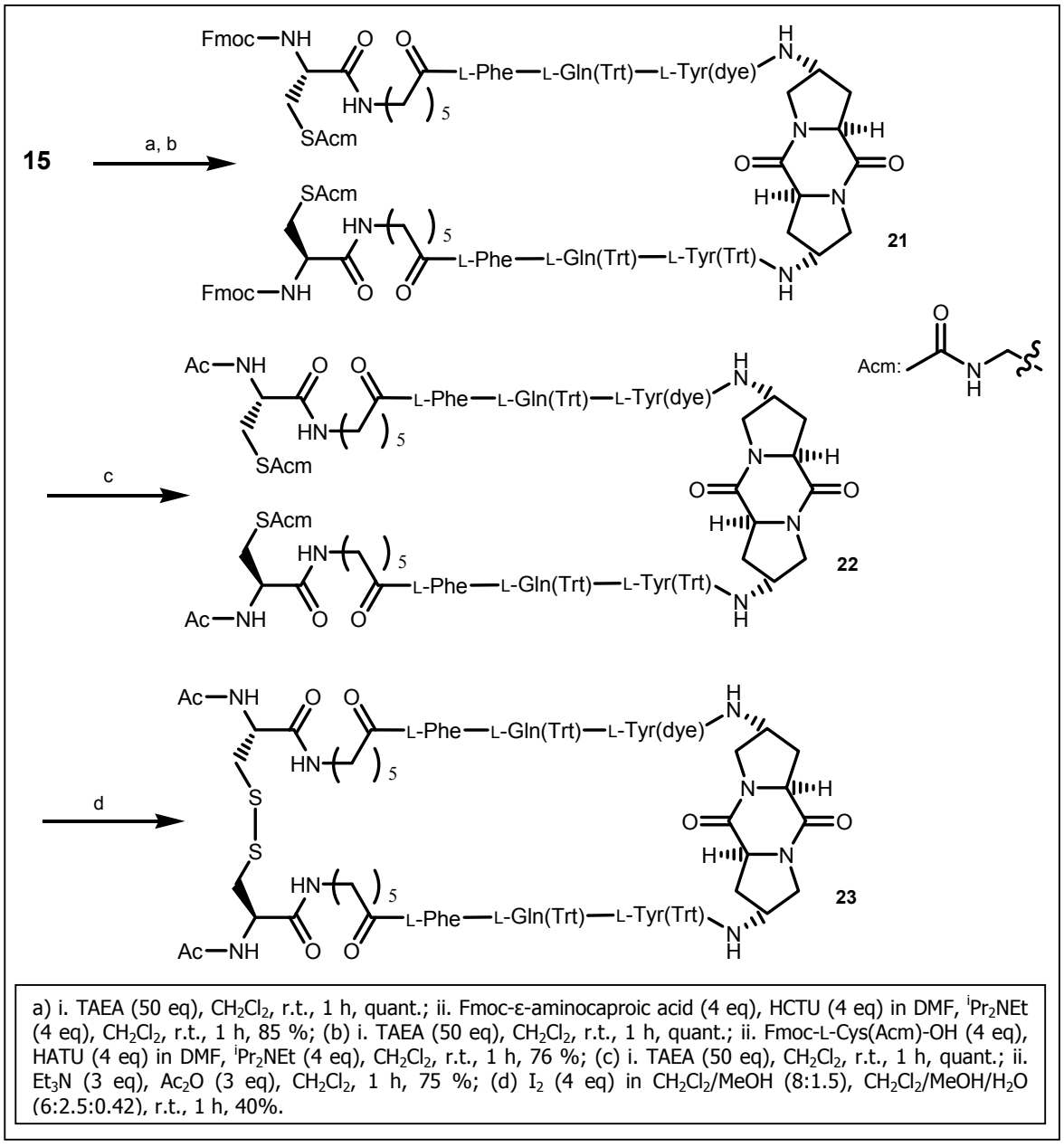
Scheme 7: *Synthesis of the macrocyclic diketopiperazine receptor **20** via amide bond formation*

2.4.3 Disulfide Bridge Formation

Disulfide bonds between cysteine residues play an important role in the folding and structural stabilization of many peptides and proteins.[67] The introduction of a disulfide bridge in our receptor is an important concept in the macrocyclization and should allow for reversible opening and closing of the cyclic receptor. Due to the complex functionality and reactivity of the individual amino acid side-chains, it is important to have a range of reagents available for disulfide bond formation. Various oxidation methods are available now, but synthetic methods for selective disulfide bond formation are still limited.[68] We tested sulfide oxidation using iodine as oxidant, with cysteine carrying various side-chain protecting groups such as acetamidomethyl (Acm), Fmoc and Trt. The best result was obtained with an Acm-side-chain protected cysteine residue.[58]

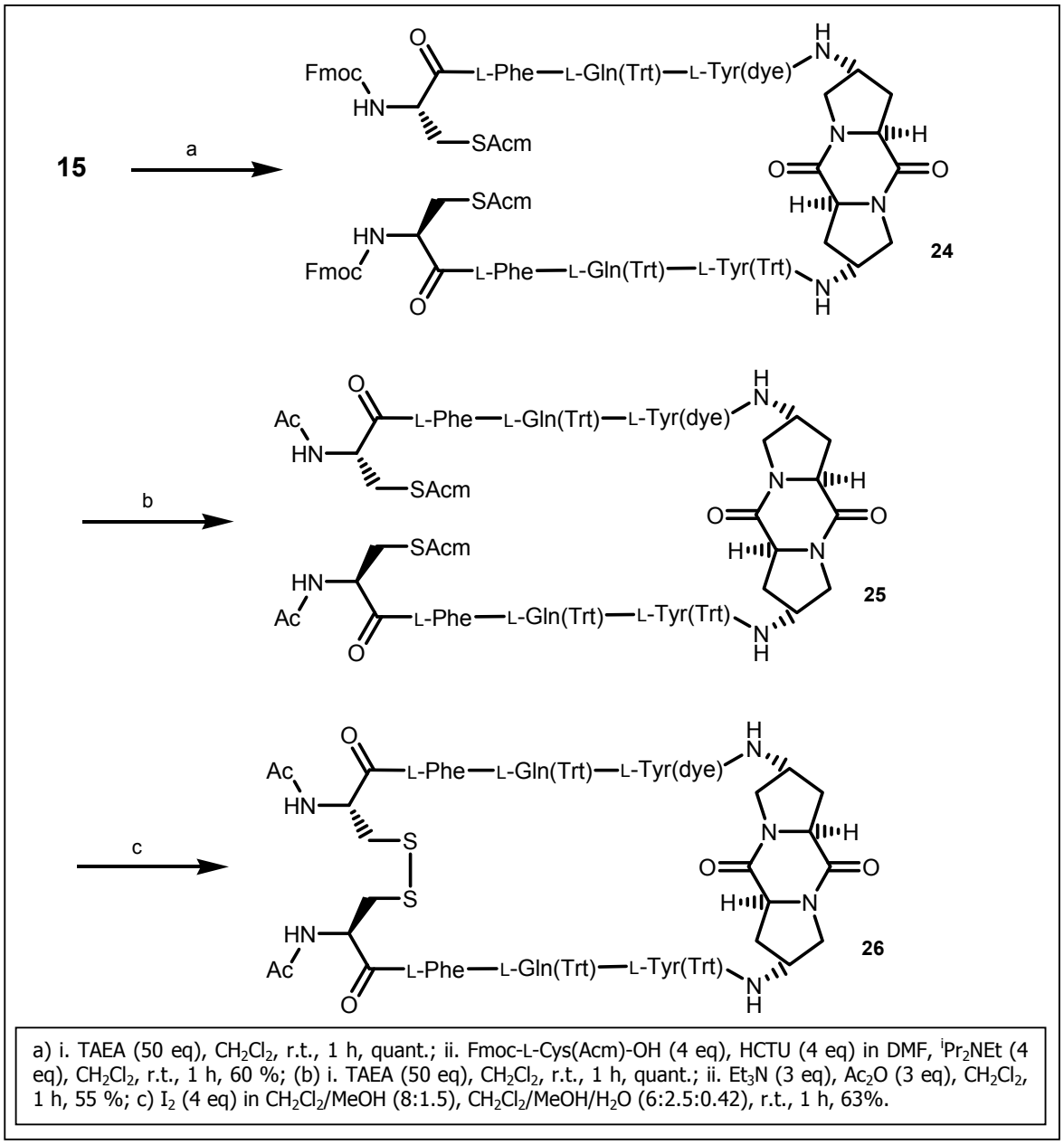
Two macrocyclic receptors were synthesized, one with a disulfide linker containing seven atoms, receptor **26**, (*Scheme 9*) and a second with a linker containing fourteen additional atoms, receptor **23**, thus, forming a larger macrocycle (*Scheme 8*).

After Fmoc-deprotection, the free amines of the two-armed receptor precursor **15** were coupled with an Acm-side-chain protected cysteine residue *via* an aminocaproic acid "spacer" to obtain the open-chain receptor prototype **21**. Direct coupling of the protected cysteine, i.e. without aminocaproic acid "spacer", lead to the receptor **24**. After Fmoc deprotection and acetylation, the disulfide bridge formation was carried out with iodine at a concentration of 1 mM in a mixture of dichloromethane, methanol and water. The disulfide bridge-containing macrocyclized receptors **23** and **26** were obtained in 40% and 63 % yield, respectively.



a) i. TAEA (50 eq), CH_2Cl_2, r.t., 1 h, quant.; ii. Fmoc-ε-aminocaproic acid (4 eq), HCTU (4 eq) in DMF, $^{i}Pr_2NEt$ (4 eq), CH_2Cl_2, r.t., 1 h, 85 %; (b) i. TAEA (50 eq), CH_2Cl_2, r.t., 1 h, quant.; ii. Fmoc-L-Cys(Acm)-OH (4 eq), HATU (4 eq) in DMF, $^{i}Pr_2NEt$ (4 eq), CH_2Cl_2, r.t., 1 h, 76 %; (c) i. TAEA (50 eq), CH_2Cl_2, r.t., 1 h, quant.; ii. Et_3N (3 eq), Ac_2O (3 eq), CH_2Cl_2, 1 h, 75 %; (d) I_2 (4 eq) in CH_2Cl_2/MeOH (8:1.5), CH_2Cl_2/MeOH/H_2O (6:2.5:0.42), r.t., 1 h, 40%.

Scheme 8: *Synthesis of the macrocyclic diketopiperazine receptors **23** via disulfide bridge formation*



a) i. TAEA (50 eq), CH_2Cl_2, r.t., 1 h, quant.; ii. Fmoc-L-Cys(Acm)-OH (4 eq), HCTU (4 eq) in DMF, $^{i}Pr_2NEt$ (4 eq), CH_2Cl_2, r.t., 1 h, 60 %; (b) i. TAEA (50 eq), CH_2Cl_2, r.t., 1 h, quant.; ii. Et_3N (3 eq), Ac_2O (3 eq), CH_2Cl_2, 1 h, 55 %; c) I_2 (4 eq) in CH_2Cl_2/MeOH (8:1.5), CH_2Cl_2/MeOH/H_2O (6:2.5:0.42), r.t., 1 h, 63%.

Scheme 9: *Synthesis of the macrocyclic diketopiperazine receptors **26** via disulfide bridge formation*

2.5 Screening of the Two-Armed Diketopiperazine Receptor Prototypes Against an Encoded Side-chain Deprotected Peptide Library

The binding properties of dye-marked receptor prototypes were tested by screening them against a side-chain deprotected acetylated tripeptide library in chloroform.[50] The same library had been used to evaluate the binding properties of diketopiperazine receptor **1**. The library was synthesized on polystyrene resin by encoded split-and-mix synthesis[33-34] using standard Fmoc-strategy[69] and had the general structure Ac-AA3-AA2-AA1-$NH(CH_2)_5CONH$-PS. Amino acid couplings were accomplished by reacting 3 eq of *N*-Fmoc-amino acid, 3 eq of diisopropylcarbodiimide (DIC) and 3 eq of *N*-hydroxybenzotriazole (HOBt) (dissolved in the smallest amount of DMF) in CH_2Cl_2 at room temperature for 2 hours. *N*-Fmoc-aminohexanoic acid (Fmoc-Ahx) was chosen as a "spacer" between the peptide sequences and the resin. 29 L- and D-amino acids were employed at each position. Thus, the library contained maximally 29^3=24389 different acetylated tripeptides (*Figure 11*).

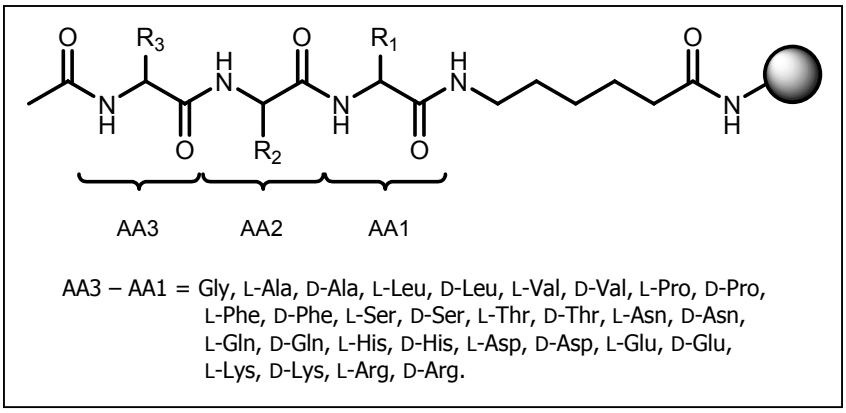


Figure 11: *General structure of the acetylated tripeptide library on polystyrene resin*

In order to ensure a representative screening result, an amount corresponding to at least five theoretical copies of the library was used per assay.[48] Upon mixing the library (~ 5 mg) with dilute solutions of receptors (50-100 µM) in $CHCl_3$ (freshly filtered through aluminium oxide) and equilibration for 1 to 3 days, several beads picked up the red color of the receptor (*Figure 12*). The structures of the peptide substrates preferentially bound to the receptor prototypes were determinated by isolating the red beads from the assays of each receptor and decoding the tags by EC-GC.

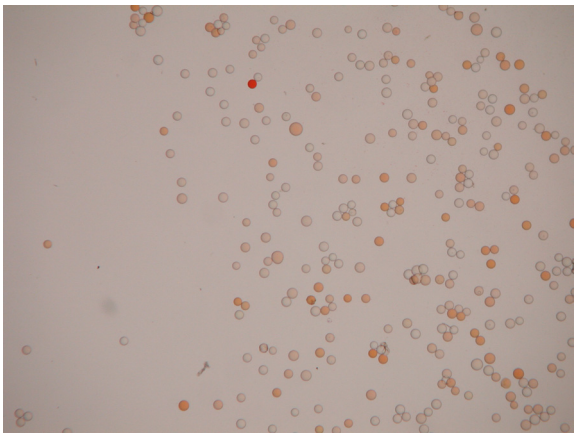


Figure 12: *Picture of a typical binging assay of macrocyclic receptor **23** with the tripeptide library*

2.5.1 Screening of Macrocyclic Diketopiperazine Receptor Prototypes

In the assays of receptors **17a** and **26**, approximately one out of 100 beads picked up the bright red color of the receptors and for the receptor **23**, one out of ~300. The assays of receptors **20** and **23** indicated an even higher level of binding selectivity since only one bead out of ~2000 and ~4000 respectively, had turned red. These qualitative results of the binding assays revealed that all macrocyclic diketopiperazine receptors are able to bind to peptides selectively. Isolation of the colored beads followed by analysis of the encoding tag-molecules revealed the amino acid sequences of the selected peptides.

Table 1 lists the most frequently occurring peptide sequences for each macrocyclic receptor prototype (*see Figure 10*).

Table 1: *Binding specificities of the macrocyclic receptors for tripeptides within the side-chain deprotected library*

Receptors	**AA3**[a]	**AA2**[a]	**AA1**[a]	freq. found [%][c]	freq. exp. [%][c]	Beads isolated
17a (metathesis)	X	D-AA	D-Pro	19	1.66	21
	L-AA	D-AA	D-/L-Lys	24	1.61	
	X	D-/L-Pro	D-Lys	14	0.24	
17b (metathesis)	X	L-Pro/ D-/L-Lys	D-/L-Pro	30	0.71	20
	D-Pro/ L-Lys	D-/L-Pro	X	20	0.48	
	D-Phe/ D-Ser	D-/L-Lys	X	20	0.48	
20 (amide bond)	L-AA	D-/L-Pro	D-/L-Pro	35	0.23	17
	D-Lys	D-Pro	L-Pro	23	0.004	
23 ("spacer" - disulfide bridge)	D-/L-Ala	L-Gln	L-Ala	17	0.008	30
	D-Gln	D-Hph[b]	D-Hph[b]	37	0.07	
	Gly/ L-Leu/ L-Ala	L-Gln	D-Hph[b]	13	0.01	
26 (disulfide bridge)	D-/L-Arg	X	D-/L-Gln	12	0.48	92
	D-/L-Thr	D-/L-Pro	D-/L-Gln	5	0.03	
	D-/L-Pro	L-Pro/ L-His	D-/L-Gln	8	0.03	
	X	L-His	D-/L-Gln	7	0.23	
	X	D-/L-Arg	D-/L-Pro	5	0.48	
	X	D-/L-Pro	D-/L-Pro	12	0.48	
	X	X	L-Arg	14	3.45	

[a] X: Random amino acids.

[b] Hph = hydrophobic amino acid can be either Gly, Ala, Val, Leu or Phe.

[c] The frequency found column lists the percentage of beads selected in the receptor binding assay for the indicated peptide sequence. The frequency expected column lists the expected frequency for the particular tripeptide sequence if the beads were picked randomly. The comparison between the percentage of "frequency found" and "frequency expected" is a measure for the selectivity level of the receptor.

2.5.2 Comparison to the Open-Chain Receptor Prototypes

After having tested the binding properties of macrocyclic dye-marked receptor prototypes, the results were compared to the binding properties of the corresponding open-chain receptors with and without different types of linkers under the same conditions (*Figure 13*). Upon mixing a dilute solution of receptor **25** in $CHCl_3$ with the 24389 membered library only a few beads (ca. one out of 1500) picked up the color of the dye-marked receptor indicating selective binding to certain members within the library. In contrast, the assays of receptors **1**, **16** and **22** indicated a particularly high level of binding selectivity, since approximately one bead out of 5000 was colored. The structures of the peptidic substrates bound to the receptors were determined by isolation of the red beads from the assay of each receptor and analysis of the encoding tag molecules was achieved by EC-GC (*Table 2*).

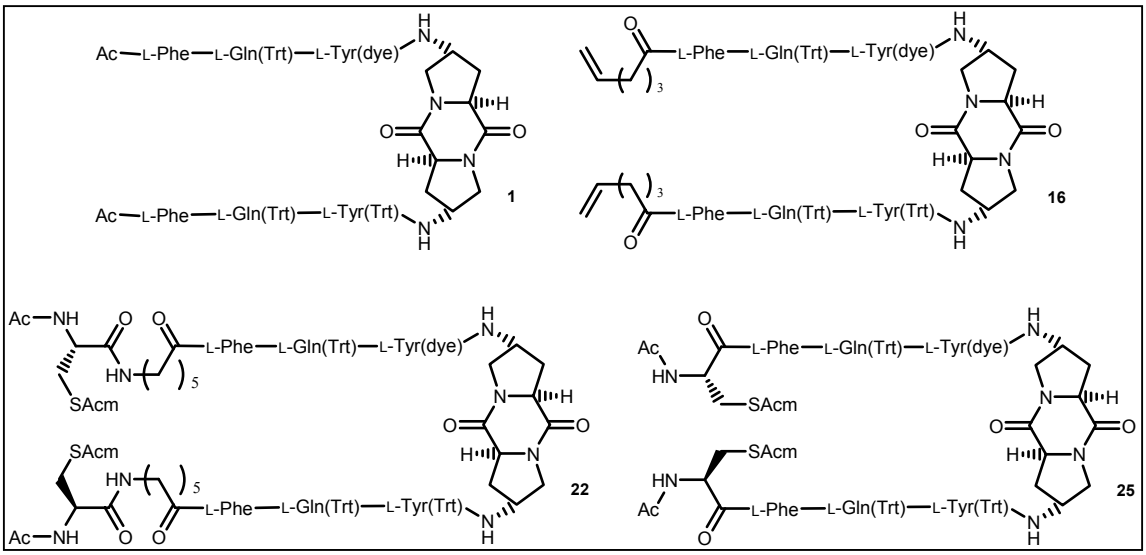


Figure 13: *Open-chain diketopiperazine receptor prototypes (dye = Disperse Red 1)*

Table 2: *Binding specificities of the open-chain receptors for tripeptides within the side-chain deprotected library*

Receptors	**AA3**[a]	**AA2**[a]	**AA1**[a]	freq. found [%][b]	freq. exp. [%][b]	Beads isolated
1	D-Ala/ D-Val	D-Hph	D-His	34	0.04	
	L-Ala/ L-Leu	L-Gln	D-Hph	37	0.04	
	D-Gln	D-Hph	D-Val/ D-Leu	20	0.04	
16 (metathesis precursor)	D-Ala	D-Phe	D-Gln	36	0.004	
	D-Ala/ Gly	L-Gln	L-Ser	29	0.008	14
	L-Hph	L-Gln	L-Ser/ D-/L-Ala	21	0.05	
22 ("spacer" – disulfide bridge precursor)	D-Gln	D-Val	D-Val	100	0.004	3
25 (disulfide bridge precursor)	D-Gln/ D-Asp	D-Phe	D-Val	12	0.008	42
	L-Ala	L-Gln	D-AA	21	0.06	
	D-/L-Pro	D-/L-Pro	D-/L-Gln	7	0.03	
	L-AA	D-/L-Pro	D-/L-Pro	14	0.23	

[a] Hph = hydrophobic amino acid can be either Gly, Ala, Val, Leu or Phe.

[b] The frequency found column lists the percentage of beads selected in the receptor binding assay for the indicated peptide sequence. The frequency expected column lists the expected frequency for the particular tripeptide sequence if the beads were picked randomly. The comparison between the percentage of "frequency found" and "frequency expected" is a measure for the selectivity level of the receptor.

2.5.3 Discussion

The analysis of the red beads revealed that, the sequence D-Hph-D-Hph-D-His, selected preferably by the open-chain receptor **1**, was not selected by any of the macrocyclic receptors nor their open-chain precursors.[50] On the other hand, the sequences D-Gln-D-Hph-D-Hph and/or L-Hph-L-Gln-D-Hph were recognized by both the macrocyclic receptor **23** and the open-chain receptors **1**, **16**, and **22**. In the case of macrocyclic receptors **17a**, **17b**, **20**, and **26**, very different amino acids were recognized compared to the open-chain receptors **1** and **16**. Strikingly, the amino acids D-/L-Pro occured frequently in the sequences, along with the hydrophilic amino acids D-/L-Lys and D-Ser for the receptor **17a**, **17b**, and **20**. Interestingly, receptor **20** has significantly higher binding selectivities compared to the isomeric receptors **17a** and **17b**, with an alkene, instead of an alkan-linker, and to receptor **26**, with a disulfide bridge in the linker. In the case of the open-chain receptor, the selected amino acid sequences are quite different since L-Ser was recognized by the receptor **16** and D-/L-Pro by the receptor **25** but not by the receptors **1** and **22**. This indicates that both the cyclization of the receptor and the choice of the linker-type lead to significant changes in their binding properties.

In contrast, the selectivity is not influenced by the disulfide bridge in the case of the receptor **23**, which has similar binding properties compared to the receptor **1**, because of the long distance between the linker and the termini of the peptidic arms. These results suggest that the ring size of the macrocycle **23** is large enough to accommodate a similar conformation as in the open-chain receptor **1** that allows for binding to the Gln-containing peptides.

In general, macrocyclic receptors bind peptides with lower selectivites compared to open-chain diketopiperazine receptors. The selectivities were found to be largely dependent on the length of the "spacer" used for the macrocyclization. Macrocyclic receptors, with a long "spacer", have binding properties that are similar to those of open-chain receptors while a short "spacer" leads to often drastically modified and typically decreased binding selectivities.

2.6 Binding Energies of the Macrocyclic Diketopiperazine Receptors and Tripeptides in $CHCl_3$

The screening results indicate that macrocyclic diketopiperazine receptors bind with selectivity to some resin-bound tripeptides. In order to analyze these selective interactions, binding studies to quantify the affinity of the substrates to the receptors were performed.[50,51,54,70] For this purpose, the selected tripeptides were re-synthesized on solid support (polystyrene, 200-400 mesh, loading 1.1 mmol g^{-1}) by employing the general procedure for solid phase synthesis, using the Fmoc-strategy. By using mixtures of *N*-Fmoc-aminohexanoic acid and acetic acid (37:63) for the initial resin functionalization, different resins with peptide loadings of ~0.3 mmol g^{-1} were prepared. The loading of each resin was determined by the quantitative Fmoc-test.[71] Diisopropylcarbodiimide and 1-hydroxybenzotriazole (HOBt) served as coupling reagents for the initial coupling as well as for the following amino acid couplings.

For the determination of the binding affinity, a precisely measured amount of the solid supported peptide was placed in a 1 ml UV-cuvette and 1 ml of the dye-marked receptor of known concentration (for receptors **17a**, **17b**, **23** and **26**, c ~ 14 µM) in $CHCl_3$ (freshly filtered through aluminium oxide) was added. The mixture was tightly sealed and allowed to equilibrate by slight agitation for at least 72 h. After this time, the UV-Vis absorbance (for Disperse Red 1 λ_{max} = 480 nm) of the remaining receptor concentration did no longer change (*Figure 14*).

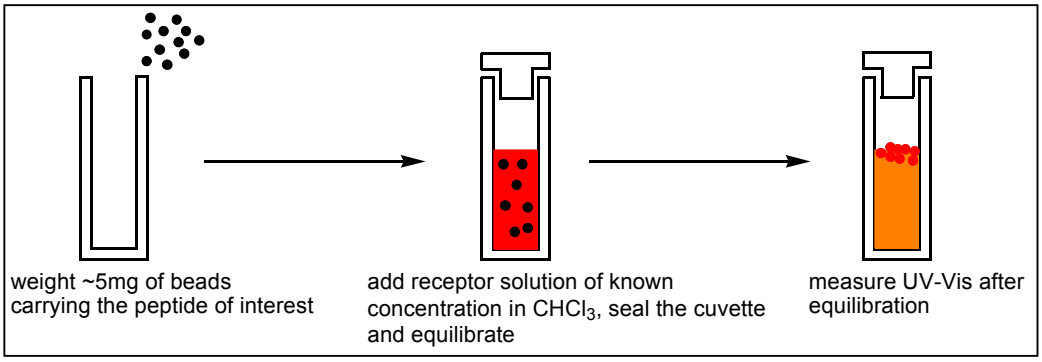


Figure 14: *Solid phase binding assay*

The binding constants and affinities were calculated under the assumption of a simple bimolecular receptor-peptide complex and participation of all peptides in the intermolecular binding by equation (**1**).

$$K_a = \frac{[RP]}{([R_0]-[RP])([P_0]-[RP]} \qquad (\mathbf{1})$$

[RP] = concentration of the receptor-peptide complex at equilibrium
$[R_0]$ = initial receptor concentration
$[R_0]$-[RP] = concentration of the receptor at equilibrium
$[P_0]$ = initial peptide concentration
$[P_0]$-[RP] = concentration of the peptide at equilibrium

Equation **1** is valid for obtaining relative binding constants, only if the resin bound peptide interacts with the receptor but not the resin itself. We checked this in a control experiment using acetylated PS resin containing no peptide. No binding was observed indicating the absence of any non-specific receptor-resin interaction. Thus, non-specific background absorption of the receptor to the resin matrix can be neglected. After the determination of K_a, the binding energy ΔG can be obtained according to equation (**2**):

$$\Delta G = -RT \ln Ka \qquad (\mathbf{2})$$

(Gas constant R = 8.314 J mol^{-1} K^{-1}, temperature T = 298 K)
All measurements were repeated multiple times to ascertain the accuracy of the binding affinities within an error of ±0.1 kcal mol^{-1}.

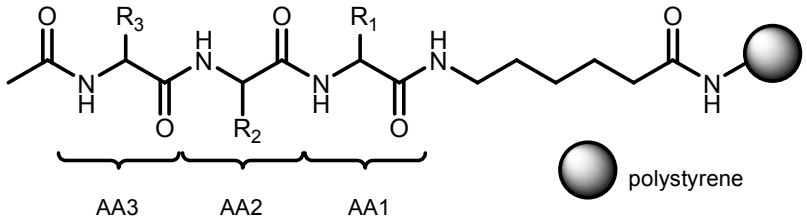
Using this protocol, the binding affinities of macrocyclic receptors towards the selected peptides are lower compared to the binding affinity with which receptor **1** binds to its selected peptide. Indeed, the association constants (K_a = 19±4 to 90±17 M^{-1}, ΔG = -1.7 to -2.7±0.1 $kcalmol^{-1}$) are ~50 times lower than the binding strength of receptor **1** (*Table 3*). This result is supported by a qualitative color comparison between the darkest beads of the macrocyclic receptor assays and those of the parent open-chain receptor **1**. None of the beads in the assays of the macrocycles was as intensely colored as the darkest beads in the assays of the open-chain receptor **1**. This indicates a somewhat weaker binding of the

macrocyclic receptors to their selected peptides, compared to the interaction between the open-chain diketopiperazine receptor **1**-peptide complexes.

The macrocyclization of diketopiperazine receptors, which rigidified the receptor and induced a higher preorganisation, leads to significant changes in the binding properties. In conclusion, the conformations of macrocyclic receptors are different compared to the open-chain diketopiperazine receptors and alter the binding selectivities and affinities of these receptors.

This demonstrates that mimicking the conformation in which the open-chain receptors bind their peptidic substrates through macrocyclization, is not trivial. It suggests that a certain degree of flexibility in the structure of the receptor is beneficial for allowing the receptor to wrap around their peptidic guests.

Table 3: *Relative binding affinities of macrocyclic receptors toward solid supported peptides: peptide-NH(CH$_2$)$_5$CONH-PS*



Receptors	Ac-AA3-AA2-AA1-linker-PS	K_a [M^{-1}]	ΔG [kcal mol^{-1}]
1	Ac-D-Val-D-Val-D-His-linker-PS Loading 0.33 mmol g^{-1}	2486±480	-4.6±0.1
17a / 17b	Ac-D-Lys-D-Pro-L-Lys-linker-PS Loading 0.34 mmol g^{-1}	59±11	-2.4±0.1
17a / 17b	Ac-L-Pro-L-Pro-D-Lys-linker-PS Loading 0.34 mmol g^{-1}	90±17	-2.7±0.1
17a / 17b	Ac-L-Ser-D-Pro-D-Pro-linker-PS Loading 0.36 mmol g^{-1}	63±12	-2.4±0.1
17a / 17b	Ac-D-Phe-L-Lys-D-Pro-linker-PS Loading 0.40 mmol g^{-1}	34±6	-2.1±0.1
17a / 17b	Ac-D-Ser-D-Lys-L-Lys-linker-PS Loading 0.41 mmol g^{-1}	38±7	-2.2±0.1
23	Ac-L-Ala-L-Gln-L-Ala-linker-PS Loading 0.34 mmol g^{-1}	51±10	-2.3±0.1
23	Ac-D-Gln-D-Ala-D-Leu-linker-PS Loading 0.33 mmol g^{-1}	19±4	-1.7±0.1
26	Ac-D-Pro-L-Pro-D-Gln-linker-PS Loading 0.33 mmol g^{-1}	25±5	-1.9±0.1

3 CARBAZOLE RECEPTORS: DEVELOPMENT OF A NOVEL CLASS OF HIGHLY SELECTIVE TWO-ARMED RECEPTORS FOR BINDING SMALL PEPTIDES

3.1 Carbazole Receptor Design

Combinatorial binding studies revealed that diketopiperazine receptors bind peptidic substrates with high selectivities and binding affinities of ΔG = -5 to -6 kcal mol^{-1} in organic and aqueous solvents[50,72] and that the tempate plays the most crucial role in allowing or preventing an intermolecular interaction of two-armed receptors toward peptides.[52] This result is supported by combinatorial binding studies with two-armed molecules based on diamines. Therefore, four different diamine templates were used: diaminocyclohexane **A**, bis-aminomethylcyclohexane **B**, diketodiazabicyclooctane **C** and 1,7-diaminoheptane **D** (*Figure 15*). The templates **A** - **D** differ not only in the distance of the two amino groups that serve as anchors for the peptidic arms but also in their rigidity. While the templates **A** and **C** are rigid, the direction of the amino groups is not fixed in **B**. The diaminoheptane **D** is completely flexible and was chosen because the distance between the two amino groups is the same as in the diketopiperazine template. Screening against the deprotected tripeptide library revealed that none of these two-armed molecules interact with any of the 24389 peptides within the tripeptide library (*see Chapter 2.5*). Thus, exchange of the template leads to significantly lower or entire loss of binding selectivity.[52]

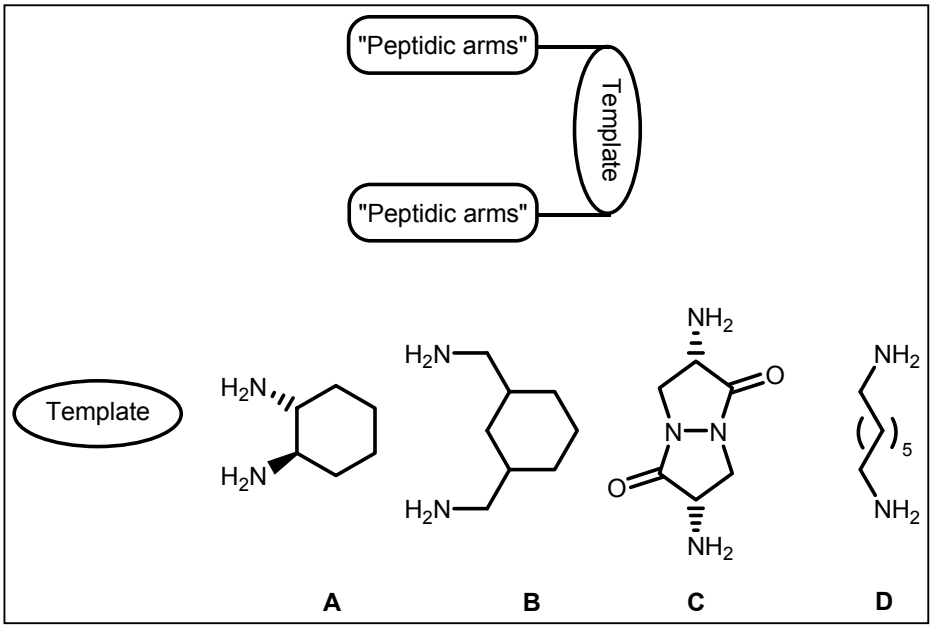


Figure 15: *Diamines used as templates for two-armed molecules*

Nevertheless, we were still interested in an alternative template since using the diketopiperazine template only allows for attachment of a dye moiety or linking to a solid suport *via* the side-chains of one of the amino acids. Attachment *via* a third functionalization site, that is opposite to the recognition elements, could be beneficial for reducing possible influence of the dye or resin onto the conformation and thereby binding properties of the receptor.

We felt that carbazole template could be such an alternative since the attachment sites for the receptor modules are separated by at least ~7.5 Å from each other by the rigid aromatic moiety and the third functionality is present in this template (*Figure 16*).

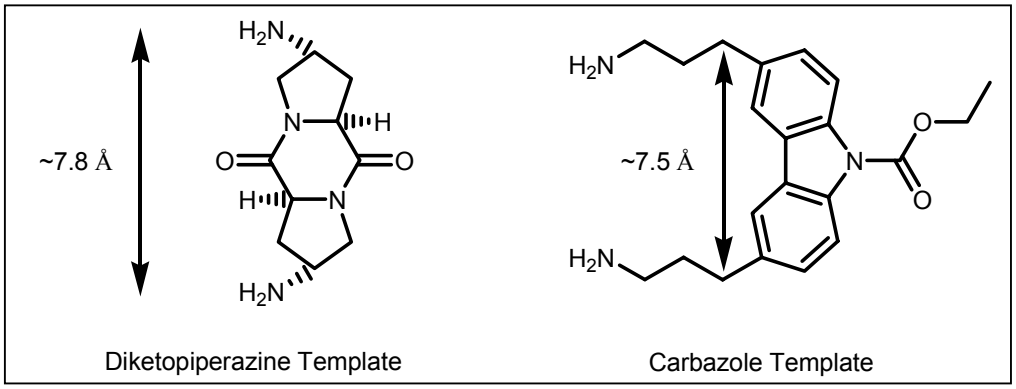


Figure 16: *Diketopiperazine and carbazole templates for two-armed molecules*

To test the binding properties of receptors based on a carbazole template, four dye-marked receptor prototypes **39** - **42** were synthesized, with side-chains which are identical to those of the parent diketopiperazine receptors **1** and **27** (*Figure 17*). These diketopiperazine receptors are known to bind tripeptides, D-Hph-D-Hph-D-His, D-Gln-D-Hph-D-Hph and L-Hph-L-Gln-D-Hph, in the case of the receptor **1** and D-Hph-D-Hph-D-His, in the case of the receptor **2**, with high selectivities and binding affinities.[50] The two symmetric tripeptidic arms contain L-Tyr(dye) or L-Tyr(tBu) at the first position of the recognition elements, L-Gln(Trt) or L-Asn(Trt) in the middle, and L-Phe at the end.

Receptor **39** is analogue to diketopiperazine receptor **1**, while receptor **40** has the dye no longer attached to the side-chains but to the additional functionalized site, the "tail" of the carbazole template. Since the solubility of these two receptors in organic solvent (e.g. $CHCl_3$) turned out to be rather low, we also prepared receptors **41** and **42** with solubilizing polyethylene glycol (PEG) moieties attached to the carbazole "tail".[56] Receptor **39**, **41** and **42** were designed to allow for a direct comparison of the binding properties of two-armed receptors based on the carbazole or diketopiperazine templates (receptor **1** and **27**).

Comparison of the binding properties of receptor **40** with those of receptors **39** or **41** should allow for an analysis of the influence of the dye moiety on the binding properties.

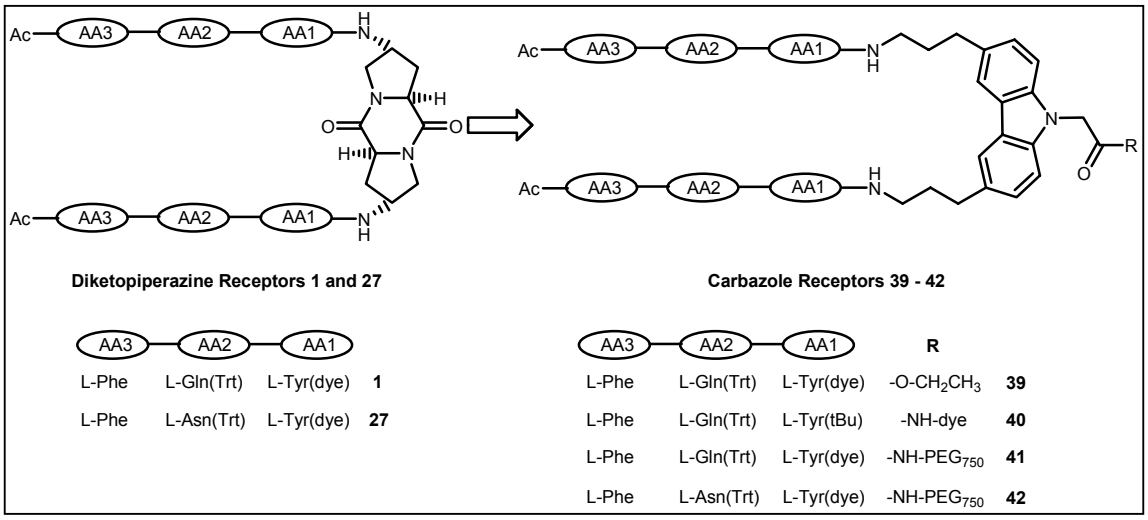
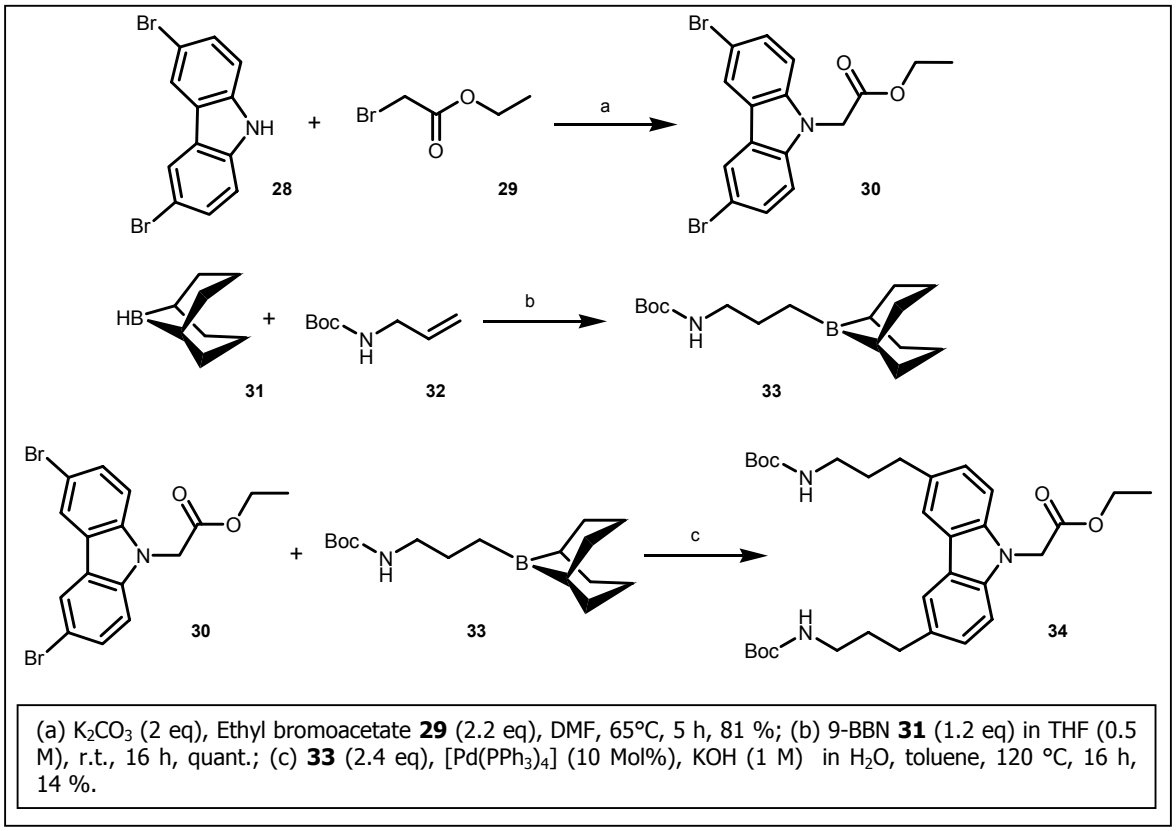


Figure 17: *Dye-Marked carbazole receptor prototypes (dye = Disperse Red 1)*

3.2 Synthesis of the Symmetric Carbazole Template

The synthesis of the carbazole template **34** (*Scheme 10*) started from the dibromocarbazole ethyl ester **30**, which was obtained by an S_N2 reaction of ethyl bromoacetate **29** with the commercially available 3,6-dibromocarbazole **28**. In another reaction, *N*-Boc-allylamin **32** was hydroborated with 9-borabicyclo[3.3.1]nonane (9-BBN) **31** and coupled via a Suzuki-Miyaura cross-coupling to dibromcarbazole ethyl ester **30** in the presence of tetrakis(triphenylphosphine)palladium ($Pd[PPh_3]_4$), leading to the *N*-Boc-protected carbazole **34** in 14 % yield.[55,73]



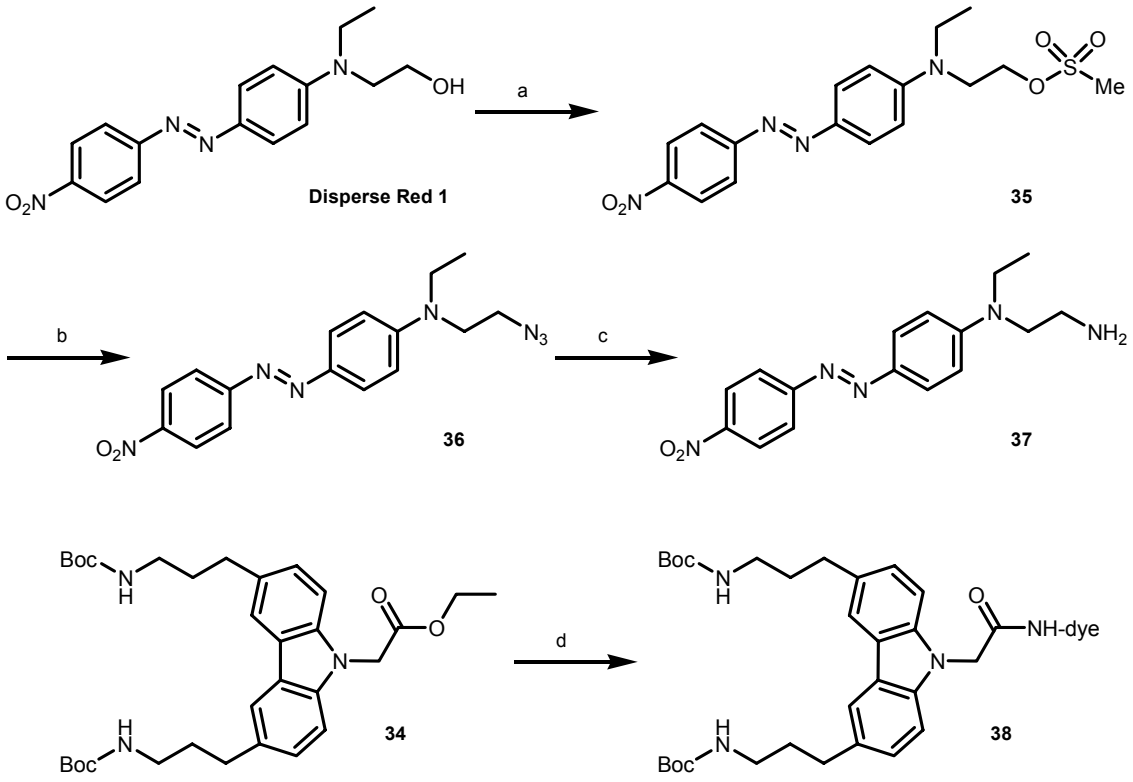
(a) K_2CO_3 (2 eq), Ethyl bromoacetate **29** (2.2 eq), DMF, 65°C, 5 h, 81 %; (b) 9-BBN **31** (1.2 eq) in THF (0.5 M), r.t., 16 h, quant.; (c) **33** (2.4 eq), $[Pd(PPh_3)_4]$ (10 Mol%), KOH (1 M) in H_2O, toluene, 120 °C, 16 h, 14 %.

Scheme 10: *Synthesis of the symmetric carbazole template*

3.3 Synthesis of Two-Armed Carbazole Receptor Prototypes

The two-armed carbazole receptor prototypes **39** – **42** were dye-labelled in two different ways: the first with the dye-marked L-tyrosine **13** coupled on free amines of the carbazole template, and the second with the amine-functionalized Disperse Red 1[74] introduced on carbazole "tail". The template was also functionalized with a PEG chain with an average molecular weight of 750 g mol^{-1} (H_2N-$(CH_2CH_2O)_{\approx 16}CH_3$).[56]

The synthesis of the amine-functionalized dye started from Disperse Red 1 which was activated as mesylate **35**, followed by the introduction of the azide-group by an S_N2 substitution with NaN_3 to obtain derivative **36** in 88 % yield (*Scheme 11*).[75] The reduction of the azide functionality under Staudinger conditions led to the amine-functionalized Disperse Red 1 **37** in 96 % yield.[76] After the hydrolytic saponification of **34**, the amine-functionalized dye was coupled using HCTU and Hünig's base to obtain the dye-marked template **38**.



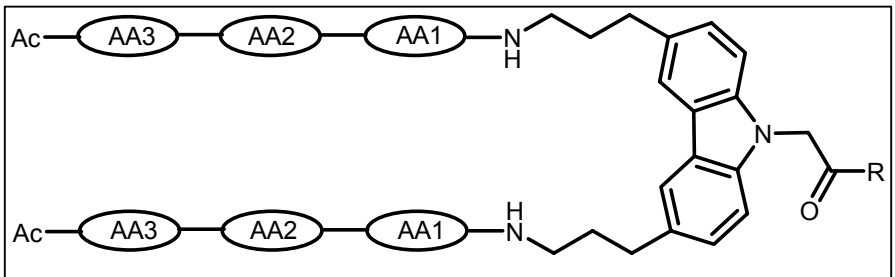
(a) MsCl (2 eq), Et_3N (2 eq), CH_2Cl_2, r.t., 1 h, quant.; (b) NaN_3 (2 eq), DMSO, 80 °C, 3 h, 88 %; (c) PPh_3 (1.6 eq), H_2O (2 eq), THF, r.t., 38 h, 96 %; (d) i. NaOH (1.4 eq) in H_2O, THF/MeOH (1:1), r.t., 4 h, quant.; ii. **37** (0.8 eq), HCTU (1.6 eq) in DMF, $^{i}Pr_2NEt$ (1.6 eq), CH_2Cl_2, r.t., 1 h, 67 %.

Scheme 11: *Synthesis of the amine-functionalized dye **37** and the dye-marked template **38***

After *N*-Boc deprotection of **34** and **38** respectively, the carbazole receptor prototypes were assembled by a similar synthetic route as described for the diketopiperazine receptors, using HCTU as coupling reagents and TAEA for Fmoc-deprotection. Acetylation with Ac_2O in the presence of Et_3N, after the final Fmoc-deprotection, led to the carbazole receptors **39** and **40** (*Figure 18*). In the case of the receptor containing a PEG chain, after assembling the peptidic arms on compound **34**, the ethyl ester functionality was converted to a PEG chain substituted amide *via* the same method as used for the amine-functionalized dye to obtain receptors **41** and **42**.

Using this synthesis route, four receptor prototypes **39** – **42** with closely related yet distinctly different structures were prepared. The two identical arms of each receptor consisted of a dye-marked L-Tyr or L-Tyr(tBu), as initial amino acid, followed by either L-Asn(*N*-trityl) or L-Gln(*N*-trityl) and as last amino acid, L-Phe. Thus, the structural differences

of the four prototypes were as subtle as a single methylene group (e.g. **41** and **42**), the position of the dye (e.g. **39** and **40**) or the presence of a PEG chain (e.g. **41** and **42**).



Carbazole Receptors 39 - 42

AA3	AA2	AA1	R	
L-Phe	L-Gln(Trt)	L-Tyr(dye)	-O-CH_2CH_3	**39**
L-Phe	L-Gln(Trt)	L-Tyr(tBu)	-NH-dye	**40**
L-Phe	L-Gln(Trt)	L-Tyr(dye)	-NH-PEG_{750}	**41**
L-Phe	L-Asn(Trt)	L-Tyr(dye)	-NH-PEG_{750}	**42**

Figure 18: *Dye-marked carbazole receptor prototypes **39** - **42***

3.4 Selective Binding of the Two-Armed Carbazole Receptors of Side-Chain Deprotected Peptides

The binding properties of the two-armed receptors **39** – **42** towards a resin-bound tripeptide library with the general structure Ac-AA3-AA2-AA1-NH$(CH_2)_5$CONH-PS (PS = polystyrene resin), were examined in chloroform: the same library as for the analysis of the binding properties of the dye-marked diketopiperazine receptors **1** and **27** was used (*Chapter 2.5*). Each combinatorial binding assay was carried out with an amount of the library that corresponded to at least five theoretical copies in order to ensure the representative screening results.[48]

Upon mixing dilute solutions (50 or 100 μM) of receptors **39** – **42** in $CHCl_3$ with the 24389 membered library, approximately one bead out of 3500 was colored, indicating a high level of selectivity comparable to what we previously observed for the diketopiperazine receptors. Isolation of the colored beads of the assays of receptors **39** – **42** followed by

analysis of the encoding tag-molecules revealed the amino acid sequences of the selected peptides (*Table 4*).

Table 4: *Binding specificities of diketopiperazine receptors **1**, **27** and carbazole receptors **39** – **42** for tripeptides within the side-chain deprotected library Ac-AA3-AA2-AA1-NH(CH$_2$)$_5$CONH-PS*

Receptors	**AA3**[a]	**AA2**[a]	**AA1**[a]	freq. found [%][b]	freq. exp. [%][b]	Beads isolated
1	D-Ala/ D-Val	D-Hph	D-His	34	0.04	
	L-Ala/ L-Leu	L-Gln	D-Hph	37	0.04	
	D-Gln	D-Hph	D-Val/ D-Leu	20	0.04	
27	D-Val/ D-Ala	D-Hph	D-His	100	0.04	
39	D-Gln	D-Phe	D-Val	60	0.004	5
	D-Gln	D-Hph	D-Hph	40	0.06	
40	L-Ala	L-Gln	D-Ala	40	0.004	
	D-Val	D-Ala/ D-Leu	D-His	20	0.008	15
	D-Gln	D-Hph	D-Hph	20	0.06	
41	D-Hph	D-Hph	D-His	36	0.06	
	D-Gln	D-Hph	D-Hph	34	0.06	42
	L-Hph	L-Gln	D-Hph	12	0.06	
42	D-Val/ D-Phe	D-Hph	D-His	100	0.04	5

[a] Hph = hydrophobic amino acid can be either Gly, Ala, Val, Leu or Phe.

[b] The frequency found column lists the percentage of beads selected in the receptor binding assay for the indicated peptide sequence. The frequency expected column lists the expected frequency for the particular tripeptide sequence if the beads were picked randomly. The comparison between the percentage of "frequency found" and "frequency expected" is a measure for the selectivity level of the receptor.

These screening results indicate that two-armed carbazole receptors bind certain resin-bound tripeptides with considerable selectivity and have similar binding selectivities as diketopiperazine receptors. Carbazole receptor **42** and diketopiperazine receptor **27** select exclusively peptides with D-His followed by two hydrophobic D-amino acids (D-Hph). Receptors **39** – **41**, with the Gln instead of Asn in the peptidic arms, have binding properties that are similar to those of diketopiperazine receptor **1**. Receptor **39** binds two of the three peptide motives that are also selected by receptor **1** preferentially. The motif, D-Hph-D-Hph-D-His, selected by receptor **1** is, however, not selected by the carbazole receptor **39**. Carbazole receptors **40** and **41** bind the same peptide sequences as diketopiperazine

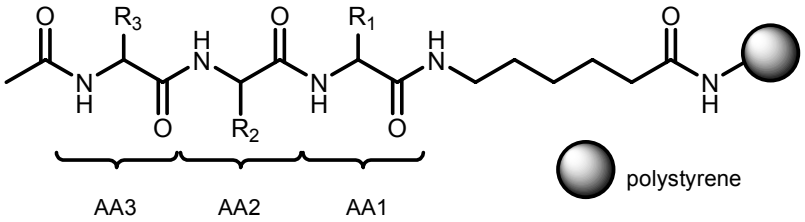
receptor **1**. This demonstrates that the carbazole template induces similar binding selectivites of two-armed receptors as diketopiperazine.

Binding properties of receptors **39** and **41** with the dye in the peptidic side-chains are similar to those of receptor **40** with the dye placed at the "tail" position of the carbazole template. This suggests that the dye is only functioning as an "innocent" spectator and is not playing a crucial role in the intermolecular interaction between two-armed receptors and their peptidic guests.

3.5 Binding Energies of the Two-Armed Carbazole Receptor Prototypes and Tripeptides in $CHCl_3$

In order to obtain a measure for the strength of the observed intermolecular association, we determined the binding affinities of carbazole receptors **39** – **42** towards the peptide, Ac-D-Val-D-Val-D-His-resin, which was selected in the one-bead assay (*Table 5*). The peptide was synthesized on polystyrene resin (200-400 mesh, loading 1.1 mmol g^{-1}) employing the general procedure for solid phase synthesis using the Fmoc-strategy. The final resin loading, after the peptide couplings, was determined by the quantitative Fmoc-test to be ~0.3 mmol g^{-1} (*see Chapter 2.6*).[71] The association constant was determined by solid phase binding of the receptors.

Table 5: *Binding affinities measured for diketopiperazine receptors **1**, **27** and carbazole receptors **39** – **42** towards the peptide selected in the on-bead assay and immobilized on PS-resin*



Receptors	Ac-AA3-AA2-AA1-linker-PS	K_a [M^{-1}]	ΔG [kcal mol^{-1}]
1	Ac-D-Val-D-Val-D-His-linker-PS Loading 0.33 mmol g^{-1}	2486±480	-4.6±0.1
27	Ac-D-Val-D-Val-D-His-linker-PS Loading 0.28 mmol g^{-1}	15000±2900	-5.7±0.1
39	Ac-D-Val-D-Val-D-His-linker-PS Loading 0.33 mmol g^{-1}	2760±530	-4.7±0.1
40	Ac-D-Val-D-Val-D-His-linker-PS Loading 0.33 mmol g^{-1}	2482±480	-4.6±0.1
41	Ac-D-Val-D-Val-D-His-linker-PS Loading 0.33 mmol g^{-1}	1852±360	-4.4±0.1
42	Ac-D-Val-D-Val-D-His-linker-PS Loading 0.33 mmol g^{-1}	1063±200	-4.1±0.1

Binding selectivities of diketopiperazine receptors and carbazole receptors were found to be comparable. Also, the binding affinities of diketopiperazine receptor **1** and carbazole receptors **39** – **41** are comparable. These results, furthermore, demonstrated that the position of the dye does affect neither the binding selectivity nor the binding affinity properties of the receptors and that the carbazole receptors **39** – **41** are not only highly selective for certain tripeptides, but also show a high binding affinity towards the selected peptide.

However, differences in the binding strength were observed for diketopiperazine receptor **27** and carbazole receptor **42**. Diketopiperazine receptor **27** was found to bind to Ac-D-Val-D-Val-D-His, that was selected preferentialy by both receptors in combinatorial on-bead assays, with a binding energy of ΔG = -5.7±0.1 kcal mol^{-1} (K_a = 15000 M^{-1}). In contrast, the corresponding carbazole receptor **42** binds this sequence less tightly with ΔG = -4.1±0.1 kcal mol^{-1} (K_a = 1063 M^{-1}).

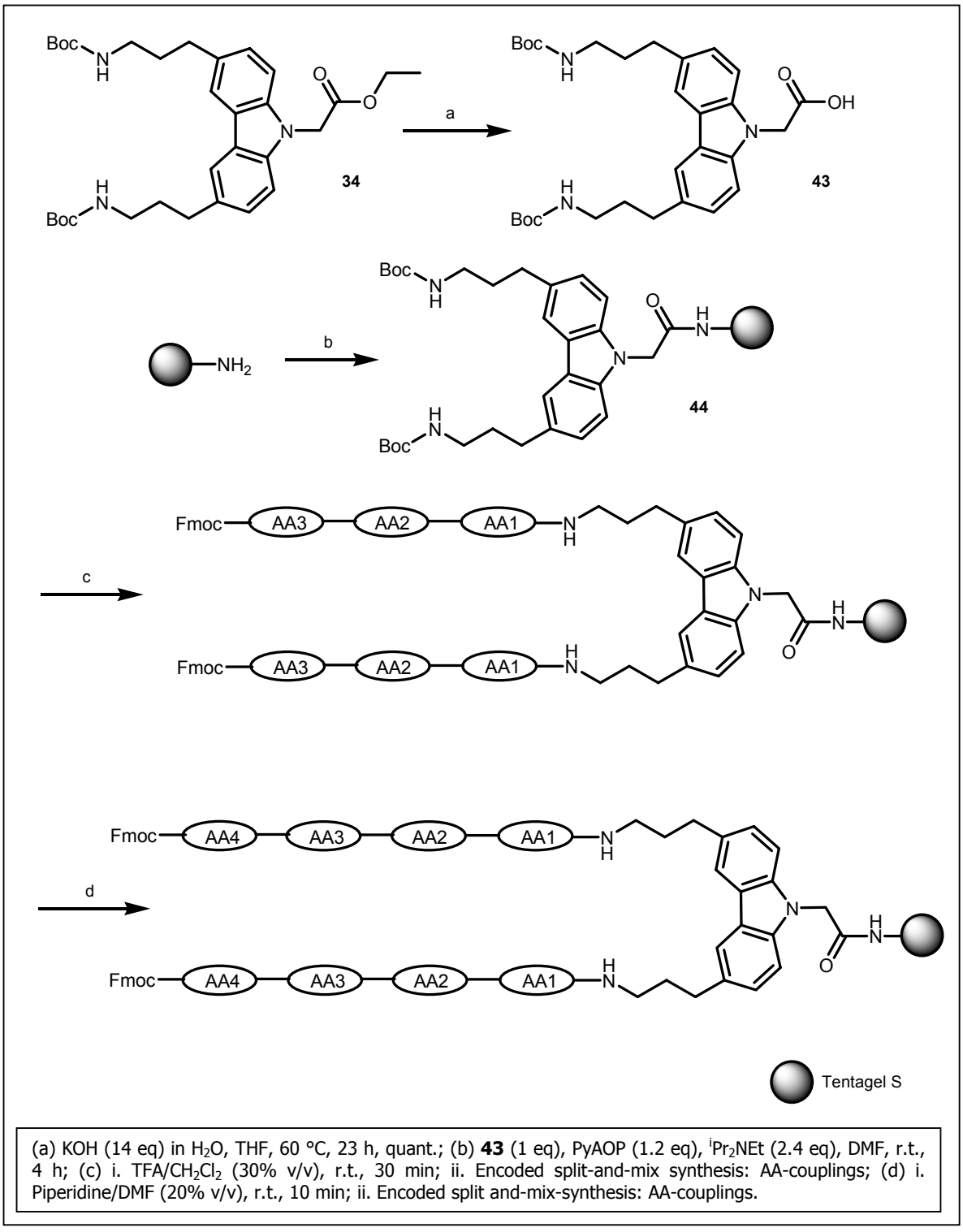
Nevertheless, the carbazole template is a choice alternative and these results justified the synthesis of a receptor library, since the carbazole receptor prototypes showed the desired properties described in chapter 2.1.

3.6 Two-Armed Carbazole Receptor Library

For the preparation of our two-armed receptor library, the symmetric carbazole scaffold **43** was chosen as initial target molecule, since it would provide an anchor point to the solid support.

After hydrolytic saponification of the carbazole template **34**, the *N*-Boc protected carbazole **43** was linked through an amide bond to the amino functionalized Tentagel S resin (loading 0.26 mmol g^{-1}) using (7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyAOP) and Hünig's base as coupling reagents (*Scheme 12*).[77] Cleavage of the Boc protecting group of **44** was performed by shaking the resin with a 30% v/v mixture of TFA/CH_2Cl_2 at r.t. for 30 minutes. After neutralization and washing steps, the resin was dried *in vacuo*. The loading of the resin was determined by quantitative Fmoc-test, by coupling Fmoc-Gly-OH on an accurate measured amount of resin. The determined loading of the resin was 0.43 mmol g^{-1}.[71]

The two symmetric 'arms' of the receptor library were built following the protocol for encoded split-and-mix synthesis using the standard Fmoc-procedure for peptide coupling (*Scheme 12*). Upon achieving synthesis of tripeptidic arms, one part of the resin was acetylated with Ac_2O in the presence of Et_3N, after the final Fmoc-deprotection, to yield the two-armed tripeptide carbazole library. The other part was coupled one-more time, using the same split-and-mix protocol, to lead to a two-armed tetrapeptide library, after final Fmoc-deprotection and acetylation. Using a part of each library, the side-chain protecting groups of peptides were cleaved by a 95:2.5:2.5 (v/v/v) mixture of TFA/H_2O/TIS at r.t. for 1 h to obtain the corresponding deprotected carbazole libraries.



(a) KOH (14 eq) in H_2O, THF, 60 °C, 23 h, quant.; (b) **43** (1 eq), PyAOP (1.2 eq), $^{i}Pr_2NEt$ (2.4 eq), DMF, r.t., 4 h; (c) i. TFA/CH_2Cl_2 (30% v/v), r.t., 30 min; ii. Encoded split-and-mix synthesis: AA-couplings; (d) i. Piperidine/DMF (20% v/v), r.t., 10 min; ii. Encoded split and-mix-synthesis: AA-couplings.

Scheme 12: *Synthesis of the tripeptide and tetrapeptide two-armed carbazole receptor libraries*

In the synthesis of the carbazole receptor libraries, 15 different D- and L- amino acids were employed in each position of the two peptidic arms. D- and L- amino acids were alternated in each position: e.g. for AA1, L-Ala was used, for AA2, D-Ala and for AA3, L-Ala (*Figure 19*). Thus, the libraries contained a maximum of 15^3 = 3375 different carbazole receptors with tripeptide arms and 15^4 = 50625 receptors with tetrapeptide arms.

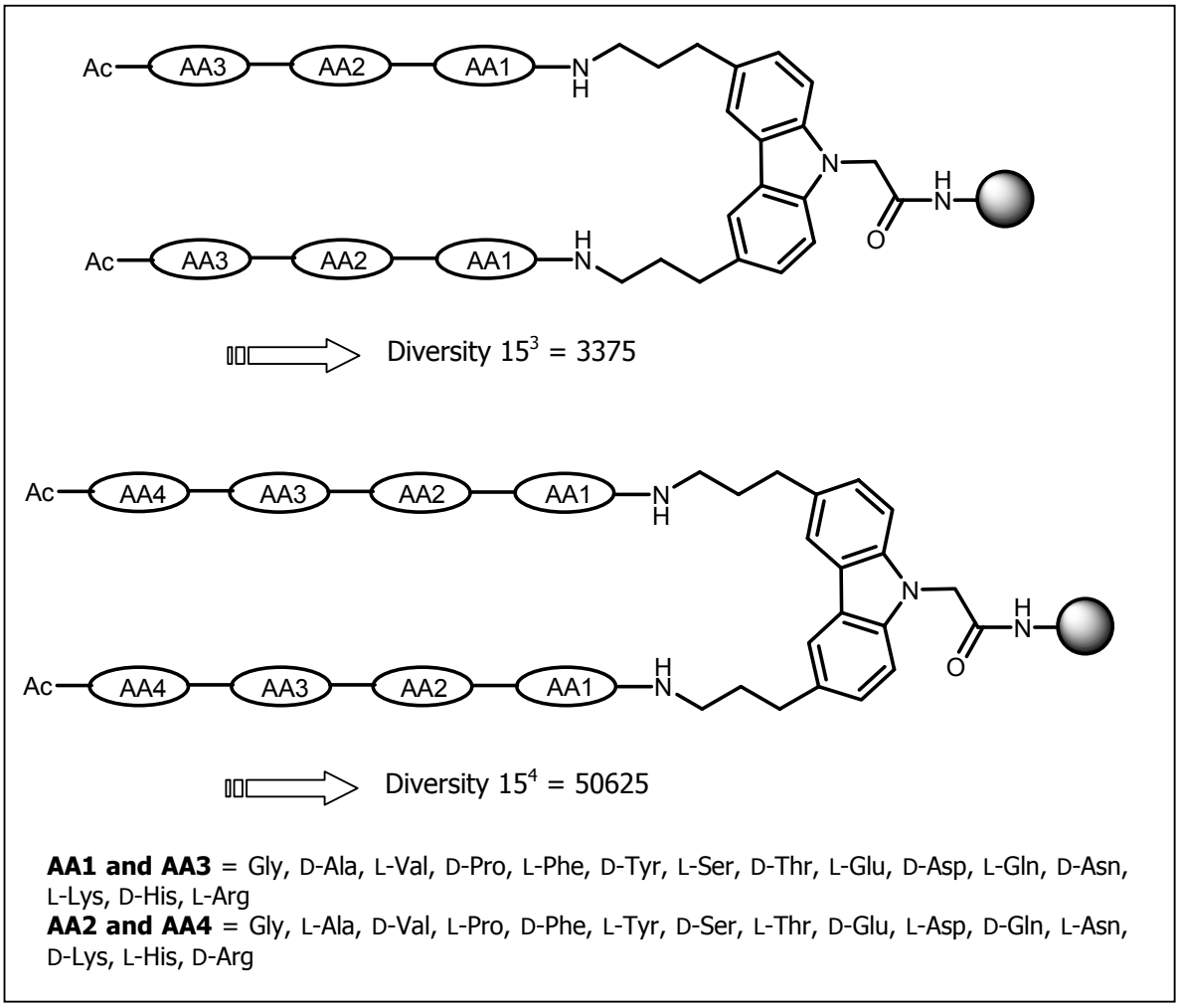


Figure 19: *Combinatorial variation of the amino acids used for the synthesis of the carbazole receptor libraries*

3.7 On-bead Screening of the Carbazole Receptor Library Against Biologically Important Peptides

3.7.1 Angiotensin II and Alzheimers' Peptide Derivatives Labelled with Fluorophores

In preliminary screenings of our carbazole receptor library, segments of Angiotensin II and Alzheimer's peptides were tested for binding to the receptors.

The goal of this screening is to find a selective receptor for the octapeptide Angiotensin II, which is responsible of an enhancement of the blood pressure and consequently leads to hypertension[78] and for a segment of β-amyloid peptide, $A\beta_{4-10}$, which is a targed for therapeutically active antibodies raised against the self-aggregation of $A\beta_{1-42}$.[79]

In order to perform screening assays with the deprotected tripeptide two-armed carbazole library, different peptides derived from Angiotensin II were labelled with a small fluorescent marker, the aminobenzoic group (Abz), a derivative of anthranilic acid. To improve the solubility in water and to reduce the influence of the fluorophore on the binding selectivity of peptides, the aminobenzoic group was attached at the *N*-terminal ends of the peptides *via* a tetraethylene glycol unit as "spacer". Using the Abz group, four different peptides were prepared on solid support using the standard Fmoc-strategy (Rink Amide AM resin or Wang resin): two peptides containing only the four *C*-terminal amino acids of Angiotensin II, **45** – **46**, and two peptides with all eight amino acids (full length Angiotensin II), **47** – **48**. One peptide in each sub-group was functionalized, at the *C*-terminus of the sequence, with a free carboxylic function (peptides **45** and **47**), the other with an amide group (peptides **46** and **48**). Our goal was to investigate the influence of these functional groups on potential binding selectivities and to discriminate between peptides of different lengths (*Figure 20*).[80]

To answer the question whether carbazole receptors are able to bind Alzheimer's peptides selectively, three fluorophore-labelled peptide derivatives **49** – **51** were screened against the side-chain deprotected acetylated carbazole library. To that effect, dansyl (DNS), carboxyfluorescein (FAM) and nitrobenzofurazan (NBD) groups were introduced in Alzheimer's peptide derivatives. Five glycine residues, between the epitope $A\beta_{4-10}$ and the fluorophores, served as "spacer" to reduce the influence of the fluorophore on the binding selectivity of the peptide. Przybylski *et al.* achieved the synthesis of the FAM-marked peptide **50** and the synthesis of peptides **49** and **51** was performed on solid support (Rink Amide AM resin) under standard Fmoc-strategy (*Figure 20*).[80]

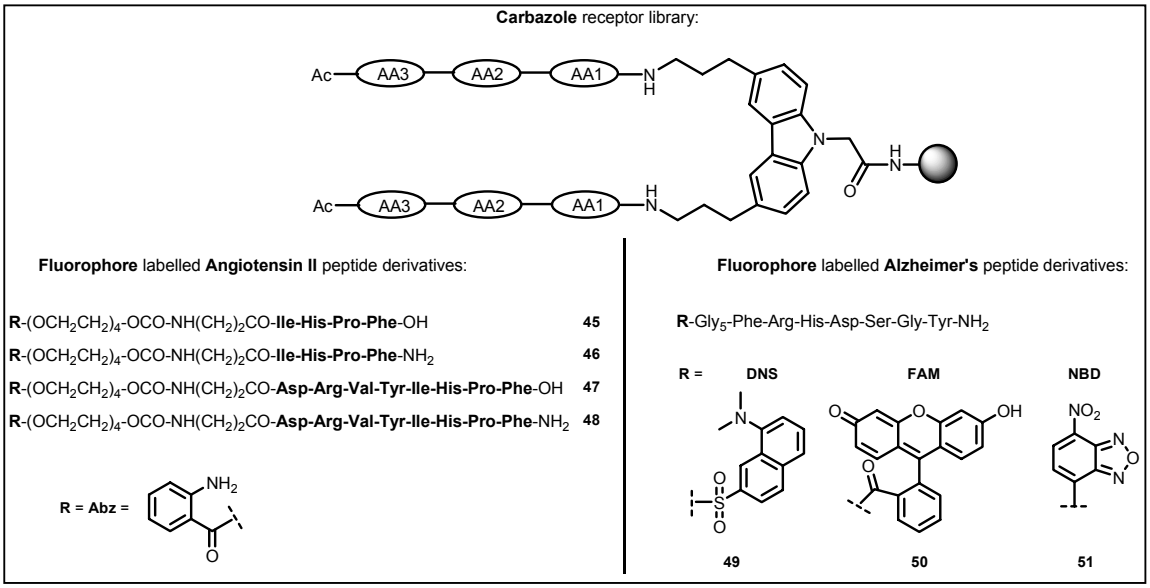


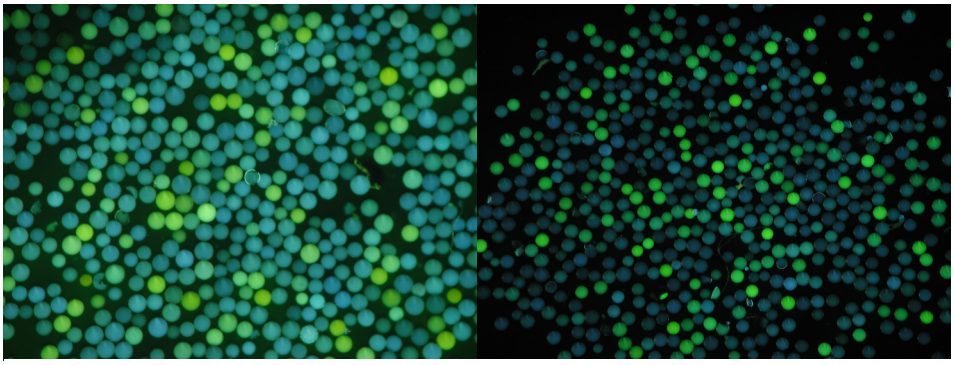
Figure 20: *Screening assays of carbazole receptor library against fluorophore labelled peptides*

3.7.2 Screening Assays of the Carbazole Receptor Library Towards Fluorophore-Labelled Peptides

A stock solution of each peptide (**45** – **48** and **49** – **51**) was prepared in a 10:90 (v/v) mixture of DMSO/H_2O, which allowed for solubilization of peptides, at a concentration of 1 mM. The fluorophore-marked peptides were screened against approx. 7 mg of the carbazole receptor library at a concentration of 100 µM in PBS buffer (10 mM, pH = 7.2) to approach physiological conditions. To obtain the desired concentration, the stock solutions of each peptide were added to this PBS buffer/carbazole receptor library mixture. The assays were allowed to shake at r.t. for 24 h. In these assays, with peptides **45** – **48**, **49** and **51** no fluorescent beads were observed. These peptides were also not recognized by any receptors in the carbazole library even after prolonged incubation (48 h), increasing the temperature (37 °C) or the concentration from 100 µM up to 200 µM, or using the two-armed tetrapeptide carbazole library. No higher concentrations have been used to run more screening assays with the different fluorophore-labelled peptides.

In the case of the FAM-marked peptide **50**, after equilibration for 24 h, several beads were found to be fluorescent. Since this result was not consistent with the other assays, some control experiments were performed using the fluorophores only (fluorescein and 5(6)-carboxyfluorescein) against the carbazole receptor, or a peptide library, under identical conditions as in the previous assays. In all assays, selective binding was observed, indicating

that the fluorophore moiety is responsible for the high interaction compared to the peptide (*Figure 21*).



Assay with **50** Assay with fluorescein

Figure 21: *Binding assays of FAM-marked peptide **50** and fluorescein with the carbazole receptor library*

These preliminary studies indicate that care has to be taken with the fluorophore as a marker in aqueous solution.[81] In order to avoid such undesired interactions, radiolabelling can be used instead. The use of ^{3}H or ^{14}C allows for marking the receptor without altering its structure. Interactions between radiolabelled receptor and 'on-bead' libraries can be detected by phosphoimaging[44,45] techniques or by microradiography.[46,47a,b] Nevertheless, the combinatorial synthesis of receptor libraries does provide a powerful method for identifying selective receptors for a chosen dye-labelled guest.

The screening assays were unfortunately not successful. However, other screening assays could be performed with these peptides in other buffers, for example 0.1 M Tris buffer (pH 7.2), or in organic solvents, like MeOH or $CHCl_3$. Nonetheless, in the future other biologically interesting targets could be screened. Other molecules can be envisaged, either with a peptide backbone (e.g. glycopeptides, PNA, RGD motif[82]) or different from peptides (e.g. oligonucleotides, cholesterol,[83] ATP,[84] c-di-GMP[85]). Some modifications on the carbazole library could be introduced to study molecular recognition, for example, the introduction of boronic acids that provide effective sugar binding sites[86] or a hydrophobic part like a phenylazobenzoyl derivative or macrocyclization of the receptor.

4 VESICLE FORMATION IN AQUEOUS SOLUTION DRIVEN BY SELECTIVE NON-COVALENT INTERACTIONS

In recent years synthetic vesicles have been developed for application, for example, in drug delivery or gene therapy.[87] The vesicular structure, generated by biological membranes for transport, consists of a spherically closed lipid bilayer, which compartmentalizes an inner aqueous pool from the physiological medium. Many approaches for the self-assembly of mechanically stable nanocontainers used amphiphilic block copolymers consisting of a hydrophilic (e.g. polyethylene glycol (PEG) or polymethacrylic acid (PMA)) and a hydrophobic (e.g. polystyrene (PS), polybutadiene) polymer block.[88] The formation of vesicles can be viewed as a two-step self-assembly process, in which the amphiphile first forms a bilayer, which then, in a second step, closes to form the vesicle. In the classical description, the factor determining the shape of self-assembled amphiphile structures is the size of the hydrophobic part relative to the hydrophilic part. It determines the curvature of the hydrophobic-hydrophilic interface.[89]

Recently, also hydrophobic and hydrophilic polypeptides have been utilized as amphiphilic block copolymers. Given the pH sensitivity of some amino acids, the resulting vesicles are responsive to their environment.[90] Another elegant approach used the formation of metal-bisterpyridine complexes for the formation of vesicles. Hydrophobic and hydrophilic polymers were functionalized with terpyridine ligands at their termini and assembled into vesicles upon addition of metal ions.[91]

Here, selective non-covalent interactions between a diketopiperazine receptor and its pegylated tripeptidic guest were used to induce the assembly of vesicles in aqueous solution. The vesicles were analyzed by a combination of light scattering, electron transmission and atomic force microscopy as well as surface pressure measurements.

4.1 Self-Assembly Process between Diketopiperazine Receptor and Pegylated Peptides in Aqueous Solution

In order to obtain a measure of the strength of the observed intermolecular interactions, we envisaged to determine the binding affinities of diketopiperazine receptor **1** towards the peptide, Ac-D-Val-D-Val-D-His-resin, selected in the on-bead assays, by solution phase binding assays, e.g. NMR spectroscopy. However, these studies of the binding mode of this receptor-peptide complex were thwarted by the poor solubility of the non-solid-supported peptide Ac-D-Val-D-Val-D-His-NH_2 in $CHCl_3$.

Initially, we attempted to increase the solubility of the peptide by conjugation with hydrophobic and/or sterically demanding residues like octylamine, 3,3-diphenylpropylamine or 3,4-dibenzyloxyphenethylamine, designed to hinder intermolecular aggregation of the peptides. However, none of these residues rendered the modified peptides significantly more soluble in chloroform. Hence, we started to explore the possibility of conjugation with PEG chains of different lengths as solubilizing moieties.

First, the peptide-PEG conjugate **2** was synthesized by conjugation in solution phase, by coupling of the peptide with a carboxylic acid at the *C*-termini with a PEG bearing a terminal amino group. For this purpose, the peptide, Ac-D-Val-D-Val-D-His(Trt)-OH, with a carboxylic acid at the *C*-termini and acid labile protecting groups on the side-chain functional groups, were prepared on a hyper acid labile solid support (2-chlorotrityl chloride resin), using the standard Fmoc-strategy. After cleavage of the peptide from the resin under mildly acid conditions (CF_3CH_2OH/AcOH/CH_2Cl_2 1:1:8, v/v/v), the side-chain protected peptide was coupled with an amino-functionalized polyethylene glycol (H_2N-$(CH_2CH_2O)_{\approx 16}CH_3$ = H_2N-PEG_{750}). However, use of the coupling reagents EDC/HOBt, HATU/Hünig's base, *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU)/Hünig's base or an activated pentafluorophenyl ester led to considerable epimerization at the chiral center of the *C*-terminal amino acid histidine. Only the use of 3-(diethyoxy-phosphoryloxy)-3*H*-benzol[*d*][1,2,3] triazin-4-one (DEPBT) and Hünig's base as coupling reagents proceeded without detectable epimerization for the coupling of peptide, Ac-D-Val-D-Val-D-His(Trt)-OH, based on analysis by ^{1}H-NMR spectroscopy.[56] After the coupling and the side-chain deprotection, the pegylated peptide **2** was soluble in $CHCl_3$ at a concentration of at least 100 mM.

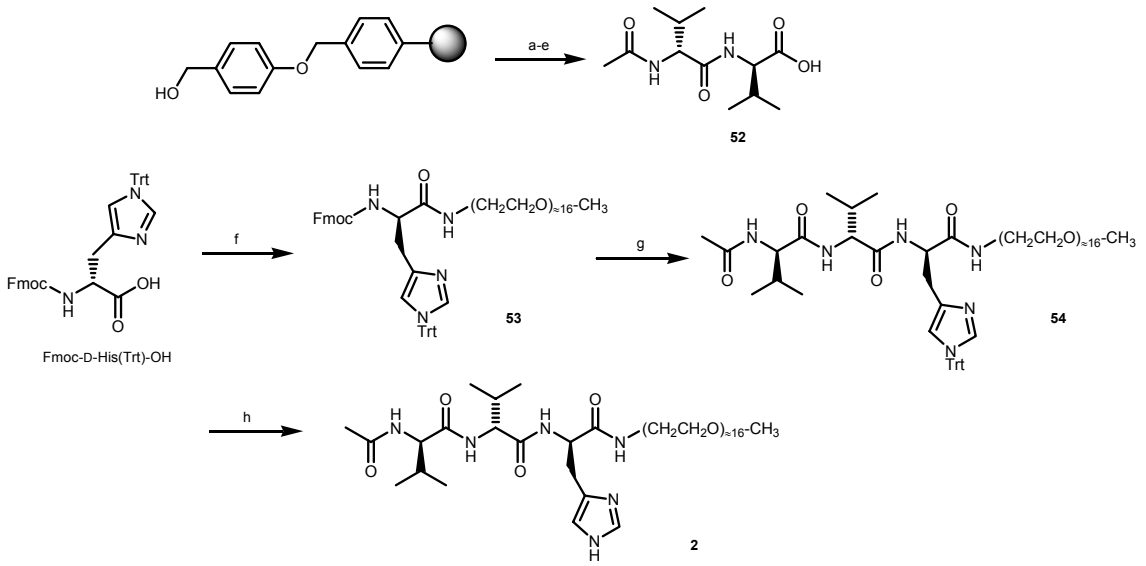
However, when we mixed peptide **2** with receptor **1** in $CDCl_3$ in an NMR tube to study the binding mode, the formation of a gel was observed, indicating the assembly of supramolecular aggregates. Since a 3D network structure occurring *via* non-covalent

interactions may explain the gel formation in chloroform, we also investigated a possible self-assembly in aqueous solution.

For this purpose, the pegylated peptides Ac-D-Val-D-Val-D-His-NH-PEG_{750} **2** was re-synthesized using a different strategy allowing to reduce the risk of epimerization and bypassing the matter of low yields obtained in the first coupling between the Fmoc-D-His-OH and the 2-chlorotrityl chloride resin otherwise. Ac-D-Val-D-Val-D-His-NH-PEG_{3200} **55**, which were selected by the diketopiperazine receptor **1**, and Ac-Lys-Lys-Lys-PEG_{850} **56** or Ac-Val-Val-Gly-PEG_{850} **57**, that did not bind to receptor **1**, were also prepared. Two strategies were pursued to obtain the pegylated peptides: (a) conjugation in solution phase for the pegylated peptide **2** and (b) synthesis of the peptide-PEG conjugates **55** - **57** on solid phase.

4.1.1 Solution Phase Synthesis of the Pegylated Peptide 2

The first strategy started with the synthesis of Ac-D-Val-D-Val-OH **52** on solid support (Wang resin) under standard Fmoc-strategy, using DIC/HOBt as coupling reagents (*Scheme 13*). After the final Fmoc-deprotection and acetylation with Ac_2O in the presence of Et_3N, the peptide was removed from the solid support using a 95:2.5:2.5 (v/v/v) mixture of TFA/H_2O/TIS at r.t. for 2 h, leading to the peptide **52** with a carboxylic acid at their *C*-termini. In a second part, the Fmoc-protected D-His(Trt) was coupled on a polyethylene glycol bearing a terminal amino group (H_2N-$(CH_2CH_2O)_{\approx 16}CH_3$ = H_2N-PEG_{750}) using DEPBT and Hünig's base as coupling reagents at r.t. for 3 h, yielding the Fmoc-protected D-His(Trt)-PEG conjugate **53**. After purification by flash chromatography and Fmoc-deprotection of **53**, the amine-functionalized D-His(Trt)-PEG conjugate was reacted with peptide **52** using DEPBT and Hünig's base at r.t. for 3 h, leading to the protected pegylated peptide **54**, which could be purified by flash chromatography on silicagel (gradient of CH_2Cl_2/MeOH/Et_3N from 99.5:0.5:0.1 to 96:4:0.1 (v/v/v)). The pegylated peptide **2** was isolated by precipitation with diethyl ether after side-chain deprotection, followed by an ion exchange chromatography (Dowex® 1x2-400, Dowex-1-chloride, strongly basic anion).



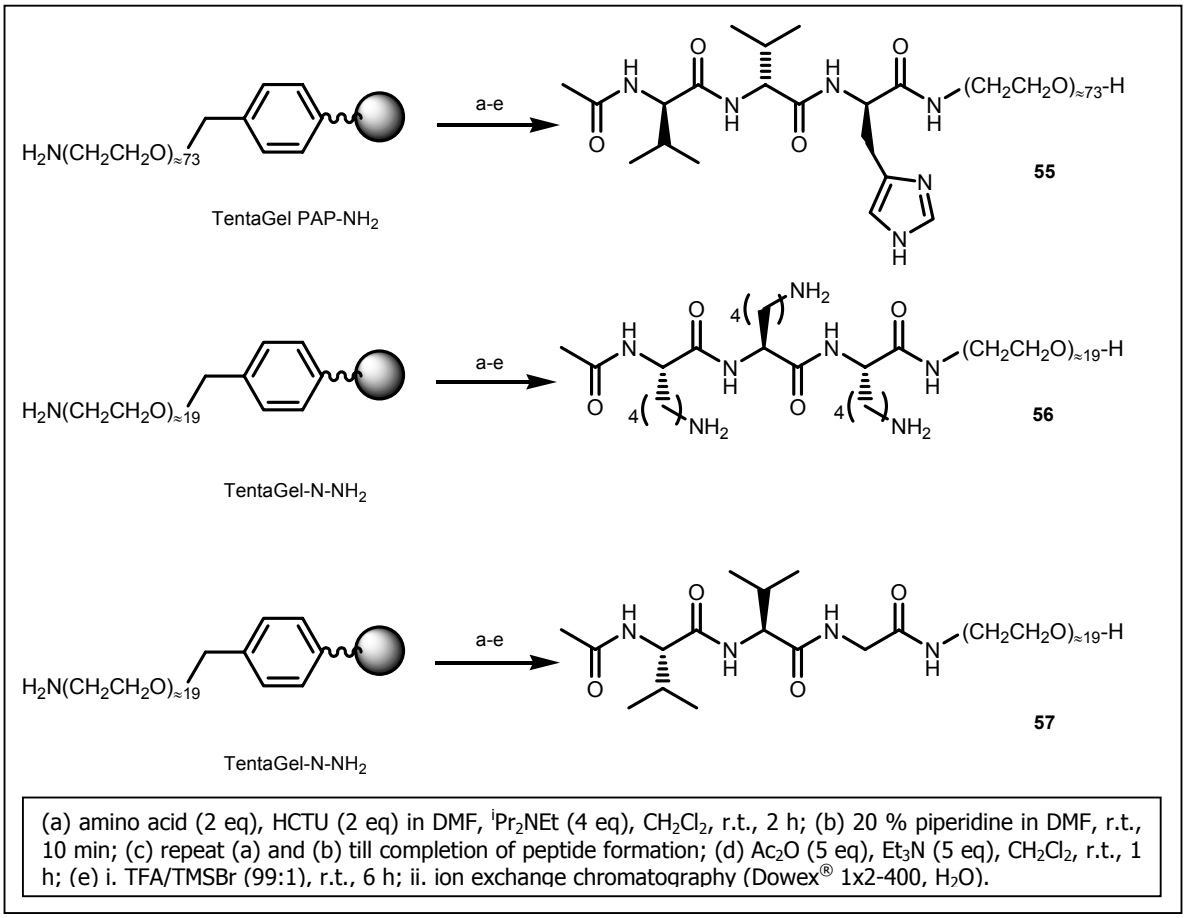
(a) Fmoc-D-Val-OH (3 eq), HOBt (3 eq) in DMF, DIC (3 eq), cat. DMAP, CH_2Cl_2, r.t., 2 h; (b) 20 % piperidine in DMF, r.t., 10 min; (c) repeat (a) and (b) without DMAP; (d) Ac_2O (5 eq), Et_3N (5 eq), CH_2Cl_2, r.t., 1 h; (e) TFA/H_2O/TIS (95:2.5:2.5), r.t., 2 h, 80%; (f) H_2N-$(CH_2CH_2O)_{\approx 16}CH_3$ (0.8 eq), DEPBT (1.2 eq), $^iPr_2NEt$ (1.6 eq), THF/CH_2Cl_2, r.t., 3 h, 95%; (g) i. diethylamine (30 eq), acetonitrile, r.t., 30 min; ii. **52** (1.2 eq), DEPBT (1.5 eq), $^iPr_2NEt$ (2 eq), THF/CH_2Cl_2, r.t., 3 h, 88%; (h) i. TFA/CH_2Cl_2 (1:5), r.t., 90 min; ii. ion exchange chromatography (Dowex® 1x2-400, H_2O), 71%.

Scheme 13: *Synthesis of the peptide-PEG conjugate **2** in solution phase*

4.1.2 Solide Phase Synthesis of the Peptide-PEG Conjugates 55 - 57

The second strategy involved the synthesis of the peptides on a polystyrene (PS) based solid support where an amino-functionalized PEG chain is attached via an acid labile benzylether linker (TentaGel® PAP-NH_2: $<M_w>$ = 3200 g mol^{-1} or TentaGel-N-NH_2: $<M_w>$ = 850 g mol^{-1}) (*Scheme 14*).[92] Peptide **55** was prepared on solid support bearing PEG_{3200} and **56** – **57**, bearing PEG_{850}. The syntheses were performed by following the standard Fmoc-strategy using HCTU/Hünig's base as coupling reagents. Side-chain deprotection and simultaneous peptide-PEG conjugate cleavage from the solid support were achieved with a 99:1 (v/v) mixture of TFA/trimethylsilylbromide (TMSBr) at r.t. for 6 h. The pegylated peptides **55** – **57** were isolated by precipitation with diethyl ether, followed by an ion exchange chromatography. As judged by NMR-spectroscopy, HPLC, and MS analysis the purity of peptide-PEG conjugates **55** – **57** was ≥ 90%. This second approach yields peptide-PEG conjugates where the PEG chain terminates with a hydroxy group, in comparison to the

methylether obtained by the conjugation of the peptides with PEG in solution phase.[56] The risk of epimerization is significantly reduced since only single amino acids are coupled, hence avoiding the risk of oxazolidinone formation.



Scheme 14: *Synthesis of the peptide-PEG conjugates **55** – **57** on solid support*

4.1.3 Receptor-Ligand Self-Assembly

Upon mixing of **2** or **55** with diketopiperazine receptor **1** in chloroform, the formation of an organogel was observed, as was expected (*Figure 22*). Gel formation was not observed with the peptide-PEG conjugates **2** and **55** themselves, or when receptor **1** was mixed with non-functionalized PEG or non-selected peptide-PEG conjugates **56** and **57**. Thus, the supramolecular assembly into an organogel is driven by selective non-covalent interactions between receptor **1** and Ac-D-Val-D-Val-D-His.

Next, the supramolecular assembly properties between receptor **1** and the peptide-PEG conjugates **2** and **55** were studied in water. Whereas peptide-PEG conjugates **2** and **55** were fully soluble in water, receptor **1** was not. To allow for a supramolecular assembly to form between receptor **1** and pegylated peptide **2**, **1** was dissolved in THF (0.4 mM) and **2** (2 mM, pH 5.5) in water. In the case of the assembly between **1** and **55**, the receptor was diluted in THF at 4.4 mM and the peptide-PEG conjugate in water at 57 µM (pH 5.5). For the studies, we prepared different peptide/receptor ratios using an accurate amount of both the stock solutions of receptor **1** and the respective peptides. If necessary, the solution was diluted with water to reach a final THF content of maximally 9% and a receptor/ligand complex concentration of maximally 1 mg ml^{-1}.

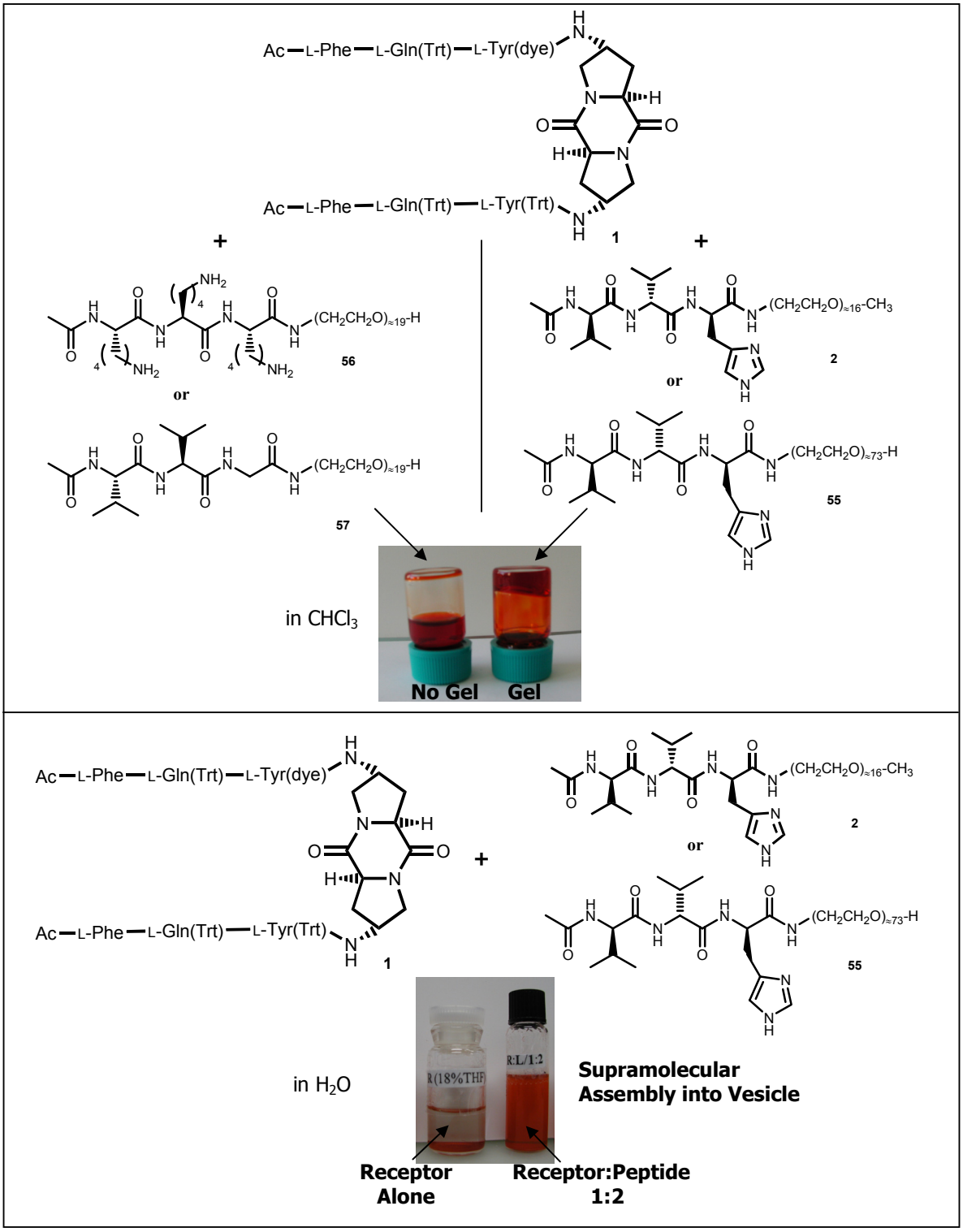


Figure 22: *Diketopiperazine receptor **1** mixed with pegylated peptides **2** and **55** – **57** in $CHCl_3$ and with **2** and **55** in H_2O*

4.2 Dynamic Light Scattering (DLS) Investigations

Dynamic Light Scattering (DLS),[93] also known as Quasi Elastic Light scattering (QELS) and Photon Correlation Spectroscopy (PCS), has the potential to yield a great deal of information about particles, including their size, over the size range from a few nanometers to a few microns, and some notion of concentration. Such information is useful in many scientific fields, and the techniques of DLS can provide such information relatively quickly and inexpensively, compared with other methods. The most direct applications for the method are for those who are interested in diffuse properties of a given solution, such as bacteria or proteins in suspension. The concept uses the idea that small particles in a suspension move in a random pattern.

According to the semi-classical light scattering theory when light (laser) impinges on matter, the electric field of the light induces an oscillating polarization of electrons in the molecules. The molecules then serve as secondary source of light and subsequently radiate (scatter) light. The frequency shifts, the angular distribution, the polarization and the intensity of the scattered light are influenced by the size, shape and molecular interactions in the scattering material. In dynamic light scattering, one measures the time dependence of the light scattered from a very small region of a solution, over a time range from tenths of a microsecond to milliseconds. These fluctuations in the intensity of the scattered light are related to the rate of diffusion of molecules in and out of the region being studied and the data can be analyzed, to directly give the diffusion coefficients of the particles doing the scattering. Traditionally, rather than presenting the data in terms of diffusion coefficients, the data are processed to give the size of the particles (radius or diameter). The relation between diffusion and particle size is based on theoretical relationships for the Brownian motion of spherical particles, which describes the way in which very small particles move in fluid suspension, where the fluid consists of molecules much smaller than the suspended particles. The hydrodynamic diameter or Stockes radius, R_h, derived from this method is the effective radius of an irregularly shaped particle that is used when describing the manner in which particles in suspension diffuse through the suspending medium. Assuming that our vesicles behave as hard spheres, the diffusion coefficient D could be related to the hydrodynamic radius R_h using the Stockes-Einstein equation:

$$\boxed{R_h = \frac{kT}{6\pi\eta D}}$$

where k is the Boltzmann constant (k = 1.3806x10^{-23} J K^{-1}), T is the temperature and η is the solvent viscosity.

For a hard sphere, the hydrodynamic radius R_h is equivalent to the gyration radius R_s (radius of the sphere). However, for other particles, we can consider R_h as the radius of a hypothetical hard sphere that diffuses with the same speed as the particle under examination. A faster one represents a smaller particle and a slower one represents a larger particle.[93]

To gain insight into our supramolecular assembly process between the diketopiperazine receptor **1** and the pegylated peptides **2** and **55**, the choice of this method was relevant.

Dynamic light scattering revealed the formation of well defined nanometer-sized structures demonstrating that receptor **1** forms supramolecular assemblies with the peptide-PEG conjugates **2** and **55** also in aqueous environment. The measurements showed that the peptide-PEG conjugate **2** with a molecular weight of PEG of ~750 g mol^{-1} yielded particles with a mean apparent hydrodynamic radius of 50 nm whereas peptide-PEG conjugate **55** with a molecular weight of PEG of ~3200 g mol^{-1} organized itself into structures with a mean apparent hydrodynamic radius of 100 nm. In both cases, the particle size does not depend on the ligand (peptide) to receptor ratio (*Figure 23*). No supramolecular assemblies were observed by the peptide-PEG conjugates **2** and **55** themselves or when receptor **1** was mixed with the peptide-PEG conjugates Ac-Lys-Lys-Lys-PEG_{850} **56** and Ac-Val-Val-Gly-PEG_{850} **57**. Thus, the assembly between receptor **1** and peptide-PEG conjugates **2** and **55** is driven by selective non-covalent interactions. The assembly might, furthermore, be strengthened by the chemical incompatibility of the hydrophobic receptor and the hydrophilic PEG segment, however, the main driving force is the selective interaction between receptor **1** and Ac-D-Val-D-Val-D-His.

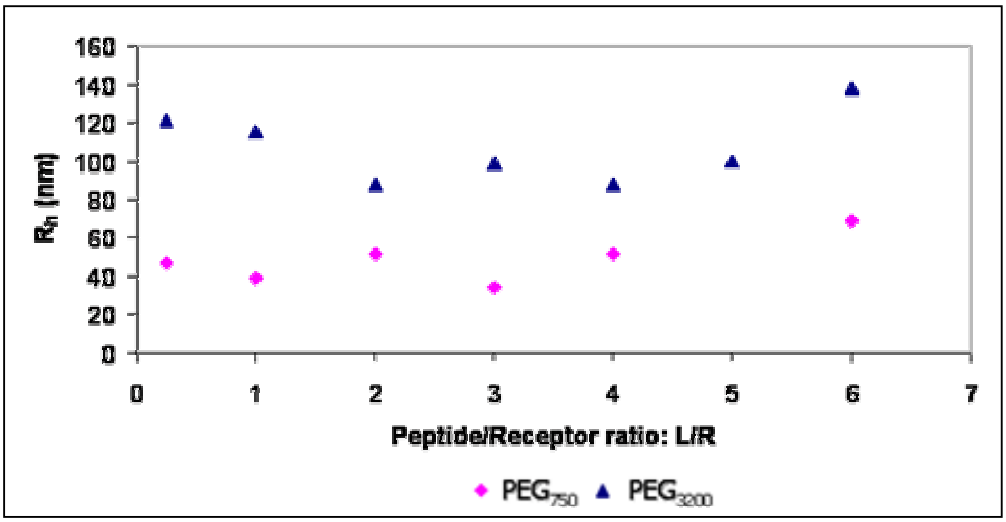


Figure 23: *The hydrodynamic radii of supramolecular assemblies between receptor **1** and peptide-PEG conjugates **2** and **55**, versus peptide/receptor ratios*

4.3 Transmission Electron (TEM) and Atomic Force (AFM) Microscopy

To further analyze the properties of the supramolecular assemblies between receptor **1** and peptide-PEG conjugates **2** and **55**, Transmission Electron Microscopy (TEM)[94] and Atomic Force Microscopy (AFM)[95] were used.

TEM is especially useful in studying the structure of cells and in crystallography. It operates on the same basic principles as the light microscope but uses electrons instead of light. What we can see with a light microscope is limited by the wavelength of light. TEMs use electrons as "light source" and their much lower wavelength make it possible to get a resolution a thousand times better than with a light microscope. In TEM, a "light source" at the top of the microscope emits the electrons that travel through vacuum in the column of the microscope (*Figure 24*). Instead of glass lenses focusing the light in the light microscope, the TEM uses electromagnetic lenses to focus electrons into a very thin beam. The electron beam then travels through the studied specimens. Depending on the density of the material present, some of the electrons are scattered and disappear from the beam. At the bottom of the microscope, the unscattered electrons hit a fluorescent screen, which gives rise to a "shadow image" of the specimen with its different parts displayed in varied darkness according to their density. The image can be studied directly by the operator or photographed with a camera.[94]

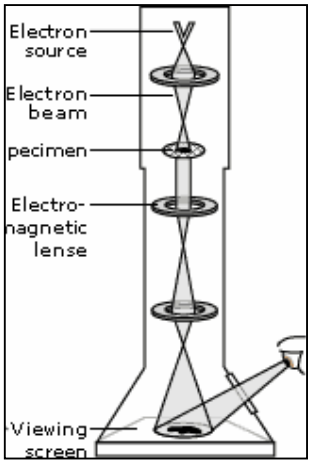


Figure 24: *Schematic presentation of Transmission Electron Microscopy*

TEM analysis of our supramolecular assemblies supported the results from the dynamic light scattering studies, showing spherical objects with a size of 100 nm and 200 nm for receptor **1** with peptide-PEG conjugates **2** and **55**, respectively (*Figure 25*). Their macroscopic shape proved to be independent of the ligand to receptor ratio and the molecular weight of the polymer.

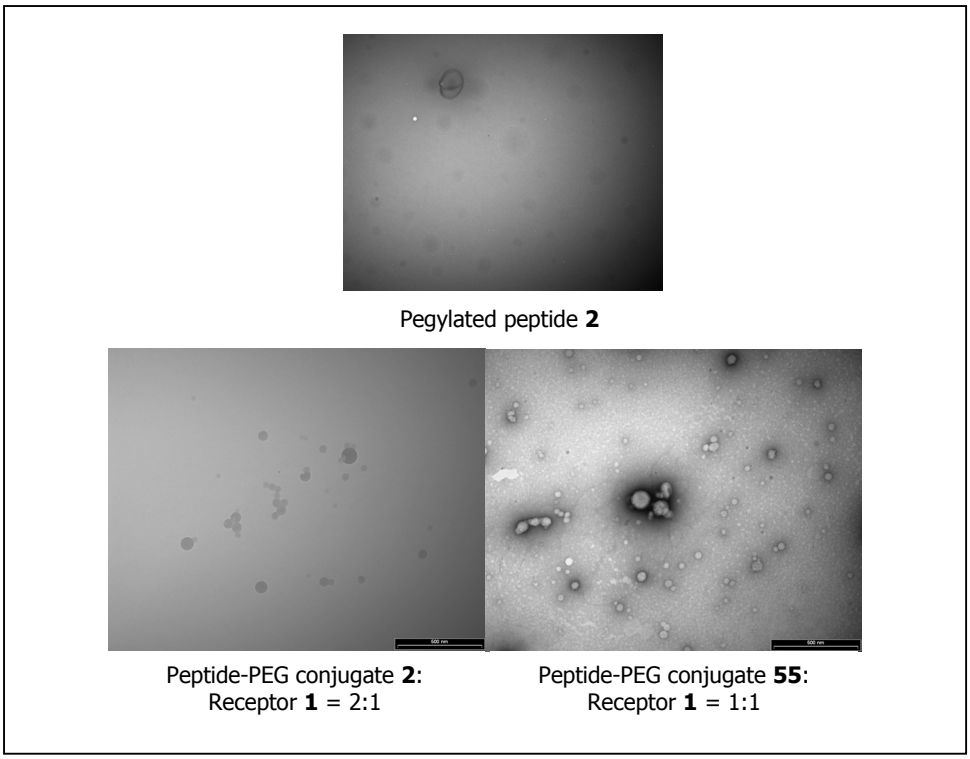


Figure 25: *TEM image of self-assembly between receptor **1** and peptides **2** and **55** in dilute aqueous solution*

To determine the morphology (vesicles or micelles) of the ligand-receptor driven organization of PEG, we studied the assemblies by AFM.

The Atomic Force Microscopy (AFM) which was invented in 1986 by Binnig, Quate and Gerber, is being used to solve processing and materials problems in a wide range of technologies including electronics, telecommunications, biological, chemical, automotive, aerospace, and energy industries. The materials being investigated include thin and thick film coatings, ceramics, composites, glasses, synthetic and biological membranes, metals, polymers, and semiconductors. The basic objective of the operation of the AFM is to measure the forces between a sharp probing tip and a sample surface. AFM works by scanning a fine ceramic or semiconductor (Si or Si_3N_4) over a surface much the same as a phonograph needle scans a record. The tip is positioned at the end of a cantilever beam shaped much like a diving board. As the tip is repelled by or attracted to the surface, the cantilever beam deflects. The magnitude of the deflection is captured by a laser that reflects at an oblique angle from the very end of the cantilever. A plot of the laser deflection versus tip position on the sample surface provides the resolution of the hills and valleys that constitute the topography of the surface (*Figure 26*). The AFM can work with the tip

touching (contact mode), or the tip can tap across the surface (tapping mode) much like the cane of a blind person. Compared with TEM, three dimensional AFM images are obtained without expensive sample preparation and yield far more complete information than the two dimensional profiles available from cross-sectioned samples.[95]

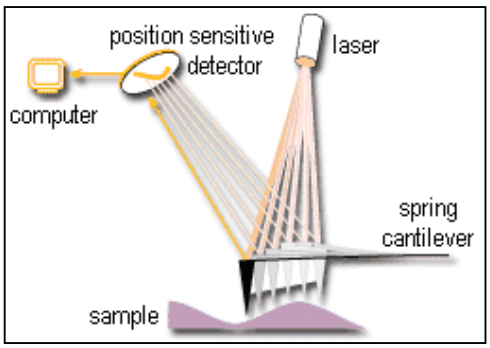


Figure 26: *Concept of Atomic Force Microscopy (AFM)*

Figure 27 shows a typical image of AFM with two spherical particles in close contact to each other. The hollow objects are indicative of former vesicles from which the interior water evaporated upon drying. The data is therefore strong evidence for the vesicular nature of the supramolecular assemblies formed between receptor **1** and the peptide-PEG conjugates. The observed heights of 4 nm at the center of the collapsed vesicle and 14 nm at the rims of the "donut shaped" structure are indicative of an average thickness of ~10 nm of two vesicle walls on top of each other. This data suggests a thickness of 5 nm for each receptor-peptide-PEG layer.

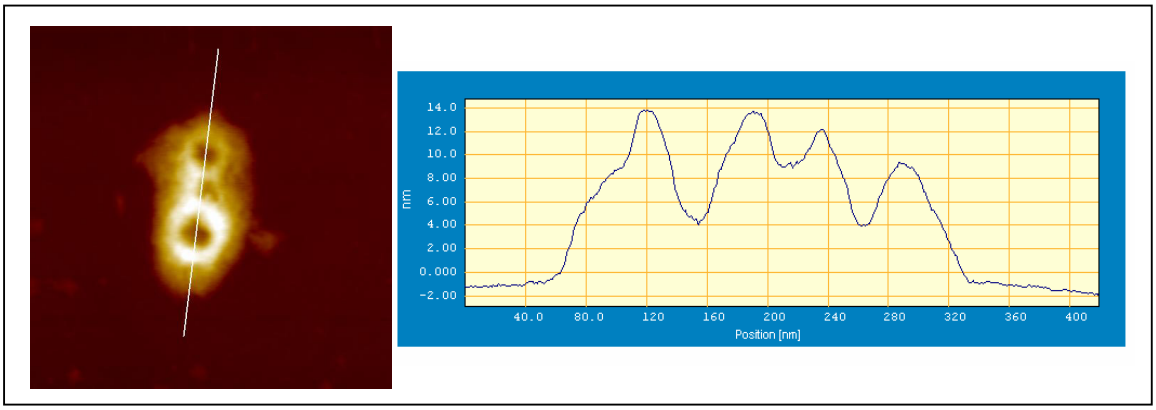


Figure 27: *Dry state AFM image of the self-assembly between receptor **1** and peptide-PEG conjugate **2** resulting from the adsorption of the vesicles from the dilute aqueous solution on silicon water and the corresponding height profile*

4.4 Other Investigations and Conclusions

To characterize the morphology of the supramolecular structure, Static Light Scattering (SLS) measurement allows us to determine the mean apparent molecular weight and the mean apparent radius of gyration of the macromolecules in the solution. From these results, one can go further and deduce for instance the aggregation number of self-assembled systems or the area occupied by a molecule inside an aggregate. Unfortunately, this measurement was not successful due to the absorption by the dye moiety of receptor **1** at the wavelength of the laser (633 nm).

To further analyze the structural properties of the vesicles formed, Langmuir-Blodgett (LB) studies were performed. These experiments were particularly motivated by the observations that a) the vesicle size had proven independent of the ligand to receptor ratio and b) the size of the vesicular structures increased from 100 nm to 200 nm when peptide-PEG conjugate **55** with the longer PEG chain instead of **2** was employed. This is in contrast to conventional amphiphilic block polymers. Their size typically decreases when the hydrophilic to hydrophobic ratio is increased and might even lead to a change in the morphology from, for example, vesicles to micelles.[96]

The LB-technique is one of the most promising techniques for preparing and studying thin films, as it enables the precise control of the monolayer thickness, homogeneous deposition of the monolayer over large areas and the possibility to make multilayer structures with varying layer composition. This method is used to create a molecular film of

amphiphile at the air-water interface. In practice, when a solution of an amphiphile in a water insoluble solvent ($CHCl_3$) is placed on a water surface, the solution spreads rapidly to cover the available area. As the solvent evaporates, a monolayer is formed. When the available area for the monolayer is large, the distance between adjacent molecules is large and their interactions are weak. The monolayer can then be regarded as a two-dimensional gas. Under these conditions, the monolayer has little effect on the surface tension of water. If the available surface area of the monolayer is reduced by a barrier system, the molecules start to exert a repulsive effect on each other. This two-dimensional analogue of a pressure is called surface pressure. The most important indicator of the monolayer properties of an amphiphilic material is given by measuring the surface pressure as a function of the area of water surface available to each molecule. This is carried out at constant temperature and is known as a surface pressure – area isotherm. Usually an isotherm is recorded by compressing the film at a constant rate while continuously monitoring the surface pressure (*Figure 28*). Furthermore, the LB technique provides information about the mean molecular area (mma) which is calculated by dividing the trough area by the number of spread molecules.[97]

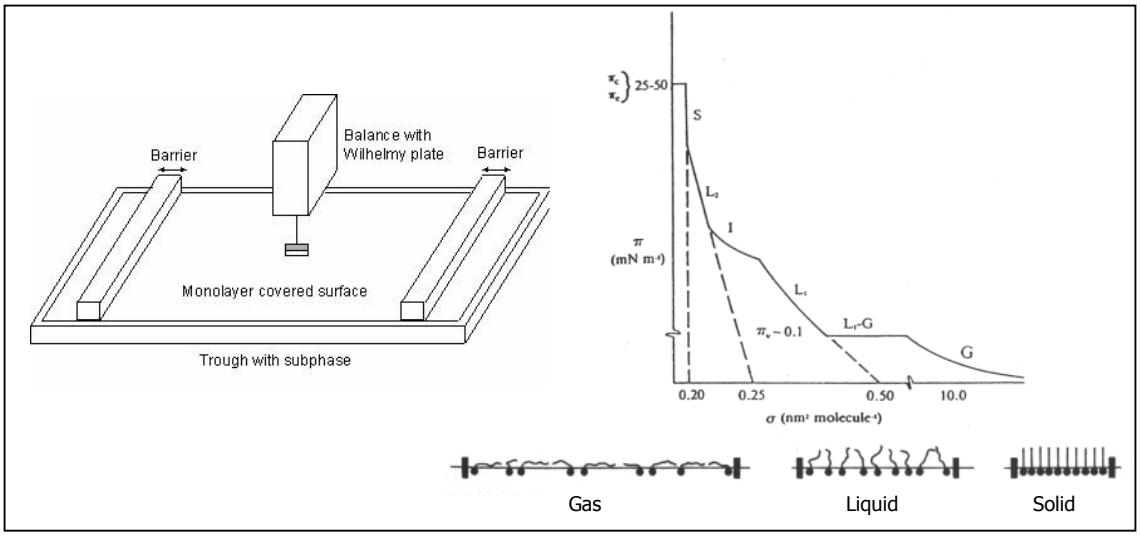


Figure 28: *Schematic illustration of Langmuir-Blodgett technique, isotherm curve and orientation of the molecules in different phases*

The surface pressure measurements demonstrated that the receptor forms a stable monolayer at the air-water interface occupying a mean molecular area of 2.2 nm^2. This area is comparable to that of low molecular weight lipids that self-assemble into liposomes (spherical lipid bilayers).[98] This suggests that the receptor organization determines the size of the vesicles by assembling into a defined layer. However, the high solubility of the peptide-PEG conjugates **2** and **55** did not allow for an analysis of the stoichiometry of binding or the kinetics, by using the LB method.

Our data, so far, suggests that upon binding of the peptide-PEG conjugates to this receptor layer, they adapt to the steric organization of the receptors. Whichever ligand to receptor ratio or molecular weight of the peptide-PEG conjugate, the same number of receptors is available / used for the molecular recognition. A larger PEG chain is forced to stretch out, compared to its lower molecular weight analogue which would explain the size increase with increased molecular weight of the PEG moiety.

Taking all of the data together, it is tempting to speculate that the vesicle walls consist of receptor-peptide-PEG layers, with the hydrophobic receptors in the inner part and the hydrophilic PEG-chains in the parts of the vesicle walls that point to the aqueous outside and inside of the vesicles (*Figure 29*).

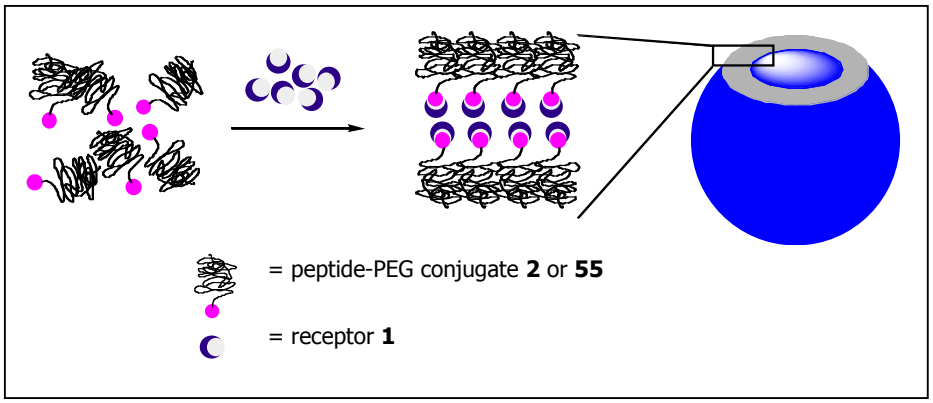


Figure 29: *Model of the proposed self-assembly between receptor **1** and peptide-PEG conjugates **2** or **55**.*

Furthermore, by changing the pH to acidic condition (pH ~3), the receptor precipitated, indicating the destruction of the vesicles. This phenomenon is due to the protonation of the histidine moiety, thereby breaking the interaction between the receptor and the pegylated peptide. This pH-sensitive behavior is interesting, for example, for the encapsulation and the delivery of drugs.

In conclusion, we have used selective non-covalent interactions between a diketopiperazine receptor and its peptidic ligand to induce supramolecular assemblies. Light scattering, TEM and AFM studies demonstrate the formation of vesicles upon mixing the receptor with the specific peptide-PEG conjugate. Their size is independent of the peptide-PEG to receptor ratio and the length of the PEG chain, suggesting that the receptor organization determines the overall shape of the vesicles. To further understand this high interaction, other investigations, like NMR tritration, are performed to determine the stoichiometry and the binding energies between the receptor and the pegylated peptides in aqueous solution.

5 CONCLUSIONS

This thesis discusses the design and synthesis of macrocyclic two-armed diketopiperazine receptors and two-armed carbazole receptors. Using encoded combinatorial peptide libraries, peptidic ligands were successfully identified for both types of receptors. Previous work had demonstrated that two-armed diketopiperazine receptors bind certain tripeptides with high binding selectivity and affinitiy. This kind of selective non-covalent interactions was the basis for other applications, like supramolecular self-assembly in organic and aqueous media.

The first part discusses the effect of macrocyclization on the binding properties of diketopiperazine receptors. Several macrocyclic receptors were prepared using different linking elements (e.g. ring closing metathesis, amide bond or disulfide bridge formation). Their binding properties were evaluated in on-bead screenings against a combinatorial split-and-mix library of 24389 different peptides. These screenings demonstrated that macrocyclic receptors bind peptides with low selectivities compared to their open-chain parent diketopiperazine receptors.

Furthermore, the binding affinities of these macrocyclic receptors were found to be weaker, in the range of ΔG = -2 to -3 kcal mol^{-1}, towards their preferred peptides, compared to the interactions between flexible diketopiperazine receptors and their selected peptides (ΔG = -5 to -6 kcal mol^{-1}). The studies also showed that the binding properties of macrocyclic receptors depend on the choice of the linker-type, with long "spacers" leading to high binding selectivities compared to short "spacers". This suggests that the conformations of macrocycles are different compared to the open-chain diketopiperazine receptor and alter the binding selectivites and affinities of these receptors. The flexibility of the open-chain receptor allows the arms to better adjust to a peptidic guest.

In the second part of this thesis, the binding properties of two-armed receptors based on a carbazole template were examined. The carbazole template was envisioned as an alternative template to the diketopiperazine template, offering an additional functionalization site for attachment of the receptors to, e.g. a dye, solid support or a soluble polymer, which is opposite to the recognition elements. Screenings of several dye-marked carbazole receptors against an encoded tripeptide library demonstrated a high level of binding selectivity of the carbazole receptors towards certain resin-bound tripeptides in chloroform.

Subsequently, solid phase binding affinity studies showed that carbazole receptors bind their selected peptides, with binding affinities in the range of ΔG = -4 to -5 kcal mol^{-1}. These binding properties are comparable to those of diketopiperazine receptors, demonstrating that the carbazole template can be a useful alternative to the diketopiperazine template. Moreover, the position of the dye - whether it is attached to the peptidic side-chains or to the additional functional site of the carbazole template – was found to neither affect the binding selectivity nor the binding affinitiy of the receptors. This suggests that the dye does not play a crucial role in the intermolecular interaction between two-armed receptors and peptides.

Based on these studies, libraries of carbazole receptors were prepared, with three or four variable side-chains, resulting in approximately 3375 carbazole receptors and 50625 receptors, respectively, for selective binding towards peptides. First screenings against fluorophore-marked Angiotensin II or segments of the Alzheimer's peptides, in aqueous solution, were unfortunately not successful.

The third part discusses the formation of vesicles between a diketopiperazine receptor and peptide-PEG conjugates in aqueous solution. The highly selective intermolecular interaction between the diketopiperazine receptor and the peptide, Ac-D-Val-D-Val-D-His-resin (resin = polystyrene), was used to induce supramolecular assemblies by functionalizing the peptide with a PEG-chain. In chloroform, the diketopiperazine receptor mixed with the pegylated peptide, forms a gel. In aqueous solution, the formation of vesicles was observed and studied using Dynamic Light Scattering, Transmission Electron and Atomic Force Microscopy, as well as surface pressure measurements. No supramolecular assemblies were observed when the receptor was mixed with non-selected peptide-PEG conjugates, demonstrating that the assembly is driven by selective non-covalent interactions.

The vesicles have a diameter of 100 nm when a small PEG-chain was attached to the peptide and 200 nm with a long PEG-chain. Furthermore, the size of the self-assembly induced with the peptide-PEG conjugates does not depend on the peptide/receptor ratio. This work is the first example of vesicle formation based on selective non-covalent interactions.

B. Experimental Section

List of Abbreviations

9-BBN	9-borabicyclo[3.3.1]nonane
AA	amino acid
Abz	2-aminobenzoyl
Ac	acetyl
Acm	acetamidomethyl
Ac_2O	acetic anhydride
AcOH	acetic acid
AFM	atomic force microscopy
Ahx	aminohexenoic acid
Ala	alanine
anh.	anhydrous
Asn	asparagine
aq.	aqueous
Boc	*tert*-butoxycarbonyl
Boc_2O	*tert*-butoxycarbonyl anhydride
calcd.	calculated
CAM	ceric ammonium molybdate
COSY	Correlated Spectroscopy
Cys	cysteine
DCC	*N,N*-dicyclohexylcarbodiimid
DIAD	diisopropyl azodicarboxylate
DIC	diisopropylcarbodiimide
DEPBT	3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(*3H*)-one
DEPT	Distortionless Enhancement by Polarization Transfer
DLS	dynamic light scattering
DMAP	*N,N*-dimethylamino pyridine
DMF	*N,N*-dimethylformamide
DMSO	dimethylsulfoxide
DNS	dansyl
EC-GC	electron capture gas chromatograpghy
EDC	1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide
ESI	electron spray ionisation

eq	equivalent
EtOAc	ethyl acetate
FAM	carboxyfluorescein
Fmoc	9-fluorenylmethoxycarbonyl
Gln	glutamine
Gly	glycine
h	hour(s)
HATU	*O*-(7-azabenzotriazol-1-yl)*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate
HCTU	*O*-(*1H*-6-chlorobenzotriazole-1-yl)1,1,3,3-tetramethyluronium hexafluorphosphate
His	histidine
HMBC	Heteronuclear Multiple Quantum Coherence
HMQC	Heteronuclear Multiple Bond Correlation
HOBt	*N*-hydroxybenzotriazole
Hph	hydrophobic amino acid
HPLC	high performance liquid chromatography
LB	Langmuir-Blodgett
Lys	lysine
MALDI-Tof	Matrix Assisted Laser Desorption Ionisation – Time of Flight
MeOH	methanol
min	minute(s)
MS	mass spectrometry
MsCl	methanesulfonyl chloride
NBD	nitrobenzofurazan
NMR	nuclear magnetic resonance
NOESY	Nuclear Overhauser Enhancement Spectroscopy
org.	organic
PAP	PEG attached products
PBS	phosphate buffer saline
PEG	polyethylene glycol
Pfp	pentafluorophenyl
Phe	phenylalanine
ppm	parts per million
PyAOP	(7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
quant.	quantitative

r.t.	room temperature
sat.	saturated
soln.	solution
TAEA	tris(2-aminoethyl)amine
TBTU	*O*-benzotriazol-1-yl-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate
TEG	tetraethylene glycol
TEM	transmission electron miscroscopy
TFA	trifluoroacetic acid
TFE	trifluoroethanol
THF	tetrahydrofuran
TIS	triisopropylsilane
TLC	thin layer chromatography
TMSBr	trimethylsilylbromide
TOCSY	Total Correlation Spectroscopy
Trt	triphenylmethyl (trityl)
*t*Bu	*tert*-butyl
Tyr	tyrosine
UV	ultra violet
Val	valine

6 GENERAL EXPERIMENTAL CONDITIONS

6.1 Analytical Methods

^{1}H NMR *Varian Gemini VXR 400* (400 MHz), *Brucker 500 DPX* (500 MHz)

Chemical shifts (δ) are indicated in ppm, relative to $SiMe_4$ (δ = 0.00 ppm) or based on the solvent signals of the partially deuterated nuclei of chloroform-d_1 (δ = 7.26 ppm), dimethyl sulfoxide-d_6 (δ = 2.50 ppm) or methanol-d_4 (δ = 3.31 ppm). All spectra are interpreted by first order, and the coupling constants (*J*) are given in Hertz (Hz). The classification of the signals was achieved by COSY, NOESY and TOCSY. The signals were abbreviated as follows: s = singlet, br. s = broad singlet, d = doublet, t = triplet, q = quartet, quint. = quintet, m = multiplet, ψ = pseudo. All solvents were purchased from *Cambridge Isotope Laboratories*, Inc.

^{13}C NMR *Varian Gemini VXR 400* (101.0 MHz), *Brucker 500 DPX* (125.6 MHz)

Chemical shifts (δ) are indicated in ppm and are relative to the following solvent signals: chloroform-d_1 (δ = 77.1 ppm), dimethyl sulfoxide-d_6 (δ = 39.5 ppm) or methanol-d_4 (δ = 49.0 ppm). The spectra are broad-band proton decoupled. The classification of the signals was achieved by DEPT, HMQC and HMBC. The signal for quaternary carbons was abbreviated as follows: Cq.

Electrospray Mass Spectrometry *Brucker esquire 3000plus*, *Finnigan MAT LCQ* (octapole mass spectrometer)

The samples were directly injected as 0.1 mg ml^{-1} solutions in MeOH/$CHCl_3$ (10:90, v/v). The ion source worked *via* electron ionization. The data are given in mass units per charge (m/z).

MALDI-TOF Mass Spectrometry (matrix-assisted laser desorption ionization time-of-flight) *Voyager-DE PRO BioSpectrometry Workstation* from *Applied Biosystems*.

2,6-dihydroxyacetophenone and α-cyano-4-hydroxycinnamic acid were used as matrix. Sample desorption and ionization was induced by a N_2-laser (337 nm, 3 ns pulses). The signals are referred to the unfragmented, single charged molecule ions $[M-H]^-$ and $[M+H]^+$. The data are given in mass units per charge (m/z).

UV-Vis Spectroscopy

Ultra violet – visible absorption spectra were recorded on a *Perkin Elmer Lambda Bio UV/Vis spectrometer* using precision cells made of quartz *SUPRASIL*® (*Hellma*, Typ 114-QS, d = 1 mm).

Electron Capture – Gas Chromatography (EC-GC)

Gas chromatography of the tags was performed on a *Hewlett Packard HP 6890 GC* system using *Agilent Techologies Ultra 1* (crosslinked methyl siloxane) column. As the solvent, DMF from *Aldrich,* was used. The tag alcohols were silylated with *N*,*O*-bis(trimethylsilyl)-acetamide from *Aldrich*.

Dynamic Light Scattering (DLS)

Dynamic Light Scattering was performed using an *ALV DLS/SLS-5022F compact goniometer system* from *ALV-Laser Vertriebs GmbH* equipped with an ALV/CGS-8F goniometer, an ALV-5000-EPP multiple tau digital correlator, ALC correlator software 3.0 and a 1145P-3083 He-Ne-laser from *JDS Uniphase* (22 mW, 633 nm) at scattering angles between 30° and 150°. Quartz cuvettes were purchased from *Hellma*.

Transmission Electron Microscopy (TEM)

TEM was carried out by Vesna Olivieri at the Microscopy Center (Pharmazentrum) at the University of Basel. The analysis were performed employing a *Philips EM 400* operated at 80 kV equipped with a *Megaview II* charge-coupled device camera (CCD) and controlled with a *Morgagni 268D* control and image acquisition software. The sample was adsorbed on a glow discharged, parlodion coated, 150 mesh copper grid. After washing cycles with water, the sample was dryed in air.

Atomic Force Microscopy (AFM)

AFM was carried out by Dipl. Ing. Olivier Casse at the Chemistry Department at the University of Basel. The analyses were conducted using a *Pico SPM LE* from *Molecular Imaging* with silicon cantilevers (C = 10-130 N/m, *Nanosensors*). Non-contact mode has been employed for the investigations of surface topography in dry state. The vesicles were deposited from aqueous solution on freshly cleaved mica. Measurements were done in the dry state, after evaporation of the solvent.

Langmuir-Blodgett Techniques (LB)

LB was carried out by Dr. Katarzyna Kita at the Chemistry Department at the University of Basel. The analyses were performed employing a *Langmuir-Blodgett mini-through* (Teflon) from *KSV* Ltd. with a total area of 243 cm^2. Symmetric compression was carried out by two Delrin barriers (compression rate: 10 mm min^{-1}) and the surface pressure was measured by Wilhelmy plate method (plate made from *ashless Whatman Chr1* chromatography paper, perimeter 20 mm, sensitivity: ±0.1 mN m^{-1}). As spreading solvent, chloroform, was used and as subphase, bi-distilled water at 20°C (pH 5.5).

6.2 Separation and purification methods

Thin Layer Chromatography (TLC) and Flash Chromatography

Reactions were monitored by thin layer chromatography (TLC) using *Merck* silica gel 60 F_{254} plates. Compounds were visualized by UV, ceric ammonium molybdate (CAM) and ninhydrin. TLC retention factors (R_f) are indicated together with the apropriate solvent mixture in brackets. Flash chromatography was performed under low pressure (~1.5 bar, membrane pump) on silica gel *Merck 60* (40-60 µm particle size). The solvents were of technical grade and were re-distilled prior to use. The mixture ratios of solvents are referred to the parts of the volume.

High Performance Liquid Chromatography (HPLC) *Shimadzu Class-VP* with UV-Vis detection. Reversed-phase HPLC analyses were carried out on *Merck LiChrosphere 100* RP-18e 5µm (250 mm x 4.6 mm) and normal-phase HPLC on a *Macherey-Nagel Nucleosil 100.5* (250 mm x 4.6 mm). As solvents, acetonitrile from *Fisher Scientific*, methanol and dichloromethane from *J.T.Backer*, were used.

Size Exclusion and Ion Exchange Chromatography

Size exclusion chromatography was performed on Sephadex LH20 resin purchased from *Sigma*. Ion exchange chromatography was performed using Dowex® 1x2-400 (Dowex-1-chloride, strongly basic anion) from *Sigma-Aldrich*.

6.3 Further Instruments

Thermomixer *Eppendorf Thermomixer confort*

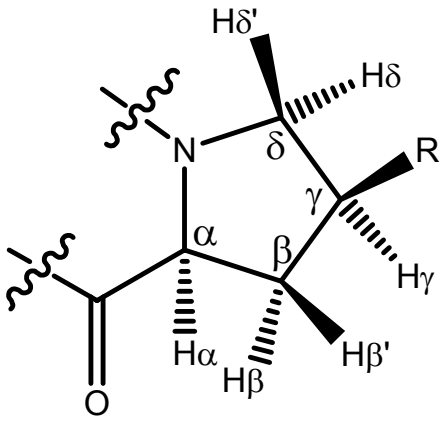
Microscope *Olympus Microscope SXZ12*

Camera (from microscope) *Olympus Camera Camedia C-3000 Zoom*

6.4 Solvents and Chemicals

Materials and reagents were of the highest commercially available grade and used without further purification. Technical grade solvents for extraction and flash column chromatography were distilled prior to use. For all other reactions in water-free environment, or for analytical purposes, absolute solvents from *Fluka* were used without further purification. The water used for reactions and HPLC was filtered over *Barnstead* ultrapure water system. TentatelGel-PAP-NH_2 was purchased from *Rapp Polymere GmbH*, Tübingen Germany and the Wang resin from *Novabiochem*. The protected amino acids were purchased from *Bachem AG* and *Novabiochem*. Coupling reagents were purchased from *Fluka*, *Iris Biotech*, *Bachem AG* or *Senn Chemicals*.

6.5 Assignment of C and H for Proline Derivatives



Preparation of PBS (phosphate buffer saline) buffer 10 mM, pH 7.20:

100 mg KCl, 100 mg KH_2PO_4, 4 g NaCl and 575 mg Na_2HPO_4 were mixed in 20 ml nanopure H_2O. The pH of the solution was adjusted to 7.20 with 1 N aq. HCl, and the volume was adjusted to 500 ml with nanopure H_2O.

Na-phosphate buffer 0.1 M, pH 5.5:

90 g NaH_2PO_4, 32.7 g Na_2HPO_4 and 500 ml of H_2O

$KMnO_4$-Dip:

9 g $KMnO_4$, 60 g K_2CO_3, 15 ml 5% NaOH and 900 ml H_2O

Ninhydrin-Dip:

0.6 g ninhydrin, 200 ml butanol and 6 ml 99% AcOH

CAM-Dip:

2.5 g $Ce(SO_4)_2$, 75 g $(NH_4)_6Mo_7O_{24}$ and 500 ml 10% H_2SO_4

α-Cyano-4-hydroxycinnamic acid matrix:

10 mg α-cyano-4-hydroxycinnamic in 1:1 (v/v) mixture of water/acetonitrile with 0.3% TFA (1 ml)

2,6-Dihydroxyacetophenone matrix:

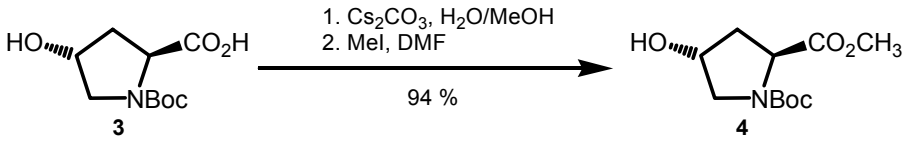
1) A small amount of 2,6-dihydroxyacetophenone in 1:1 (v/v) mixture of H_2O/acetonitrile (1 ml)

2) 92 mg ammonium tartrate in H_2O (1 ml)

3) 600 µl of **1** mixed with 300 µl of **2**

7 SYNTHESIS OF THE SYMMETRIC DIKETOPIPERAZINE TEMPLATE

7.1 *N*-Boc-*trans*-γ-hydroxy-L-proline methyl ester 4



To a solution of 20.0 g (86.45 mmol, 1 eq) *N*-Boc-*trans*-γ-hydroxy-L-proline **3** in MeOH (100 ml), 15.6 g (47.56 mmol, 0.55 eq) Cs_2CO_3, dissolved in H_2O (20 ml), were added. After stirring for 1 h at r.t., MeOH was removed under reduced pressure and the residue was co-evaporated 5 times with toluene (20 ml). The resulting precipitate was dissolved in DMF (60 ml) and treated with 11 ml (172.93 mmol, 2 eq) MeI. After 30 min in the sonicator at r.t., the reaction mixture was first filtered (formation of CsI-salt) and then extracted with Et_2O (6x160ml). The combined org. phases were dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. 20.0 g (81.55 mmol, 94%) *N*-Boc-trans-γ-hydroxy-L-proline methyl ester **4** were isolated as a white/yellow solid. (^{1}H and ^{13}C NMR-spectra show a double set of peaks (~ 1:2) due to the s-*cis* and s-*trans* conformers on the tertiary amide and the carbamate).

$C_{11}H_{19}NO_5$: 245.13

TLC: Pentane/EtOAc (1/1); R_f: 0.29 (Ninhydrin).

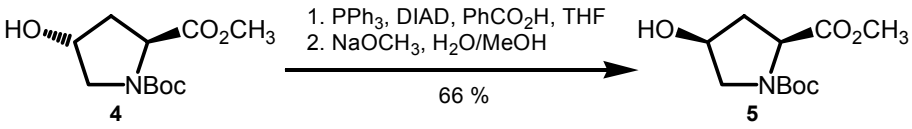
^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ(ppm) = 4.43 (m, 2H; Hα, Hγ, major + minor), 3.71 (s, 3H; -CO_2CH_3, major + minor), 3.53 (m, 2H; Hδ, Hδ', major + minor), 2.25 (m, 1H; Hβ, major + minor), 2.01 (m, 1H; Hβ', major + minor), 1.44 (s, 9H; -*t*Bu, minor), 1.45 (s, 9H; -*t*Bu, major).

^{13}C-NMR (101 MHz, $CDCl_3$, 25°C): δ(ppm) **major** = 173.6 (Cq;-CO_2CH_3), 153.9 (Cq; CO_{Boc}), 80.3 (Cq; -*t*Bu), 69.3 (CH; Cγ), 57.9 (CH; Cα), 54.6 (CH_2; Cδ, Cδ'), 52.0 (CH_3; -CO_2CO_3), 39.0 (CH_2; Cβ, Cβ'), 28.2 (CH_3; -*t*Bu).

^{13}C-NMR (101 MHz, $CDCl_3$, 25°C): δ(ppm) **minor** = 173.4 (Cq;-*C*O_2CH_3), 154.5 (Cq; *C*O_{Boc}), 80.2 (Cq; -*t*Bu), 70.0 (CH; Cγ), 57.4 (CH; Cα), 54.7 (CH_2; Cδ, Cδ'), 52.2 (CH_3; -CO_2*C*O_3), 38.4 (CH_2; Cβ, Cβ'), 28.3 (CH_3; -*t*Bu).

ESI-MS: m/z: calcd for $C_{11}H_{19}NO_5Na$: 268 $[M+Na]^+$; found: 268.

7.2 *N*-Boc-*cis*-γ-hydroxy-L-proline methyl ester 5



To a suspension of 20.0 g (81.55 mmol, 1 eq) *N*-Boc-*trans*-γ-hydroxy-L-proline methyl ester **4** in THF (135 ml), 11.5 g (93.78 mmol, 1.15 eq) benzoic acid and 24.8 g (94.4 mmol, 1.16 eq) PPh_3 were added. The mixture was cooled with an ice bath before 17.4 ml (89.70 mmol, 1.1 eq) DIAD were added and allowed to stir at r.t. for 2.5 h. After removal of the solvent under reduced pressure, the oily residue was co-evaporated three times with CH_2Cl_2 (70 ml) and then triturated 2 times with a mixture of hexane and EtOAc to yield a white solid (triphenylphosphine oxide). The white solid was filtered off and then washed with hexane. The hexane-phases were combined and, after removal of the solvent under reduced pressure, the residue was purified by flash chromatography (gradient of pentane/EtOAc from 9:1 to 4:1 (v/v) with 10% CH_2Cl_2) to afford 27.4 g (78.58 mmol, 96%) of the benzoic acid ester of **4** as white solid. The product was subsequently dissolved in MeOH (112 ml) and allowed to react with 4.0 g (74.67 mmol, 0.95 eq) $NaOCH_3$ dissolved in MeOH (22 ml). After 15 min. the solvent was removed under reduced pressure and the residue was diluted in CH_2Cl_2 and washed with water. The aq. phase was extracted again with CH_2Cl_2 and the combined org. phases were dried over Na_2SO_4. After removal of all volatiles under reduced pressure, flash chromatography (gradient of pentane/EtOAc from 3:1 to 2:3 (v/v)) afforded 12.7 g (51.81 mmol, 66%) *N*-Boc-*cis*-γ-hydroxy-L-proline methyl ester **5** as a white solid. (^{1}H and ^{13}C NMR-spectra show a double set of peaks (~ 1:1) due to the s-*cis* and s-*trans* conformers on the tertiary amide and the carbamate).

$C_{11}H_{19}NO_5$: 245.13

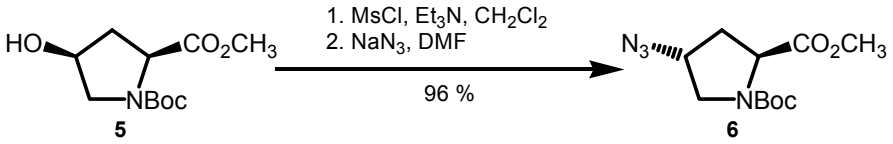
TLC: Pentane/EtOAc (1/1); R_f: 0.17 (Ninhydrin).

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ(ppm) = 4.33 (m, 2H; Hα, Hγ), 3.78 (s, 3H; -CO_2CH_3), 3.61 (m, 2H; Hδ, Hδ'), 2.33 (m, 2H; Hβ), 2.08 (m, 2H; Hβ'), 1.48 (s, 9H; -*t*Bu), 1.37 (s, 9H; -*t*Bu).

^{13}C-NMR (101 MHz, $CDCl_3$, 25°C): δ(ppm) = 175.3/175.1 (Cq;-CO_2CH_3), 154.4/153.6 (Cq; CO_{Boc}), 80.3 (Cq; -*t*Bu), 71.0/69.9 (CH; Cγ), 57.8/57.6 (CH; Cα), 55.7/55.1 (CH_2; Cδ, Cδ'), 52.6/52.3 (CH_3; -CO_2CO_3), 38.5/35.3 (CH_2; Cβ, Cβ'), 28.3/28.1 (CH_3; -*t*Bu).

ESI-MS: m/z: calcd for $C_{11}H_{19}NO_5Na$: 268 $[M+Na]^+$; found: 268.

7.3 *N*-Boc-*trans*-γ-azido-L-proline methyl ester 6



To a solution of 12.5 g (50.99 mmol, 1 eq) *N*-Boc-*cis*-γ-hydroxy-L-proline methyl ester **5** in CH_2Cl_2 (145 ml), 8.5 ml (61.19 mmol, 1.2 eq) Et_3N were added. The mixture was cooled with an ice bath and then 4.7 ml (61.19 mmol, 1.2 eq) MsCl were slowly added. After 30 min. the reaction mixture was washed with sat. $NaHCO_3$-soln. and extracted three times with CH_2Cl_2. The combined org. phases were dried over Na_2SO_4. Filtration and removal of the solvent under reduced pressure yielded a yellow oily residue that was dissolved in DMF (145 ml) and treated with 16.6 g (254.97 mmol, 5 eq) NaN_3 at 80°C. After 2 h, the reaction mixture was washed with $NaHCO_3$-soln. and extracted three times with Et_2O, the combined org. phases were dried over Na_2SO_4. After removal the solvent under reduced pressure and drying *in vacuo*, 13.2 g (48.86 mmol, 96%) *N*-Boc-*trans*-γ-azido-L-proline methyl ester **6** were isolated as a white solid. (^{1}H and ^{13}C NMR-spectra show a double set of peaks (~ 2:3) due to the s-*cis* and s-*trans* conformers on the tertiary amide and the carbamate).

$C_{11}H_{18}N_4O_4$: 270.13

TLC: Pentane/EtOAc (1/1); R_f: 0.7 (Ninhydrin).

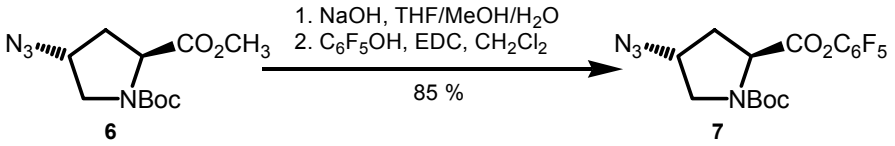
^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ(ppm) = 4.38 (t, J = 7.1 Hz, 1H; Hα, minor), 4.29 (t, J = 7.6 Hz, 1H; Hα, major), 4.17 (m, 1H; Hγ, major + minor), 3.71 (s, 3H; -CO_2CH_3, major + minor), 3.66 (m, 1H; Hδ, major + minor), 3.50 (m, 1H; Hδ', major + minor), 2.30 (m, 1H; Hβ, major + minor), 2.14 (m, 2H; Hβ', major + minor), 1.43 (1s, 9H; -*t*Bu, minor), 1.38 (1s, 9H; -*t*Bu, major).

^{13}C-NMR (101 MHz, $CDCl_3$, 25°C): δ(ppm) **major** = 172.9 (Cq;-CO_2CH_3), 153.0 (Cq; CO_{Boc}), 80.1 (Cq; -*t*Bu), 58.5 (CH; Cα), 57.4 (CH; Cγ), 51.8 (CH_2; Cδ, Cδ'), 50.9 (CH_3; -CO_2CO_3), 35.9 (CH_2; Cβ, Cβ'), 27.9 (CH_3; -*t*Bu).

^{13}C-NMR (101 MHz, $CDCl_3$, 25°C): δ(ppm) **minor** = 172.4 (Cq;-CO_2CH_3), 153.7 (Cq; CO_{Boc}), 80.0 (Cq; -*t*Bu), 59.0 (CH; Cα), 57.1 (CH; Cγ), 52.0 (CH_2; Cδ, Cδ'), 51.1 (CH_3; -CO_2CO_3), 35.0 (CH_2; Cβ, Cβ'), 28.0 (CH_3; -*t*Bu).

ESI-MS: m/z: calcd for $C_{11}H_{18}N_4O_4Na$: 293 $[M+Na]^+$; found: 293.

7.4 *N*-Boc-*trans*-γ-azido-L-proline pentafluorophenyl ester 7



7.0 g (25.91 mmol, 1 eq) *N*-Boc-*trans*-γ-azido-L-proline methyl ester **6** were dissolved in a 1:1 (v/v) mixture of THF/MeOH (50 ml). After the addition of 2.0 g (46.64 mmol, 1.8 eq) NaOH in water (12.5 ml), the mixture was stirred for 1.5 h at r.t. The reaction mixture was then carefully acidified with 1 M HCl to pH 3. The mixture was diluted in CH_2Cl_2 and washed with water. The aq. phase was extracted with EtOAc, the org. phases were washed with brine and dried over Na_2SO_4. Filtration and evaporation of the solvent under reduced pressure yielded a yellow oily residue which was dissolved in CH_2Cl_2 (67 ml).

After addition of 5.3 g (28.50 mmol, 1.1 eq) 2,3,4,5,6-pentafluorophenol and 7.4 g (38.87 mmol, 1.5 eq) EDC, the solution was stirred at r.t. for 1 h and then acidified with 1 M HCl to pH 3. The reaction mixture was extracted with water and EtOAc. The aq. phase was extracted again with EtOAc and the org. phases were washed with brine and dried over Na_2SO_4. Filtration and removal of all volatiles under reduced pressure yielded 8.7 g (20.60

mmol, 80%) of the pentafluorophenylester **7** as a white solid. (^{1}H and ^{13}C NMR-spectra show a double set of peaks (~ 2:1) due to the s-*cis* and s-*trans* conformers on the tertiary amide and the carbamate).

$C_{16}H_{15}F_5N_4O_4$: 422.31

TLC: Pentane/EtOAc (5/1); R_f: 0.7 (Ninhydrin).

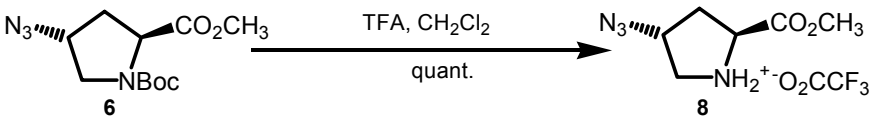
^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ(ppm) = 4.72 (m, 1H; Hα, major + minor), 4.26 (m, 1H; Hγ, major + minor), 3.72 (m, 1H; Hδ, major + minor), 3.56 (m, 1H; Hδ', major + minor), 2.55 (m, 1H; Hβ, major + minor), 2.38 (m, 1H; Hβ', major + minor), 1.48 (s, 9H; -*t*Bu, minor), 1.46 (1s, 9H; -*t*Bu, major).

^{13}C-NMR (101 MHz, $CDCl_3$, 25°C): δ(ppm) **major** = 168.5 (Cq;-*C*$O_2C_6F_5$), 153.3 (Cq; *C*O_{Boc}), 82.0 (Cq; -*t*Bu), 58.6 (CH; Cα), 57.3 (CH; Cγ), 51.3 (CH_2; Cδ, Cδ'), 36.7 (CH_2; Cβ, Cβ'), 28.0 (CH_3; -*t*Bu).

^{13}C-NMR (101 MHz, $CDCl_3$, 25°C): δ(ppm) **minor** = 168.3 (Cq;-*C*$O_2C_6F_5$), 153.9 (Cq; *C*O_{Boc}), 81.5 (Cq; -*t*Bu), 59.3 (CH; Cα), 57.2 (CH; Cγ), 51.4 (CH_2; Cδ, Cδ'), 35.4 (CH_2; Cβ, Cβ'), 28.2 (CH_3; -*t*Bu).

ESI-MS: m/z: calcd for $C_{16}H_{15}F_5N_4O_4Na$: 445 $[M+Na]^+$; found: 445.

7.5 TFA-*trans*-γ-azido-L-proline methyl ester 8



5.6 g (20.57 mmol, 1 eq) of the *N*-Boc protected proline methylester **6** were dissolved in a 1:3 (v/v) mixture of TFA and CH_2Cl_2 (21.3 ml) and allowed to stir at r.t. for 1.5 h. After removal of all volatiles under reduced pressure the oily residue was triturated with Et_2O and isolated by decantation followed by removal of all residual volatiles *in vacuo* to yield 5.6 g (20.57 mmol, quant.) of the TFA-salt **8** as a white solid.

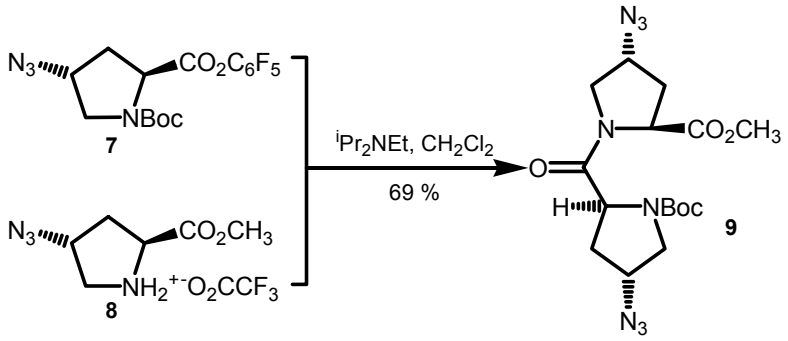
$C_9H_{11}F_3N_4O_4$: 284.2

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ(ppm) = 4.56 (m, 1H; Hα), 4.49 (m, 1H; Hγ), 3.81 (s, 3H; -CO_2CH_3), 3.76 (m, 1H; Hδ), 3.47 (m, 1H; Hδ'), 2.49 (m, 2H; Hβ), 2.40 (m, 2H; Hβ').

^{13}C-NMR (101 MHz, $CDCl_3$, 25°C): δ(ppm) = 168.4 (Cq;-CO_2CH_3), 59.4/58.2 (CH; Cα), 53.7 (CH; Cγ), 50.7 (CH_2; Cδ, Cδ'), 34.6 (CH_2; Cβ, Cβ').

ESI-MS: m/z: calcd for $C_9H_{10}N_4O_2$: 170 $[M]^+$; found: 170.

7.6 *N*-Boc-(*trans*-γ-azido-L-Pro)$_2$-OCH$_3$ 9



8.7 g (20.57 mmol, 1 eq) of the pentafluorophenol-ester **7** were added to a 5.8 g (20.57 mmol, 1 eq) TFA-salt **8** and 6.1 ml (41.14 mmol, 2 eq) ^{i}Pr$_2$Net solution in CH_2Cl_2 (27 ml). The solution was slowly concentrated using a N_2 flow and the residual colorless oil stirred for 15 h. The reaction mixture was then acidified with 1 M HCl to pH 3 and extracted with H_2O and EtOAc. The aq. phase was extracted with EtOAc, the org. phases were washed with brine and dried over Na_2SO_4. Filtration and evaporation of the solvent under reduced pressure yielded the crude product, which was purified by flash chromatography on silica gel (gradient of CH_2Cl_2/MeOH from 100:0 to 98:2 (v/v)) to yield 5.8 g (14.23 mmol, 69%) of the dipeptide **9** as a white solid. (^{1}H and ^{13}C NMR-spectra show a double set of peaks (~ 2:1) due to the s-*cis* and s-*trans* conformers on the tertiary amide and the carbamate).

$C_{16}H_{24}N_8O_5$: 408.41

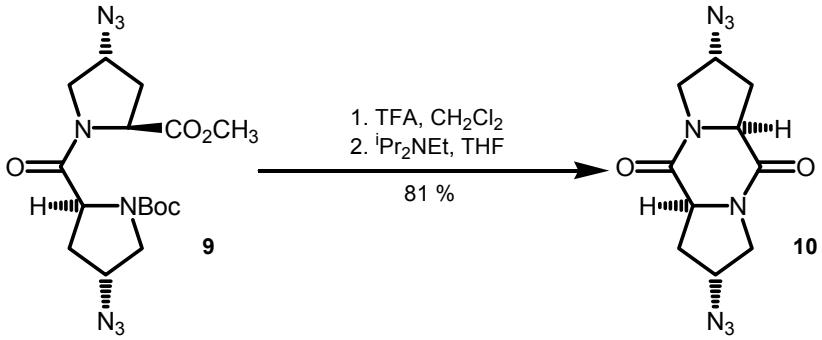
TLC: MeOH/CH_2Cl_2 (10:90); R_f: 0.6 (Ninhydrin).

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ(ppm) = 4.56 (m, 2H; Hα, major + minor), 4.30 (m, 2H; Hγ, major + minor), 3.83 (m, 2H; Hδ, major + minor), 3.67 (1s, 3H; -CO_2CH_3, minor), 3.66 (1s, 3H; -CO_2CH_3, major), 3.62 (m, 2H; Hδ', major + minor), 2.23 (m, 4H; Hβ, Hβ', major + minor), 1.37 (1s, 9H; -*t*Bu, major), 1.34 (s, 9H; -*t*Bu, minor).

^{13}C-NMR (101 MHz, $CDCl_3$, 25°C): δ(ppm) = 171.8/171.6/170.8/170.7 (Cq; Cε, -CO_2CH_3, major + minor), 153.9/153.1 (Cq; CO_{Boc}, major + minor), 80.8 (Cq; -*t*Bu, minor), 80.1 (Cq; -*t*Bu, major), 59.7/59.5/59.3/58.9 (CH; Cα, major + minor), 57.7/57.6/56.3/56.0/ (CH; Cγ, major + minor), 52.6/52.5 (CH_2; Cδ, Cδ', major + minor), 51.6/51.5/51.3 (CH_3; -CO_2CO_3, major + minor), 35.6/34.9/34.1 (CH_2; Cβ, Cβ', major + minor), 28.3 (CH_3; -*t*Bu, major), 28.2 (CH_3; -*t*Bu, minor).

ESI-MS: m/z: calcd for $C_{16}H_{24}N_8O_5Na$: 431 $[M+Na]^+$; found: 431.

7.7 Cyclo-(*trans*-γ-azido-L-Pro)$_2$ 10



5.8 g (14.24 mmol, 1 eq) of the dipeptide **9** were dissolved in a 1:3 (v/v) mixture of TFA and CH_2Cl_2 (46 ml) and allowed to stir at r.t. for 1.5 h. After removal of all volatiles under reduced pressure, the oily residue was triturated with Et_2O to yield a white solid which was isolated by decantation, followed by removal of all residual volatiles *in vacuo*. The residue was dissolved in THF (53 ml), 12.2 ml (71.20 mmol, 5 eq) $^iPr_2NEt$ were added and the mixture was stirred at r.t. for 16 h. The reaction mixture was acidified with 1 M HCl to pH 3 and extracted with H_2O and CH_2Cl_2. The aq. phase was extracted with CH_2Cl_2, the org. phases were washed with brine and dried over Na_2SO_4. After filtration and evaporation of the solvent under reduced pressure, the crude product was purified by flash chromatography on silica gel (gradient of CH_2Cl_2/MeOH from 97:3 to 96:4 (v/v)) to afford 3.2 g (11.51 mmol, 81%) of the diketopiperazine **10** as a white solid.

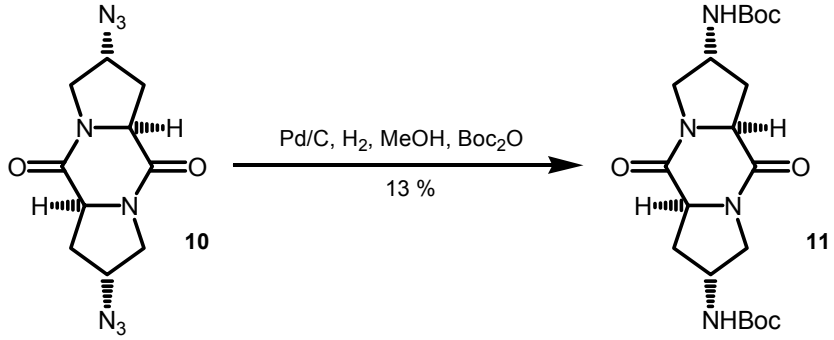
$C_{10}H_{12}N_8O_2$: 276.25

TLC: MeOH/CH_2Cl_2 (5:95); R_f: 0.5 (Ninhydrin).

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ(ppm) = 4.47 (dd, J = 10.6, 6.7 Hz, 2H; Hα), 4.36 (m, 2H; Hγ), 3.71 (dd, J = 12.6, 4.5 Hz, 2H; Hδ), 3.62 (m, 2H; Hδ'), 2.45 (ddd, J = 13.6, 6.6, 1.5 Hz, 2H; Hβ), 2.30 (ddd, J = 15.7, 10.6, 5.0 Hz, 2H; Hβ').

^{13}C-NMR (101 MHz, $CDCl_3$, 25°C): δ(ppm) = 165.7 (Cq; Cε), 58.7/58.6 (CH; Cα, Cγ), 50.8 (CH_2; Cδ, Cδ'), 33.9 (CH_2; Cβ, Cβ').

7.8 Cyclo-(*trans*-γ-*N*-Boc-L-Pro)$_2$ 11



360 mg (10% of **10**) Palladium on carbon were added to a solution of 3.2 g (11.51 mmol, 1 eq) of the diazide **10** and 10.3 g (47.19 mmol, 4.1 eq) Boc_2O in MeOH (123 ml). The black suspension was evacuated, flushed with hydrogen and allowed to stir for 3 h at r.t. After filtration over celite, the residue was washed with a 10:90 (v/v) mixture of MeOH/CH_2Cl_2. After removal of the solvent under reduced pressure, the residue was purified by flash chromatography over silica gel (gradient of CH_2Cl_2/MeOH from 100:0 to 95:5 (v/v)) to afford 4.4 g (1.53 mmol, 13%) of the diketopiperazine **11** as a white solid.

$C_{20}H_{32}N_4O_6$: 424.23

TLC: MeOH/CH_2Cl_2 (10:90); R_f: 0.3 (Ninhydrin).

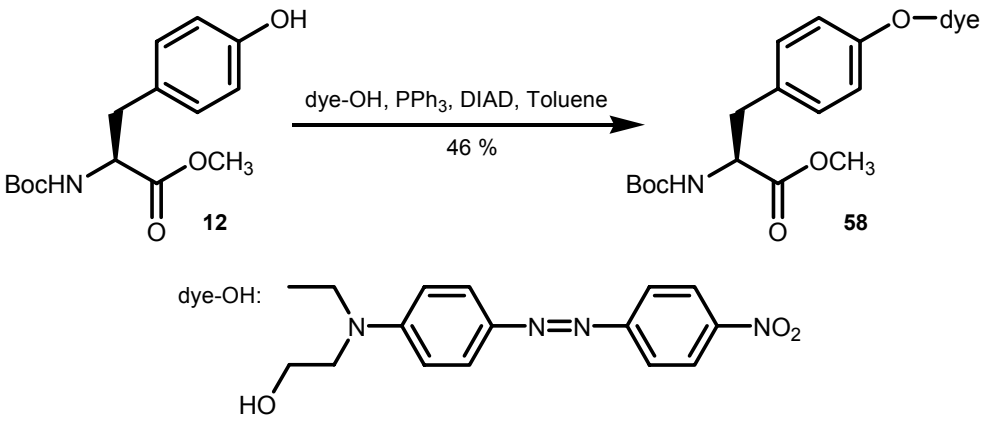
^{1}H-NMR (500 MHz, DMSO, 25°C): δ(ppm) = 4.42 (ψt, J = 8.6 Hz, 2H; Hα), 4.01 (m, 2H; Hγ), 3.61 (m, 2H; Hδ), 3.21 (m, 2H; Hδ'), 2.15 (m, 2H; Hβ), 2.08 (m, 2H; Hβ'), 1.39 (s, 18H; -*t*Bu).

^{13}C-NMR (125.6 MHz, DMSO, 25°C): δ(ppm) = 166.4 (Cq; Cε), 155.1 (Cq; CO_{Boc}), 58.2 (CH; Cα, Cγ), 51.4 (CH_2; Cδ, Cδ'), 33.1 (CH_2; Cβ, Cβ'), 28.2 (CH_3; -*t*Bu).

ESI-MS: m/z: calcd for $C_{20}H_{32}N_4O_6Na$: 447 $[M+Na]^+$; found: 447.

8 SYNTHESIS OF THE TWO-ARMED DIKETOPIPERAZINE RECEPTOR PRECURSOR

8.1 *N*-Boc-L-Tyr(dye)-OCH_3 58



2.0 g (6.77 mmol, 1 eq) *N*-Boc-L-tyrosine-methylester, 2.1 g (6.77 mmol, 1 eq) Disperse Red 1 (dye) and 1.8 g (6.77 mmol, 1 eq) PPh_3 were dissolved in toluene (136 ml). 1.3 ml (6.77 mmol, 1 eq) DIAD were added dropwise in 15 min and the mixture was allowed to stir at r.t. for 16 h. After removal of the solvent under reduced pressure, flash chromatography over silica gel (gradient of CH_2Cl_2/Acetone from 100:0 to 97:3 (v/v)) afforded 1.9 g (3.16 mmol, 46%) *N*-Boc-L-Tyr(dye)-OCH_3 **58** as a red solid.

$C_{31}H_{37}N_5O_7$: 591.65

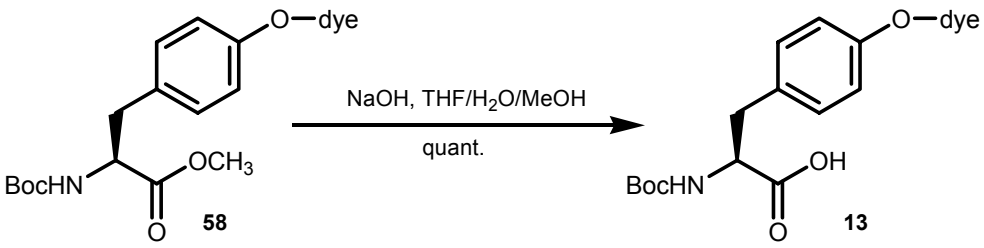
TLC: Pentan/EtOAc (2:1); R_f: 0.3.

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ(ppm) = 8.32 (d, *J* = 9.1 Hz, 2H; H_{dye}), 7.92 (d, *J* = 9.1 Hz, 2H; H_{dye}), 7.90 (d, *J* = 9.0 Hz, 2H; H_{dye}), 7.02 (d, *J* = 8.6 Hz, 2H; H_{Tyr}), 6.81 (m, 4H; H_{dye}, H_{Tyr}), 4.95 (br d, *J* = 8.6 Hz, 1H; -N*H*$_{Boc}$), 4.53 (dd, *J* = 13.7, 6.1 Hz, 1H; Hα), 4.16 (t, *J* = 6.1 Hz, 2H; -N-CH_2CH_2-O-$_{dye}$), 3.84 (t, *J* = 5.9 Hz, 2H; -N-CH_2CH_2-O-$_{dye}$), 3.70 (s, 3H; -CO_2CH_3), 3.61 (q, *J* = 7.1 Hz, 2H; -N-CH_2CH_{3dye}), 3.05 (dd, *J* = 14.2, 6.2 Hz, 1H; Hβ), 3.02 (dd, *J* = 14.0, 6.0 Hz, 1H; Hβ'), 1.41 (s, 9H; -*t*Bu), 1.29 (t, *J* = 7.0 Hz, 3H; -N-CH_2CH_{3dye}).

^{13}C-NMR (101 MHz, $CDCl_3$, 25°C): δ(ppm) = 172.3 (Cq; *C*O), 156.8/151.2/147.4/143.7/ 128.6 (Cq; dye, Tyr), 130.4/126.3/124.7/122.6/114.5/111.4 (CH; dye, Tyr), 79.9 (Cq; $C_{-t\mathrm{Bu}}$), 65.3 (CH_2; -N-CH_2*C*H_2-O-$_{dye}$), 54.5 (CH; Cα), 52.2 (CH_3; -CO_2*C*H_3), 49.9 (CH_2; -N-*C*H_2CH_2-O-$_{dye}$), 46.2 (CH_2; -N-*C*H_2CH_{3dye}), 37.5 (CH_2; Cβ, Cβ'), 28.3 (CH_3; $C_{-t\mathrm{Bu}}$), 12.3 (CH_3; -N-CH_2*C*H_{3dye}).

ESI-MS: m/z: calcd for $C_{31}H_{37}N_5O_7Na$: 614 $[M+Na]^+$; found: 614.

8.2 *N*-Boc-L-Tyr(dye)-OH 13



837 mg (1.41 mmol, 1 eq) *N*-Boc-L-Tyr(dye)-OCH_3 **58** were dissolved in a 1:1 (v/v) mixture of THF and MeOH (2.6 ml). After the addition of 102 mg (2.55 mmol, 1.8 eq) NaOH in water (1 ml), the mixture was stirred overnight at r.t. The reaction mixture was then carefully acidified to pH 3 with 1 M HCl. CH_2Cl_2 and water were added and the mixture was extracted. The aq. phase was extracted again with CH_2Cl_2 and the org. phases were washed with brine and dried over Na_2SO_4. Filtration and evaporation of the solvent under reduced pressure yielded 814 mg (1.41 mmol, quant.) *N*-Boc-L-Tyr(dye)-OH **13** as red solid.

$C_{30}H_{35}N_5O_7$: 577.53

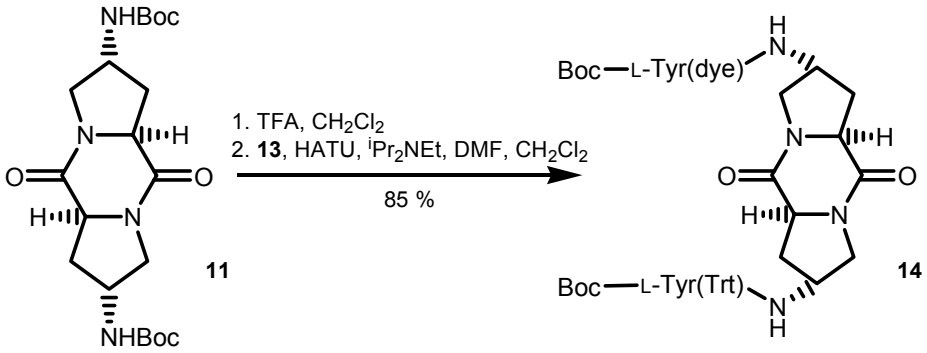
TLC: MeOH/CH_2Cl_2 (10:90); R_f: 0.6.

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ(ppm) = 8.32 (d, *J* = 9.1 Hz, 2H; H_{dye}), 7.92 (d, *J* = 9.1 Hz, 2H; H_{dye}), 7.90 (d, 2H; H_{dye}), 7.09 (d, *J* = 8.7 Hz, 2H; H_{Tyr}), 6.81 (m, 4H; H_{dye}, H_{Tyr}), 4.90 (br d, *J* = 8.0 Hz, 1H; -N*H*$_{Boc}$), 4.53 (m, 1H; Hα), 4.16 (t, *J* = 5.6 Hz, 2H; -N-CH_2C*H*$_2$-O-$_{dye}$), 3.84 (t, *J* = 5.4 Hz, 2H; -N-C*H*$_2CH_2$-O-$_{dye}$), 3.60 (q, *J* = 7.1 Hz, 2H; -N-C*H*$_2CH_{3dye}$), 3.11 (dd, *J* = 14.1, 5.6 Hz, 1H; Hβ), 3.02 (dd, *J* = 14.1, 5.4 Hz, 1H; Hβ'), 1.41 (s, 9H; -*t*Bu), 1.28 (t, *J* = 7.0 Hz, 3H; -N-CH_2C*H*$_{3dye}$).

^{13}C-NMR (101 MHz, $CDCl_3$, 25°C): δ(ppm) = 167.1 (Cq; *C*O), 157.6 (Cq; *C*O_{Boc}), 156.8/ 151.2/147.4/143.7 (Cq; dye, Tyr), 130.5/126.3/124.7/122.6/114.5/111.4 (CH; dye, Tyr), 80.3 (Cq; C_{-tBu}), 65.3 (CH_2; -N-CH_2*C*H_2-O-$_{dye}$), 54.5 (CH; Cα), 49.8 (CH_2; -N-*C*H_2CH_2-O-$_{dye}$), 46.1 (CH_2; -N-*C*H_2CH_{3dye}), 37.0 (CH_2; Cβ, Cβ'), 28.3 (CH_3; C_{-tBu}), 12.3 (CH_3; -N-CH_2*C*H_{3dye}).

ESI-MS: m/z: calcd for $C_{30}H_{35}N_5O_7Na$: 600 $[M+Na]^+$; found: 600.

8.3 Synthesis of 14



The Boc-protecting groups of 168 mg (396.7 µmol, 1 eq) of the bis-*N*-Boc-protected diketopiperazine **11** were removed with a 1:3 (v/v) mixture of TFA and CH_2Cl_2 (2.4 ml) as described for the formation of the TFA-salt **11**. The residue was dissolved in CH_2Cl_2 (5.5 ml) followed by addition of 150 µl (707.5 µmol, 2 eq) $^iPr_2NEt$. The solution was cooled with an ice bath before a mixture of 603 mg (1.59 mmol, 4 eq) HATU in DMF (1 ml), 150 µl (707.5 µmol, 2 eq) $^iPr_2NEt$ and 916 mg (1.59 mmol, 4eq) *N*-Boc-L-Tyr(dye)-OH **13** in CH_2Cl_2 (5.5 ml), was added and stirred at r.t. for 1 h. The reaction mixture was extracted three times with 0.1 M Na-phosphate buffer (pH 5.5) and CH_2Cl_2. The aq. phases were extracted again with CH_2Cl_2. The org. phases were extracted with 0.5 M NaOH and CH_2Cl_2. The aq. phase was extracted again with CH_2Cl_2. The org. phases were washed with brine and dried over Na_2SO_4. After filtration and evaporation of the solvent under reduced pressure the crude product was purified by flash chromatography on silica gel (gradient of CH_2Cl_2/MeOH from 99.5:0.5 to 99:1 (v/v) with 0.5% of triethylamine) and by another flash chromatography on silica gel (gradient CH_2Cl_2/MeOH from 99.5:0.5 to 97:3 (v/v)) and finally by preparative-TLC (CH_2Cl_2/MeOH, 90:10, v/v) to afford 451 mg (336.6 µmol, 85%) of the diketopiperazine with attached *N*-Boc protected tyrosine **14**.

$C_{70}H_{82}N_{14}O_{14}$: 1343.49

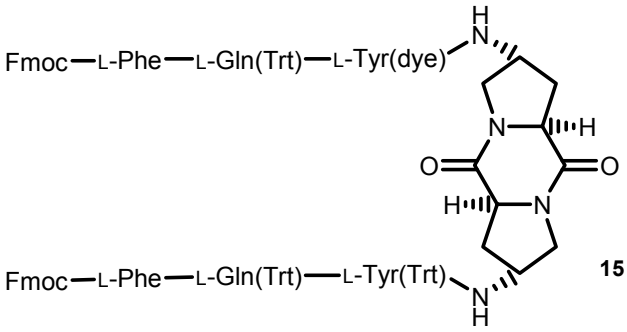
TLC: MeOH/CH_2Cl_2 (10:90); R_f: 0.54.

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ(ppm) = 8.30 (d, *J* = 9.1 Hz, 4H; H_{dye}), 7.90 (m, 8H; H_{dye}), 7.11 (d, *J* = 8.3 Hz, 4H; H_{Tyr}), 6.81 (m, 8H; H_{dye}, H_{Tyr}), 5.49 (br s, 2H; -N*H*$_{Boc}$), 4.32 (m, 2H; Hα$_{Tyr}$), 4.16 (m, 8H; -N-CH_2CH_2-O-$_{dye}$, Hα, Hγ), 3.82 (t, *J* = 5.3 Hz, 4H; -N-C*H*$_2$$CH_2$-O-$_{dye}$), 3.76 (m, 2H; Hδ), 3.60 (q, *J* = 7.0 Hz, 4H; -N-C*H*$_2$$CH_{3dye}$), 3.40 (dd, *J* = 12.7, 1.9 Hz, 2H; Hδ'), 2.92 (m, 4H; Hβ$_{Tyr}$, Hβ'$_{Tyr}$), 2.26/2.02 (m, 4H; Hβ, Hβ'), 1.38 (s, 18H; -*t*Bu), 1.27 (t, *J* = 7.0 Hz, 9H; -N-CH_2C*H*$_{3dye}$).

^{13}C-NMR (101 MHz, $CDCl_3$, 25°C): δ(ppm) = 171.3 (Cq; *C*O$_{Tyr}$), 165.9 (Cq; *C*O), 157.4 (Cq; *C*O$_{Boc}$), 156.7/151.3/147.3/143.6/128.1 (Cq; dye, Tyr), 130.4/124.6/122.5/114.4/111.4 (CH; dye, Tyr), 65.3 (CH_2; -N-CH_2*C*H_2-O-$_{dye}$), 58.4 (CH; Cα, Cα$_{Tyr}$), 50.9 (CH_2; Cδ, Cδ'), 49.7 (CH_2; -N-*C*H_2CH_2-O-$_{dye}$), 47.2 (CH; Cγ), 46.0 (CH_2; -N-*C*H_2CH_{3dye}), 37.9 (CH_2; Cβ$_{Tyr}$, Cβ'$_{Tyr}$), 33.5 (CH_2; Cβ, Cβ'), 28.1 (CH_3; C$_{-t}$$_{Bu}$), 12.4 ($CH_3$; -N-$CH_2$*C*$H_{3dye}$).

ESI-MS: m/z: calcd for $C_{70}H_{82}N_{14}O_{14}$: 1343 $[M]^+$; found: 1343.

8.4 General Fmoc-strategy for the Synthesis of the Two-Armed Diketopiperazine Receptor Precursor in Solution



8.4.1 Diketopiperazine Receptor with Attached Fmoc-Protected Gln

451 mg (335.7 µmol, 1 eq) of the diketopiperazine precursor **14** were dissolved in MeOH (7.5 ml) and treated with a solution of 4 M HCl in Dioxan (20 ml) at r.t. for 1 h. After removal of all volatiles under reduced pressure, the residue was triturated with Et_2O to yield a red solid which was isolated by decantation and dried *in vacuo*. The corresponding HCl-salt of **14** was then dissolved in dry CH_2Cl_2 (5 ml) followed by addition of 115 µl (671.4 µmol, 2 eq) $^{i}Pr_2NEt$, ready for the next coupling.

The solution was cooled with an ice bath before a mixture of 511 mg (1.34 mmol, 4 eq) HATU in DMF (1ml), 230 µl (1.34 mmol, 4 eq) $^{i}Pr_2NEt$ and 820 mg (1.34 mmol, 4 eq) Fmoc-L-Gln(Trt)-OH in CH_2Cl_2 (5 ml), was added and stirred at r.t. for 1 h. The reaction mixture was extracted three times with 0.1 M Na-phosphate buffer (pH 5.5) and CH_2Cl_2. The aq. phases were extracted again with CH_2Cl_2. The org. phases were washed with brine and dried over Na_2SO_4. After filtration and evaporation of the solvent under reduced pressure, the crude product was purified by flash chromatography on silica gel (gradient of CH_2Cl_2/MeOH from 99.5:0.5 to 97:3 (v/v)) and preparative-TLC (CH_2Cl_2/MeOH, 90:10, v/v) to afford 697 mg (299.7 µmol, 89%) of the diketopiperazine precursor with attached Fmoc-protected Gln.

$C_{138}H_{130}N_{18}O_{18}$: 2328.62

TLC: MeOH/CH_2Cl_2 (10:90); R_f: 0.68.

ESI-MS: m/z: calcd for $C_{138}H_{129}N_{18}O_{18}Na$: 2350 $[M-H+Na]^+$; found: 2350.

8.4.2 Two-Armed Diketopiperazine Precursor 15

697 mg (299.7 µmol, 1 eq) of *N*-α-Fmoc-protected receptor precursor were dissolved in CH_2Cl_2 (36 ml) followed by addition of 2.2 ml (14.98 mmol, 50 eq) TAEA, which caused the formation of a precipitate after 2-5 min. The suspension was stirred at r.t. for 30 min and then extracted three times with 0.1 M Na-phosphate buffer (pH 5.5) followed by brine. The aq. phases were washed once with CH_2Cl_2 and the org. phases were dried over Na_2SO_4 to yield the corresponding diamine after filtration and removal of all volatiles under reduced pressure.

The diamine was dissolved in dry CH_2Cl_2 (5 ml) followed by addition of 102 µl (599.5 µmol, 2 eq) $^{i}Pr_2NEt$. The solution was cooled with an ice bath before a mixture of 496 mg (1.20 mmol, 4 eq) HCTU in DMF (1.5 ml), 205 µl (1.20 mmol, 4 eq) $^{i}Pr_2NEt$ and 464 mg (1.20 mmol, 4 eq) Fmoc-L-Phe-OH in CH_2Cl_2 (5 ml), was added and stirred at r.t. for 1 h. The reaction mixture was extracted three times with 0.1 M Na-phosphate buffer (pH 5.5) and CH_2Cl_2. The aq. phases were extracted again with CH_2Cl_2 and the org. phases were washed with brine and dried over Na_2SO_4. After filtration and evaporation of the solvent under reduced pressure, the crude product was purified by flash chromatography on silica gel (gradient of CH_2Cl_2/MeOH from 99.5:0.5 to 97:3 (v/v)) and preparative-TLC (CH_2Cl_2/MeOH, 90:10, v/v) to afford 606 mg (231.4 µmol, 77%) of the two-armed diketopiperazine precursor **15**.

$C_{156}H_{148}N_{20}O_{20}$: 2622.97

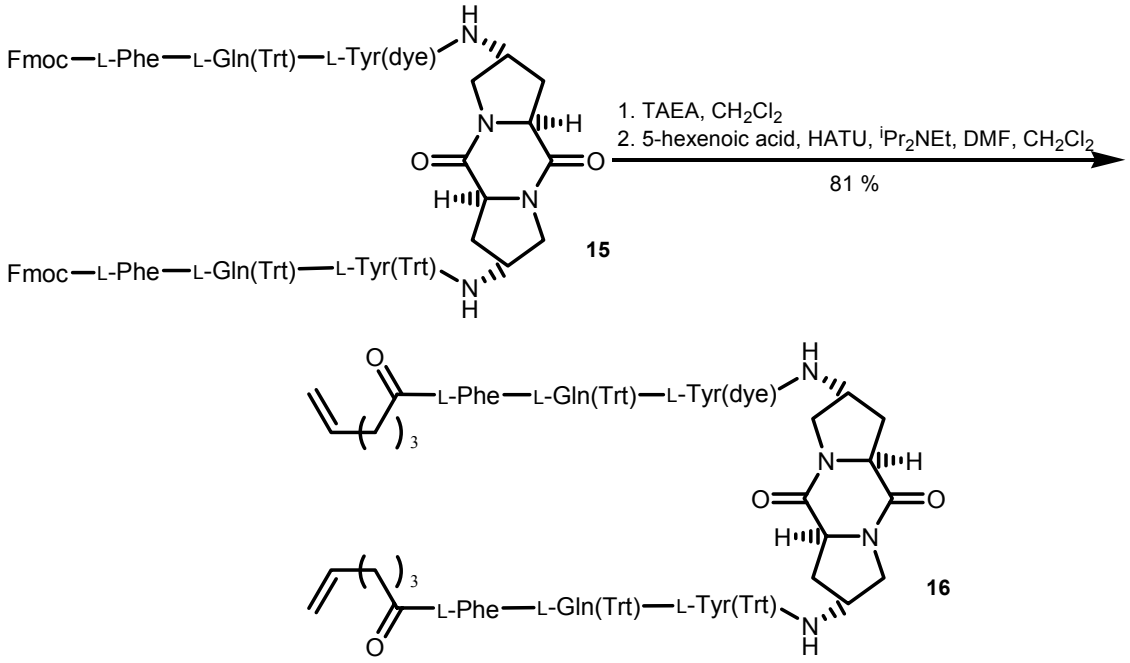
TLC: MeOH/CH_2Cl_2 (5:95); R_f: 0.23.

ESI-MS: m/z: calcd for $C_{156}H_{147}N_{20}O_{20}Na$: 2644 $[M-H+Na]^+$; found: 2644.

9 SYNTHESIS OF MACROCYCLIC DIKETOPIPERAZINE RECEPTOR PROTOTYPES

9.1 Ring Closing Metathesis

9.1.1 Two-Armed Diketopiperazine Receptor Precursor 16



100 mg (38.2 µmol, 1 eq) of the diketopiperazine receptor precursor **15** were dissolved in CH_2Cl_2 (4.5 ml) followed by addition of 0.2 ml (1.92 mmol, 50 eq) TAEA which caused the formation of a precipitate after 2-5 min. The suspension was stirred at r.t. for 30 min and then extracted three times with 0.1 M Na-phosphate buffer (pH 5.5). The aq. phases were washed once with CH_2Cl_2. The org. phases were washed with brine and dried over Na_2SO_4 to yield the corresponding diamine after filtration and removal of all volatiles under reduced pressure.

The diamine was dissolved in dry CH_2Cl_2 (1 ml) followed by addition of 15 µl (76.5 µmol, 2 eq) $^iPr_2NEt$. The solution was cooled with an ice bath before a mixture of 60 mg (152.8 µmol, 4 eq) HATU in DMF (0.5 ml), 15 µl (76.5 µmol, 2 eq) $^iPr_2NEt$ and 20 µl (152.8 µmol, 4eq) 5-hexenoic acid in CH_2Cl_2 (1 ml), was added and stirred at r.t. for 1 h. The reaction mixture was extracted three times with 0.1 M Na-phosphate buffer (pH 5.5) and CH_2Cl_2. The aq. phases were extracted again with CH_2Cl_2 and the org. phases were washed with brine and dried over Na_2SO_4. After filtration and evaporation of the solvent at reduced pressure, the crude product was purified by flash chromatography on silica gel (gradient of

CH_2Cl_2/MeOH from 99.5:0.5 to 96:4 (v/v)), preparative-TLC (CH_2Cl_2/MeOH, 90:10, v/v) and gel filtration (LH 20, CH_2Cl_2/MeOH, 90:10, v/v) to afford 73 mg (30.9 µmol, 81%) of the two-armed diketopiperazine precursor **16**.

$C_{138}H_{144}N_{20}O_{18}$: 2370.74

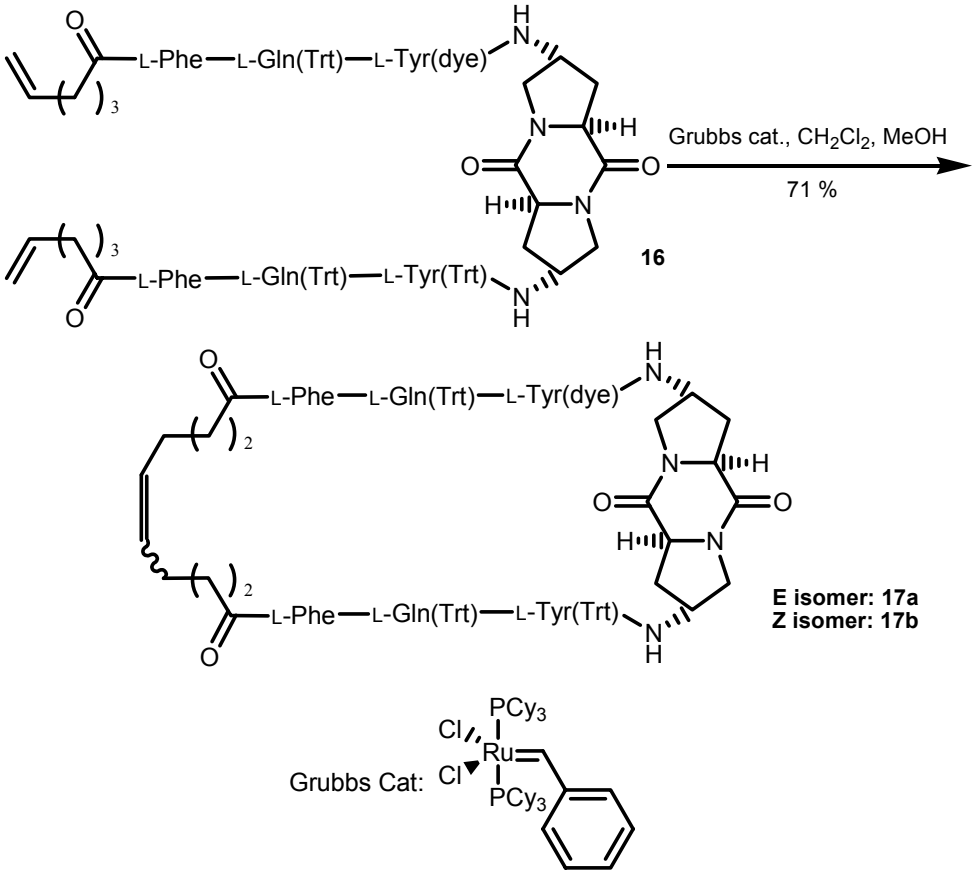
TLC: MeOH/CH_2Cl_2 (10:90); R_f: 0.70.

^{1}H-NMR (500 MHz, 5% CD_3OD in $CDCl_3$, 25°C): δ(ppm) = 8.23 (d, J = 8.4 Hz, 4H; H_{dye}), 7.85 (d, J = 8.8 Hz, 8H; H_{dye}), 7.29 – 7.04 (m, 44H, H_{Tyr}, H_{Phe}, H_{Trt}), 6.74 (d, J = 9.0 Hz, 4H; H_{Tyr}), 6.70 (d, J = 8.6 Hz, 4H; H_{Tyr}), 5.66 (ddt, J = 17.0, 10.3 Hz, 2H; $H\delta_{Hex}$), 4.82 (m, 4H; $H\varepsilon_{Hex}$), 4.49 (dd, J = 10.6, 4.0 Hz, 2H; $H\alpha_{Tyr}$), 4.43 (m, 2H; Hγ), 4.39 (m, 2H; Hα), 4.14 (dd, J = 10.4, 4.2 Hz, 2H; $H\alpha_{Phe}$), 3.98 (m, 4H; -N-CH_2-CH_2-O-$_{dye}$), 3.88 (m, 2H; $H\alpha_{Gln}$), 3.68 (t, J = 5.5 Hz, 4H; -N-CH_2-CH_2-O-$_{dye}$), 3.61 (dd, J = 12.5, 5.9 Hz, 2H; Hδ), 3.50 (q, J = 7.1 Hz, 2H; -N-CH_2-CH_{3dye}), 3.46 (m, 2H; Hδ'), 3.24 (m, 2H; $H\beta_{Tyr}$), 2.93 (dd, J = 14.2, 4.0 Hz, 2H; $H\beta_{Phe}$), 2.81 (dd, J = 14.3, 11.0 Hz, 2H; $H\beta'_{Tyr}$), 2.57 (dd, J = 14.1, 10.1 Hz, 2H; $H\beta'_{Phe}$), 2.26 (m, 2H; Hβ), 2.15 (m, 2H; Hβ'), 2.06 (m, 2H;$H\gamma_{Gln}$), 1.96 (m, 2H;$H\gamma'_{Gln}$), 1.83 (m, 4H; $H\alpha_{Hex}$), 1.76 (m, 4H; $H\gamma_{Hex}$), 1.68 (m, 4H; $H\beta_{Gln}$, $H\beta'_{Gln}$), 1.40 (tt, J = 7.1, 6.7 Hz, 4H; $H\beta_{Hex}$), 1.19 (t, J = 7.1 Hz, 6H; -N-CH_2-CH_{3dye}).

^{13}C-NMR (125.6 MHz, 5% CD_3OD in $CDCl_3$, 25°C): δ(ppm) = 175.2/173.9/173.2/171.6/ 170.9 (Cq; *C*O), 167.3 (Cq; Cε), 157.1 (Cq; Tyr), 155.5/152.1/147.1 (Cq; dye), 144.2 (Cq; Trt), 143.0 (Cq; dye), 137.6 (CH, *C*δ_{Hex}), 136.4 (Cq; Phe), 130.2 (Cq; Tyr), 128.9 (CH; *C*H_{Tyr}), 128.6 (CH; *C*H_{Phe}), 127.9 (CH; *C*H_{Trt}), 127.1/124.7/122.1 (CH; dye), 115.1 (CH_2; $C\varepsilon_{Hex}$), 112.3 (CH; *C*H_{Tyr}), 112.1 (CH; dye), 70.5 (Cq; Trt), 65.2 (CH_2; -N-CH_2-*C*H_2-O-$_{dye}$), 58.8 (CH, Cα), 55.8 (CH; $C\alpha_{Phe}$), 55.2 (CH; $C\alpha_{Gln}$), 54.6 (CH; $C\alpha_{Tyr}$), 50.1 (CH_2; Cδ, Cδ'), 49.9 (CH_2; -N-*C*H_2-CH_2-O-$_{dye}$), 48.0 (CH; Cγ), 46.3 (CH_2; -N-*C*H_2-CH_{3dye}), 36.5 (CH_2; $C\beta_{Phe}$, $C\beta'_{Phe}$), 35.8 (CH_2; $C\beta_{Tyr}$, $C\beta'_{Tyr}$), 34.9 (CH_2; $C\alpha_{Hex}$), 33.6 (CH_2; Cβ, Cβ'), 32.9 (CH_2; $C\gamma_{Gln}$, $C\gamma'_{Gln}$), 32.8 (CH_2; $C\gamma_{Hex}$), 25.7 (CH_2; $C\beta_{Gln}$, $C\beta'_{Gln}$), 24.2 (CH_2; $C\beta_{Hex}$), 12.2 (CH_3; -N-CH_2-*C*H_{3dye}).

ESI-MS: m/z: calcd for $C_{138}H_{143}N_{20}O_{18}Na$: 2392 $[M-H+Na]^+$; found: 2392.

9.1.2 Macrocyclic Diketopiperazine Receptor Prototype 17



73.3 mg (30.9 µmol, 1 eq) of the diketopiperazine precursor **16** were dissolved in a 99:1 (v/v) mixture of CH_2Cl_2 and MeOH (30 ml). The mixture was heated under reflux for 1 h. A solution of 11 mg (13.3 µmol, 0.4 eq) of Grubbs cat. in CH_2Cl_2 (2 ml) was added and heating was continued for 24 h. The same amount of Grubbs cat. (13.3 µmol, 0.4 eq) in CH_2Cl_2 (2 ml) was added again and reflux maintained for 24 h. After removal of all volatiles under reduced pressure, preparative-TLC (MeOH/CH2Cl2, 10:90, v/v) afforded 51.1 mg (21.8 µmol, 71%) of a mixture of E- and Z-isomers **17a** and **17b** as a red solid, which were separated by normal-phase HPLC chromatography (*Macherey-Nagel Nucleosil 100.5* (250 mm x 4.6 mm), gradient of CH_2Cl_2/MeOH from 95:5 to 90:10 (v/v) in 30 min).

$C_{136}H_{140}N_{20}O_{18}$: 2342.69

TLC: MeOH/CH_2Cl_2 (10:90); R_f: 0.75.

17a:

^{1}H-NMR (600 MHz, DMSO, 25°C): δ(ppm) = 8.56 (s, 2H; -N*H*$_{Trt}$*)*, 8.37 (d, *J* = 9.1 Hz, 4H; H$_{dye}$), 8.05 (m, 2H; -N*H*$_{Gln}$), 7.97 (m, 2H; -N*H*$_{Phe}$), 7.95 (m, 2H; -N*H*$_{Pro}$), 7.94 (d, *J* = 9.1 Hz, 4H; H$_{dye}$), 7.88 (m, 2H; -N*H*$_{Tyr}$), 7.84 (d, *J* = 9.1 Hz, 4H; H$_{dye}$), 7.30-7.15 (m, 40H, H$_{Phe}$, H$_{Trt}$), 7.08 (d, *J* = 8.5 Hz, 4H; H$_{Tyr}$), 6.91 (d, *J* = 9.2 Hz, 4H; H$_{dye}$), 6.79 (d, *J* = 8.5 Hz, 4H; H$_{Tyr}$), 5.21 (m, 2H; Hδ$_{Hex}$), 4.45 (m, 2H; Hα$_{Phe}$), 4.36 (t, *J* = 7.9 Hz, 2H; Hα), 4.28 (m, 2H; Hα$_{Tyr}$), 4.11 (m, 2H; Hγ), 4.09 (m, 4H; -N-CH$_2$-C*H*$_2$-O-$_{dye}$), 4.04 (m, 2H; Hα$_{Gln}$), 3.80 (m, 4H; -N-C*H*$_2$-CH$_2$-O-$_{dye}$), 3.62 (m, 2H; Hδ), 3.56 (m, 4H; -N-C*H*$_2$-CH$_{3dye}$), 3.18 (br d, *J* = 11.3 Hz, 2H; Hδ'), 3.03 (m, 2H; Hβ$_{Phe}$), 2.96 (m, 2H; Hβ$_{Tyr}$), 2.82 (m, 2H; Hβ'$_{Tyr}$), 2.74 (m, 2H; Hβ'$_{Phe}$), 2.26 (m, 4H;Hγ$_{Gln}$,, Hγ'$_{Gln}$,), 2.13 (m, 2H; Hβ), 2.05 (m, 2H; Hβ'), 2.00 (m, 4H; Hα$_{Hex}$), 1.81 (m, 4H; Hβ$_{Gln}$,), 1.78 (m, 4H; Hγ$_{Hex}$), 1.72 (m, 4H; Hβ'$_{Gln}$,), 1.39 (m, 4H; Hβ$_{Hex}$), 1.17 (t, *J* = 7.1 Hz, 6H; -N-CH$_2$-C*H*$_{3dye}$).

^{13}C-NMR (125.6 MHz, DMSO, 25°C): δ(ppm) = 157 (Cq; Tyr), 156/151/146 (Cq; dye), 145 (Cq; Trt), 143 (Cq; dye), 138 (Cq; Phe), 130 (CH; *C*H$_{Tyr}$), 130 (CH; Cδ$_{Hex}$), 130 (Cq; Tyr), 126 – 129 (CH; *C*H$_{Phe}$, *C*H$_{Trt}$), 125/124/122 (CH; dye), 114 (CH; *C*H$_{Tyr}$), 112 (CH; dye), 70 (Cq; Trt), 65 (CH$_2$; -N-CH$_2$-*C*H$_2$-O-$_{dye}$), 57 (CH, Cα), 54 (CH; Cα$_{Tyr}$), 53 (CH; Cα$_{Phe}$), 52 (CH; Cα$_{Gln}$), 50 (CH$_2$; Cδ, Cδ'), 49 (CH$_2$; -N-*C*H$_2$-CH$_2$-O-$_{dye}$), 47 (CH; Cγ), 45 (CH$_2$; -N-*C*H$_2$-CH$_{3dye}$), 37 (CH$_2$; Cβ$_{Phe}$, Cβ'$_{Phe}$), 36 (CH$_2$; Cβ$_{Tyr}$, Cβ'$_{Tyr}$), 35 (CH$_2$; Cα$_{Hex}$), 33 (CH$_2$; Cβ, Cβ'), 32 (CH$_2$; Cγ$_{Gln}$, Cγ'$_{Gln}$), 31 (CH$_2$; Cγ$_{Hex}$), 27 (CH$_2$; Cβ$_{Gln}$, Cβ'$_{Gln}$), 25 (CH$_2$; Cβ$_{Hex}$), 12 (CH$_3$; -N-CH$_2$-*C*H$_{3dye}$).

17b:

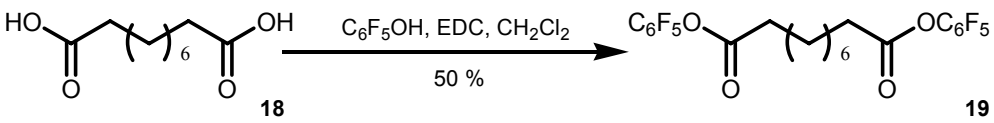
^{1}H-NMR (600 MHz, DMSO, 25°C): δ(ppm) = 8.54 (s, 2H; -N*H*$_{Trt}$*)*, 8.35 (d, *J* = 9.1 Hz, 4H; H$_{dye}$), 8.06 (m, 2H; -N*H*$_{Gln}$), 7.93 (m, 2H; -N*H*$_{Phe}$), 7.92 (d, *J* = 9.1 Hz, 4H; H$_{dye}$), 7.89 (m, 2H; -N*H*$_{Pro}$), 7.87 (m, 2H; -N*H*$_{Tyr}$), 7.83 (d, *J* = 9.3 Hz, 4H; H$_{dye}$), 7.30-7.15 (m, 40H, H$_{Phe}$, H$_{Trt}$), 7.07 (d, *J* = 8.7 Hz, 4H; H$_{Tyr}$), 6.91 (d, *J* = 9.2 Hz, 4H; H$_{dye}$), 6.78 (d, *J* = 8.5 Hz, 4H; H$_{Tyr}$), 5.21 (m, 2H; Hδ$_{Hex}$), 4.44 (m, 2H; Hα$_{Phe}$), 4.37 (t, *J* = 8.2 Hz, 2H; Hα), 4.23 (m, 2H; Hα$_{Tyr}$), 4.08 (m, 2H; Hγ), 4.05 (m, 4H; -N-CH$_2$-C*H*$_2$-O-$_{dye}$), 3.98 (m, 2H; Hα$_{Gln}$), 3.76 (m, 4H; -N-C*H*$_2$-CH$_2$-O-$_{dye}$), 3.61 (m, 2H; Hδ), 3.53 (m, 4H; -N-C*H*$_2$-CH$_{3dye}$), 3.18 (br d, *J* = 11.0 Hz, 2H; Hδ'), 3.02 (m, 2H; Hβ$_{Phe}$), 2.95 (m, 2H; Hβ$_{Tyr}$), 2.82 (m, 2H; Hβ'$_{Tyr}$), 2.71 (m, 2H; Hβ'$_{Phe}$), 2.21 (m, 4H;Hγ$_{Gln}$, Hγ'$_{Gln}$,), 2.15 (m, 2H; Hβ), 2.05 (m, 2H; Hβ'), 2.00 (m, 4H; Hα$_{Hex}$), 1.80 (m, 4H; Hβ$_{Gln}$,), 1.78 (m, 4H; Hγ$_{Hex}$), 1.72 (m, 4H; Hβ'$_{Gln}$,), 1.38 (m, 4H; Hβ$_{Hex}$), 1.12 (t, *J* = 6.9 Hz, 6H; -N-CH$_2$-C*H*$_{3dye}$).

^{13}C-NMR (125.6 MHz, DMSO, 25°C): δ(ppm) = 130 (CH; *C*H$_{Tyr}$), 130 (CH; Cδ$_{Hex}$), 130 (Cq; Tyr), 126–129 (CH; *C*H$_{Phe}$, *C*H$_{Trt}$), 126/125/122 (CH; dye), 114 (CH; *C*H$_{Tyr}$), 111.5 (CH; dye), 65 (CH$_2$; -N-CH$_2$-*C*H$_2$-O-$_{dye}$), 58 (CH, Cα), 55 (CH; Cα$_{Tyr}$), 53.5 (CH; Cα$_{Phe}$), 52 (CH; Cα$_{Gln}$), 50.5 (CH$_2$; Cδ, Cδ'), 48 (CH$_2$; -N-*C*H$_2$-CH$_2$-O-$_{dye}$), 47 (CH; Cγ), 45 (CH$_2$; -N-*C*H$_2$-CH$_{3dye}$), 37 (CH$_2$; Cβ$_{Phe}$, Cβ'$_{Phe}$), 36 (CH$_2$; Cβ$_{Tyr}$, Cβ'$_{Tyr}$), 36 (CH$_2$; Cα$_{Hex}$), 32.5 (CH$_2$; Cβ, Cβ'), 32 (CH$_2$; Cγ$_{Gln}$, Cγ'$_{Gln}$), 27.5 (CH$_2$; Cβ$_{Gln}$, Cβ'$_{Gln}$), 26 (CH$_2$; Cγ$_{Hex}$), 25 (CH$_2$; Cβ$_{Hex}$), 12 (CH$_3$; -N-CH$_2$-*C*H$_{3dye}$).

ESI-MS: m/z: calcd for $C_{136}H_{140}N_{20}O_{18}Na$: 2365 $[M+Na]^+$; found: 2365.

9.2 Amide Bond Formation

9.2.1 Sebacic Acid Activated as Pentafluorophenol esters 19



18 **19**

10 mg (49.4 µmol, 1 eq) sebacic acid **18** were dissolved in CH_2Cl_2 (0.1 ml) followed by addition of 20 mg (108.7 µmol, 2.2 eq) 2,3,4,5,6-pentafluorophenol and 14 mg (74.1 µmol, 1.5 eq) EDC, and the mixture was allowed to stir at r.t. for 1 h. The reaction mixture was then carefully acidified to pH 3 with 1 M HCl and extracted with CH_2Cl_2. The aq. phase was extracted again with CH_2Cl_2 and the org. phases were washed with brine and dried over Na_2SO_4. Filtration and evaporation of the solvent under reduced pressure yielded the crude product which was purified by flash chromatography on silica gel (gradient of EtOAc/Pentane from 0.5:99.5 to 2:98 (v/v)) to yield 13 mg (24.7 µmol, 50%) of the activated sebacic acid **19** as a white solid.

$C_{22}H_{16}F_{10}O_4$: 534.34

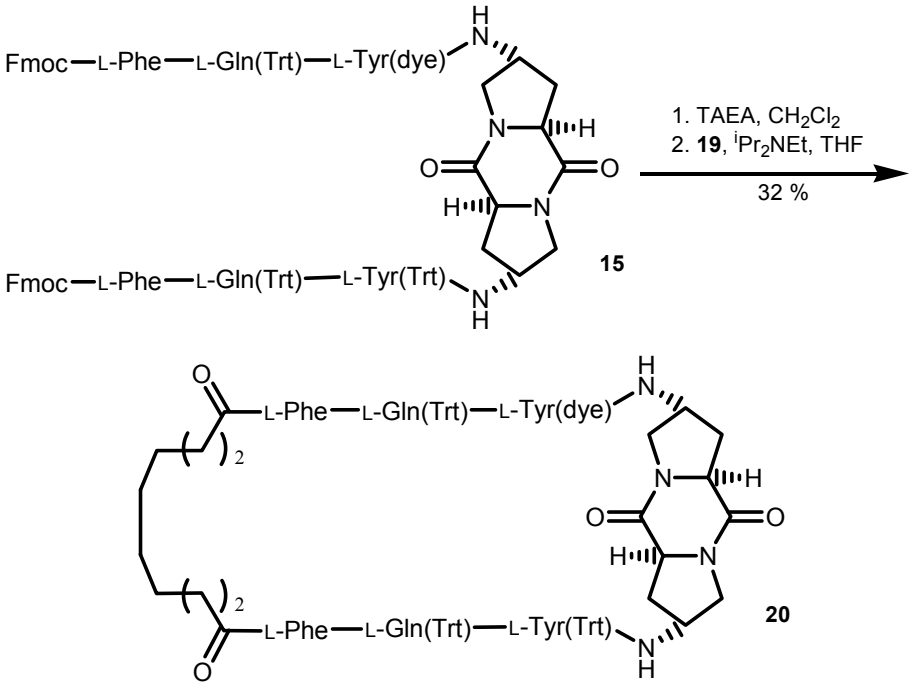
TLC: EtOAc/Pentane (2:98); R_f: 0.59 ($KMnO_4$).

^{1}H-NMR (400 MHz, $CDCl_3$, 25°C): δ(ppm) = 2.67 (t, J = 7.0 Hz, 4H; Hα$_{Seb}$), 1.78 (quin, J = 7.6 Hz, 4H; Hβ$_{Seb}$), 1.41 (m, 8H; Hγ$_{Seb}$, Hδ$_{Seb}$).

^{13}C-NMR (101 MHz, $CDCl_3$, 25°C): δ(ppm) = 169.5 (Cq; *C*O), 33.3 (CH_2; $C\alpha_{Seb}$), 28.9/28.7 (CH_2; $C\gamma_{Seb}$, $C\delta_{Seb}$), 24.7 (CH_2; $C\beta_{Seb}$).

ESI-MS: m/z: calcd for $C_{22}H_{16}N_{10}O_4Na$: 557 $[M+Na]^+$; found: 557.

9.2.2 Macrocyclic Diketopiperazine Receptor Prototype 20



50 mg (19.1 µmol, 1 eq) of the diketopiperazine receptor precursor **15** were dissolved in CH_2Cl_2 (2.3 ml) followed by addition of 143 µl (955.0 µmol, 50 eq) TAEA which caused the formation of a precipitate after 2-5 min. The suspension was stirred at r.t. for 30 min and then extracted three times with 0.1 M Na-phosphate buffer (pH 5.5). The aq. phases were washed once with CH_2Cl_2 and the org. phases were washed with brine and dried over Na_2SO_4 to yield the corresponding diamine, after filtration and removal of all volatiles under reduced pressure.

A solution of the diamine in THF (5 ml) and a solution of 12 mg (22.9 µmol, 1.2 eq) of the activated sebacic acid **19** in THF (5 ml) were simultaneously added dropwise over a period of 5 h to a solution of 20 µl (114.6 µmol, 6 eq) $^iPr_2NEt$ in THF (10 ml) at r.t. After stirring for another 17 h, the reaction mixture was extracted three times with 0.1 M Na-phosphate buffer (pH 5.5) and CH_2Cl_2. The aq. phases were extracted again with CH_2Cl_2. The

org. phases were washed with brine and dried over Na_2SO_4. After filtration and evaporation of the solvent under reduced pressure, the crude product was purified by preparative-TLC (CH_2Cl_2/MeOH, 90:10, v/v) and gel filtration (LH 20, CH_2Cl_2/MeOH, 90:10, v/v) to yield 14 mg (6.0 µmol, 32%) of the macrocyclic diketopiperazine receptor **20** as a red solid.

$C_{136}H_{142}N_{20}O_{18}$: 2344.71

TLC: CH_2Cl_2/MeOH (90:10); R_f: 0.5.

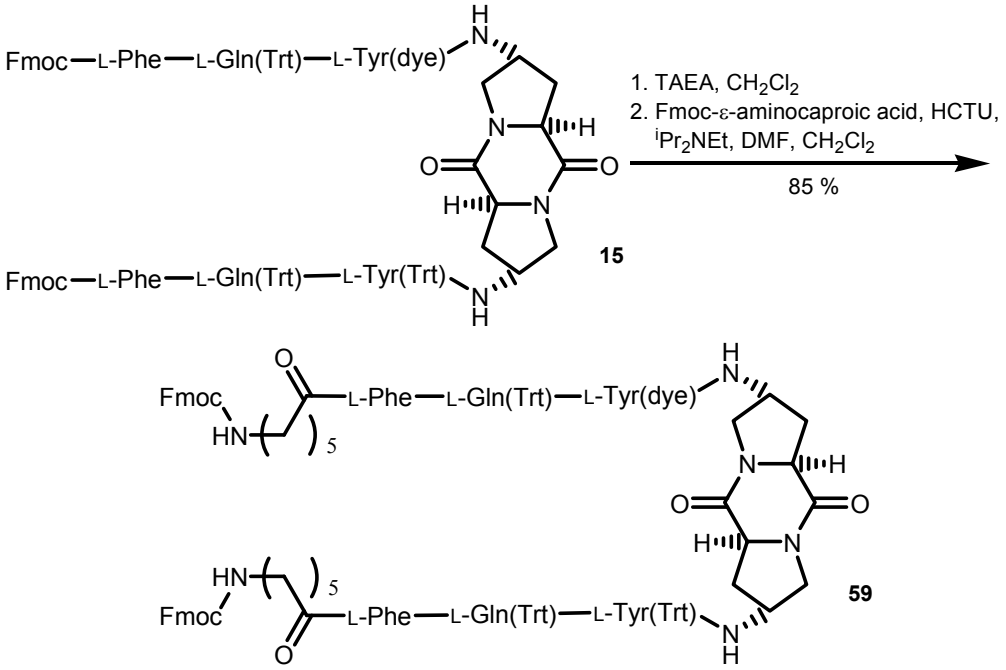
^{1}H-NMR (500 MHz, DMSO, 25°C): δ(ppm) = 8.55 (s, 2H; -N*H*$_{Trt}$*)*, 8.35 (d, *J* = 9.1 Hz, 4H; H_{dye}), 8.02 (m, 2H; -N*H*$_{Pro}$), 8.00 (m, 2H; -N*H*$_{Gln}$), 7.99 (m, 2H; -N*H*$_{Tyr}$), 7.93 (d, *J* = 9.1 Hz, 4H; H_{dye}), 7.92 (m, 2H; -N*H*$_{Phe}$), 7.83 (d, *J* = 9.2 Hz, 2H; H_{dye}), 7.22 (m, 40H, H_{Phe}, H_{Trt}), 7.07 (d, *J* = 8.8 Hz, 4H; H_{Tyr}), 6.90 (d, *J* = 9.3 Hz, 4H; H_{dye}), 6.78 (d, *J* = 8.6 Hz, 4H; H_{Tyr}), 4.42 (m, 2H; Hα$_{Tyr}$), 4.37 (t, *J* = 8.1 Hz, 2H; Hα), 4.28 (dd, *J* = 5.7 Hz, 2H; Hα$_{Phe}$), 4.10 (m, 2H; Hγ), 4.07 (t, *J* = 5.1 Hz, 4H; -N-CH_2-C*H*$_2$-O-$_{dye}$), 4.05 (m, 2H; Hα$_{Gln}$), 3.78 (t, *J* = 4.9 Hz, 4H; -N-C*H*$_2$-CH_2-O-$_{dye}$), 3.59 (m, 2H; Hδ), 3.54 (q, *J* = 7.1, 4H; -N-C*H*$_2$-CH_{3dye}), 3.17 (br d, *J* = 11.3 Hz, 2H; Hδ'), 3.02 (m, *J* = 10.2 Hz, 2H; Hβ$_{Tyr}$), 2.94 (m, *J* = 8.9 Hz, 2H; Hβ$_{Phe}$), 2.76 (dd, 2H; Hβ'$_{Phe}$), 2.74 (dd, *J* = 7.9, 5.7 Hz, 2H; Hβ'$_{Tyr}$), 2.25 (m, 4H; Hγ$_{Gln}$, Hγ'$_{Gln}$), 2.12 (m, 2H; Hβ), 2.01 (m, 2H; Hβ'), 1.97 (m, 4H; Hα$_{Seb}$), 1.77 (m, 4H; Hβ$_{Gln}$, Hβ'$_{Gln}$), 1.30 (m, 4H; Hβ$_{Seb}$), 1.14 (t, *J* = 7.0 Hz, 6H; -N-CH_2-C*H*$_{3dye}$), 1.04 (m, 8H; Hγ$_{Seb}$, Hδ$_{Seb}$).

^{13}C-NMR (125.6 MHz, DMSO, 25°C): δ(ppm) = 172.7/171.8/171.5/170.9/170.8 (Cq; *C*O), 165.5 (Cq; Cε), 156.9 (Cq; Tyr), 156.2/151.6/146.8 (Cq; dye), 144.8 (Cq; Trt), 142.8 (Cq; dye), 137.8 (Cq; Phe), 130.1 (CH; *C*H$_{Tyr}$), 129.7 (Cq; Tyr), 129.6/128.0 (CH; *C*H$_{Phe}$), 128.5/127.5 (CH; *C*H$_{Trt}$), 126.3/125.0/122.5 (CH; dye), 114.0 (CH; *C*H$_{Tyr}$), 111.7 (CH; dye), 69.2 (Cq; Trt), 65.2 (CH_2; -N-CH_2-*C*H_2-O-$_{dye}$), 58.3 (CH, Cα), 54.6 (CH; Cα$_{Phe}$), 54.3 (CH; Cα$_{Tyr}$), 52.9 (CH; Cα$_{Gln}$), 50.8 (CH_2; Cδ, Cδ'), 49.1 (CH_2; -N-*C*H_2-CH_2-O-$_{dye}$), 47.2 (CH; Cγ), 45.3 (CH_2; -N-*C*H_2-CH_{3dye}), 37.0 (CH_2; Cβ$_{Tyr}$, Cβ'$_{Tyr}$), 36.1 (CH_2; Cβ$_{Phe}$, Cβ'$_{Phe}$), 35.2 (CH_2; Cα$_{Seb}$), 32.8 (CH_2; Cβ, Cβ'), 32.4 (CH_2; Cγ$_{Gln}$, Cγ'$_{Gln}$), 28.6/28.5 (CH_2; Cγ$_{Seb}$, Cδ$_{Seb}$), 27.9 (CH_2; Cβ$_{Gln}$, Cβ'$_{Gln}$), 25.1 (CH_2; Cβ$_{Seb}$), 12.0 (CH_3; -N-CH_2-*C*H_{3dye}).

ESI-MS: m/z: calcd for $C_{136}H_{142}N_{20}O_{18}Na$: 2367 $[M+Na]^+$; found: 2367.

9.3 Disulfide Bridge Formation

9.3.1 Two-Armed Diketopiperazine Receptor Precursor 21



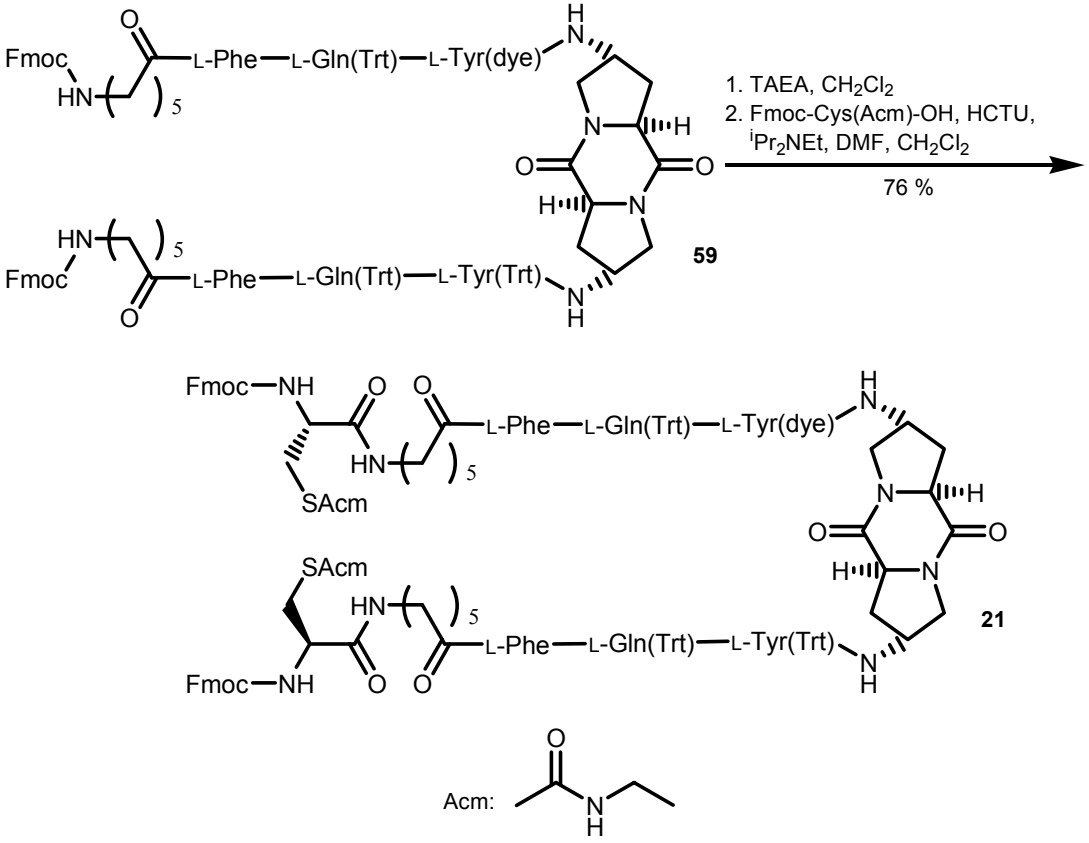
310 mg (118.3 µmol, 1 eq) of the diketopiperazine receptor precursor **15** were dissolved in CH_2Cl_2 (10 ml) followed by addition of 0.9 ml (5.92 mmol, 50 eq) TAEA which caused the formation of a precipitate after 2-5 min. The suspension was stirred at r.t. for 30 min and then extracted three times by 0.1 M Na-phosphate buffer (pH 5.5). The aq. phases were washed once with CH_2Cl_2 and the org. phases were washed with brine and dried over Na_2SO_4 to yield the corresponding diamine, after filtration and removal of all volatiles under reduced pressure.

The diamine was dissolved in dry CH_2Cl_2 (3.5 ml) followed by addition of 41 µl (236.6 µmol, 2 eq) $^iPr_2NEt$. The solution was cooled with an ice bath before a mixture of 196 mg (473.2 µmol, 4 eq) HCTU in DMF (1 ml), 41 µl (236.6 µmol, 2 eq) $^iPr_2NEt$ and 167 mg (473.2 µmol, 4eq) Fmoc-ε-aminocaproic acid in CH_2Cl_2 (3.5 ml), was added and stirred at r.t. for 1 h. The reaction mixture was extracted three times with 0.1 M Na-phosphate buffer (pH 5.5) and CH_2Cl_2. The aq. phases were extracted again with CH_2Cl_2. The org. phases were washed with brine and dried over Na_2SO_4. After filtration and evaporation of the solvent under reduced pressure, the crude product was purified by flash chromatography on silica gel (gradient of CH_2Cl_2/MeOH from 99.5:0.5 to 96:4 (v/v)) to afford 286 mg (100.5 µmol, 85%) of the Fmoc-protected diketopiperazine precursor **59**.

$C_{168}H_{170}N_{22}O_{22}$: 2849.28

TLC: MeOH/CH_2Cl_2 (10:90); R_f: 0.54.

ESI-MS: m/z: calcd for $C_{168}H_{169}N_{22}O_{22}Na$: 2871 $[M-H+Na]^+$; found: 2871.



143 mg (50.3 µmol, 1 eq) of the diketopiperazine receptor precursor **59** were dissolved in CH_2Cl_2 (5 ml) followed by addition of 0.4 ml (2.51 mmol, 50 eq) TAEA which caused the formation of a precipitate after 2-5 min. The suspension was stirred at r.t. for 30 min and then extracted three times with 0.1 M Na-phosphate buffer (pH 5.5). The aq. phases were washed once with CH_2Cl_2 and the org. phases were washed with brine and dried over Na_2SO_4 to yield the corresponding diamine, after filtration and removal of all volatiles under reduced pressure.

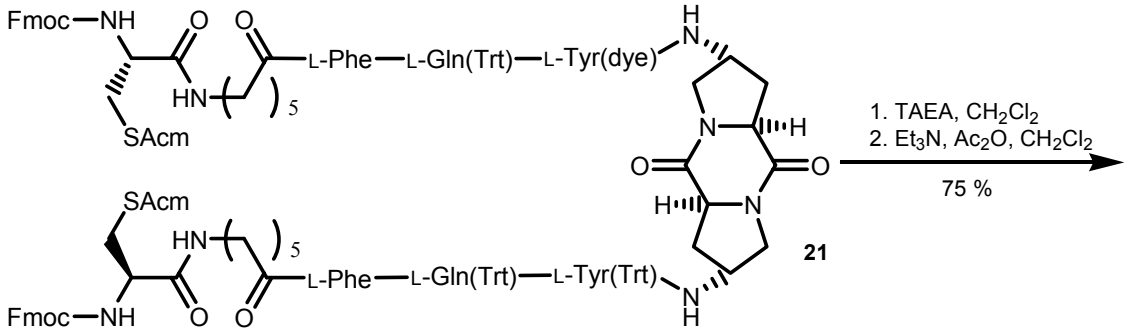
The diamine was dissolved in dry CH_2Cl_2 (2 ml) followed by addition of 17 µl (100.6 µmol, 2 eq) $^iPr_2NEt$. The solution was cooled with an ice bath before a mixture of 83 mg (201.2 µmol, 4 eq) HCTU in DMF (0.5 ml), 17 µl (100.6 µmol, 2 eq) $^iPr_2NEt$ and 83 mg (201.2 µmol, 4eq) Fmoc-Cys(Acm)-OH in CH_2Cl_2 (2 ml), was added and stirred at r.t. for 1 h. The reaction mixture was extracted three times with 0.1 M Na-phosphate buffer (pH 5.5)

and CH_2Cl_2. The aq. phases were extracted again with CH_2Cl_2. The org. phases were washed with brine and dried over Na_2SO_4. After filtration and evaporation of the solvent under reduced pressure, the crude product was purified by flash chromatography on silica gel (gradient of CH_2Cl_2/MeOH from 99.5:0.5 to 94:6 (v/v)) to yield 121 mg (38.0 µmol, 76%) of the Fmoc-protected diketopiperazine precursor **21**.

$C_{180}H_{190}N_{26}O_{26}S_2$: 3197.72

TLC: MeOH/CH_2Cl_2 (10:90); R_f: 0.63.

ESI-MS: m/z: calcd for $C_{180}H_{190}N_{26}O_{26}S_2Na$: 3220 $[M+Na]^+$; found: 3220.



121 mg (38.0 µmol, 1 eq) of the diketopiperazine receptor precursor **21** were dissolved in CH_2Cl_2 (5 ml) followed by addition of 0.3 ml (1.90 mmol, 50 eq) TAEA which caused the formation of a precipitate after 2-5 min. The suspension was stirred at r.t. for 30 min and then extracted three times with phosphate buffer (pH 5.5). The aq. phases were washed once with CH_2Cl_2 and the org. phases were washed and dried over Na_2SO_4 to yield the corresponding diamine, after filtration and removal of all volatiles under reduced pressure.

The diamine was dissolved in dry CH_2Cl_2 (5 ml) followed by addition of 16 µl (114.0 µmol, 3 eq) Et_3N and 11 µl (114.0 µmol, 3 eq) Ac_2O. After stirring for 30 min, the reaction mixture was extracted three times with 0.1 M Na-phosphate buffer (pH 5.5) and CH_2Cl_2. The aq. phases were extracted again with CH_2Cl_2. The org. phases were washed with brine and dried over Na_2SO_4. After filtration and evaporation of the solvent under reduced pressure, the crude product was purified by preparative-TLC (CH_2Cl_2/MeOH, 90:10, v/v) and gel filtration (LH 20, CH_2Cl_2/MeOH, 90:10, v/v) to afford 81 mg (28.4 µmol, 75%) of the two-armed diketopiperazine precursor **22**.

$C_{154}H_{174}N_{26}O_{24}S_2$: 2837.32

TLC: MeOH/CH_2Cl_2 (10:90); R_f: 0.47.

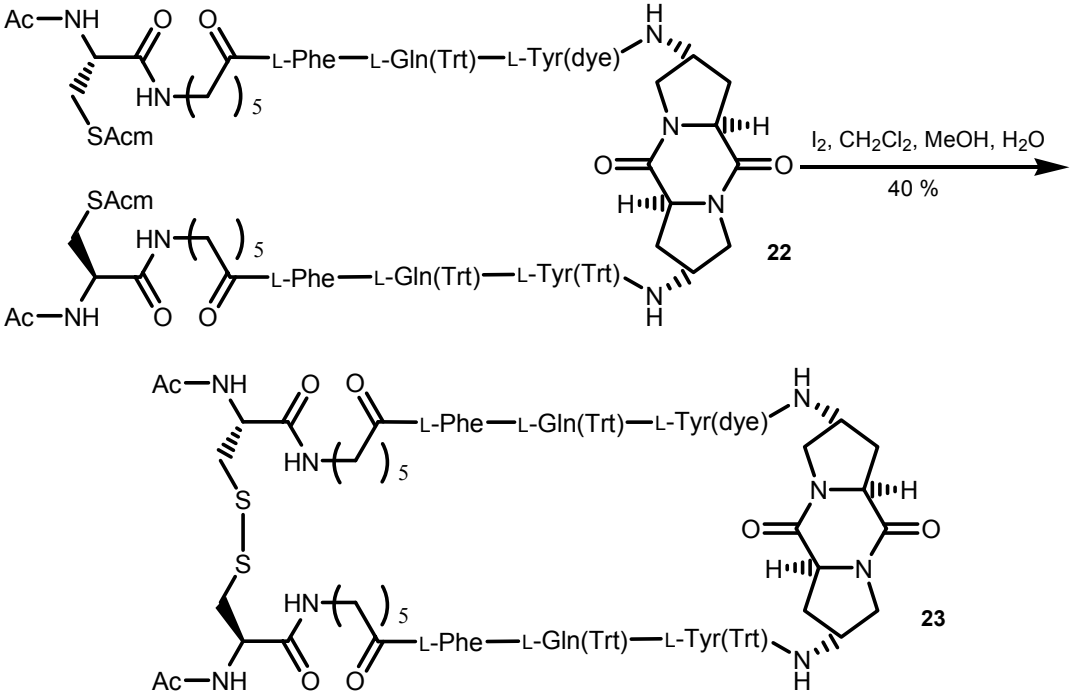
^{1}H-NMR (500 MHz, DMSO, 25°C): δ(ppm) = 8.58 (s, 2H; -N*H*$_{Trt}$), 8.55 (t, *J* = 6.0, 2H; -N*H*$_{Acm}$), 8.35 (d, *J* = 8.9 Hz, 4H; H_{dye}), 8.32 (m, 2H; -N*H*$_{Pro}$), 8.17 (d, *J* = 7.1 Hz, 2H; -N*H*$_{Gln}$), 8.14 (d, *J* = 8.4 Hz, 2H; -N*H*$_{Tyr}$), 7.98 (d, *J* = 7.9 Hz, 2H; -N*H*$_{Phe}$), 7.93 (d, *J* = 8.9 Hz, 4H; H_{dye}), 7.91 (m, 2H; -N*H*$_{Ahx}$), 7.89 (m, 2H; -N*H*$_{Cys}$), 7.84 (d, *J* = 9.0 Hz, 4H; H_{dye}), 7.23-7.12 (m, 40H, H_{Phe}, H_{Trt}), 7.09 (d, *J* = 8.3 Hz, 4H; H_{Tyr}), 6.91 (d, *J* = 9.2 Hz, 4H; H_{dye}), 6.79 (d, *J* = 8.4 Hz, 4H; H_{Tyr}), 4.51 (m, 2H; Hα$_{Phe}$), 4.43 (m, 2H; Hα$_{Tyr}$), 4.37 (m, 2H; Hα$_{Cys}$), 4.27 (m, 2H; Hα), 4.23 (m, 2H; C*H*$_{2Acm}$), 4.19 (m, 2H; Hα$_{Gln}$), 4.17 (m, 2H; C*H*$_{2Acm}$), 4.14 (m, 2H; Hγ), 4.08 (m, 4H; -N-CH$_2$-C*H*$_2$-O-$_{dye}$), 3.78 (m, 4H; -N-C*H*$_2$-CH$_2$-O-$_{dye}$), 3.61 (m, 2H; Hδ), 3.55 (q, *J* = 6.8 Hz, 4H; -N-C*H*$_2$-CH$_{3dye}$), 3.15 (m, 2H; Hδ'), 2.97 (m, 2H; Hβ$_{Phe}$), 2.95 (m, 4H; Hα$_{Ahx}$), 2.87 (m, 2H; Hβ$_{Cys}$), 2.82 (dd, *J* = 13.5, 5.3 Hz, 2H; Hβ$_{Tyr}$), 2.76 (m, 2H; Hβ'$_{Cys}$), 2.67 (m, 2H; Hβ'$_{Phe}$), 2.65 (m, 2H; Hβ'$_{Tyr}$), 2.29 (m, 4H; Hγ$_{Gln}$, Hγ'$_{Gln}$), 2.10 (m, 2H; Hβ), 1.96 (m, *J* = 5.7 Hz, 4H; Hε$_{Ahx}$), 1.85 (m, 2H; Hβ'), 1.84 (s, 3H; -COC*H*$_3$), 1.83 (s, 3H; -COC*H*$_3$), 1.81 (m, 2H; Hβ$_{Gln}$), 1.69 (m, 2H; Hβ'$_{Gln}$), 1.31 (m, 4H; Hδ$_{Ahx}$), 1.28 (m, 4H; Hβ$_{Ahx}$), 1.15 (t, *J* = 6.8 Hz, 6H; -N-CH$_2$-C*H*$_{3dye}$), 1.04 (m, *J* = 14.9, 7.7 Hz, 4H; Hγ$_{Ahx}$).

^{13}C-NMR (125.6 MHz, DMSO, 25°C): δ(ppm) = 72.2/171.8/171.6/171.1/170.6/ 170.0/169.6/169.3 (Cq; *C*O), 165.7 (Cq; Cε), 156.9 (Cq; Tyr), 156.2/151.7/146.9 (Cq; dye), 144.9 (Cq; Trt), 142.8 (Cq; dye), 138.1 (Cq; Phe), 130.3 (CH; *C*H$_{Tyr}$), 129.5 (Cq; Tyr), 129.2 (CH; *C*H$_{Phe}$), 128.5/127.5 (CH; *C*H$_{Trt}$), 127.9 (CH; *C*H$_{Phe}$), 126.4/125.0/124.9/122.5 (CH; dye), 113.9 (CH; *C*H$_{Tyr}$), 111.7 (CH; dye), 69.2 (Cq; Trt), 65.1 (CH$_2$; -N-CH$_2$-C*H*$_2$-O-$_{dye}$), 58.1 (CH, Cα), 53.9 (CH; Cα$_{Cys}$), 53.8 (CH; Cα$_{Phe}$), 53.4 (CH; Cα$_{Tyr}$, Cα$_{Gln}$), 50.4 (CH$_2$; Cδ, Cδ'), 49.1 (CH$_2$; -N-C*H*$_2$-CH$_2$-O-$_{dye}$), 46.9 (CH; Cγ), 45.3 (CH$_2$; -N-*C*H$_2$-CH$_{3dye}$), 40.3 (CH$_2$, *C*H$_{2Acm}$), 38.5

(CH_2; $C\alpha_{Ahx}$), 37.5 (CH_2; $C\beta_{Phe}$, $C\beta'_{Phe}$), 37.0 (CH_2; $C\beta_{Cys}$, $C\beta'_{Cys}$), 35.1 (CH_2; $C\varepsilon_{Ahx}$), 33.0 (CH_2; $C\beta$, $C\beta'$), 32.8 (CH_2; $C\beta_{Tyr}$, $C\beta'_{Tyr}$), 32.6 (CH_2; $C\gamma_{Gln}$, $C\gamma'_{Gln}$), 28.7 (CH_2; $C\beta_{Ahx}$), 28.1 (CH_2; $C\beta_{Gln}$, $C\beta'_{Gln}$), 25.8 (CH_2; $C\gamma_{Ahx}$), 24.9 (CH_2; $C\delta_{Ahx}$), 22.6 (CH_3; -CO*C*H_3), 22.6 (CH_3; -CO*C*H_3), 12.0 (CH_3; -N-CH_2-*C*H_{3dye}).

ESI-MS: m/z: calcd for $C_{154}H_{173}N_{26}O_{24}S_2Na$: 2859 $[M-H+Na]^+$; found: 2859.

9.3.2 Macrocyclic Diketopiperazine Receptor Prototype 23



78 mg (27.4 µmol, 1 eq) of the diketopiperazine receptor precursor **22** were dissolved in a 6:2.5:0.42 (v/v/v) mixture of CH_2Cl_2, MeOH and H_2O (22 ml) followed by addition of 28 mg (109.6 µmol, 4 eq) I_2 in a 8:1.5 (v/v) mixture of CH_2Cl_2 and MeOH (5 ml). After stirring for 1 h, the excess of I_2 was quenched with a 1 M ascorbic acid. The mixture was then extracted three times with 0.1 M Na-phosphate buffer (pH 5.5) and CH_2Cl_2. The aq. phases were extracted again with CH_2Cl_2. The org. phases were washed with brine and dried over Na_2SO_4. After filtration and evaporation of the solvent under reduced pressure, the crude product was purified by preparative-TLC (CH_2Cl_2/MeOH, 90:10, v/v) and gel filtration (LH 20, CH_2Cl_2/MeOH, 90:10, v/v) to yield 30 mg (11.0 µmol, 40%) of the macrocyclic diketopiperazine prototype **23**.

$C_{148}H_{162}N_{24}O_{22}S_2$: 2693.15

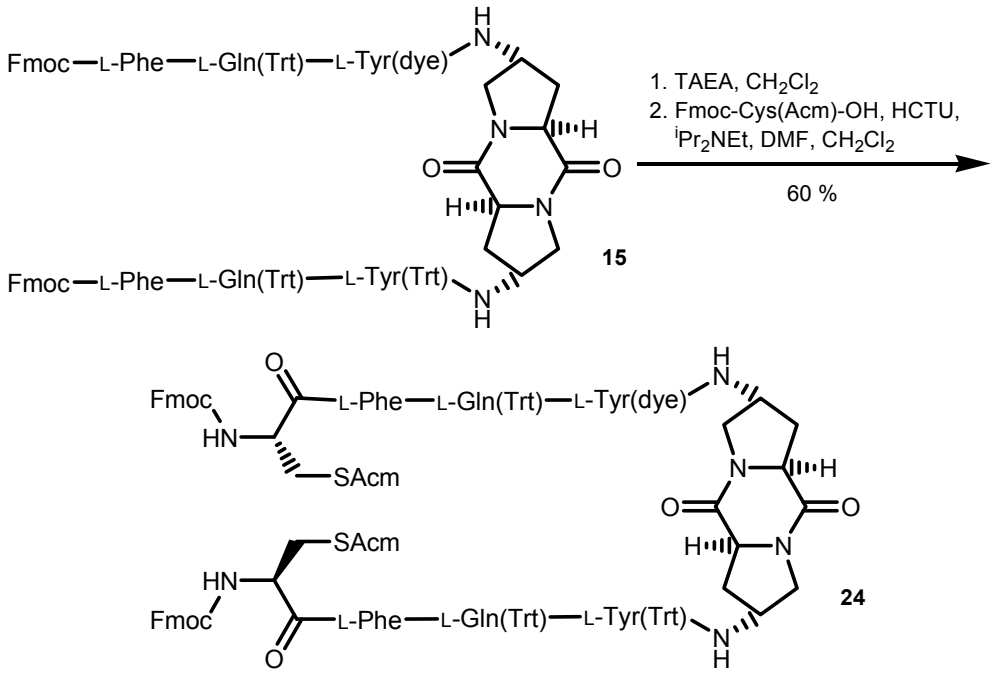
TLC: MeOH/CH_2Cl_2 (10:90); R_f: 0.49.

^{1}H-NMR (500 MHz, DMSO, 25°C): δ(ppm) = 8.59 (s, 2H; -N*H*$_{Trt}$), 8.35 (d, *J* = 8.9 Hz, 4H; H_{dye}), 8.19 (d, *J* = 8.1 Hz, 2H; -N*H*$_{Cys}$), 8.12 (m, 2H; -N*H*$_{Pro}$), 8.06 (m, 2H; -N*H*$_{Gln}$), 8.00 (m, 2H; -N*H*$_{Ahx}$), 7.95 (m, 2H; -N*H*$_{Phe}$), 7.91 (d, *J* = 8.8 Hz, 4H; H_{dye}), 7.88 (m, 2H; -N*H*$_{Tyr}$), 7.83 (d, *J* = 8.9 Hz, 4H; H_{dye}), 7.29-7.12 (m, 40H, H_{Phe}, H_{Trt}), 7.07 (d, *J* = 8.3 Hz, 4H; H_{Tyr}), 6.90 (d, *J* = 9.1 Hz, 4H; H_{dye}), 6.78 (d, *J* = 8.3 Hz, 4H; H_{Tyr}), 4.50 (m, 2H; Hα$_{Cys}$), 4.46 (m, 2H; Hα$_{Phe}$), 4.33 (m, 2H; Hα$_{Tyr}$), 4.31 (m, 2H; Hα), 4.12 (m, 2H; Hα$_{Gln}$), 4.10 (m, 2H; Hγ), 4.06 (m, 4H; -N-CH_2-C*H*$_2$-O-$_{dye}$), 3.78 (m, 4H; -N-C*H*$_2$-CH_2-O-$_{dye}$), 3.62 (m, 2H; Hδ), 3.54 (m, 4H; -N-C*H*$_2$-CH_3), 3.17 (m, 2H; Hδ′), 3.03 (m, 2H; Hβ$_{Cys}$), 3.00 (m, 2H; Hβ$_{Phe}$), 2.95 (m, 4H; Hα$_{Ahx}$), 2.91 (m, 2H; Hβ$_{Tyr}$), 2.50 (m, 2H; Hβ′$_{Cys}$), 2.78 (m, 2H; Hβ′$_{Tyr}$), 2.71 (m, 2H; Hβ′$_{Phe}$), 2.28 (m, 4H; Hγ$_{Gln}$, Hγ′$_{Gln}$), 2.13 (m, 2H; Hβ), 1.97 (m, 4H; Hε$_{Ahx}$), 1.93 (m, 2H; Hβ′), 1.84 (s, 6H; -COC*H*$_3$), 1.81 (m, 2H; Hβ$_{Gln}$), 1.73 (m, 2H; Hβ′$_{Gln}$), 1.31 (m, 4H; Hδ$_{Ahx}$), 1.27 (m, 4H; Hβ$_{Ahx}$), 1.12 (t, *J* = 6.8 Hz, 6H; -N-CH_2-C*H*$_{3dye}$), 1.04 (m, 4H; Hγ$_{Ahx}$).

^{13}C-NMR (125.6 MHz, DMSO, 25°C): δ(ppm) = 172.4/171.8/171.6/170.9/170.7/169.7/169.5 (Cq; *C*O), 165.7 (Cq; Cε), 156.9 (Cq; Tyr), 156.2/151.6/146.8 (Cq; dye), 144.9 (Cq; Trt), 142.8 (Cq; dye), 137.9 (Cq; Phe), 130.2 (CH; *C*H$_{Tyr}$), 129.6 (Cq; Tyr), 129.1 (CH; *C*H$_{Phe}$), 128.5/126.4 (CH; *C*H$_{Trt}$), 127.5 (CH; *C*H$_{Phe}$), 126.3/126.1/125.0/122.5 (CH; dye), 113.9 (CH; *C*H$_{Tyr}$), 111.7 (CH; dye), 69.2 (Cq; Trt), 65.1 (CH_2; -N-CH_2-C*H*$_2$-O-$_{dye}$), 58.2 (CH, Cα), 53.3 (CH; Cα$_{Tyr}$), 54.0 (CH; Cα$_{Phe}$), 52.7 (CH; Cα$_{Gln}$), 52.0 (CH; Cα$_{Cys}$), 50.6 (CH_2; Cδ, Cδ′), 49.1 (CH_2; -N-C*H*$_2$-CH_2-O-$_{dye}$), 47.1 (CH; Cγ), 45.3 (CH_2; -N-*C*H$_2$-CH_{3dye}), 40.9 (CH_2;Cβ$_{Cys}$, Cβ′$_{Cys}$), 38.7 (CH_2; Cα$_{Ahx}$), 37.3 (CH_2; Cβ$_{Phe}$, Cβ′$_{Phe}$), 33.6 (CH_2; Cβ$_{Tyr}$, Cβ′$_{Tyr}$), 35.1 (CH_2; Cε$_{Ahx}$), 32.9 (CH_2; Cβ, Cβ′), 32.5 (CH_2; Cγ$_{Gln}$, Cγ′$_{Gln}$), 28.7 (CH_2; Cβ$_{Ahx}$), 27.8 (CH_2; Cβ$_{Gln}$, Cβ′$_{Gln}$), 25.8 (CH_2; Cγ$_{Ahx}$), 24.8 (CH_2; Cδ$_{Ahx}$), 22.6 (CH_3; -CO*C*H$_3$), 12.0 (CH_3; -N-CH_2-*C*H$_{3dye}$).

ESI-MS: m/z: calcd for $C_{148}H_{161}N_{24}O_{22}S_2Na$: 2715 $[M-H+Na]^+$; found: 2715.

9.3.3 Two-Armed Diketopiperazine Receptor Precursor 25



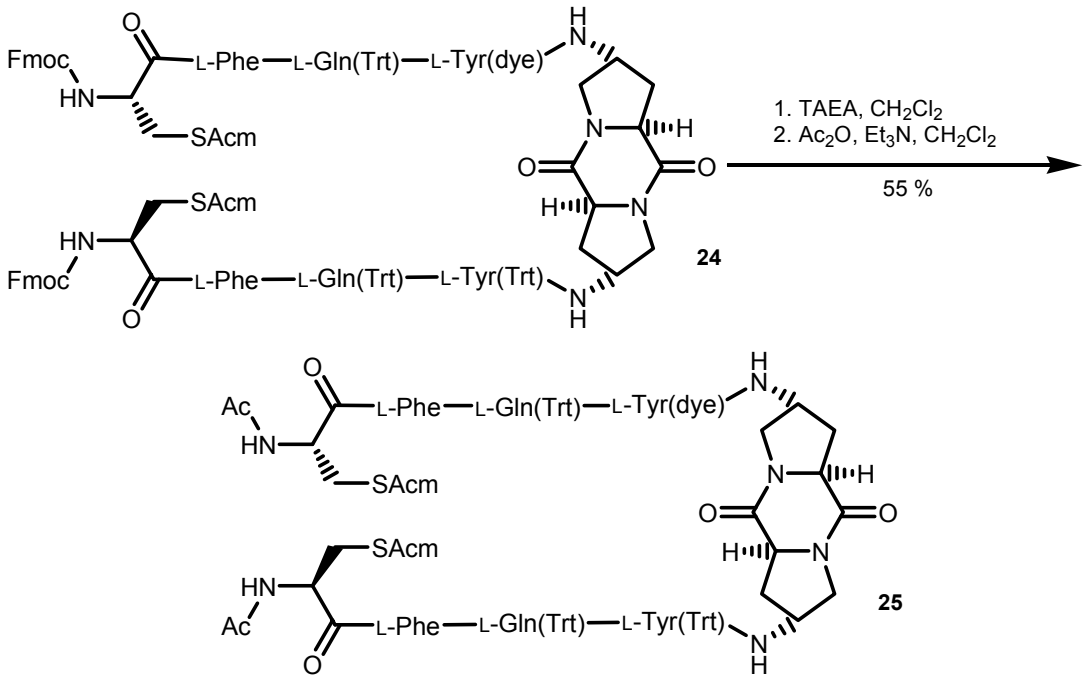
200 mg (76.3 µmol, 1 eq) of the diketopiperazine receptor precursor **15** were dissolved in CH_2Cl_2 (10 ml) followed by addition of 0.6 ml (3.82 mmol, 50 eq) TAEA which caused the formation of a precipitate after 2-5 min. The suspension was stirred at r.t. for 30 min and then extracted three times with 0.1 M Na-phosphate buffer (pH 5.5). The aq. phases were washed once with CH_2Cl_2 and the org. phases were washed and dried over Na_2SO_4 to yield the corresponding diamine, after filtration and removal of all volatiles under reduced pressure.

The diamine was dissolved in dry CH_2Cl_2 (2.5 ml) followed by addition of 26 µl (152.7 µmol, 2 eq) $^{i}Pr_2NEt$. The solution was cooled with an ice bath before a mixture of 116 mg (305.5 µmol, 4 eq) HCTU in DMF (0.5 ml), 26 µl (152.7 µmol, 2 eq) $^{i}Pr_2NEt$ and 127 mg (305.5 µmol, 4eq) Fmoc-Cys(Acm)-OH in CH_2Cl_2 (2.5 ml), was added and stirred at r.t. for 1 h. The reaction mixture was extracted three times with 0.1 M Na-phosphate buffer (pH 5.5) and CH_2Cl_2. The aq. phases were extracted again with CH_2Cl_2. The org. phases were washed with brine and dried over Na_2SO_4. After filtration and evaporation of the solvent under reduced pressure, the crude product was purified by flash chromatography on silica gel (gradient of CH_2Cl_2/MeOH from 99.5:0.5 to 95:5 (v/v)) to afford 137 mg (46.2 µmol, 60%) of the Fmoc-protected diketopiperazine precursor **24**.

$C_{168}H_{168}N_{24}O_{24}S_2$: 2971.41

TLC: MeOH/CH_2Cl_2 (10:90); R_f: 0.46.

ESI-MS: m/z: calcd for $C_{168}H_{167}N_{24}O_{24}S_2Na$: 2993 $[M-H+Na]^+$; found: 2993.



137 mg (46.2 µmol, 1 eq) of the diketopiperazine receptor precursor **24** were dissolved in CH_2Cl_2 (5 ml) followed by addition of 0.3 ml (2.31 mmol, 50 eq) TAEA which caused the formation of a precipitate after 2-5 min. The suspension was stirred at r.t. for 30 min and then extracted three times with 0.1 M Na-phosphate buffer (pH 5.5). The aq. phases were washed once with CH_2Cl_2 and the org. phases were washed with brine and dried over Na_2SO_4 to yield the corresponding diamine, after filtration and removal of all volatiles under reduced pressure.

The diamine was dissolved in dry CH_2Cl_2 (5 ml) followed by addition of 19 µl (138.6 µmol, 3 eq) Et_3N and 13 µl (138.6 µmol, 3 eq) Ac_2O. After stirring for 30 min, the reaction mixture was extracted three times with 0.1 M Na-phosphate buffer (pH 5.5) and CH_2Cl_2. The aq. phases were extracted again with CH_2Cl_2. The org. phases were washed with brine and dried over Na_2SO_4. After filtration and evaporation of the solvent under reduced pressure, the crude product was purified by preparative-TLC (CH_2Cl_2/MeOH, 90:10, v/v) and gel filtration (LH 20, CH_2Cl_2/MeOH, 90:10, v/v) to yield 70 mg (25.6 µmol, 55%) of the two-armed diketopiperazine precursor **25**.

$C_{142}H_{152}N_{24}O_{22}S_2$: 2611.00

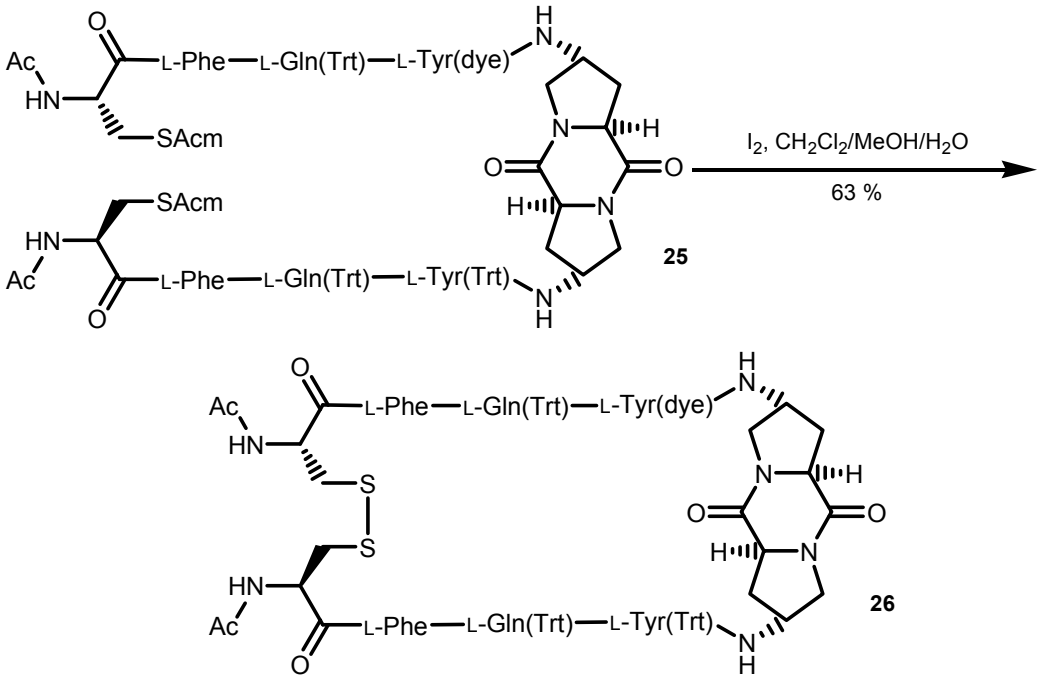
TLC: MeOH/CH_2Cl_2 (10:90); R_f: 0.28.

^{1}H-NMR (500 MHz, DMSO, 25°C): δ(ppm) = 8.58 (s, 2H; -N*H*$_{Trt}$), 8.51 (t, *J* = 6.3, 2H; -N*H*$_{Acm}$), 8.35 (d, *J* = 8.9 Hz, 4H; H_{dye}), 8.31 (m, 2H; -N*H*$_{Pro}$), 8.12 (m, 2H; -N*H*$_{Cys}$), 8.09 (m, 2H; -N*H*$_{Gln}$), 7.95 (m, 2H; -N*H*$_{Tyr}$), 7.92 (d, *J* = 8.8 Hz, 4H; H_{dye}), 7.89 (d, *J* = 7.8 Hz, 2H; -N*H*$_{Phe}$), 7.83 (d, *J* = 9.0 Hz, 4H; H_{dye}), 7.29-7.12 (m, 40H, H_{Phe}, H_{Trt}), 7.10 (d, *J* = 8.1 Hz, 4H; H_{Tyr}), 6.91 (d, *J* = 9.0 Hz, 4H; H_{dye}), 6.79 (d, *J* = 8.1 Hz, 4H; H_{Tyr}), 4.46 (m, 2H; Hα$_{Phe}$), 4.44 (m, 2H; Hα$_{Cys}$), 4.38 (dd, *J* = 15.6, 8.6 Hz, 2H; Hα$_{Tyr}$), 4.28 (m, 2H; Hα), 4.26 (m, 2H; C*H*$_{2Acm}$), 4.19 (dd, *J* = 18.8, 7.6 Hz, 2H; Hα$_{Gln}$), 4.12 (m, 2H; Hγ), 4.10 (m, 2H; C*H*$_{2Acm}$), 4.06 (m, 4H; -N-CH_2-C*H*$_2$-O-$_{dye}$), 3.77 (m, 4H; -N-C*H*$_2$-CH_2-O-$_{dye}$), 3.60 (m, 2H; Hδ), 3.53 (m, 4H; -N-C*H*$_2$-CH_{3dye}), 3.14 (m, 2H; Hδ'), 3.00 (m, *J* = 10.7 Hz, 2H; Hβ$_{Phe}$), 2.86 (m, 2H; Hβ$_{Tyr}$), 2.83 (m, 2H; Hβ$_{Cys}$), 2.77 (m, 2H; Hβ'$_{Tyr}$), 2.73 (m, 2H; Hβ'$_{Phe}$), 2.53 (m, 2H; Hβ'$_{Cys}$), 2.29 (m, 4H; Hγ$_{Gln}$, Hγ'$_{Gln}$), 2.10 (m, 2H; Hβ), 1.84 (m, 2H; Hβ'), 1.85 (s, 3H; -COC*H*$_3$), 1.78 (s, 3H; -COC*H*$_3$), 1.78 (m, 2H; Hβ$_{Gln}$), 1.69 (m, 2H; Hβ'$_{Gln}$), 1.14 (t, *J* = 6.9 Hz, 6H; -N-CH_2-C*H*$_{3dye}$).

^{13}C-NMR (125.6 MHz, DMSO, 25°C): δ(ppm) = 171.5/171.0/170.8/170.6/170.5/169.9/169.5 (Cq; *C*O), 165.7 (Cq; Cε), 156.9 (Cq; Tyr), 156.2/151.6/146.9 (Cq; dye), 144.9 (Cq; Trt), 142.8 (Cq; dye), 137.6 (Cq; Phe), 130.3 (CH; *C*H$_{Tyr}$), 129.3 (Cq; Tyr), 128.5/127.5 (CH; *C*H$_{Trt}$), 126.4 (CH; *C*H$_{Phe}$), 126.3/126.2/125.0/122.5 (CH; dye), 113.9 (CH; *C*H$_{Tyr}$), 111.7 (CH; dye), 69.2 (Cq; Trt), 65.1 (CH_2; -N-CH_2-C*H*$_2$-O-$_{dye}$), 58.1 (CH, Cα), 54.0 (CH; Cα$_{Tyr}$), 53.9 (CH; Cα$_{Phe}$), 52.4 (CH; Cα$_{Gln}$), 52.2 (CH; Cα$_{Cys}$), 50.4 (CH_2; Cδ, Cδ'), 49.1 (CH_2; -N-C*H*$_2$-CH_2-O-$_{dye}$), 46.8 (CH; Cγ), 45.3 (CH_2; -N-*C*H$_2$-CH_{3dye}), 39.5 (CH_2, *C*H$_{2Acm}$), 37.3 (CH_2; Cβ$_{Phe}$, Cβ'$_{Phe}$), 37.1 (CH_2; Cβ$_{Tyr}$, Cβ'$_{Tyr}$), 33.2 (CH_2; Cβ, Cβ'), 32.7 (CH_2; Cγ$_{Gln}$, Cγ'$_{Gln}$), 32.3 (CH_2; Cβ$_{Cys}$, Cβ'$_{Cys}$), 28.3 (CH_2; Cβ$_{Gln}$, Cβ'$_{Gln}$), 28.6 (CH_3; -CO*C*H$_3$), 28.5 (CH_3; -CO*C*H$_3$), 12.0 (CH_3; -N-CH_2-*C*H$_{3dye}$).

ESI-MS: m/z: calcd for $C_{142}H_{150}N_{24}O_{22}S_2Na$: 2632 $[M-2H+Na]^+$; found: 2632.

9.3.4 Macrocyclic diketopiperazine receptor prototype 26



70 mg (25.6 µmol, 1 eq) of the diketopiperazine receptor precursor **24** were dissolved in a 6:2.5:0.42 (v/v/v) mixture of CH_2Cl_2, MeOH and H_2O (20 ml) followed by addition of 26 mg (102.5 µmol, 4 eq) I_2 in a 8:1.5 (v/v) mixture of CH_2Cl_2 and MeOH (5 ml). After stirring for 1 h, the excess of I_2 was quenched with a 1 M ascorbic acid. The mixture was then extracted three times with 0.1 M Na-phosphate buffer (pH 5.5) and CH_2Cl_2. The aq. phases were extracted again with CH_2Cl_2. The org. phases were washed with brine and dried over Na_2SO_4. After filtration and evaporation of the solvent under reduced pressure, the crude product was purified by preparative-TLC (CH_2Cl_2/MeOH, 90:10, v/v) and gel filtration (LH 20, CH_2Cl_2/MeOH, 90:10, v/v) to yield 40 mg (16.1 µmol, 63%) of the macrocyclic diketopiperazine prototype **26**.

$C_{136}H_{140}N_{22}O_{20}S_2$: 2466.83

TLC: MeOH/CH_2Cl_2 (10:90); R_f: 0.48.

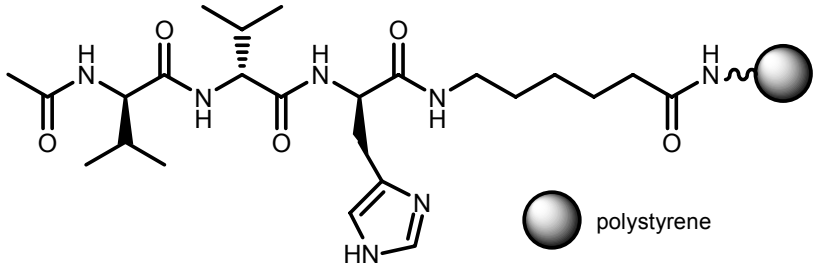
^{1}H-NMR (500 MHz, DMSO, 25°C): δ(ppm) = 8.56 (s, 2H; -N*H*$_{Trt}$), 8.36 (d, *J* = 8.9 Hz, 4H; H_{dye}), 8.15 (m, 2H; -N*H*$_{Gln}$), 8.12 (d, *J* = 7.7 Hz, 2H; -N*H*$_{Cys}$), 8.05 (m, 2H; -N*H*$_{Phe}$), 7.93 (d, *J* = 8.9 Hz, 4H; H_{dye}), 7.90 (m, 2H; -N*H*$_{Tyr}$), 7.83 (d, *J* = 9.1 Hz, 4H; H_{dye}), 7.67 (m, 2H; -N*H*$_{Pro}$), 7.29-7.12 (m, 40H, H_{Phe}, H_{Trt}), 7.08 (d, *J* = 8.5 Hz, 4H; H_{Tyr}), 6.89 (d, *J* = 9.2 Hz, 4H;

H_{dye}), 6.78 (d, J = 8.4 Hz, 4H; H_{Tyr}), 4.51 (m, 2H; $H\alpha_{Cys}$), 4.48 (m, 2H; $H\alpha_{Phe}$), 4.44 (m, 2H; Hα), 4.21 (dd, J = 10.4, 5.5 Hz, 2H; $H\alpha_{Tyr}$), 4.13 (m, 2H; Hγ), 4.04 (m, 4H; -N-CH_2-CH_2-O-$_{dye}$), 3.91 (m, 2H; $H\alpha_{Gln}$), 3.77 (m, 4H; -N-CH_2-CH_2-O-$_{dye}$), 3.60 (m, 2H; Hδ), 3.52 (m, 4H; -N-CH_2-CH_{3dye}), 3.12 (m, 2H; Hδ'), 3.04 (m, 2H; $H\beta_{Tyr}$), 3.01 (*m*, 2H; $H\beta_{Phe}$), 2.96 (m, 2H; $H\beta_{Cys}$), 2.89 (m, 2H; $H\beta'_{Tyr}$), 2.77 (m, 2H; $H\beta'_{Phe}$), 2.69 (m, 2H; $H\beta'_{Cys}$), 2.26 (m, 4H; $H\gamma_{Gln}$, $H\gamma'_{Gln}$), 2.18 (m, 2H; Hβ), 2.09 (m, 2H; Hβ'), 1.77 (m, 2H; $H\beta_{Gln}$, $H\beta'_{Gln}$), 1.78 (s, 6H; -COCH_3), 1.13 (t, J = 6.9 Hz, 6H; -N-CH_2-CH_{3dye}).

^{13}C-NMR (125.6 MHz, DMSO, 25°C): δ(ppm) = 171.8/171.3/171.0/170.7/170.2/169.7 (Cq; *C*O), 165.8 (Cq; Cε), 156.8 (Cq; Tyr), 156.2/151.6/146.9 (Cq; dye), 144.9 (Cq; Trt), 142.8 (Cq; dye), 137.3 (Cq; Phe), 130.2 (Cq; Tyr), 130.1 (CH; *C*H_{Tyr}), 128.5/127.5 (CH; *C*H_{Trt}), 126.3 (CH; *C*H_{Phe}), 126.1/125.0/122.5 (CH; dye), 114.1 (CH; *C*H_{Tyr}), 111.7 (CH; dye), 69.2 (Cq; Trt), 65.2 (CH_2; -N-CH_2-CH_2-O-$_{dye}$), 58.3 (CH, Cα), 54.9 (CH; $C\alpha_{Tyr}$), 53.9 (CH; $C\alpha_{Phe}$), 53.6 (CH; $C\alpha_{Gln}$), 51.7 (CH; $C\alpha_{Cys}$), 50.5 (CH_2; Cδ, Cδ'), 49.1 (CH_2; -N-CH_2-CH_2-O-$_{dye}$), 47.3 (CH; Cγ), 45.3 (CH_2; -N-*C*H_2-CH_{3dye}), 39.8 (CH_2;$C\beta_{Cys}$, $C\beta'_{Cys}$), 36.9 (CH_2; $C\beta_{Phe}$, $C\beta'_{Phe}$), 35.2 (CH_2; $C\beta_{Tyr}$, $C\beta'_{Tyr}$), 32.7 (CH_2; Cβ, Cβ'), 32.4 (CH_2; $C\gamma_{Gln}$, $C\gamma'_{Gln}$), 27.3 (CH_2; $C\beta_{Gln}$, $C\beta'_{Gln}$), 22.4 (CH_3; -CO*C*H_3), 12.0 (CH_3; -N-CH_2-*C*H_{3dye}).

ESI-MS: m/z: calcd for $C_{136}H_{140}N_{22}O_{20}S_2$: 2466 $[M]^+$; found: 2466.

10 GENERAL FMOC-STRATEGY FOR THE SYNTHESIS OF SIDE-CHAIN DEPROTECTED PEPTIDES ONTO AMINO-FUNCTIONALIZED RESIN EXEMPLIFIED BY THE SYNTHESIS OF Ac-D-Val-D-Val-D-His-NH(CH_2)$_5$CONH-Resin



10.1 Coupling onto Amino-Functionalized Resin

7.0 g (7.70 mmol, 200-400 mesh, loading 1.1mmol g^{-1}) amino methyl polystyrene resin were placed in a Merrifield vessel and washed three times with CH_2Cl_2 for 1 min. The resin was then suspended in the smallest amount dry CH_2Cl_2 (30 ml) to allow efficient shaking. 3.0 g (8.47 mmol, 1.1 eq) *N*-α-Fmoc-aminocaproic acid and 0.8 ml (14.63 mmol, 1.9 eq) acetic acid, along with 3.1 g (23.1 mmol, 3 eq) HOBt in DMF (7 ml) were added and the mixture was shaken for 5 min before 3.6 ml (23.10 mmol, 3 eq) DIC were added. The mixture was shaken for 2 h and then washed three times with DMF and five times with CH_2Cl_2 for 1 min each. After this a few resin beads were sampled to a small glass tube for the Kaiser test (qualitative test for the presence or absence of free amino groups).[99] If the Kaiser test was positive (blue color) the Fmoc protecting groups were removed and the next amino acid was coupled. If the Kaiser test was negative (yellow color) the coupling was repeated. At the end, the loading of the resin was determined, by the quantitative Fmoc-test, to be 0.41 mmol g^{-1}.[71]

10.2 Fmoc-Deprotection

200 mg (82.0 µmol, 200-400 mesh, loading 0.41 mmol g^{-1}) of the Fmoc-functionalized resin was washed three times with DMF for 1 min. The deprotection was achieved by shaking the resin with a mixture of 20% (v/v) piperidine in DMF (10 ml), first for 2 min and then for 10 min. After washing the resin three times with DMF and five times with CH_2Cl_2 1 min each, the Kaiser test was performed to ensure the successful Fmoc-deprotection. Consecutive deprotection, then coupling and deprotection cycles employing

152 mg (246.0 µmol, 3 eq) Fmoc-D-His(Trt)-OH, 83 mg (246.0 µmol, 3 eq) Fmoc-D-Val-OH, 33 mg (246.0 µmol, 3 eq) HOBt and 40 µl (246.0 µmol, 3 eq) DIC yielded Fmoc-D-Val-D-Val-D-His(Trt)-NH$(CH_2)_5$CONH-resin. After another Fmoc-test was done, the new loading of the resin post coupling was determined to be 0.33 mmol g^{-1}.

10.3 Acetylation of Free Amines

After the final Fmoc-deprotection, the resin was suspended in dry CH_2Cl_2 (5 ml) and approx. 56 µl (410.0 µmol, 5 eq) Et_3N, before 39 µl (410.0 µmol, 5 eq) Ac_2O were added. The mixture was shaken for 1 h and then washed three times with DMF and five times with CH_2Cl_2 for 1 min each. The yellow color of the Kaiser test confirmed the completion of the reaction.

10.4 Cleavage of the Side-Chain Protecting Groups

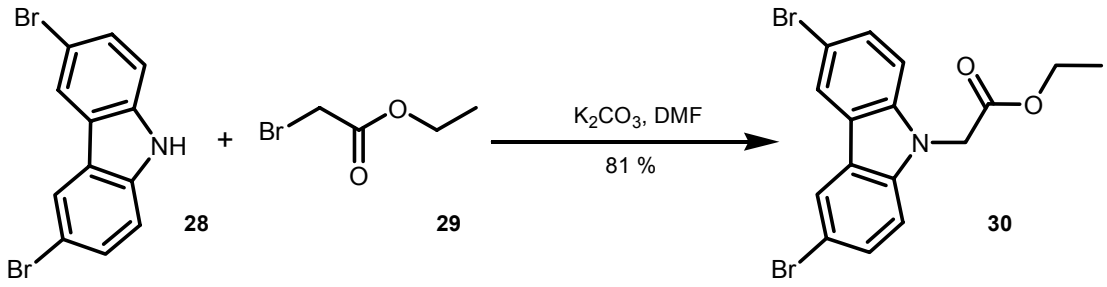
Cleavage of the side-chain protecting groups was achieved by shaking the resin with a 1:1:0.1 (v/v/v) mixture of TFA/CH_2Cl_2/TIS (10 ml), first for 2 min and then again for 1 h. The resin was then washed three times with DMF, three times with CH_2Cl_2, three times with a 8:2 (v/v) mixture of CH_2Cl_2/Et_3N and three times with CH_2Cl_2 for 1 min each. Ac-D-Val-D-val-D-His-NH$(CH_2)_5$CO-NH-Resin was finally removed, dried *in vacuo* for at least 4 h and then stored at low temperature (4°C).

Using the same pathway, the following peptides were synthesized on amino methyl polystyrene resin which was functionalized with *N*-α-Fmoc-aminocaproic acid (200-400 mesh, loading 0.41 mmol g^{-1}):

Ac-L-Ala-L-Gln-L-Ala-$NH(CH_2)_5CO$-NH-Resin: loading 0.33 mmol g^{-1}
Ac-D-Ala-L-Gln-L-Ala-$NH(CH_2)_5CO$-NH-Resin: loading 0.34 mmol g^{-1}
Ac-D-Gln-D-Ala-D-Leu-$NH(CH_2)_5CO$-NH-Resin: loading 0.33 mmol g^{-1}
Ac-D-Gln-D-Phe-D-Val-$NH(CH_2)_5CO$-NH-Resin: loading 0.33 mmol g^{-1}
Ac-L-Ala-L-Gln-D-Phe-$NH(CH_2)_5CO$-NH-Resin: loading 0.33 mmol g^{-1}
Ac-D-Gln-D-Phe-D-Leu-$NH(CH_2)_5CO$-NH-Resin: loading 0.33 mmol g^{-1}
Ac-D-Gln-D-Leu-D-Leu-$NH(CH_2)_5CO$-NH-Resin: loading 0.33 mmol g^{-1}
Ac-L-Leu-L-Gln-D-Phe-$NH(CH_2)_5CO$-NH-Resin: loading 0.33 mmol g^{-1}
Ac-D-Phe-D-Val-D-Gln-$NH(CH_2)_5CO$-NH-Resin: loading 0.33 mmol g^{-1}
Ac-D-Lys-D-Pro-L-Pro-$NH(CH_2)_5CO$-NH-Resin: loading 0.35 mmol g^{-1}
Ac-L-Pro-L-Pro-D-Lys-$NH(CH_2)_5CO$-NH-Resin: loading 0.35 mmol g^{-1}
Ac-L-Ser-D-Pro-D-Pro-$NH(CH_2)_5CO$-NH-Resin: loading 0.36 mmol g^{-1}
Ac-D-Phe-L-Lys-D-Pro-$NH(CH_2)_5CO$-NH-Resin: loading 0.40 mmol g^{-1}
Ac-D-Ser-D-Lys-L-Lys-$NH(CH_2)_5CO$-NH-Resin: loading 0.41 mmol g^{-1}
Ac-D-Pro-L-Pro-D-Gln-$NH(CH_2)_5CO$-NH-Resin: loading 0.33 mmol g^{-1}

11 SYNTHESIS OF THE SYMMETRIC CARBAZOLE TEMPLATE

11.1 3,6-Dibromo-carbazole-9-acetic acid-ethyl ester 30



A mixture of 10.0 g (30.77 mmol, 1.0 eq) 3,6-dibromocarbazole **28** and 8.6 g (61.54 mmol, 2 eq) potassium carbonate was evacuated and flushed with nitrogen three times. After the addition of anhydrous DMF (170 ml), the yellow suspension was stirred for 3.5 h at 65°C. 7.5 ml (67.69 mmol, 2.2 eq) ethyl bromoacetate **29** were then added and the mixture was allowed to stir at 60°C for 2 h. After cooling to r.t., water (170 ml) was added and a white precipitate was observed. After cooling in an ice bath, the suspension was filtered, washed with 1 l of cold water and dried overnight *in vacuo*. After recrystallisation of the precipitate from 190 ml of Hexane/Chloroforme (1:1, v/v), 10.5 g (25.54 mmol, 81 %) 3,6-dibromocarbazolethylester **30** were obtained as a white solid.

$C_{16}H_{13}Br_2NO_2$: 411.09 g/mol

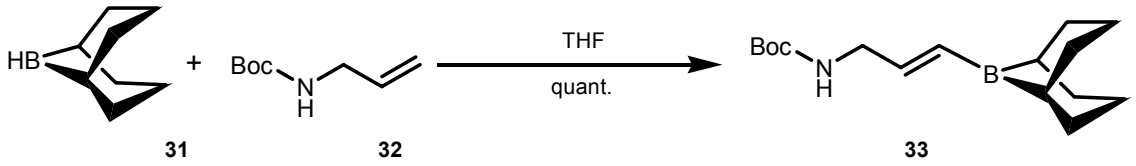
TLC: Pentane/EtOAc (7:1); R_f: 0.49.

^{1}H-NMR (500 MHz, $CDCl_3$, 25°C): δ(ppm) = 8.14 (m, 2H; 4, 4'), 7.57 (d, J = 1.7 Hz, 1H; 2), 7.55 (d, J = 1.7 Hz, 1H; 2'), 7.20 (d, J = 8.6 Hz, 2H; 1, 1'), 4.93 (s, 2H; -N-C*H*$_2$-CO_2-), 4.20 (q, J = 7.1 Hz, 2H; CH_3-C*H*$_2$-CO_2-), 1.22 (t, J = 7.1 Hz, 3H; C*H*$_3$-CH_2-CO_2-).

^{13}C-NMR (125.6 MHz, $CDCl_3$, 25°C): δ(ppm) = 167.9 (Cq; -*C*O_2-), 139.6 (Cq; $C_{3,3'}$), 129.5 (CH; $C_{2,2'}$), 124.0 (Cq; *C*$_{5,5'}$), 123.6 (CH; $C_{4,4'}$), 113.1 (Cq; $C_{6,6'}$), 110.3 (CH; $C_{1,1'}$), 60.1 (CH_2; CH_3-*C*H_2-CO_2-), 45.0 (CH_2; -N-*C*H_2-CO_2-), 14.3 (CH_3; *C*H_3-CH_2-CO_2-).

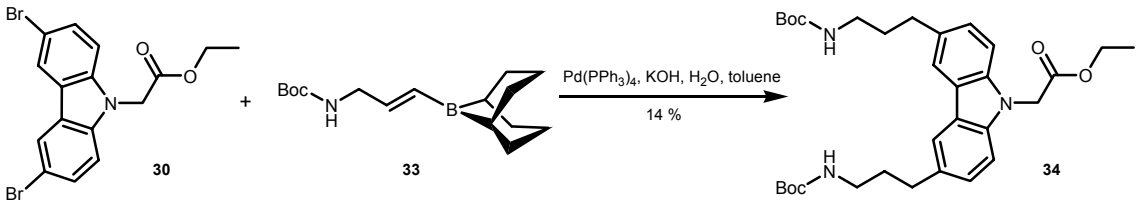
ESI-MS: m/z: calcd for $C_{16}H_{13}Br_2NO_2Na$: 434 $[M+Na]^+$; found: 434.

11.2 *N-tert*-Boc Protected 3,6-bis(3-aminopropyl)-carbazole-9-acetic acid-ethyl ester 34



31 **32** **33**

5.7 g (36.26 mmol, 2.4 eq) *tert*-butyl *N*-allylcarbamante **32** were evacuated and flushed with argon three times. After cooling in an ice bath, 85.0 ml (42.30 mmol, 2.8 eq) of 0.5 M solution of 9-BBN **31** in THF were added slowly and the mixture was allowed to stir overnight at r.t. Under argon, removal of the solvent under reduced pressure yielded a yellow oily residue **33**.



30 **33** **34**

Under Argon, toluene (310 ml) and a 1 M solution of KOH (91 ml) were added to the oily residue **33**. The mixture was degassed two times. 6.2 g (15.11 mmol, 1.0 eq) 3,6-dibromo-carbazole-9-acetic acid-ethyl ester **30** were then added and the mixture was degassed two times. After the addition of 1.8 g (1.50 mmol, 10 mol %) $Pd[PPh_3]_4$, the yellow solution was then degassed three times and allowed to stir at 120°C overnight. After cooling to r.t., the reaction mixture was washed with sat. $NaHCO_3$-soln. and with brine and dried over Na_2SO_4. The aq. phase was slightly evaporated at reduced pressure to eliminate the toluene and then extracted with chloroform (3x150 ml) and the org. phases were dried over Na_2SO_4. After filtration of the toluene- and the chloroform-phases and removal of all volatiles under reduced pressure, flash chromatography (gradient of pentane/EtOAc from 5:1 to 3:1 (v/v)) afforded 1.2 g (2.11 mmol, 14 %) of the *N-tert*-Boc protected 3,6-bis(3-aminopropyl)-carbazole-9-acetic acid-ethyl ester **34** as a white solid.

$C_{32}H_{45}N_3O_6$: 567.72 g/mol

TLC: Pentane/EtOAc (3:1); R_f: 0.29.

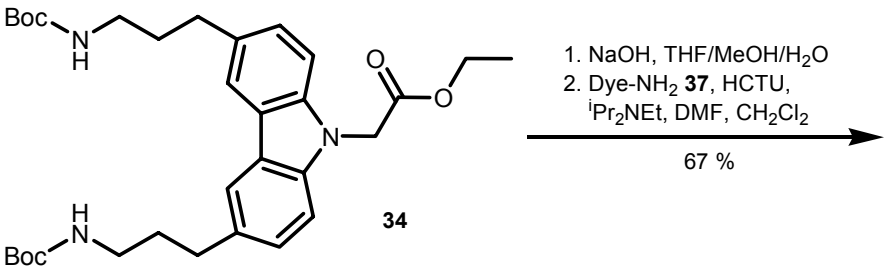
^{1}H-NMR (500 MHz, $CDCl_3$, 25°C): δ(ppm) = 7.85 (s, 2H; 4, 4'), 7.26 (d, J = 1.7 Hz, 1H; 2), 7.25 (d, J = 1.6 Hz, 1H; 2'), 7.21 (d, J = 8.6 Hz, 2H; 1, 1'), 4.93 (s, 2H; -N-CH_2-CO_2-), 4.19 (q, J = 7.1 Hz, 2H; CH_3-CH_2-CO_2-), 3.19 (t, J = 7.0 Hz, 4H; -CH_2-CH_2-CH_2-NH-), 2.81 (t, J = 7.5 Hz, 4H; -CH_2-CH_2-CH_2-NH-), 1.90 (tt, J = 7.6, 7.2 Hz, 4H; -CH_2-CH_2-CH_2-NH-), 1.45 (s, 18H; CH_{3Boc}), 1.22 (t, J = 7.1 Hz, 3H; CH_3-CH_2-CO_2-).

^{13}C-NMR (125.6 MHz, $CDCl_3$, 25°C): δ(ppm) = 168.6 (Cq; -CO_2-), 156.1 (Cq; CO_{Boc}), 139.5 (Cq; $C_{5,5'}$), 132.7 (Cq; $C_{3,3'}$), 126.5 (CH; $C_{2,2'}$), 123.2 (Cq; $C_{6,6'}$), 119.8 (CH; $C_{4,4'}$), 108.2 (CH; $C_{1,1'}$), 79.3 (Cq; C_{Boc}), 61.6 (CH_2; CH_3-CH_2-CO_2-), 44.9 (CH_2; -N-CH_2-CO_2-), 40.4 (CH_2; -CH_2-CH_2-CH_2-NH-), 33.1 (CH_2; -CH_2-CH_2-CH_2-NH-), 32.5 (CH_2; -CH_2-CH_2-CH_2-NH-), 28.5 (CH_3; C_{Boc}), 14.2 (CH_3; CH_3-CH_2-CO_2-).

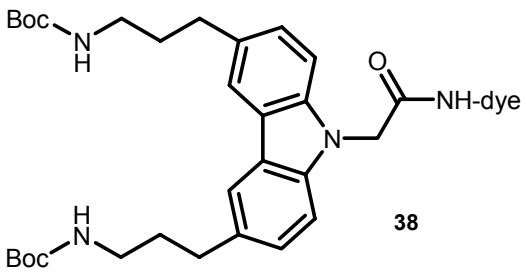
ESI-MS: m/z: calcd for $C_{32}H_{45}N_3O_6Na$: 590 $[M+Na]^+$; found: 590.

12 SYNTHESIS OF TWO-ARMED CARBAZOLE RECEPTOR PROTOTYPES

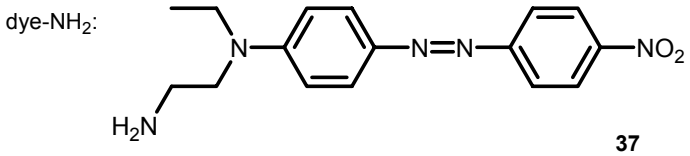
12.1 Dye-Marked Carbazole Precursor 38



34



38



37

500 mg (880.7 µmol, 2 eq) of the *N-tert*-Boc protected 3,6-bis(3-aminopropyl)-carbazole-9-acetic acid-ethyl ester **34** were dissolved in a 1:1 (v/v) mixture of THF and MeOH (3 ml). After the addition of 1.5 M NaOH (0.7 ml), the mixture was stirred for 4 h at r.t. The reaction mixture was then carefully acidified to pH 3 with 1 M HCl. CH_2Cl_2 and water were added and the mixture was extracted. The aq. phase was extracted again with CH_2Cl_2 and the org. phases were washed with brine and dried over Na_2SO_4. Filtration and evaporation of the solvent under reduced pressure yielded a red solid which was mixed with 364 mg (880.7 µmol, 2.0 eq) HCTU in DMF (0.5 ml), 151 µl (880.7 µmol, 2 eq) $^{i}Pr_2NEt$ and CH_2Cl_2 (2 ml). This mixture was stirred at r.t. for 1 min and added to a cold mixture of 138 mg (440.4 µmol, 1 eq) of the Dye-NH_2 **37** and 151 µl (880.7 µmol, 2 eq) $^{i}Pr_2NEt$ in CH_2Cl_2 (2 ml). The reaction mixture was stirred at r.t. for 2 h. The reaction mixture was extracted three times with 0.1 M Na-phosphate buffer (pH 5.5) and CH_2Cl_2. The aq. phases were extracted again with CH_2Cl_2. The org. phases were washed with brine and dried over Na_2SO_4. After filtration and evaporation of the solvent under reduced pressure the crude product was

purified by preparative-TLC (CH_2Cl_2/MeOH, 90:10, v/v) to yield 247 mg (295.8 µmol, 67 %) of the dye-marked carbazole precursor **38**.

$C_{46}H_{58}N_8O_7$: 835.02

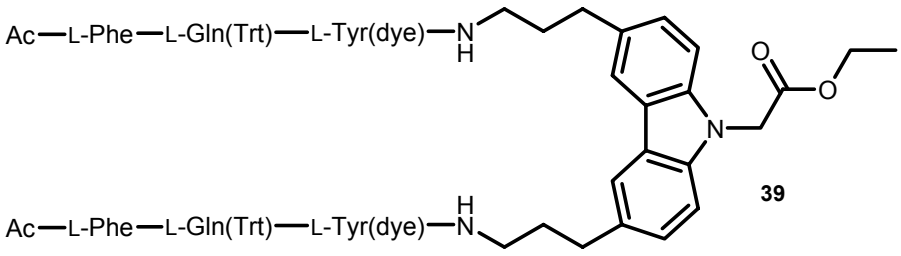
TLC: MeOH/CH_2Cl_2 (5:95): 0.48.

^{1}H-NMR (500 MHz, 5% CD_3OD in $CDCl_3$, 25°C): δ(ppm) = 8.23 (d, J = 9.0 Hz, 2H; H_{dye}), 7.86 (d, J = 8.9 Hz, 2H; H_{dye}), 7.79 (m, 2H; H_{dye}), 7.78 (s, 2H; $H_{4,4'}$), 7.17 (d, J = 8.6 Hz, 2H; $H_{2,2'}$), 7.08 (d, J = 8.2 Hz, 2H; $H_{1,1'}$), 6.68 (d, J = 8.6 Hz, 2H; H_{dye}), 4.78 (s, 2H; -N-C*H*$_2$-CO-), 3.40 (m, 2H; -N-C*H*$_2$-C*H*$_2$-NH-$_{dye}$), 3.33 (m, 2H; -N-C*H*$_2$-C*H*$_2$-NH-$_{dye}$), 3.26 (q, J = 6.8 Hz, 2H; -N-C*H*$_2$-CH_{3dye}), 3.03 (t, J = 6.7 Hz, 4H; -CH_2-CH_2-C*H*$_2$-NH-), 2.73 (t, J = 7.4 Hz, 4H; -C*H*$_2$-CH_2-CH_2-NH-), 1.81 (tt, J = 7.5, 7.2 Hz, 4H; -CH_2-C*H*$_2$-CH_2-NH-), 1.39 (s, 18H; C*H*$_{3Boc}$), 1.04 (t, J = 6.9 Hz, 3H; -N-CH_2-C*H*$_{3dye}$).

^{13}C-NMR (125.6 MHz, 5% CD_3OD in $CDCl_3$, 25°C): δ(ppm) = 169.5 (Cq; *C*O), 156.3 (Cq; CO_{Boc}), 155.4/152.2/147.1/142.9 (Cq; dye), 139.1 (Cq; $C_{5,5'}$), 133.3 (Cq; $C_{3,3'}$), 127.4 (CH; dye), 126.9 (CH; $C_{2,2'}$), 124.8 (CH; dye), 123.3 (Cq; $C_{6,6'}$), 122.0 (CH; dye), 119.9 (CH; $C_{4,4'}$), 112.1 (CH; dye), 108.3 (CH; $C_{1,1'}$), 79.3 (Cq, *C*H$_{Boc}$), 48.9 (CH_2; -N-C*H*$_2$-CH_2-N-$_{dye}$), 46.8 (CH_2; -N-*C*H$_2$-CO-), 45.5 (CH_2; -N-*C*H$_2$-CH_{3dye}), 39.9 (CH_2; -CH_2-CH_2-*C*H$_2$-NH-), 37.0 (CH_2; -N-C*H*$_2$-CH_2-N-$_{dye}$), 32.9 (*C*H$_2$; -*C*H$_2$-CH_2-CH_2-NH-), 32.2 (CH_2; -CH_2-*C*H$_2$-CH_2-NH-), 28.3 (CH_3; C_{Boc}), 12.1 (CH_3; -N-CH_2-*C*H$_{3dye}$).

ESI-MS: m/z: calcd for $C_{46}H_{58}N_8O_7$: 835 $[M]^+$; found: 835; calcd for $C_{46}H_{57}N_8O_7Na$: 857 $[M-H+Na]^+$; found: 857; calcd for $C_{46}H_{57}N_8O_7K$: 873 $[M-H+K]^+$; found: 873.

12.2 General Fmoc-Strategy for the Synthesis of the Two-Armed Carbazole Receptors in Solution, Exemplified by the Synthesis of the Receptor 39



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12.2.1 First coupling

150 mg (264.4 µmol, 1 eq) of the bis-*N*-Boc-protected carbazole precursor **34** were dissolved in a 1:3 (v/v) mixture of TFA and CH_2Cl_2 (2.2 ml) and allowed to stir at r.t. for 1 h. After the removal of all volatiles under reduced pressure, the residue was triturated with Et_2O to yield a red solid which was isolated by decantation and dried *in vacuo*. The corresponding TFA-salt of **34** was then dissolved in dry CH_2Cl_2 (2.5 ml) followed by addition of 91 µl (528.8 µmol, 2 eq) $^{i}Pr_2NEt$, ready for the next coupling.

The solution was cooled with an ice bath before a mixture of 437 mg (1.06 mmol, 4 eq) HCTU in DMF (2 ml), 91 µl (528.8 mmol, 2 eq) $^{i}Pr_2NEt$ and 610 mg (1.06 mmol, 4 eq) Fmoc-L-Tyr(dye)-OH **13** in CH_2Cl_2 (2.5 ml), was added and stirred at r.t. for 1 h. The reaction mixture was extracted three times with 0.1 M Na-phosphate buffer (pH 5.5) and CH_2Cl_2. The aq. phases were extracted again with CH_2Cl_2. The org. phases were washed with brine and dried over Na_2SO_4. After filtration and evaporation of the solvent under reduced pressure, the crude product was purified by flash chromatography on silica gel (gradient of CH_2Cl_2/MeOH from 99.5:0.5 to 98:2 (v/v)) to afford 227 mg (152.7 µmol, 60%) of the dye-marked bis-*N*-Boc-protected receptor precursor.

$C_{82}H_{95}N_{13}O_{13}$: 1486.71

TLC: MeOH/CH_2Cl_2 (3:97); R_f: 0.62.

ESI-MS: m/z: calcd for $C_{82}H_{95}N_{13}O_{13}$: 1486 $[M]^+$; found: 1486, calcd for $C_{82}H_{94}N_{13}O_{13}Na$: 1508 $[M\text{-}H+Na]^+$; found: 1508.

12.2.2 Second coupling

227 mg (152.7 µmol, 1 eq) of the dye-marked bis-*N*-Boc-protected receptor precursor were dissolved in MeOH (3 ml) and treated with a solution of 4 M HCl in dioxan (9.5 ml) at r.t. for 1 h. After removal of all volatiles under reduced pressure, the residue was triturated with Et_2O to yield a red solid which was isolated by decantation and dried *in vacuo*. The corresponding HCl-salt was dissolved in dry CH_2Cl_2 (5 ml) with 52 µl (305.5 µmol, 2 eq) $^{i}Pr_2Net$.

This solution was cooled with an ice bath before a mixture of 253 mg (610.8 mmol, 4 eq) HCTU in DMF (2 ml), 52 µl (305.5 mmol, 2 eq) $^{i}Pr_2NEt$ and 373 mg (610.8 mmol, 4eq) Fmoc-L-Gln(Trt)-OH in CH_2Cl_2 (5 ml), was added and stirred at r.t. for 1 h. The reaction mixture was extracted three times with 0.1 M Na-phosphate buffer (pH 5.5) and CH_2Cl_2. The aq. phases were extracted again with CH_2Cl_2. The org. phases were washed with brine and dried over Na_2SO_4. Filtration and evaporation of the solvent at reduced pressure yielded the crude product which was triturated with MeOH to yield a red solid. The solid was isolated by filtration and dried *in vacuo* to obtain 376 mg (152.7 µmol, 100%) of the dye-marked bis-*N*-Fmoc-protected receptor precursor.

$C_{150}H_{143}N_{17}O_{18}$: 2471.84

TLC: MeOH/CH_2Cl_2 (5:95); R_f: 0.19.

ESI-MS: m/z: calcd for $C_{150}H_{143}N_{17}O_{18}Na$: 2494 $[M+Na]^+$; found: 2494.

12.2.3 Third coupling

376 mg (152.7 µmol, 1 eq) of the dye-marked bis-*N*-Fmoc-protected receptor precursor were dissolved in DMF (2 ml) followed by addition of 474 µl (4.58 mmol, 30 eq) diethylamine. The reaction mixture was stirred at r.t. for 30 min. After removal of DMF under reduced pressure, the crude product was co-evaporated three times with *n*-heptane. The residue was dried *in vacuo* and then dissolved in a mixture of dry CH_2Cl_2 (1 ml) and DMF (2 ml) with 52 µl (305.5 µmol, 2 eq) $^{i}Pr_2NEt$, ready for the last amino acid coupling.

This solution was cooled with an ice bath before a mixture of 253 mg (610.8 mmol, 4 eq) HCTU in DMF (2 ml), 52 µl (305.5 mmol, 2 eq) $^{i}Pr_2NEt$ and 236 mg (610.8 mmol, 4eq) Fmoc-L-Phe-OH in CH_2Cl_2 (1 ml), was added and stirred at r.t. for 1 h. The reaction mixture was extracted three times with 0.1 M Na-phosphate buffer (pH 5.5) and CH_2Cl_2. The aq. phases were extracted again with CH_2Cl_2. The org. phases were washed with brine and dried over Na_2SO_4. Filtration and evaporation of the solvent at reduced pressure yielded the crude product which was triturated with MeOH to yield a red solid. The solid was isolated by filtration and dried *in vacuo* to obtain 372 mg (134.5 µmol, 88%) of the dye-marked bis-*N*-Fmoc-protected receptor precursor which contained two tripeptidic arms.

$C_{168}H_{161}N_{19}O_{20}$: 2766.19

TLC: MeOH/CH_2Cl_2 (5:95); R_f: 0.49.

ESI-MS: m/z: calcd for $C_{168}H_{160}N_{19}O_{20}Na$: 2788 $[M-H+Na]^+$; found: 2788.

12.2.4 Acetylation

After the final Fmoc-deprotection using 479 µmol (4.04 mmol, 30 eq) diethylamine in DMF (4 ml), the diamine was dissolved in a mixture of CH_2Cl_2 (6 ml) and DMF (6 ml) followed by the addition of 56 µl (403.9 µmol, 3 eq) Et_3N and 38 µl (403.9 µmol, 3 eq) Ac_2O. After stirring the mixture for 30 min, the reaction mixture was extracted three times with 0.1 M Na-phosphate buffer (pH 5.5) and CH_2Cl_2. The aq. phases were extracted again with CH_2Cl_2. The org. phases were washed with brine and dried over Na_2SO_4. After filtration and evaporation of the solvent under reduced pressure the crude product was purified by

preparative-TLC (CH_2Cl_2/MeOH, 90:10, v/v) and gel filtration (LH 20, CH_2Cl_2/MeOH, 90:10, v/v) to yield 172 mg (71.5 µmol, 53%) of the two-armed carbazole receptor **39**.

$C_{142}H_{145}N_{19}O_{18}$: 2405.79

TLC: MeOH/CH_2Cl_2 (5:95); R_f: 0.29.

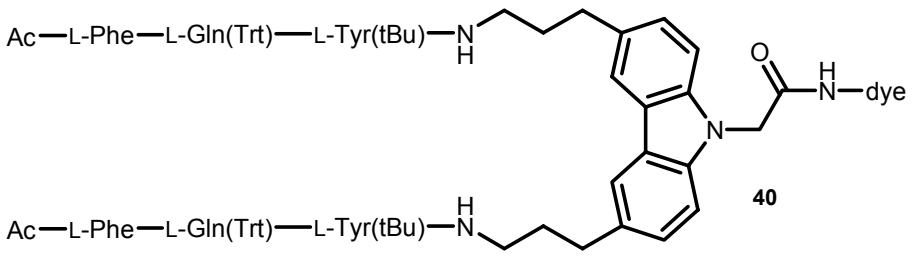
^{1}H-NMR (500 MHz, DMSO, 25°C): δ(ppm) = 8.50 (s, 2H; -N*H*$_{Trt}$), 8.29 (d, *J* = 9.1 Hz, 4H; H_{dye}), 8.13 (d, *J* = 7.7 Hz, 2H; -N*H*$_{Gln}$), 8.02 (d, *J* = 8.1 Hz, 2H; -N*H*$_{Tyr}$), 7.91 (m, 2H; -CH_2-CH_2-CH_2-N*H*-), 7.87 (d, *J* = 9.1 Hz, 4H; H_{dye}), 7.81 (s, 2H; $H_{4,4'}$), 7.79 (m, 2H; -N*H*$_{Phe}$), 7.76 (d, *J* = 9.2 Hz, 4H; H_{dye}), 7.34 (m, 2H; $H_{2,2'}$), 7.22-7.09 (m, 42H, H_{Phe}, H_{Trt}, $H_{1,1'}$), 7.07 (d, *J* = 8.6 Hz, 4H; H_{Tyr}), 6.79 (d, *J* = 9.3 Hz, 4H; H_{dye}), 6.73 (d, *J* = 8.6 Hz, 4H; H_{Tyr}), 5.18 (s, 2H; -N-C*H*$_2$-CO_2-), 4.44 (m, 2H; Hα$_{Tyr}$), 4.37 (dd, *J* = 14.3, 7.7 Hz, 2H; Hα$_{Phe}$), 4.15 (dd, *J* = 13.5, 7.6 Hz, 2H; Hα$_{Gln}$), 4.07 (q, *J* = 7.1 Hz, 2H; CH_3-C*H*$_2$-CO_2-), 3.94 (m, 4H; -N-CH_2-C*H*$_2$-O-$_{dye}$), 3.64 (m, 4H; -N-C*H*$_2$-CH_2-O-$_{dye}$), 3.42 (q, *J* = 7.0 Hz, 4H; -N-C*H*$_2$-CH_{3dye}), 3.04 (m, 2H; -CH_2-CH_2-C*H*$_2$-NH-), 2.94 (m, 2H; -CH_2-CH_2-C*H*$_2$-NH-), 2.91 (m, 2H; Hβ$_{Phe}$), 2.85 (m, 2H; Hβ$_{Tyr}$), 2.73 (dd, *J* = 13.0, 7.8 Hz, 2H; Hβ'$_{Tyr}$), 2.65 (dd, *J* = 13.9, 10.3 Hz, 2H; Hβ'$_{Phe}$), 2.56 (m, 4H; -C*H*$_2$-CH_2-CH_2-NH-), 2.25 (m, 4H; Hγ$_{Gln}$, Hγ'$_{Gln}$), 1.77 (m, 2H; Hβ$_{Gln}$), 1.67 (m, 2H; Hβ'$_{Gln}$), 1.64 (s, 6H; -COC*H*$_3$), 1.62 (m, 4H; -CH_2-C*H*$_2$-CH_2-NH-), 1.14 (t, *J* = 7.1 Hz, 3H; C*H*$_3$$CH_2$-$CO_2$-), 1.05 (t, *J* = 6.9 Hz, 6H; -N-CH_2-C*H*$_{3dye}$).

^{13}C-NMR (125.6 MHz, DMSO, 25°C): δ(ppm) = 172.1/171.9/171.3/170.9/169.8 (Cq; *C*O), 169.3 (Cq; -CO_2-), 157.3 (Cq; Tyr), 156.6/152.0/147.3 (Cq; dye), 145.3 (Cq; Trt), 143.2 (Cq; dye), 139.6 (Cq; $C_{5,5'}$), 138.5 (Cq; Phe), 132.8 (Cq; $C_{3,3'}$), 130.7 (CH; *C*H$_{Tyr}$), 130.2 (Cq; Tyr), 129.6/128.4 (CH; *C*H$_{Phe}$), 128.9/127.9 (CH; *C*H$_{Trt}$), 126.7 (CH; dye), 126.5 (CH; $C_{1,1'}$), 125.4/122.9 (CH; dye), 122.7 (Cq; $C_{6,6'}$), 120.0 (CH; $C_{4,4'}$), 114.4 (CH; *C*H$_{Tyr}$), 112.1 (CH; dye), 109.4 (CH; $C_{2,2'}$), 69.6 (Cq; Trt), 65.6 (CH_2; -N-CH_2-*C*H_2-O-$_{dye}$), 61.3 (CH_3-*C*H_2-CO_2-), 54.6 (CH; Cα$_{Phe}$), 54.5 (CH; Cα$_{Tyr}$), 52.8 (CH; Cα$_{Gln}$), 49.5 (CH_2; -N-*C*H_2-CH_2-O-$_{dye}$), 45.7 (CH_2; -N-*C*H_2-CH_{3dye}), 44.5 (CH_2; -N-*C*H_2-CO_2-), 38.7 (CH_2; -CH_2-CH_2-*C*H_2-NH-), 37.8 (CH_2; Cβ$_{Phe}$, Cβ'$_{Phe}$), 37.5 (CH_2; Cβ$_{Tyr}$, Cβ'$_{Tyr}$), 33.0 (CH_2; Cγ$_{Gln}$, Cγ'$_{Gln}$), 32.9 (*C*H_2; -*C*H_2-CH_2-CH_2-NH-), 31.9 (CH_2; -CH_2-*C*H_2-CH_2-NH-), 28.4 (CH_2; Cβ$_{Gln}$, Cβ'$_{Gln}$), 22.8 (CH_3; -CO*C*H_3), 14.5 (CH_3; *C*H_3-CH_2-CO_2-), 12.4 (CH_3; -N-CH_2-*C*H_{3dye}).

ESI-MS: m/z: calcd for $C_{142}H_{144}N_{19}O_{18}Na$: 2427 $[M-H+Na]^+$; found: 2427.

12.2.5 Two-Armed Carbazole Receptor 40

(**40** was obtained in analogy to the procedures described in 12.2.1 – 12.2.4)



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First coupling: 200 mg (239.5 µmol, 1 eq) of receptor precursor **38**
14 ml of 4 M HCl in Dioxan
Methanol (4 ml)
440 mg (958.0 µmol, 4 eq) Fmoc-L-Tyr(tBu)-OH
396 mg (958.0 µmol, 4 eq) HCTU in DMF (1 ml)
164 µl (958.0 µmol, 4 eq) $^{i}Pr_2Net$
CH_2Cl_2 (2 ml)
Yield: 174 mg (114.9 µmol, 48%)

$C_{92}H_{96}N_{10}O_{11}$: 1517.81

TLC: MeOH/CH_2Cl_2 (5:95); R_f: 0.74.

ESI-MS: m/z: calcd for $C_{92}H_{96}N_{10}O_{11}Na$: 1540 $[M+Na]^+$; found: 1540.

Second coupling: 859 µl (5.75 mmol, 50 eq) TAEA
CH_2Cl_2 (10 ml) and DMF (4 ml)
281 mg (459.7 µmol, 4 eq) Fmoc-L-Gln(Trt)-OH
190 mg (459.7 µmol, 4 eq) HCTU in DMF (1 ml)
79 µl (459.7 µmol, 4 eq) $^{i}Pr_2Net$
CH_2Cl_2 (4 ml)
Yield: 259 mg (114.9 µmol, 100%)

$C_{140}H_{140}N_{14}O_{15}$: 2258.69

TLC: MeOH/CH_2Cl_2 (5:95); R_f: 0.35.

ESI-MS: m/z: calcd for $C_{140}H_{140}N_{14}O_{15}$: 2258 $[M]^+$; found: 2258, calcd for $C_{140}H_{139}N_{14}O_{15}Na$: 2280 $[M-H+Na]^+$; found: 2280, calcd for $C_{140}H_{139}N_{14}O_{15}K$: 2296 $[M-H+K]^+$; found: 2296.

Third coupling: 859 µl (5.75 mmol, 50 eq) TAEA
CH_2Cl_2 (10 ml) and DMF (4 ml)
178 mg (459.7 µmol, 4 eq) Fmoc-L-Phe-OH
190 mg (459.7 µmol, 4 eq) HCTU in DMF (1 ml)
79 µl (459.7 µmol, 4 eq) $^iPr_2Net$
CH_2Cl_2 (4 ml)
Yield: 204 mg (79.9 µmol, 70%)

$C_{158}H_{158}N_{16}O_{17}$: 2553.04

TLC: MeOH/CH_2Cl_2 (5:95); R_f: 0.15.

ESI-MS: m/z: calcd for $C_{158}H_{156}N_{16}O_{17}Na$: 2574 $[M-2H+Na]^+$; found: 2574.

Acetylation: 597 µl (3.40 mmol, 50 eq) TAEA
CH_2Cl_2 (9 ml) and DMF (1 ml)
33 µl (239.7 µmol, 3 eq) Et_3N
23 µl (239.7 µmol, 3 eq) Ac_2O
CH_2Cl_2 (5 ml)
Yield: 95 mg (43.4 µmol, 54%) of the two-armed carbazole receptor **40**

$C_{132}H_{142}N_{16}O_{15}$: 2192.64

TLC: MeOH/CH_2Cl_2 (10:90); R_f: 0.73.

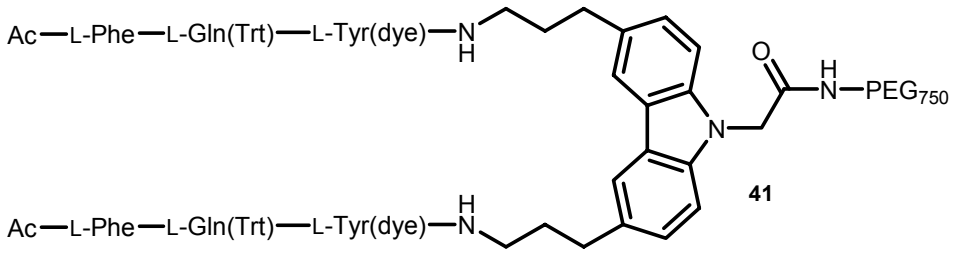
^{1}H-NMR (500 MHz, 5% CD_3OD in $CDCl_3$, 25°C): δ(ppm) = 8.22 (d, J = 8.9 Hz, 2H; H_{dye}), 7.85 (d, J = 8.6 Hz, 2H; H_{dye}), 7.82 (s, 2H; $H_{4,4'}$), 7.79 (m, 2H; H_{dye}), 7.27-6.90 (m, 48H, H_{Phe}, H_{Trt}, H_{Tyr}, $H_{1,1'}$, $H_{2,2'}$), 6.78 (d, J = 8.3 Hz, 4H; H_{Tyr}), 6.71 (m, 2H; H_{dye}), 4.70 (s, 2H; -N-CH_2-CO-), 4.62 (m, 2H; Hα_{Tyr}), 4.16 (dd, J = 10.9, 3.5 Hz, 2H; Hα_{Phe}), 4.02 (m, 2H; Hα_{Gln}), 3.41 (dd, J = 14.5, 3.8 Hz, 2H; Hβ_{Tyr}), 3.33 (m, 2H; -N-CH_2-CH_2-NH-$_{dye}$), 3.28 (m, 2H; -N-CH_2-CH_{3dye}), 3.26 (m, 2H; -N-CH_2-CH_2-NH-$_{dye}$), 3.19 (m, 4H; -CH_2-CH_2-CH_2-NH-), 3.05 (dd, J = 13.9, 2.9 Hz, 2H; Hβ_{Phe}), 2.78 (m, 2H; Hβ'_{Tyr}), 2.76 (m, 4H; -CH_2-CH_2-CH_2-NH-), 2.63 (t, 2H; Hβ'_{Phe}), 2.04 (m, 2H; Hγ_{Gln}), 1.93 (m, 2H; Hβ_{Gln}), 1.89 (m, 4H; -CH_2-CH_2-CH_2-NH-), 1.62 (m, 2H; Hβ'_{Gln}), 1.43 (s, 6H; -COCH_3), 1.19 (m, 2H;Hγ'_{Gln}), 1.17 (s, 18H; CH_{3tBu}), 1.06 (t, 3H; -N-CH_2-CH_{3dye}).

^{13}C-NMR (125.6 MHz, 5% CD_3OD in $CDCl_3$, 25°C): δ(ppm) = 174.3/174.1/173.2/ 171.4/171.3/169.7 (Cq; CO), 153.1 (Cq; Tyr), 146.8 (Cq; dye), 144.0 (Cq; Trt), 142.1 (Cq; dye), 139.0 (Cq; $C_{5,5'}$), 136.5 (Cq; Phe), 133.8 (Cq; Tyr), 133.5 (Cq; $C_{3,3'}$), 129.6 (CH; CH$_{Tyr}$), 128.6/127.9 (CH; CH$_{Trt}$), 128.5 (CH; CH$_{Phe}$), 126.9 (CH; dye), 126.9 (CH; $C_{2,2'}$), 124.9 (CH; dye), 124.4 (CH; CH$_{Tyr}$), 123.4 (Cq; $C_{6,6'}$), 121.4 (CH, dye), 120.2 (CH; $C_{4,4'}$), 112.9 (CH; dye), 108.1 (CH; $C_{1,1'}$), 78.9 (Cq, CH$_{3tBu}$), 70.3 (Cq; Trt), 56.9 (CH; Cα_{Phe}), 54.6 (CH; Cα_{Tyr}), 54.5 (CH; Cα_{Gln}), 49.9 (CH_2; -N-CH_2-CH$_2$-NH-$_{dye}$), 46.8 (CH_2; -N-CH$_2$-CO-), 45.9 (CH_2; -N-CH$_2$-CH_{3dye}), 39.2 (CH_2; -CH_2-CH_2-CH$_2$-NH-), 36.9 (CH_2; -N-CH$_2$-CH_2-NH-$_{dye}$), 36.4 (CH_2; Cβ_{Phe}, Cβ'_{Phe}), 36.1 (CH_2; Cβ_{Tyr}, Cβ'_{Tyr}), 33.0 (CH$_2$; -CH$_2$-CH_2-CH_2-NH-), 31.4 (CH_2; Cγ_{Gln}, Cγ'_{Gln}), 31.2 (CH_2; -CH_2-CH$_2$-CH_2-NH-), 28.6 (CH_3; C_{tBu}), 25.5 (CH_2; Cβ_{Gln}, Cβ'_{Gln}), 22.4 (CH_3; -COCH$_3$), 12.2 (CH_3; -N-CH_2-CH$_{3dye}$).

MS (MALDI-ToF): m/z: calcd for $C_{132}H_{143}N_{16}O_{15}$: 2193 $[M+H]^+$; found: 2193, m/z: calcd for $C_{132}H_{143}N_{16}O_{15}Na$: 2216 $[M+H+Na]^+$; found: 2216.

12.2.6 Two-Armed Carbazole Receptor 41

(**41** was obtained in analogy to the procedures described in 12.2.1 – 12.2.4)



100 mg (41.6 µmol, 1.2 eq) of the carbazole receptor **39** were dissolved in a 1:1 (v/v) mixture of THF and MeOH (10 ml), DMF was added until complete solubilization. After the addition of 150 µl (312.0 µmol, 9 eq) 2 M NaOH, the mixture was stirred for 1 h at r.t. The reaction mixture was extracted three times with 0.1 M Na-phosphate buffer (pH 5.5) and CH_2Cl_2. The aq. phases were extracted again with CH_2Cl_2. The org. phases were washed with brine and dried over Na_2SO_4. Filtration and evaporation of the solvent under reduced pressure yielded a red solid which was mixed with 286 mg (69.3 µmol, 2.0 eq) HCTU in DMF (1 ml), 12 µl (69.3 µmol, 2 eq) $^{i}Pr_2NEt$ and CH_2Cl_2 (2.5 ml). This mixture was stirred at r.t. for 1 min and added to a cold mixture of 26 mg (34.6 µmol, 1 eq) $H_2N\text{-}(CH_2CH_2O)_{\approx 16}CH_3$ ($H_2N\text{-}PEG_{750}$), which was before melted at 80°C *in vacuo* for 30 min, in order to remove traces of moisture, and 12 µl (69.3 µmol, 2eq) $^{i}Pr_2NEt$ in CH_2Cl_2 (2.5 ml). The reaction mixture was stirred at r.t. for 1 h, and then extracted three times with 0.1 M Na-phosphate buffer (pH 5.5) and CH_2Cl_2. The aq. phases were extracted again with CH_2Cl_2. The org. phases were washed with brine and dried over Na_2SO_4. After filtration and evaporation of the solvent under reduced pressure, the crude product was purified by preparative-TLC (CH_2Cl_2/MeOH, 90:10, v/v) to afford 75 mg (24.2 µmol, 70 %) of the dye-marked carbazole precursor **41**.

$C_{173}H_{208}N_{20}O_{33}$: 3095.62

TLC: MeOH/CH_2Cl_2 (10:90); R_f: 0.52.

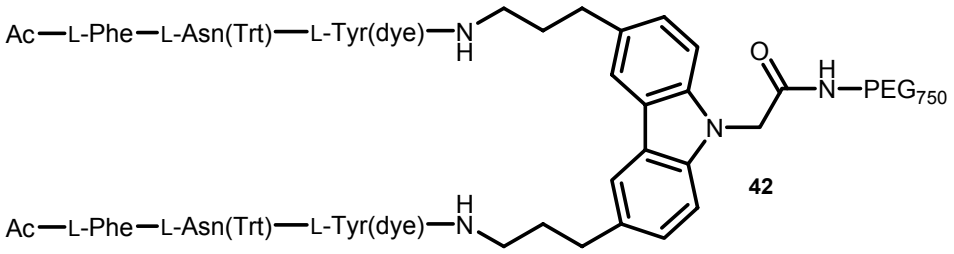
^{1}H-NMR (500 MHz, DMSO, 25°C): δ(ppm) = 8.53 (s, 2H; -N*H*$_{Trt}$), 8.34 (d, *J* = 8.9 Hz, 4H; H$_{dye}$), 8.31 (s, 1H; -N*H*-$(CH_2CH_2O)_{\approx 16}$-CH_3), 8.16 (d, *J* = 7.6 Hz, 2H; -N*H*$_{Gln}$), 8.06 (d, *J* = 8.1 Hz, 2H; -N*H*$_{Phe}$), 7.95 (m, 2H; -CH_2-CH_2-CH_2-N*H*-), 7.91 (d, *J* = 8.9 Hz, 4H; H$_{dye}$), 7.84 (s, 2H; H$_{4,4'}$), 7.83 (m, 2H; -N*H*$_{Tyr}$), 7.79 (d, *J* = 9.0 Hz, 4H; H$_{dye}$), 7.36 (d, *J* = 8.2 Hz, 2H; H$_{2,2'}$), 7.22-7.11 (m, 42H, H$_{Phe}$, H$_{Trt}$, H$_{1,1'}$), 7.10 (d, *J* = 8.5 Hz, 4H; H$_{Tyr}$), 6.83 (d, *J* = 9.2 Hz, 4H; H$_{dye}$), 6.76 (d, *J* = 8.4 Hz, 4H; H$_{Tyr}$), 4.93 (s, 2H; -N-C*H*$_2$-CO-), 4.48 (m, 2H; Hα$_{Phe}$), 4.41 (dd, *J* = 14.2, 7.6 Hz, 2H; Hα$_{Tyr}$), 4.19 (dd, *J* = 12.9, 7.3 Hz, 2H; Hα$_{Gln}$), 3.97 (m, 4H; -N-CH_2-C*H*$_2$-O-$_{dye}$), 3.67 (m, 4H; -N-C*H*$_2$-CH_2-O-$_{dye}$), 3.58-3.30 (m, 64H; -NH-(C*H*$_2$C*H*$_2$O)$_{\approx 16}$-CH_3), 3.47 (m, 4H; -N-C*H*$_2$-CH_{3dye}), 3.22 (s, 3H; -NH-$(CH_2CH_2O)_{\approx 16}$-C*H*$_3$), 3.08 (m, 2H; -CH_2-CH_2-C*H*$_2$-NH-), 2.99 (m, 2H; -CH_2-CH_2-C*H*$_2$-NH-), 2.94 (m, 2H; Hβ$_{Phe}$), 2.89 (m, 2H; Hβ$_{Tyr}$), 2.77 (m, 2H; Hβ'$_{Tyr}$), 2.69 (m, 2H; Hβ'$_{Phe}$), 2.60 (m, 4H; -C*H*$_2$-CH_2-CH_2-NH-), 2.29 (m, 4H; Hγ$_{Gln}$, Hγ'$_{Gln}$), 1.81 (m, 2H; Hβ$_{Gln}$), 1.71 (m, 2H; Hβ'$_{Gln}$), 1.69 (s, 6H; -COC*H*$_3$), 1.66 (m, 4H; -CH_2-C*H*$_2$-CH_2-NH-), 1.08 (t, *J* = 6.8 Hz, 6H; -N-CH_2-C*H*$_{3dye}$).

^{13}C-NMR (125.6 MHz, DMSO, 25°C): δ(ppm) = 171.7/171.5/170.9/170.5/169.4/167.6 (Cq; *C*O), 156.8 (Cq; Tyr), 156.2/151.6/146.8 (Cq; dye), 144.9 (Cq; Trt), 142.8 (Cq; dye), 139.4 (Cq; C$_{5,5'}$), 138.1 (Cq; Phe), 132.1 (Cq; C$_{3,3'}$), 130.2 (CH; *C*H$_{Tyr}$), 129.7 (Cq; Tyr), 129.1/128.0 (CH; *C*H$_{Phe}$), 128.5/127.5 (CH; *C*H$_{Trt}$), 126.3/126.2 (CH; dye), 126.1 (CH; C$_{1,1'}$), 125.0/122.5 (CH; dye), 122.2 (Cq; C$_{6,6'}$), 119.3 (CH; C$_{4,4'}$), 114.0 (CH; *C*H$_{Tyr}$), 111.6 (CH; dye), 109.0 (CH; C$_{2,2'}$), 71.3/69.8/69.6(CH_2; -NH-(*C*H_2*C*H_2O)$_{\approx 16}$-CH_3), 69.6 (Cq; Trt), 69.2/69.0 (CH_2; -NH-(*C*H_2*C*H_2)$_{\approx 16}$-OCH_3), 65.2 (CH_2; -N-CH_2-*C*H_2-O-$_{dye}$), 58.1 (CH_3; -NH-$(CH_2CH_2O)_{\approx 16}$-*C*H_3), 54.2 (CH; Cα$_{Tyr}$), 54.1 (CH; Cα$_{Phe}$), 52.4 (CH; Cα$_{Gln}$), 49.0 (CH_2; -N-*C*H_2-CH_2-O-$_{dye}$), 45.6 (CH_2; -N-*C*H_2-CO-), 45.2 (CH_2; -N-*C*H_2-CH_{3dye}), 38.7 (CH_2; -NH-(*C*H_2*C*H_2O)$_{\approx 16}$-CH_3), 38.3 (CH_2; -CH_2-CH_2-*C*H_2-NH-), 37.4 (CH_2; Cβ$_{Phe}$, Cβ'$_{Phe}$), 37.1 (CH_2; Cβ$_{Tyr}$, Cβ'$_{Tyr}$), 32.6 (CH_2; Cγ$_{Gln}$, Cγ'$_{Gln}$), 32.5 (*C*H_2; -*C*H_2-CH_2-CH_2-NH-), 31.5 (CH_2; -CH_2-*C*H_2-CH_2-NH-), 28.0 (CH_2; Cβ$_{Gln}$, Cβ'$_{Gln}$), 22.4 (CH_3; -CO*C*H_3), 11.9 (CH_3 ; -N-CH_2-*C*H_{3dye}).

MS (MALDI-ToF): m/z: calcd for $C_{173}H_{207}N_{20}O_{33}Na$: 3117 $[M-H+Na]^+$; found: 3117 (distribution of peaks due to the polydispersity of the PEG).

12.2.7 Two-Armed Carbazole Receptor 42

(**42** was obtained in analogy to the procedures described in 12.2.1 – 12.2.4)



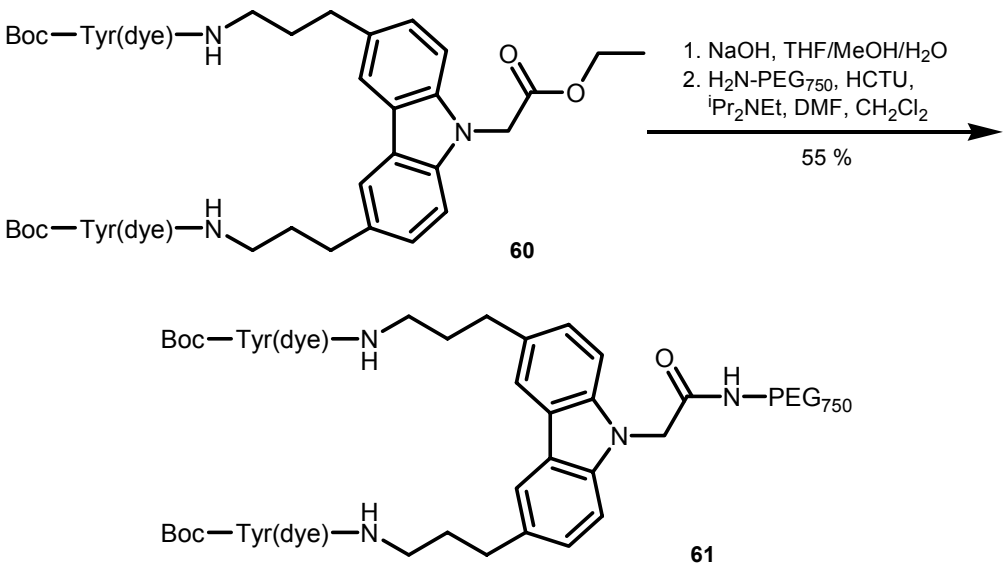
42

First coupling: 300 mg (528.8 µmol, 1 eq) of receptor precursor **34**
1:3 (v/v) mixture of TFA and CH_2Cl_2 (4 ml)
1.22 mg (2.11 mmol, 4 eq) Fmoc-L-Tyr(dye)-OH
874 mg (2.11 mmol, 4 eq) HCTU in DMF (2 ml)
362 µl (2.11 mmol, 4 eq) $^{i}Pr_2Net$
CH_2Cl_2 (10 ml)
Yield: 174 mg (373.3 µmol, 71%)

$C_{82}H_{95}N_{13}O_{13}$: 1486.71

TLC: MeOH/CH_2Cl_2 (3:97); R_f: 0.62.

ESI-MS: m/z: calcd for $C_{82}H_{95}N_{13}O_{13}$: 1486 $[M]^+$; found: 1486, calcd for $C_{82}H_{94}N_{13}O_{13}Na$: 1508 $[M-H+Na]^+$; found: 1508.



PEG coupling: 500 mg (336.3 µmol, 1.2 eq) of receptor precursor **60**
21 ml of a 2 M solution of NaOH
1:1 (v/v) mixture of THF and MeOH (160 ml)
210 mg (280.6 µmol, 1 eq) H_2N-PEG_{750}
232 mg (560.5 µmol, 2 eq) HCTU in DMF (1 ml)
96 µl (560.5 µmol, 2 eq) $^{i}Pr_2Net$
CH_2Cl_2 (15 ml) and DMF (15 ml)
Yield: 336 mg (154.4 µmol, 55%)

$C_{113}H_{158}N_{14}O_{29}$: 2176.54

TLC: MeOH/CH_2Cl_2 (10:90); R_f: 0.50.

^{1}H-NMR (500 MHz, DMSO, 25°C): δ(ppm) = 8.35 (d, J = 8.9 Hz, 4H; H_{dye}), 8.30 (m, 1H; -N*H*-$(CH_2CH_2O)_{\approx 16}$-CH_3), 7.92 (d, J = 8.9 Hz, 4H; H_{dye}), 7.90 (m, 2H; -CH_2-CH_2-CH_2-N*H*-), 7.87 (s, 2H; $H_{4,4'}$), 7.82 (d, J = 9.1 Hz, 4H; H_{dye}), 7.38 (d, J = 8.4 Hz, 2H; $H_{2,2'}$), 7.22 (d, J = 8.2 Hz, 2H, $H_{1,1'}$), 7.15 (d, J = 9.1 Hz, 4H; H_{Tyr}), 6.89 (d, J = 9.1 Hz, 4H; H_{dye}), 6.83 (m, 2H; -N*H*$_{Tyr}$), 6.82 (d, J = 8.0 Hz, 4H; H_{Tyr}), 4.94 (s, 2H; -N-C*H*$_2$-CO-), 4.10 (m, 2H; Hα_{Tyr}), 4.07 (m, 4H; -N-CH_2-C*H*$_2$-O-$_{dye}$), 3.77 (m, 4H; -N-C*H*$_2$-CH_2-O-$_{dye}$), 3.54 (m, 4H; -N-C*H*$_2$-CH_{3dye}), 3.52-3.26 (m, 64H; -NH-(C*H*$_2$C*H*$_2$O)$_{\approx 16}$-CH_3), 3.22 (s, 3H; -NH-$(CH_2CH_2O)_{\approx 16}$-C*H*$_3$), 3.08 (m, 4H; -CH_2-CH_2-C*H*$_2$-NH-), 2.85 (dd, J = 13.5, 5.1 Hz, 2H; Hβ_{Tyr}), 2.69 (m, 2H; Hβ'_{Tyr}), 2.65 (m,

4H; -C*H*$_2$-CH$_2$-CH$_2$-NH-), 1.72 (m, 4H; -CH$_2$-C*H*$_2$-CH$_2$-NH-), 1.29 (s, 18H; C*H*$_{3Boc}$), 1.14 (t, J = 6.9 Hz, 6H; -N-CH$_2$-C*H*$_{3dye}$).

^{13}C-NMR (125.6 MHz, DMSO, 25°C): δ(ppm) = 171.5/167.6 (Cq; *C*O), 156.8 (Cq; Tyr), 156.2 (Cq; dye), 155.2 (Cq; CO$_{Boc}$), 151.6/146.9/142.8 (Cq; dye), 139.5 (Cq; C$_{5,5'}$), 132.2 (Cq; C$_{3,3'}$), 130.4 (CH; *C*H$_{Tyr}$), 130.3 (Cq; Tyr), 126.2 (CH; C$_{1,1'}$), 126.1/125.0/122.5 (CH; dye), 122.2 (Cq; C$_{6,6'}$), 119.3 (CH; C$_{4,4'}$), 114.0 (CH; *C*H$_{Tyr}$), 111.7 (CH; dye), 109.1 (CH; C$_{2,2'}$), 71.3/69.8/69.6/69.5/69.0 (CH$_2$; -NH-(*C*H$_2$*C*H$_2$O)$_{\approx 16}$-CH$_3$), 65.3 (CH$_2$; -N-CH$_2$-*C*H$_2$-O-$_{dye}$), 58.1 (CH$_3$; -NH-(CH$_2$CH$_2$O)$_{\approx 16}$-*C*H$_3$), 56.1 (CH; Cα$_{Tyr}$), 49.2 (CH$_2$; -N-*C*H$_2$-CH$_2$-O-$_{dye}$), 46.0 (CH$_2$; -N-*C*H$_2$-CO-), 45.3 (CH$_2$; -N-*C*H$_2$-CH$_{3dye}$), 38.7 (CH$_2$; -NH-(*C*H$_2$*C*H$_2$O)$_{\approx 16}$-CH$_3$), 38.2 (CH$_2$; -CH$_2$-CH$_2$-*C*H$_2$-NH-), 36.9 (CH$_2$; Cβ$_{Tyr}$, Cβ'$_{Tyr}$), 32.5 (*C*H$_2$; -*C*H$_2$-CH$_2$-CH$_2$-NH-), 31.7 (CH$_2$; -CH$_2$-*C*H$_2$-CH$_2$-NH-), 28.2 (CH$_3$; C$_{Boc}$), 12.0 (CH$_3$; -N-CH$_2$-*C*H$_{3dye}$).

ESI-MS: m/z: calcd for C$_{113}$H$_{157}$N$_{14}$O$_{29}$Na: 2198 [M-H+Na]$^+$; found: 2198 (distribution of peaks due to the polydispersity of the PEG).

Second coupling: 136.7 mg (62.8 µmol, 1 eq) of **61**
4 ml of 4M HCl in Dioxan
Methanol (2 ml)
150 mg (251.2 µmol, 4 eq) Fmoc-Asn(Trt)-OH
104 mg (251.2 µmol, 4 eq) HCTU in DMF (1 ml)
43 µl (251.2 µmol, 4 eq) ^{i}Pr$_2$Net
CH_2Cl_2 (5 ml)
Yield: 167.6 mg (53.5 µmol, 85%)

C$_{183}$H$_{210}$N$_{18}$O$_{35}$: 3221.73

TLC: MeOH/CH_2Cl_2 (10:90); R_f: 0.49

ESI-MS: m/z: calcd for C$_{183}$H$_{209}$N$_{18}$O$_{35}$Na: 3243 [M-H+Na]$^+$; found: 3243 (distribution of peaks due to the polydispersity of the PEG).

Third coupling: 148 mg (47.1 µmol, 1 eq) of receptor precursor
146 µl (1.41 mmol, 30 eq) diethylamine
DMF (4 ml)
73 mg (188.8 µmol, 4 eq) Fmoc-L-Phe-OH
78 mg (188.8 µmol, 4 eq) HCTU in DMF (2 ml)
32 µl (188.8 µmol, 4 eq) $^{i}Pr_2Net$
CH_2Cl_2 (5 ml)
Yield: 155 mg (45.3 µmol, 96%)

$C_{201}H_{228}N_{20}O_{37}$: 3516.07

TLC: MeOH/CH_2Cl_2 (10:90); R_f: 0.52.

ESI-MS: m/z: calcd for $C_{201}H_{226}N_{20}O_{37}Na$: 3537 $[M-2H+Na]^+$; found: 3537 (distribution of peaks due to the polydispersity of the PEG).

Acetylation: 106 mg (31.0 µmol, 1 eq) of receptor precursor
97 µl (931.1 µmol, 30 eq) diethylamine
DMF (4 ml)
13 µl (93.1 µmol, 3 eq) Et_3N
9 µl (93.1 µmol, 3 eq) Ac_2O
CH_2Cl_2 (4 ml)
Yield: 77 mg (25.1 µmol, 81%) of the two-armed carbazole receptor **42**

$C_{175}H_{212}N_{20}O_{35}$: 3155.67

TLC: MeOH/CH_2Cl_2 (10:90); R_f: 0.46.

^{1}H-NMR (500 MHz, DMSO, 25°C): δ(ppm) = 8.79 (s, 2H; -N*H*$_{Trt}$), 8.39 (d, *J* = 8.2 Hz, 2H; -N*H*$_{Asn}$), 8.34 (d, *J* = 9.1 Hz, 4H; H$_{dye}$), 8.31 (s, 1H; -N*H*-(CH$_2$CH$_2$)$_{16}$-OCH$_3$), 8.07 (d, *J* = 7.7 Hz, 2H; -N*H*$_{Tyr}$), 8.01 (d, *J* = 8.3 Hz, 2H; -N*H*$_{Phe}$), 7.91 (d, *J* = 9.1 Hz, 4H; H$_{dye}$), 7.80 (d, *J* = 9.3 Hz, 4H; H$_{dye}$), 7.79 (m, 2H; -CH$_2$-CH$_2$-CH$_2$-N*H*-), 7.78 (m, 2H; H$_{4,4'}$), 7.38 (d, *J* = 8.3 Hz, 2H; H$_{2,2'}$), 7.26-7.11 (m, 42H, H$_{Phe}$, H$_{Trt}$, H$_{1,1'}$), 7.09 (d, *J* = 8.6 Hz, 4H; H$_{Tyr}$), 6.81 (d, *J* = 9.3 Hz, 4H; H$_{dye}$), 6.72 (d, *J* = 8.6 Hz, 4H; H$_{Tyr}$), 4.96 (s, 2H; -N-C*H*$_2$-CO-), 4.55 (m, 2H; Hα$_{Asn}$), 4.52 (m, 2H; Hα$_{Phe}$), 4.32 (dd, *J* = 13.6, 8.4 Hz, 2H; Hα$_{Tyr}$), 3.87 (m, 4H; -N-CH$_2$-C*H*$_2$-O-$_{dye}$), 3.60 (m, 4H; -N-C*H*$_2$-CH$_2$-O-$_{dye}$), 3.52-3.30 (m, 64H; -NH-(C*H*$_2$C*H*$_2$)$_{16}$-OCH$_3$), 3.42 (m, 4H; -N-C*H*$_2$-CH$_{3dye}$), 3.22 (s, 3H; -NH-(CH$_2$CH$_2$)$_{16}$-OC*H*$_3$), 3.02 (m, 2H; Hβ$_{Tyr}$), 2.93 (m, 2H; Hβ$_{Phe}$), 2.85 (m, 2H; -CH$_2$-CH$_2$-C*H*$_2$-NH-), 2.79 (m, 2H; Hβ$_{Asn}$), 2.72 (m, 2H; Hβ'$_{Tyr}$), 2.68 (m, 2H; Hβ'$_{Phe}$), 2.64 (m, 2H; -CH$_2$-CH$_2$-C*H*$_2$-NH-), 2.61 (m, 2H; Hβ'$_{Asn}$), 2.51 (m, 4H; -C*H*$_2$-CH$_2$-CH$_2$-NH-), 1.71 (s, 6H; -COC*H*$_3$), 1.51 (m, 4H; -CH$_2$-C*H*$_2$-CH$_2$-NH-), 1.09 (t, *J* = 6.9 Hz, 6H; -N-CH$_2$-C*H*$_{3dye}$).

^{13}C-NMR (125.6 MHz, DMSO, 25°C): δ(ppm) = 171.4/170.5/170.2/169.7/169.0/167.6 (Cq; *C*O), 156.7 (Cq; Tyr), 156.2/151.5/146.8 (Cq; dye), 144.7 (Cq; Trt), 142.8 (Cq; dye), 139.4 (Cq; C$_{5,5'}$), 138.1 (Cq; Phe), 132.1 (Cq; C$_{3,3'}$), 130.3 (Cq; Tyr), 130.1 (CH; *C*H$_{Tyr}$), 129.2/127.9 (CH; *C*H$_{Phe}$), 128.5/127.5 (CH; *C*H$_{Trt}$), 126.3 (CH; C$_{1,1'}$), 126.2/125.0/122.5 (CH; dye), 122.2 (Cq; C$_{6,6'}$), 119.3 (CH; C$_{4,4'}$), 113.9 (CH; *C*H$_{Tyr}$), 111.6 (CH; dye), 109.0 (CH; C$_{2,2'}$), 71.3/69.8/69.6(CH$_2$; -NH-(*C*H$_2$*C*H$_2$)$_{16}$-OCH$_3$), 69.6 (Cq; Trt), 69.4/69.0 (CH$_2$; -NH-(*C*H$_2$*C*H$_2$)$_{16}$-OCH$_3$), 65.1 (CH$_2$; -N-CH$_2$-*C*H$_2$-O-$_{dye}$), 58.1 (CH$_3$; -NH-(CH$_2$CH$_2$)$_{16}$-O*C*H$_3$), 54.4 (CH; Cα$_{Tyr}$), 53.7 (CH; Cα$_{Phe}$), 49.7 (CH; Cα$_{Asn}$), 49.0 (CH$_2$; -N-*C*H$_2$-CH$_2$-O-$_{dye}$), 45.5 (CH$_2$; -N-*C*H$_2$-CO-), 45.2 (CH$_2$; -N-*C*H$_2$-CH$_{3dye}$), 38.8 (CH$_2$; -NH-(*C*H$_2$*C*H$_2$)$_{16}$-OCH$_3$), 38.6 (CH$_2$; Cβ$_{Asn}$, Cβ'$_{Asn}$), 38.3 (CH$_2$; -CH$_2$-CH$_2$-*C*H$_2$-NH-), 37.9 (CH$_2$; Cβ$_{Phe}$, Cβ'$_{Phe}$), 36.2 (CH$_2$; Cβ$_{Tyr}$, Cβ'$_{Tyr}$), 32.5 (*C*H$_2$; -*C*H$_2$-CH$_2$-CH$_2$-NH-), 31.3 (CH$_2$; -CH$_2$-*C*H$_2$-CH$_2$-NH-), 22.4 (CH$_3$; -CO*C*H$_3$), 11.9 (CH$_3$; -N-CH$_2$-*C*H$_{3dye}$).

ESI-MS: m/z: calcd for C$_{175}$H$_{211}$N$_{20}$O$_{35}$Na: 3177 [M-H+Na]$^+$; found: 3177 (distribution of peaks due to the polydispersity of the PEG).

13 GENERAL PROTOCOL FOR THE SYNTHESIS OF AN ENCODED COMBINATORIAL TWO-ARMED CARBAZOLE RECEPTOR LIBRARY USING POLYCHLORINATED AROMATICS AS TAG MOLECULES

13.1 Splitting and Encoding of the Resin

5.2 g (2.26 mmol, loading 0.43 mmol g^{-1}) Tentagel S functionalized with *N*-*tert*-Boc protected carbazole template **44**[77] were placed in 150 ml Merrifield vessel and washed three times with CH_2Cl_2. Cleavage of the Boc protecting group was achieved by shaking the resin with a 30% (v/v) mixture of TFA/CH_2Cl_2 for 30 min. The resin was then washed three times with CH_2Cl_2, five times with a 30% (v/v) mixture of $^{i}Pr_2NEt$/ CH_2Cl_2 and then three times with CH_2Cl_2 for 1 min each. Finally, the resin was removed and dried for at least 2 h. Subsequently, the resin was split into fifteen equal portions of 350 mg (0.15 mmol) each and placed into fifteen 25 ml Merrifield shaking vessels. The resin was then suspended in dry CH_2Cl_2 (5 ml). 2 mol% of each tag were dissolved in 1 ml DMF and added separately into the fifteen reaction vessels along with 20 mg (0.15 mmol, 1 eq) HOBt, per tag, in 0.5 ml DMF. The mixtures were shaken for at least 5 min to ensure an equal distribution of the tags. After the addition of 35 µl (0.23 mmol, 1.5 eq) DIC, per tag, to each reaction vessel the mixture was shaken immediately and the reaction allowed to proceed overnight. The fifteen portions of resin were then washed once with DMF and three times with CH_2Cl_2 for 1 min each.

13.2 Check of Successful Encoding

In order to check the success of the tag coupling, three beads from each of the fifteen vessels were isolated in micropipette. The beads were washed two times with DMF, then 1.5 µl DMF was added and the micropipettes were sealed. The tag alcohols were released by photolysis using a UV lamp (366 nm) for 2 h and analyzed by EC-GC analysis. If EC-GC detection of the tags could be achieved, the resin was ready for coupling, if not, the tag coupling process was repeated.

13.3 Synthesis of the Two Peptidic Arms

After checking the success of the first encoding process, the coupling of the fifteen amino acids was performed with 3 eq of each amino acid, 61 mg (0.45 mmol, 3 eq) HOBt in 0.5 ml DMF and 69 µl (0.45 mmol, 3 eq) DIC following the general procedure for Fmoc-synthesis (for the amount of each amino acid for each step, *see* Chapter 13.7, experimental section). After washing the beads three times with DMF and five times with CH_2Cl_2 for 1 min each, the resin portions were recombined in a 150 ml Merrifield vessel. The Fmoc-protecting group was removed as described in the general procedures for Fmoc-synthesis (*see* Chapter 10.2, experimental section). The resin was then dried for at least 2 h after the last CH_2Cl_2 wash and split into fifteen equal portions again.

Splitting, encoding of the resin and coupling of each amino acid cycles were repeated using the protocol described above until the two tripeptidic arms were assembled.

13.4 Synthesis of the Side-Chain Deprotected Carbazole Receptor Library

Fmoc-deprotection and acetylation of 1.0 g (0.43 mmol) protected receptor-resin was achieved following the procedure described in Chapter 10 (experimental section).

Cleavage of the side-chain protecting groups was achieved by shaking 410 mg of acetylated protected receptor-resin with a 95:2.5:2.5 (v/v/v) mixture of TFA/H_2O/TIS (10 ml) first for 5 min, 15 min and then again 45 min. The resin was then washed ten times with CH_2Cl_2, ten times with a 30% (v/v) mixture of Et_3N/CH_2Cl_2 and finally twenty times with CH_2Cl_2 for 1 min each. The resin was dried *in vacuo* and then stored at low temperature (4°C).

13.5 Synthesis of the Tetrapeptide Two-Armed Carbazole Library

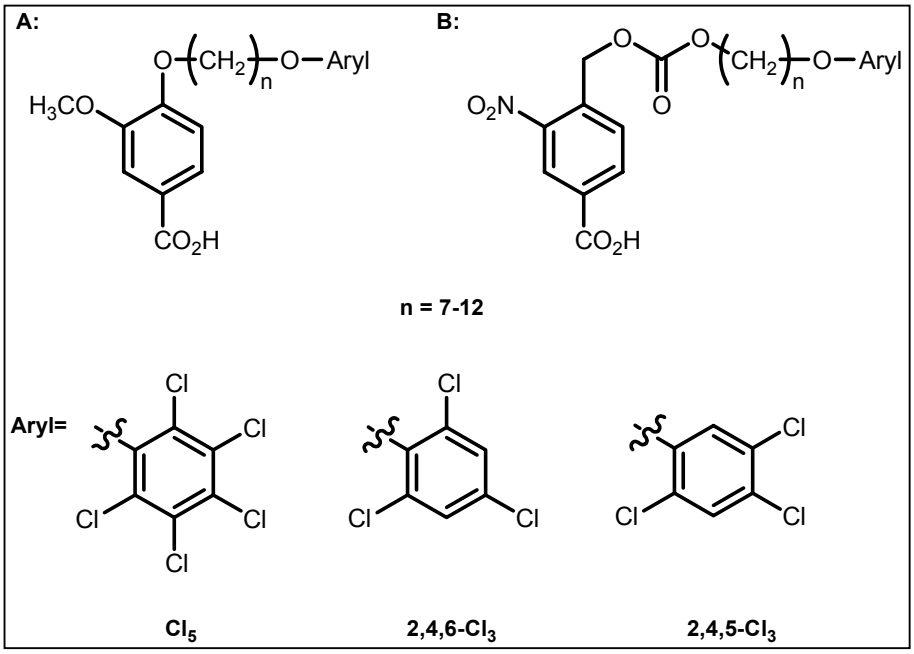
After the amino acid coupling at the third position, Fmoc-deprotection of 3.1 g (1.33 mmol) protected receptor-resin was achieved and the resin dried for at least 2 h. Subsequently, the resin was split into fifteen equal portions of 207 mg (0.09 mmol) each and placed into fifteen 25 ml Merrifield shaking vessels. The resin was then suspended in dry CH_2Cl_2 (5 ml). 2 mol% of each tag were dissolved in 1 ml DMF and added separately into the

fifteen reaction vessels along with 12 mg (0.09 mmol, 1 eq) HOBt, per tag, in 0.5 ml DMF. The mixtures were shaken for at least 5 min to ensure an equal distribution of the tags. After the addition of 21 µl (0.13 mmol, 1.5 eq) DIC, per tag, to each reaction vessel the mixture was agitated immediately and the reaction allowed to proceed overnight. The fifteen portions of resin were then washed once with DMF and three times with CH_2Cl_2 for 1 min each. Encoding of the resin and checking for successful encoding was performed as described in Chapter 13.2.

The coupling of the fifteen amino acids was performed with 3 eq of each amino acid, 41 mg (0.26 mmol, 3 eq) HOBt in 0.5 ml DMF and 42 µl (0.26 mmol, 3 eq) DIC following the general procedure for Fmoc-synthesis (for the amount of each amino acid for each step, *see* Chapter 13.7, experimental section). After washing the beads three times with DMF and five times with CH_2Cl_2 for 1 min each, the resin portions were recombined in a 150 ml Merrifield vessel.

Fmoc-deprotection and acetylation of 1.7 g (0.73 mmol) protected receptor-resin was achieved following the procedure described in Chapter 10 (experimental section). Cleavage of the side-chain protecting groups was achieved by shaking 800 mg of acetyl protected receptor-resin with a 95:2.5:2.5 (v/v/v) mixture of TFA/H_2O/TIS (10 ml) first for 5 min, 15 min and then again 45 min. The resin was then washed ten times with CH_2Cl_2, ten times with a 30% (v/v) mixture of Et_3N/ CH_2Cl_2 and finally twenty times with CH_2Cl_2 for 1 min each. The resin was dried *in vacuo* and then stored at low temperature (4°C).

13.6 Tags Used for the Described Library



*Oxidatively (**A**) and Photolabile (**B**) Cleavage Tag Molecules*

1st step: $(CH_2)_{12}$-Cl_5 (2.03 mg, 3.01 µmol) = tag No 1 in step 1 (**T_1**)
$(CH_2)_{11}$-Cl_5 (1.99 mg, 3.01 µmol) = tag No 2 in step 1 (**T_2**)
$(CH_2)_{10}$-Cl_5 (1.95 mg, 3.01 µmol) = tag No 3 in step 1 (**T_3**)
$(CH_2)_9$-Cl_5 (1.90 mg, 3.01 µmol) = tag No 4 in step 1 (**T_4**)

2nd step: $(CH_2)_{12}$-2,4,5-Cl_3 (1.82 mg, 3.01 µmol) = tag No 5 in step 2 (**T_5**)
$(CH_2)_{12}$-2,4,6-Cl_3 (1.82 mg, 3.01 µmol) = tag No 6 in step 2 (**T_6**)
$(CH_2)_{11}$-2,4,5-Cl_3 (1.78 mg, 3.01 µmol) = tag No 7 in step 2 (**T_7**)
$(CH_2)_{11}$-2,4,6-Cl_5 (1.78 mg, 3.01 µmol) = tag No 8 in step 2 (**T_8**)

3rd step: $(CH_2)_{10}$-2,4,5-Cl_3 (1.74 mg, 3.01 µmol) = tag No 9 in step 3 (**T_9**)
$(CH_2)_{10}$-2,4,6-Cl_3 (1.74 mg, 3.01 µmol) = tag No 10 in step 3 (**T_{10}**)
$(CH_2)_9$-2,4,5-Cl_3 (1.69 mg, 3.01 µmol) = tag No 11 in step 3 (**T_{11}**)
$(CH_2)_9$-2,4,6-Cl_5 (1.69 mg, 3.01 µmol) = tag No 12 in step 3 (**T_{12}**)

4th step: $(CH_2)_8$-2,4,5-Cl_3 (0.98 mg, 1.78 µmol) = tag No 13 in step 4 (**T_{13}**)

$(CH_2)_8$-2,4,6-Cl_3 (0.98 mg, 1.78 µmol) = tag No 14 in step 4 (**T_{14}**)

$(CH_2)_7$-2,4,5-Cl_3 (0.95 mg, 1.78 µmol) = tag No 15 in step 4 (**T_{15}**)

$(CH_2)_7$-2,4,6-Cl_5 (0.95 mg, 1.78 µmol) = tag No 16 in step 4 (**T_{16}**)

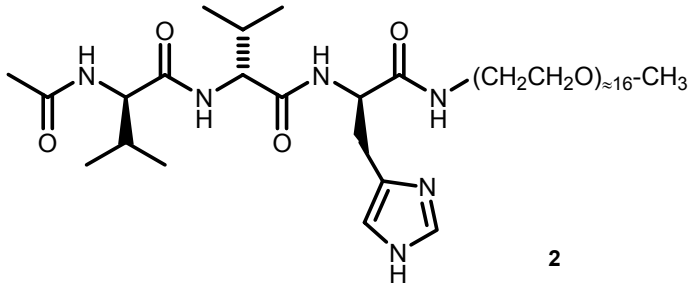
13.7 Encoding Scheme and Amounts of Amino Acids Used

Step 1:	**M.W.**	**0.45 mmol**	**Tag**	**M.W.:**	**Charge**	**Code**
Fmoc-Gly	297.31	134 mg	T_1	673.79	2 mol%	0001
Fmoc-D-Ala	311.34	140 mg	T_2	660.77	"	0010
Fmoc-L-Val	339.39	152 mg	T_3	646.74	"	0100
Fmoc-D-Pro	337.38	152 mg	T_4	632.72	"	1000
Fmoc-L-Phe	387.44	174 mg	$T_1 + T_2$		"	0011
Fmoc-D-Tyr(tBu)	459.54	206 mg	$T_2 + T_3$		"	0110
Fmoc-L-Ser(tBu)	383.44	172 mg	$T_3 + T_4$		"	1100
Fmoc-D-Thr(tBu)	397.5	178 mg	$T_1 + T_3$		"	0101
Fmoc-L-Glu(OtBu)	425.48	191 mg	$T_2 + T_4$		"	1010
Fmoc-D-Asp(OtBu)	411.46	185 mg	$T_1 + T_4$		"	1001
Fmoc-L-Gln(Trt)	610.71	275 mg	$T_1 + T_2 + T_3$		"	0111
Fmoc-D-Asn(Trt)	596.68	268 mg	$T_2 + T_3 + T_4$		"	1110
Fmoc-L-Lys(Boc)	468.55	210 mg	$T_1 + T_2 + T_4$		"	1011
Fmoc-D-His(Trt)	619.72	278 mg	$T_1 + T_3 + T_4$		"	1101
Fmoc-L-Arg(Pbf)	648.78	292 mg	$T_1 + T_2 + T_3 + T_4$		"	1111
Step 2:	**M.W.**	**0.45 mmol**	**Tag**	**M.W.:**	**Charge**	**Code**
Fmoc-Gly	297.31	134 mg	T_5	604.90	2 mol%	0001
Fmoc-L-Ala	311.34	140 mg	T_6	604.90	"	0010
Fmoc-D-Val	339.39	152 mg	T_7	590.88	"	0100
Fmoc-L-Pro	337.38	152 mg	T_8	590.88	"	1000
Fmoc-D-Phe	387.44	174 mg	$T_5 + T_6$		"	0011
Fmoc-L-Tyr(tBu)	459.54	206 mg	$T_6 + T_7$		"	0110
Fmoc-D-Ser(tBu)	383.44	172 mg	$T_7 + T_8$		"	1100
Fmoc-L-Thr(tBu)	397.5	178 mg	$T_5 + T_7$		"	0101
Fmoc-D-Glu(OtBu)	425.48	191 mg	$T_6 + T_8$		"	1010
Fmoc-L-Asp(OtBu)	411.46	185 mg	$T_5 + T_8$		"	1001
Fmoc-D-Gln(Trt)	610.71	275 mg	$T_5 + T_6 + T_7$		"	0111

Fmoc-L-Asn(Trt)	596.68	268 mg	$T_6 + T_7 + T_8$		"	1110
Fmoc-D-Lys(Boc)	468.55	210 mg	$T_5 + T_6 + T_8$		"	1011
Fmoc-L-His(Trt)	619.72	278 mg	$T_5 + T_7 + T_8$		"	1101
Fmoc-D-Arg(Pbf)	648.78	292 mg	$T_5 + T_6 + T_7 + T_8$		"	1111
Step 3:	**M.W.**	**0.45 mmol**	**Tag**	**M.W.:**	**Charge**	**Code**
Fmoc-Gly	297.31	134 mg	T_9	576.85	2 mol%	0001
Fmoc-D-Ala	311.34	140 mg	T_{10}	576.85	"	0010
Fmoc-L-Val	339.39	152 mg	T_{11}	562.85	"	0100
Fmoc-D-Pro	337.38	152 mg	T_{12}	562.85	"	1000
Fmoc-L-Phe	387.44	174 mg	$T_9 + T_{10}$		"	0011
Fmoc-D-Tyr(tBu)	459.54	206 mg	$T_{10} + T_{11}$		"	0110
Fmoc-L-Ser(tBu)	383.44	172 mg	$T_{11} + T_{12}$		"	1100
Fmoc-D-Thr(tBu)	397.5	178 mg	$T_9 + T_{11}$		"	0101
Fmoc-L-Glu(OtBu)	425.48	191 mg	$T_{10} + T_{12}$		"	1010
Fmoc-D-Asp(OtBu)	411.46	185 mg	$T_9 + T_{12}$		"	1001
Fmoc-L-Gln(Trt)	610.71	275 mg	$T_9 + T_{10} + T_{11}$		"	0111
Fmoc-D-Asn(Trt)	596.68	268 mg	$T_{10} + T_{11} + T_{12}$		"	1110
Fmoc-L-Lys(Boc)	468.55	210 mg	$T_9 + T_{10} + T_{12}$		"	1011
Fmoc-D-His(Trt)	619.72	278 mg	$T_9 + T_{11} + T_{12}$		"	1101
Fmoc-L-Arg(Pbf)	648.78	292 mg	$T_9 + T_{10} + T_{11} + T_{12}$		"	1111
Step 4:	**M.W.**	**0.26 mmol**	**Tag**	**M.W.:**	**Charge**	**Code**
Fmoc-Gly	297.31	79 mg	T_{13}	548.80	2 mol%	0001
Fmoc-L-Ala	311.34	83 mg	T_{14}	548.80	"	0010
Fmoc-D-Val	339.39	91 mg	T_{15}	534.80	"	0100
Fmoc-L-Pro	337.38	90 mg	T_{16}	534.80	"	1000
Fmoc-D-Phe	387.44	103 mg	$T_{13} + T_{14}$		"	0011
Fmoc-L-Tyr(tBu)	459.54	122 mg	$T_{14} + T_{15}$		"	0110
Fmoc-D-Ser(tBu)	383.44	102 mg	$T_{15} + T_{16}$		"	1100
Fmoc-L-Thr(tBu)	397.5	105 mg	$T_{13} + T_{15}$		"	0101
Fmoc-D-Glu(OtBu)	425.48	112 mg	$T_{14} + T_{16}$		"	1010
Fmoc-L-Asp(OtBu)	411.46	109 mg	$T_{13} + T_{16}$		"	1001
Fmoc-D-Gln(Trt)	610.71	163 mg	$T_{13} + T_{14} + T_{15}$		"	0111
Fmoc-L-Asn(Trt)	596.68	159 mg	$T_{14} + T_{15} + T_{16}$		"	1110
Fmoc-D-Lys(Boc)	468.55	124 mg	$T_{13} + T_{14} + T_{16}$		"	1011
Fmoc-L-His(Trt)	619.72	165 mg	$T_{13} + T_{15} + T_{16}$		"	1101
Fmoc-D-Arg(Pbf)	648.78	173 mg	$T_{13} + T_{14} + T_{15} + T_{16}$		"	1111

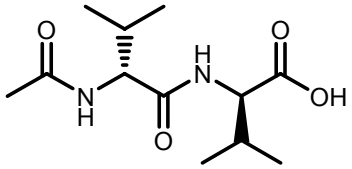
14 SYNTHESIS OF THE PEGYLATED TRIPEPTIDES IN SOLUTION PHASE AND ON SOLID SUPPORT

14.1 Synthesis of the Peptide-PEG Conjugate 2 in Solution Phase



2

14.1.1 General Fmoc-Strategy for the Synthesis of Peptides onto Hydroxy-Functionalized Resin Exemplified by the Synthesis of Ac-D-Val-D-Val–OH 52



52

2.5 g (2.50 mmol, 100-200 mesh, loading 1 mmol g^{-1}) alkoxy-benzyl alkohol resin (Wang resin) were placed in a 25 ml Merrifiel vessel and washed three times with CH_2Cl_2 for 1 min each. The resin was then suspended in smallest amount of dry CH_2Cl_2 to allow efficient shaking (15 ml). 2.5 g (7.50 mmol, 3 eq) Fmoc-D-Val-OH, 1.0 g (7.50 mmol, 3 eq) HOBt dissolved in the smallest amount possible of dry DMF (3 ml) and 30 mg (0.1 eq relative to the resin loading) DMAP were added and the mixture was shaken 5 min before 1.2 ml (7.50 mmol, 3 eq) DIC were added. The mixture was shaken for 2 h and then washed three times with DMF and five times with CH_2Cl_2 for 1 min each. Semiquantitative Fmoc-test was used to determine the loading after the coupling.[71] If the value obtained was less than 70% the first residue attachment procedure was repeated. For the Fmoc-deprotection the resin was washed three times with DMF for 1 min each. The deprotection was achieved by shaking the resin with a mixture of 20% (v/v) piperidine in DMF (30 ml), first for 2 min and then for 10 min. After washing the resin three times with DMF and five times with CH_2Cl_2, 1 min each, the resin was ready for the next coupling. Both the amino acid coupling and the Fmoc-deprotection were closely monitored by the Kaiser test for free amines.[99] Consecutive coupling without DMAP and deprotection cycle employing 2.5 g (7.50 mmol, 3 eq) Fmoc-D-Val-OH yielded H-D-Val-D-val-resin. The resin was suspended in 15 ml dry CH_2Cl_2 and 1.8 ml

(12.50 mmol, 5 eq) Et_3N, before 1.2 ml (12.50 mmol, 5 eq) Ac_2O were added. The mixture was allowed to shake for 1 h and then washed three times with DMF and five times with CH_2Cl_2 1 min each. The Kaiser test was performed to ensure the success of acetylation. Cleavage of the side-chain protected peptide from the solid support was achieved by treating the resin two times with a 95:2.5:2.5 (v/v/v) mixture of TFA/H_2O/TIS (30 ml) at r.t. for 1 h each. The resin was then washed three times with CH_2Cl_2 (30 ml). After removal of the solvent, the oily residue was triturated with Et_2O to yield a white solid which was isolated by decantation followed by removal of all volatiles *in vacuo*. 514 mg (1.99 mmol, 80%) Ac-D-Val-D-Val-OH **52** were obtained without further purification.

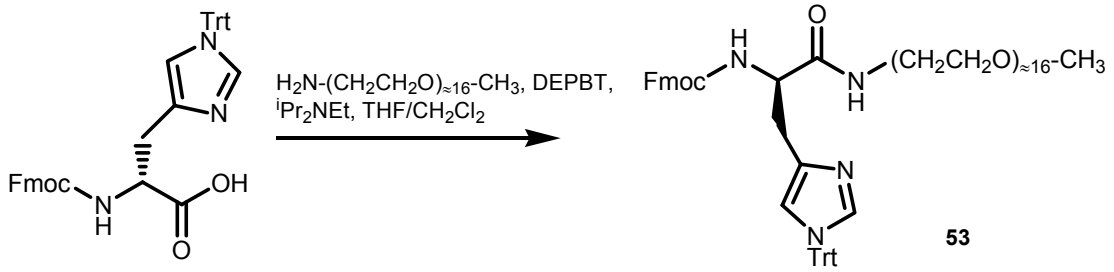
$C_{12}H_{22}N_2O_4$: 258.31 g/mol

^{1}H-NMR (500 MHz, CD_3OD, 25°C): δ(ppm) = 4.34 (d, J = 5.7 Hz, 1H; $H\alpha_{D\text{-}Val}$), 4.25 (d, J = 7.7 Hz, 1H; $H\alpha_{D\text{-}Val}$), 2.20 (dsept, J = 6.8, 5.8 Hz, 1H; $H\beta_{D\text{-}Val}$), 2.03 (septd, J = 7.7, 6.8 Hz, 1H; $H\beta_{D\text{-}Val}$), 2.00 (s, 3H; -$COCH_3$), 1.02-0.97 (m, 12H; $H\gamma_{D\text{-}Val}$).

^{13}C-NMR (125.6 MHz, CD_3OD, 25°C): δ(ppm) = 173.1/172.6/171.9 (Cq; *C*O), 58.9 (CH; $C\alpha_{D\text{-}Val}$), 57.5 (CH; $C\alpha_{D\text{-}Val}$), 30.3/30.3 (CH; $C\beta_{D\text{-}Val}$), 20.9 (CH_3; -CO*C*H_3), 18.3/18.1/17.4/16.9 (CH_3; $C\gamma_{D\text{-}Val}$).

ESI-MS: m/z: calcd for $C_{12}H_{22}N_2O_4Na$: 281 $[M+Na]^+$; found: 281, calcd for $C_{24}H_{44}N_4O_8Na$: 539 $[2M+Na]^+$; found: 539, calcd for $C_{12}H_{21}N_2O_4Na$: 257 $[M-H]^-$; found: 257, calcd for $C_{24}H_{43}N_2O_4$: 515 $[2M-H]^-$; found: 515.

14.1.2 Synthesis of PEG-Chain Functionalized Fmoc-D-His(Trt)



53

1.5 g (2.42 mmol, 1.2 eq) Fmoc-D-His(Trt)-OH were preactivated with 898 mg (3.02 mmol, 1.5 eq) DEPBT and 685 µl (4.03 mmol, 2 eq) $^{i}Pr_2NEt$ dissolved in a 1:1 (v/v) mixture of THF/CH_2Cl_2 (14 ml) for 1 min at r.t. and then added to a solution of dry CH_2Cl_2 (28 ml) containing 1.5 g (2.02 mmol, 1 eq) H_2N-$(OCH_2CH_2)_{\approx 16}$-OCH_3 which was before melted at 80°C *in vacuo* for 30 min, in order to remove traces of moisture. The reaction mixture was allowed to stir at r.t. for 3 h. After removal of the solvent under reduced pressure, the residue was purified by flash chromatography on silica gel (gradient of CH_2Cl_2/MeOH from 99.5:0.5 to 98:2 (v/v)) to afford 2.6 g (1.91 mmol, 95%) Fmoc-D-His(Trt)-NH-$(CH_2CH_2O)_{\approx 16}$-CH_3 **53** as a white oil.

$C_{73}H_{100}N_4O_{19}$: 1336.59 g/mol.

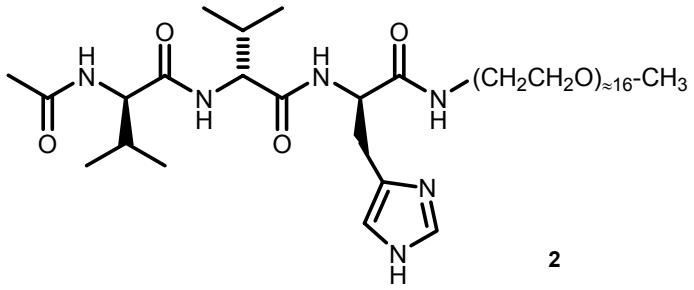
TLC: MeOH/CH_2Cl_2 (10:90); R_f: 0.56.

^{1}H-NMR (500 MHz, CD_3OD, 25°C): δ(ppm) = 7.76 (d, J = 7.6 Hz, 2H; H_{Fmoc}), 7.58 (d, J = 7.5 Hz, 2H; H_{Fmoc}), 7.34 (m, 2H; H_{Fmoc}), 7.33 (s, 1H; H_{Imi}), 7.28-7.20/7.06-7.01 (m, 17H; H_{Trt}/H_{Fmoc}), 6.75 (s, 1H; H_{Imi}), 4.41 (dd, J = 4.9, 9.1 Hz, 1H; $H\alpha_{D\text{-}His}$), 4.21 (m, J = 7.2 Hz, 2H; CH_{2Fmoc}), 4.07 (t, J = 7.1 Hz, 1H; CH_{Fmoc}), 3.59-3.51 (m, 64H; -NH-(CH_2-CH_2O)$_{\approx 16}$-CH_3), 3.29 (s, 3H; -NH-(CH_2-CH_2O)$_{\approx 16}$-CH_3), 3.03 (dd, J = 4.8, 14.8 Hz, 1H; $H\beta_{D\text{-}His}$), 2.84 (dd, J = 9.2, 14.7 Hz, 1H; $H\beta'_{D\text{-}His}$).

^{13}C-NMR (125.6 MHz, CD_3OD, 25°C): δ(ppm) = 172.5 (Cq; *C*O), 156.6 (Cq; -*C*O_2-), 143.9/143.8 (Cq; C_{Fmoc}), 142.2 (Cq; C_{Trt}), 141.2/141.1 (Cq; C_{Fmoc}), 138.0 (CH; *C*H_{Imi}), 136.6 (Cq; C_{Imi}), 129.5/128.1/128.0 (CH; *C*H_{Trt}), 127.6/127.0/125.1/119.8 (CH; *C*H_{Fmoc}), 119.7 (CH; *C*H_{Imi}), 75.4 (Cq; C_{Trt}), 71.6/70.2/70.0/69.9/60.1 (CH_2; -NH-(*C*H_2-*C*H_2O)$_{\approx 16}$-CH_3), 66.7 (CH_2; *C*H_{2Fmoc}), 57.8 (CH_3; -NH-(CH_2-CH_2O)$_{\approx 16}$-*C*H_3), 55.18 (CH; $C\alpha_{D\text{-}His}$), 47.0 (CH; *C*H_{Fmoc}), 39.3/39.1 (CH_2; -NH-(*C*H_2-*C*H_2O)$_{\approx 16}$-CH_3) 30.8 (CH_2; $C\beta_{D\text{-}His}$, $C\beta'_{D\text{-}His}$).

ESI-MS: m/z: calcd for $C_{73}H_{100}N_4O_{19}Na$: 1359 $[M+Na]^+$; found 1359 (distribution of peaks due to the polydispersity of the PEG).

14.1.3 Coupling of 52 and Side-Chain Deprotection



2

1.8 g (1.30 mmol, 1 eq) Fmoc-D-His(Trt)-NH-$(CH_2CH_2O)_{\approx 16}$-CH_3 **53** were dissolved in acetonitrile (64 ml) followed by addition of 4.1 ml (39.10 mmol, 30 eq) diethylamine and allowed to stir at r.t. for 1 h. After removal of all volatiles under reduced pressure, the oily residue was co-evaporated 3 times with *n*-heptane to yield the corresponding amine as a white oil, which was dissolved in CH_2Cl_2 (18 ml). 403 mg (1.56 mmol, 1.2 eq) Ac-D-Val-D-Val-OH **52** were preactivated with 583 mg (1.95 mmol, 1.5 eq) DEPBT and 445 µl (2.60 mmol, 2 eq) $^iPr_2NEt$ dissolved in a 1:1 (v/v) mixture of THF/CH_2Cl_2 (12.5 ml) for 1 min at r.t. and then added to the solution. The reaction mixture was allowed to stir at r.t. for 4 h. After removal of the solvent under reduced pressure, the residue was purified by flash chromatography on silica gel (gradient of CH_2Cl_2/MeOH/Et_3N from 99.5:0.5:0.1 to 96:4:0.1 (v/v/v)) to afford 1.57 g (1.14 mmol, 88 %) Ac-D-Val-D-Val-D-His(Trt)-NH-$(CH_2CH_2O)_{\approx 16}$-CH_3 **54** as a white oil.

1.6 g (1.14 mmol) of the protected pegylated tripeptide **54** were dissolved in a 1:5 (v/v) mixture of TFA/CH_2Cl_2 (78.5 ml) and allowed to stir at r.t. for 1 h. After removal of all volatiles under reduced pressure, the oily residue was triturated with cold Et_2O to yield a white solid which was isolated by decantation followed by removal of all residual volatiles under reduced pressure. The TFA-salt peptide was treated with ion-exchange chromatography (Dowex® 1x2-400, H_2O) affording 921 mg (827.2 µmol, 71%) of the desalted pegylated tripeptide **2**, as a colorless sticky oil.

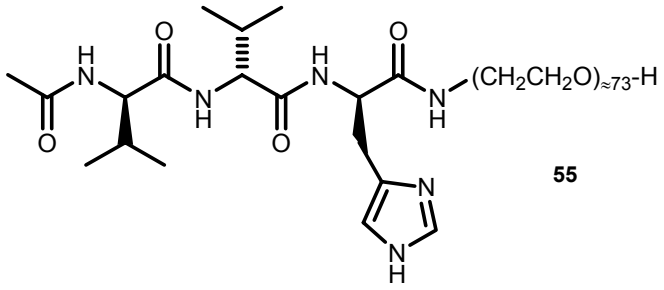
$C_{51}H_{96}N_6O_{20}$: 1113.34 g/mol.

^{1}H-NMR (500 MHz, CD_3OD, 25°C): δ(ppm) = 7.29 (s, 1H; H_{Imi}), 6.56 (s, 1H; H_{Imi}), 4.26 (dd, J = 1.5, 7.8 Hz, 1H; $H\alpha_{D\text{-}His}$), 3.85 (d, J = 3.8 Hz, 1H; $H\alpha_{D\text{-}Val}$), 3.84 (d, J = 3.7 Hz, 1H; $H\alpha_{D\text{-}Val}$), 3.31-2.94 (m, 64H; -NH-(C*H*$_2$-C*H*$_2$O)$_{\approx 16}$-CH_3), 3.04 (s, 3H; -NH-(CH_2-CH_2O)$_{\approx 16}$-C*H*$_3$), 2.73 (dd, J = 6.2, 14.9 Hz, 1H; $H\beta_{D\text{-}His}$), 2.63 (dd, J = 7.9, 14.8 Hz, 1H; $H\beta'_{D\text{-}His}$), 1.74-1.69 (m, 2H; $H\beta_{D\text{-}Val}$), 1.68 (s, 3H; -COC*H*$_3$), 0.62-0.57 (m, 12H; $H\gamma_{D\text{-}Val}$).

^{13}C-NMR (125.6 MHz, CD_3OD, 25°C): δ(ppm) = 173.9/173.4/173.1/173.0 (Cq; *C*O), 136.3 (CH; *C*H_{Imi}), 72.9/71.5/71.3/71.3/70.4 (CH_2; -NH-(*C*H_2-*C*H_2O)$_{\approx 16}$-CH_3), 60.5/60.2 (CH; $C\alpha_{D\text{-}Val}$), 59.1 (CH_2; -NH-(CH_2-CH_2O)$_{\approx 16}$-*C*H_3), 54.7 (CH; $C\alpha_{D\text{-}His}$), 40.4 (CH_2; -NH-(*C*H_2-*C*H_2O)$_{\approx 16}$-CH_3), 31.9 (CH; $C\beta_{D\text{-}Val}$), 31.6 (CH; $C\beta_{D\text{-}Val}$), 30.4 (CH_2; $C\beta_{D\text{-}His}$, $C\beta'_{D\text{-}His}$), 22.4 (CH_3; -CO*C*H_3), 19.8/19.7/18.9/18.8 (CH_3; $C\gamma_{D\text{-}Val}$).

ESI-MS: m/z: calcd for $C_{51}H_{96}N_6O_{20}Na$: 1136 $[M+Na]^+$; found 1136 (distribution of peaks due to the polydispersity of the PEG).

14.2 General Fmoc-Strategy for the Synthesis of Peptides onto Tentagel PAP Resin, Exemplified by the Synthesis of Ac-D-Val-D-Val-D-His-NH-$(CH_2CH_2O)_{\approx 73}$-H 55



55

14.2.1 Coupling onto Tentagel PAP Resin

2.5 g (600.0 µmol, loading 0.24 mmol g^{-1}) of the Tentagel PAP resin were placed in a clean dry Merrifield vessel and washed three times with CH_2Cl_2 for 1 min. The resin was then suspended in smallest amount dry CH_2Cl_2 to allow efficient shaking (15 ml). 740 mg (1.20 mmol, 2 eq) Fmoc-D-His(Trt)-OH along with 497 mg (1.20 mmol, 2 eq) HCTU in DMF (2 ml) were added and the mixture was shaken for 5 min before 412 µl (2.40 mmol, 4 eq) $^{i}Pr_2NEt$ were added. The mixture was shaken for 2 h and then washed three times with DMF and five times with CH_2Cl_2 for 1 min each. The amino acid coupling was monitored by the Kaiser test.

14.2.2 Fmoc-Deprotection

The Fmoc-functionalized resin was washed three times with DMF (1 min) for 1 min each. The deprotection was achieved by shaking the resin with a mixture of 20% (v/v) piperidine in DMF (20 ml, v/v) first for 2 min and then for 10 min. After washing the resin three times with DMF (5 ml) and five times with CH_2Cl_2 (5 ml) for 1min each, the Kaiser test was performed to ensure the successful Fmoc-deprotection. Consecutive coupling and deprotection cycles employing 407 mg (1.20 mmol, 2 eq) Fmoc-D-Val-OH yielded H-D-Val-D-Val-D-His(Trt)-PEG-resin.

14.2.3 Acetylation of Free Amines

The resin was suspended in 15 ml dry CH_2Cl_2 and 412 µl (3.00 mmol, 5 eq) Et_3N, before 287 µl (3.00 mmol, 5 eq) Ac_2O were added. The mixture was allowed to shake at r.t. for 1 h and then washed three times with DMF (5 ml) and five times with CH_2Cl_2 (5 ml) for 1 min each.

14.2.4 Removal of Peptide from Tentagel PAP Resin

The solid-supported peptide on Tentagel PAP resin was first dried *in vacuo* and treated with a 99:1 (v/v) mixture of TFA/TMSBr (30 ml) at r.t. for 6 h. Following filtration, the resin was then washed once with the cleavage mixture (30 ml), TFA (30 ml) and three times with CH_2Cl_2 (15 ml). Filtrates were combined and after removal of the solvent, the oily residue was triturated with Et_2O to yield a white solid which was isolated by decantation followed by removal of all residual volatiles under reduced pressure. The TFA-salt of the peptide was treated with ion-exchange chromatography (Dowex® 1x2-400, H_2O) affording, after concentration of the fractions, 1.6 g (451.5 µmol, 75%) of the desalted pegylated tripeptide **55**, as a colorless sticky oil.

$C_{164}H_{322}N_6O_{77}$: 3610.31 g/mol.

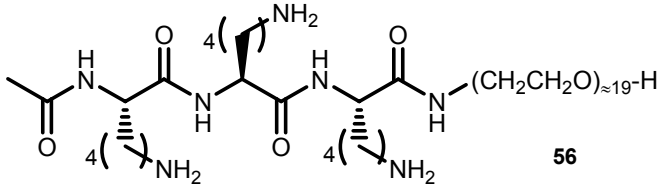
^{1}H-NMR (500 MHz, CD_3OD, 25°C): δ(ppm) = 8.90 (s, 1H; H_{Imi}), 7.43 (s, 1H; H_{Imi}), 4.66 (ψt, J = 6.6 Hz, 1H; $H\alpha_{D\text{-}His}$), 4.17 (d, J = 7.6 Hz, 1H; $H\alpha_{D\text{-}Val}$), 4.10 (d, J = 7.5 Hz, 1H; $H\alpha_{D\text{-}Val}$), 3.82-3.76/3.72-3.55/3.54-3.47/3.25 (m, 292H; -NH-(C*H*$_2$-C*H*$_2$O)$_{\approx 73}$-H), 3.23 (dd, J = 6.5, 14.9 Hz, 1H; $H\beta_{D\text{-}His}$), 3.12 (dd, J = 6.3, 15.1 Hz, 1H; $H\beta'_{D\text{-}His}$), 2.08-2.02 (m, 2H; $H\beta_{D\text{-}Val}$), 2.01 (s, 3H; -COC*H*$_3$), 0.99-0.93 (m, 12H; $H\gamma_{D\text{-}Val}$).

^{13}C-NMR (125.6 MHz, CD_3OD, 25°C): δ(ppm) = 174.4/173.7/173.6/171.7 (Cq; *C*O), 135.9 (CH; *C*H_{Imi}), 130.5 (Cq; C_{Imi}), 119.1 (CH; *C*H_{Imi}), 74.0/71.7/71.5/70.6/62.5 (CH_2; -NH-(*C*H_2-*C*H_2O)$_{\approx 73}$-H), 60.5/60.8 (CH; $C\alpha_{D\text{-}Val}$), 53.8 (CH; $C\alpha_{D\text{-}His}$), 40.7 (CH_2; -NH-(O*C*H_2-*C*H_2O)$_{\approx 73}$-H), 31.9/31.9 (CH; $C\beta_{D\text{-}Val}$), 28.6 (CH_2; $C\beta_{D\text{-}His}$, $C\beta'_{D\text{-}His}$), 22.7 (CH_3; -CO*C*H_3), 20.1/20.0/19.3/19.2 (CH_3; $C\gamma_{D\text{-}Val}$).

ESI-MS: m/z: calcd for $C_{164}H_{321}N_6O_{77}$: 3609 $[M-H]^+$; found 3609 (distribution of peaks due to the polydispersity of the PEG).

14.3 Synthesis of Ac-Lys-Lys-Lys-NH-$(CH_2CH_2O)_{\approx 19}$-H 56

(**56** was obtained in analogy to the procedure described in 14.2)



56

$C_{58}H_{117}N_7O_{23}$: 1280.58 g/mol.

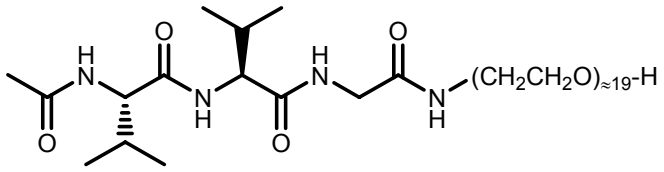
^{1}H-NMR (500 MHz, CD_3OD, 25°C): δ(ppm) = 4.38-4.25 (m, 3H; $H\alpha_{Lys}$), 3.85-3.40/3.39-3.19 (m, 76H; -NH-(CH_2-$CH_2O)_{\approx 19}$-H), 2.98 (m, 6H; $H\varepsilon_{Lys}$), 2.00 (s, 3H; -$COCH_3$), 1.84 (m, 3H; $H\beta_{Lys}$), 1.74 (m, 3H; $H\beta'_{Lys}$), 1.70 (m, 6H; $H\delta_{Lys}$), 1.49 (m, 6H; $H\gamma_{Lys}$).

^{13}C-NMR (125.6 MHz, CD_3OD, 25°C): δ(ppm) = 170.9/170.8/170.6 (Cq; *C*O), 70.7/68.6/68.2/68.0/67.5/59.2 (CH_2; -NH-(*C*H_2-*C*$H_2O)_{\approx 19}$-H), 52.0/51.6/51.5 (CH; $C\alpha_{Lys}$), 46.9 (CH_2; -NH-(*C*H_2-*C*$H_2O)_{\approx 19}$-H), 37.8/37.6/37.3 (CH_2; $C\varepsilon_{Lys}$), 29.8/29.5/29.3 (CH_2; $C\beta_{Lys}$, $C\beta'_{Lys}$), 25.2/25.2/25.0 (CH_2; $C\delta_{Lys}$), 20.9/20.8/20.7 (CH_2; $C\gamma_{Lys}$), 19.5 (CH_3; -CO*C*H_3).

ESI-MS: m/z: calcd for $C_{58}H_{117}N_7O_{23}$: 1280 $[M]^+$; found 1280 (distribution of peaks due to the polydispersity of the PEG).

14.4 Synthesis of Ac-Val-Val-Gly-NH-$(CH_2CH_2O)_{\approx 19}$-H 57

(**57** was obtained in analogy to the procedure described in 14.2)



57

$C_{52}H_{102}N_4O_{23}$: 1151.38 g/mol.

^{1}H-NMR (500 MHz, CD_3OD, 25°C): δ(ppm) = 4.16 (d, J = 7.7 Hz, 1H; $H\alpha_{Val}$), 4.07 (d, J = 7.6 Hz, 1H; $H\alpha_{Val}$), 3.91 (ψd, J = 16.7 Hz, 1H; $H\alpha_{Gly}$), 3.77 (ψd, J = 16.7 Hz, 1H; $H\alpha_{Gly}$), 3.72-3.39/3.37-3.24 (m, 76H; -NH-(CH_2-CH_2O)$_{\approx 19}$-H), 2.14-2.02 (m, 2H; $H\beta_{Val}$), 1.99 (s, 3H; -$COCH_3$), 0.99-0.94 (m, 12H; $H\gamma_{Val}$).

^{13}C-NMR (125.6 MHz, CD_3OD, 25°C): δ(ppm) = 174.2/174.0/173.5/171.4 (Cq; CO), 73.6/71.6/71.5/71.5/71.4/71.3/70.4/62.2 (CH_2; -NH-(OCH_2-CH_2)$_{\approx 19}$-OH), 60.9/60.4 (CH; $C\alpha_{Val}$), 49.6 (CH_2; -NH-(CH_2-CH_2O)$_{\approx 19}$-H), 43.4 (CH; $C\alpha_{Gly}$), 40.4 (CH_2; -NH-(CH_2-CH_2O)$_{\approx 19}$-H), 31.7/31.5 (CH; $C\beta_{Val}$), 22.4 (CH_3; -$COCH_3$), 19.8/19.7/19.0/18.9 (CH_3; $C\gamma_{Val}$).

ESI-MS: m/z: calcd for $C_{52}H_{102}N_4O_{23}Na$: 1174 $[M+Na]^+$; found 1174 (d distribution of peaks due to the polydispersity of the PEG).

C. Notes and References

[1] For reviews see: a) J.J. Lavigne, E. V. Anslyn, *Angew. Chem. Int. Ed.* **2001**, *40*, 3118; b) M. W. Peczuh, A. D. Hamilton, *Chem. Rev.* **2000**, *100*, 2479; c) N. Srinivasan, J. D. Kilburn, *Curr. Opin. Chem. Biol.* **2004**, *8*, 305.

[2] a) O. Middel, W. Reinhoudt, *Eur. J. Org. Chem.* **2002**, *15*, 2587; b) S. Rensing, T. Schrader, *Org. Lett.* **2002**, *4*, 2161; c) O. Rusin, K. Lang, V. Kral, *Chem. Eur. J.* **2002**, *8*, 655; d) S. Rensing, M. Arendt, A. Springer, T. Grawe, T. Schrader, *J. Org. Chem.* **2001**, *66*, 5814; e) G. Giraudi, C. Giovannoli, C. Tozzi, C. Baggiani, L. Anfossi, *Chem. Commun.* **2000**, *13*, 1135.

[3] a) J. Kehler, B. Ebert, O. Dahl, Povl. Krogsgaard-Larsen, *Tetrahedron* **1999**, *55*, 771; b) S. Patterson, S. Bradley, R. E. Taylor, *Tetrahedron Lett.* **1998**, *39*, 3111; c) H. Shinmori, M. Takeuchi, S. Shinkai, *J. Chem. Soc., Perkin Transaction 2* **1998**, *4*, 847; d) H. Fenniri, M. W. Hosseini, J. M. Lehn, *Helv. Chim. Acta* **1997**, *80*, 786; e) M. Mazik, W. Sicking, *Chemistry* **2001**, *7*, 664.

[4] a) B. Hinzen, P. Seiler, F. Diederich, *Helv. Chim. Acta* **1996**, *79*, 942; b) C. L. Nesloney, J. W. Kelly, *J. Am. Chem. Soc.* **1996**, *118*, 5836; c) M. W. Peczuh, A. D. Hamilton, J. Sanchez-Quesada, J. de Mendoza, T. Haack, E. Giralt, *J. Am. Chem. Soc.* **1997**, *119*, 9327; d) M. Torneiro, W. C. Still, *Tetrahedron* **1997**, *53*, 8739; e) M. A. Hossain, H.-J. Schneider, *J. Am. Chem. Soc.* **1998**, *120*, 3536; f) R. Xu, G. Greiveldinger, L. E. Marenus, A. Cooper, J. A. Ellman, *J. Am. Chem. Soc.* **1999**, *121*, 4898.

[5] a) C. Chamorro, R. M. J. Liskamp, *Tetrahedron* **2004**, *60*, 11145; b) C. Chamorro, J.-W. Hofman, R. M. J. Liskamp, *Tetrahedron* **2004**, *60*, 8691; c) C. Schmuck, M. Heil, *Org. Biomol. Chem.* **2003**, *1*, 633; d) C. Schmuck, M. Heil, *ChemBioChem* **2003**, *4*, 1232; e) C. Schmuck, L. Geiger, *J. Am. Chem. Soc.* **2004**, *126*, 8898; f) M. Wehner, D. Janssen, G. Schäder, T. Schrader, *Eur. J. Org. Chem.* **2006**, 138; g) C. Schmuck, M. Heil, J. Scheiber, K. Baumann, *Angew. Chem. Int. Ed.* **2005**, *44*, 7208; h) C. Schmuck, P. Wich, *Angew. Chem. Int. Ed.* **2006**, *45*, 4277; i) C. Schmuck, M. Heil, *Chem. Eur. J.* **2006**, *12*, 1339.

[6] a) E. J. Iorio, Y. Shao, C.T. Chen, H. Wagner, W. C. Still, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1635; b) L. Baldini, A. J. Wilson, J. Hong, A. D. Hamilton, *J. Am. Chem. Soc.* **2004**, *126*, 5656; c) M. Mauer, T. Schrader, *Angew. Chem. Int. Ed.* **2005**, *44*, 2265; d) W. L. Wong, K.-H. Huang, P.-F. Tei, C.-S. Lee, H.-L. Kwong, *Chem.*

Commun. **2005**, 384; e) Z. Zhong, E. V. Anslyn, *J. Am. Chem. Soc.* **2002**, *124*, 9014; f) S. E. Schneider, S. N. O'Neil, E. V. Anslyn, *J. Am. Chem. Soc.* **2000**, *122*, 542; g) K. Niikura, A. Metzger, E. V. Anslyn, *J. Am. Chem. Soc.* **1998**, *120*, 8533.

[7] a) F. Gasparrini, D. Misiti, W. C. Still, C. Villani, H. Wennemers, *J. Org. Chem.* **1997**, *62*, 8221; b) F. Gasparrini, D. Misiti, C. Villani, A. Borchardt, M. T. Burger, W. C. Still, *J. Org. Chem.* **1995**, *60*, 4314.

[8] a) J. P. Waltho, J. Cavanagh, W. H. Dudley, *J. Chem. Soc.* **1988**, *11*, 707; b) G. E. Hawkes, H. Molinari, S. Singh, L. Y. Lian, *J. Magnet. Res.* **1987**, *74*, 188; c) D. H. Williams, B. Bardsley, *Angew. Chem. Int. Ed.* **1999**, *38*, 1172; d) T. D. H. Bugg, G. D. Wright, S. Dutka-Mallen, P. Courvalin, C. T. Walsh, *Biochemistry* **1991**, *30*, 10408; e) R. D. Süssmuth, *ChemBioChem* **2002**, *3*, 295; f) K. C. Nicolaou, C. N. C. Boddy, S. Bräse, N. Winssinger, *Angew. Chem. Int. Ed.* **1999**, *38*, 2096.

[9] a) J. C. J. Barne, D. H. Williams, *Annu. Rev. Microbiol.* **1984**, *38*, 339; b) D. H. Williams, *Nat. Prod. Rep.* **1996**, *13*, 469; c) M. L. Cohen, *Science* **1992**, *257*, 1050; d) H. C. Neu, *Science* **1992**, *257*, 1064.

[10] C. T. Walsh, S. L. Fisher, I. S. Park, M. Prahalad, *Chem. Biol.* **1996**, *3*, 21.

[11] a) B. P. Morgan, J. M. Scholtz, M. D. Ballinger, I. D. Zipkin, P. A. Bartlett, *J. Am. Chem. Soc.* **1991**, *113*, 297; b) C. C. McComas, B. M. Crowley, D. L. Boger, *J. Am. Chem. Soc.* **2003**, *125*, 9314; c) C. Schmuck, D. Rupprecht, W. Wienand, *Chem. Eur. J.* **2006**, *12*, 9186.

[12] M. C. F. Monnee, A. J. Brouwer, R. M. J. Liskamp, *QSAR Comb. Sci.* **2004**, *23*, 546.

[13] C. W. Chen, H. W. Jr. Whitlock, *J. Am. Chem. Soc.* **1978**, *100*, 4921.

[14] a) W. C. Still, *Acc. Chem. Res.* **1996**, *29*, 155; b) C. Gennari, H. P. Nestler, U. Piarulli, B. Salom, *Liebigs Ann. / Recueil* **1997**, 637.

[15] a) A. T. Wright, E. V. Anslyn, *Chem. Soc. Rev.* **2006**, *35*, 14; b) A. T. Wright, E. V. Anslyn, J. T. McDevitt, *J. Am. Chem. Soc.* **2005**, *127*, 17405.

[16] a) H. Wennemers, S. S. Yoon, W. C. Still, *J. Org. Chem.* **1995**, *60*, 1108; b) M. Torneiro, W. C. Still, *J. Am. Chem. Soc.* **1995**, *117*, 5887; c) Y. Shao, W. C. Still, *J. Org. Chem.* **1996**, *61*, 6086; d) Z. Pan, W. C. Still, *Tetrahedron Lett.* **1996**, *37*, 8699.

[17] a) R. C. Boyce, G. Li, H. P. Nestler, T. Suenaga, W. C. Still, *J. Am. Chem. Soc.* **1994**, *116*, 7955; b) Y. Cheng, T. Suenaga, W. C. Still, *J. Am. Chem. Soc.* **1996**, *118*, 1813; c) H. Hioki, T. Yamada, C. Fujioka, M. Kodama, *Tetrahedron Lett.* **1999**, *40*, 6821; d) H. De Muynck, A. Madder, N. Farcy, P.J. De Clercq, M.N. Pérez-Payán, L. M. Öhberg, A. P. Davis, *Angew. Chem. Int. Ed.* **2000**, *39*, 145.

[18] a) C. Gennari, H. P. Nestler, B. Salom, W. C. Still, *Angew. Chem. Int. Ed.* **1995**, *34*, 1765; b) D. W. P. M. Löwik, M. D. Weingarten, M. Broekema, A. J. Brouwet, W. C. Still, R. M. J. Liskamp, *Angew. Chem. Int. Ed.* **1998**, *37*, 1846; c) D. W. P. M. Löwik, S. J. E. Mulders, Y. Cheng, Y. Shao, R. M. J. Liskamp, *Tetrahedron Lett.* **1996**, *37*, 8253.

[19] a) M. Bonnat, M. Bradley, J. D. Kilburn, *Tetrahedron Lett.* **1996**, *37*, 5409; b) M. Davies, M. Bonnat, F. Guillier, J. D. Kilburn, M. Bradley, *J. Org. Chem.* **1998**, *63*, 8696; c) T. Fessmann, J. D. Kilburn, *Angew. Chem. Int. Ed.* **1999**, *38*, 1993; d) T. Braxmeier, M. Demarcus, T. Fessmann, S. McAteer, J. D. Kilburn, *Chem. Eur. J.* **2001**, *7*, 1889; e) E. Botana, S. Ongeri, R. Arienzo, M. Demarcus, J. G. Frey, U. Piarulli, D. Potenza, J. D. Kilburn, C. Giennari, *Eur. J. Org. Chem.* **2001**, 4625; f) R. Arienzo, J. D. Kilburn, *Tetrahedron* **2002**, *58*, 711; g) E. Botana, S. Ongeri, M. Demarcus, J. G. Frey, U. Piarulli, D. Potenza, C. Gennari, J. D. Kilburn, *Chem. Commun.* **2001**, 1358; h) K. B. Jensen, T. M. Braxmeier, M. Demarcus, J. G. Frey, J. D. Kilburn, *Chem. Eur. J.* **2002**, *8*, 1300; i) J. Shepherd, T. Gale, K.B. Jensen, J. D. Kilburn, *Chem. Eur. J.* **2006**, *12*, 713.

[20] a) Y. R. De Miguel, J. K. M. Sanders, *Curr. Opin. Chem. Biol.* **1998**, *2*, 417; b) H. P. Nestler, R. Liu, *Comb. Chem. High Throughput Screening* **1998**, *1*, 113.

[21] a) G. Jung, '*Combinatorial Chemistry: Synthesis, Analysis, Screening*', WILEY-VCH, **1999**; b) B. Linton, A. D. Hamilton, *Curr. Opin. Chem. Biol.* **1999**, *3*, 307; c) S. Otto, R. L. E. Furlan, J. K. M. Sanders, *Curr. Opin. Chem. Biol.* **2002**, *6*, 321; d) S. J. Rowan, S. J. Cantrill, G. R. L. Cousins, J. K. M. Sanders, *Angew. Chem. Int. Ed.* **2002**, *41*, 898; e) C. Schmuck, P. Wich, *New. J. Chem.* **2006**, *30*, 1377.

[22] a) E. W. McFarland, W. H. Weiberg, *Trends in Biotechnology* **1999**, *17*, 107; b) D. Batra, K. J. Shea, *Curr. Opin. Chem. Biol.* **2003**, *7*, 43.

[23] a) M. T. Reetz, *Angew. Chem. Int. Ed.* **2001**, *40*, 284; b) F. L. Tietze, N. Rackelmann, G. Sekar, *Ang. Chem. Int. Ed.* **2003**, *42*, 4254; c) A. Berkessel, *Curr. Opin. Chem. Biol.* **2003**, *7*, 409; d) S. J. Miller, *Acc. Chem. Res.* **2004**, *37*, 601; e) G. T. Copeland, S. J. Miller, *J. Am. Chem. Soc* **2001**, *123*, 6496; f) K. S. Griswold, S. J. Miller, *Tetrahedron* **2003**, *59*, 8869; g) M. Hechavarria Fonseca, B. List, *Curr. Opin. Chem. Biol.* **2004**, *8*, 319; h) M. Meldal, *Curr. Opin. Chem. Biol.* **2004**, *8*, 238.

[24] a) P. Krattiger, C. Mc. Carthy, A. Pfaltz, H. Wennemers, *Angew. Chem. Int. Ed.* **2003**, *42*, 1722; b) J. D. Revell, D. Gantenbein, P. Krattiger, H. Wennemers, *Biopolymers* **2006**, *84*, 105; c) P. Krattiger, R. Kovasy, J. D. Revell, H. Wennemers, *QSAR Comb. Sci.* **2005**, *24*, 1158; d) P. Krattiger, R. Kovasy, J. D. Revell, S. Ivan, H. Wennemers,

Org. Lett. **2005**, *7*, 1101; e) H. Wennemers, *Comb. Chem. & High Throughput Screening* **2001**, *4*, 273.

[25] R. A. Houghten, C. Pinilla, S. E. Blondelle, J. R. Appel, C. T. Dooley, J. H. Cuervo, *Nature* **1991**, *354*, 84.

[26] A. Furka, F. Sebestyén, M. Asgedom, G. Dibo, *Int. J. Pept. Protein Res.* **1991**, *37*, 487.

[27] K. S. Lam, S. E. Salmon, E. M. Hersh, V. J. Hruby, W. M. Kazmierski, R. J. Knapp, *Nature* **1991**, *354*, 82.

[28] M. Lebl, V. Krchnak, N. F. Sepetov, B. Seligmann, P. Strop, S. Felder, K. S. Lam, *Biopolymers* **1995**, *7*, 58.

[29] H. M. Geysen, R. H. Meloen, S. J. Barteling, *Proc. Natl. Acad. Sci. USA* **1984**, *81*, 3398.

[30] S. P. A. Fodor, J. L. Read, M. C. Pirrung, L. Stryer, A. T. Lu, D. Solas, *Science* **1991**, *251*, 767.

[31] a) J. Wang, M. Uttamchandani, H. Sun, S. Q. Yao, *QSAR Comb. Sci.* **2006**, *25*, 1009; b) K. S. Lam, M. Renil, *Curr. Opin. Chem. Biol.* **2002**, *6*, 353.

[32] P. Edman, G. Begg, *Eur. J. Biochem.* **1967**, *1*, 80.

[33] N. F. Sepetov, O. L. Issakova, M. Lebl, K. Swiderek, D. C. Stahl, T. D. Lee, *Rapid Comm. Mass Spectrom.* **1993**, *7*, 58.

[34] J. Metzger, S. Stevanovic, J. Brünjes, K.-H. Wiesmüller, *Methods* **1994**, *6*, 425.

[35] B. J. Egner, G. J. Langley, M. Bradley, *J. Org. Chem.* **1995**, *60*, 2652.

[36] a) O. Lorthioir, R. A. E. Carr, M. S. Congreve, M. H. Geysen, C. Kay, P. Marshall, S. C McKeown, N. J. Parr, J. J. Scicinski, S. P. Watson, *Anal. Chem.* **2001**, *73*, 963; b) M. G. Paulick, K. M. Hart, K. M. Brinner, M. Tjandra, D. H. Charych, R. N. Zuckermann, *J. Comb. Chem.* **2006**, *8*, 417; c) A. Song, J. Zhang, C. B. Lebrilla, K. S. Lam, *J. Am. Chem. Soc.* **2003**, *125*, 6180; d) A. H. Franz, R. Liu, A. Song, K. S. Lam, C. B. Lebrilla, *J. Comb. Chem.* **2003**, *5*, 125.

[37] R. C. Anderson, J. P. Stokes, M. J. Shapiro, *Tetrahedron Lett.* **1995**, *36*, 5311.

[38] J. K. Chen, S. L. Schreider, *Angew. Chem. Int. Ed.* **1995**, *90*, 953.

[39] a) M. C. Pirrung, K. Park, L. N. Tumey, *J. Comb. Chem.* **2002**, *4*, 329; b) N. J. Wells, M. Davies, M. Bradley, *J. Org. Chem.* **1998**, *63*, 6430; c) T. Groth, M. Renil, E. Meinjohanns, *Combinatorial Chemistry & High Throughput Screening* **2003**, *6*, 589.

[40] S. Brenner, R. A. Lerner, *Proc. Natl. Acad. Sci. USA* **1993**, *89*, 5381.

[41] a) M. H. J. Ohlmeyer, R. N. Swanson, L. W. Dillard, J. C. Reader, G. Asouline, R. Kobayashi, M. Wigler, W. C. Still, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 10922; b) H. P. Nestler, P. A. Bartlett, W. C. Still, *J. Org. Chem.* **1994**, *59*, 4723.

[42] J. K. Chen, W. S. Lane, A. W. Brauer, A. Tanaka, S. L. Schreiber, *J. Am. Chem. Soc.* **1993**, *115*, 12591.

[43] R. Frank, *Tetrahedron* **1992**, *48*, 9217.

[44] K. S. Lam, J. Wu, *Methods* **1994**, *6*, 401.

[45] C. W. Turk, *Methods* **1994**, *6*, 396.

[46] H. P. Nestler, H. Wennemers, R. Sherlock, D. L.-Y. Dong, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1327.

[47] a) K. C. Nicolaou, J. A. Pfefferkorn, H. J. Mitchell, A. J. Roecker, S. Barluenga, G.6Q. Cao, R. L. Affleck, J. E. Lillig, *J. Am. Chem. Soc.* **2000**, *122*, 9954; b) X.-Y. Xiao, Z. Paradoosh, M. P. Nova, *J. Am. Chem. Soc.* **1997**, *62*, 6059; c) A. W. Czarnik, *Curr. Opin. Chem. Biol.* **1997**, *1*, 60.

[48] P.-L. Zhao, R. Zambias, J. A. Bolognese, D. Boulton, K. T. Chapman, *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 10212.

[49] K. Burgess, A. I. Liaw, N. Wang, *J. Med. Chem.* **1994**, *37*, 2985.

[50] H. Wennemers, M. Conza, M. Nold, P. Krattiger, *Chem. Eur. J.* **2001**, *7*, 3342.

[51] M. Conza, H Wennemers, *J. Org. Chem.* **2002**, *67*, 2696.

[52] H. Wennemers, M. Nold, K. J. Kulicke, M. Neuburger, *Chem. Eur. J.* **2003**, *9*, 442.

[53] H. Wennemers, *Chimia* **2003**, *57*, 237.

[54] M. Conza, H. Wennemers, *Chem. Commun.* **2003**, *7*, 866.

[55] D. Eckhardt, M. Groenewolt, E. Krause, H. G. Börner, *Chem. Commun.* **2005**, *22*, 2814.

[56] J. Grun, J. D. Revell, M. Conza, H. Wennemers, *Bioorg. Med. Chem.* **2006**, *14*, 6197.

[57] a) A. Fürstner, K. Langemann, *J. Am. Chem. Soc.* **1997**, *119*, 9130; b) S. Miller, H. J. Blackwell, R. H. Grubbs, *J. Am. Chem. Soc.* **1996**, *118*, 9606; c) R. H. Grubb, *Prix Nobel* **2006**, *2005*, 194; d) R. H. Grubbs, T. M. Trnka, *Ruthenium in Organic Synthesis* **2004**, 153; e) R. H. Grubbs, *Tetrahedron* **2004**, *60*, 7117; f) R. R. Schrock, *Prix Nobel* **2006**, *2005*, 216; g) T. A. Kirkland, R. H. Grubbs, *J. Org. Chem.* **1997**, *62*, 7310.

[58] a) P. Gomez-Martinez, F. Guibé, F. Albericio, *Letters in Peptide Science* **2000**, *7*, 187; b) T. Maruyama, T. Ikeo, M. Ueki, *Tetrahedron Lett.* **1999**, *40*, 5031; c) A. Hartmann, K. Eisler, B. Riniker, H. H. Rink, P. Sieber, W. Rittel, *Helv. Chim. Acta* **1980**, *63*, 899.

[59] **3**: CAS [13726-69-7]; a) B. P. Gangamini, V. A. Kumar, K. N. Ganesh, *Tetrahedron* **1996**, *52*, 15017; b) M. L. Petersen, R. Vince, *J. Med. Chem.* **1989**, *32*, 1754; c) K. E. Ng, L. E. Orgel, *J. Med. Chem.* **1989**, *55*, 721.

[60] a) B. C. Borer, S. Deerenberg, H. Bieräugel, U. K. Pandit, *Tetrahedron Lett.* **1994**, *35*, 3191; b) S. J. Miller, R. H. J. Grubbs, *J. Am. Chem. Soc.* **1995**, *117*, 5855; c) T. D. Clark, M. R. Ghadiri, *J. Am. Chem. Soc.* **1995**, *117*, 12364; d) B. König, C. Horn, *Synlett* **1996**, 1013; e) M. A. McKervey, M. Pitarch, *J. Chem. Soc.*, *Chem. Commun.* **1996**, 1689; f) Z. Yang, Y. He, D. Vourloumis, H. Vallberg, K. C. Nicolaou, *Angew. Chem.* **1997**, *109*, 170; g) K. C. Nicolaou, Y. He, D. Vourloumis, H. Vallberg, Z. Yang, *Angew. Chem.* **1996**, *108*, 2554; h) D. Schinzer, A. Limberg, A. Bauer, O. M. Böhm, M. Cordes, *Angew. Chem.* **1997**, *109*, 543; i) P. Bertiano,, E. J. Sorensen, D. Meng, S. J. Danishefsky, *J. Org. Chem.* **1996**, *61*, 8000; j) A. F. Houri, Z. Xu, D. A. Cogan, A. H. Hoveyda, *J. Am. Chem. Soc.* **1995**, *117*, 2943; k) S. F. Martin, Y. Liao, H.-J. Chen, M. Pätzel, M. N. Ramser, *Tetrahedron Lett.* **1994**, *35*, 6005; l) G. Descotes, J. Ramza, J.-M. Basset, S. Pagano, E. Gentil, J. Banoub, *Tetrahedron* **1996**, *52*, 10903.

[61] a) A. Fürstner, K. Langemann, *J. Org. Chem.* **1996**, *61*, 3942; b) A. Fürstner, K. Langemann, *J. Org. Chem.* **1996**, *61*, 8746; c) A. Fürstner, N. Kindler, *Tetrahedron Lett.* **1996**, *37*, 7005; d) A. Fürstner, K. Langemann, *Synthesis* **1997**, 792; e) A. Fürstner, T. Müller, *Synlett* **1997**, 1010.

[62] R. H. Grubbs, S. J. Miller, G. C. Fu, *Acc. Chem. Res.* **1995**, *28*, 446.

[63] P. E. Schwab, R. H. Grubbs, J. W. Ziller, *J. Am. Chem. Soc.* **1996**, *118*, 100.

[64] R. R. Schrock, J. S. Murdzek, G. C. Bazan, J. Robbins, M. DiMare, M. O'Regan, *J. Am. Chem. Soc.* **1990**, *112*, 3875.

[65] a) S. F. Martin, Y. Liao, T. Rein, *Tetrahedron Lett.* **1994**, *35*, 691; b) C. M. Huwe, S. Blechert, *etrahedron Lett.* **1995**, *36*, 1621; c) H. S. Overkleeft, U. K. Pandit, *etrahedron Lett.* **1996**, *37*, 547.

[66] a) R. H. Grubbs, S. Chang, *Tetrahedron* **1998**, *54*, 4413; b) G. C. Fu, S. T. Nguyen, R. H. J. Grubbs, *J. Am. Chem. Soc.* **1993**, *115*, 9856.

[67] a) J. M. Thornton, *J. Mol. Biol.* **1981**, *151*, 261; b) S.F. Betz, *Protein Sci.* **1993**, *2*, 1551; c) R. T. Raines, *Nat. Struct. Biol.* **1997**, *4*, 424.

[68] a) L. Annis, B. Hargittai, G. Barany, *Methods in Enzymology* **1997**, *289*, 198; b) J. Eichler, R. A. Houghten, *Protein and Peptide Lett.* **1997**, *4*, 157; c) T. Shi, D. L. Rabenstein, *J. Org. Chem.* **1999**, *64*, 4590; d) T. Shi, D. L. Rabenstein, *J. Am. Chem. Soc.* **2000**, *122*, 6809.

[69] M. Bodansky, A. Bodansky, *the Practice of Peptide Synthesis*, 2nd Ed.; Springer-Verlag: New York, **1994**.

[70] a) P. W. Smith, G. Chang, W. C. Still, *J. Org. Chem.* **1998**, *53*, 1587; b) M. C. F. Monnee, A. J. Brouwer, L. M. Verbeek, A. M. A. Van-Wageningen, R. M. J. Liskamp, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1521.

[71] a) J. M. Stewart, J. D. Young, Solid Phase Peptide Synthesis, Pierce Chemical Company, Rockford, IL, **1984**; b) Merck-Biosciences (Novabiochem) Catalog **2003**, Method 3-6.

[72] a) P. Krattiger, H. Wennemers, *Synlett* **2005**, *4*, 706; b) A. R. Stettler, P. Krattiger, H. Wennemers, M. Schwarz, *Electrophoresis, in press*.

[73] A. Kamatani, L. E. Overman, *J. Org. Chem.* **1999**, *64*, 8743.

[74] For preparation of **37** see: PhD work of Philipp Krattiger, Organic Chemistry at University of Basel.

[75] J. Zhang, G. Dugeon, N. L'hermite, *Tetrahedron Lett.* **2001**, *41*, 3599.

[76] a) C. W. Lindsley, *Tetrahedron Lett.* **2002**, *43*, 4467; b) M. Vaultier, *Tetrahedron Lett.* **1983**, *24*, 763.

[77] Preparation of **44**: Group of H. G. Börner, Max Planck Institute of Colloids and Interfaces, Golm, Germany.

[78] a) I. A. Reid, *Advan. Physiol. Educ.* **1998**, *20*, 236; b) E. Hackenthal, M. Paul, D. Ganten, R. Taugner, *Physiol. Rev.* **1990**, *70*, 1067.

[79] a) M. Manea, G. Mezö, F. Hudecz, M. Przybylski, *Biopolymers* **2004**, *76*, 503; b) J. McLaurin, R. Cecal, M. E. Kierstead, X. Tian, A. L. Phinney, M. Manea, J. E. French, M. H. L. Lambermon, A. A. Darabie, M. E. Brown, C. Janus, M. A. Chishti, P. Horne, D. Westaway, P. E. Fraser, H. T. J. Mount, M. Przybylski, P. St George-Hyslop, *Nature Med.* **2002**, *8*, 1263.

[80] Thanks to Dr Patricia Durieux for the preparation of **48** – **54** and **56**.

[81] H. Wennemers, W. C. Still, *Tetrahedron Lett.* **1994**, *35*, 6413.

[82] a) M. Kantlehner, D. Finsinger, J. Meyer, P. Schaffner, A. Jonczyk, B. Diefenbach, B. Nies, H. Kessler, *Angew. Chem. Int. Ed.* **1999**, *38*, 560; b) C. D. Buckley, D. Pilling, N. V. Henriquez, G. Parsonage, K. Threlfall, D. Scheel-Toellner, D. L. Simmons, A. N. Akbar, J. M. Lord, M. Salmon, *Nature* **1999**, *397*, 534; c) M. J. Janssen, W. J. Oyen, I. Dijkgraaf, L. F. Massuger, C. Frielink, D. S. Edwards, M. Rajopadhye, H. Bonnstra, F. H. Corstens, O. C. Boerman, *Cancer Research* **2002**, *62*, 6146; d) R. Haubner, H. J. Wester, F. Burkhart, R. Senekowitsch-Schmidtke, W. Weber, S. L. Goodman, H. Kessler, M. Schwaiger, *J. Nucl. Med.* **2001,** *42*, 326; e) S. L. Hart, A. M. Knight, R. P.

Harbottle, A. Mistry, H.-D. Hunger, D. F. Cutler, R. Williamson, C. Coutelle, *J. Biol. Chem.* **1994**, *17*, 12468; f) P. Lanza, B. Felding-Habermann, Z. M. Ruggeri, M. Zanetti, R. Billetta, *Blood Cells, Molecules, and Diseases* **1997**, *23*, 230.

[83] B. R. Peterson, F. Diederich, *Angew. Chem. Int. Ed.* **1994**, *33*, 1625.

[84] S. C. McCleskey, M. J. Griffin, S. E. Schneider, J. T. McDevitt, E. V. Anslyn, *J. Am. Chem. Soc.* **2003**, *125*, 1114.

[85] U. Römling, M. Gomelsky, M. Y. Galperin, *Mol. Microbiol.* **2005**, *57*, 629.

[86] A. T. Wright, M. J. Griffin, Z. Zhong, S. C. McCleskey, E. V. Anslyn, J. T. McDevitt, *Angew. Chem. Int. Ed.* **2005**, *44*, 6375.

[87] a) K. Kita-Tokarczyk, J. Grumelard, T. Haefele, W. Meier, *Polymer* **2005**, *46*, 3540; b) A. Graff, S. Benito, C. Vebert, W. Meier, *Nanobiotechnology* **2004**, 168.

[88] a) C. Nardin, D. Bolikal, J. Kohn, *Langmuir* **2004**, *20*, 11721; b) G. Battaglia, A. J. Rian, *Macromolecules* **2006**, *39*, 798; c) H. Bermudez, H. Aranda-Espinoza, D. A. Hammer, D. E. Discher, *Europhys. Lett.* **2003**, *64*, 550; d) J. Wu, A. Eisenberg, *J. Am. Chem. Soc.* **2006**, *128*, 2880; e) S. Jain, F. S. Bates, *Macromolecules* **2004**, *37*, 1511; f) S. Schrage, R. Sigel, H. Schlaad, *Macromolecules* **2003**, *36*, 1717.

[89] a) PhD work of Alexandra Graff, Physical Chemistry at University of Basel; b) X. Chen, X. Ding, Z. Zheng, Y. Peng, *New J. Chem.* **2006**, *30*, 577; c) A. Choucair, C. Lavigueur, A. Eisenberg, *Langmuir* **2004**, *20*, 3894; d) F. T. Liu, A. Eisenberg, *J. Am. Chem. Soc.* **2003**, *125*, 15059; e) O. Terreau, L. B. Luo, A. Eisenberg, *Langmuir* **2003**, *19*, 5601; f) D. E. Discher, A. Eisenberg, *Science* **2002**, *297*, 967.

[90] a) S. Lecommandoux, O. Sandre, F. Chécot, J. Rodriguez-Hernandez, R. Perzynski, *Adv. Mater.* **2005**, *17*, 712; b) F. Chécot, S. Lecommandoux, Y. Gnanou, H.-A. Klok, *Angew. Chem. Int. Ed.* **2002**, *41*, 1340; c) J. Rodriguez-Hernandez, S. Lecommandoux, *J. Am. Chem. Soc.* **2005**, *127*, 2026.

[91] J.-F. Gohy, G. G. Bas Lohmeijer, U. S. Schubert, *Macromolecules* **2002**, *35*, 4560.

[92] a) Rapp Polymere, Tübingen (Germany); b) D. Hudson, *J. Comb. Chem.* **1999**, *1*, 403.

[93] a) M. Sartor, *Dynamic Light Scattering*, University of California San Diego; b) B. J. Berne, R. Pecora, "*Dynamic Light Scattering: with Applications to Chemistry, Biology, and Physics*" John Wiley **1975**; c) www.ap-lab.com; d) B. H. Zimm, *J. Chem. Phys.* **1948**, *16*, 1093.

[94] a) E. Ruska, *Rev. Mod. Phys.* **1987**, *59*, 627; b) E. F. Burton, J. Hillier, A. Prebus, *Physical Review* **1939**, *56*, 1171; c) http://nobelprize.org.

[95] a) T. R. Albrecht, S. Akamine, T. E. Carver, C. F. Quate, *J. Vac. Sci. Technol. A* **1990**, *8*, 3386; b) T. R. Albrecht, P. Grütter, D. Horne, D. Rugar, *J. Appl. Phys.* **1991**, *69*, 668; c) S. Alexander, L. Hellemans, O. Marti, J. Schneir, V. Elings, P. K. Hansma, M. Longmiro, J. Gurley, *J. Appl. Phys.* **1989**, *65*, 164; d) G. Binnig, C. F. Quate, Ch. Gerber, *Phys. Rev. Lett.* **1986**, *56*, 930; e) G. Meyer, N. M. Amer, *Appl. Phys. Lett.* **1988**, *53*, 1045; f) G. Meyer, N. M. Amer, *Appl. Phys. Lett.* **1990**, *57*, 2089; g) C. A. J. Putman, B. G. De Grooth, N. F. Van Hulst, J. Greve, *J. Appl. Phys.* **1992**, *72*, 6; h) A. L. Weisenhorn, P. K. Hansma, T. R. Albrecht, C. F. Quate, *Appl. Phys. Lett.* **1989**, *54*, 2651; i) http://www.chembio.uoguelph.ca/educmat/chm729/afm/firstpag.htm; j) http://www.che.utoledo.edu/nadarajah/webpages/whatsafm.html.

[96] a) L. Chen, H. Shen, A. Eisenberg, *J. Phys. Chem. B* **1999**, *103*, 9488; b) Y. Yu, L. Zhang, A. Eisenberg, *Langmuir* **1997**, *13*, 2578.

[97] a) I. Langmuir, *J. Am. Chem. Soc.* **1917**, *39*, 1848; b) K. B. Blodgett, *J. Am. Chem. Soc.* **1935**, *57*, 1007; c) G. L. Gaines, "*Insoluble Monolayers at the Liquid-Gas Interface*" **1966**, Wiley-Interscience, New York; d) B. P. Binks, *Adv. Colloid Interface Sci.* **1991**, *34*, 343.

[98] D. D. Lasic, F. J. Martin, *Editors. Stealth liposomes* **1995**.

[99] E. Kaiser, R. L. Colescott, C. D. Bossinger, P. I. Cook, *Anal. Biochem.* **1970**, *34*, 595.

D. Listing of the Sequences Found in the Screenings of the Peptide Libraries

Sequences found in the screening of the side-chain deprotected tripeptide library Ac-AA3-AA2-AA1-NH$(CH_2)_5$CONH-PS with macrocyclic diketopiperazine receptors **17a**, **17b**, **20**, **23** and **26**.

Macrocyclic diketopiperazine receptor **17a**:

50 µmol in $CHCl_3$, 4 days

	AA3	**AA2**	**AA1**
Ac	L-Ser	L-Pro	L-Pro
Ac	L-Ser	D-Pro	D-Pro
Ac	D-Gln	D-Pro	D-Pro
Ac	D-His	D-Phe	D-Pro
Ac	L-Phe	L-Leu	L-Asn

50 µmol in $CHCl_3$, 7 days

	AA3	**AA2**	**AA1**
Ac	Gly	D-Lys	D-Pro
Ac	Gly	D-Val	D-Gln
Ac	L-Val	L-Phe	L-Gln
Ac	L-Phe	D-Phe	L-Lys
Ac	D-Pro	L-Pro	L-Lys
Ac	L-Pro	D-Pro	D-Lys
Ac	L-Thr	L-Pro	D-Lys
Ac	D-Gln	L-Pro	D-Lys
Ac	L-Pro	D-Lys	D-Lys
Ac	L-Ser	D-Ala	D-Lys
Ac	L-Ala	D-His	D-Lys
Ac	L-Arg	D-His	D-Lys
Ac	L-Val	L-Asn	D-Lys
Ac	L-Arg	L-Asn	L-Lys
Ac	D-Lys	L-Ala	L-Arg
Ac	D-Glu	D-Arg	D-Glu

Macrocyclic diketopiperazine receptor **17b**:

100 µmol in $CHCl_3$

	AA3	**AA2**	**AA1**
Ac	D-Arg	D-Thr	D-Phe
Ac	D-Phe	D-Thr	D-Gln
Ac	D-Phe	L-Phe	L-Lys
Ac	D-Phe	L-Lys	L-Phe
Ac	D-Phe	L-Lys	D-Pro
Ac	L-Pro	D-Phe	L-Lys
Ac	L-Leu	D-Lys	L-Pro
Ac	D-Lys	L-Pro	L-Val
Ac	D-Lys	L-Pro	L-Lys
Ac	L-Lys	L-Pro	L-Pro
Ac	D-Pro	D-Lys	D-Pro
Ac	D-Pro	D-Pro	L-Gln
Ac	D-Pro	L-Pro	D-Gln
Ac	D-Gln	L-Pro	D-Pro
Ac	D-Ala	L-Pro	D-Pro
Ac	D-Ser	D-Gln	D-Pro
Ac	D-Ser	D-Lys	L-Lys
Ac	D-Ser	D-Lys	D-Lys
Ac	D-Arg	D-Ser	L-Gln
Ac	D-His	L-Arg	L-Lys

Macrocyclic diketopiperazine receptor **20**

50 µmol in $CHCl_3$

	AA3	**AA2**	**AA1**
Ac	L-Asp	L-Glu	L-Gln
Ac	D-Asp	L-Pro	L-Gln
Ac	D-Thr	L-Pro	L-Lys
Ac	D-Pro	D-Lys	L-Pro

100 µmol in $CHCl_3$

	AA3	**AA2**	**AA1**	
Ac	D-Lys	D-Pro	L-Pro	4x
Ac	D-Thr	D-Pro	D-Pro	
Ac	L-Ala	L-Arg	L-Pro	
Ac	L-Leu	L-Pro	D-Pro	
Ac	L-Lys	D-Pro	D-Pro	
Ac	L-Lys	L-Pro	L-Pro	
Ac	L-Lys	D-Pro	D-Phe	
Ac	L-Pro	L-Pro	D-Pro	
Ac	L-Thr	D-Pro	L-Pro	
Ac	L-Thr	L-Pro	D-Pro	

Macrocyclic diketopiperazine receptor **23**

30 µM in $CHCl_3$

	AA3	**AA2**	**AA1**	
Ac	L-Ala	L-Asp	D-Phe	
Ac	L-Ala	L-Asp	L-Lys	
Ac	D-Ala	L-Asp	L-Ala	
Ac	D-Gln	D-Phe	D-Val	
Ac	D-Gln	D-Phe	D-Thr	
Ac	D-Gln	D-Lys	D-Phe	
Ac	D-Gln	D-Leu	D-Phe	
Ac	D-Gln	D-Leu	L-Gln	

100 µM in $CHCl_3$, 2 days

	AA3	**AA2**	**AA1**	
Ac	D-Ala	L-Gln	L-Ala	
Ac	D-Gln	D-Leu	D-Leu	
Ac	D-Gln	D-Ala	D-Phe	
Ac	D-Gln	D-Ala	D-Leu	3x
Ac	L-Leu	L-Gln	D-Ala	

100 µM in $CHCl_3$, 3 days

	AA3	**AA2**	**AA1**	
Ac	D-Ala	L-Gln	L-Ala	
Ac	L-Ala	L-Gln	L-Ala	3x
Ac	L-Ala	L-Gln	D-Ala	
Ac	D-Gln	D-Val	D-Val	
Ac	D-Gln	D-Phe	D-Val	
Ac	D-Gln	D-Phe	D-Leu	
Ac	D-Gln	D-Ala	D-Leu	
Ac	L-Ala	L-Gln	D-Leu	
Ac	L-Arg	L-Gln	L-Leu	
Ac	L-Leu	L-Arg	D-Ser	
Ac	Gly	L-Gln	L-Ala	
Ac	Gly	L-Gln	D-Ala	
Ac	Gly	L-Gln	D-Phe	

Macrocyclic diketopiperazine receptor **26**

100 µM in $CHCl_3$

	AA3	**AA2**	**AA1**	
Ac	D-His	L-Lys	L-Gln	
Ac	D-Arg	L-Lys	D-Gln	
Ac	L-Arg	D-Gln	D-Leu	
Ac	L-Arg	D-Gln	L-Leu	
Ac	L-Arg	L-Pro	D-Gln	
Ac	D-Arg	D-Pro	D-Gln	
Ac	D-Arg	L-Ser	D-Gln	
Ac	L-Gln	L-Arg	D-Gln	
Ac	D-Thr	L-Gln	L-Arg	
Ac	D-Arg	L-Thr	L-Arg	
Ac	D-His	L-Ser	L-Arg	
Ac	D-Pro	L-Leu	L-Arg	2x
Ac	L-Ser	D-Arg	L-Arg	
Ac	D-Ser	Gly	D-Arg	

50 µM in $CHCl_3$

	AA3	**AA2**	**AA1**
Ac	L-Thr	D-Gln	D-Arg
Ac	L-Lys	L-His	D-Gln
Ac	D-Val	D-Ala	D-Gln
Ac	L-Thr	L-Pro	D-Gln
Ac	D-Thr	L-Asn	D-Gln
Ac	D-Leu	D-Asn	L-Gln
Ac	D-Gln	L-Asn	L-Gln
Ac	D-Arg	L-Pro	L-Gln
Ac	L-Arg	L-Arg	L-Gln
Ac	D-Pro	D-Arg	L-Gln
Ac	D-Gln	D-Arg	D-Pro
Ac	D-Phe	L-Arg	L-Pro
Ac	D-Arg	D-Asn	L-Pro
Ac	L-Gln	L-Leu	L-Asn

100 µM in $CHCl_3$, 2 days

	AA3	**AA2**	**AA1**
Ac	L-Ser	L-Pro	L-Pro
Ac	D-Thr	L-Pro	L-Pro
Ac	L-Phe	D-Pro	D-Pro
Ac	D-Glu	D-Pro	D-Pro
Ac	D-Pro	L-Pro	D-Gln
Ac	D-Pro	L-His	D-Gln

100 µM in $CHCl_3$, 3 days

	AA3	**AA2**	**AA1**	
Ac	L-Phe	L-Ser	D-Gln	
Ac	L-Thr	D-Pro	D-Gln	
Ac	D-Pro	L-Pro	L-Gln	2x
Ac	L-Arg	L-Pro	L-Gln	
Ac	D-Pro	L-His	L-Gln	
Ac	D-Thr	L-His	L-Gln	
Ac	D-Thr	L-His	D-Gln	
Ac	L-Lys	D-Val	D-Gln	
Ac	D-Pro	L-Arg	L-Pro	
Ac	D-Asp	L-Pro	L-Pro	

100 µM in $CHCl_3$, 4 days

	AA3	**AA2**	**AA1**
Ac	L-Phe	L-Val	L-Gln
Ac	L-Arg	D-Ala	D-Gln
Ac	Gly	L-His	D-Gln
Ac	D-Lys	L-His	D-Gln
Ac	D-Gln	L-His	L-Arg
Ac	L-Thr	D-Lys	L-Arg
Ac	L-Asn	L-Ala	L-Arg
Ac	D-Phe	L-Ala	L-Arg
Ac	D-Val	L-Pro	L-Arg
Ac	D-Val	D-Pro	L-Arg
Ac	L-Asn	D-Arg	D-Val
Ac	L-Phe	D-Arg	D-Asn
Ac	L-Lys	D-Arg	L-Pro
Ac	L-Thr	D-Arg	D-Pro

50 µM in $CHCl_3$, 2 days

	AA3	**AA2**	**AA1**
Ac	L-Ser	L-Pro	L-Pro
Ac	D-Thr	L-Pro	L-Pro

50 µM in $CHCl_3$, 3 days

	AA3	**AA2**	**AA1**	
Ac	L-Thr	L-Pro	L-Gln	
Ac	D-Pro	L-Pro	L-Gln	
Ac	D-Pro	L-Pro	L-Asn	
Ac	L-Pro	D-Pro	D-Val	
Ac	L-Pro	L-His	L-Gln	
Ac	L-Pro	D-Gln	D-Ala	
Ac	L-Asp	D-Pro	D-Pro	
Ac	D-Pro	L-Pro	D-Pro	2x

50 µM in $CHCl_3$, 4 days

	AA3	**AA2**	**AA1**
Ac	D-Asn	L-Phe	L-Phe
Ac	D-Arg	L-Val	L-Arg
Ac	D-Arg	D-Pro	L-Arg
Ac	D-Arg	L-Gln	L-Phe
Ac	D-Arg	D-Val	L-Gln
Ac	L-Arg	D-Arg	L-Gln
Ac	D-Thr	D-Pro	D-Gln
Ac	D-Pro	D-Asn	D-Gln
Ac	D-Pro	D-Arg	D-Gln
Ac	L-Leu	D-Val	D-Gln
Ac	D-His	D-Arg	D-Val
Ac	L-Gln	D-Phe	L-Phe

Sequences found in the assays of the side-chain deprotected tripeptide library and open-chain diketopiperazine receptors **16**, **22** and **25**.

Open-chain diketopiperazine receptor **16**:

100 µmol in $CHCl_3$

	AA3	**AA2**	**AA1**	
Ac	D-Gln	D-Ala	D-Val	
Ac	D-Gln	D-Phe	D-Ser	
Ac	D-Ala	L-Gln	L-Ser	2x
Ac	Gly	L-Gln	L-Ser	2x
Ac	L-Ala	L-Gln	D-Ser	
Ac	L-Leu	L-Gln	D-Ala	
Ac	L-Ala	L-Gln	L-Ala	
Ac	D-Ala	D-Phe	D-Gln	5x

Open-chain diketopiperazine receptor **22**:

100 µmol in $CHCl_3$

	AA3	**AA2**	**AA1**	
Ac	D-Gln	D-Val	D-Val	3x

Open-chain diketopiperazine receptor **25**:

100 µmol in $CHCl_3$

	AA3	**AA2**	**AA1**	
Ac	D-Val	D-Val	D-His	
Ac	L-Arg	L-Leu	D-Gln	
Ac	L-Ala	L-Gln	D-Thr	2x
Ac	D-Ala	L-Gln	Gly	
Ac	Gly	L-Gln	Gly	
Ac	L-Ala	L-Ser	L-Leu	
Ac	D-Asn	L-Asn	L-Gln	
Ac	D-Val	D-Gln	L-Asn	
Ac	Gly	L-Val	D-Phe	

50 µmol in $CHCl_3$

	AA3	**AA2**	**AA1**	
Ac	D-Gln	D-Phe	D-Val	3x
Ac	D-Gln	D-Val	D-Leu	
Ac	L-Leu	L-Gln	D-Ser	
Ac	L-Val	L-Ala	D-Asn	
Ac	Gly	D-Asn	D-Leu	

100 µmol in $CHCl_3$, 2 days

	AA3	**AA2**	**AA1**
Ac	D-Pro	L-Gln	D-Pro
Ac	D-Pro	D-Gln	D-Pro
Ac	L-Pro	D-Leu	D-Asn
Ac	L-Phe	D-Pro	D-Asn
Ac	L-Ala	L-Asn	L-Phe
Ac	D-Phe	L-Pro	L-Leu
Ac	L-Pro	D-Pro	D-Pro

100 µmol in $CHCl_3$, 3 days

	AA3	**AA2**	**AA1**
Ac	L-Ala	L-Gln	D-Thr
Ac	L-Ala	L-Gln	D-Ala
Ac	L-Pro	D-Pro	D-Pro

100 µmol in $CHCl_3$, 4 days

	AA3	**AA2**	**AA1**	
Ac	D-Asp	D-Phe	D-Val	2x

50 µmol in $CHCl_3$, 2 days

	AA3	**AA2**	**AA1**
Ac	L-Gln	L-Pro	L-Pro
Ac	D-Ala	L-Pro	L-Gln
Ac	L-Pro	D-Pro	L-Gln
Ac	D-Pro	L-Pro	D-Gln
Ac	L-Thr	L-Pro	L-Pro

50 µmol in $CHCl_3$, 3 days

	AA3	**AA2**	**AA1**
Ac	L-Ala	L-Gln	D-Leu
Ac	L-Ala	L-Gln	D-Ser
Ac	L-Asp	D-Pro	D-Pro
Ac	L-Phe	D-Pro	D-Pro
Ac	D-Pro	L-Pro	D-Pro
Ac	L-Pro	L-Pro	L-Gln

50 µmol in $CHCl_3$, 4 days

	AA3	**AA2**	**AA1**
Ac	L-Ala	L-Gln	D-Ser
Ac	L-Ala	L-Gln	D-Ala

Sequences found in the screening of the side-chain deprotected tripeptide library Ac-AA3-AA2-AA1-$NH(CH_2)_5CONH$-PS with two-armed carbazole receptors **39** - **42**.

Two-armed carbazole receptor **39**:

50 µmol in $CHCl_3$

	AA3	**AA2**	**AA1**	
Ac	D-Gln	D-Phe	D-Val	
Ac	D-Gln	D-Leu	D-Leu	

100 µmol in $CHCl_3$

	AA3	**AA2**	**AA1**	
Ac	D-Gln	D-Val	D-Phe	
Ac	D-Gln	D-Phe	D-Val	2x

Two-armed carbazole receptor **40**:

100 µmol in $CHCl_3$

	AA3	**AA2**	**AA1**	
Ac	L-Ala	L-Gln	D-Ala	2x
Ac	D-Gln	D-Phe	D-Ala	
Ac	L-Ala	L-Ala	Gly	
Ac	D-Val	D-Leu	D-Leu	

50 µmol in $CHCl_3$

	AA3	**AA2**	**AA1**	
Ac	L-Ala	L-Gln	D-Ala	3x
Ac	D-Val	D-Ala	D-His	
Ac	D-Val	D-Leu	D-His	

1 mmol in $CHCl_3$

	AA3	**AA2**	**AA1**	
Ac	D-His	L-Gln	D-Phe	
Ac	L-Ala	L-Gln	D-Ala	
Ac	D-Val	D-Leu	D-His	
Ac	D-Gln	D-Leu	D-Val	
Ac	D-Gln	D-Val	D-Leu	

Two-armed carbazole receptor **41**:

100 µmol in $CHCl_3$, 2 days

	AA3	**AA2**	**AA1**	
Ac	D-Ala	D-Val	D-His	
Ac	D-Val	D-Leu	D-His	
Ac	D-Val	D-Val	D-His	2x
Ac	D-Val	D-Phe	D-His	2x
Ac	D-Ala	D-Phe	D-His	
Ac	D-Leu	D-Phe	D-His	
Ac	D-Phe	D-Val	D-His	3x
Ac	D-His	D-Ala	D-Val	2x
Ac	D-Ala	D-Phe	D-His	
Ac	D-Gln	D-Phe	D-Phe	2x
Ac	D-Gln	D-Phe	D-Val	3x
Ac	D-Gln	D-Phe	D-Leu	

50 µmol in $CHCl_3$, 2 days

	AA3	**AA2**	**AA1**	
Ac	D-Val	D-Ala	D-His	
Ac	D-Phe	D-Phe	D-His	
Ac	D-Phe	D-Val	D-His	
Ac	D-Gln	D-Phe	D-Val	2x
Ac	D-Gln	D-Phe	D-Leu	
Ac	D-Gln	D-Val	D-Leu	2x
Ac	D-Val	D-Ala	L-Ser	

50 µmol in $CHCl_3$, 5 days

	AA3	**AA2**	**AA1**	
Ac	D-Val	D-Leu	D-His	
Ac	D-Val	D-Val	D-His	
Ac	D-Gln	D-Phe	D-Val	
Ac	D-Gln	D-Val	D-Leu	
Ac	D-Gln	D-Ala	D-Val	
Ac	L-Val	L-Gln	D-Thr	
Ac	L-Val	L-Gln	D-Val	3x
Ac	L-Ala	L-Gln	D-Ala	2x
Ac	L-His	L-Gln	L-Ala	
Ac	L-Gln	L-Ser	D-Ser	

Two-armed carbazole receptor **42**:

100 µmol in $CHCl_3$

	AA3	**AA2**	**AA1**
Ac	D-Val	D-Ala	D-His
Ac	D-Val	D-Val	D-His
Ac	D-Val	D-Phe	D-His
Ac	D-Phe	D-Val	D-His
Ac	D-Phe	D-Phe	D-His

Dipl. Ing Jessica Grun

Eidesstattliche Erklärung

Ich erkläre, dass die Dissertation mit dem Titel:
"**Two-Armed Molecular Receptors –**
Peptide Recognition and Vesicle Formation
Driven by Selective Non-Covalent Interactions"
nur mit der darin angegebenen Hilfe verfasst
und bei keiner anderen Fakultät eingereicht wurde.

Basel, den 30. Januar 2007 Jessica Grun

The following lecturers contributed to my education:

University of Mulhouse: D. Bolmont, J. Brendlé-Miéhé, L. Delfosse, Z. Gabelica, M. Hamieh, B. Muckensturm, J. Schultz.

Ecole Nationale Supérieure de Chimie de Mulhouse: A. Accary, H. Aleboyed, X. Allonas, P. Caullet, J.M. Chezeau, P. Dumas, J. Eustache, J.P. Fouassier, A. Kalt, G. Killé, R. Le Dred, C. Le Drian, A. Louati, J.C. Mougenel, S. Neunlist, E. Penigault, J. Streith, C. Tarnus, D. Lougnot.

University of Basel: H. Wennemers.

Curriculum Vitae

- **Nationality:** French
- **Place of birth:** Mulhouse (France)
- **Date of birth:** 1st of October 1978

EDUCATION

- **Nov. 2002 – Feb. 2007**: **PhD Student in Organic Chemistry**, Basel/Switzerland

  > **Supervisor:** Prof. Dr. H. Wennemers

- **June 2002**: **DEA in Chemistry**, Mulhouse/France

  > Diplôme d'Etudes Approfondies specialising in Organic and Bioorganic Chemistry at UHA (Université de Haute-Alsace) in Mulhouse

- **Sept. 1998 - June 2002**: **Chemical Engineering Diploma**, Mulhouse/France

  > 4 years at the Ecole Nationale Supérieure de Chimie de Mulhouse, ENSCMu (Higher National School in Muhlouse)

- **June 2000**: **Maîtrise in Chemistry**, Mulhouse/France

  > MSc. Equivalent specialising in Physical Chemistry at UHA (Université de Haute-Alsace) in Mulhouse

- **June 1999**: **Licence in Chemistry**, Mulhouse/France

  > BSc. Equivalent in Physical Chemistry at UHA (Université de Haute-Alsace) in Mulhouse

- **Sept. 1996 - June 1998**: **DEUG in Sciences**, Mulhouse/France

  > Diplôme d'Etude Universitaire Général, 2-year university diploma specialising in Maths, Chemistry and Physics, at Faculté des Sciences de Mulhouse

- **June 1996**: **Baccalauréat**, Mulhouse/France

  > Equivalent 'A' levels majoring in biology, chemistry and physics

PROFESSIONAL EXPERIENCE

- **Feb. 2002 - Oct. 2002**: **Training Course**, Basel/Switzerland

  > Supervisor: Dr. T. Masquelin, Department of Combinatorial Chemistry at Basilea Pharmaceutica, Basel/Switzerland

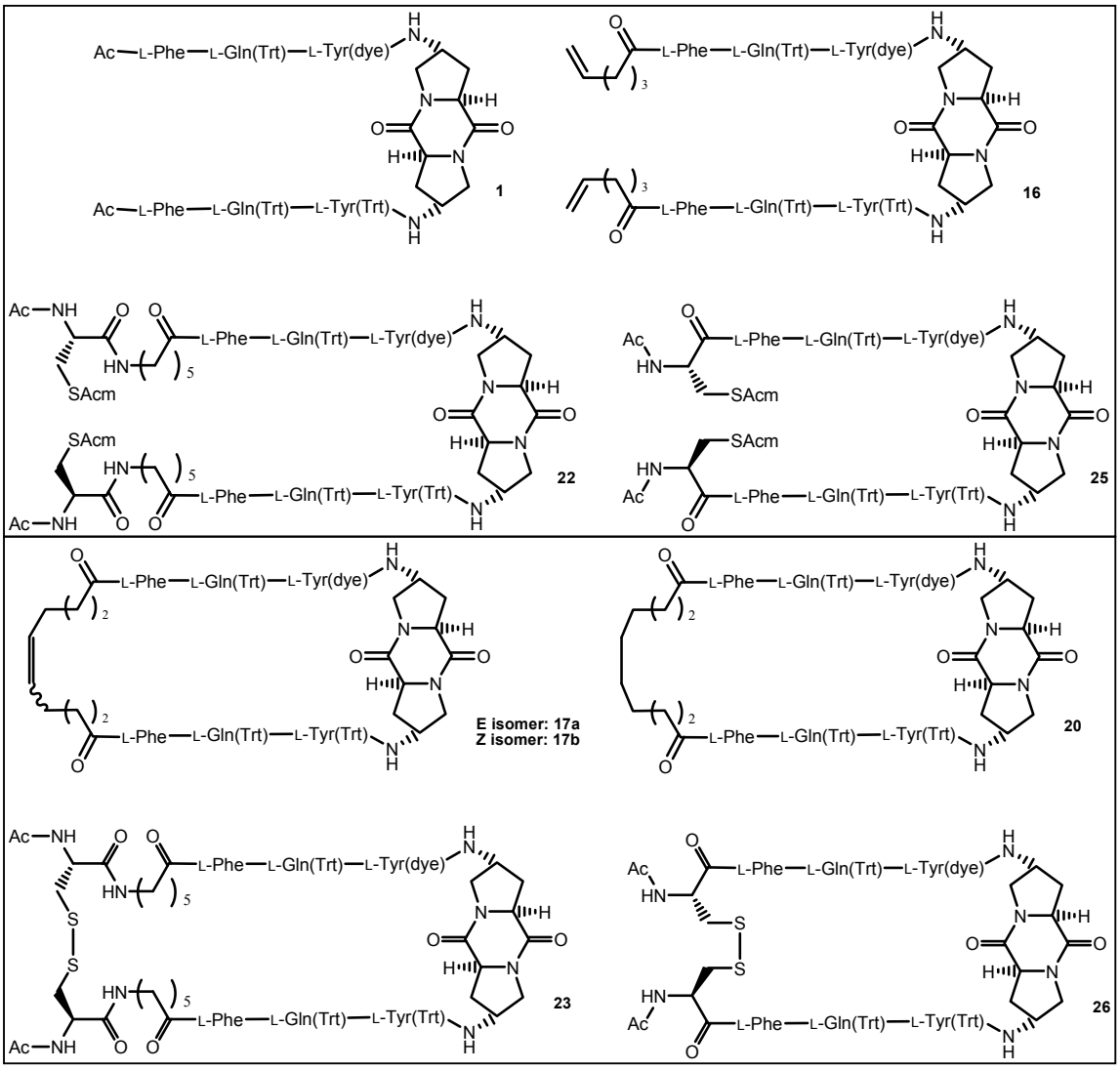
- **July 2000 - August 2001**: **Training Course**, Basel/Switzerland

  > Supervisor: Dr. M. Stumpf, Research and Development at Clariant AG, Basel/Switzerland

- **January 2004 – June 2007**: **Teaching assistant**

  > Teaching assistant for the mandatory organic practical course for students in Biochemistry, Biology and Pharmacy.

Chapter 2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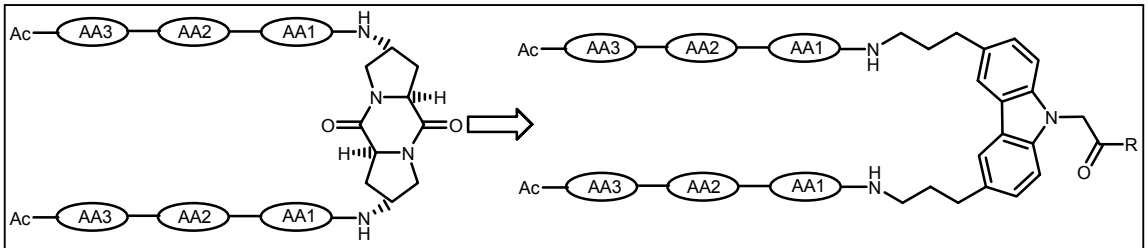
Ac—L-Phe—L-Gln(Trt)—L-Tyr(Trt)
1
L-Phe—L-Gln(Trt)—L-Tyr(dye)
L-Phe—L-Gln(Trt)—L-Tyr(Trt)
16
SAcm
22
25
E isomer: 17a
Z isomer: 17b
20
23
26

Chapter 3

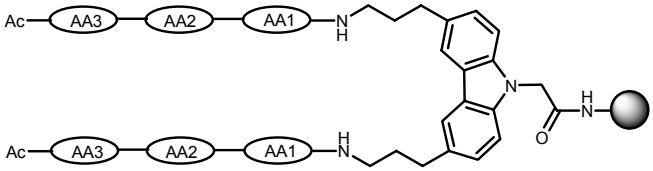


Diketopiperazine Receptors 1 and 27

Carbazole Receptors 39 - 42

AA3	AA2	AA1	R	
L-Phe	L-Gln(Trt)	L-Tyr(dye)		**1**
L-Phe	L-Asn(Trt)	L-Tyr(dye)		**27**
L-Phe	L-Gln(Trt)	L-Tyr(dye)	$-O-CH_2CH_3$	**39**
L-Phe	L-Gln(Trt)	L-Tyr(tBu)	-NH-dye	**40**
L-Phe	L-Gln(Trt)	L-Tyr(dye)	$-NH-PEG_{750}$	**41**
L-Phe	L-Asn(Trt)	L-Tyr(dye)	$-NH-PEG_{750}$	**42**

Carbazole receptor library:



Fluorophore labelled **Angiotensin II** peptide derivatives:

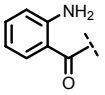
R-$(OCH_2CH_2)_4$-OCO-NH$(CH_2)_2$CO-**Ile-His-Pro-Phe**-OH **45**

R-$(OCH_2CH_2)_4$-OCO-NH$(CH_2)_2$CO-**Ile-His-Pro-Phe**-NH_2 **46**

R-$(OCH_2CH_2)_4$-OCO-NH$(CH_2)_2$CO-**Asp-Arg-Val-Tyr-Ile-His-Pro-Phe**-OH **47**

R-$(OCH_2CH_2)_4$-OCO-NH$(CH_2)_2$CO-**Asp-Arg-Val-Tyr-Ile-His-Pro-Phe**-NH_2 **48**

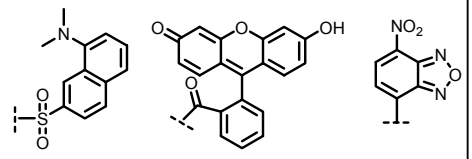
R = Abz =



Fluorophore labelled **Alzheimer's** peptide derivatives:

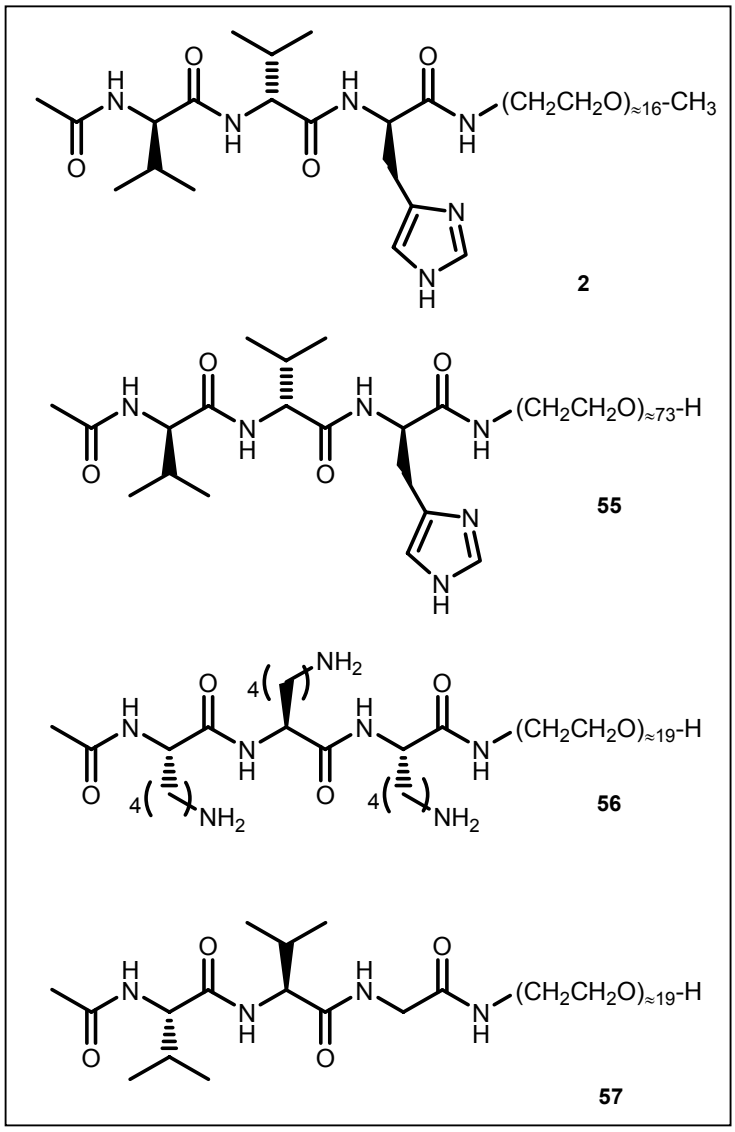
R-Gly_5-Phe-Arg-His-Asp-Ser-Gly-Tyr-NH_2

R = **DNS** **FAM** **NBD**



49 **50** **51**

Chapter 4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2
(CH2CH2O)≈73-H
55
NH2
(CH2CH2O)≈19-H
56
(CH2CH2O)≈19-H
57